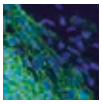
Research Report 2016

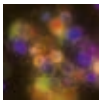
MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Image Captions

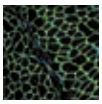


Cover: What looks like coral reefs and brightly glowing fishes in this picture are actually induced pluripotent stem cells in a pluripotency test. The cells are stained with a fluorescent dye (Lin28-DAPI).
Credit: Gizem Inak (Lab of Erich Wanker, Group of Alessandro Prigione)

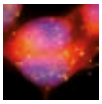
Cardiovascular and Metabolic Disease: These fireworks and balloons are fatty cells. Insulin receptors appear red and early endosomes green. Nuclei are blue and lipid droplets white.
Credit: Vanessa Schmidt (Lab of Thomas Willnow)



Cancer Research: The colorful confetti of tumor cells which has been stained with different fluorescent dyes.
Credit: Matthias Leisegang (formerly Lab of Wolfgang Uckert)

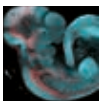


Diseases of the Nervous System: Under the microscope the cross-section of mouse skeletal muscle looks has a sponge-like structure. Muscle stem cells stained by an antibody against Pax7 (blue) are located under the basal lamina (green).
Credit: Dominique Bröhl (formerly Lab of Carmen Birchmeier)



BIMSB: Reminiscent of faraway galaxies: cancer cells found in malignant brain tumors produce circular RNA (circRNA), a type of molecule we know relatively little about. CircRNAs in yellow next to the pink cell bodies with blue nuclei.
Credit: Christin Stottmeister (Lab of Nikolaus Rajewsky)

ECRC: This image depicts human myoblasts and myotubes derived from a biopsy. Myosin heavy chain (MyHC) protein of fused muscle cells is stained with a green fluorescent dye; nuclei appear in blue.
Credit: Eric Metzler, PhD student (Lab of Simone Spuler)



Technology Platforms: The 3-D-mouse embryo is a reconstruction made up of 360 individual images, the developing nervous system appears in orange.
Credit: Julian Heuberger (Lab of Walter Birchmeier), Anje Sporbert (Advanced Light Microscopy (ALM) technology platform)

Research Report 2016
(covers the period 2014-2016)

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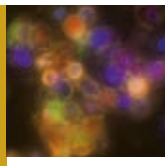

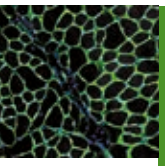

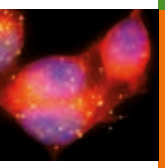

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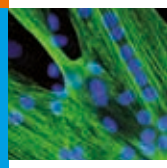

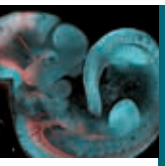

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Introduction by the Scientific Director

Prof. Dr. Martin Lohse

Life in our labs deals with fundamental questions of health and disease, while at the same time focusing on small details – such as the intricate workings of an organism, a cell and even an ion channel or receptor. At first sight, such basic research may seem abstract. However, medicine cannot advance without uncovering the basic processes that underlie life.

Exploring these mechanisms and translating our findings as quickly as possible into clinical applications has been our mission from the very beginning. As we celebrate our 25th anniversary in 2017, we are reflecting on our achievements and formulating a vision for the future.

Health research has traditionally been approached in an organ-specific manner. In line with this, we have been active in cardiovascular and neuroscience research as well as cancer research. However, most severe diseases cannot be understood or treated if we consider just the organ that seems to be primarily affected. A particularly good example is chronic heart failure, where we find not only a weak heart, but also changes in blood vessels and hormonal systems, in kidneys and brain and even in the immune system. And as a consequence, heart failure therapies work only if we consider the organism as a whole and address all of these complex and interrelated mechanisms.

At the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), we bring together a wide range of expertise and technology. To fully exploit our scientific potential, we aim to integrate our activities in one common research program with a focus on system-wide and cardiovascular disease mechanisms.

Within this program, we will concentrate on five topics that reflect different perspectives on system-wide diseases, namely:

- cardiovascular disease and metabolism, which inherently affect the whole organism;
- developmental biology, which includes a better understanding of inherited disorders and how diseases may have their roots early in life, and furthers regenerative medicine;
- immunology and inflammation, which are key players in the pathogenesis of many major diseases and can be harnessed to develop novel therapies;
- gene regulation including systems biology approaches, which often form the fundamental basis of diseases; and
- homeostasis and cell communication, which explore how cells and organs regulate and maintain their functions, and how they go wrong in disease.

MDC researchers cover the entire range of methods and levels of analysis – from basic to translational science – in each topic. They collaborate closely with each other to develop diagnostics and preventive and therapeutic approaches that embrace the complexity of diseases. Indeed, highly interdisciplinary work has long been a hallmark of MDC science.

At the frontiers of science, we must constantly adopt new technologies and develop them ourselves. Two types of new developments will be of particular importance for the MDC. The first example involves new imaging methods. Recent advances in light and electron microscopy provide entirely new perspectives on health and disease processes, by visualizing structures and processes that have so far been beyond our reach. Therefore, we aim to establish an Optical Imaging Center, where physicists, biologists and other experts can further this field as innovators and expand the MDC's scientific profile.


Photo: Bettina Flitner

The second example is single-cell biology, a field made possible by recent technological advances. It carries human biology as well as disease mechanisms to the identity, function and structure of individual cells. The MDC aims to be a major player in advancing these methods and in contributing to the Human Cell Atlas that would give us a unique ID card for all cell types. This is a worldwide project that will rival the Human Genome Project in scale.

Biomedical research commonly crosses disciplinary and institutional boundaries. The MDC has embraced these developments by readily incorporating new fields and forming new collaborations such as within the Berlin Institute of Health (BIH). This strengthens our longstanding relationship with the Charité – Universitätsmedizin Berlin. In the context of the BIH, our main goal for the next years will be to recruit outstanding translational researchers in stem cell research, regenerative medicine and personalized medicine.

All these developments would not be possible without attracting the brightest minds to the MDC – graduate students, postdoctoral fellows as well as established researchers. We aim to support these scientists in their individual development and to aid them at their respective levels on their paths to scientific independence and excellence.

MDC and the Campus Berlin-Buch offer ideal conditions for translation. These include close cooperation with clinical researchers at the Experimental and Clinical Research Center (ECRC), a common endeavor with the Charité; close collaboration with the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), including our large common Screening Unit; as well as all the options available at the BiotechPark Berlin-Buch to start new companies.

Our technology transfer office is actively scouting for ideas and projects, and supports the process of turning these ideas into reality. During the last two years, two new drugs have reached the market in Europe and the United States that represent entirely new therapeutic principles based on research at the MDC: the cancer drug Blincyto and a new treatment for bleeding disorders, VONVENDI. We are proud of these recent successes and constantly strive for new opportunities.

This research report showcases our work during the past three years. We hope that it makes you realize how our research relates fundamental principles to clinical problems, and how our strategies for the future link past knowledge with open scientific issues. We also hope that it conveys some of the fascination and excitement that characterize our work.

Prof. Martin Lohse
Scientific Director of the MDC

Photo: Katharina Bohm / MDC

The MDC

The MDC

Breaking down barriers in molecular medicine

Research at the MDC

Without the knowledge of cells, it is impossible to know anything about the biology of life. The simplest creatures are single-celled organisms, and an adult human contains 100 trillion of these tiny units. All cells can produce energy, are capable of dividing, and perform a very specific task. They also each have a DNA data set that contains all the instructions they need in order to function.

For more than 150 years now, researchers in Berlin have been trying to understand how cells interact. In 1858, Rudolf Virchow noted that all cells come from cells. Some 70 years later, theoretical physicists such as Niels Bohr became interested in discovering the secret of life. Bohr's enthusiasm proved contagious for Max Delbrück: "It's strange when you think about it: Humans produce humans, cats produce cats, and corn produces corn. This doesn't seem to be how physics or chemistry works. Atoms don't make the same atoms."

Photo: Katharina Bohm / MDC

Delbrück, a native Berliner, often visited the biologist Nikolay Timofeev-Ressovsky and his collaborator Karl Günther Zimmer. The researchers were producing mutations in fruit flies by exposing them to X-rays in their laboratory in Berlin-Buch. They discovered that increasing the radiation dose led to more mutations. Taking a physicist's perspective on these observations, Delbrück helped make the concept of the gene tangible. If genes were complex clusters of atoms arranged linearly along the chromosomes, then the X-rays could strike random points and cause a break that would split a gene. The three researchers published their study in 1936. As the first work to consider the molecular level, it signaled the start of modern genetics.

When the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC) was founded in 1992, it was easy to decide whom to name it after. The German-American biophysicist and Nobel laureate stands for the courage to pursue one's own ideas (no matter how unusual), the ability to think beyond the borders of individual disciplines, and the open-mindedness of an international perspective.

Twenty-five years later, the MDC – home to roughly 1,600 employees and guests – is one of the world's leading biomedical research institutes. Its scientists come from 60 different countries and work together in around 70 research groups. Their aim is to shed light on the complex causes of widespread diseases, such as cardiovascular disease, metabolic disease, cancer, and diseases of the nervous system. Their approaches include methods used in medical systems biology. Most of the researchers are biologists or chemists, while others are bioinformaticians, mathematicians, physicians, or physicists.

Diversity at the MDC: 47 percent of our scientists come from abroad

For these experts, molecular medicine is about combining the different perspectives of physicians and scientists. Instead of categorizing patient groups according to their symptoms, the researchers start from the molecular biological processes occurring in the cells. Their methods range from structural biology, to omics technologies and animal models – in which they can use new gene-editing tools to simulate diseases even more realistically. Their experiments show that a mechanism can have completely different effects on the body and, conversely, that seemingly identical symptoms can have very different causes.

This provides the researchers with fundamental insights into what happens in the body when it is healthy and when something goes wrong (see the chapter "Discoveries" for several highlights from 2014–2016). Above all, though, these findings should benefit patients as soon as possible. They should improve diagnoses and therapies, and help healthy people avoid getting ill. This has been the MDC's mission from the very outset.

The extent of the scientific success can be seen in just a few selected highlights. 16 scientists who won highly sought-after ERC grants from the European Research Council are currently working at the MDC. Four ERC grants were awarded in 2016 alone. The same year saw the number of publications rise to 463 (from 416 in 2015), with 88 (2015: 82) appearing in outstanding journals with an impact factor above ten. Some 60 percent of all studies were co-authored by researchers from other countries. In the past two years, two drugs have been approved that are based on work done at the MDC. MDC researchers are also playing a leading role in clinical studies and spin-offs related to innovative T-cell therapies – an immunotherapy used to treat cancer.

As one of five health research centers within the Helmholtz Association, the MDC is part of Germany's largest scientific organization. The MDC offers excellent conditions for both established and up-and-coming researchers. They can use state-of-the-art biomedical technology platforms (see the chapter "Scientific Infrastructures & Tech-

nology Platforms") and have the opportunity to develop their own technologies. Researchers have access to strong clinical partnerships, such as within the Berlin Institute of Health or the German Center for Cardiovascular Research (see the chapter "From bedside to bench and back again), as well as a network of international collaborations.

EU-LIFE, an alliance of 13 renowned research centers for the life sciences, is just one example of many. The spectrum of the MDC's international collaborations includes joint research groups such as the Helmholtz-China group on optogenetic evaluation of central cardiovascular control (2015–2018) and the MDC-INSERM group on stem cell and macrophage biology (2012–2017). In addition, PhD students have the opportunity to participate in international training programs (see chapter "Promoting young researchers").

In Berlin, the MDC works closely with its neighbors on campus such as the FMP and the city's universities. Through the NeuroCure Cluster of Excellence, the MDC was involved in setting up the Einstein Center for Neurosciences. It has also played a role in establishing the Einstein Center Digital Future and the Einstein Center for Regenerative Therapies. For the Integrative Research Institute for the Life Sciences, which is part of the Institutional Strategy that Humboldt-Universität zu Berlin (HU) drew up under the German government's Excellence Initiative, a bridging professorship was established at HU and the MDC. All of this helps attract world-class researchers to the German capital – and as they come together here and engage with each other – creates an environment where new ideas can emerge.

When James Watson and Francis Crick finally presented the structure of DNA in 1953, Max Delbrück was disappointed. The idea that our genetic blueprint was a hereditary molecule of just four letters combined to form a twisted rope ladder – the double helix – seemed a little dull to him. The complexity only gradually became clear as new technologies provided ever more detailed insights into cell interiors.

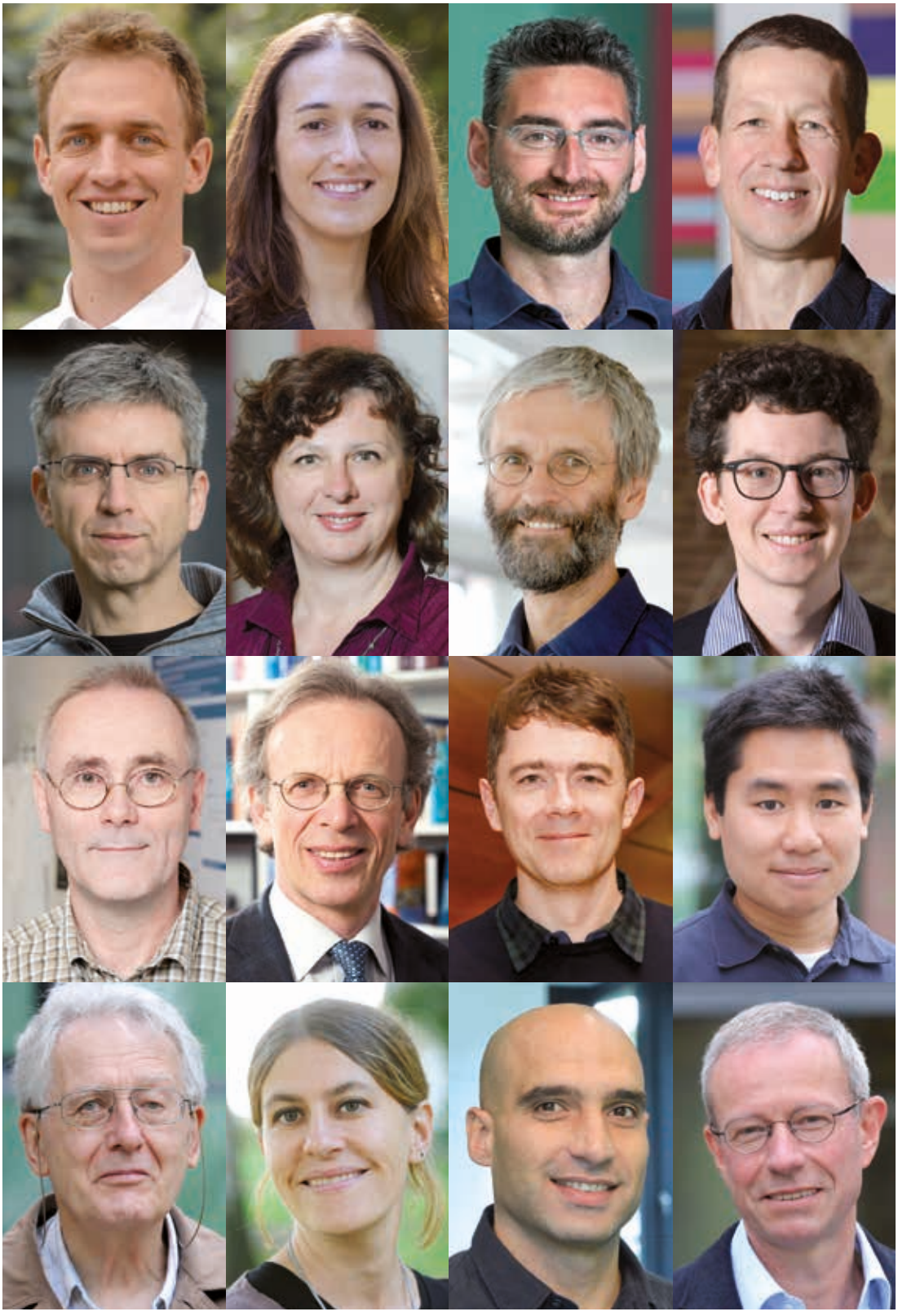
Genetic blueprints are written in the same language for every living thing. They even have many matching text blocks – the match rate between humans and mice is 97.5 percent. However, the differences between species, between individuals, and between health and illness do not just arise from our genes; they are also defined by the way in which genes are switched on and off in an organism, and by how the DNA strands are folded. You can think of DNA as a kind of library. Different areas of the body are activated to perform certain tasks, in much the same way as you would take a specific textbook off the shelf if you wanted to learn about that subject. And every now and again, mistakes can occur in the process.

The methodological search for the connections is now leading researchers away from focusing on one gene and one protein. At the Berlin Institute for Medical Systems Biology (BIMSB), scientists from different disciplines are investigating the interplay of genes and networks in cells, organs, and organisms. The BIMSB was founded in 2008 as an extension of the MDC's research program. The MDC has been receiving fixed institutional funding for its working groups – which now number 14 – since 2012. The more details they discover using high-throughput omics methods, the more complex the picture becomes. We are still far from solving the great puzzle of life.

Change at the MDC's helm

Since March 1, 2014, Dr. Heike Wolke has served as Administrative Director of the Max Delbrück Center, after previously working at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI) in Bremerhaven. The Würzburg pharmacologist Professor Martin Lohse took over on April 1, 2016 as Chair of the Board of Directors and Scientific Director of the MDC. He succeeded Professor Walter Rosenthal, who had become President of the Friedrich Schiller University Jena on October 15, 2014.

16 scientists currently working at the MDC have won highly sought-after ERC grants.



From bedside to bench and back again

Clinical translation at the MDC

As the sun shines down on the long balconies, it is easy to imagine this once being a place where patients could relax under the colorful awnings and forget, for a brief moment, about the routine of hospital life. "The laboratories behind us were once four-bed hospital rooms, and my own office formed part of the nurses' station," explains Friedrich Luft, director of what is now the Experimental and Clinical Research Center (ECRC).

The ECRC building breathes history. From its inception, this was a place where basic research and patient care were closely interwoven. Neuroscientists Oskar and Cécile Vogt set up the clinic in the 1920s, positioning it directly next to their laboratory building. "This was a visionary concept," explains Luft. To this day, he believes this bedside-to-bench and bench-to-bedside approach is the only way to conduct effective research in molecular medicine.

The original hospital wards may have closed long ago, but the facilities are now home to twelve of the Charité's university outpatient clinics. Many of the patients that come here are participating in trials. "We want to know what people really need," says Luft. The ECRC acts as an interface between the Charité and MDC. The former hospital rooms were converted into research facilities and, most recently, a new stem cell laboratory was added. In May 2017, additional demolition and reconstruction work began – this time for the Berlin Institute of Health (BIH).

The BIH was founded in 2013, and made an autonomous corporation under public law in 2015 thanks to the Law on the Foundation of Berlin Institute of Health. With its funding programs, large-scale collaborative projects and joint appointments, the BIH has served to considerably strengthen the partnership between the Charité and MDC (see box). Significantly more basic researchers from the MDC are now working with physicians to address systems medicine issues. This allows discoveries made in molecular biology to be more swiftly translated into patient care.

One main focal area is personalized medicine – an approach whereby instead of treating all patients with the same method, and thus in some cases having to deal with unbeneficial side effects, each patient receives specially tailored treatment. This means administering the right molecule or the correct cell therapy or immunotherapy at just the right time.

Under construction: With the new building, a new BIH location will arise on the Buch campus.

Photo: kleyer.koblitz.letzel.freivogel.architekten Gesellschaft von Architekten mbH, Berlin

Berlin Institute of Health (BIH)

The founding of the BIH in 2013 triggered a number of translational activities, ranging from them BIH Twinning Research Grants to the Clinical Research Unit. In 2015, changes at the top ushered in the next phase. Erwin Böttinger from New York's Icahn School of Medicine at Mount Sinai took up the post of Chief Executive Officer. Rolf Zettl, until then Managing Director of the Helmholtz Association, assumed responsibility for business and administrative duties. Axel Radlach Pries, Dean of the Charité, und Martin Lohse, Chairman of the MDC's Board of Directors, also joined the Executive Board of the BIH.

Two BIH appointments have been particularly important for the MDC: Holger Gerhardt has since 2014 been a research group leader at the MDC and BIH as well as Professor of Cardiovascular Research at the Charité. He is also integrated into the activities of the German Center for Cardiovascular Research (DZHK). The angiogenesis specialist is investigating new ways to stop pathological blood vessel growth. Ralf Kühn, an expert in genome engineering and stem cell biology, came to Berlin as a guest researcher at the MDC, where he leads the BIH working group on iPS cells and heads the MDC Transgenic Core Facility.

One example from oncology demonstrates just how urgently this approach is required. When a young child develops a neuroblastoma of the autonomic nervous system, physicians are currently unable to tell parents exactly what to expect. In some children, spontaneous regression of the tumor occurs. In others, this malignant growth – which usually develops in the medulla (inner part) of the adrenal gland or next to the spinal cord – must be surgically removed. Then there are those who require treatment with a combination of aggressive chemotherapy, surgery and radiation, or perhaps even a bone marrow transplant. For all parents, a year of hope and fear follows as they wait to see if the cancer has been truly defeated. Only one in ten children survives a relapse.

Charité oncologist Angelika Eggert and MDC proteomics expert Matthias Selbach have set out to analyze the different guises of this disease and to characterize each tumor by integrating all available data: data on the genome, transcriptome, proteome and metabolome. The researchers are hoping that such molecular profiling will enable them to define risk groups and develop targeted therapy strategies. What makes the tumor aggressive? When do tiny fragments of the tumor tend to remain, in spite of all the medication? Can a liquid biopsy be used to predict metastases? In addition, genetically modified T-cells may soon be able to better combat the tumor. Currently, 27 scientists are involved in this interdisciplinary research, working in eight subprojects. Thanks to the BIH Collaborative Research Grant "Terminate NB," they have secured funding of 3.1 million euros until 2019.

This project is just one example of many. Beyond the Charité outpatient clinics, there are plans to set up additional patient cohorts aimed at gathering samples from a large number of patients who suffer from the same illness. Scientists will analyze these samples with the help of high-throughput technologies, looking for individual alterations in genetic material, enzymes, proteins or metabolites and comparing these with samples from healthy subjects. This will allow researchers to filter out all the molecular determinants (biomarkers) that suggest the occurrence of abnormal processes in the body. These biomarkers not only identify where in the organism something has gone wrong; they can also provide information on the course of the disease or indicate where new targeted medication could be used.

These samples are stored in biobanks – one of which was established at the Berlin-Buch campus in 2016. The centerpiece of the building is a room that houses two cooling tanks, both containing 2.5 million liquid samples stored in nitrogen. Blood and urine samples, for example, sit here in minus 160 degrees Celsius, and are continually supplemented over the course of long-term studies. This room has become a treasure chamber for researchers.

One of these long-term studies is the German National Cohort, led by the Helmholtz Association's health centers together with university and non-university partners.

The nationwide study began its main phase in 2014, and is documenting the health and lifestyle of 200,000 adults. For 20 years these individuals will undergo regular medical examinations and answer questions on their living conditions and habits, in order to help scientists better research the emergence of widespread diseases.

Six thousand of the participants will receive full-body magnetic resonance imaging (MRI) scans. These images allow physicians to see, for example, if there are fatty deposits in the liver, which can increase a person's risk of diabetes. MDC is responsible for all these MRI scans, as well as for coordinating the Berlin-Brandenburg Cluster and the decentralized storage of biological samples. Another 10,000 participants are to be recruited at the MDC's Berlin-Nord study center.

It is clear that cooperative research provides a better approach for investigating widespread diseases, including cardiovascular diseases and their risk factors. With the founding of the German Center for Cardiovascular Research (DZHK), MDC has made a significant contribution to better connecting this area of research in Germany. A total of 28 institutions are working together at seven locations in an effort to improve the prevention, diagnosis and treatment of cardiovascular diseases. An intermediary review in 2014 attested that the DZHK was off to an impressive start, displaying fantastic development and excellent preclinical research. Reviewers were very impressed with the central infrastructure for clinical studies that the DZHK had set up in such a short period of time.

Photo: Horst Krüger / MDC

The MDC is not only represented on the DZHK's Board of Directors by Thomas Sommer; there are altogether 58 MDC scientists involved in the DZHK. These scientists are responsible for organizing project groups such as "Genetics of Congenital Heart Disease" and "Nutrition," in which specialists can exchange knowledge and ideas and make their expertise – particularly in terms of technology and infrastructure – available to DZHK's partners. Michael Bader, for example, offers expertise on the generation and phenotyping of transgenic rats, while Norbert Hübner's specialist field is genomics and proteomics.

Structures like the DZHK offer a framework to facilitate cooperative research. However, some projects require a great deal of patience on the side of the researcher before the molecular cause of a disease is found. One example of such a case is the unusual story of a Turkish family who lived from a village by the Black Sea. Whenever a child was born into this family, the first thing the relatives would do was examine the child's fingers. If they appeared to not be growing normally and the hands remained small, the child would be expected to die young.

It emerged that a single gene within the family was causing hereditary hypertension, with recorded values of up to 270/160 mmHg. Researchers led by Friedrich Luft hoped they could identify this suspicious gene. Little did they know this family would be the focus of a study that was to last over 20 years. It was only in 2015 that the researchers announced in Nature Genetics that they had found the guilty suspect: a mutated form of the enzyme PDE3A. This enzyme not only regulates blood pressure, but also bone growth.

Luft believed at this point that the genetic sequence had been cracked and the problem was solved. "But projects like these don't go away," he says. His group has now modified the same gene in animals to see if they too develop hypertension. In addition, several other families with similar symptoms came forward. The scientists decoded their genetic material, too. And rather than only looking at the gene sequence, they also want to work together with structural biologists to work out the three-dimensional form of the protein and uncover the mechanism that leads to the problems.

"Sometimes science is like looking for a black cat in a dark room," reflects Luft. "And often we don't even know if the cat is there." It is only bit by bit, he says, that we are starting to realize the many unknowns that exist in science that have so far been completely overlooked.


Photo: Maj-Britt Hansen

Okan Toka, a former member of Fred Luft's team at the MDC, speaks to one of his Turkish patients.

Discoveries

Selected Research Highlights from MDC Labs

Pressure relief valve discovered in the cell membrane

What causes cells to shrink? This question has vexed biologists for decades. But now, after four years of research, a team of scientists led by Thomas Jentsch have found the answer. Together with the Screening Unit, which is jointly run by the FMP and the MDC, the scientists identified a previously unknown gene as an essential constituent of the volume-regulated anion channel (VRAC). The gene is called leucine-rich repeat-containing 8A (LRRC8A). Using a one-by-one approach in a large-scale cell culture experiment, Jentsch's team transiently silenced approximately 20,000 human genes. In an automated screening process the researchers also investigated which of the genes are responsible for the swelling-activated chloride flux across the cell membrane. The approximately 130,000 time-dependent measurements were statistically analyzed with help from the Bioinformatics Group led by Miguel Andrade (until April 2014 at the MDC; now at the University of Mainz). Jentsch's team went on to show that LRRC8A requires other members of the LRRC8 gene family to form VRAC.

Voss FK, Ullrich F, Münch J, Lazarow K, Lutter D, Mah N, Andrade-Navarro MA, von Kries JP, Stauber T, Jentsch TJ. Science 344 (6184): 634–638 (9 May 2014).

Why the heart fails

Defects in splicing are implicated in a number of serious genetic diseases, like the heart disease dilated cardiomyopathy (DCM). This condition is marked by an enlarged, weakened heart and is responsible for about a third of deaths from congestive heart failure. MDC scientists have shown that mutations in the protein RBM20 (RNA binding motif protein 20) probably contribute to DCM by affecting the splicing of crucial molecules in the heart. They provided a detailed account of the operation of RBM20, which is preferentially present in the heart and orchestrates a large number of target molecules. Studying RBM20's target pattern revealed that it was located in introns, from where it tells the spliceosome to remove a nearby exon, thus shortening the molecule. But the defective RBM20 version fails to remove the exon and instead creates RNA with extra segments, resulting in proteins that are too long. In the case of titin, an essential heart protein, this creates a molecular spring that is too slack and muscles that don't contract efficiently. Ultimately, the heart is forced to work harder and becomes enlarged. Further study will show if these findings are clinically relevant.

Maatz H, Jens M, Liss M, Schafer S, Heinig M, Kirchner M, Adami E, Rintisch C, Dauksaite V, Radke MH, Selbach M, Barton PJR, Cook SA, Rajewsky N, Gotthardt M, Landthaler M, Hübner N. Journal of Clinical Investigation 124 (8): 3419–3430 (1 August 2014).

DNA transcription: Start region shown to be unidirectional

To enable enzymes to grab ahold and start transcribing a gene at the right place, the DNA contains recognition sequences, so-called promoters, which are located immediately upstream. Ever since researchers began using high-throughput sequencing technology to precisely investigate gene expression patterns, they have believed that a large percentage of promoters are not unidirectional. But researchers at the MDC and the University of California in San Diego have now discovered that this isn't so. Their studies show that a central part of promoters, the core promoter, is intrinsically unidirectional, and that transcripts of the opposing DNA strand arise from their own core promoters. Using high-throughput experiments and various analytical procedures, the researchers determined that in fact about 40 percent of the genes have two opposite core promoters at variable distances. Through the copy of the opposing strand a long non-coding RNA arises (lncRNA), i.e. a transcript, which is not translated into a protein and whose function has yet to be elucidated. Since reverse-directed core promoters are common but not universal, the researchers presume that these at least partially help to regulate gene transcription.

Duttke SH, Lacadie SA, Ibrahim MM, Glass CK, Corcoran DL, Benner C, Heinz S, Kadonaga JT, Ohler U. Molecular Cell (Jan 2015).

Making genome editing more efficient

With the help of molecular tricks, researchers at BIH and the MDC have managed to significantly improve the efficiency of genome editing using CRISPR-Cas9 "gene scissors." The CRISPR-Cas9 technology allows researchers to create DNA double-strand breaks at specific locations within the genome. These are repaired in cells by using one of two naturally occurring mechanisms – either by homologous recombination, which allows researchers to make very precise changes to genome sequences, or by non-homologous end joining, which is more efficient but less precise, since it frequently deletes DNA sequences. The BIH and MDC researchers have now used nature's bag of tricks to suppress cellular non-homologous end joining, which makes it possible to increase the efficiency of homologous recombination and thus of precise genetic modifications using CRISPR-Cas9 by up to eightfold.

Chu VT, Weber T, Wefers B, Wurst W, Sander S, Rajewsky K, Kühn R. Nat Biotechnol (24 Mar 2015).

Photo: Horst Krüger / MDC

What hypertension and brachydactyly have in common

After more than 20 years of research, a team of scientists led by Friedrich Luft at the Experimental and Clinical Research Center (ECRC), a joint cooperation between the MDC and the Charité, have found the cause of a rare disease suffered by a Turkish family. The affected individuals have hereditary hypertension, unusually short fingers and are small in stature. They typically die before the age of fifty if their hypertension goes untreated. This disease, which afflicts this and five other families not related to each other, causes six different point mutations in the PDE3A gene. These mutations lead to high blood pressure and shortened bones of the extremities, particularly the metacarpal and metatarsal bones. The scientists have thus discovered the first Mendelian hypertension form not based on salt reabsorption in the kidneys, but instead is more directly related to the structure and function of the vascular wall.

Maass PG, Aydin A, Luft FC, Schächterle C, Weise A, Stricker S, Lindschau C, Vaegler M, Qadri F, Toka HR, Schulz H, Krawitz PM, Parkhomchuk D, Hecht J, Hollfinger I, Wefeld-Neuenfeld Y, Bartels-Klein E, Mühl A, Kann M, Schuster H, Chitayat D, Bialer MG, Wienker TF, Ott J, Rittscher K, Liehr T, Jordan J, Plessis G, Tank J, Mai K, Naraghi R, Hodge R, Hopp M, Hattenbach LO, Busjahn A, Rauch A, Vandeput F, Gong M, Rüschendorf F, Hübner N, Haller H, Mundlos S, Bilginturan N, Movsesian MA, Klussmann E, Toka O, Bähring S. Nat Genet 47(6): 647–53 (Jun 2015).

Fighting tumors with T cells

The consortium "Targeting somatic mutations in human cancer by T cell receptor gene therapy" has made a major advance in the development of cancer immunotherapy. The researchers have successfully modified immune cells (T cells) to recognize and specifically target cancer cells in mice. They were able to analyze the antigens and clearly distinguish between "good" and "bad" T cell targets by using a humanized mouse model. This animal model can be used to test the therapeutic suitability of T cell receptors and antigens, which is an important prerequisite for the development of clinical applications.

Targeting human melanoma neoantigens by T cell receptor gene therapy. Leisegang M, Kammertöns T, Uckert W, Blankenstein T. Journal of Clinical Investigation (25 Jan 2016).

Publications 2014-2016

	Total	> Impact Factor 10	Ø Impact Factor	% in Tier 10*
2016	490	77	6,39	38 %
2015	432	68	6.32	33 %
2014	402	67	6.54	40 %

* Tier 10: Number of publications which appeared in the top-tier journals of their respective fields (top 10 %).

Focus on international collaboration: ISRAEL

What happens within a cell when it has to process information and make decisions? This question is at the core of Nir Friedman's research. In 2015, the Israeli systems biologist was granted a **Humboldt Research Award** that enabled him to continue his work in Berlin. Nir Friedman (Hebrew University in Jerusalem) and Nikolaus Rajewsky (BIMSB / MDC) are jointly investigating new regulatory signals at the RNA level – a collaboration fostered through the SignGene Research School. Moreover, Prof. Yehudit Bergman, also from the Hebrew University in Jerusalem, received in 2015 the **Helmholtz International Fellow Award**, an honor aimed at fostering her collaboration with MDC scientists and within the SignGene program.

SignGene – the **German-Israeli Helmholtz Research School "Frontiers in Cell Signaling & Gene Regulation"** – is a joint endeavor of the MDC, Humboldt University and Charité in Berlin, the Technion in Haifa and the Hebrew University in Jerusalem. Since 2013, 37 principal investigators and 20 PhD students have been involved, resulting in 21 papers so far. For their thesis projects, students have a main supervisor at their home institute and a co-mentor abroad. Visits in the co-mentor's lab can last up to six months. Joint events such as symposia, winter schools and PhD retreats are intended to offer a space for the two scientific cultures to meet and benefit from each other. Among SignGene faculty members is Nobel laureate Aaron Ciechanover from the Technion.

Israel might not be a large country, but it is a powerhouse in competitive biomedical research. Many principal investigators at the MDC have forged collaborations with Israeli scientists. Apart from short-term visits, they are actively contributing to the **Helmholtz-Israeli Cooperation in Personalized Medicine** through two projects:

- "Natural RNA circles: An emerging group of biomarkers for personalized cancer medicine" (2015-2017), Nikolaus Rajewsky (MDC) with Yosef Yarden (Weizmann Institute of Science)
- "Network-based identification of personalized targets for Huntington's disease" (2015-2017), Erich Wanker (MDC) with Roded Sharan (Tel Aviv University)

Two other projects have been funded by the **German-Israeli Foundation**:

- "Mechanisms of isoform-specific regulation of L-type Ca channels by protein kinases" (2013-2016), Nathan Dascal and Sharon Weiss (Tel Aviv University) with Hannelore Haase and Enno Klussmann (MDC)
- "Molecular dissection of the epithelial-mesenchymal cross-talk in establishing final pancreas shape" (2015-2018), Francesca Spagnoli (MDC) with Limor Landsman (Tel Aviv University)

Jan Philipp Junker of the MDC and Erez Levanon of Bar-Ilan University in Israel are co-developing the first-ever high-resolution maps of changes in certain gene transcripts. The **ARCHES Prize**, conferred in 2016 and endowed with a total of 200,000 euros over a period of five years, will enable them to combine their expertise and tackle this challenging task.

Development aid

Technology transfer at the MDC

Everyone knows about omega-3 fatty acids; they are found in oily fish such as salmon and are believed to protect against heart disease. But not all patients will benefit from a change of diet. This is because the omega-3 fatty acids first have to be converted into active metabolites in the body, as Wolf-Hagen Schunck's team at the Max Delbrück Center for Molecular Medicine (MDC) has discovered.

The biochemist and his colleagues are now developing a substance called OMT-28 at OMEICOS, a MDC spin-off. It is supposed to bypass the intermediate step and act directly on the receptors of heart muscle cells. The researchers hope that this will prevent atrial fibrillation and reduce the risk of heart failure and strokes. The substance has already proved effective in animal models. A phase 1 study on healthy human volunteers began in March 2017.

Photo: blümchen36 / photocase.com

This is the MDC's latest coup in technology transfer, but not the only one. MDC researchers made a key contribution to two drugs that came on the market in 2015: VONVENDI (Baxalta Inc., now: Shire) and Blincyto (Amgen). VONVENDI is the only recombinant treatment for adults living with von Willebrand disease (VWD), an inherited bleeding disorder. Patients lack a protein – known as von Willebrand factor – that is crucial for normal blood clotting. VONVENDI provides a substitute and therefore helps control bleeding episodes.

Blincyto, on the other hand, is an immunotherapy against a very aggressive form of blood cancer (B-cell acute lymphoblastic leukemia). The bone marrow of these patients produces too many immature white blood cells. Rather than maturing into functional cells, these lymphoblasts rapidly reproduce, suppressing normal blood formation. The drug enlists the body's own T-cells to destroy this cancer.

The royalties from these successes are now helping to translate other basic research ideas into applications and find new precompetitive funding formats, as well as expand advisory services for start-ups in consultation with Berlin Institute of Health (BIH). The MDC law states that technology transfer is a core mission of the Center. At present, 20 research groups are working on new medical therapies or diagnostics intended for commercialization. Each project receives comprehensive support.

The first step is to secure the patent at an early stage—without intellectual property rights, nothing happens in biomedical marketing. In the last three years alone, 24 new patents have been filed. Ascenion and a technology transfer company based on the Buch campus which supports numerous institutions in the life sciences sector, plays a key role in this process. The technology transfer office and Ascenion jointly examine the market potential of the discovery and bring in external consultants to take the project to the next step, or explore opportunities for cooperation with other institutes and industry. They determine whether a spin-off would be appropriate or whether the drug should be out-licensed soon.

Photo: Monique Wuestenhagen

MDC's internal funding programs can give projects a boost. Since 2008, for example, "PreGo Bio" has been making advances possible by providing 150,000 euros annually for equipment and personnel for up to three years. Project teams can also receive coaching and advice from industry experts. To complement these opportunities, since the spring of 2016 the funding program "Boost" has been helping early-stage projects – whose potential still needs to be evaluated – get off the ground. In addition, the campus provides access to valuable expertise, for example, from clinicians at the Experimental and Clinical Research Center (see chapter on ECRC) or from the Screening Unit operated jointly with the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) and the Chemical Biology Platform.

MDC researchers have no shortage of ideas. Berlin Cures, founded as a spin-off of the MDC and the Charité in 2014, is developing a drug called Aptamer BC007 to treat the causes of chronic heart failure. It is currently being tested on healthy volunteers.

Also in 2016, for the first time a German team won at Onestart – the world's largest start-up competition in the life sciences. Captain T Cell, a MDC spin-off, won against 400 international start-ups. With the 100,000 pounds prize money, the team will continue to develop a cancer immunotherapy that involves a platform to produce personalized, cancer-specific T-cell receptors. With the help of these receptors, the body's own T-cells should be able to find the cancer, and systematically destroy it.

In the spring of 2017, the Max Delbrück Center Cell Engineering Lab (MD-CEL), a platform for transposon-based cell engineering sponsored by the Helmholtz Association, began to work closely with science and industry. It has its roots in Wolfgang Uckert's and Zsuzsanna Izsvak's research teams at the MDC.

The road through the "valley of death" from the basic research to market approval is long and stony. In the case of a promising discovery in Wolf-Hagen Schunck's laboratory, a substance that could be used to treat atrial fibrillation, this process took ten years. And the risk of failure is still 90 percent. Schunck is well aware of this. But right now he is especially proud to be starting tests on human volunteers: "That's a dream come true."

Promoting young researchers

Training at the MDC

For Douaa AS Mugahid, the road to Boston went through Berlin. Between 2011 and 2015, the Egyptian researcher worked as a PhD student in Michael Gotthardt's lab at the MDC. With her wide range of interests, open-mindedness, and helpful and diplomatic nature, she immediately caught the attention of her colleagues. They found her to be just as capable of discussing problems as she was of talking about scientific theories. Although she was both a PhD student representative and an active member of the Helmholtz Juniors network, she still found time to help refugees.

"I'm not sure if the challenges I faced were that much different from the challenges every graduate student faces: getting the experiments to work, dealing with large data sets and making sense of them within the context of the biological questions I was addressing," says Mugahid. "Also, pursuing a few different lines of investigation in parallel meant that I had to learn the art of multitasking." Her next step proves just how well she succeeded: In 2015, Mugahid began a postdoctoral fellowship in the Department of Systems Biology at Harvard Medical School.

Over 360 PhD students and around 230 postdocs are currently engaged in research at the MDC. More than half of them come from outside of Germany, with 42 different countries represented on campus. The students are equally varied in terms of subject: Their backgrounds cover everything from molecular biology, biochemistry, and physics, to computer science, and medicine.

With the Helmholtz Graduate School in Molecular Cell Biology, doctoral students benefit from a structured PhD program that the MDC set up in collaboration with Humboldt-Universität zu Berlin, Freie Universität Berlin, and Charité – Universitätsmedizin Berlin. In addition to their lab-based projects, students can acquaint themselves with new technologies in workshops, attend seminars and lectures, and network with each other at events such as retreats.

Some students also participate in the MDC's thematically focused programs – such as the Helmholtz Research Schools Translational Cardiovascular and Metabolic Medicine (TransCard), Frontiers in Cell Signaling and Gene Regulation (SignGene) – or

Molecular biology in schools

Biomedical knowledge is growing exponentially. Neither textbooks nor carefully developed curriculum frameworks can keep pace with the latest developments and methods. This is why the MDC launched the Lab Meets Teacher training program (Labor trifft Lehrer, LTL) in 2011. The program brings teachers into the lab and allows them to spend a day immersing themselves in the world of research. They conduct experiments, listen to talks, and discuss the latest topics with researchers. All courses are recognized as advanced teacher training by the Berlin Senate Department for Education, Youth and Science, and by the Brandenburg Ministry of Education, Youth and Sport. The Lab Meets Teacher courses also produce teaching materials that are freely available as open educational resources.

The MDC has also spent the past 15 years working directly with young people in schools to get them excited about science. Its efforts here include running the Life Science Learning Lab, a learning center that gives over 12,000 school pupils an insight into biomedical research each year. It also sends MDC lecturers into schools to give talks, and does much more besides.

Photo: Harry Schnitger / MDC

in the MDC-NYU PhD Exchange Program in Systems Biology, an international program run by the Berlin Institute for Medical Systems Biology. The Berlin Institute of Health's PhD scholarships for medical students and its Clinician Scientist Program help lay the foundations for collaboration beyond the borders of basic research and medicine. The schemes give physicians the space they need to do research during their training.

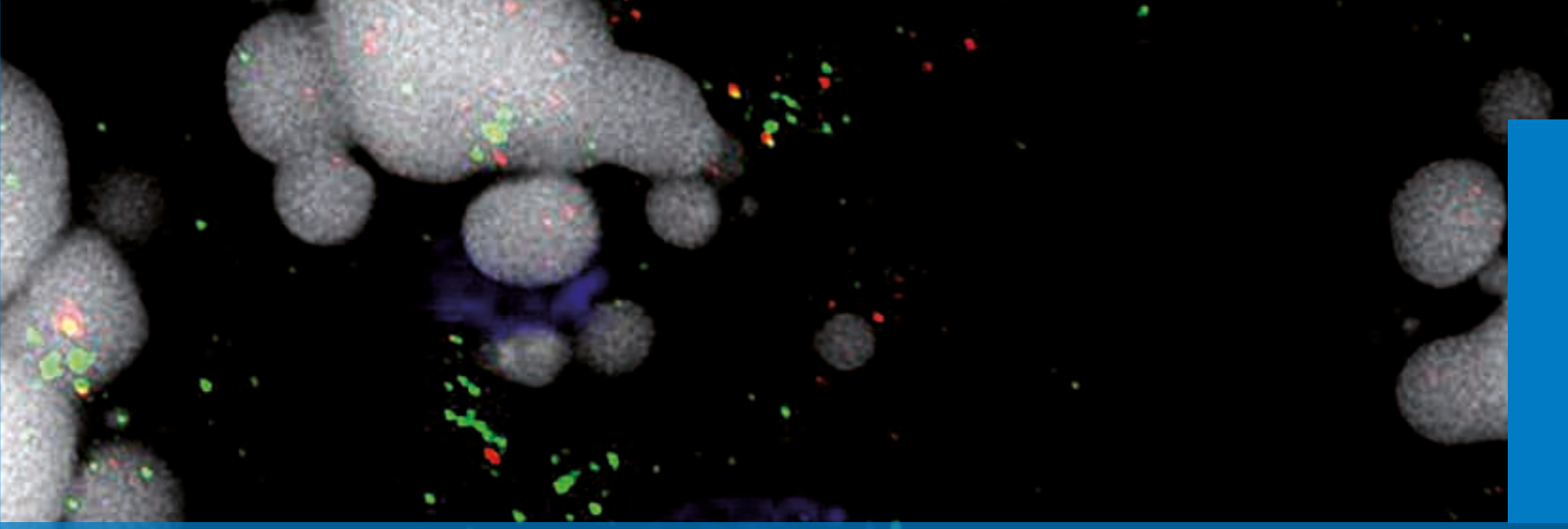
"The MDC is a wonderful center. It brings together many disciplines and gives young researchers real opportunities to work independently," says Martin Lohse, scientific director of the MDC. However, he adds, working in a center of this size means students first need to acquaint themselves with it and then quickly work out what they want to achieve during their time here. This applies to postdocs in particular.

Internationally, just 4.4 percent of candidates go on to earn the coveted title of professor. It is therefore crucial that students prepare themselves for the leap into academic independence. However, this is not a leap everyone wants to take. The MDC Mentoring Program is designed to help postdocs make a well-informed decision about their career.

In the program, an experienced mentor from research, academia, or industry, and a variety of workshops help postdocs to identify and develop their professional goals and personal skills. They can also apply for various Helmholtz programs. The MDC is a partner in the LIBRA project, an EU-wide network designed to support women on the path to leadership positions in science.

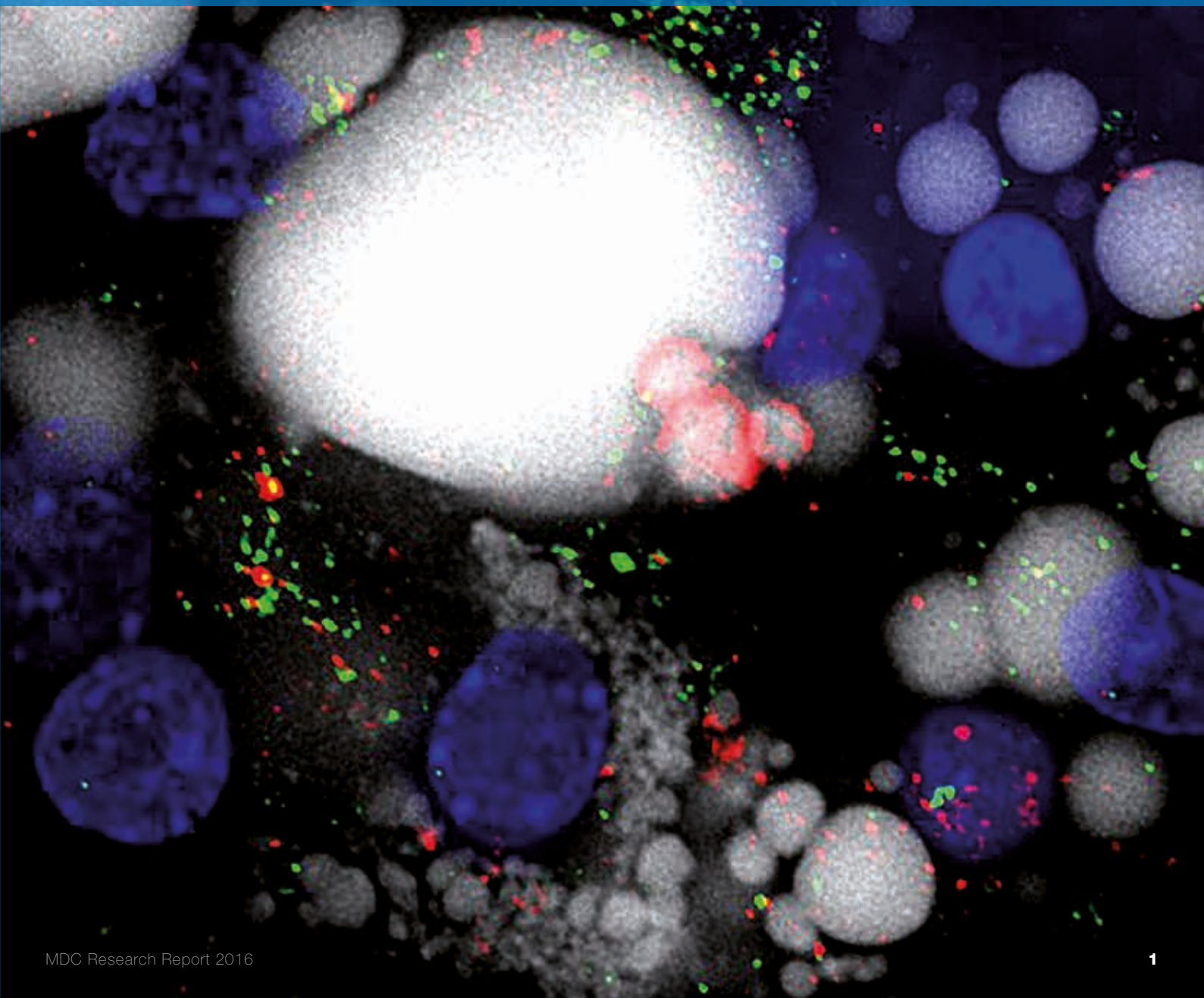
Since 2016, the MDC Postdoctoral Association has been holding Networking Lunchtime Seminars, which are informal and interactive. During the events, young group leaders and former postdocs who now work in industry talk about their career paths. The annual MDC Career Day allows PhD students and postdocs to make initial contact with companies. And in the Career Pathway lectures, alumni reflect on their time at the MDC and on what came afterwards.

"I never wanted to be an academic for life," says Mugahid. She often considering switching to industry, but then she realized that science still offered many opportunities to grow professionally. Her advice: "Learn to trust yourself and your instincts. Many breakthroughs are made by young people who have not yet become sceptics by listening too much to others in the field."



Cardiovascular and Metabolic Disease

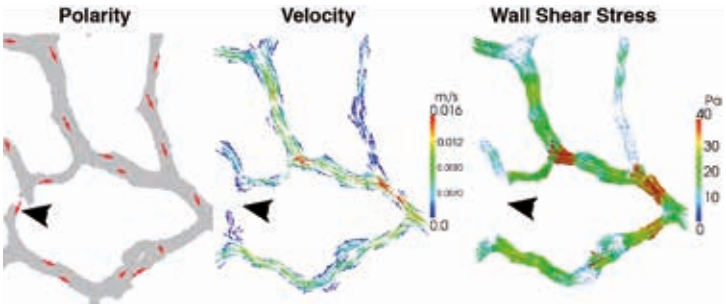
Coordinator: Norbert Hübner



Cardiovascular and Metabolic Disease

Coordinator: Norbert Hübner

Cardiovascular and metabolic diseases are the most common cause of death worldwide, increasing burden on society. Most cases of cardiovascular diseases are likely the result of contributions from multiple genes, and apparently also complex epigenetic mechanisms may lead to cardiovascular disease. Getting an experimental handle on these topics and improving therapeutics still is the major challenge for the next decade. An increasing number of animal models – particularly strains of rats and mice – are available and necessary to convert findings into relevant clinical tools. Research in this field sometimes is hampered by special problems including finding patients with a particular profile, extrapolating from a very small number of cases, and understanding complex interactions within cells and cell types. We are dealing with these challenges while constantly developing new types of modeling and through a thorough integration of bioinformatics with clinical and experimental approaches. The molecular analysis of cardiovascular and metabolic functions at the cellular level is a prerequisite for a mechanistic understanding and forms a basis for the rational implementation of novel therapies to target cardiovascular and metabolic disorders. The Cardiovascular and Metabolic Diseases program has revealed some considerable key molecules and pathways involved in major processes of the cardiovascular and metabolic systems. For example, the group of Michael Gotthardt studies the role of titin in the regulation of contractility in cardiac, skeletal, and smooth muscle. Using their animal models they were able to elucidate titin's role in sarcomere assembly, to link titin to adrenergic signal transduction, lateral growth of the sarcomere, and the hypertrophy response. Their work emphasized the role of post-transcriptional regulation in the etiology of myocardial pathology. RBM20 interacts not only with titin but potentially in a network of 30 genes previously linked to cardiomyophathy, ion-homeostasis, and sarcomere biology. The group of Thomas Jentsch has a long standing interest in investigating the volume-regulated anion channel (VRAC) channel and the relevant subunits ranging from biophysics, structure function, to cell biology, mouse models and medicine with implications for cardiovascular outcomes. Recently, they could identify LRRC8 heteromers as an essential component of VRAC. Metabolic disturbances are emerging as major risk factors not only of cardiovascular but also neurodegenerative and oncological diseases. However, the molecular mechanisms that link the control of metabolism to other biological systems remain poorly understood. The group of Thomas Willnow is studying endocytic receptors to uncover their roles in normal physiological processes and in human disease. They demonstrated that LRP2, a member of the LDL receptor gene family, balances signaling of the morphogen sonic hedgehog in the developing retina, and that lack of this activity results in severe overgrowth of the eyes in mouse models and patients. Organogenesis involves multiple sequential events such as fate specification, growth, differentiation, and morphogenesis, which ultimately result in the development of a functional mature organ. This step-wise process relies on a coordinated and highly complex interplay



Endothelial axial polarity measured by the position of the golgi to the nucleus of each endothelial cells (red, left panel) points against the direction of blood flow (compare to arrows signifying flow velocity, middle panel). The axial polarity against flow is a response of endothelial cells to wall shear stress (right panel). Black arrowhead points to region of low flow, low wall shear and consequently divergent polarity leading to migration of cells out of the low flow segment and therefore branch regression.

Image courtesy of Claudio Franco and Miguel Bernabeu (Franco et al, 2015 PLoS Biology) / AG Holger Gerhardt

of signaling events and transcriptional networks. Importantly, aberrant developmental processes or a loss of their coordination are common causes of congenital defects of the cardiovasculature, liver, or pancreas. In cardiac biology Ca^{2+} ions play pivotal roles in a multitude of cellular processes, ranging from excitation-contraction coupling to the regulation of hormone secretion and gene expression. The research of Daniela Pánáková aims to uncover the molecular mechanisms that mediate the crosstalk between calcium signaling and other physiological stimuli with major morphogenetic pathways such as Wnt signaling during cardiovascular development as well as in disease states in zebrafish. Learning to treat diabetes and various types of cardiovascular lesions will require a deeper knowledge of the genetic profile of myocardial or pancreatic progenitor cells. Deciphering the molecular signatures of "stem cell-ness" of particular cell types of the heart, liver, and pancreas, will allow us to isolate such cells for therapeutic purposes and possibly to instruct stem cells or progenitor cells to activate appropriate differentiation programs. Using latest NGS technology, Francesca Spagnoli aims at elucidating how distinct cell types like liver and pancreas can arise from common progenitors. Her findings on organogenesis fate will contribute knowledge necessary to close the gap between basic model research and applied clinics. The vascular biologist Holger Gerhardt, who joined the program in 2015, identified how developmental vessel regression is driven by cell migration, where cells are attracted to vessel segments that have higher blood flow, activily moving out of segments with poor flow. Genetic variability among individuals plays an important role in the development and progression of cardiovascular disease. Genetic and genomic studies in human populations are investigated by Young-Ae Lee to understand the etiology of chronic inflammation that is often shared by other forms of common human cardiovascular and metabolic diseases. She conducted the first GWAS for the atopic march and showed that there are specific genetic loci influencing the march's unfortunate course. The group of Michael Bader has also a strong emphasis in the field of rat embryology and stem cell research. So far, they targeted genes for the insulin receptor, the (pro)renin receptor, apolipoprotein E, and the LDL receptor. CRISPR/Cas technology has also been established and successfully used to ablate genes in rats. Understanding diseases at the molecular and cellular level is an important step in elucidating mechanisms that ultimately have their effects at the level of organs and organisms. Following the idea of translational medicine the MDC cooperates with the Charité through the Experimental and Clinical Research Center (ECRC) on several topics. The group of Thoralf Niendorf concentrates on the development of MR technology enabling new ways of mapping/probing morphology, function, physiology and metabolism including cardiac and metabolic imaging.
We further strengthened our collaborations within the German Center for Cardiovascular Research (DZHK) and the Berlin Institute of Health (BIH) which is highlighted not only by joint recruitments (like Holger Gerhardt) but also by the growing number of scientific projects. The challenge remains to understand the regulatory genetic networks that influence cardiovascular and metabolic disease risk, to link molecular networks with physiological data, and to identify key regulatory nodes that can be targeted therapeutically to reduce the burden of cardiovascular and metabolic diseases in patients.

Scientific Management:
Kathrin Saar

Photo: David Ausserhofer/MDC

Michael Bader

Molecular Biology of Peptide Hormones

The group focuses on the molecular biology and function of hormone systems involved in cardiovascular regulation. The physiological functions of these systems are analyzed by the production and analysis of transgenic and gene-targeted animal models. Among the hormones studied, serotonin is of special interest, since it is not only involved in vascular homeostasis and other peripheral functions, but also serves as potent and multifunctional neurotransmitter in the brain. In addition, the group is interested in embryology and stem cell research, in particular applying these fields to the rat.

Renin-angiotensin system

The renin-angiotensin system (RAS) is of central importance in blood pressure regulation and in the initiation of target organ damage. In particular, local angiotensin-II generating systems in tissues such as brain, heart, vessels, and kidney are involved in these processes. Therefore, transgenic rats with local up- or downregulation of RAS components in these organs, e.g. by the local expression of antisense-RNA or of a peptide-liberating protein, were produced and analyzed to clarify the local functions of angiotensin II. Other genetically altered mouse and rat models for non-classical RAS components such as neurolysin, ACE2, the (pro)renin receptor, angiotensin(1-7) and its receptor Mas, have elucidated the physiological function of these molecules. Moreover, a novel peptide of the RAS, alamandine, with its receptor, MrgD, was discovered and functionally analyzed. Together with transgenic rats overexpressing ACE2 or angiotensin(1-7), ACE2- and Mas-knockout mice characterized the ACE2/angiotensin(1-7)/Mas system as a protective axis that counteracts the classical RAS effects in cardiovascular and muscular diseases and in fat homeostasis (Fig. 1). Furthermore, these animals showed that angiotensin(1-7) and Mas are important for insulin sensitivity and the pathogenesis of metabolic syndrome. In a newly established transgenic rat model with inducible diabetes mellitus type 2 a novel oral formulation of angiotensin(1-7) was shown to exert potent antidiabetic actions revealing the therapeutic potential of the protective RAS axis. In addition, ACE2 was found to be pivotal for serotonin generation in brain and periphery by its function as part of the uptake system for tryptophan in the gut. This may also be the reason for the blunted exercise-induced hippocampal neurogenesis which we observed in ACE2-deficient mice.

Kallikrein-kinin system and chemokines

The kallikrein-kinin system (KKS) is an important hormone system for cardiovascular regulation also mostly counteracting the effects of the RAS. As models for the functional analysis of the KKS in intact animals, transgenic rats were generated expressing different components of the system, such as tissue kallikrein, the kinin B1 or the B2 receptor either ubiquitously or specifically in cardiovascular organs. These animals supported the protective role of the KKS in kidney and heart against ischemic, diabetic, and hypertrophic injury. Knockout mice for the kinin B1 receptor and mice lacking both kinin receptors were generated and revealed important functions of these proteins in pain perception and inflammation. Moreover, kinin receptors turned out to be involved in arteriogenesis, sepsis, stroke, multiple sclerosis and high-fat diet induced obesity.

Moreover, downstream mediators of kinins such as the chemokine CXCL5 and its receptor CXCR1 as well as other chemokines, such as CXCL12, are functionally analyzed

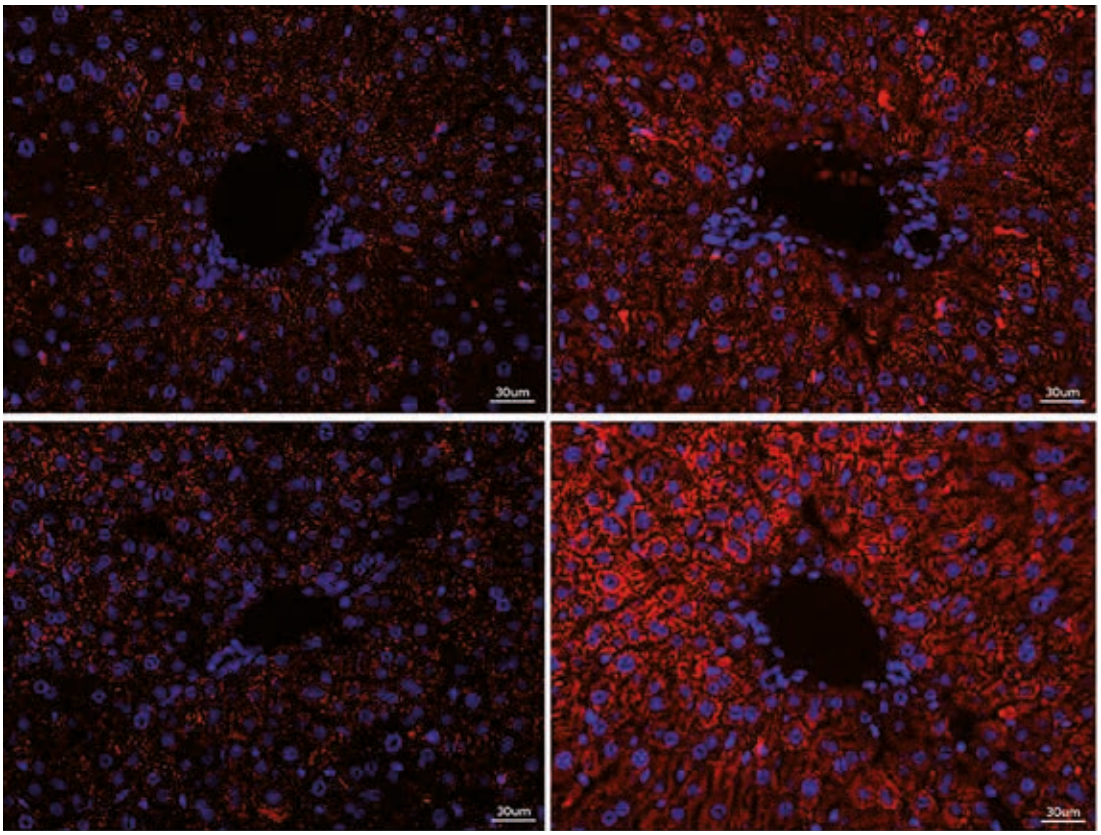


Figure 1: Mice deficient for angiotensin converting enzyme 2 (ACE2) develop liver steatosis with age. *Liver tissue sections of ACE-knockout (lower panels) and wildtype (upper panels) mice at 3 (left) and 6 months (right) of age were stained for adipophilin, which labels lipid droplets in the cells.*

in newly developed knockout mouse models. Mice with a local deletion of CXCL12 in cardiomyocytes and transgenic rats overexpressing the chemokine in this cell type revealed an unexpected deteriorating role of this chemokine for heart function after ischemic damage. CXCL5 was characterized as major determinant of macrophage foam cell formation in atherosclerosis.

Serotonin system

Serotonin is a monoamine which functions as an important neurotransmitter in the central nervous system and as a major peripheral mediator produced by enterochromaffin cells of the gut and transported and released by platelets in the circulation. We discovered that vertebrates have two tryptophan hydroxylases, the rate limiting enzymes in serotonin synthesis, TPH1 and TPH2. Mice deficient in TPH1, the isoform responsible for the synthesis of serotonin in the gut, showed that peripheral serotonin is involved in thrombosis, pulmonary hypertension, remodelling of mammary glands, tumor angiogenesis, liver regeneration, and hepatitis, but not in bone metabolism as previously suggested. Mice deficient in TPH2, the isoform responsible for the synthesis of serotonin in the brain, were surprisingly viable and fertile, despite a near complete lack of serotonin in the brain, and showed growth retardation and altered autonomic control leading to impairment of sleep, respiration, cardiovascular and metabolic parameters. We also revealed that exercise-induced adult neurogenesis is abolished in these animals. In addition, TPH2-deletion is correlated with increased aggression, maternal neglect, and reduced aversive and anxiety-like behavior. Moreover, lack of TPH2 evoked increase in sexual activity of female mice. Since further behavior analysis of this model was hampered by poor ability of the mouse for complex social interactions and learning capacity, we now perform these studies in TPH2-deficient rats generated via zinc-finger-nuclease based gene-deletion (Fig. 2) and in rats with inducible knockdown of TPH2. First data indicated communication deficit and impairment in ultrasonic vocalization in juvenile serotonin-deficient rats. To study role of serotonin in embryonic development we have additionally generated TPH1/2 double knockout mice.

Since serotonin is a major determinant in the pathogenesis of several diseases, such as pulmonary hypertension, we developed and patented modulators of TPH activity as novel therapeutic drugs together with the high-throughput screening platform and the medicinal chemistry department of the Research Institute for Molecular Pharmacology (FMP) and the Helmholtz Protein Sample Production Facility (PSPF).

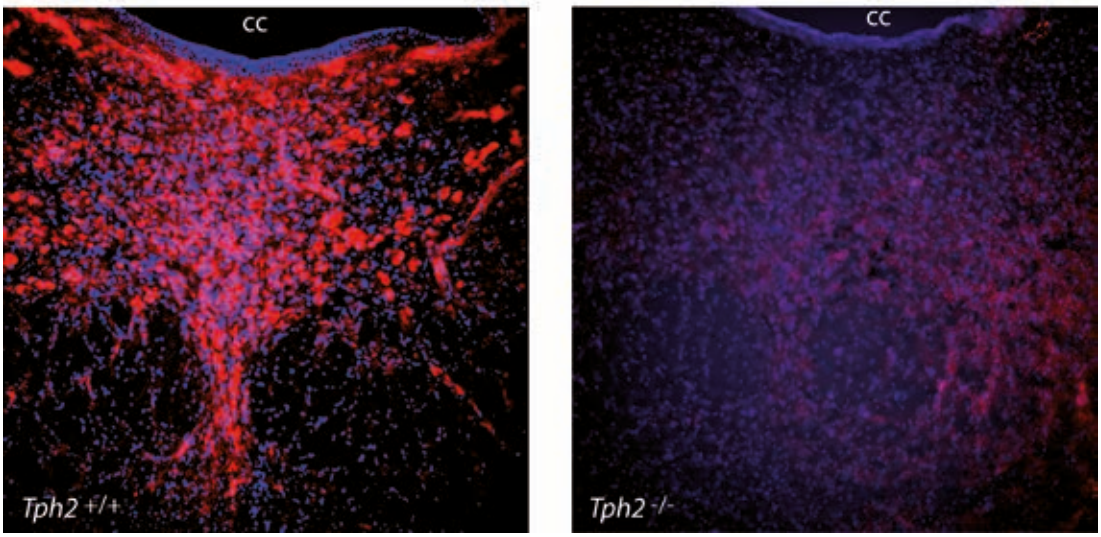


Figure 2: Rats deficient for tryptophan hydroxylase 2 (TPH2) lack serotonin in the brain. *Hindbrain sections of wild-type (TPH2+/+, left) and TPH2-knockout rats (TPH2-/-, right) were stained with anti-serotonin antibodies.*

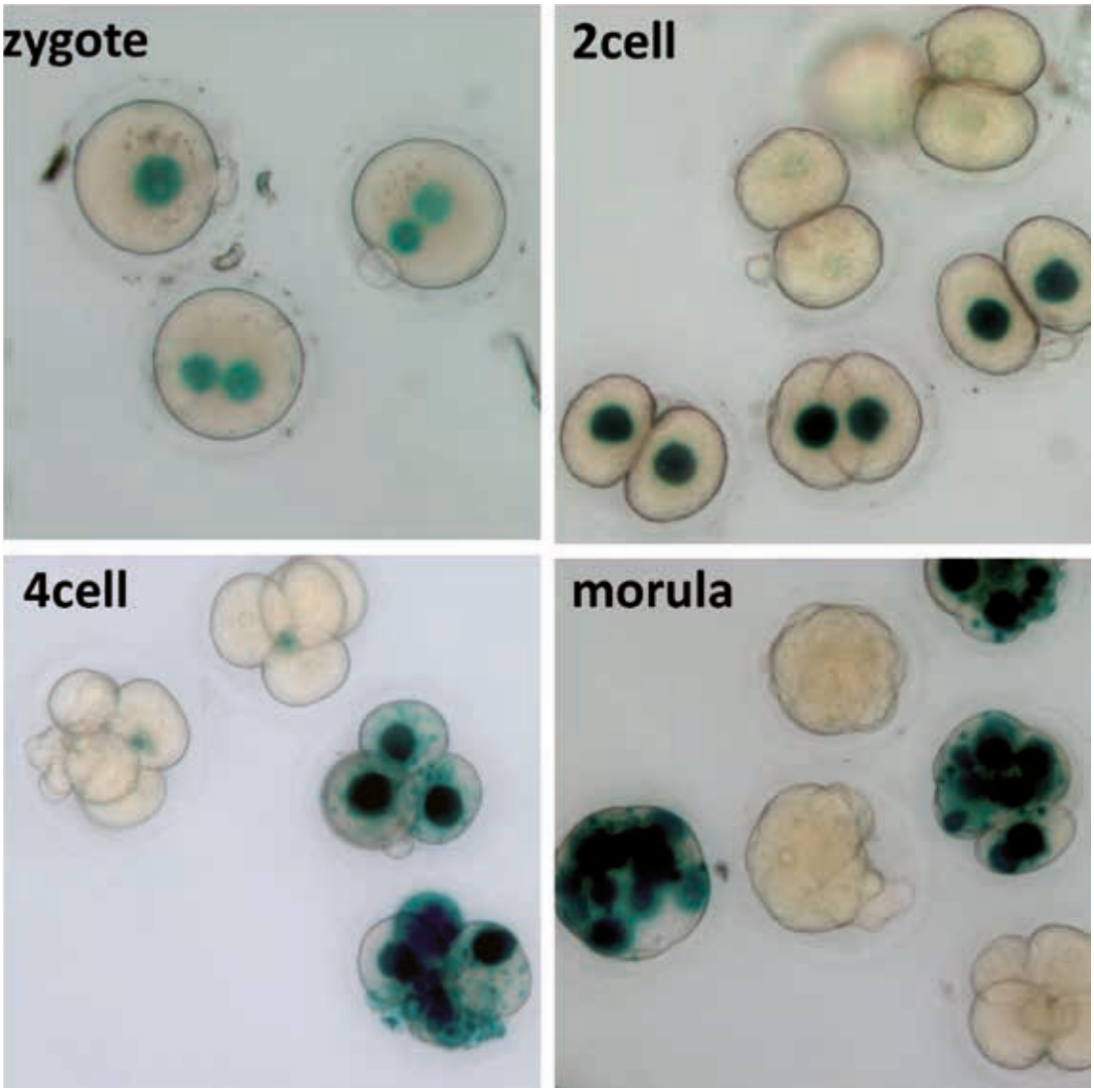


Figure 3: Xgal staining in importin α1 (KPNA2) deficient embryos. *Female KPNA2 deficient mice are infertile. To assess KPNA2 expression in early embryos, females heterozygous for a lacZ genetrap in the KPNA2 locus were crossed to wildtype males. Heterozygous embryos show Xgal staining from 2cell stage on, wildtype embryos don't. The weak staining in all zygotes is caused by maternal KPNA2 mRNA. Thus, KPNA2 is a maternal effect gene and a marker of zygotic genome activation.*

Androgen receptor

The group generated and characterized animal models with altered androgen receptor expression in distinct cell types. Transgenic rats overexpressing the androgen receptor in cardiomyocytes exhibited marked alterations in cardiac rhythmicity probably due to dysregulation of potassium channels and mice lacking the receptor in macrophages revealed its function in salt homeostasis and blood pressure control.

Importins

Importins are essential components of the machinery that transports proteins into the nucleus of eukaryotic cells. In a collaborative approach with the University of Lübeck to study the physiological functions of alpha importins we have generated knockout mice for five paralogs. The most obvious phenotype was discovered in mice lacking importin alpha1 and alpha7: In both lines the females are infertile. The female infertility of importin alpha7 knockout mice is based on its essential function during zygotic genome activation of developing embryos. For importin alpha1 the molecular basis is still under investigation (Fig. 3), as is the cause for the male infertility which is additionally observed in importin alpha7 knockout mice. Furthermore, importin alpha7 is involved in Influenza and Ebola virus infection of cells. In parallel, proteomic analyses were performed identifying the specific import cargos of single alpha importin paralogs.

Transgenic and stem cell technology

The group has also a strong emphasis in the field of rat embryology and stem cell research. The rat is the preferred species in physiological and behavioural studies. In order to obtain rat pluripotent stem cells two methodologies were applied in our group: isolation of ES cells from rat preimplantation embryos and generation of induced pluripotent stem (iPS) cells from fibroblasts upon infection with lentiviruses carrying pluripotency genes. Furthermore, transgenic rats have been produced carrying constructs, which express small interference RNAs suited to downregulate specific genes. The first target genes were the insulin receptor, the (pro)renin receptor, apolipoprotein E, and the LDL receptor. CRISPR/Cas technology has also been established and successfully used to ablate genes in rats.

Patents / Patent applications

Method for diagnosing neuronal diseases and for the treatment of deficient primary hemostasis, US7049336 (B2) (23.05.2006)

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Photo: David Ausserhofer/MDC

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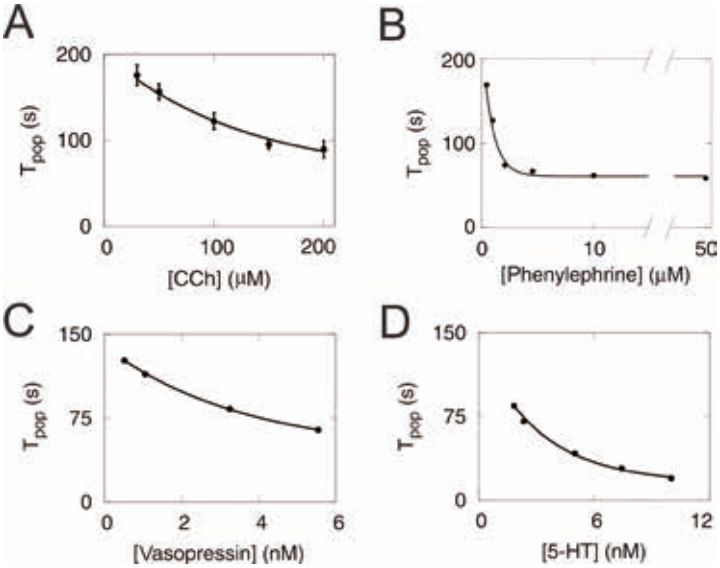
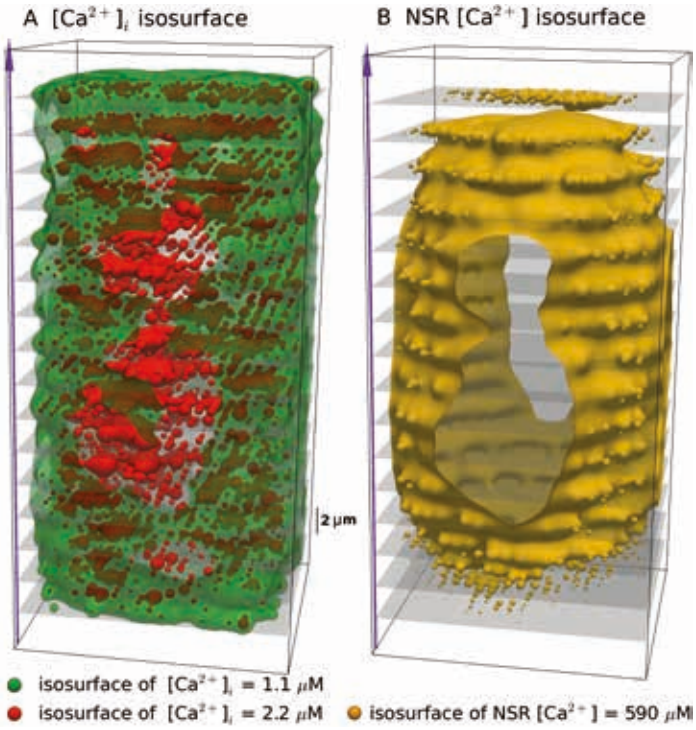
Mathematical Cell Physiology

The group develops mathematical models for cell biology from single molecules, via protein complexes to cell level. Modelling serves to test and formulate biological hypotheses in a quantitative way as well as to predict the behavior of complex pathways and networks. Current projects comprise analysis and modelling of IP_3 induced Ca^{2+} spiking and excitation contraction coupling in cardiac myocytes.

Ca^{2+} is a ubiquitous intracellular messenger that regulates diverse cellular activities. Extracellular stimuli often evoke sequences of intracellular Ca^{2+} spikes, and spike frequency may encode stimulus intensity. However, the timing of spikes within a cell is random because each interspike interval has a large stochastic component. In human embryonic kidney 293 cells and rat primary hepatocytes, we also found that the average interspike interval varied between individual cells (1). To evaluate how individual cells reliably encoded stimuli when Ca^{2+} spikes exhibited such unpredictability, we combined Ca^{2+} imaging of single cells with mathematical analyses of the Ca^{2+} spikes evoked by receptors that stimulate formation of inositol 1,4,5-trisphosphate (IP_3). This analysis revealed that signal-to-noise ratios were improved by slow recovery from feedback inhibition of Ca^{2+} spiking operating at the whole-cell level, and they were robust against perturbations of the signaling pathway. Despite variability in the frequency of Ca^{2+} spikes between cells, steps in stimulus intensity caused the stochastic period of the interspike interval to change by the same factor in all cells. These fold changes



Fold changes determine a universal exponential concentration-response relation for the average interspike interval of Ca^{2+} spikes evoked by stimulation of GPCR with the concentrations given in the figures. **The** *lines are single exponential functions fit to the experimental data.(***A***) HEK cells stimulated with CCh,* **(B)** *hepatocytes stimulated with phenylephrine,* **(C)** *hepatocytes stimulated with vasopressin,* **(D)** *salivary gland stimulated with 5-HT.*



Three dimensional visualisation of spatially resolved $[Ca^{2+}]$ at 70.0 ms after depolarisation of the membrane potential. **(A)** *Isosurfaces show $[Ca^{2+}]_i$ in green for $[Ca^{2+}]_i$ = 0.6 µM and red for $[Ca^{2+}]_i$ = 2.4 µM .* **(B)** *The yellow isosurface shows free sarcoplasmic $[Ca^{2+}]$ = 430 µM. There are 320 CRU per z-disc, amounting to 5160 CRUs in total, with an average of 50 RyR and 12.5 LCC per Ca^{2+} release unit (diadic cleft).*

reliably encoded changes in stimulus intensity, and they resulted in an exponential dependence of average interspike interval on stimulation strength. We conclude that Ca^{2+} spikes enable reliable signaling in a cell population despite randomness and cell-to-cell variability, because global feedback reduces noise, and changes in stimulus intensity are represented by fold changes in the stochastic period of the interspike interval.

Mathematical modelling of excitation-contraction coupling (ECC) in ventricular cardiac myocytes is a multiscale problem, and it is therefore difficult to develop spatially detailed simulation tools. ECC involves gradients on the length scale of 100 nm in dyadic spaces and concentration profiles along the 100 µm of the whole cell, as well as the submillisecond time scale of local concentration changes and the change of lumenal Ca^{2+} content within tens of seconds. Our concept for a multiscale mathematical model of Ca^{2+}-induced Ca^{2+} release (2) (CICR) and whole cardiomyocyte electrophysiology incorporates stochastic simulation of individual LC- and RyR-channels, spatially detailed concentration dynamics in dyadic clefts, rabbit membrane potential dynamics and a system of partial differential equations for myoplasmic and lumenal free Ca^{2+} and Ca^{2+} -binding molecules in the bulk of the cell. We developed a novel computational approach to resolve the concentration gradients from dyadic space to cell level by using a quasistatic approximation within the dyad (3) and finite element methods for integrating the partial differential equations. We simulate whole cell Ca^{2+} concentration profiles using three previously published RyR-channel Markov schemes.

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Photo: David Ausserhofer/MDC

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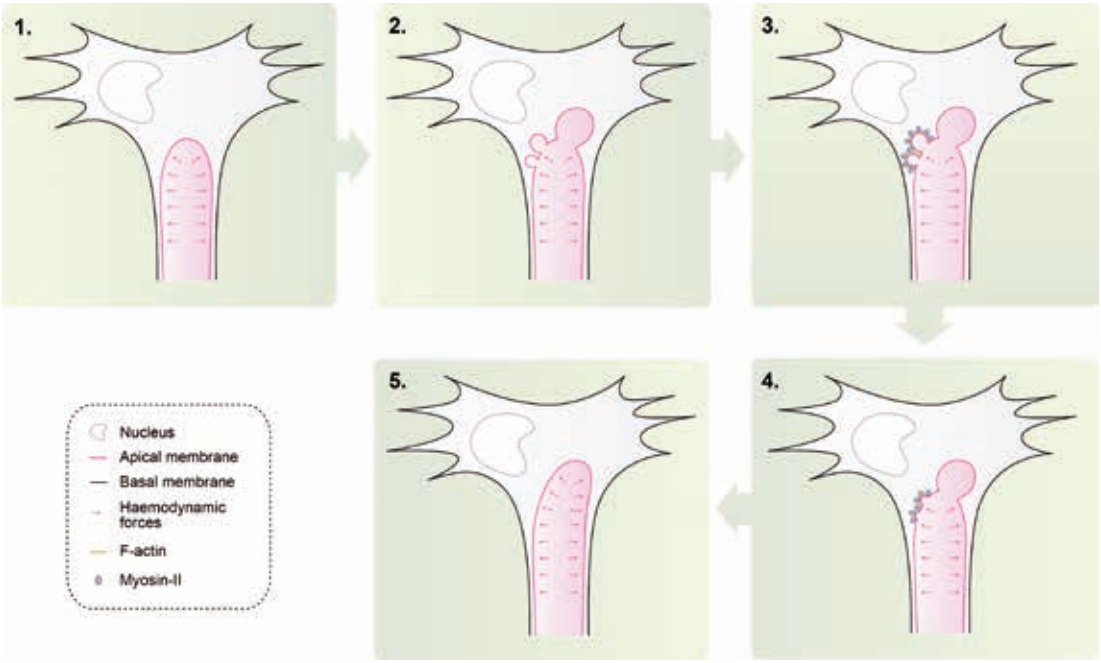
Integrative Vascular Biology Laboratory

The Integrative Vascular Biology Laboratory aims to unravel the fundamental principles and molecular/genetic regulation of functional vascular network formation in development and disease. The ultimate goal of our research activities is to advance our understanding of mechanisms and molecules controlling formation and patterning of a hierarchically branched vascular network such that we can inform, innovate and implement therapeutic approaches to mitigate cardiovascular complications, and establish or restore tissue homeostasis in compromised patients. We develop and use genetic tools to image and manipulate molecules, complexes and cells in spatiotemporal controlled manner in vivo in mouse and zebrafish models. We further use iteration between predictive computational modeling and experimentation to develop hypothesis and systems level understanding of the processes at play.

The years 2015 and 2016 mark our first full years at the MDC following relocation from the Francis Crick Institute in London. Many projects in our team currently converge on investigating how endothelial cells collectively coordinate functional vessel remodeling, i.e. the transition from a primitive and poorly perfused vessel plexus to the fully remodeled network with hierarchical diameter control. We are also beginning to address the mechanisms regulating maintenance and plasticity of vascular networks in adult physiology, and principles of functional adaptation, as well as maladaptation in vascular disease.
The present report however will focus on the identification of a highly unexpected and novel mechanism of lumen formation in angiogenesis and its implications for vessel plasticity.

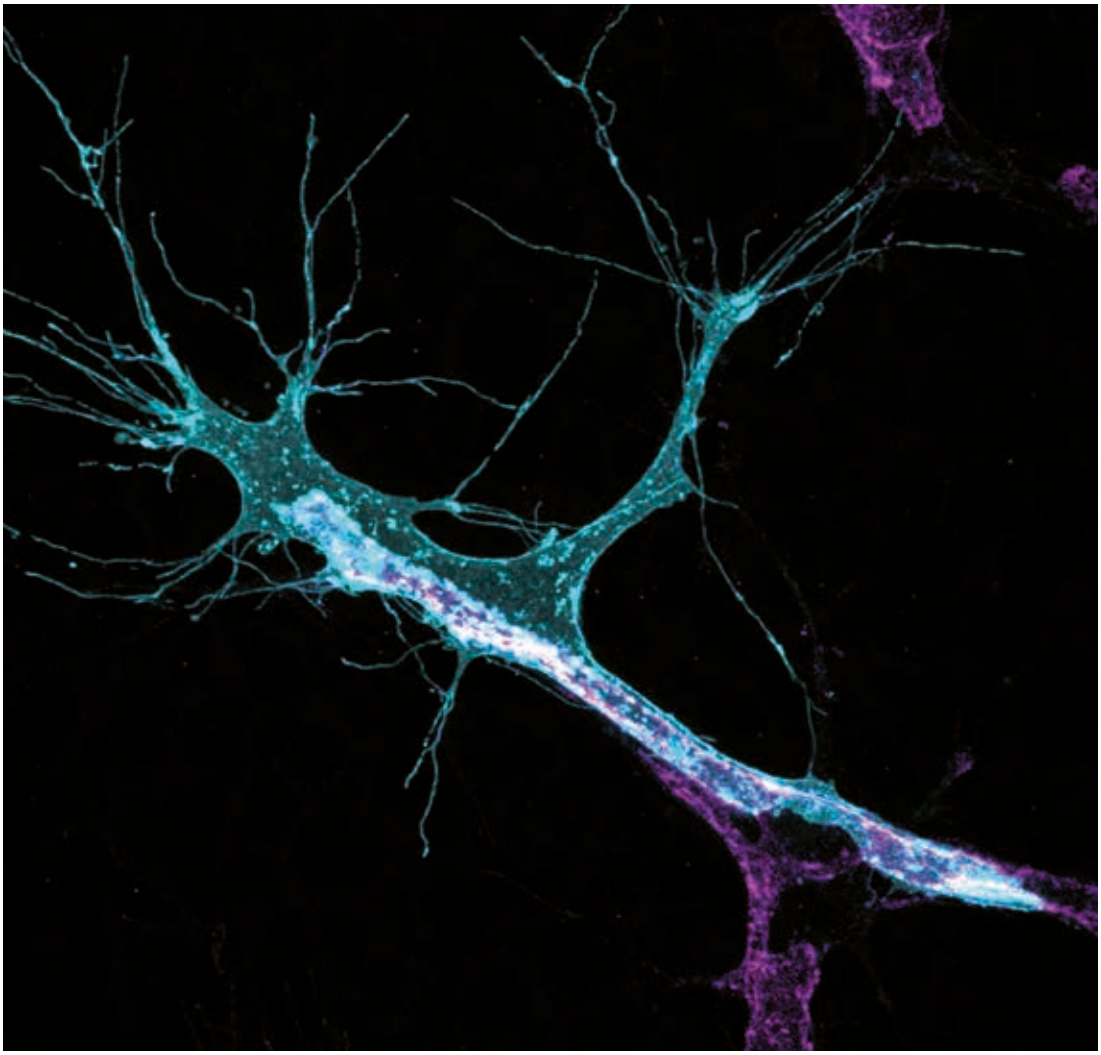
Previous studies in zebrafish models of angiogenesis and in 3D-cultured endothelial cells identified intracellular vacuole formation and coalescence as a mechanism of lumen formation during blood vessel development. Indeed, the earliest vascular lumens that form in the embryo before heartbeat commences, as well as lumens that form in cell culture in the absence of flow, can form de novo through this mechanism. Most vascular networks in vertebrates however form and extend after cardiac activity and blood flow are established, following a process known as sprouting angiogenesis, and lumen formation mechanisms in these conditions are not fully understood. We established fluorescent reporters for the apical membrane (i.e. lining the lumen) of endothelial cells to allow detailed dynamic imaging of lumen formation in the intersegmental vessels of zebrafish embryos. By pushing the limits of spatial and temporal resolution using state-of-the art spinning disc microscopy, we found no evidence for



Model of lumen formation by inverse membrane blebbing during sprouting angiogenesis.

de novo vacuole formation during sprouting angiogenesis. Instead we found that lumen expands through a mechanism that we termed 'inverse membrane blebbing'. The haemodynamic forces applied on the apical membrane of endothelial cells as blood pushes into new vessels during sprouting induce spherical deformations of the apical membrane reminiscent of the membrane blebs observed during cell migration or cell division (Charras and Paluch, 2008). However, unlike classical blebs, the apical membrane deformations display an inverted polarity, with the membrane protruding into the cell. Interestingly, by following the dynamics of the actomyosin cytoskeleton during this process, we found that lumen expansion by inverse blebbing is tightly regulated through local and transient recruitment and contraction of actomyosin at the surface of the blebs, thus allowing controlled growth of new lumen branches. Using inducible single-cell loss-of-function approaches, we identified that endothelial cells with impaired contractility fail to expand lumens or show dilated, multi-branched lumens. Together, these results unravel a novel mechanism of lumen formation in blood vessels, where a tight balance between the pushing forces exerted by the blood on the membrane of endothelial cells and the endothelial cells own contractile responses is required for shaping new vascular tubes (Gebala et al, 2016). Future work will need to establish how this exquisite elasticity of the apical membrane and its underlying actin cortex contractility is regulated during the angiogenic process, as vessels mature and are subjected to higher and changing levels of blood pressure.

Interestingly, the nascent lumen appears to be highly instable as cessation of blood flow or increased endothelial contractility lead to lumen collapse. This is in marked contrast to mature vascular networks, which experience changing pressure and flow regimes in the course of physiological flow regulation. Thus mature lumens must somehow be stabilized, yet the mechanisms are unclear.



Single endothelial tip cell (cyan, cell membrane) undergoing lumen formation (magenta, apical/luminal membrane) at the sprouting front of a mouse retina vasculature at post-natal day 6 (P6).

A second project in our team on endothelial actin regulation uncovered one important mechanism of lumen stabilization – endothelial rearrangement and junctional actin nucleation. Whereas nascent lumens can form within single endothelial cells or between two cells, the mature endothelial configuration in capillaries shows generally two endothelial cells aligning so that parallel junctional profiles run along the length of each vessel. The configuration is achieved through dynamic cell rearrangements that require regulated junctional VE-cadherin turnover (Bentley et al, 2014) and junctional actin nucleation through the formin Fmnl3 (Phng et al, 2015). Live imaging in zebrafish embryos illustrated that Fmnl3 localizes to endothelial junctions, and inducible endothelial expression of dominant negative Fmnl3 causes lumen collapse and failed endothelial rearrangement. Instead of forming parallel junctions, endothelial cells lacking Fmnl3, or exposed to chemical fomin inhibitors, maintained isolated ring-like junctions. Recent work by the team of Markus Affolter suggest that the c-terminal binding of actin to VE-cadherin is critical for junctional force transmission that drives endothelial cell shape changes and rearrangements during vessel elongation and lumen stabilization (Sauteur et al 2014).

How Fmnl3 activity and localization is regulated, and whether blood flow itself is involved in its recruitment to the junction remains to be shown. Our future work will need to unravel the complexity and dynamics of the junctional complexes at play during these morphogenic processes in development. From a clinical perspective, we will seek to establish whether and how endothelial reactivation in adult physiology or inflammation might destabilize vascular lumens and therefore contribute to small vessel disease.

Perfused blood vessels in the trunk of a transgenic zebrafish embryo expressing the actin marker Lifeact specifically in endothelial cells. Note the distinct actin accumulation along endothelial junctions visible as thin lines running across and along the vessels. Image courtesy of Li-Kun Phng

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(Aspalter, Gordon et al., 2015, Franco, Jones et al., 2016, Gebala, Collins et al., 2016, Phng, Gebala et al., 2015, Ubezio, Blanco et al., 2016)

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Gebala V, Collins R, Geudens I, Phng LK, Gerhardt H (2016) Blood flow drives lumen formation by inverse membrane blebbing during angiogenesis in vivo. Nature cell biology 18: 443-50

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Photo: David Ausserhofer/MDC

Michael Gotthardt

Neuromuscular and Cardiovascular Cell Biology

The long-term goal of the Gotthardt-lab is to develop a basic understanding of the transition from cardiovascular health to disease at the molecular, cellular, organ and systems level and use this information to improve patient care. Specifically, we are interested in understanding how increased filling of the cardiac ventricle leads to improved contraction (Frank-Starling mechanism of the heart), how alternative splicing relates to diastolic heart failure, and how the electrical properties of the heart and mechanoelectrical coupling are regulated. The group focuses on titin, the largest protein in the human body, and the multifunctional coxsackie- and adenovirus receptor (CAR). We utilize animal models and differentiated stem cells combined with cell biological, biochemical, genetic, pharmacological, and omics tools to establish titin as a mechanosensor and study sarcomere dynamics. With a loss of function approach, we have demonstrated that CAR is crucial for embryonic development, determines the electrical properties of the heart, and can serve as a therapeutic target to improve cardiac remodeling. Currently our main translational focus is on harnessing alternative splicing to improve cardiac function using a pharmacological screen with custom splice reporter assays. This work aims to identify and characterize patients with splice related heart disease towards improved personalized diagnostics and therapy. An overview of our scientific approach is depicted in Figure 1.

Titin in sarcomere assembly and heart disease

Titin is a giant protein found in vertebrate muscle, where it extends over the half-sarcomere and integrates into the Z-disc and M-band to form a continuous filament. The titin gene expresses differentially spliced mRNAs of up to 100 kb that encode for proteins of up to 33,000 amino acids. Titin contains a PEVK segment, N2B-, and Ig regions, which provide elastic diversity through alternative splicing and posttranslational modifications. Its C-terminus contains a serine/threonine kinase domain, which has been implicated in mechanotransduction. Using our animal models, we were able to elucidate titin's role in sarcomere assembly and to link titin to adrenergic signal transduction, lateral growth of the sarcomere, and the hypertrophy response. We study the role of titin in cardiac adaptation and how it converts mechanical input into a molecular signal.

The Coxsackie- and Adenovirus Receptor (CAR)

Both adenovirus and coxsackievirus are common pathogens associated with myocarditis and dilated cardiomyopathy (DCM). They infect cardiomyocytes using CAR and its co-receptors integrin αvß3 und αvß5. When we started the CAR conditional knockout project, the analysis of CAR in muscle and heart disease had been restricted to basic expression and localization analysis, which indicated an important role in remodeling after myocardial infarction. Accordingly, CAR expression is low in the adult but CAR is upregulated in areas surrounding myocardial infarction. Our conditional knockout provided novel insights into the role of CAR in arrhythmia and established its role in myocarditis and myocardial infarction. On the cellular level, we study how

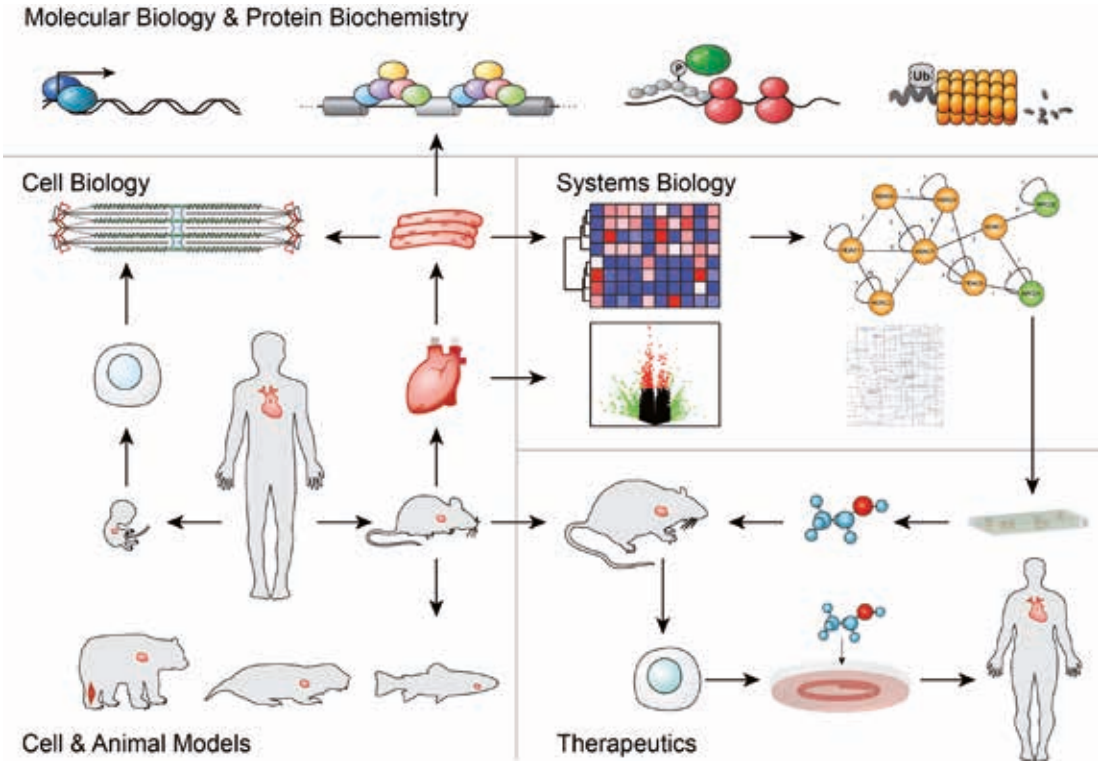

Figure 1: *Experimental approach. We use cell- and animal models to understand the molecular and cellular basis of heart disease. Focusing on the sarcomeric protein titin and cardiac splicing, we integrate a candidate- with a systems biology approach to derive splice regulatory networks and therapeutic targets. With high throughput screening to identify splice regulators and validation in cell and animal models, we aim to develop novel therapeutic strategies for cardiac disease.*

CAR effects the cytoskeleton, endocytosis, and signal transduction using inducible CAR deficient ES and iPS-cells, cardiomyocytes, and neurons (Figure 2). We translate our findings and develop biologicals to interfere with CAR function and improve the outcome of mice with myocardial infarction by improving the response to ischemia.

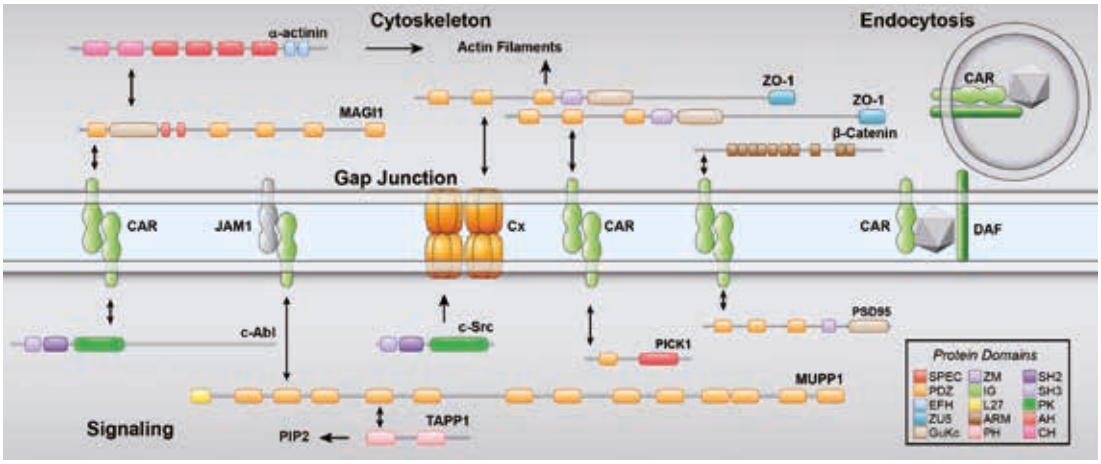

Figure 2: *CAR is a multifunctional protein. CAR (light green) is involved in cell contact formation, actin filament assembly, ion homeostasis (gap junctions), endocytosis, and several signal transduction pathways. It interacts with multiple extra- and intracellular proteins.*

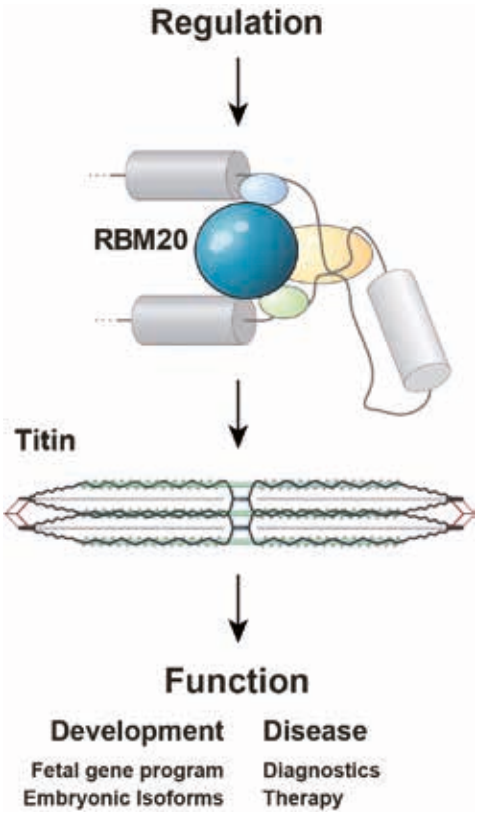

Figure 3: *Regulation of cardiac function via alternative splicing. RBM20 is a cardiac splice factor that determines the elastic properties of the sarcomeric protein titin. Its substrate spectrum is now well characterized and RBM20 has been linked to human cardiomyopathy with fibrosis, arrhythmia, and sudden death.*

Alternative splicing and regulation of titin isoform expression in health and disease

Alternative splicing has emerged as a major source of proteome diversity and as a central regulator of protein isoform expression in development and disease. In an integrative approach, we have used rodent and human genetics, cardiac physiology, and functional genomics to identify RBM20 as a novel *trans*- acting factor regulating the differential splicing across a broad range of transcripts during normal development (Figure 3). Notably, this is one of the few examples of a human disease mechanism involving an effect in *trans*. Our extended splicing analysis suggests that RBM20 targets a subset of cardiac genes that concertedly affect biomechanics, electrical activity, and signal transduction. This analysis of cardiac isoform expression does not only offer insights into the coordination of splicing events, but provides novel diagnostic and therapeutic targets for cardiac disease. In addition to evaluating RBM20 as a therapeutic target, we have established a dual luciferase assay and identified molecules that inhibit RBM20 activity to interfere with titin splicing and thus the diastolic properties of the heart. Using genetic engineering *in vivo* and the CRISPR/Cas9 system, we have identified novel titin splice factors, which are currently evaluated for their substrate profile and regulation.

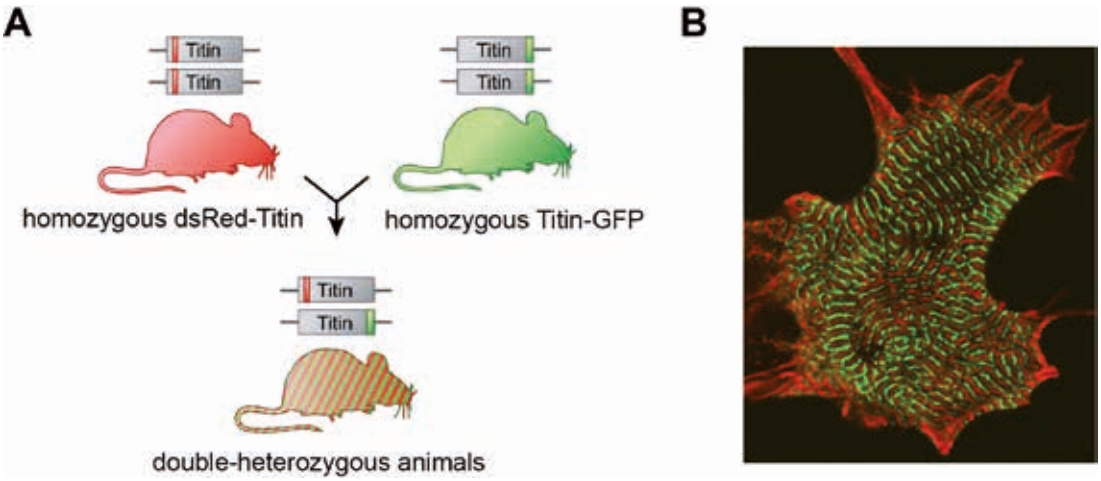

Figure 4: *Visualizing sarcomere dynamics. A) Generation of titin knock-in mice expressing RFP-Titin and Titin-GFP fusion proteins. B) Titin fusion proteins are properly integrated into the sarcomere to produce the characteristic periodic Z-disc (Red) and M-band staining (green).*

Advancing technology and model systems

To lay the foundation for the *in vivo* analysis of titin's multiple signaling, elastic, and adaptor domains, we have generated various titin deficient mice and established surgical and mechanical protocols to induce atrophy and hypertrophy. Recently, we have added a naturally occurring animal model to our portfolio that exhibits little muscle atrophy despite extended periods of immobilization – namely the hibernating grizzly bear. Using an omics and mathematical modeling approach, we derived novel therapeutic targets to help prevent muscle atrophy in aging, immobilization, and inherited muscle disease.

In a gain of function approach we engineered mice to express a combination of fluorescently labeled titin molecules that enable the analysis of titin mobility, sarcomere assembly, remodeling, and turnover under different mechanical loads, in embryonic versus postnatal development, and in cardiac and skeletal muscle disease (Figure 4). To evaluate compartmentalized mechanosignaling and follow changes in protein composition along the sarcomere in health and disease, we have now established localization proteomics *in vivo* using the BioID system. This approach has already provided us with insights into subnuclear compartmentalization of splicing, and now enables the quantification of protein phosphorylation and -complex formation at subsarcomeric resolution. This work complements our live imaging analysis of sarcomere assembly in titin GFP and RFP knockin mice towards understanding the plasticity of myofilaments.

We will build on our expertise in generating and analyzing animal models to study the molecular basis of cardiovascular, skeletal muscle and neurological disease, identify therapeutic targets, and to evaluate novel therapeutic strategies. To advance patient care, we will improve our interaction with clinicians at the Charité and the DZHK and scientists at the FMP. This includes the E:Bio initiative of the BMBF for personalized diagnostics and therapy of splice related cardiac disease as well as the FMP-screening and drug development infrastructure and outsourcing of the production of biologicals under GMP conditions.

Patents / Patent applications

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Photo: David Ausserhofer/MDC

Norbert Hübner

Genetics and Genomics of Cardiovascular Diseases

A major challenge today is to understand systems perturbations that are relevant to complex diseases in human populations and that are due to subtle changes induced by naturally occurring DNA variation or environmental conditions. Often such perturbations of biological networks, induced by complete knockout or over-expression of individual genes, have been used to help understanding the molecular basis of human diseases. We are applying novel large scale technologies and advanced computation in multidisciplinary approaches to expand the systems concept and to accelerate discovery of gene networks underlying complex cardiovascular and metabolic disease phenotypes in the rat model, and we translate the findings to the molecular pathology of related human disorders. Our particular interests are inflammatory and metabolic genetic risk factors that lead to cardio- and cerebrovascular target organ damage. Any layer of regulation can affect expression of a gene, and we are more and more interested to look not only at the genetic variation regulation of gene expression but also at effects in post-transcriptional and epigenetic processes of complex disease. We are interested in all levels of regulation in the rat and human. We are using integrated approaches involving methods like RNA-seq, ChIPseq and ribosome profiling, and we have already shown that translational regulation is extensive in the left ventricles and livers of the BN-Lx and SHR rat strains.

Genetic regulation on multiple layers

We are interested in the identification of genes and pathways that predict aberrant physiology in complex disease or metabolic control. The spontaneously hypertensive rat (SHR) is the most widely studied animal model of human hypertension and it displays a large number of other physiological and pathophysiological phenotypes, including stroke, cardiac hypertrophy and failure, and features of the human metabolic syndrome. We study the SHR and SHR derived substrains using genetic linkage and previously identified thousands of genetic control points for gene expression – or expression quantitative trait loci (eQTLs) – across the SHR genome. We extended our studies on the transcriptional level and included 7 tissues from the BXH/HXB panel of recombinant inbred (RI) strains bred from SHR and BN founders for comprehensive expression studies. We constantly seek to improve our strategies and methods using deep re-sequencing and statistical approaches for the identification of key genetic regulators in the animal model. The next step for us was to extend our studies to the regulation on the translational level. Several genetic disorders are caused by mutations that affect protein translation, but so far genome-wide protein synthesis rates have not been studied in complex disease models. Therefore, we adapted a new method, ribosome profiling (Ribo-seq), and performed genome-wide RNA sequencing and ribosome profiling in heart and liver tissues to investigate strain-specific translational regulation in the spontaneously hypertensive rat. With this technique, only the parts of mRNA molecules that are occupied by translating ribosomes are captured and sequenced. In our lab, we optimized this method to make

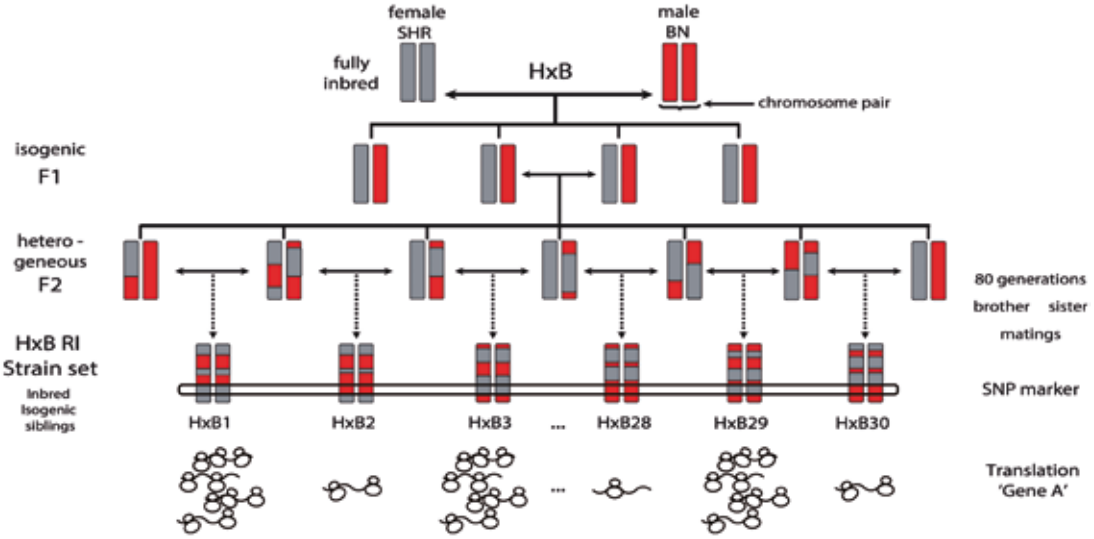

Figure 1 *The HxB/BxH recombinant inbred (RI) panel and riboQTL mapping for identification of genetic loci responsible for translational differences. Scheme of the RI panel showing alleles from the BN rat (red) and the SHR strain (grey). RNA-seq and Ribo-seq data from left ventricular and liver tissue was integrated with genotyping information for eQTL and riboQTL mapping. QTLs are genetically variable loci that strongly link to transcription and translation differences.*

it applicable to mammalian tissues, including the left ventricle. We found hundreds of strain-specific differences in translation, almost doubling the number of differentially expressed genes.

We extend this approach to liver and left-ventricular tissue of all 30 animals of the RI panel. We integrate these data with panel-wide genotype information to perform quantitative trait loci (QTL) mapping. The integration of genetic, transcriptional and translational data sets reveals distinct signatures in 3'UTR variation, RNA-binding protein motifs and miRNA expression associated with translational regulation of gene expression. Using both transcriptional and translational information we can identify local (cis) and distant (trans) effectors of both levels of gene expression regulation and discriminate genetic loci regulating transcript abundance (eQTLs) from genetic loci acting purely at the translational level (riboQTLs). We find that strain-specific differences on the transcriptional level tend to be equally apparent on the translational level. Remarkably, Ribo-seq data also expose strain-specific differences in translation, which are invisible at the transcriptome level. They do influence final cellular protein abundance, and almost double the number of differentially expressed genes between the strains. Translational regulation influenced the expression levels of many genes with potential relevance to the cardio-metabolic disease phenotype of the SHR rat, including the cardiac disease genes Myh6 and Fads1 and genes regulating metabolite levels (e.g. Acadl and Tat). Moreover, several human GWAS candidates were found to be primarily translationally regulated in the rat model. These data provide insight in the wide variety of genetic mechanisms that introduce variation in gene expression levels, possibly contributing to complex disease. Recently, we have adapted this technique to human left-ventricular biopsies of dilated cardiomyopathy (DCM) patients. This resulted in an unprecedented assessment of translated sequences in the human diseased heart, including evidence that ribosomes translate truncated titin (TTN) isoforms up to the truncating mutation, and occasionally even beyond (~5-10% read-through). When comparing DCM patients and controls on the transcriptional level, we find many differentially expressed genes including master regulators of cardiac transcription such as TBX20. Interestingly, we also find 437 differentially expressed long noncoding RNAs (lncRNAs), of which 257 (~59%) appear to be translated. The latter could be an indication of a regulatory function for these lncRNAs during translation or the differential translation of so far unknown micropeptides potentially playing a role in DCM. Combined, these data suggest a variety of potential novel disease mechanisms driven by transcriptional and translational regulation in the human heart.

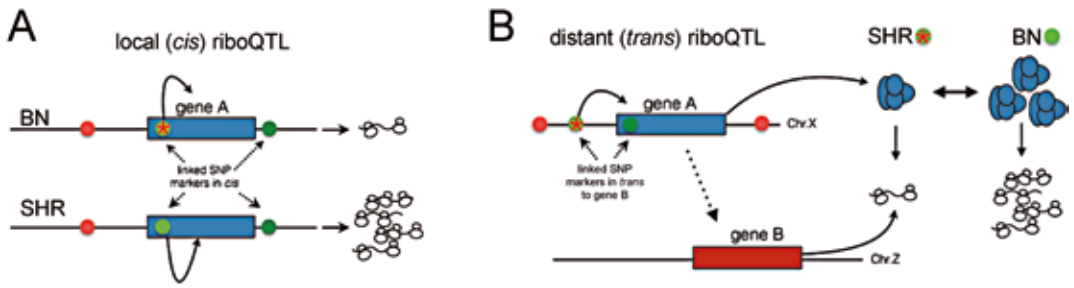

Figure 2 *Identifying local and distant riboQTLs as cis or trans regulators of differential translation. (A) In cis, a genetic variant may change specific transcript properties that affect translational efficiency. (B) In trans, a distant variant may locally change the expression of 'gene A', which regulates the translation of 'gene B' at a distant locus.*

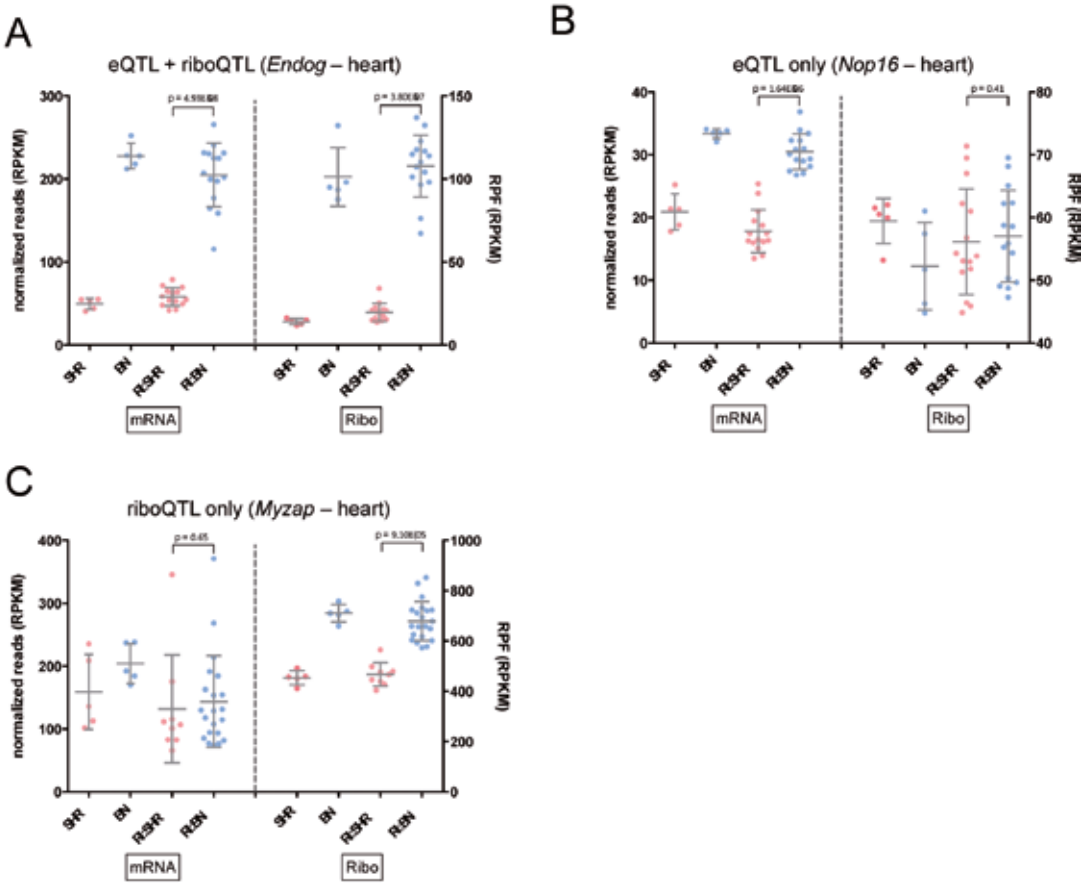

Figure 3 *(A) The Endog gene is differentially transcribed and differentially translated. A clear difference in expression is indicative of a strong cis eQTL (left) and riboQTL (right). (B) An example of a gene (Nop16) with transcriptional regulation in cis, this effect is absent at the translational level (translational buffering). (C) The Myzap gene has no local regulator of transcription, but does have a local genetic variant strongly linked to a translational difference.*

Integrated genomic approaches for identification of gene networks

We have identified mutations in RNA binding proteins that contribute to pathological posttranscriptional regulation of an entire network of target genes that lead to cardiomyopathy and heart failure. In collaboration with Michael Gotthardt (MDC) we used genome-wide approaches in a rat strain deficient in titin splicing and found a loss-of-function mutation in RBM20 as the underlying cause for the pathological isoform expression of titin. Mutations in RBM20 in humans have previously been shown to disrupt isoform transitions of a network of genes with essential cardiac functions and cause dilated cardiomyopathy. We showed that RBM20 deficient rats resembled the human disease phenotype. Together with Markus Landthaler and Nikolaus Rajewsky, we investigated the role of RBM20 as a cardiac specific splicing factor. We identified directly RBM20 regulated transcripts by applying state-of-the-art RNA-protein cross-linking and immunoprecipitation coupled with high-throughput sequencing (CLIP-seq) experiments. The identification of direct targets revealed that RBM20 acts as a splicing repressor in the heart. Using SILAC based proteomics we demonstrated direct interaction with important spliceosomal components and showed how mutations within RBM20 may disrupt its repressor function. We further revealed that not only genetic variation, but also the modulation of RBM20 gene expression levels is operative in the regulation of its network and may be an attractive target for therapeutic intervention. These binding data will be used to extend our studies determining the impact of genomic variability on posttranscriptional modifications in humans. Our studies emphasize the importance of posttranscriptional regulation in cardiac function and provide novel mechanistic insights into the pathogenesis of human heart failure.

Epigenetic control

In addition to the identification of thousands of gene expression traits under genetic control, we recently described the genetic impact on histone modifications in rat tissue. Several histone marks were associated to functional genomic regions or gene expression states. We quantified the genome-wide distribution of H3K4me3 and H3K27me3, two methylation marks associated with actively transcribed promoters and silent genes, respectively. In rat tissue, we could show that 14 % (liver) -21 % (heart) of mapped H3K4me3 marks were under genetic influence and segregating within the RI panel. A high proportion of 33 to 49 % of H3K4me3 marks were under distant genetic control and one major H3K4me3 regulating genomic region was identified impacting hundreds of H3K4me3 sites. As the examined histone marks were known predictors of gene expression states, data on genetic variation underlying histone marks could significantly increase the number of eQTLs. Hence, we could show that genetic variation has a great impact on histone modifications in rat tissue that might further help to elucidate so far unknown genotype-phenotype relationships in the heart.

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Zsuzsanna Izsvák

Mobile DNA

Transposable elements (TEs) are discrete segments of DNA that have the distinctive ability to move from one genetic location to another in a genome. Although, large fractions of genomes can be composed of TE-derived sequences (~55% of the human genome), the vast majority of these elements are not essential to the host cell, and often referred as 'junk DNA' As an attempt to turn "junk" into treasure, the molecular reconstruction of ancient transposable elements, including Sleeping Beauty (SB) represents a milestone in applying transposition-mediated gene delivery in vertebrate species. Our focus is to understand the basic mechanism of transposition and the interplay between TEs and host cells in mammals. The Mobile DNA team integrates basic knowledge with technologies that are revolutionizing genomic manipulations in vertebrate species, including gene therapy, transgenesis, cancer research and functional genomics. Our studies also focus on evolutionary processes that seem to "recycle" inactive TEs and co-opt them for novel cellular function, and argue against the dogma that views them exclusively as parasites or "junk" DNA.

The impact of domesticated TE-derived sequences

J. Wang, M. Singh, A. Szvetnik, T. Rasko, C. Sun, V. Raghunathan

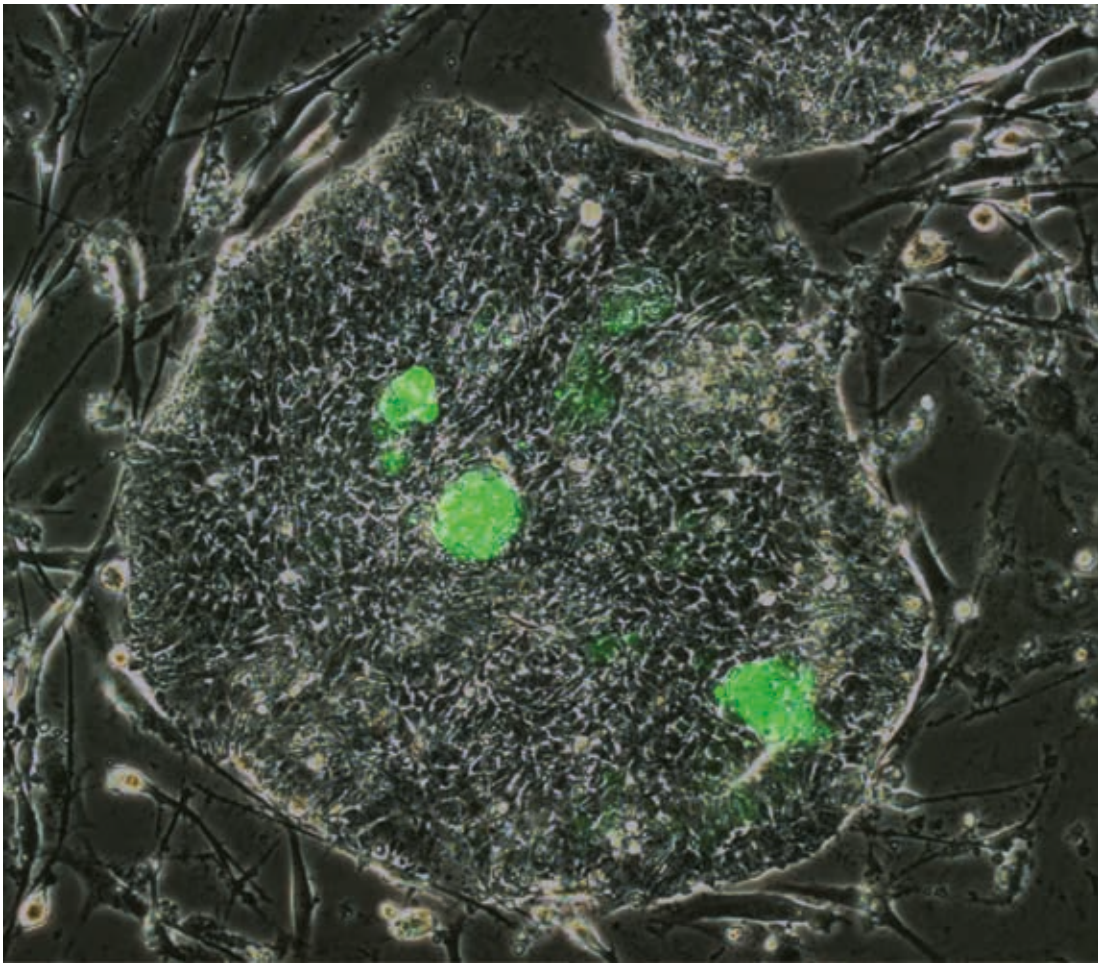
Occasionally transposons are co-opted for cellular functions. A high throughput protein interaction screen identified cellular interacting partners of the Sleeping Beauty and piggyBac transposases. Our data suggest an important ramification for the presence of domesticated piggyBac-like elements in the human genome.

Recent studies indicate that some families of ancient endogenous retroviruses (ERVs), such as HERVH has a role in the acquisition and maintenance of pluripotency in primates. Using human pluripotent stem cells as models, we aim at deciphering the potential biological functions of TEs in human pluripotency (see press release: *https://www.mdc-berlin.de/43776186/*).

Recently, TFCP2l1 (LBP9) has been recognized as a potential transcription factor that in conjunction with HERVH is involved in human pluripotent network. LBP9 belongs to the CP2 family of transcription factors. We study how the CP2 family is involved in the self-renewal and differentiation potential in human pluripotent cells.

We investigate the impact of conserved TEs during primate evolution (collab. with L. D. Hurst, U. Bath, UK). Transcriptional expression of TEs is tightly controlled during development and seems to be deregulated in certain diseases.

TEs have likely played a key role in distributing non-coding, regulatory elements in the vertebrate genome. We investigate the potential impact of the evolutionary conserved Medium Reiterated frequency repeats (MERs).



The image shows typical human embryonic stem cell (hESC) colony. The heterogenous colony consist of primed (grey) and naïve-like cells (green). The naive-like cells are expressing HERVH–driven GFP reporter. The reporter-based strategy can be used to derive homogenous, naïve-like colonies from conventional human pluripotent cultures.
https://insights.mdc-berlin.de/en/2016/01/how-to-detect-and-preserve-human-stem-cells-in-the-lab/
Shortener Version: https://goo.gl/J1T7XF

The impact of TE-derived activities on disease mechanisms

J. Rugor, M. Singh, H. Cai, N. Fuchs, R. Anwar*

Global epigenetic changes during early development resets the epigenetic status resulting in transcriptional activation of certain TE families, including LINE-1 and various HERV families. We investigate the risk of genetic destabilization inflicted by upregulation of human endogenous TEs in cancer.
In comparison to other tissues, the genomic noise, generated by various TEs is relatively high in placenta. In collaboration with Our aim is to investigate the epigenetic regulation and imprinting abnormalities in the pathogenesis of preeclampsia, a pregnancy disorder associated with cardiovascular diseases of the mother (collab. R. Dechend, Charité and L. D. Hurst , U. Bath, UK).

Transposon-host interaction

S. Narayanvari, A. Szvetnik, G. Yong, A.Schmitt, A. Devaraj*, D. Grzela*,*

TEs are best described as molecular parasites with the potential to give rise to a variety of genetic alterations. Such alterations include mutational damage to gene function, but can also provide useful genetic variability in host genomes. Thus, in contrast to most viruses, TEs and the host have coevolved in a way that permits propagation of the transposon, but minimizes damage to the host. TEs make several interactions to host cellular machineries, piggyback and modify certain basic mechanisms of the host organism. Our studies indicate that transposition is sensitive to stress, and stress-response involves a complex, interactive regulatory platform involving evolutionary conserved cellular mechanisms.

We aim at validating that transcription factor, HMGXB4 (a component of the Wnt-signalling pathway) targets SB transposition to a well-defined developmental phase.
The SB transposition seems to piggyback the heat shock response pathway that normally reactivates heat-aggregated, nuclear proteins. Thus, transposons might exist in a "latent" form in the genome and are able to sense developmental and environmental changes and manipulate stress signalling.

Translating experience accumulated in TE research to cutting-edge technologies

H. Cai, H. Escobar, A. García Pérez, I. Bilic, E. Nagy, SS. Chilkunda,Z. Zhou, J. Menyhert*, I. Klinge*

TE-based, non-viral integrating vector systems represent a novel technology that opens up new possibilities for gene therapy. The plasmid-based hyperactive SB100X transposon system (Molecule of the Year, 2009, Press Release MDC https://goo.gl/0oCvLQ) has become a popular tool for non-viral, therapeutic transgene delivery. The SB100X transposon has a favourable safety profile as compared to widely used retro/lentiviral approaches. The SB100X system provides long-term expression in various primary and stem cells. As an important issue regarding the implementation of clinical trials, transposon vectors can be maintained and propagated as plasmid DNA, making them simple and inexpensive to manufacture (e.g. GMP vector production).
In order to fill the gap between the recent vector development and clinical trials we (1) try the SB system in disease models that were already on clinical trials using retroviral vectors, but where safety was a serious issue; (2) try the SB system in disease models that were already on clinical trials using retroviral vectors, but feasibil-

ity was an issue – engineered T cells for personalized cancer immunotherapy; BIH consortium; (3) try the SB system in disease models that were already on clinical trials, using non-viral approaches, but efficacy was a limiting factor – Age-related Macular Degeneration (AMD), TargetAMD consortium [collab. G. Thumann (U. Hospital Geneva, Switzerland)]; Von Willebrand disease Type III, TRANSPOSMART, ERARE consortium; (4) include models where the transposon-based regenerative technology has a potential – cell-based pacemaker [collaboration M. Morad (U. of South Carolina)], muscle stem cells, dysferlinopathy [collab. S. Spuler (ECRC) (see press release: https://www.mdc-berlin.de/43612981), MyoGrad)]; (5) combine the plasmid-based integration vectors with the cutting-edge DNA delivery strategies;

We develop the SB as a novel tool for chromatin 3D mapping. This chromatin mapping approach is based on the 'local hopping' feature of SB. A great advantage of the SB-based method would be that chromatin regions are signed under physiological conditions avoiding chemical fixation and limitations of cross-linking.
The SB transposon is suitable for somatic mutagenesis and emerged as a powerful tool in cancer research. We utilize SB in a rat model to decipher gene regulatory networks cooperating in hormone- dependent breast cancer development, progression and metastasis (collab. M. Bader (MDC), D. Largaespada (U. Minnesota), J. Shull (U. Wisconsin).
Transposons can be harnessed as vehicles for introducing mutations into genes. By combining transposon mutagenesis with the innate potency of spermatogonia, comprehensive libraries of gene knockouts are used to study fertility and behaviour (collab. K. Hamra, UTSW, USA).
We use hiPSCs and genome engineering in a Kcnq1 model of type 2 diabetes aiming drug screening.

* former students

Selected Publications

Wang Y.; Pryputniewicz-Dobrinska D.; Nagy E.E.; Kaufman C.D.; Singh M.; Yant S.; Wang J.; Dalda A.; Kay M.A.;Ivics Z.; Izsvak Z. Regulated complex assembly safeguards the fidelity of Sleeping Beauty transposition. Nucleic Acids Research. (2017-01-09) Nucleic Acids Res. 2017 Jan 9;45(1):311-326. doi: 10.1093/nar/gkw1164. PMID: 27913727

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Anchored Signalling

Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger that directs a plethora of cellular processes. In the cardiovascular system, cAMP, for example modulates contractility of cardiac myocytes and participates in the control of blood pressure through vascular smooth muscle cells. The Anchored Signalling group aims to elucidate molecular mechanisms conferring specificity to cAMP signalling.

AKAPs and PDEs define cAMP signalling compartments

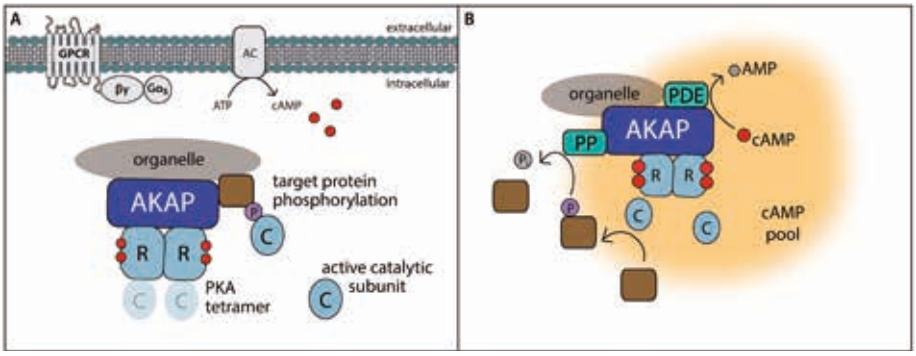
Stimulation of various G protein-coupled receptors (GPCRs) induces adenylyl cyclases (AC) to synthesise cAMP. cAMP could theoretically diffuse freely throughout the cell. However, this does not occur due to its degradation by cyclic nucleotide phosphodiesterases (PDEs). PDEs hydrolyse cAMP to 5'AMP and are the only means for terminating cAMP signalling. There are 21 genes encoding 11 families of PDEs, PDE1–11. The genes give rise to >100 mRNAs and thus to >100 isozymes. The PDEs of the PDE4, PDE7 and PDE8 families hydrolyse cAMP, those of the PDE5, PDE6 and PDE9 families hydrolyse cGMP, while the members the PDE1, PDE2, PDE3, PDE10 and PDE11 families hydrolyse both cAMP and cGMP. PDEs are constitutively active enzymes that are located in compartments, and thus establish gradients of cyclic nucleotides.

The cAMP gradients are sensed by cAMP effectors, amongst them protein kinase A (PKA). PKA holoenzyme consists of a dimer of regulatory R subunits and two catalytic (C) subunits. cAMP activates the kinase, which then phosphorylates nearby substrates. PKA directly interacts with A-kinase anchoring proteins (AKAPs), a family of around 50 scaffolding proteins, that tether the enzyme to defined cellular compartments and thereby limit the kinase's access to only a subset of its substrates. Phosphatases dephosphorylate target proteins, counteracting PKA activity. Compartmentalized PKA in concert with PDEs and phosphatases facilitates coordinated phosphorylation of target proteins in response to stimulation of a specific GPCR. AKAPs possess specific protein interaction domains for binding PKA, PKA substrates, PDEs, phosphatases and further signalling proteins such as additional kinases and may constitute the platforms for signalling hubs that facilitate specific cellular responses to specific extracellular cues (figure 1).

Functions of phosphodiesterase 3 in cardiac myocytes and vascular smooth muscle cells

Two PDE3 subfamilies, PDE3A and PDE3B, have been described, which both hydrolyse cAMP and cGMP competitively. PDE3A expression is higher in cardiac myocytes, platelets, vascular smooth muscle cells (VSMC), and oocytes. PDE3A exists in three isoforms, PDE3A1, PDE3A2 and PDE3A3, which are located in different cellular compartments. PDE3A1 is a membrane protein, while PDE3A2 and PDE3A3 are both cytosolic and membrane-associated in human myocardium. PDE3A2 is the dominant isoform in human VSMC. PDE3A1, A2 and A3 all contain the same catalytic region and are similar concerning catalytic activity and inhibitor sensitivity.

Ca^{2+} release from the sarcoplasmic reticulum (SR) into the cytosol of cardiac myocytes stimulates contraction, while its removal from the cytosol causes relaxation of the myocyte. We had identified AKAP18δ in the rat and observed that it participates in the control of Ca^{2+} reuptake into the SR of cardiac myocytes during diastole through direct interactions with PKA and phospholamban (PLN). In collaboration with the groups of V. Magianello, NIH, USA, and M. Movsesian, University of



The interplay of cAMP synthesis and degradation and compartmentalisation of relevant enzymes confers specificity to cAMP signalling. A. Activation of cAMP signalling through G protein-coupled receptors (GPCRs). B. Termination of cAMP signalling through phosphodiesterases (PDEs) and phosphatases. AKAPs bind directly PKA, PKA substrates, PDEs, phosphatases and further signalling proteins and coordinate cAMP signalling (fig. adapted from Dema et al. Cell Signal. 272474-87).

Utah, USA, we recently showed that in human cardiac myocytes PDE3A1 phosphorylation-dependently interacts with a multi-protein complex, which contains AKAP18γ, the human ortholog of AKAP18δ, and SERCA2A and controls Ca^{2+} reuptake into the SR.

In collaboration with F. Luft and S. Bähring, ECRC, we analysed six missense mutations in the gene encoding PDE3A from six unrelated families with hypertension and brachydactyly type E (HTNB). The mutations are adjacent to each other and responsible for amino acid substitutions in a conserved region that is present in PDE3A1 and PDE3A2 and to which a function has not been ascribed, but which is in close proximity to the catalytic domain. We observed that the mutations lead to increased phosphorylation of PDE3A1 and PDE3A2 by PKA, which in turn renders the enzymes hyperactive. In VSMC the presence of hyperactive PDE3A is associated with increased proliferation. Together with increased constriction this is likely causative for the increased peripheral vascular resistance in the patients and explains the hypertensive phenotype.

The new AKAP GSKIP facilitates crosstalk with GSK3β and controls palatal bone fusion

AKAPs may interact not only with PKA, PKA substrates or PDEs but also with further signalling proteins and thereby facilitate crosstalk with other signalling systems. We have identified GSK3β interaction protein (GSKIP) as a new AKAP, and have shown that it binds PKA and a second kinase, glycogen synthase kinase (GSK)3β. GSK3β is ubiquitously expressed, a component of multiple signaling systems like canonical Wnt, insulin, Hedgehog, Notch, and TGFβ signaling, and is involved in the regulation of different biological processes, including embryonic development, cell cycle progression, glycogen metabolism and immune regulation. Deregulation of GSK3β is associated with diseases such as cancer or cardiac hypertrophy. Our in vitro analyses had indicated that GSKIP facilitates the phosphorylation and thus inhibition of GSK3β by PKA. In order to gain insight into the physiological function of GSKIP we generated a mouse model where expression of the Gskip gene was abolished through a gene targeting approach. The loss of GSKIP leads to aberrant regulation of GSK3β during development, and is associated with the inability to close the palatal shelves. The loss of GSKIP expression in mice causes a cleft palate resembling Hemifacial microsomia (Goldenhar syndrome) in humans.

Patents / Patent applications

Patent No.: US 9,102,667 B2
N-Arylaminomethylene benzothiophenones for treatment of Cardiovascular Disease
Inventors: E. Klussmann, W. Rosenthal, J. Milic, M. Bergmann

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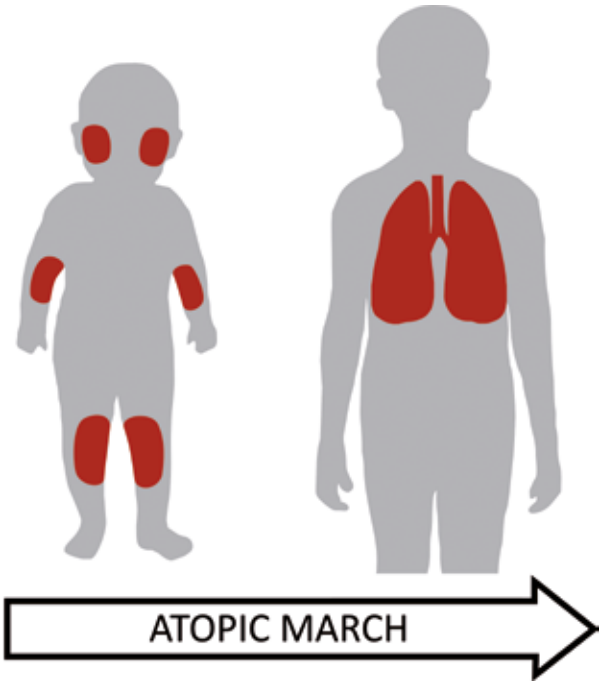
Molecular genetics of chronic inflammation and allergic disease

The allergic disorders, eczema, asthma, and hay fever, are the most common chronic diseases in childhood. The prevalence of allergic diseases has increased to epidemic dimensions over the past decades. In the industrialized countries, 25-30% of the population are affected.
The goal of our group is to use genetic and genomic approaches in large study groups of affected patients and families to identify genes and genetic variants that predispose to eczema, asthma, and chronic inflammation. We complement clinical phenotypes with precise molecular phenotypes to characterize underlying functional pathways. Enhanced understanding of how the interaction between environmental factors, genetics, and the immune system results in inflammation will enable the development of novel diagnostic and predictive clinical tools, and of targets for innovative therapies.

Chronic inflammation has become one of the core paradigms of chronic human disease underlying numerous conditions including allergic and autoimmune disorders, cardiovascular, and neurological disease. Regardless of the mechanism of inflammation, inappropriate inflammatory responses to environmental, microbial or self antigens contribute to dysfunction and destruction of target organs. Epidemiologic data provide strong evidence for a steady rise in the incidence of diverse types of chronic inflammatory disorders in industrialized countries over the past decades, indicating that genetic variants that have evolved to arm our immune system against multiple infectious/environmental exposures now influence immune response thresholds to favor chronic inflammatory disease in the hygienic environment of modern civilization.

Our recent studies highlight the genetic overlap between risk loci for allergic disease with those for other chronic inflammatory conditions that have previously been thought to be unrelated. Recently, the largest genome-wide association study on eczema comprising over 21,000 cases and 95,000 controls identified 10 novel loci bringing the total number of eczema susceptibility loci to 31 (1). Notably, 80% of the eczema loci also confer risk for other chronic inflammatory diseases including inflammatory bowel disease, psoriasis, ankylosing spondylitis, multiple sclerosis, rheumatoid arthritis, or type 1 diabetes, pointing to common mechanisms involved in eczema and other immune-mediated traits. Molecular pathways involved in innate immune signaling and T-cell polarization were significantly enriched. While the importance of the skin barrier defect and of an exaggerated immune response to environmental allergens in eczema is well established, all new susceptibility loci were related to (auto-) immune regulation, in particular innate signaling and T-cell activation and specification. In summary, our results suggest that targeted immune modulation could be another promising approach for the treatment of eczema.

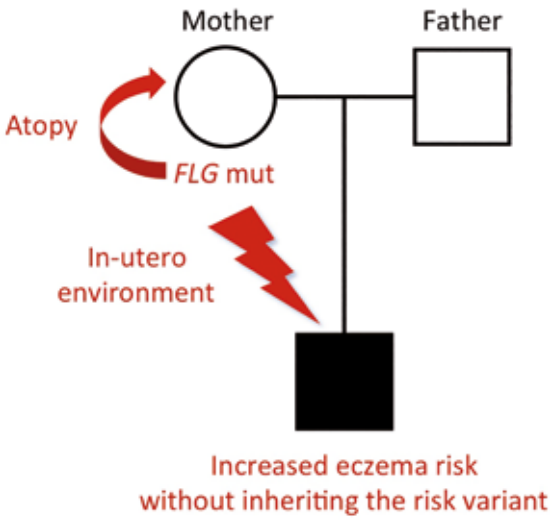


Eczema usually develops in early infancy and is characterized by inflammatory and itchy lesions at typical sites of the body. Children with eczema have an increased risk to develop allergic asthma later in life in a disease course referred to as the "atopic march". We analyzed the genetic determinants underlying disease progression from eczema to asthma.

Clinical and molecular subphenotyping

To better understand the clinical diversity and temporal trajectory of allergic disease we evaluate patients in our research outpatient clinic (Hochschulambulanz) for pediatric allergy at the Experimental and Clinical Research Center (ECRC of Charité and MDC). Over the years, we have recruited several thousand study participants. In many cases, we have obtained prospective clinical data of the of allergic disease course and sensitization patterns.

Recently, we used the longterm clinical data to perform the first genome-wide analysis of allergic disease prognosis. Infantile eczema is considered a "gateway" condition that often heralds a lifetime of allergic disease. In an age-dependent pattern known as the "atopic march", a susceptible child commonly passes a characteristic sequence of transient or persistent disease stages that begins with eczema in the young infant and continues with the development of respiratory airways disease later in childhood and adulthood. We have recently initiated a worldwide collaboration of research groups to perform the first genome-wide association study for the "atopic march". Focussing on children with longitudinal data who strictly met our phenotype definition of early eczema followed by asthma, we studied 2,400 cases and 17,000 controls and identified 7 regions in the human genome which were associated with the atopic march at genome-wide significance ($P < 10^{-8}$). While four of them, FLG (1q21.3), IL4/KIF3A (5q31.1), AP5B1/OVOL1 (11q13.1), and C11orf30/LRRC32 (11q13.5), were known susceptibility factors for eczema and one region harbored an asthma locus (IKZF3, 17q21), we discovered two new regions (EFHC1 at 6p12.3 and SLC6A15 at 12q21.3) that were not previously known to be involved in any allergic/chronic inflammatory condition. One of the novel loci contained the EF-hand domain containing protein 1 gene (EFHC1)



Loss-of-function mutations in the filaggrin gene (FLG) cause skin barrier deficiency and strongly predispose to eczema. We showed that having a FLG carrier mother increased the eczema risk of the child even when the mutations were not transmitted. The FLG maternal effect was observed only when mothers had allergic sensitization, suggesting that FLG mutation-induced systemic immune responses in the mother may influence eczema risk in the child.

which has been involved in ciliary dysfunction in brain and lung providing a potential link to asthma. The second novel locus included the solute carrier family 6, member 15 gene (SLC6A15). Selective inhibition of SLC6A15 by the histamine H1 receptor antagonist loratadine which is clinically used for the treatment of allergic disease, was recently reported.

Both novel loci specifically increased the risk for the atopic march without any effects on eczema alone or asthma alone. Of the five known risk loci, eczema loci were much more prominent than asthma loci. Supporting these results, almost 90% of all known eczema loci were found to be associated with the atopic march, whereas only 25% of the known asthma loci were identified. Our results suggest that genes triggering eczema are the main genetic determinants of the atopic march. This finding highlights the role of the skin as the interface between host and environment in shaping not only local, but also systemic immune responses that induce chronic inflammation and may affect distant organs in the host. A causal relationship between eczema and this asthma subtype has important implications for interventional strategies. Since no cure for asthma exists, the modulation of skin immune homeostasis early in infancy could be an effective strategy to prevent not only eczema but also the disease progression along the atopic march.

Parent-of-origin effects

Epidemiological studies on allergic diseases have shown that maternal allergy is a stronger risk factor for the child than paternal allergy. The molecular basis of this preferential maternal transmission of allergy risk is currently unknown but it can potentially occur through two different biological mechanisms: genomic imprinting and direct maternal genotype effect. In genomic imprinting, either the maternally or the paternally inherited allele is expressed and the other allele is silenced. Thus, the effect of an allele depends on parental origin resulting in phenotypic differences between reciprocal heterozygotes (parent-of origin effects). Alternatively, maternal genotype effects occur when the maternal genotype directly influences the child's phenotype. This effect is independent of the child's own genotype and occurs through the maternally provided environment during prenatal development.

We have now investigated parent-of-origin effects for the loss-of-function mutations in the filaggrin gene (FLG) which are the strongest genetic risk factors for eczema known to date and provided the first molecular evidence linking skin barrier deficiency to eczema (3). We analyzed the four most prevalent European FLG mutations in 759 complete nuclear families with eczema from Central Europe. For the analysis, we

applied the PREMIM/EMIM tool which uses a multinomial and maximum-likelihood approach for flexible modelling of parent-of-origin effects.

Our study replicated the known effect of the children's genotype on eczema, yielding a relative risk (RR) of 3.1 for heterozygous and of 10.5 for homozygous FLG mutation carriers. By including the genotype of the mother in our model, we identified a significantly increased eczema risk (RR, 1.55) for children having a FLG mutation carrying mother which was independent of the children's genotype. Importantly, this maternal effect was replicated in another 450 European families and it was not observed for the paternal genotype. Our study revealed, for the first time, a direct influence of a maternal mutation on the child's risk for a common disease. The maternal FLG effect was only found when the mothers were allergic, and was absent in families of non-allergic mothers. Our finding suggests that FLG-induced changes in the maternal immune response shape the child's immune system during pregnancy and increase the child's risk for eczema. Our study indicates that maternal FLG mutations act as strong environmental risk factors for the child and highlights the potential of family-based studies in uncovering novel disease mechanisms in medical genetics.

Functional analysis of disease genes

A major goal is to characterize the effects of the susceptibility genes for allergic and chronic inflammatory diseases (4) in order to better understand the earliest events in human physiology that lead to this type of inflammatory response. We integrate gene-expression data from relevant target tissues to characterize the molecular mechanisms underlying disease associations. In order to identify the functional effect of small structural variants (short insertions or deletions, indels) within the human genome, the association of these variants with gene expression levels (expression quantitative trait loci, eQTLs) from multiple tissues was analyzed. Previous studies have demonstrated that SNPs with an effect on gene expression were enriched in introns and in regions close to UTRs. Indel eQTLs were even more enriched in these regions suggesting that indels are more likely to be functional because they have a more deleterious effect on regulatory elements and splice sites. Interestingly, a substantial proportion of SNPs previously found in GWAS on disease traits were correlated with indels with larger effects on eQTLs, supporting the hypothesis that indels are more likely to be causal.

Selected Publications

The EArly Genetics and Lifecourse Epidemiology (EAGLE) Eczema Consortium [MDC contribution: Esparza-Gordillo J, Marenholz I, Kalb B, Matanovic A, Rüschendorf F, Bauerfeind A, Hübner N, Lee YA] Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. **Nature Genetics** epub ahead of print. 2015.

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Receptor signalling

Our group focuses on the mechanisms of activation and signalling by of G-protein coupled receptors (GPCR), and subsequent downstream events which lead to alterations in cell function.
G protein-coupled receptors (GPCRs) are a large family of approximately 800 members, which include receptors for light, taste and smell, but also many receptors for transmitters and hormones. They are also the most important group of targets for drugs, for example beta blockers, antihistamines and opiates. All GPCRs share common structural features, presumably also common activation mechanisms, and they all signal via heterotrimeric G proteins (Gαβγ) and – at later points upon activation, via β-arrestins. We use a variety of biochemical and pharmacological methods as well as new microscopic techniques to study the mechanisms of their function and their potential to serve as targets for innovative drug therapies.

Current Projects

Spatio-temporal Patterns of GPCR Activation

GPCR activation is triggered by binding of a ligand (e.g. adrenaline, glutamate) to a specific site of a receptor. This is followed by a conformational change in the receptor, which enables it to bind to and activate G-proteins. We have developed methods to monitor these signalling events via fluorescence microscopy, and we aim to study where and when in a cell these signals occur. We use techniques to trigger receptor activation by light to monitor signalling with sub-millisecond resolution.
We are also working on techniques to adapt such measurements to high throughput screening in order to search for compounds with unusual signalling properties, which might make them new classes of drugs with only a subset of effects compared to conventional drugs.

Spatial Patterns of cAMP Signalling

cAMP is a second messenger downstream of GPCR activation, which plays an important role in many physiological processes, ranging form regulation of heartbeat and force to synaptic plasticity. Although cAMP appears to be a freely diffusible substance, recent evidence suggests that it may act in cells in a highly localized manner. We are attempting to visualize such local effects and are searching for mechanisms that might lead to the postulated nano-compartments. To do so, we use models ranging from the design and expression of specific cAMP sensor proteins to studies in transgenic fly and mouse models, where we attempt to image local cAMP signals (Figure 1).

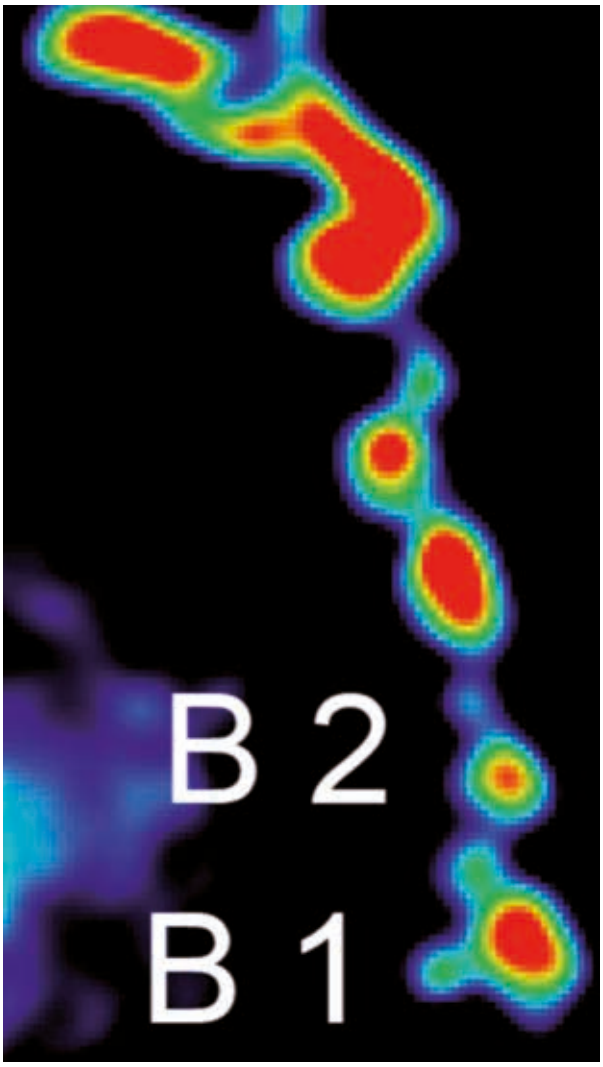

Figure 1
Imaging of cAMP in Drosophila *larvae. Shown is the end of a motor neuron from a transgenic fly expressing a cAMP sensor. cAMP levels are indicated as a graded color response derived from fluorescence resonance energy transfer (FRET).* Adapted from Maiellaro et al. Cell Rep. 2016.

Signalling by β-arrestins

Ever since their discovery more than two decades ago, β-arrestins have surprised us with new functions and properties. Originally thought to represent just inhibitors of receptor signalling, they have now been identified as signalling proteins in their own right. When they bind to receptors, they terminate receptor signalling to G proteins and at the same time trigger a number of subsequent steps: receptor internalization and activation of downstream protein kinases. Recent data indicate that during this process β-arrestins undergo an activation/deactivation cycle (Figure 2).

Dynamics of GPCR Oligomerization

GPCRs can form supramolecular complexes (i.e. di-/oligomers) on the cell surface. However, their size, nature and dynamics as well as their physiological and pharmacological implications are still largely unknown. We focus on investigating the dimerization of receptors (especially adrenergic, opiate and chemokine) on the surface of intact cells as well as evaluating the role of dimerization on receptor activation and downstream signalling. We do so by a variety of biochemical and microscopic techniques, notably brightness analyses and single particle microscopy.

Mechanisms of heart failure

GPCRs are important for a plethora of physiological functions, one of them being the regulation of cardiac frequency and contractility. We are interested in the biochemical mechanisms that underlie the development of heart failure, and in potential strategies to combat this sequence of events. We do so by studying heart failure in mouse models and search for transgenic as well as pharmacological strategies for its treatment and prevention (Figure 3).

Outlook

Our group is in the process of translocating from the University of Würzburg to the MDC, and will focus initially on the spatiotemporal aspects of GPCR signalling and their analysis by advanced microscopy. Senior members of the team are leaving to assume leading positions elsewhere: Kristina Lorenz has become director at the Leibniz Institute of Analytical Sciences in Dortmund, while Carsten Hoffmann and Davide Calebiro will assume chairs at the universities of Jena and Birmingham.

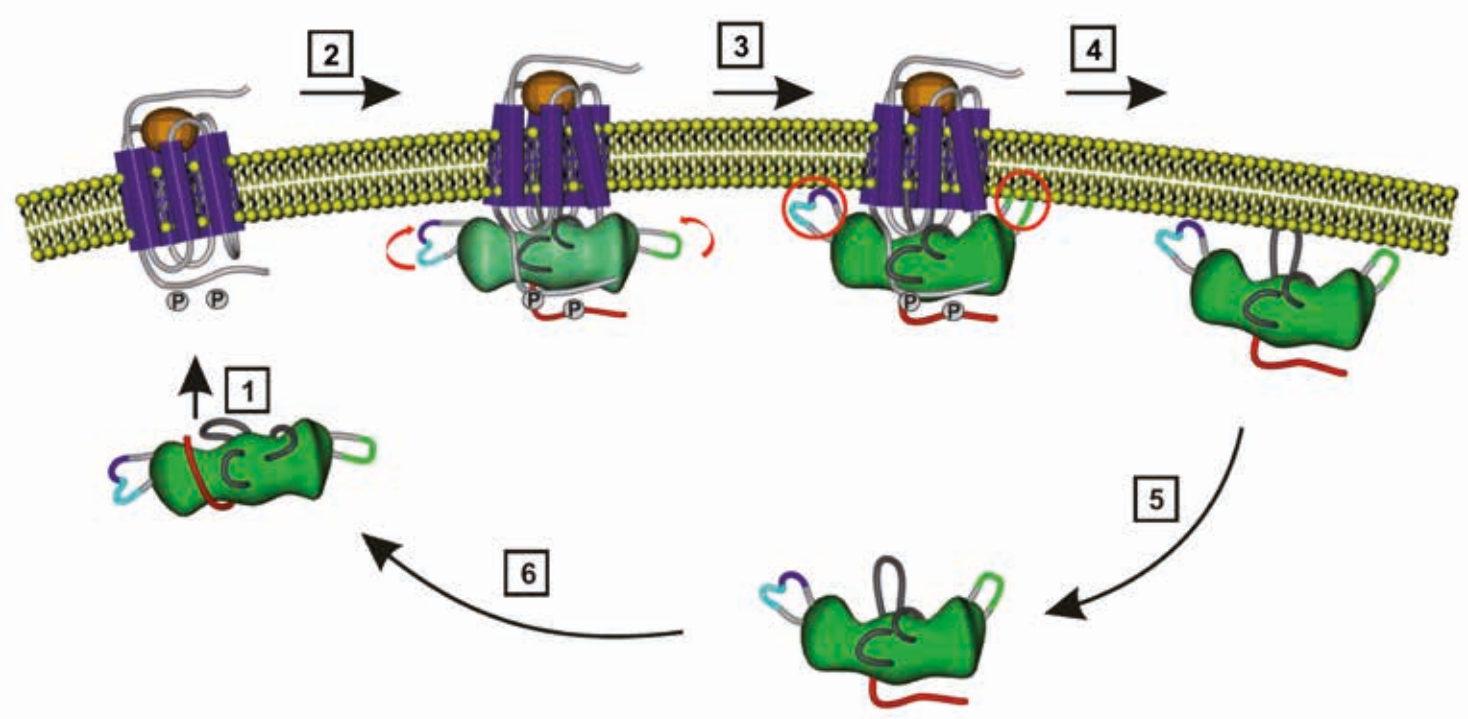

Figure 2
An activation/deactivation cycle of β-arrestin. Upon binding to an active phosphorylated receptor (1) β-arrestin adopts an activated conformation (2) that might facilitate fitting to the activated, phosphorylated receptor surface. Parts of C- and/or N-domain undergo further movements (3) to bring β-arrestin into a receptor-specific activated conformation. Upon agonist removal, β-arrestin dissociates from the receptor (4) and remains active for some time (5) before its active state is reversed (6). Adapted from Nuber et al. Nature 2016.

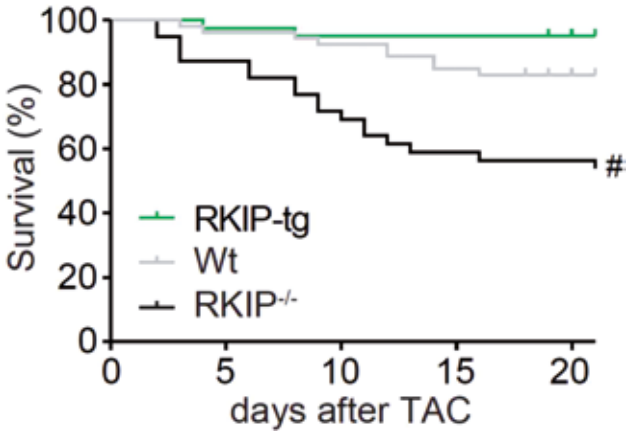

Figure 3
RKIP is a protein that protects the heart. Shown is the survival of several mouse lines after cardiac stress (induced by a high blood pressure). Mice transgenic for RKIP in the heart (RKIP-tg) survive better, and mice lacking RKIP (RKIP$^{-/-}$) survive less than controls. Adapted from Schmid et al. Nature Medicine, 2015.

Selected Recent Publications

Maiellaro I, Lohse MJ, Kittel RJ, Calebiro D. cAMP Signals in Drosophila Motor Neurons Are Confined to Single Synaptic Boutons. *Cell Reports*. 2016;17:1238-1246.

Nuber S, Zabel U, Lorenz K, et al. β-Arrestin biosensors reveal a rapid, receptor-dependent activation/deactivation cycle. *Nature*. 2016;531:661-664.

Schmid E, Neef S, Berlin C, et al. (2015) Cardiac RKIP induces a beneficial β-adrenoceptor-dependent positive inotropy. *Nat. Medicine*. 2015;21:1298-1306

Lohse MJ, Hoffmann KP. Spatial and temporal aspects of signaling by G-protein-coupled receptors. *Mol Pharmacol*. 2015;88(3):572-578.

Calebiro D, Hannawacker A, Lyga S, et al. Mutations of PKA catalytic subunit in adrenocortical Cushing´s adenomas impair association with the regulatory subunit. *Nature Comm*. 2014;5:5680.

Start of the Group: Januar 2017

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Photo: David Ausserhofer/MDC

Friedrich Luft

Hypertension Genetics, Regulators, and Environment

Gene laboratory

Our goal is to reduce cardiovascular morbidity and mortality. Hypertension is the most important risk factor. We investigate genetic, mechanistic, regulatory, and environmental influences. Since 1994, we have pursued a Mendelian form of hypertension that cosegregates with brachydactyly type E (HTNB). We recently reported six missense mutations in *PDE3A* (encoding phosphodiesterase 3A) in six unrelated families with HTNB. The syndrome features brachydactyly type E (BDE), severe salt-independent but age-dependent hypertension, an increased fibroblast growth rate, neurovascular contact at the rostral-ventrolateral medulla, altered baroreflex blood pressure regulation, and death from stroke before age 50 years when untreated. In vitro analyses of mesenchymal stem cell-derived vascular smooth muscle cells (VSMCs) and chondrocytes provided insights into molecular pathogenesis. The mutations increased protein kinase A-mediated PDE3A phosphorylation and resulted in gain of function, with increased cAMP-hydrolytic activity and enhanced cell proliferation. Levels of phosphorylated VASP (vasodilator-stimulated phosphoprotein) were diminished, and PTHrP (parathyroid hormone-related peptide) levels were dysregulated. We suggested that the identified PDE3A mutations cause the syndrome. VSMC-expressed PDE3A deserves scrutiny as a therapeutic target for the treatment of hypertension. (Figure 1)

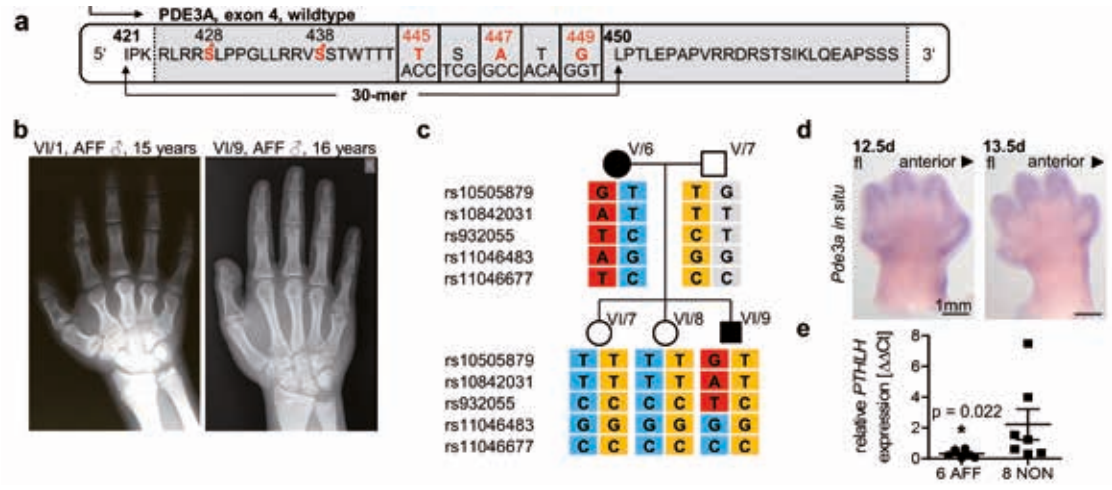


Fig. 1: ***(a)*** *Peptide sequence encoded by PDE3A exon 4 that harbors the mutations (which alter amino acids 445, 447, 449) and phosphorylation sites Ser428 and Ser438 near the altered residues. Mutations cause a gain-of-function effect.****(b)*** *Mild BDE of VI/9 compared tomore severe BDE of cousin VI/1.****(c)*** *Haplotype analysis identified a new recombination event.****(d)*** *Chondrogenic Pde3a expression in developing mice. PTHLH expression exhibits a downregulation responsible for BDE.*

We also conducted clinical studies of vascular function, cardiac functional imaging, platelet function in affected and nonaffected persons, and performed cell-based assays. Large-vessel and cardiac functions seemed to be preserved in HTNB. The platelet studies showed normal platelet function. Cell-based studies demonstrated that available phosphodiesterase 3A inhibitors suppressed the mutant isoforms, but less effective. However, increasing cGMP to indirectly inhibit the enzyme seemed to have particular utility. Our results shed more light on phosphodiesterase 3A activation and could be relevant to the treatment of severe hypertension in the general population.

Eicosanoid regulators

Cytochrome P450 (CYP)-dependent metabolites of arachidonic acid (AA) contribute to the regulation of cardiovascular function. CYP enzymes also accept eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to yield more potent vasodilatory and potentially anti-arrhythmic metabolites, suggesting that the endogenous CYP-eicosanoid profile can be favorably shifted by dietary omega-3 fatty acids. To test this hypothesis, we treated 20 healthy volunteers with an EPA/DHA supplement and analyzed for concomitant changes in the circulatory and urinary levels of AA-, EPA-, and DHA-derived metabolites produced by the cyclooxy-

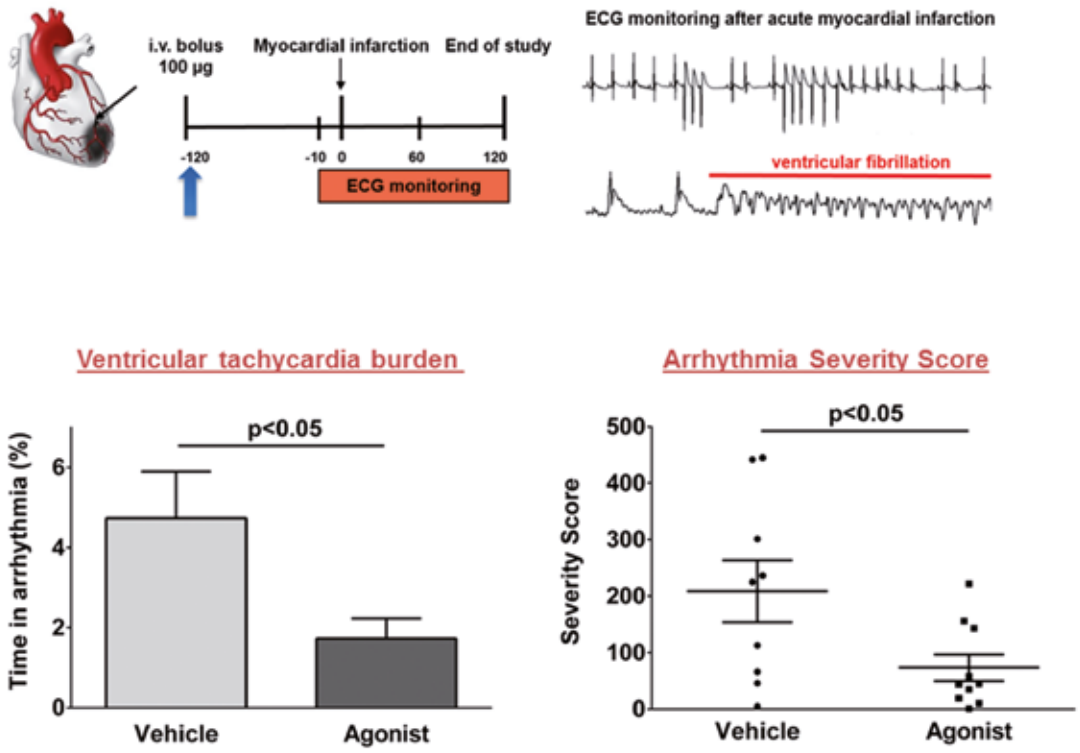


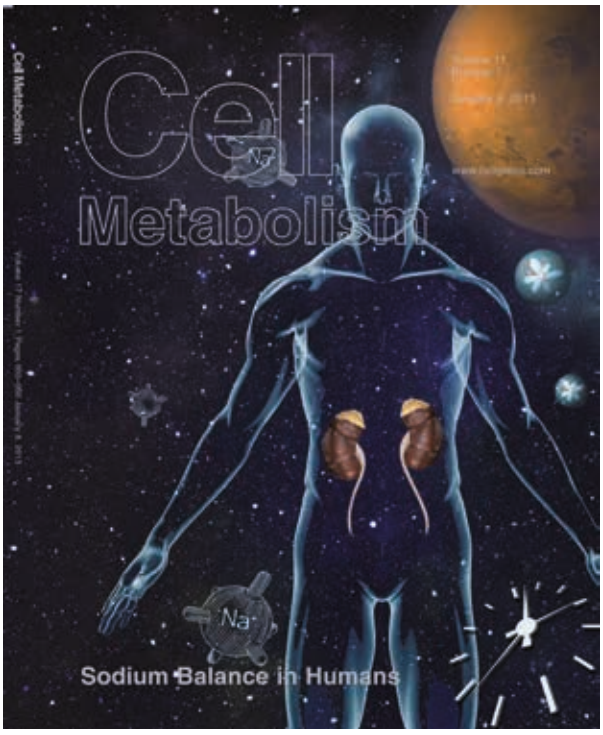
Fig. 2: A synthetic analog of omega-3 fatty acid-derived epoxyeicosanoids protects against cardiac arrhythmia in a rat model of myocardial infarction (MI). *The animals (n= 9-10 per group) received the analog or its vehicle two hours before inducing MI by coronary artery ligation. The compound reduced both the duration and severity of MI-elicited spontaneous ventricular arrhythmias as monitored by continuous ECG recording.*

genase-, lipoxygenase (LOX)-, and CYP-dependent pathways. Raising the Omega-3 Index from about four to eight primarily resulted in a large increase of EPA-derived CYP-dependent epoxy-metabolites followed by increases of EPA- and DHA-derived LOX-dependent monohydroxy-metabolites including the precursors of the resolvin E and D families; resolvins themselves were not detected. The metabolite/precursor fatty acid ratios indicated that CYP epoxygenases metabolized EPA with an 8.6-fold higher efficiency and DHA with a 2.2-fold higher efficiency than AA. Effects on leukotriene, prostaglandin E, prostacyclin, and thromboxane formation remained rather weak. We propose that CYP-dependent epoxy-metabolites of EPA and DHA may function as mediators of the vasodilatory and cardioprotective effects of omega-3 fatty acids and could serve as biomarkers in clinical studies investigating the cardiovascular effects of EPA/DHA supplementation. We collaborate with John R. Falck (UT Southwestern, Dallas) in developing CYP-eicosanoid-derived pharmacological approaches to prevent cardiovascular disease. OMEICOS Therapeutics is a spin-off company of the MDC on the BioTech-Campus Berlin-Buch (*http://www.omeicos.com*) that resulted from this work. (Figure 2)

Environment/salt intake

Dietary salt intake is said to be responsible for salt-sensitive hypertension. We found earlier that sodium follows a three-compartment, rather than a two-compartment model in mammals. The ion is bound to negatively charged proteoglycans that are widely distributed, but largely reside in skin. In two very long balance studies at 6, 9, and 12 g/day salt intakes, we found that sodium is excreted according to circaseptan rhythms regulated by aldosterone. Our 2013 report in Cell Metab is in strong running for "cover of the decade".

Urinary recovery of dietary salt was 92% of recorded intake, indicating long-term steady-state sodium balance in both studies. Even at fixed salt intake, 24-hour urine collection for sodium excretion (UNaV) showed infradian rhythmicity. We defined a ±25 mmol deviation from the average difference between recorded sodium intake and UNaV as the prediction interval to accurately classify a 3-g difference in salt intake. Because of the biological variability in UNaV, only every other daily urine sample correctly classified a 3-g difference in salt intake (49%). By increasing the observations to 3 consecutive 24-hour collections and sodium intakes, classification accuracy improved to 75%. Collecting seven 24-hour urines and sodium intake samples improved classification accuracy to 92%. We conclude that single 24-hour urine collections at intakes ranging from 6 to 12 g salt per day were not suitable to detect a 3-g difference in individual salt intake. Repeated measurements of 24-hour UNaV improve precision. This knowledge could be relevant to patient care and the conduct of intervention trials.



We believe there are teleologically sound reasons why sodium might be stored in skin. The microdomain interface between stored sodium bound to proteoglycans is hypertonic to plasma and interstitial fluid. Immune cells regulate a hypertonic microenvironment in the skin; however, the biological advantage of increased skin sodium concentrations is unknown. We found that sodium accumulated at the site of bacterial skin infections in humans and in mice. We used the protozoan parasite Leishmania major as a model of skin-prone macrophage infection to test the hypothesis that skin-sodium storage facilitates antimicrobial host defense. Activation of macrophages in the presence of high NaCl concentrations modified epigenetic markers and enhanced p38 mitogen-activated protein kinase (p38/MAPK)-dependent nuclear factor of activated T cells 5 (NFAT5) activation. This high-salt response resulted in elevated type-2 nitric oxide synthase (Nos2)-dependent nitric oxide (NO) production and improved *Leishmania major control*. Finally, we found that increasing sodium content in the skin by a high-salt diet boosted activation of macrophages in an Nfat5-dependent manner and promoted cutaneous antimicrobial defense. We suggested that the hypertonic microenvironment could serve as a barrier to infection.

Perspectives

We will reproduce HTNB in mouse and rat models and will pursue cell-based studies to elucidate the function of PDE3A further. This work will be performed in cooperation with Enno Klussmann and his laboratory at the MDC. Wolf-Hagen Schunck's group is the first (and only) clinically based research group that had the courage to establish an independent company. This company is now developing synthetic analogs of omega-3 fatty acid-derived epoxyeicosanoids for the prevention and treatment of cardiovascular diseases including atrial fibrillation. In conjunction with Jens Titze at Vanderbilt University, where Friedrich C. Luft also has an adjunct appointment, we will pursue novel avenues of sodium metabolism including new findings regarding sodium-based effects on metabolism.

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Maass PG, Aydin A, Luft FC, Schächterle C, Weise A, Stricker S, Lindschau C, Vaegler M, Qadri F, Toka HR, Schulz H, Krawitz PM, Parkhomchuk D, Hecht J, Hollfinger I, Wefeld-Neuenfeld Y, Bartels-Klein E, Mühl A, Kann M, Schuster H, Chitayat D, Bialer MG, Wienker TF, Ott J, Rittscher K, Liehr T, Jordan J, Plessis G, Tank J, Mai K, Naraghi R, Hodge R, Hopp M, Hattenbach LO, Busjahn A, Rauch A, Vandeput F, Gong M, Rüschendorf F, Hübner N, Haller H, Mundlos S, Bilginturan N, Movsesian MA, Klussmann E, Toka O, Bähring S. PDE3A mutations cause autosomal dominant hypertension with brachydactyly. Nat Genet 2015;47:647-53.

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Dr. Atakan Aydin
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Yvette Wefeld-Neuenfeld
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Christel Andrée

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Photo: David Ausserhofer/MDC

Ingo Morano

Molecular Muscle Physiology

We are studying the functional roles of subunits of key proteins of Ca^{2+}-handling and force generation in muscles. Further, our lab aims to understand the effects and mechanisms of sex and estrogen in the heart in more detail. Particularly, we are screening new protein binding factors of estrogen receptor and determine their effects on estrogen receptor actions in the heart.

Structural and functional roles of the myosin essential light chain (ELC) in the cardiac contraction

Two myosin heavy chains (MyHC) and four non-covalently linked myosin light chains, two essential myosin light chains (ELC) and two regulatory light chains (RLC), form the native Type II myosin molecule, which drives muscle contraction (Figure 1). The primary structure of cardiac ELC isoforms [ALC-1 (atrial ELC) and VLC-1 (ventricular ELC)] presents with an elongated N-terminal, and a dumbbell-like C-terminal domain ELCs bind with the utmost lysine-rich N-terminus to actin and with their C-terminal domain to the myosin lever arm, RLC and myosin motor domain (Figure 1). ELCs are involved in modulation of force generation of myosin motors and cardiac contraction, while its mechanism of action remained elusive. To assess whether ELC modulates myosin stiffness, and thus its force production and subsequently the cardiac contraction, we generated heterologous transgenic mouse (TgM) strains with cardiomyocyte-specific expression of ELC with human VLC-1 ($TgM^{hVLC\text{-}1}$) or E56G-mutated hVLC-1 ($hVLC\text{-}1^{E56G}$; TgM^{E56G}). Our data showed that stiffness and actin sliding velocity of myosin from $TgM^{hVLC\text{-}1}$ were significantly higher than myosin from TgM^{E56G} or myosin with mouse VLC-1 (mVLC-1) prepared from C57BL/6. Additionally, we could show that max. left ventricular pressure development of isolated perfused hearts from $TgM^{hVLC\text{-}1}$ were significantly higher than hearts from TgM^{E56G} or C57BL/6. These findings showed that ELCs decreased myosin stiffness, in vitro motility, and thereby cardiac contraction.

The sticky N-terminus of ELC binds to a cluster of acidic residues at the C-terminus of actin. We could show that although both hALC-1 and hVLC-1 have similar secondary structure, but the actin affinity of the N-terminus of hALC-1 was significantly weaker than actin affinity of hVLC-1, which may provide a lower mechanical load placed on the cross-bridges than the hVLC-1, thus allowing a higher maximal shortening velocity. Hence, differential expression of ELC isoforms could modulate muscle contractile activity via distinct α-actin interactions. Re-expression of the ALC-1 in the hypertrophied human ventricle, therefore, seems to be an auto-regulatory mechanism of the human heart to adapt to an increased work demand.

Human ALC-1 as a co-repressor of ERα-mediated transcription in human heart

We showed that E2 attenuated hALC-1 expression in human atrial tissues of both sexes and in AC16 cells (a human ventricular cell line). E2 induced the nuclear translocation of estrogen receptor alpha (ERα) and hALC-1 in AC16 cells, where they cooperatively regulate the transcriptional activity of hALC-1 promoter. E2-activated ERα reduces the transcriptional activity of hALC-1 promoter. This inhibitory effect was significantly potentiated in the presence of hALC-1, and thus, hALC-1 acts as

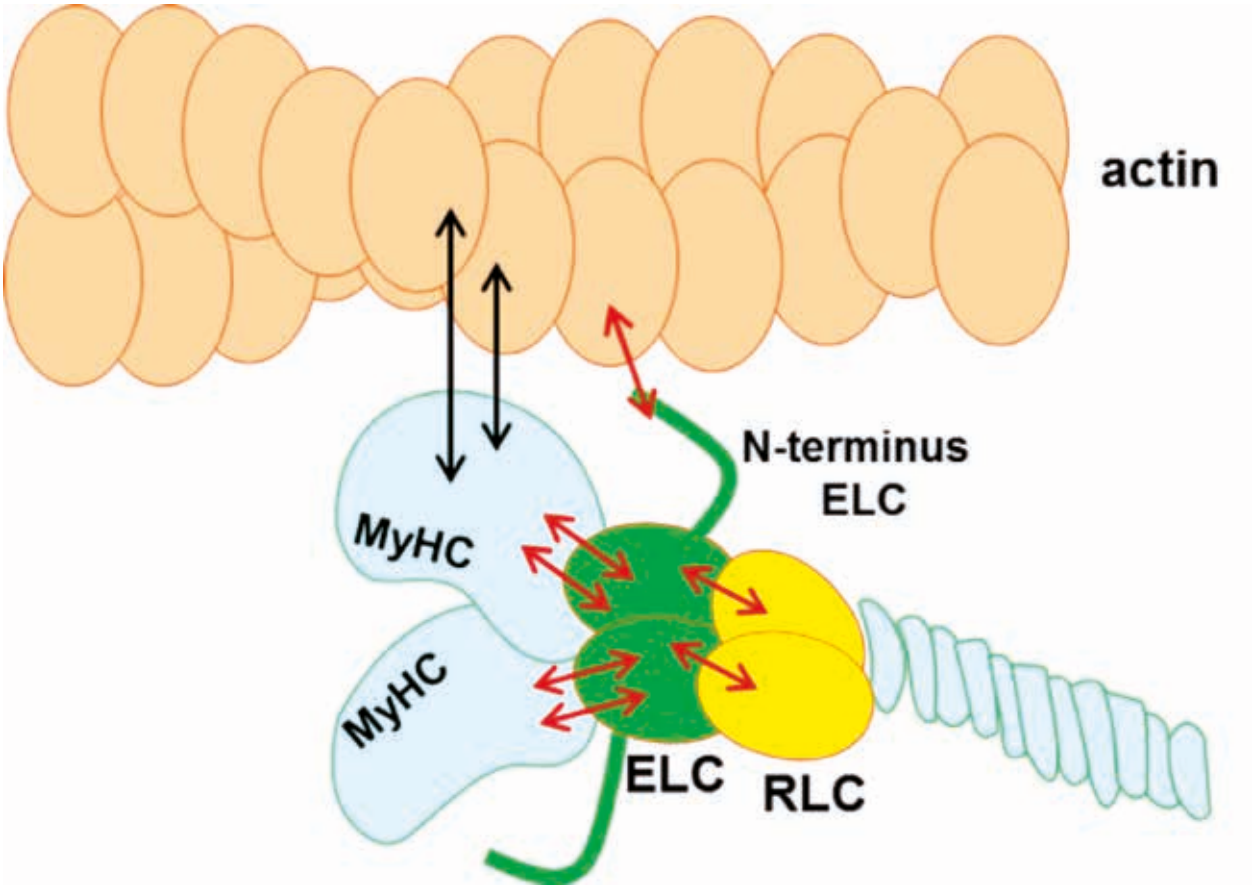

Figure 1: *Schematic interaction interfaces of myosin essential light chain (ELC). The interaction domains of ELC (green) with the MyHC, actin, and regulatory light chain (RLC) (red double arrows).*

a co-repressor of ERα-mediated transcription. Additionally, we showed that ERα interacts physically with hALC-1 in the presence of E2. As a further novel effect, we showed that chronic E2-treatment of adult mouse cardiomyocytes overexpressing hALC-1 resulted in reduced cell-shortening amplitude and twitching kinetics of these cells independent of Ca^{2+} activation levels. Our findings show that an E2 treatment counteracts the hALC-1-mediated improvement of contractile function in mouse cardiomyocytes. This could be of importance for the compensation process in overloaded hearts and preservation of cardiac function.

Effects of sex and 17β-Estradiol on nuclear translocation of the cardiac L-type calcium channel C-terminus

The cardiac L-type Ca^{2+} channel (Cav1.2) constitutes the main entrance gate for Ca2+ that triggers cardiac contraction. The distal C-terminus fragment of Cav1.2 α1C subunit (α1C-dCT) is proteolytically cleaved and shuttles between the plasma membrane and the nucleus, which is regulated both developmentally and by Ca^{2+}. We could show for the first time that the nuclear shuttling of α1C-dCT in cardiomyocytes is regulated in a sex-dependent manner. The ratio of nuclear to cytosolic intensity of α1C-dCT

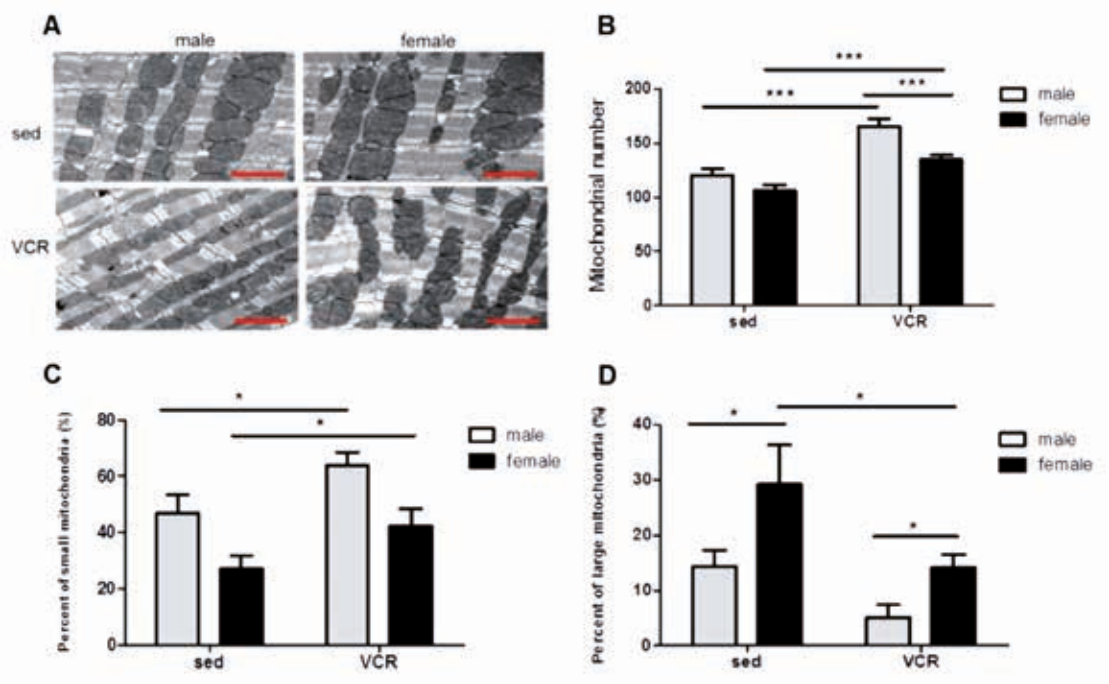

Figure 2: *Pronounced mitochondrial remodelling in female WT mice after VCR. A) Representative electron micrographs of mitochondria; X11.700 magnification; scale bar: 2µm. B) Mitochondrial number per unit area is significantly increased after VCR in both sexes. (C) Both sexes show a significant increase in small mitochondria, but (D) only in female mice, large mitochondria are significantly reduced. n = 4–6 / group and sex. 2-way ANOVA: *P ≤ 0.05; ***P ≤ 0.001. VCR: voluntary cage wheel running; sed: sedentary. Small mitochondria: ≤0.5 µm², large mitochondria: ≥1 µm².*

(I_{nuc}/I_{cyt}) was significantly higher in isolated female cardiomyocytes compared to male cardiomyocytes. Furthermore, we found a significant decrease in nuclear staining intensity of α1C-dCT in both female and male cardiomyocytes upon serum withdrawal. Interestingly, subsequent E2 treatment for 8h and 45min normalized the intracellular distribution of α1C-dCT in male cardiomyocytes, but not in female cardiomyocytes. This effect of E2 was revised by ICI 182780, an ER-inhibitor, indicating the involvement of ER in this signaling pathway. This identified novel signaling mechanism may explain, at least partly, the observed sex differences in the regulation of cardiac Cav1.2 channel activity.

Effects of sex on cardiac mitochondrial remodeling

In a recent study, we showed that sex differences exist in exercise-induced physiological myocardial hypertrophy (MH). Female mice showed a higher increase in cardiac mass compared with males, independent of forced or voluntary exercise character. During exercise-induced physiological MH, mitochondrial adaptations are needed to cope with the heart's increased energy demand, which can be achieved by increased mitochondrial biogenesis or mitochondrial remodelling. We hypothesized that sex differences in mitochondrial adaptation could contribute to a sexual dimorphism in physiological MH. Indeed, we showed that the

expression level of mitochondrial key modulators, such as NRF-1, NRF-2, Mef2a and Atp5k are significantly increased only in female WT mice after voluntary cage wheel running (VCR) (Figure 2). Furthermore, our data showed that although the number of smaller mitochondria (≤0.5 μm^2) in both sexes increased, the number of large mitochondria (≥1 µm2), however, decreased significantly only in female WT mice after VCR, and this was in line with the significant reduction in Mfn-2 protein only in females (Figure 2). These data indicate that exercise increases mitochondrial number and remodelling in both sexes, with a shift from large towards smaller mitochondria only in female VCR WT mice. Ongoing studies will clarify in which extent E2/ER are involved in sexually dimorphic exercise-induced cardiac mitochondrial remodeling.

The functional role of the ahnak protein family in skeletal muscle fibers

In the heart, ahnak1 is located at the sarcolemma and T-tubuli of cardiomyocytes indirectly associated with the voltage- dependent L-type Ca^{2+} channel (L-VDCC) via its β2-subunit (ß2), where it stimulates L-VDCC current. AHNAK function and subcellular localization in skeletal muscle are unclear. Co-localization of ahnak1 and ahnak2 with vinculin clearly demonstrates that both proteins are components of the costameric network. In contrast, no ahnak expression was detected in the T-tubule system. A laser wounding assay with ahnak1-deficient fibers suggests that ahnak1 is not involved in membrane repair. Using atomic force microscopy (AFM), we observed a significantly higher transverse stiffness of ahnak1-/- fibers. These findings suggest novel functions of ahnak proteins in skeletal muscle. Interestingly, human muscle biopsy specimens obtained from patients with limb girdle muscular dystrophy showed that ahnak1 lost its sarcolemmal localization and appeared in the extracellular space in both muscular dystrophies.

Selected Publications

Duft K, Schanz M, Pham H, Abdelwahab A, Schriever C, Kararigas G, Dworatzek E, Davidson MM, Regitz-Zagrosek V, **Morano I**, **Mahmoodzadeh S**. 17β-Estradiol-induced interaction of estrogen receptor α and human atrial essential myosin light chain modulates cardiac contractile function. Basic Res Cardiol. 2017; 112(1):1. Epub 2016 Nov 11.

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Photo: David Ausserhofer/MDC

Thoralf Niendorf

Ultrahigh Field Magnetic Resonance (UHF-MR)

Our research concentrates on the development of MR technology enabling new ways of mapping/probing morphology, function, physiology and metabolism together with explorations into the benefits of ***ultrahigh field (UHF) MR****. These efforts are designed to characterize (patho)physiological and biophysical processes to harmonize basic research with clinical science. To meet this goal small animal (9.4 T) and whole-body human (7.0T & 3.0T) MR are advanced. Our research is built on collaborations with the Charité, the PTB, Siemens, Bruker Biospin and other (inter)national institutions. The group forms a key/integral part of major research initiatives including the Helmholtz Alliance for Imaging and Curing Environmental Diseases (ICEMED), the population imaging program of the German National Cohort (GNC)* ***(1)****,* the DFG research group *FOR 1368 on Hemodynamics of Acute Kidney Injury, the imaging program of the German Center for Cardiovascular Research (DZHK), the individualized Medicine (iMED) initiative of the Helmholtz Association, the BMBF project on targeted radiofrequency heating (THERAHEAT), the BMBF funded validation project on the diagnosis of renal diseases (renalMROXY), the German Ultrahigh Field Imaging (GUFI) network, the EU COST Action PARENCHIMA on renal imaging, the DFG funded project on Fluorine MR technologies to study cellular therapies, the Marie Sklodowska Curie project SODIUMMRI-4-EU (EU project 752489), the DFG funded graduate school on quantitative imaging (BIOQIC) and the European Ultrahigh-Field Imaging Network in Neurodegenerative Diseases (EUFIND). Our initiatives resulted in 65 peer-reviewed publications during 2014-16, 70 abstract contributions to the 2014/16 meetings of the International Society of Magnetic Resonance in Medicine plus one US patent. Thoralf Niendorf recently received an ERC advanced grant by the European Research Council for his project ThermalMR: A New Instrument to Define the Role of Temperature in Biological Systems and Disease for Diagnosis and Therapy which eloquently speaks about the excellence of our science and the translational and cross-domain nature of our collaborations.*

Technology Focus: Enabling Methodology for UHF-MR

The sensitivity gain afforded by UHF-MR is the driving force behind our technological developments. We pioneered multi-channel radiofrequency (RF) coil technology including the development and clinical evaluation of 6-, 12-, 16- and 32 channel RF coil configurations tailored for clinical cardiovascular, brain and ophthalmic MRI **(2)** at 7.0 T **(Figure 1)**. To stay at the forefront of research we developed a modular 16 channel bow tie antenna RF coil array **(3) (Figure 1)**. Going beyond conventional proton (^{1}H) MR we are exploring MRI of sodium, fluorine, and other nuclei to gain a better insight into metabolic and (nano)molecular processes. ^{23}Na, ^{19}F RF coil arrays **(Figure 1)** designed for (pre)clinical applications were developed to form the basis for translational research. We were able to demonstrate the feasibility of sodium MR imaging of the

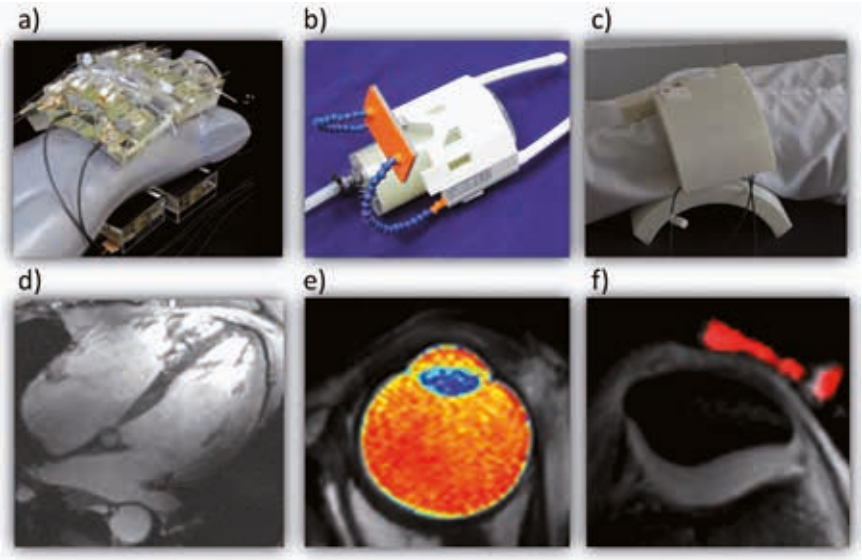

Figure 1: Enabling Methodology for UHF-MR
RF coil technology including **(a)** *16-channel,* **(b)** *6-channel and* **(c)** *8-channel transceiver arrays designed, and clinically evaluated by our group which were put to clinical use for* **(d)** *high fidelity imaging of the heart,* **(e)** *for ophthalmic imaging and* **(f)** *for in vivo ^{19}F MRT of fluorinated drugs.*

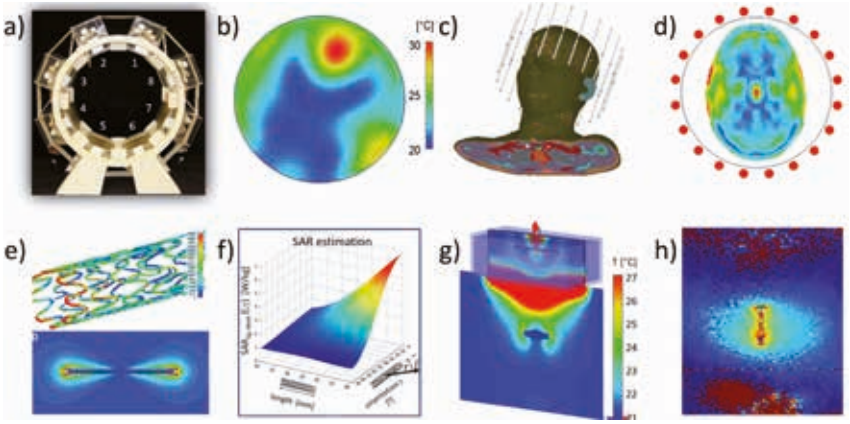

Figure 2: Next Generation of MR Technology and Applications
Our group explores innovative MR applications that are not clinical mainstream yet. We developed a novel instrument for Thermal Magnetic Resonance which supports targeted RF heating at 7.0 T **(a,b)**. *We extended our simulations to a 20 T class MR system (1 GHz)* **(c,d)**. *Assessment of MR safety of passive conductive implants including stents constitutes another research focus of our group* **(e-h)**.

human heart and showed proof-of-principle for high spatial resolution in vivo sodium imaging of the human skin. Our group was the first that facilitated human ^{19}F MRI at 7.0 T for the in vivo study of pharmacokinetics of ^{19}F-containing drugs **(Figure 1)**.

The group also focuses on developing novel MR applications. These efforts include our push towards a Thermal Magnetic Resonance (ThermoMR) instrument **(4)** for the study of the role of temperature in biological systems and disease. For this project, Thoralf Niendorf was rewarded with an **ERC advanced grant** by the European Research Council. Our numerical simulations showed how to adapt an UHF-MR instrument by installing high-density arrays of RF antennae that will permit us to generate heat in highly focused regions of tissue along with temperature mapping and diagnostic imaging **(Figure 2)**. This new instrument provides a revolutionary method for focal, *in vivo* temperature manipulations **(Figure 2)**. To master the physics of even higher magnetic fields we devoted our research to numerical electromagnetic field (EMF) simulations up to B_0=23 T (1GHz) **(4)**. MR safety is also one of our focuses, to gain a better insight into RF heating of stents and other medical devices, which is of paramount relevance for driving UHF-MR into the clinic **(Figure 2)**.

Clinical Focus: Explorations into Cardio- and Neurovascular Diseases Using UHF-MRI

The fields of cardio- and neurovascular MR are in the spotlight of our group's clinical applications-oriented research. En route to novel imaging-guided diagnostic and therapeutic approaches we strive towards the non-invasive assessment and early diagnosis of

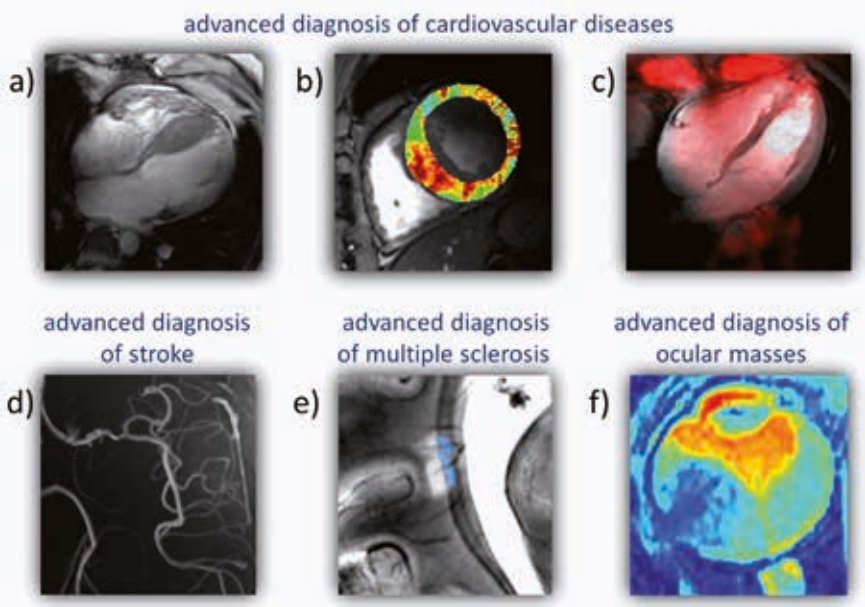

Figure 3: UHF-MRI of Cardio- and Neurovascular Diseases
TOP: *En route to novel diagnostic approaches we strive for high spatial resolution and micro-structural imaging of the heart including ^{1}H* **a)** *anatomical MRI,* **b)** *parametric imaging using MR biomarker and* **c)** *sodium MRI.* **BOTTOM:** *Examples of clinical studies of neurovascular diseases including* **d)** *diagnostic MRI of stroke* **e)** *differential diagnosis of neuroinflammatory pathologies and* **f)** *early diagnosis of ocular masses.*

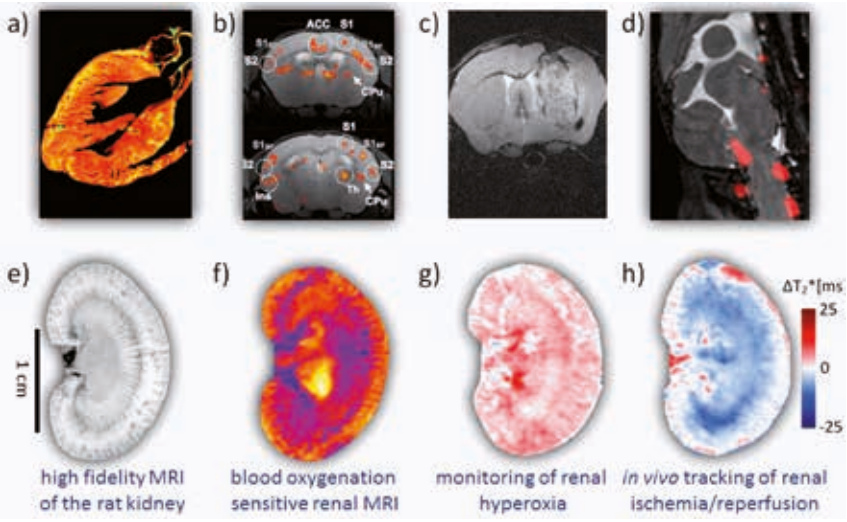

Figure 4: Quantitative Mapping and (Nano)Molecular Probing
TOP: a) *Microstructural mapping of mice heart,* **b)** *functional MRI of mice brain,* **c)** *monitoring of brain tumour growth and* **d)** *tracking of nanoparticlelabeled cell migration.* **BOTTOM:** *Understanding the mechanisms of acute kidney injury (AKI) is our motivation for changing renal diagnostics with* **e)** *anatomic MRI and with* **f)** *blood oxygenation level sensitive MRI to* **(g)** *monitor interventions and* **(h)** *to assess ischemia.*

cardiac and neurovascular diseases through MR-biomarker based tissue characterization, quantitative parametric mapping, high spatial resolution and microstructural imaging of the heart, brain and other target anatomy **(Figure 3)**. We have been able to conduct the world's first cardiac MR patient study at 7.0, which showed an increase in the MR relaxation parameter T_2* for patients suffering from hypertrophic cardiomyopathy. Our research results warrant an early diagnosis of pathologies (e.g. hypertrophic cardiomyopathy), advances diagnostic imaging (e.g. of stroke and small vessel disease, **Figure 3**), secures a differential diagnosis of pathologies (e.g. multiple sclerosis versus orphan neuroinflammatory diseases **(Figure 3)** and enables emerging clinical applications (e.g. diagnosis of ocular masses, **Figure 3**).

Experimental Focus: Quantitative Mapping and (Nano)Molecular Probing

The ultimate aim of the group is to harmonize research carried out in the area of preclinical animal imaging with clinical imaging. We are recognized leaders in the field of high fidelity *in vivo* and *ex vivo* anatomical, functional and microstructural imaging of the heart using cryo-cooled RF coil technology **(Figure 4)**. With this boon we developed a setup for normothermic functional brain MRI in mice during acute focal thermostimulation for probing nociception **(Figure 4)**. Our achievements supported in vivo and non-invasive monitoring of tumor expansion **(5) (Figure 4**). Our achievements supported *in vivo* and non-invasive monitoring of tumor expansion

(5) (Figure 4). We made major progress to study brain inflammation during the pathogenesis of autoimmune encephalomyelitis (EAE). Our know-how on fluorine (^{19}F) and physicochemical characteristics of nanoparticles is being leveraged to follow inflammatory processes and cell migration in real time with greater sensitivity *in vivo* by boosting cellular uptake of fluorinated nanoparticles **(Figure 4)**.

Gaining a better understanding of the mechanisms behind acute kidney injury (AKI) is in the center of our experimental research radar screen. We successfully combined MRI **(Figure 4)** and quantitative physiological techniques to monitor renal hemodynamics in an innovative hybrid setup (MR-PHYSIOL). This is now taken to the next level by incorporating near infrared spectroscopy (NIRS) as part of our translational project renalMROXY with the goal to translate the diagnostic capabilities of parametric MRI from experimental research to clinical imaging.

Future Directions:

Our strategic research will continue to be formed around MR physics to drive/support translational explorations into cardio- and neurovascular diseases. We are dedicated to throw further weight behind the solution of the remaining underserved clinical needs. To meet this goal MR technology remains to be developed and perfected. The development of next generation RF coil technology and imaging techniques will be tailored to demonstrate the clinical value of UHF-MR. On the MR physics side we strive to extend our activities and master MR electrodynamics by progressing our numerical simulations up to 70.0 T to support explorations into extreme field MR. For this reason we got heavily engaged in the Helmholtz application for a 14 T and 20 T class whole body MR system submitted to the **National Roadmap for Research Infrastructure** initiative of the Federal Ministry of Education and Research. Our **ThermalMR** approach for thermal interventions will eliminate the main barriers to the study and use of temperature - a critical dimension of life that is of intense clinical interest, but very poorly understood so far. Our approach opens an entire new research field of **thermal phenotyping** as a link to individualised medicine and is in full alignment with the mission and need to setup a precision medicine ecosystem at the Campus Berlin-Buch. To meet this goal our research is aligned along the mantra of the great physicist Freeman Dyson who stated that "New directions in science are launched by new tools much more often than by new concepts."

Patents / Patent applications

US patent: US 8,842,897 B2

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Electrochemical signaling in development and disease

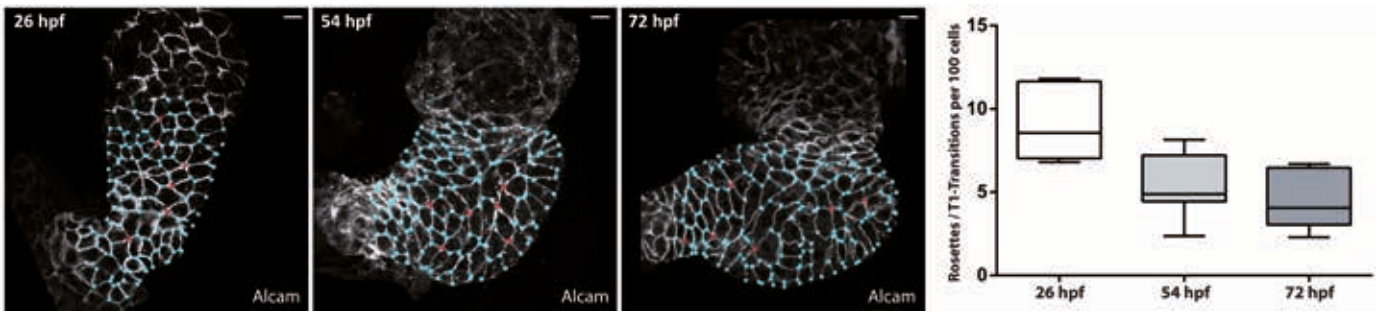
Ca^{2+} homeostasis is central to signaling pathways that control cell fate, differentiation, and organogenesis. In cardiac biology Ca^{2+} ions play pivotal roles in a multitude of cellular processes, ranging from excitation-contraction coupling to the regulation of hormone secretion and gene expression. Our research aims to uncover the molecular mechanisms that mediate the crosstalk between calcium signaling and other physiological stimuli with major morphogenetic pathways such as Wnt signaling during cardiovascular development as well as in disease states in zebrafish. We have recently shown that the calcium-mediated reactive oxygen species metabolism is essential for robust activation of Wnt/ß-catenin signal transduction ensuring more efficient and sustained signaling required for cell differentiation (JBC 2014). Currently, we pursue three lines of research. First, we investigate the molecular mechanisms that lead to the attenuation of calcium fluxes through the L-type Ca^{2+} channel by non-canonical Wnt signals during heart development. Next, we study the role of calcium fluxes through L-type Ca^{2+} channel in angiogenesis. Last, we investigate the mechanisms that govern the development of the functional cardiac syncytium. Our work expands the understanding of fundamental molecular processes that lead to proper formation of functional cardiovascular system, and their relevance in disease.

Molecular bases of interactions between Wnt non-canonical pathway and L-type Ca^{2+} channel

The L-Type Ca^{2+}-channel (LTCC) has an important function in cardiac biology. It belongs to a family of voltage-gated Ca^{2+} channels that are the major point of entry for Ca^{2+} ions into the cytoplasm of cardiomyocytes. Its conductance is regulated by membrane depolarization or by β-adrenergic/Protein kinase A (PKA) pathway. The dysregulation of the channel results in arrhythmias as well cardiac hypertrophy, underlying pathologies leading to sudden cardiac death. We reported that the Wnt11 non-canonical pathway regulates the intercellular electrical coupling in the developing zebrafish heart by attenuating the LTCC conductance (Nature 2010). However, the molecular mechanisms by which Wnt11 signaling might modify LTCC, and thus regulates its activity are unclear. Our results suggest that Wnt11 neither alters the LTCC at the transcriptional level, nor exerts any effect on the localization or abundance of the main pore-forming subunit of the LTCC. Interestingly, we found that Wnt11 signaling regulates the generation of two C-terminal isoforms of the α1C channel subunit: the distal C-terminal isoform (dCt), and a novel proximal C-terminal isoform (pCt). We observed that Wnt11 signaling affects the essential interaction between A-kinase anchoring protein (AKAP) and PKA, required for the formation of two C-terminal isoforms. Our data indicate that the Wnt11 signaling pathway regulates the LTCC conductance by preventing the generation of the distinct C-termini. However, how exactly and which AKAP has a key role in this process has to be determined.

Role of L-type calcium channel during angiogenesis

Several calcium channels are responsible for the intracellular calcium oscillations in endothelial cells. Although, the increase in



Epithelial remodeling guides early cardiogenesis. (a-c) *Confocal images of wild type hearts at 26 hpf (***a***), 54hpf (***b***) and 72 hpf (***c***) stained for Alcam as membrane marker with transition states in red dots.* **(d)** *Graph showing the reduction of transition states over time. 9.1 transition states per 100 cells are at 26 hpf (n=11), 5.2 at 54 hpf (n=10), 4.5 per 100 cells at 72 hpf (n=6). Scale bar = 10 µm. *** = p<0.001.*

intracellular Ca^{2+} concentrations is essential for physiological function of arteries, the role of Ca^{2+} fluxes in regulating angiogenic processes is unclear. We first showed that the voltage gated L-Type Ca^{2+} channel encoded by the *cacna1c* gene is expressed in endothelial cells. To better understand what processes are regulated by the Ca^{2+} fluxes through LTCC during angiogenic sprouting, we downregulated or stimulated the LTCC in the zebrafish and monitored the outgrowth of intersomitic vessels (ISVs). We were able to show that LTCC modulate the angiogenic sprouting of ISVs. Furthermore, we showed that LTCC and TRPC1 (transient receptor potential type C) channels interact genetically indicating the redundancy of different plasma membrane calcium channels in regulating vessel growth. Our data also suggest that Ca^{2+} fluxes in general are implicated in modulating the endothelial cell motility, and that the distinct intracellular calcium concentrations are crucial for the proper ISV development.

PCP pathway drives cardiac remodeling

The profound morphogenetic changes that lead to the looped and chambered vertebrate heart occur concomitantly with the establishment and refinement of intercellular communication between cardiomyocytes. However, the molecular and cellular mechanisms that underlie the patterning of functional myocardial syncytium during early embryonic development are not thoroughly explored. During vertebrate cardiogenesis the two-chambered looped heart is formed from a simple linear tube. Changes in tissue architecture are controlled by junctional remodeling, cell intercalations, and collective cell migration. Wnt/Planar cell polarity (PCP) signaling plays a crucial role in guiding the tissue remodeling. We showed that the non-canonical ligands Wnt11 and Wnt5b and the PCP core components Fzd7, Vangl2, Dvl2, and Pk1 are involved in the regulation of cell re-arrangements during cardiac chamber formation. Downstream effectors of the PCP pathway target cell adhesion, cytoskeleton, and migration. We showed that deficient PCP signaling affects the cardiac chamber architecture through changes in actomyosin organization. We observed specific changes in the localization of the phosphorylated non-muscle myosin II regulatory light chain that are regulated by the PCP pathway core components. Moreover, this process is accompanied by impaired SRF signaling. Taken together, our data indicate that the Wnt/PCP pathway regulates cardiac chamber remodeling by modulating actomyosin activity that is coupled to mechanosensitive signaling controlling cardiomyocyte differentiation.

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Photo: David Ausserhofer/MDC

Tobias Pischon

Molecular Epidemiology

The Molecular Epidemiology Group studies at the molecular level the relationship between lifestyle, diet, genetic, metabolic, and environmental factors with risk and outcome of chronic diseases in human populations. The focus is on biomarkers for accurate and precise assessment of exposure, intermediary effects, and early disease development. Our aims are to contribute to the understanding of disease etiology, to allow more precise disease prediction, and to improve the identification of high risk individuals as well as the quantification of the effect of interventions, thus envisioning a reduction of chronic disease risk through targeted prevention.

Metabolic dysfunction and risk of chronic disease

Abdominal obesity is associated with abnormal glucose metabolism, elevated blood pressure, dyslipidemia, and type 2 diabetes mellitus (T2D), which cluster within the Metabolic Syndrome (MetS). Components of MetS may not only be risk factors for cardiovascular disease (CVD), but also for certain cancers, particularly colorectal and liver (Nimptsch & Pischon, Horm Mol Biol Clin Invest 2015). As part of the Helmholtz Portfolio topic "Metabolic Dysfunction" we investigate associations of metabolic factors with chronic disease risk. For **colorectal cancer (CRC)** we found the association with MetS largely accounted for by abdominal obesity and abnormal glucose metabolism (Aleksandrova et al, Cancer Prev Res 2011). Abdominal obesity is mostly accounted for by visceral fat, which secretes a variety of cytokines and hormones. Within the large **European Prospective Investigation into Cancer and Nutrition (EPIC)**, we evaluated in collaboration with the German Institute of Human Nutrition and other partners the extent to which 11 biomarkers may mediate the association of abdominal adiposity with colon cancer risk (Aleksandrova et al, Int J Cancer 2014). The positive association was mostly accounted for by 3 biomarkers, HDL-cholesterol, non-HMW adiponectin and soluble leptin receptor, suggesting that alterations in levels of these biomarkers may represent a mechanism of action.

Visceral adipose tissue derived cytokines may induce hepatic secretion of acute phase proteins, including **C-reactive protein (CRP)**. We previously found higher CRP levels associated with higher CRC risk (Aleksandrova et al, Am J Epidemiol 2010). In a **Mendelian Randomization** approach we observed CRP genetic variability leading to raised CRP concentrations associated with higher risk of CRC (Nimptsch et al, Int J Cancer 2015), supporting the hypothesis that circulating CRP may play a causal role.
The liver is central in human metabolism, and obesity is associated with non-alcoholic steatosis, steatohepatitis, liver cirrhosis, and liver cancer. **Fetuin-A** is a liver protein that inhibits insulin actions, and studies found fetuin-A levels related to risk of T2D and CVD. Hyperinsulinemia is a possible risk factor for CRC, but the role of fetuin-A was unclear. We found higher circulating fetuin-A levels associated with a higher risk of CRC (Nimptsch et al, Int J Cancer 2015). Although fetuin-A genetic variation explained a large proportion of fetuin-A levels, genetically determined higher fetuin-A was not associated with CRC, suggesting that fetuin-A may not be causally related to CRC.

We also studied prospectively the association of inflammatory and metabolic biomarkers with risks of **liver and bilary tract cancers** (Aleksandrova et al, Hepatology 2014). Higher concentrations of CRP, IL-6, C-peptide, and non-HMW adiponectin were associated with higher risk of hepatocellular carcinoma (HCC), suggesting that elevated levels of biomarkers of inflammation and hyperinsulinemia are associated with a higher risk of HCC, independent of obesity and established liver cancer risk factors, and may be able to improve risk assessment.

The **insulin-like growth factor (IGF) axis** mainly regulates cellular proliferation, differentiation and apoptosis, but has also been linked to **type 2 diabetes mellitus** (T2D). IGF binding protein 3 (IGFBP-3) may increase T2D risk via binding of IGF-1. We investigated together with partners from the German Institute of Human Nutrition serum concentrations of IGF-1, IGFBP-3 and their ratio in relation to T2D incidence within **EPIC-Potsdam** (Drogan et al, Am J Epidemiol 2016). Our findings did not confirm an association between IGF-1 and risk of T2D, whereas higher IGFBP-3 levels may increase T2D risk independent of IGF-1 levels.

Metabolomics is the simultaneous study of numerous low-molecular weight compounds. We previously identified 14 metabolites independently associated with risk of T2D, including sugar metabolites, amino acids, and choline-containing phospholipids (Floegel et al, Diabetes 2013). We currently investigate to what extent metabolites are associated with risk of **cardiovascular disease**.

Obesity has also been linked to **cognitive impairment**, although mechanisms are unclear. Post-operative cognitive dysfunction (POCD) is a frequent condition after surgery among persons within the general elderly population. POCD can also be considered a model to study the influences on cognitive impairment in general. In a meta-analysis, we found only a few studies that have investigated the association of obesity with risk of POCD and the results provide only limited support that obesity increases risk (Feinkohl et al, Diabetes Metab Res Rev 2016). As part of the project **"BioCog – Biomarker Development for Postoperative Cognitive Impairment in the Elderly"**, supported within FP7 by the European Union we investigate associations of obesity and metabolic dysfunction with risk of POCD in detail.

Methodological studies

Abdominal obesity, as assessed by waist circumference, is closely associated with metabolic dysfunction. Manual measurements have so far been the method of choice for body circumference assessment. **3D body surface scanners** facilitate computation of body measures, but limited information is available on validity and reliability. We found strong correlations between automated measurements with the bodyscanner VitussmartXXL and manual measurements (Jaeschke et al, PLOS ONE 2015). Reliability was high and correlations with parameters of MetS were similar between automated and manual measurements. Supported by the Federal Ministry for Economic Affairs and Energy (BMWi), we investigate in the **MetSScan Study** to what extent automated measures of abdominal volume may improve detection of MetS.

Analysis of complex data from omics studies requires appropriate **statistical approaches**. We propose a biological framework and a statistical model incorporating multi-level biological measures, which leads to more powerful genetic asso-

ciation analyses (Konigorski et al, BMC Proc 2016). We investigated the effect of single nucleotide variants on blood pressure and gene expression, while considering the non-directional dependence between both, using copula functions. Our results show that such tests have smaller p-values than univariate single or multi marker tests.

Diet, Nutrition, Physical Activity

Circulating **fetuin-A**, a marker for hepatic fat accumulation, has been related to a higher risk of T2D and CVD. Little is known about **dietary or nutritional determinants** of fetuin-A concentrations. Together with partners from the Helmholtz Center Munich, we found in the **Bavarian Food Consumption Survey II (BVSII)** higher energy intake nonsignificantly associated with higher fetuin-A concentrations (Nimptsch et al, Br J Nutr 2015). There was no clear association for energy-providing nutrients, but higher alcohol intake and milk and dairy products were associated with lower fetuin-A. Our data indicate that among dietary factors particularly alcohol consumption and potentially dairy intake may be related to fetuin-A concentrations. **Paraoxonase (PON1) and arylesterase (AE)** are functions of the enzyme paraoxonase, synthesized by the liver and circulating in plasma to protect lipoproteins against oxidative modification. Excessive alcohol consumption may reduce serum PON1 and AE activities but less is known for habitual alcohol intake. In BVSII we found no strong association between alcohol and PON1 and AE (Schwedhelm et al, Br J Nutr 2016). PON1 activity was lowest in non-drinkers and highest with medium alcohol consumption. AE activity increased with alcohol consumption. Associations were attenuated after adjustment for HDL concentrations. These results do not support the hypothesis that habitual alcohol consumption is related to important alterations in PON1 and AE activities.

Physical activity (PA) is an important factor in the etiology of chronic diseases, but less is known for **activity related energy expenditure** (AEE). Traditionally, AEE has been estimated from questionnaires. Accelerometers might help to objectively measure PA but it is unclear to what extent this may explain the variance in AEE. Further, variability in PA, observational time needed to estimate habitual PA, and reliability are unknown. In a systematic review, the variance of AEE explained by accelerometer assessed PA was 4-80% (Jeran et al, Int J Obes 2016). Inclusion of other predictors significantly increased it to 13-41%. Thus, data on predicted AEE based on accelerometry assessed PA need to be interpreted cautiously. As part of the **ActivE Study**, we aim to derive more precise prediction models. We assessed 24-h-PA in participants wearing accelerometers over two weeks (Jaeschke et al) and found individual PA highly variable between days, but the day of assessment or the day of the week explained only small parts of this variance. Our data indicate that one week of assessment is necessary for reliable estimation of habitual PA.

Assessment of the association between diet, nutrition, and metabolic factors on a population level can best be achieved with the use of well-designed epidemiologic studies. We participate in the **European Nutritional Phenotype Assessment and Data Sharing Initiative (ENPADASI)**, supported by the Federal Ministry of Food and Agriculture (BMEL) within the Joint Programming Initiative (JPI) "A Healthy Diet for a Healthy Life" (HDHL). The objective is to deliver a research infrastructure with data from a variety of nutritional studies. This will foster the analyses of diet, nutrition, and metabolic factors in different populations.

Although diet and physical activity are among the main risk factors for chronic diseases, their determinants are less clear. Within the Knowledge Hub **Determinants of Diet and Physical Activity Choice (DEDIPAC)**, which is part of the European JPI HDHL we investigate determinants of diet and physical activity, supported by the Federal Ministry of Education and Research (BMBF) (Condello et al., BMC Public Health 2016).

The National Cohort (NAKO)

With the aim to develop new strategies for risk assessment, early detection, and prevention of major chronic diseases, a network of German research institutions,

including the MDC, has started a large prospective cohort, supported by the Federal Ministry of Education and Research (BMBF), the Helmholtz Association, and the States of Germany. The National Cohort will include 200,000 participants from the general population of Germany, recruited through 18 study centers (German National Cohort (GNC) Consortium, Eur J Epidemiol 2014). All participants undergo baseline examination and 5-year re-examination. Every 2-3 years questionnaires on lifestyle changes and new major diseases will be collected. Mortality follow-up and systematic linkage with disease registries will be performed. Biomaterials are stored in a centralized biobank and in decentralized storages. The Molecular Epidemiology Group coordinates the Cluster Berlin-Brandenburg, including the MDC, the Charité-Universitätsmedizin Berlin, and the German Institute of Human Nutrition (Steinbrecher et al, Berliner Ärzte 2015). The Cluster includes 3 **study centers**, recruiting 30,000 participants into the cohort. Samples from these participants are stored in a **biobank** at the MDC. At MDC's Berlin Ultrahigh Field Facility (B.U.F.F.), the Molecular Epidemiology Group, the Experimental Ultrahigh-Field MR group, and the Cardiac MRI group conduct **MRI measurements** of 6.000 study participants (Bamberg et al, Radiology 2015).

With support from the German Center for Cardiovascular Research (DZHK) we investigate the association of lifestyle and metabolic factors with risk of secondary events in patients with prior myocardial infarction, which may form the basis for secondary prevention measures.

Selected Publications

Nimptsch K, Aleksandrova K, Boeing H, Janke J, Lee YA, Jenab M, Kong SY, Tsilidis KK, Weiderpass E, Bueno-De-Mesquita HB, Siersema PD, Jansen EH, Trichopoulou A, Tjonneland A, Olsen A, Wu C, Overvad K, Boutron-Ruault MC, Racine A, Freisling H, Katzke V, Kaaks R, Lagiou P, Trichopoulos D, Severi G, Naccarati A, Mattiello A, Palli D, Grioni S, Tumino R, Peeters PH, Ljuslinder I, Nystrom H, Brandstedt J, Sanchez MJ, Gurrea AB, Bonet CB, Chirlaque MD, Dorronsoro M, Quiros JR, Travis RC, Khaw KT, Wareham N, Riboli E, Gunter MJ, Pischon T. Plasma fetuin-A concentration, genetic variation in the AHSG gene and risk of colorectal cancer. Int J Cancer 2015;137:911-20

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Matthew Poy

microRNA and Molecular Mechanisms of Metabolic Diseases

Type II diabetes has finally become recognized as a major challenge to global health. It is imperative to improve our understanding of the molecular mechanisms behind this disorder and develop new drug therapies. The pathophysiology of diabetes is undoubtedly complex, typically characterized by hyperglycemia resulting from varying states of insulin resistance and impaired b-cell function. Oftentimes, the failure to regulate circulating blood glucose levels is a consequence of the inability to produce sufficient amounts of insulin by the pancreatic β-cells. In our group, we focus on fundamental pathways regulating glucose metabolism and how altered pancreatic islet physiology contributes to metabolic disorders such as type 2 diabetes.

To date, numerous counter-regulatory mechanisms have been characterized in the cell illustrating the dynamic nature of the intracellular environment. Among the more recently discovered is the microRNA (miRNA) pathway and as of this writing, 2578 mature human miRNAs have been annotated in the miRBase database which now catalogs 206 different plant and animal species. Importantly, since their identification in C.elegans, a canonical pathway has now been identified to describe how miRNAs are processed and incorporated into Argonaute-containing complexes to maintain their effect on gene expression. Many insights into the functional role of miRNAs have been recently made, improving our understanding of how these small RNAs integrate into the already complex landscape of regulating gene expression. Most notably, based on a number of miRNA knockout mouse models showing subtle phenotypes under steady state conditions, a hypothesis has gradually emerged suggesting the miRNA pathway contributes to cellular stress responses. Moreover, these observations are further supported by our recent study focusing on the role of Argonaute2 (Ago2) in the compensatory proliferation of the pancreatic β-cell during insulin resistance. These recent findings culminate over 10 years since our initial study which identified miR-375, a miRNA now established among the highest expressed sequences in this cell type.

The very first loss and gain-of-function studies revealed this miRNA as a negative regulator of insulin release via the direct targeting of the gene myotrophin. The role of miR-375 in insulin release was further confirmed in vivo using the total mouse knockout (375KO). Furthermore, this model facilitated the identification of several additional targets of miR-375 including Elavl4/HuD, Cadm1, Gphn, and Rasd1. Moreover, 375KO mice exhibited hyperglycemia and decreased β-cell mass, and these effects were further exacerbated after crossing the knockout onto the leptin-deficient ob/ob background. This result indicated that miR-375 plays an essential role in the compensatory β-cell proliferation induced by insulin-resistance in ob/ob mice. While the precise causes are not understood, insulin resistance is one of the most common metabolic stress conditions and a consequence of chronic over-nutrition. Moreover, our recent observations showing loss of Ago2 also blocked proliferation during insulin resistance further supports a role for the miRNA pathway in mediating cellular stress responses. Importantly, we showed that Ago2 is up-regulated in the pancreatic islets of both genetic and diet-induced models of insulin resistance and obesity as a result of the silencing of miR-

184. Furthermore, the inverse relationship between Ago2 and miR-184 was confirmed in islets of human subjects further underlining the relevance of studying β-cell function in these mouse models. Of note, miR-184 was shown to target the gene Slc25a22, a mitochondrial glutamate transporter, indicating miRNAs may regulate many stages of the canonical pathway in the β-cell leading to insulin secretion.

While miR-184 has been shown to potently regulate both growth and secretion of the β-cell, it remains to be determined how changes in insulin sensitivity as shown in the models of obesity may contribute to the direct regulation and function of all miRNAs in this cell type. Meanwhile, it is unclear whether miRNA function in the β-cell may also change in response to alterations within its metabolic environment such as levels of extracellular nutrients (glucose, amino acids or fatty acids), signaling hormones, neurotransmitters, or perturbations in cell-to-cell contact with neighboring endocrine, endothelial, mesenchymal, or neuronal cells. Moreover, key transcriptional regulators of miRNAs in the β-cell as well as RNA-binding proteins that may specifically bind miR-184 and target it for degradation remain unidentified. It is unclear how the additional abundant β-cell miRNAs may coordinately target genes to promote insulin release as well as facilitate compensatory expansion of the β-cell as metabolic demand increases. While miR-184 was the most significantly regulated miRNA in the islets of insulin-resistant mice, additional miRNAs have been shown to participate in the β-cell growth using human islet cells8. It will presumably be determined that numerous miRNAs act in a concerted fashion to regulate multiple targets that mediate cell growth and the continual recruitment of insulin-containing granules to the plasma membrane. Interestingly, it is still not known whether the same miRNAs perform an identical regulatory function in different cell types. As Ago2-associated sequences such as miR-375 are expressed in several unique tissues such as the pituitary and adrenal gland, it is unclear how this miRNA may regulate growth and secretion in these other neuroendocrine cell types.

In summary, in light of recent findings identifying components of the RNAi machinery in the nucleus including Ago2, Dicer, and GW182, it appears only a matter of time before new mechanistic insights into the role of this pathway are discovered. While the identification of direct targets of the miRNAs and their biological significance remains to be studied in greater detail, the role of small RNAs in the nucleus also remains to be described. The story of non-coding RNAs and their functional role in the pancreatic β-cell is far from complete. In an exciting time of rapidly evolving technologies, the next 10 years are certain to bring clarity to this expanding narrative.

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Photo: Feriet Tunç/BIH

Kai Schmidt-Ott

Molecular and Translational Kidney Research

Kidney disease affects millions of people and causes substantial morbidity and mortality. Our research group studies the molecular and cellular mechanisms that govern kidney development in the embryo and kidney disease later in life. We strive to discover novel diagnostic markers and therapeutic targets, and to better understand the molecular networks that govern tissue differentiation, homeostasis, and regeneration. We conduct clinical studies and utilize model organisms employing a wide spectrum of cell and molecular biology techniques, as well as systems biology approaches. Our central goal is to improve the diagnosis and therapy of kidney disease.

Background

The kidney is a central organ in cardiovascular diseases. It excretes toxins, regulates blood pressure and solute homeostasis, and produces hormones. High blood pressure (hypertension) and chronic kidney disease rank among the top risk factors for cardiovascular end organ damage. The kidney is composed of structural units called nephrons, which consist of more than 20 different types of epithelial cells that facilitate transport between the urinary compartment and the interstitium. In the embryo, kidney development is initiated during mid-embryogenesis, when the ureteric bud, an epithelial tubule extending from the nephric duct, interacts with an adjacent cell population of committed stem cells in the metanephric mesenchyme. The ureteric bud undergoes branching morphogenesis to give rise to the ureter, renal pelvis and collecting duct system, while the metanephric mesenchyme progenitors generate many additional cell types of the nephron. The molecular and cellular events underlying kidney development are intricately linked to kidney disease. Exogenous or genetic perturbations of kidney development result in urogenital malformations, cystic kidney diseases and other types of congenital kidney disease. Furthermore, adult kidney epithelia preserve the ability to reactivate molecular pathways from earlier developmental stages in certain disease states, including acute kidney injury, kidney fibrosis, and kidney tumors.

Gene Regulatory Networks in Kidney Epithelial Development and Homeostasis

Gene expression control governs development, homeostasis and adaptive responses. We discover and characterize novel molecular pathways that regulate epithelial morphogenesis and differentiation during kidney development and their response to renal injury. Our past and ongoing studies include the WNT, BMP, NF-κB pathways and their downstream transcriptional targets. In addition, we study Grainyhead type transcription factors, which we found to govern critical aspects of epithelial morphogenesis and differentiation, but which have also been implicated in epithelial injury. The projects benefit from our collaborative network which includes groups from Columbia University (Jonathan Barasch) and MDC (Carmen Birchmeier, Thomas Willnow, Michael Bader). Within research unit 1368 (Hemodynamic mechanisms of acute kidney injury) we study the role of the BMP and NF-kB pathways in kidney regeneration (with Dominik Müller, ECRC, and Ruth Schmidt-Ullrich, MDC).

Systems biology of kidney diseases

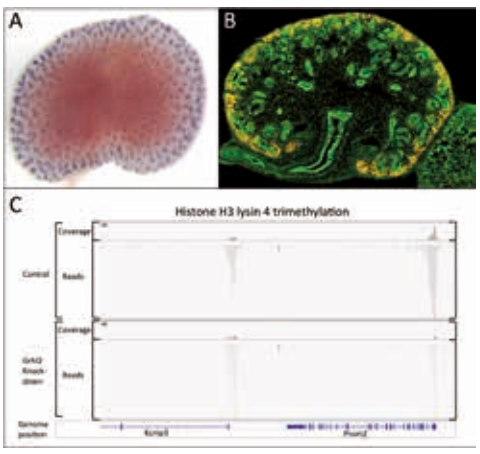
Kidney injury and phenotpyical transformations of kidney epithelia in congenital and neoplastic kidney diseases are associated with widespread alterations of the genome and its expression. We are combining next generation sequencing based techniques and bioinformatics to map alterations of the genome, epigenome, and transcription factor binding sites

in normal and diseased kidney cells. For this purpose we are collaborating with the Department of Urology at Charité (Kurt Miller), with the group of Walter Birchmeier (MDC) and with systems biology groups at the Berlin Institute of Medical Systems Biology (BIMSB). We believe that a detailed elucidation of the genome-wide alterations in diseased renal cells will yield an unprecedented opportunity to understand in detail the molecular mechanisms of renal disease. These studies are supported by the Urological Research Foundation.

Molecular Diagnosis of Kidney Injury

We aim to identify molecular signatures or markers that are linked with specific aspects of the kidney's response to injury. We have extensively studied neutrophil gelatinase-associated lipocalin (NGAL) and calprotectin (S100A8/S100A9), two proteins that are specifically expressed in the kidney in response to structural tissue tissue injury and that quickly get released into the urine. Our studies indicate that NGAL and calprotectin are valuable non-invasive biomarkers of kidney injury. In collaboration with Jonathan Barasch (Columbia University, New York), Timm Westhoff (University of Bochum), Klemens Budde (Department of Nephrology, Charité) and Friedrich C. Luft (MDC, ECRC), we are conducting basic and clinical studies to study the biology and clinical applications of NGAL and calprotectin in kidney injury. We develop diagnostic algorithms that utilize these markers to predict renal injury and to differentiate renal injury from related clinical entities. We also aim to identify additional novel and phase-specific biomarkers of kidney injury and compare them to the available diagnostic methods in nephrology. These studies are supported by the Deutsche Forschungsgemeinschaft within Research Unit 1368 (Hemodynamic mechanisms of AKI).

Developing mouse kidneys are analyzed by whole-mount in situ hybridization (A) or immunofluorescent staining (B). (C) shows an example of an experiment to detect genome-wide DNA modifications by next generation sequencing. Reads and coverage depict histone H3K4 trimethylation in collecting duct cells following knockdown of the transcription factor Grhl2 (Grhl2-Knockdown) and in control cells. Note differential H3K4 trimethylation at the promoter of the gene encoding Prom2.



Acknowledgements

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For additional information please visit:
www.mdc-berlin.de/schmidt-ott

Selected patents /patent applications

Determining the temporal phase of acute kidney injury involves obtaining test sample from subject, and determining the expression level of at least one biomarker e.g. cation transport regulator-like protein 1. Patent Number: WO2011157828-A1; EP2582840-A1; US2013165338-A1. Publ. Date: 12/22/2011. Inventors: Kai M. Schmidt-Ott, Anne Wuebken (via Max Delbrueck Center)

Diagnosing acute kidney injury in a subject comprises obtaining a urine sample and determining amount of neutrophil gelatinase-associated lipocalin- and kidney injury molecule-1 protein; Patent Number(s): WO2012068545-A1. Publ. Date: 05/24/2012. Inventors: Jonathan Barasch, Thomas L. Nickolas, Kai M. Schmidt-Ott (via Columbia University)

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Aue A*, Hinze C*, Walentin K, Ruffert J, Yurtdas ZY, Werth M, Chen W, Rabien A, Kilic E, Schulzke JD, Schumann M, Schmidt-Ott KM. A grainyhead-like 2/ovo-like 2 pathway regulates renal epithelial barrier function and lumen expansion. **Journal of the American Society of Nephrology.** 2015, 26:2704-15.* Equal contribution.

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Proteome Dynamics

Understanding how the genomic information is interpreted to yield a specific phenotype is perhaps the most important question in the post-genomic era. Proteins are the central players in this process: First, they are the final product of most genes. Therefore, understanding gene expression control requires analysis of protein synthesis and degradation. Second, function and dysfunction of proteins is directly responsible for cellular phenotypes. Studying protein function can therefore provide novel insights into biological processes in health and disease. We are using quantitative mass spectrometry-based proteomics as our central technology to study proteome dynamics on a global scale. The lab is interested in two major questions. First, how is the genomic information processed to yield a specific proteome? To answer this question we are studying protein synthesis and degradation. Second, how do proteins that are expressed at a specific cellular condition affect the phenotype? We are investigating this by analysing protein-protein interactions and posttranslational modifications.

Introduction

Mass spectrometry-based ("shotgun") proteomics is now a well-established technology that can identify essentially all proteins expressed in a mammalian cell. In addition, different methods have been developed to quantify proteins by mass spectrometry. The combination of mass spectrometry with quantification makes it possible to obtain precise functional information and to monitor temporal changes in the proteome. In one approach, named SILAC (for stable-isotope labelling with amino acids in cell culture), cells are differentially labelled by cultivating them in the presence of either normal or a heavy isotope–substituted amino acids. Due to their mass difference, pairs of chemically identical peptides of different stable-isotope composition can be distinguished in a mass spectrometer. The ratio of intensities for such peptide pairs accurately reflects the abundance ratio for the corresponding proteins. Classical SILAC can be used to quantify differences in steady-state protein levels (Fig. 1, left). In addition, we developed pulsed SILAC (pSILAC) as a novel variant to measure differences in protein synthesis (Fig. 1, middle). Finally, dynamic SILAC can reveal protein turnover (Fig. 1, right). SILAC is thus a versatile quantification method that can provide information about different aspects of cellular proteome dynamics.

Gene expression control

Many researchers use the term "gene expression" when they refer to mRNA levels. However, it is important to keep in mind that proteins are the final products of most genes. Therefore, understanding gene expression control requires information about protein synthesis and degradation. It is now clear that all steps in the gene expression cascade can be regulated.

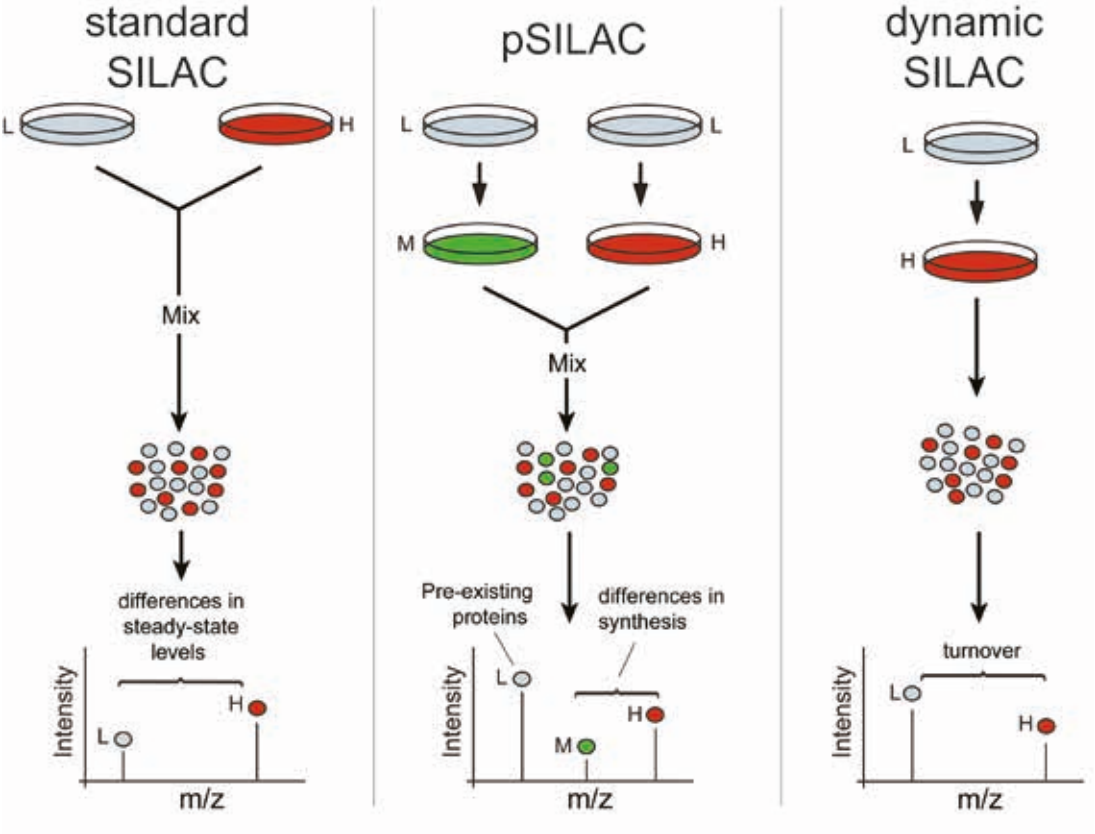

Fig. 1: Different variants of SILAC for the analysis of cellular proteome dynamics. *Cells are metabolically labelled with light (L), medium-heavy (M) or heavy (H) stable isotope-encoded amino acids. Differentially labelled peptides can be distinguished by mass spectrometry. Standard SILAC reveals relative differences in protein levels. pSILAC quantifies changes in protein production. Dynamic SILAC provides information about protein turnover.*

However, little is known about how the combined effect of all regulatory events shapes gene expression. The fundamental question of how genomic information is processed to obtain a specific cellular proteome is therefore still largely unresolved.

We are using metabolic pulse labelling approaches to comprehensively quantify gene expression at the protein level. For example, we developed pSILAC to directly quantify protein translation on a proteome-wide scale (Schwanhäusser et al., 2009, doi:10.1002/pmic.200800275). This method enabled us to quantify the impact of microRNAs on protein synthesis (Selbach et al., 2008; collaboration with the N. Rajewsky lab). We also used metabolic labeling of newly synthesized mRNAs (using 4-thiouridine) and proteins ("dynamic SILAC") to obtain the first global view of mammalian gene expression control at all major levels (Schwanhäusser et al., 2011; collaboration with W. Chen and J. Wolf labs). Our results indicated that gene expression in mouse fibroblasts is predominantly controlled at the level of translation. We also found that translation is actively regulated

during Schwann cell development in vivo (Sheean et al., 2014; collaboration with the C. Birchmeier lab). Most recently, we have used pulse chase experiments to study the kinetics of cellular protein degradation (McShane et al., 2016). The data revealed that many proteins are less stable in the first few hours of their life and stabilize with age. This so-called non-exponential degradation (NED) is common, conserved and has important consequences for protein complex formation and regulation of protein abundance.

Protein-protein interactions

Proteins typically interact with other proteins to exert a specific cellular function. Identifying interaction partners therefore provides direct insights into protein function and can reveal disease mechanisms. We are using quantitative interaction proteomics as a very versatile technology to study protein-protein interactions (Meyer and Selbach, 2015; doi:10.3389/fgene.2015.00237). This approach has two unique advantages. First, accurate quantification allows us to distinguish specific interaction partners from background contaminants with very high confidence. Second, quantification reveals how interactions change in response to perturbation. Therefore, this method can assess how disease-associated mutations and cell signaling events affect protein-protein interactions.

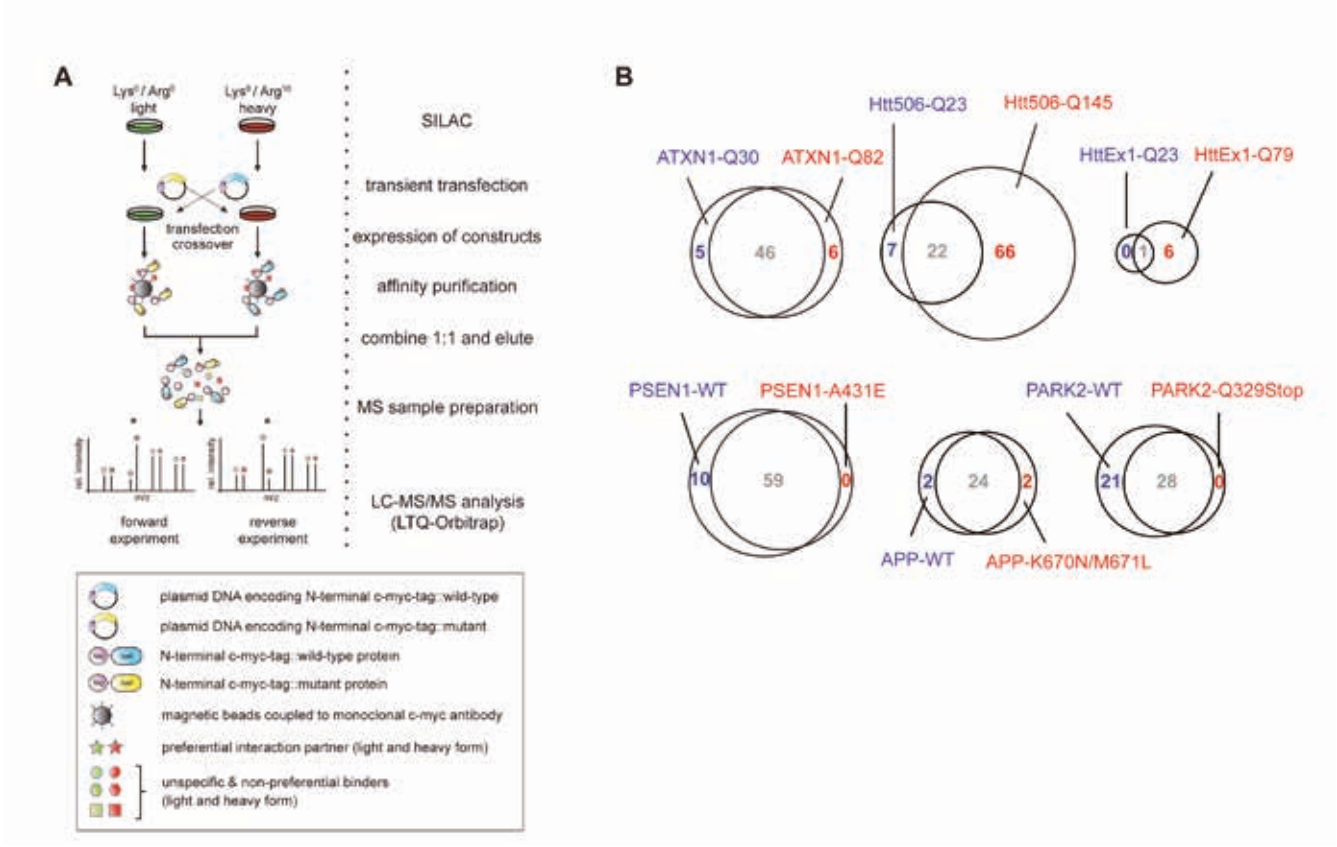

Fig. 2: Mapping interactions of neurodegenerative disease proteins. *(A) Stable isotope-labeled cells are transfected with plasmids encoding tagged wild-type or mutant bait proteins. Quantitative proteomics identifies differential binders. (B) Comparison of preferential interactors for wild-type (blue) or disease-associated variants (red) of ataxin-1 (ATXN1), huntingtin (HTT), presenilin-1 (PSEN1), amyloid precursor protein (APP) and parkin-2 (PARK2). From Hosp et al., 2015.*

We recently used quantitative interaction proteomics to identify binding partners of neurodegenerative disease proteins (Hosp et al., 2015). Functional follow-ups showed that identified interaction partners are linked to disease phenotypes in animal models and in human patients. Moreover, the direct comparison of wild-type proteins and disease-associated variants allowed us to identify binders involved in pathogenesis, which highlights the power of differential interactome mapping (Fig. 2). Systematic mapping of protein-protein interactions in diseases can thus help bridging the gap between identification of disease-associated variants and disease phenotypes. We are therefore currently upscaling this approach and applying it to different diseases. Moreover, we are also using quantitative interaction proteomics to explore the function of influenza A virus proteins and previously uncharacterized human proteins.

In vivo quantitative proteomics

Cell culture-based experiments cannot recapitulate all of the complex interactions among different cell types and tissues that occur in vivo. Small animal models such as worms and fruit flies are attractive alternatives that are extensively used in many areas of biomedical research, especially in genetics and development. We have extended the SILAC technology to Drosophila melanogaster (Sury et al., 2010, doi:10.1074/mcp.M110.000323) and Caenorhabditis elegans (Grun et al., 2014, doi:10.1016/j.celrep.2014.01.001, collaboration with N. Rajewsky lab). Moreover, we have used these model systems to study protein-protein interactions in vivo (Chen et al., 2016, doi:10.1074/mcp.M115.053975; collaboration with B. Tursun and K. Gunsalus).

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Molecular and Cellular Basis of Embryonic Development

Understanding the establishment of cellular identity is a major goal in stem cell biology and regenerative medicine. The endoderm germ layer gives rise to a number of vital organs, including the lungs, liver, pancreas and intestine. This remarkable diversity derives from a homogenous population of multipotent cells. The aim of our research is to elucidate the fundamental principles that establish and maintain pancreatic cell identity. One of the challenges in my laboratory is to understand how distinct cell types, such as liver and pancreas, arise from common endoderm progenitors in the embryo and acquire specialized shape to form functional organs. This fascinating question in developmental biology has direct clinical relevance. In the long run, our investigations will have implications in developing novel strategies to generate pancreatic ß-cells either from programming of stem cells or re-programming of hepatic cells, closing the gap between studies of basic processes in model systems and clinical research.

Comprehensive analysis of the hepatic and pancreatic lineage divergence

The pancreas consists of two organs in one: the exocrine pancreas, which produces digestive enzymes, and the endocrine compartment, which houses the insulin-secreting β-cells and is critical for blood glucose homeostasis. The β-cells are key metabolic regulators, and their loss or dysfunction results in diabetes, a still incurable disease. Finding ways to restore pancreatic β-cells in patients with diabetes is a challenge of top clinical priority. The adult pancreas has a very limited capacity for spontaneous regeneration and, therefore, represents an ideal target for lineage reprogramming approaches (Heinrich et al. 2015). The liver is an attractive tissue source for generating new pancreatic β-cells, due to its close developmental origin with the pancreas, regenerative capacity and accessibility. Thus, a successful reprogramming strategy of liver to pancreas potentially offers a solution to the problem, providing a renewable source of β-cells for cell-therapy of diabetes. Lineage reprogramming has its fundaments in developmental biology and is based on the knowledge of transcriptional networks underlying the establishment of cellular identity. We used RNASeq to define the molecular identity of liver and pancreas progenitor cells isolated from the mouse embryo at the time of their lineage divergence (Fig. 1). By integrating temporal and spatial gene expression profiles, we defined mutually exclusive signaling signatures in hepatic and pancreatic progenitors (Rodriguez-Seguel et al., 2013). Our results have provided us with guidelines for generating hepatocytes and pancreatic cells from ES and iPS cells and also for formulating liver-to-pancreas reprogramming strategies. Notably, we identified a unique non-canonical Wnt signaling signature in the emergence of pancreas versus liver from endoderm progenitors. In addition, we found transcriptional co-regulators, such as the TALE homeoproteins TGIF and Pbx, being differentially expressed between pancreatic and hepatic progenitor cells; epigenetic modulators (e.g. Setd7 histone methyltransferase) and lncRNAs, as potential dynamic regulators of cell identity. Finally, our results provided an invaluable resource for lineage tracing analysis to pinpoint the exact origin of the hepato-pancreatic lineage and isolate progenitor cell populations. Ongoing genetic lineage tracing experiments in the mouse aim at establishing the exact contribution of distinct progenitor domains to liver and pancreas.

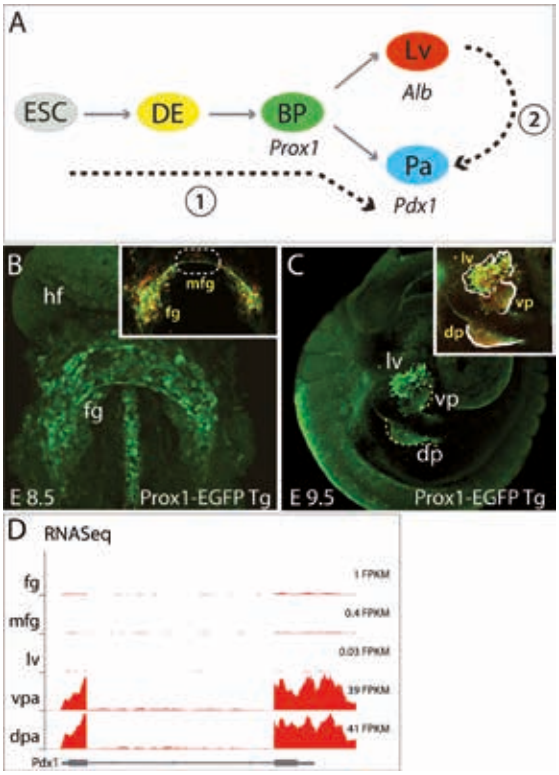

Figure 1. *(A) Schematic of hepatic and pancreatic cell lineage specification (1) and conversion (2). (B-D) RNASeq profiling of foregut, pancreatic and hepatic progenitors isolated from Tg(Prox1-EGFP) mouse embryos by FACS at distinct developmental stages (E8.5-E10.5 interval). Abbreviations: BP, bipotent progenitor; fg, foregut; hf, head fold; lv, liver; pa, pancreas; dp, dorsal pancreas; vp, ventral pancreas. Adapted from Rodriguez-Seguel et al. 2013.*

Lineage reprogramming to generate functional pancreatic cells

Our previous studies laid the basis for formulating reprogramming strategies between liver and pancreatic cells. Targeted functional studies of developmental regulators of the liver and pancreas fate decision and their lineage reprogramming activity are ongoing in my group. We identified the TALE homeoprotein TGIF2 as an intrinsic regulator of the pancreas versus liver fate decision in the embryo. Consistently, forced expression of Tgif2 is sufficient to convert differentiated liver cells to a pancreatic progenitor state both in vivo and ex vivo (Cerda-Esteban et al. 2017). This stepwise TGIF2-dependent fate conversion represents a novel strategy for controlled generation of pancreatic progenitors from liver and a starting point for production of β-cells (European Patent EP14177287.1). Moreover, this is an ideal platform for dissecting the intermediate steps of liver-to-pancreas interconversion and understanding the molecular basis of cellular identity and plasticity. In a long-term perspective, we will build on this knowledge **i.** to improve the efficiency of reprogramming and maturation of pancreatic-reprogrammed cells; and **ii.** to apply our findings to human hepatocyte models for studying fate conversion.

Tissue-architecture in the developing pancreas

During organogenesis, tissue-architecture defines particular local microenvironments, which in turn impact progenitor specification and subsequent differentiation. We, and others, showed that cells at the branched tips of the pancreatic epithelium undergo self-renewal ensuring the expansion of the progenitor reservoir, while cells at the trunk become restricted toward the endocrine lineage, giving rise to β-cells (Petzold et al. 2013). Thus, it is conceivable that different access to the surroundings underlies the fate differences between tip and trunk pancreatic cell populations. To date, how specialized "mesenchymal niches" influence lineage allocation within the pancreas epithelium is largely unknown. Novel lines of research in my laboratory are aimed at **i.** investigating how intrinsic epithelium modulators influence the crosstalk with its surrounding mesenchyme and **ii.** addressing the requirement of mesenchymal cells in epithelial morphogenesis and β cell differentiation. Ultimately, better comprehension of these concepts will have practical implications in future regenerative therapies of diabetes.

Patents / Patent applications

TGIF2-INDUCED REPROGRAMMING OF HEPATIC CELLS TO PANCREATIC PROGENITOR CELLS AND MEDICAL USES THEREOF

Lead Inventor: Dr. Francesca M. Spagnoli.

European Patent EP14177287.1, filed on July 2014.

Selected Publications

Cerdá-Esteban N, Naumann N, Ruzzittu, S, Cozzitorto, C., Pongrac IM, Hommel A, Mah N, Andrade-Navarro MA, Bonifacio E, **Spagnoli FM**. Step-wise reprogramming of liver to pancreas progenitor state by a single transcriptional regulator. **Nature Communications**. 2017 8:14127 doi:10.1038/ncomms14127.

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Mathias Treier

Genetics of Metabolic and Reproductive Disorders

Our research is focused on the regulation of mammalian physiology by genetic variation. Transcriptional regulators constitute the cornerstones of our research activities. In particular, we try to understand how metabolic changes modulate the transcriptional response of organs in a quest for maintaining body homeostasis during adaptation to novel environmental conditions. We utilize a plethora of mouse models for human diseases that we create ourselves that allow us to dissect even complicated physiological questions at the organismal level.

FGWAS using CRISPR/Cas9 transgenic technology

Most cases of noncommunicable or chronic diseases including cardiovascular disease and diabetes are the result of mainly unknown combinations of polygenetic variations in the genome of the affected individuals rather than the consequence of a single gene disruption which is the hallmark of rare diseases. Although *GenomeWideAssociationStudies (GWAS)* have identified a plethora of genetic polymorphisms associated with the development of noncommunicable diseases their predictive value for development of the disease later in life remains limited. This is mainly due to the fact that it was not possible to experimentally validate which combination of predisposing genetic polymorphisms are most deleterious for disease development.

Modifying the genome of mice has become a routine procedure which has made the mouse the most genetically studied mammalian organism despite the fact that until recently genome engineering in mice has been very time consuming. The discovery of the CRISPR/Cas9 system and its implementation for mammalian genome editing has turned out to be a game changer with respect to speed and sophistication in generating DNA modifications in the mouse genome. Employment of the CRISPR/Cas9 system has reduced the time for generating germ line mutations down to the generation time of the animal species investigated. With this technology it is now possible to study polygenetic traits by simultaneously introducing multiple genetic polymorphisms in a single step into the murine genome without leaving a molecular fingerprint behind. This application that we term *FunctionalGenomeWideAssociationStudies (FGWAS)*, will allow us in the future to directly evaluate the contribution of various combinations of genetic polymorphisms in an isogenic background with respect to development and progression of chronic diseases.

Genetic dissection of the renocardiac syndrome

Cardiovascular disease is still the major noncommunicable disease type in western societies. The CardioRenal Syndrome (CRS) is within the cardiovascular disease complex a subclass of disease entities that is defined as a complex pathophysiological disorder of the heart and the kidneys in which acute or chronic dysfunction in one organ affects the function of the other. CRS can be subdivided in five major classes one of which is the renocardiac syndrome where heart damage is indirectly elicited through acute or chronic kidney disease. The underlying molecular mechanisms of this organ crosstalk being still incomplete understood.

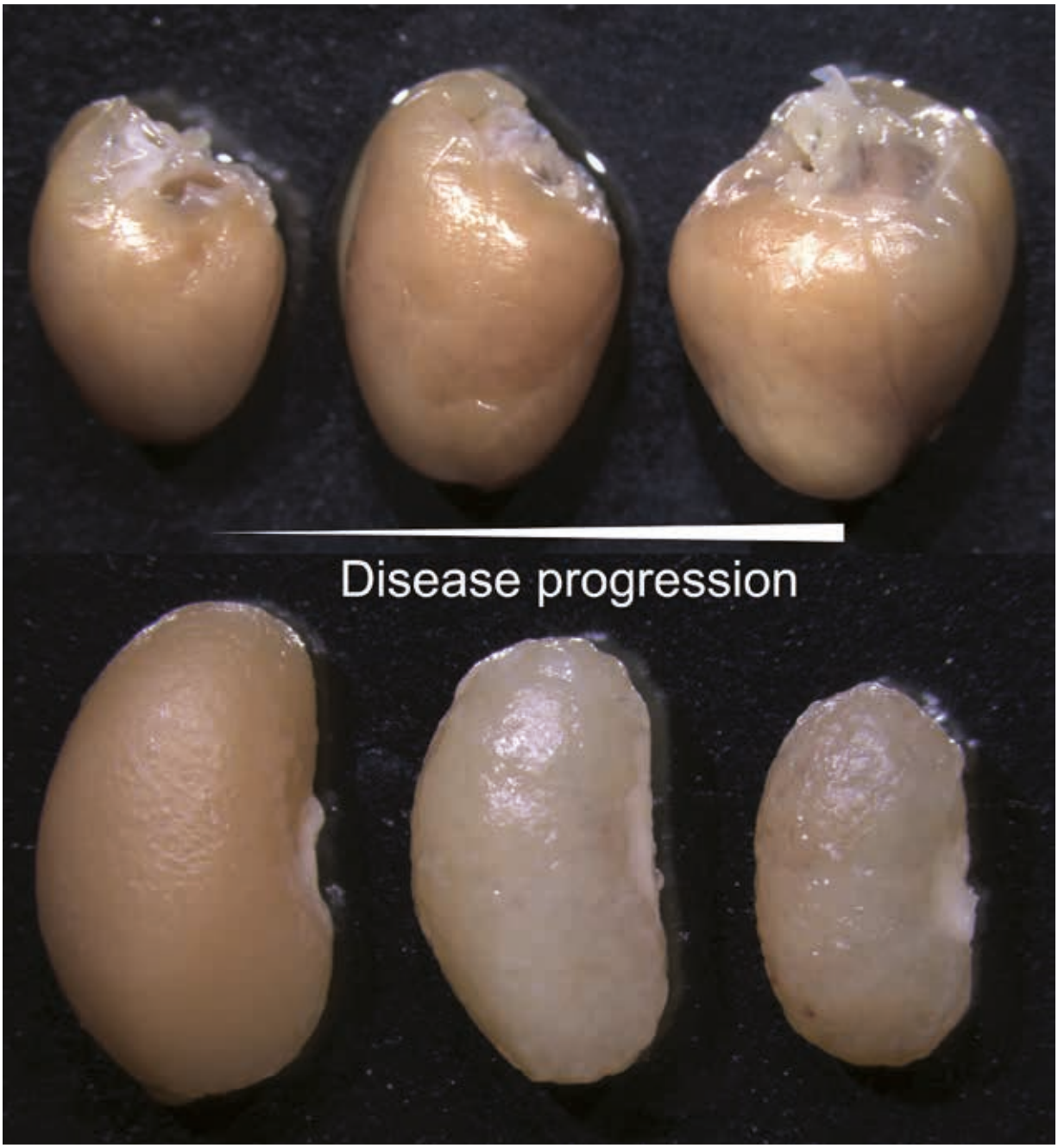

Fig. 1: *Disease modeling of the renocardiac syndrome in mice. The degeneration of the kidney overtime is mirrored by increased hyperplasia of the heart (heart top row, kidney below at different time points of disease progression).*

Based on our discovery that mutations in GLIS2, a zinc finger transcriptional regulator, causes nephronophthisis, a progressive chronic kidney disease (CKD), in mice and men, we have started to screen for modifier genes of the renocardiac disease spectrum based on the concept of synthetic lethality. Synthetic lethality describes the observation that the combination of mutations in at least two genes is lethal whereas each mutation on its own is said to be viable. We are in particular interested to identify novel genetic mutations whose lethality is dependent on loss of GLIS2 function. We have successfully identified novel genetic modifiers that lead to acceleration of CKD progression which allows us now to model the chronic renocardiac syndrome in mice efficiently (Fig. 1). Employing *FGWAS* and CRISPR/Cas9 transgenic technology, we are now able to systematically dissect any CRS subtype on a genetic level that should lead to the identification of novel targets for pharmacological intervention in cardiovascular disease.

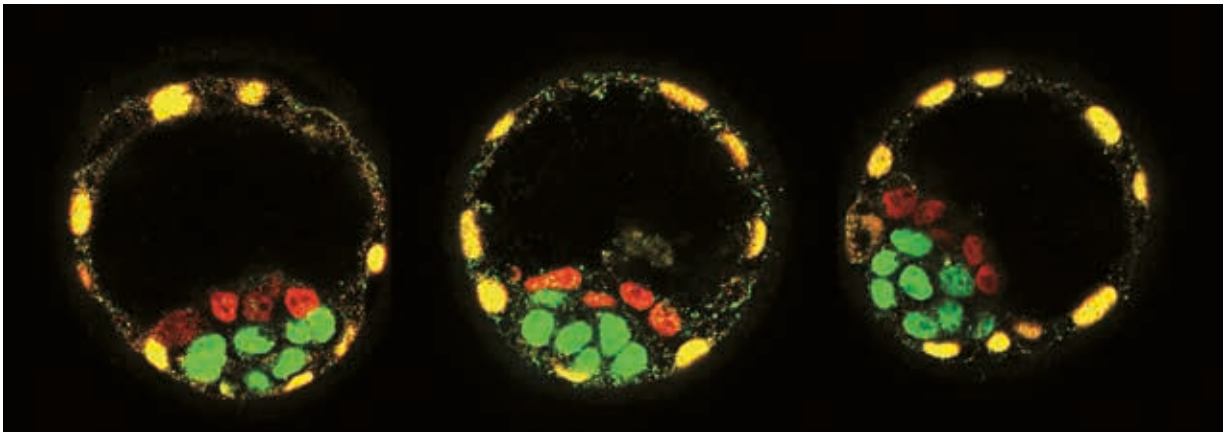

Fig. 2: *Murine blastocysts stained with antibodies marking the three cell lineages of the pre-implantation embryo: epiblast (Nanog/green), primitive endoderm (Gata6/red), trophoectoderm (Cdx2/yellow).*

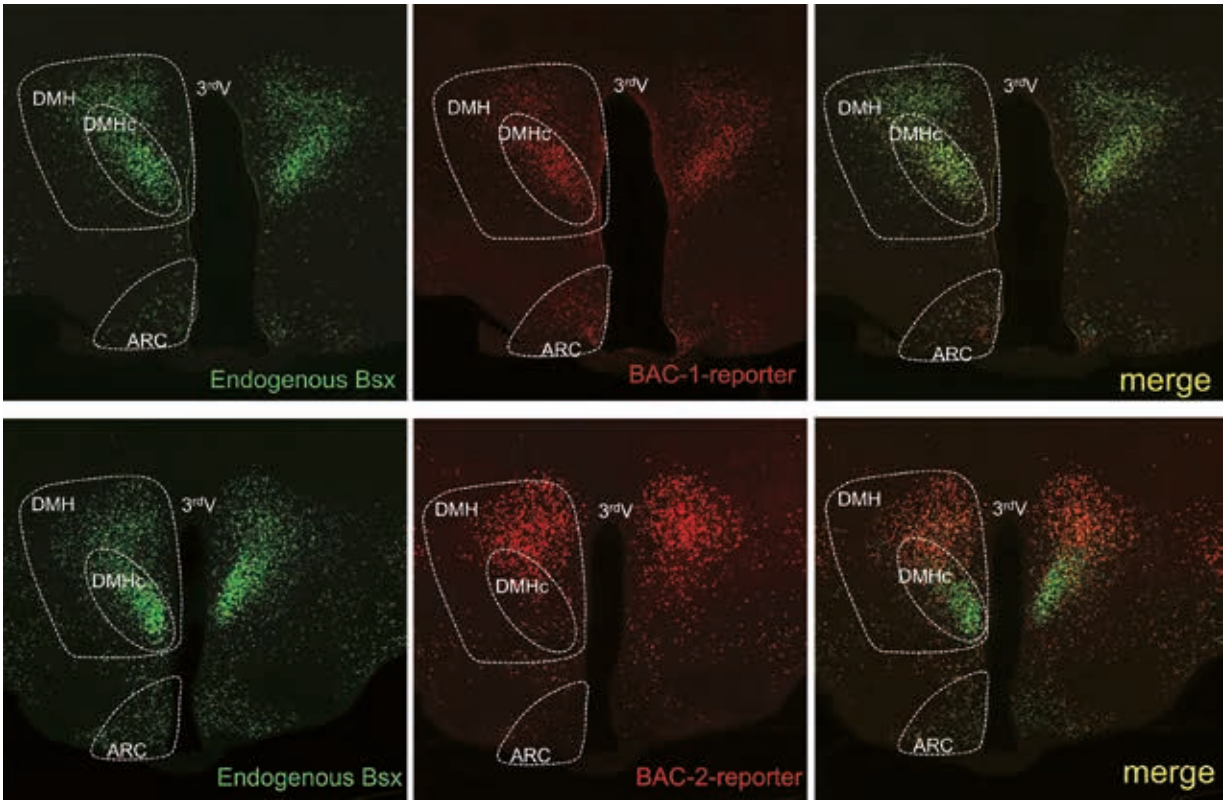

Fig. 3: *Identification of neuronal enhancer elements employing BAC (Bacterial Artifical Chromosome) mouse transgenesis. Shown is the endogenous expression pattern of BSX in the mouse hypothalamus visualized by Histone2B-GFP expression with superimposed expression of BAC reporters (Histone2B-RFP) harboring the BSX locus. Note, the enhancer element for the DMHc (compact region of the Dorsomedial hypothalamus) is missing on the BAC-2 reporter (ARC = arcuate nucleus).*

Stem cells, plasticity and regeneration

The great interest in stem cell biology is mainly due to the intrinsic ability of stem cells to indefinitely self-renew and their potential to differentiate into all cell types of the body. The study of early mouse development has revealed a lot of basic insights into the molecular mechanisms underlying stem cell self-renewal and plasticity (Fig. 2). These insights are now applied in adult stem cell biology and utilized in regenerative medicine for the extrinsic activation of endogenous organ repair processes. Along these lines we have been among the first showing that cells can functionally be reprogrammed *in vivo* through the demonstration of somatic sex reversal in adult female mice. Furthermore, we identified with SALL4 the central transcriptional regulator of stem cell pluripotency. Utilizing a proteomic approach we have delineated a SALL4 centered protein network in stem cells and currently investigate the molecular function of so far uncharacterized proteins in this network. This work has led to the identification of embryonic stem cells with extended developmental potential, which now allows us to study in greater detail the epigenetic modifications underlying embryonic stem cell plasticity which may have important implications for applied stem cell therapy in regenerative medicine.

Voluntary physical activity and sleep disorder

Obesity is the major risk factor for development of cardiovascular disease and diabetes. Physical activity is a key component of energy expenditure and therefore of energy balance. Sports-like exercise has a strong obesity protective effect in humans. However, why some people voluntary exercise and others do not remains an open question. We have proposed in our initial work on the analysis of *BSX* (*B*rain-*S*pecific *H*omeobox) gene function that differences in voluntary physical activity may have an underlying genetic cause. *BSX* was therefore named by others the "*fidgeting*" gene. *BSX* expression marks a neuronal network within the hypothalamus of the brain that orchestrates eating behavior, sleep and higher cognitive functions. Currently, we are interested in the neuronal circuits of the central nervous system (CNS) that are involved in the (dys)regulation of physical activity and sleep, major factors that can contribute to the development of obesity in humans. In order to deconstruct the BSX CNS network we combine light sheet microscopy with tissue clearing and enhancer mapping using CRISPR/Cas9 and BAC transgenic technology to delineate a structure-function correlation with respect to neuronal populations that modulate physical activity and sleep patterns (Fig. 3). We functionally probe the identified neuronal subpopulations using *DREADD* technology (*Designer Receptors Exclusively Activated by Designer Drugs*). DREADDs are engineered G-protein coupled receptors which are activated by otherwise inert drug-like small molecules. This technology allows us to activate or silence neuronal networks in free running animals thereby directly testing their involvement in the regulation of physical activity and sleep.

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Photo: David Ausserhofer/MDC

Thomas Willnow

Molecular Cardiovascular Research

We study endocytic receptors to uncover their roles in normal physiological processes and in human disease. We demonstrated that LRP2, a member of the LDL receptor gene family, balances signaling of the morphogen sonic hedgehog in the developing retina, and that lack of this activity results in severe overgrowth of the eyes in mouse models and patients. We also studied SORLA, an endocytic receptor expressed in neurons. We showed that this receptor controls distinct steps in the catabolism of Aβ, the causative agent in Alzheimer's disease, and that SORLA dysfunction is a cause common to sporadic and familial forms of this disease.

Introduction

Receptor-mediated endocytosis is the main mechanism for selective transport of macromolecules into cells. Significant progress has been made in elucidating the steps of endocytosis at the cellular level. However, the physiological relevance of many endocytic pathways for organ (dys)function remains elusive.

The main class of endocytic receptors is the LDL receptor gene family that mediates uptake of lipoproteins in many cell types (Fig. 1). Surprisingly, previous studies uncovered additional functions performed by these receptors, changing our perception of lipoprotein receptors from mere cargo transporters to key regulators of many physiological processes. In particular, the significance of these receptors for embryonic development is noteworthy. Yet, studies so far provided just a glimpse at their manifold contributions to developmental processes, with many details still to be uncovered.

Whereas studies on the LDL receptor gene family yielded insights into the significance of endocytosis for cell signaling, identification of another group of receptors, called VPS10P domain receptors, directed our attention to intracellular protein transport as a cause of human disease. VPS10P domain receptors share structural similarity to sorting receptors in yeast, suggesting their involvement in intracellular protein transport in mammalian cell types. While this hypothesis was confirmed by earlier studies, including our own work, the many functions of VPS10P domain receptors in human (patho)physiology still remain to be identified.

LRP2 is an auxiliary SHH receptor essential for eye development

Annabel Christ, Julia Klippert, Anna Christa

LRP2 is a receptor expressed in the neural tube, the embryonic tissue that gives rise to the CNS. Early on, we recognized the importance of LRP2 for brain development, as mice deficient for this receptor suffer from holoprosencephaly, a defect in separation of the forebrain hemispheres and the most common forebrain anomaly in humans. The significance of this receptor in humans was confirmed by others, demonstrating LRP2 defects as the cause of Donnai-Barrow syndrome, a monogenic disorder characterized by forebrain malformations. In our earlier work, Annabel Christ identified the molecular mechanism of LRP2 action in brain development by demonstrating that it acts as an auxiliary receptor for the morphogen sonic hedgehog (SHH). In the neural tube, LRP2 facilitates signaling of SHH through the receptor Patched 1, a step essential for proper forebrain patterning (Christ et al., Dev Cell 2012).

Now, Annabel and her colleagues showed that the role of LRP2 in SHH signaling extends to eye development (Christ et al., Dev

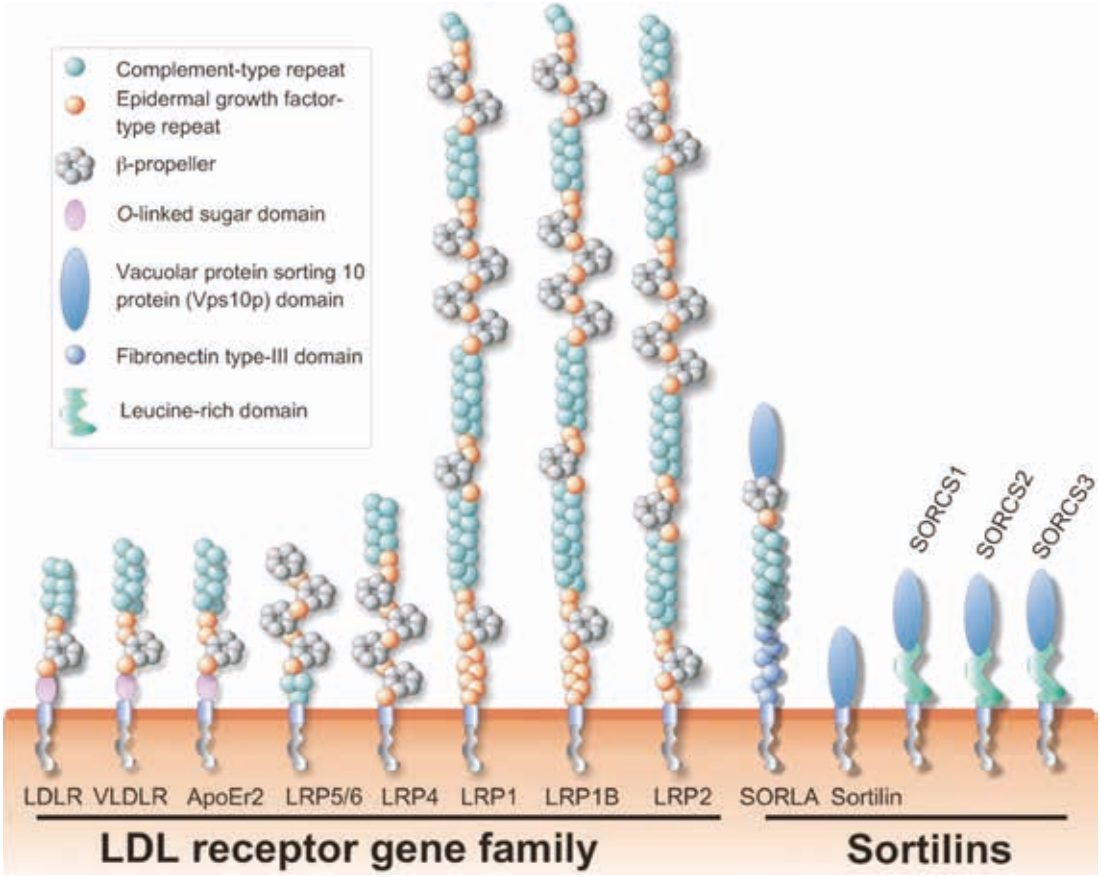

Fig. 1: *Structural organization of mammalian members of the LDL receptor and VPS10 domain receptor (Sortilins) families.*

Cell 2015). They identified that in the developing retina, LRP2 mediates endocytic clearance of SHH to antagonize the action of this morphogen. LRP2-mediated clearance prevents spread of SHH from the central retina into the retinal margin, and protects quiescent progenitor cells in this ciliary marginal zone (CMZ) from mitogenic stimuli. Loss of LRP2 in mice increases the sensitivity of the CMZ for SHH, causing aberrant expansion of the retinal progenitor cell pool and hyperproliferation of the retinal margin. As a consequence, the size of the eye globe increases, a phenotype referred to as buphthalmos. These findings document the ability of LRP2 to act, in a context dependent manner, as an activator or inhibitor of the SHH pathway. Furthermore, they uncovered LRP2 activity as the molecular mechanism imposing quiescence of the retinal margin in the mammalian eye, and they suggest SHH-induced proliferation of the retinal margin as cause of the large eye phenotype in patients with Donnai-Barrow syndrome.

SORLA controls production of neurotoxic Aβ peptides

Safak Caglayan, Vanessa Schmidt

Aβ is a neurotoxic peptide produced by proteolytic processing of the amyloid precursor protein (APP). Excessive production of Aβ is responsible for the synaptic dysfunction and memory impairment in patients with Alzheimer's disease (AD). Accordingly, mechanisms that reduce Aβ levels represent promising targets for prevention of this devastating disease. Previously, Vanessa Schmidt in the lab documented the ability of SORLA to act as an intracellular sorting receptor for APP in neurons that prevents accumulation of APP in endosomes where processing into Aβ occurs. These findings

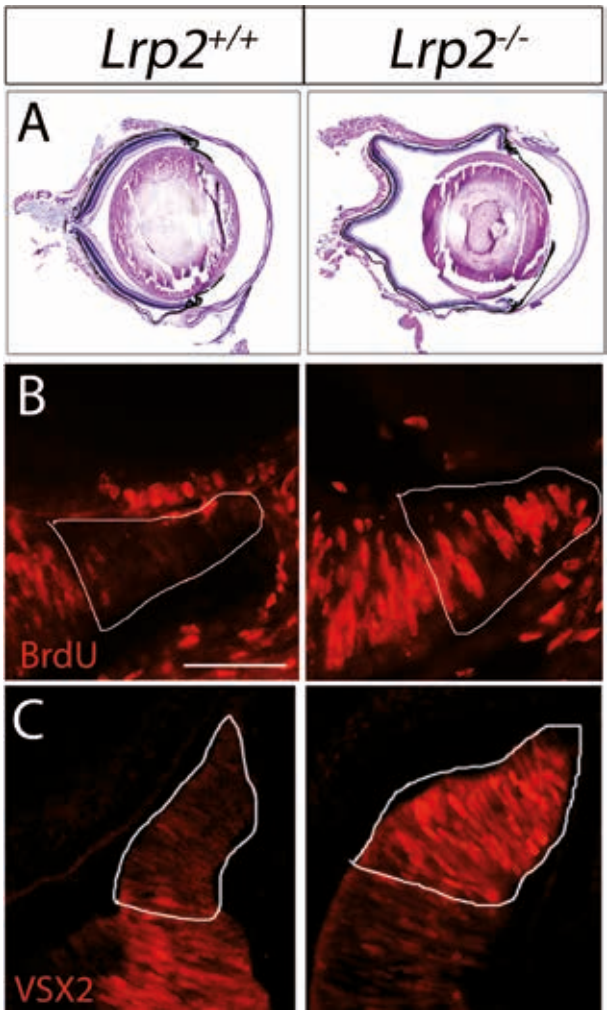

Fig. 2: Expansion of retinal progenitor cell pool and eye overgrowth in LRP2-deficient mice. *(**A**) Increased size of the eye globe in adult mice lacking LRP2 ($Lrp2^{-/-}$) compared to wild type animals ($Lrp2^{+/+}$). (**B, C**) Increase in progenitor cell number and proliferative capacity in the ciliary marginal zone (encircled) of $Lrp2^{-/-}$ compared to $Lrp2^{+/+}$ mouse embryos (embryonic day 16.5) as shown by BrdU incorporation (B) and staining for marker VSX2 (C). Scale bars: 50 µm.*

suggested low SORLA levels seen in individuals with AD as the underlying cause of enhanced Aβ production (Schmidt et al., EMBO J 2012).

To provide proof of concept that overexpression of SORLA reduces Aβ levels in vivo and represents a therapeutic target for AD, Safak Caglayan generated mice that overexpress this receptor. In line with our hypothesis, increasing levels of SORLA in the brain profoundly reduced Aβ burden in these animals. Exploring the underlying molecular mechanism, Safak, Vanessa, and their colleagues made the surprising observation that SORLA not only acts as sorting receptor for APP but also moves newly synthesized Aβ molecules to lysosomes for degradation, further reducing the amount of neurotoxic peptides released by neurons (Caglayan et al., Science Trans Med, 2014). They also documented that the ability of SORLA to sort Aβ is lost in a receptor mutant expressed in a familial form of AD. Taken together, these findings substantiated SORLA as a potential drug target in AD and identified loss of the receptor's Aβ sorting function as a possible cause of early-onset familial AD.

SORLA-mediated trafficking is a common cause of sporadic and familial forms of AD

Sonya Dumanis, Tilman Burgert, Safak Caglayan, Anne-Sophie Carlo

Given the importance of SORLA for amyloidogenic processes, Sonya Dumanis, Tilman Burgert, and Safak Caglayan set out to elucidate the molecular mechanisms controlling SORLA-dependent sorting of APP and Aβ in the brain in vivo. Their studies were based on our earlier observations in cell lines that the interaction of SORLA with two cytosolic adaptors, termed retromer and GGA, is required for receptor sorting to and from the trans-Golgi network (TGN). The TGN is a central hub for protein sorting. However, the relevance of anterograde or retrograde trafficking at this organelle for SORLA activity in vivo and for progression of AD remained unexplored. Now, Sonya and

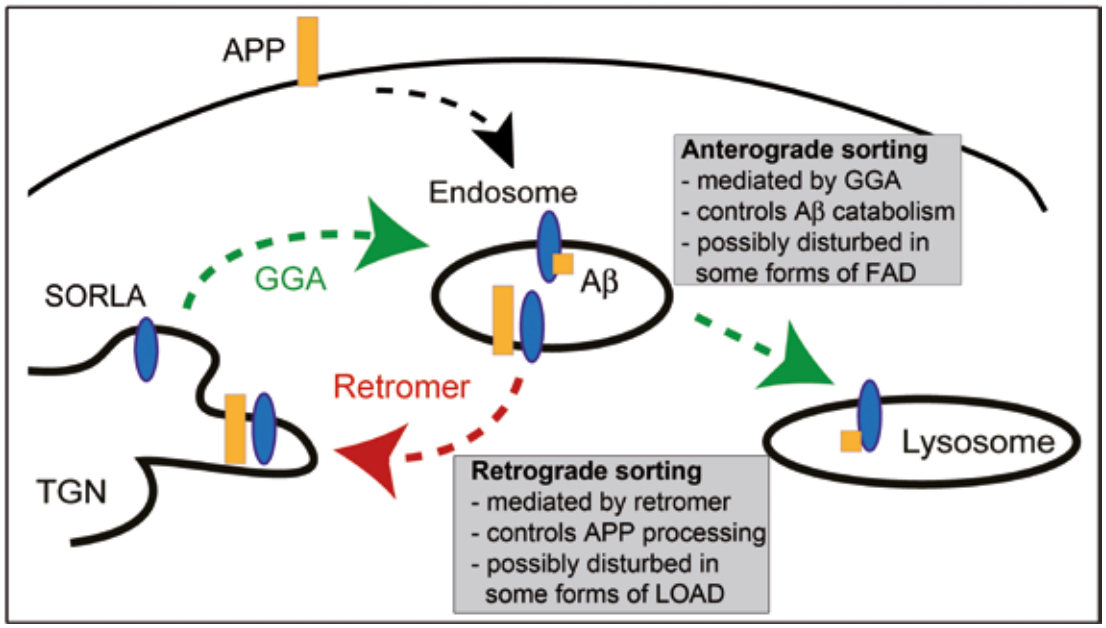

Fig. 3: SORLA trafficking pathways in Alzheimer's disease. *GGAs mediate anterograde sorting of SORLA and promote Aβ catabolism in lysosomes. A mutation in SORL1, disrupting Aβ binding, causes a familial form of AD (FAD). Retromer mediates retrograde sorting of SORLA/APP complexes to the trans-Golgi network (TGN). Disrupting this pathway enhances APP processing in endosomes. Low levels of SORLA or retromer components are risk factors for late-onset AD (LOAD).*

her collaborators generated novel mouse models expressing SORLA variants lacking binding sites for GGA or retromer to query this concept. Disruption of retromer binding resulted in a retrograde sorting defect with accumulation of SORLA in endosomes and depletion from the TGN, and in an overall enhanced APP processing rate. In contrast, disruption of the GGA interaction did not impact APP processing but caused increased brain Aβ levels, a mechanism attributed to a defect in anterograde lysosomal targeting of Aβ. Our new findings substantiated the significance of adaptor-mediated sorting for SORLA activities in vivo, and they uncovered that anterograde and retrograde sorting paths serve discrete receptor functions in amyloidogenic processes.

Outlook

Dysregulation of protein uptake and intracellular transport emerges as a molecular mechanism of major importance in many human disease processes. Among other functions, faulty protein transport impacts cellular signal reception (as for SHH in brain and eye) or results in abnormal protein homeostasis (as for Aβ catabolism in the brain). Our data have shown that endocytic receptors play key roles in these pathological processes. Our future work aims at refining the molecular details of how these receptors affect trafficking and functional expression of target molecules, and how receptor dysfunction contributes to the most devastating defects in brain development and function inflicting humans.

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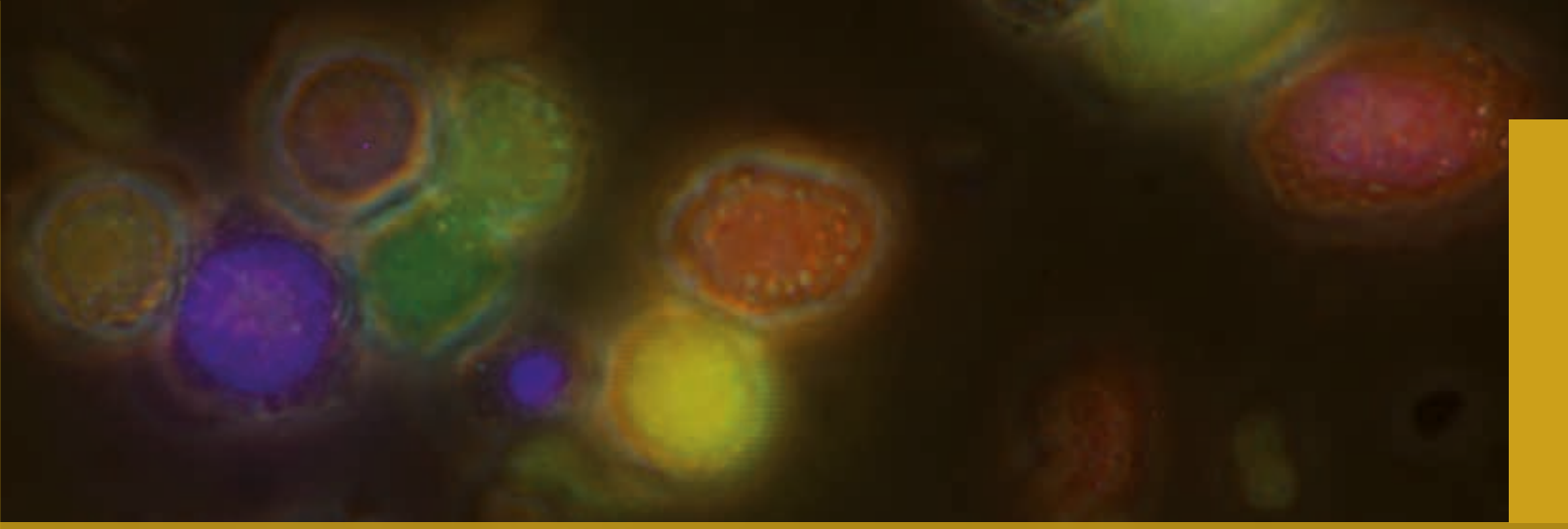
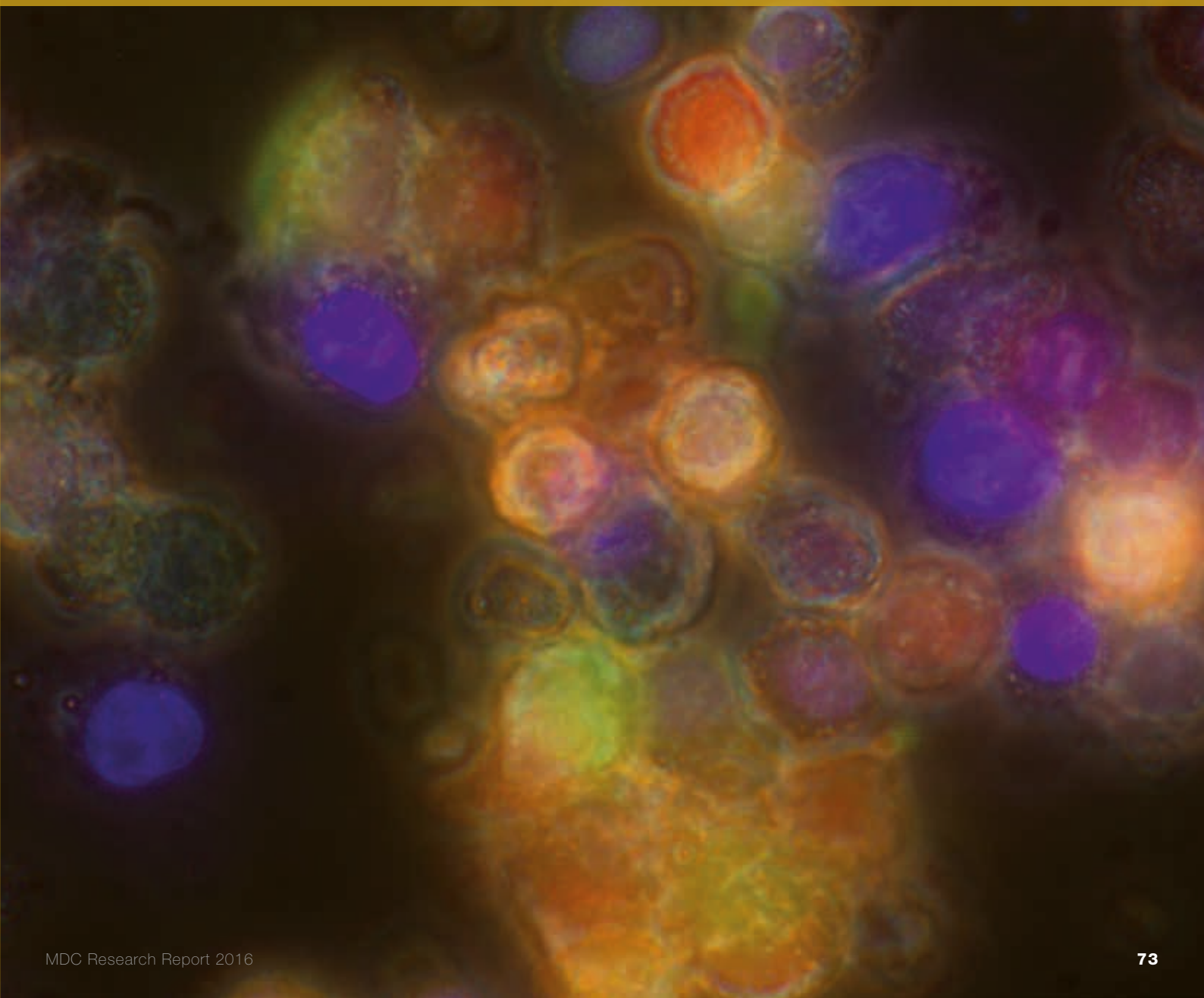


Cancer Research

Coordinator: Claus Scheidereit



Cancer Research

Claus Scheidereit

Cancer is a serious health burden with nearly half a million cases diagnosed in Germany each year, making advancements in diagnosis, treatment and prevention of cancer a major challenge. Our mission is to understand the principles of cancer development and progression to provide the basis for improved diagnosis and treatment. One aim is to discover and characterize genes and cellular components that are important in the development and treatment of cancer and that are promising as novel targets for therapies. Another aim is to understand and therapeutically exploit interactions of the immune system with tumors.

Cancer Research at the MDC covers the topics (i) signaling pathways, cell biology and cancer, (ii) structural and functional genomics and (iii) tumor immunology and combines a spectrum of interdisciplinary basic research laboratories and clinically oriented groups.

In the reporting period, **Michela Di Virgilio** and **Gaetano Gargiulo** were recruited as Helmholtz junior research group leaders from The Rockefeller University in New York and the Netherlands Cancer Institute in Amsterdam, respectively. Both also received European Research Council (ERC) starting grants. Michela di Virgilio has made important contributions in the field of DNA double strand break repair mechanisms. Her research is relevant to the understanding of the pathogeneses of immunodeficiencies and cancer. Gaetano Gargiulo established new procedures to characterize cancer genes in gliomagenesis and contributed to understanding the role of tumor cell identity in lung cancer. He now develops new approaches to combat glioblastoma. In 2014, the MDC- and Inserm/CNRS-associated immunologist **Michael Sieweke** has been elected member of the European Molecular Biology Organization (EMBO). **Martin Lipp** received the German Cancer Award (2017) in recognition of his seminal contributions to our understanding of chemokine receptors in immune reaction, lymphoid organogenesis and metastasis.

Selected scientific highlights

As an exciting new genome editing tool, CRISPR-Cas9 is faster and cheaper than previous gene targeting methods. Nonetheless, most mutagenic events are small deletions, while sequence replacements, required for a precise creation or correction of e.g. cancer related mutations, occur at lower frequency. The groups of **Klaus Rajewsky** and **Ralf Kühn** found a way of favoring the homology-directed introduction of precise DNA modifications by suppression of the competing NHEJ DNA repair pathway which causes sequence deletions (Chu et al., Nature Biotechnol., 2015).

The group of **Walter Birchmeier** found that impairment of the tyrosine phosphatase Shp2 in PyMT mammary glands of mice induces senescence, which blocks tumor formation. Src, Fak and Mapk inhibit senescence by activating expression of Skp2, Aurka, and Delta-like 1, which block p27 and p53. Expression of Shp2 and of target genes predicts human breast cancer outcome. Therapies that rely on senescence induction may block breast cancer (Lan et al., EMBO J, 2015).

The RNA destabilizing protein RC3H1 (also known as ROQUIN) represses autoimmunity. The groups of **Udo Heinemann** and **Markus Landthaler** presented the crystal structure of a Roquin domain, gaining insight into the mode of RNA binding (Schuetz et al., Nature Commun., 2014). In a collaboration with several MDC groups, they demonstrated that RC3H1 modulates the activity of the IKK/NF-κB pathway and contributes post-transcriptional regulation of the DNA damage response (Murakawa et al., Nature Commun., 2015).

Processing of the precursor proteins p100 and p105 is fundamental to activation of NF-κB. The groups of **Claus Scheidereit, Gunnar Dittmar, Jana Wolf** could demonstrate that p100 acts upstream of p105, resulting in concurrent stimulus-induced production of p50 and p52. Both precursors bind to segregase (p97/VCP), which promotes proteasomal processing. The findings are supported by mass spectrometry-based quantitative mathematical models (Yilmaz et al., Cell Reports, 2014).

At the endoplasmic reticulum (ER), aberrantly folded proteins are recognized and exported for degradation by proteasomes. The group of **Thomas Sommer** has shown how substrates are targeted for destruction. A key ER membrane component is Der1 which threads misfolded proteins from substrate receptors in the ER lumen through the membrane to ubiquitylation enzymes in the cytoplasm (Mehnert et al. Nat Cell Biol., 2014).

The mechano-chemical GTPase dynamin oligomerizes around the neck of clathrin-coated vesicles and catalyzes membrane scission in a GTPase-dependent fashion. The group of **Oliver Daumke** has explored structure and function of the dynamin tetramer, providing detailed insights into its assembly mode and regulation. The study may explain how dynamin mutations can cause Centro-Nuclear Myopathy or Charcot-Marie-Tooth Disease (Reubold et al., Nature, 2015).

Dendritic cells (DCs) have pro- and anti-tumorigenic functions. Using a Myc-driven B cell lymphoma model, **Uta Höpken**, **Armin Rehm** and coworkers showed that DCs upregulate immunomodulatory cytokines, growth factors and the CCAAT/enhancer binding protein β (C/EBPβ). They found that C/EBPβ-controlled DC functions are essential for the creation of a lymphoma growth promoting and immunosuppressive niche (Rehm et al., Nature Commun., 2014).

Macrophages can repopulate themselves by self-renewing, but the underlying genetic programs have remained unknown. **Michael Sieweke** and colleagues could demonstrate that the transcription factors MafB and c-Maf repress the enhancers of genes regulating self-renewal. When macrophages need to proliferate, they transiently decrease expression of Maf transcription factors. A parallel pathway also operates to control the self-renewal of embryonic stem cells (Soucie et al., Science, 2016).

Adoptive T cell therapy is a promising alternative in the fight against cancer. T cells recognize malignant cells via abnormal surface epitopes generated by proteasomal degradation of mutant proteins. **Thomas Blankenstein** and colleagues found that certain epitopes require interferon-γ dependent trimming. Tumor cells with impaired interferon-γ response do not produce such epitopes and thereby escape T-cell recognition. Thus, future T cell therapy applications should utilize epitopes that are independent of interferon-γ mediated trimming (Textor et al., J Exp Med, 2016).

The group of **Clemens Schmitt** identified small molecules that promote re-expression of B-cell phenotype markers that are lost in classical Hodgkin's lymphoma (cHL). When applied in a combination treatment, cHL cells became susceptible to CD20 antibody-mediated apoptosis (Du, et al., Blood, 2016).

The group of **Oliver Rocks** identified a novel PKA antagonist, the Rho GTPase activating protein ARHGAP36. It inhibits the PKA catalytic subunit as a pseudosubstrate and targets it for rapid ubiquitin-mediated endolysosomal degradation. PKA inhibition by ARHGAP36 promotes derepression of Hedgehog signaling, thereby providing a simple rationale for the upregulation of ARHGAP36 in medulloblastoma (Eccles et al., Nat Commun, 2016).

Photo: David Ausserhofer/MDC

Walter Birchmeier

Signal Transduction in Development and Cancer

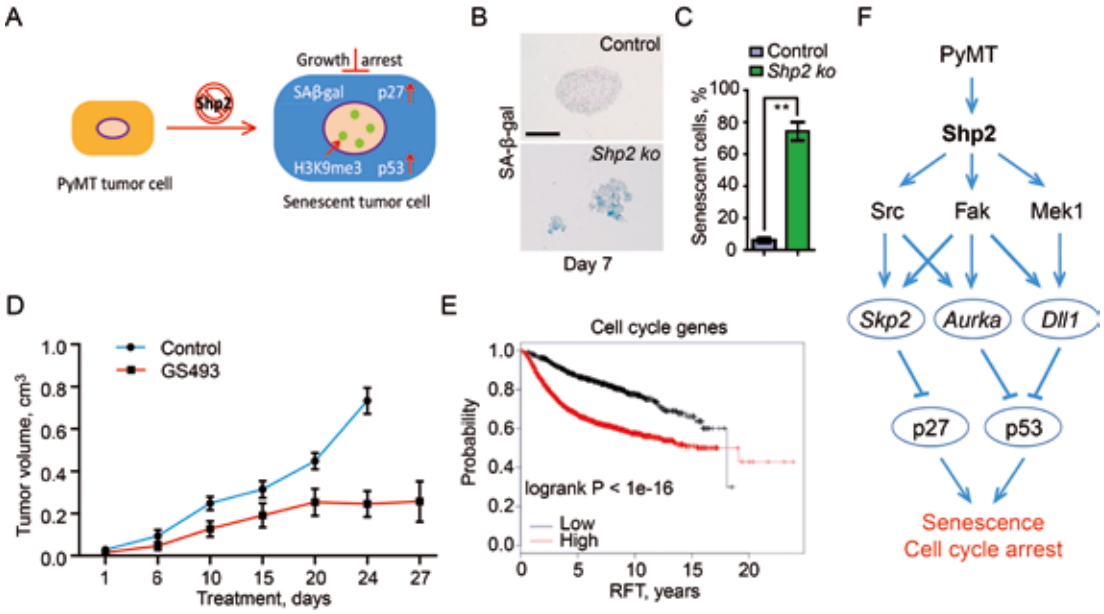
Our research is primarily focused on two signaling systems: Wnt/beta-catenin and receptor tyrosine kinase signaling. Components of both signaling systems are frequently mutated or deregulated in a variety of developmental disorders and cancers in humans. In the previous years, the laboratory has studied adhesion and signaling of E-cadherin/Wnt/beta-catenin by biochemical means. We have shown that beta-catenin binds to the transcription factors Lef1/Tcf, and that this translocates beta-catenin to the nucleus. Beta-catenin is recruited to its degradation complex containing Axin2/Conductin and APC. We have also investigated the role of scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, the Met tyrosine kinase, in the morphogenesis of epithelial cells. Signals of Met are transmitted by the cytoplasmic multi-adapter Gab1 and the tyrosine phosphatase Shp2. Conventional ablations of beta-catenin and Gab1 in mice result in gastrulation defects and embryonic organ failures, respectively. In recent years, we have examined the role of Wnt/beta-catenin and Met/Gab1/Shp2 by conditional mutagenesis in mice. Beta-catenin regulates precursor and stem cells in the nervous system, the hair and the heart; Gab1 and Shp2 control precursor and stem cells in skin, limbs, liver and kidney. Met regulates wound healing in the skin. Activation of beta-catenin and HGF/Met in adult mouse tissues induces tumors and cancer stem cells.

In the present report period, we have published the following investigations:

Shp2/Mapk signaling controls goblet/paneth cell fate decisions in the intestine

J. Heuberger, F. Kosel, J. Qi, K. Grossmann, K. Rajewsky, and W. Birchmeier

In the development of the mammalian intestine, Notch and Wnt/beta-catenin signals control stem cell maintenance and their differentiation into absorptive and secretory cells. Mechanisms that regulate differentiation of progenitors into the three secretory lineages, goblet, paneth or enteroendocrine cells, are not fully understood. Using conditional mutagenesis in mice, we observed that Shp2-mediated Mapk signaling determines the choice between paneth and goblet cell fates, and also affects Lgr5+ stem cells. Ablation of the tyrosine phosphatase Shp2 in the intestinal epithelium reduced Mapk signaling and led to a reduction of goblet cells while promoting paneth cell development. Conversely, conditional Mek1 activation rescued the Shp2 phenotype, promoted goblet cell and inhibited paneth cell generation. The Shp2 mutation also expanded Lgr5+ stem cell niches, which could be restricted by activated Mek1 signaling. Changes of Lgr5+ stem cell quantities were accompanied by alterations of paneth cells, indicating that Shp2/Mapk signaling might affect stem cell niches directly or via paneth cells. Remarkably, inhibition of Mapk signaling in intestinal organoids and cultured cells changed the relative abundance of Tcf4 isoforms and by this, promoted Wnt/beta-catenin activity. The data thus show that Shp2-mediated Mapk signaling controls the choice between goblet and paneth cell fates by regulating Wnt/beta-catenin activity (published in Proc. Natl Acad. Sci. USA 111, 3472-3477, 2014).



A. *PyMT mammary gland tumors undergo senescence on Shp2 ablation.* **B**,**C**. *SA-beta-gal staining of spheres by control and Shp2 knock-out cells and quantification of senescence.* **D**. *Growth of tumors in control and GS493-treated mice.* **E**. *Kaplan-Meier survival analysis of human breast cancer patients based on cell cycle genes regulated by Shp2.* **F**. *Scheme of Shp2-dependent signaling systems and target genes involved in senescence and cell cycle programs (EMBO J. 2015).*

Shp2 signaling suppresses senescence in PyMT-induced mammary gland cancer in mice

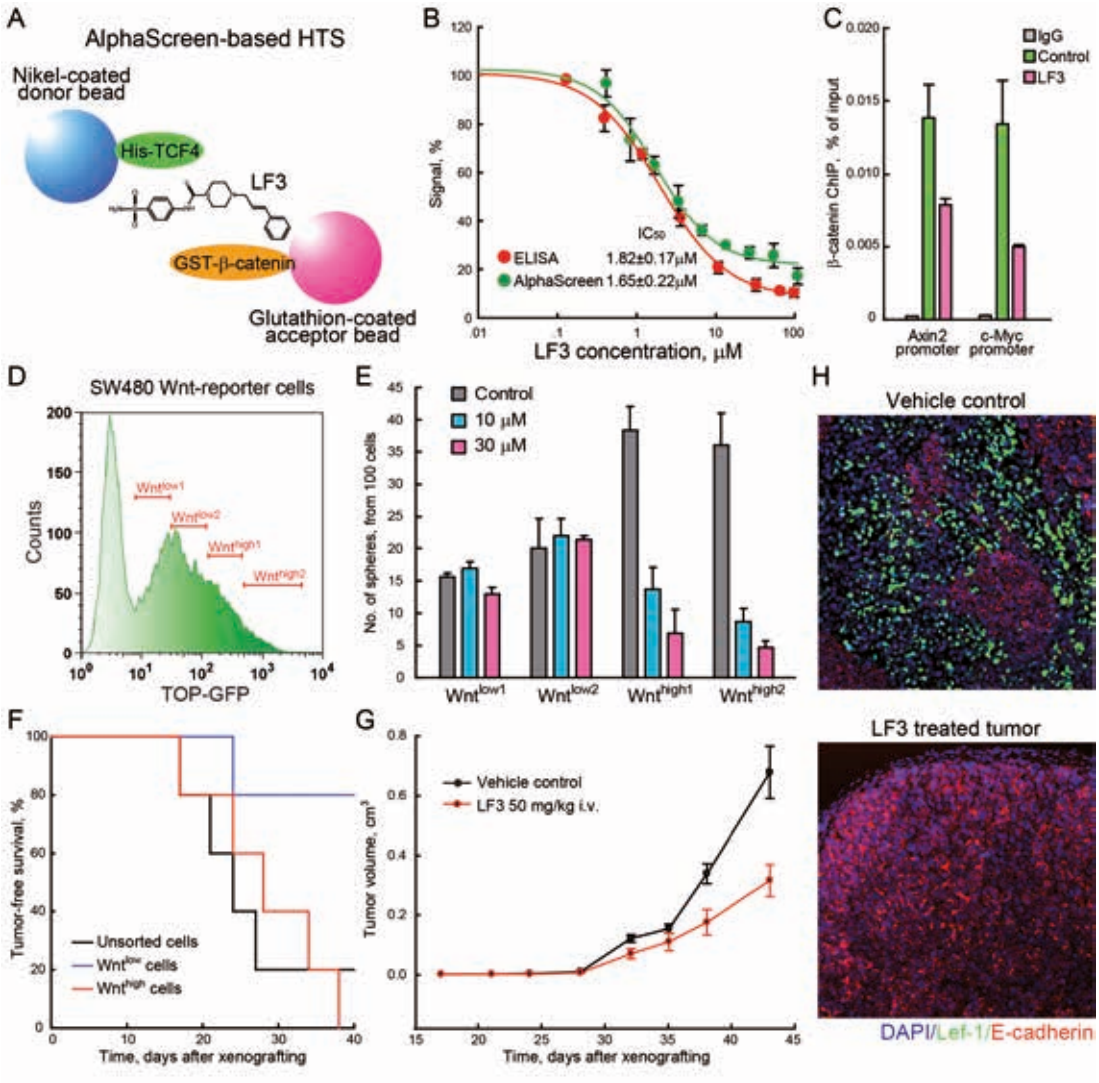
L. Lan, J.D. Holland, J. Qi, S. Grosskopf, J. Rademann, R. Vogel, B. Györffy, A. Wulf-Goldenberg, and W. Birchmeier

In this study, we have used techniques from cell biology, biochemistry, and genetics to investigate the role of the tyrosine phosphatase Shp2 in tumor cells of MMTV-PyMT mouse mammary glands. Genetic ablation or pharmacological inhibition of Shp2 induces senescence, as determined by the activation of senescence-associated beta-gal (SA-beta-gal), cyclin-dependent kinase inhibitor 1B (p27), p53, and histone 3 trimethylated lysine 9 (H3K9me3). Senescence induction leads to the inhibition of self-renewal of tumor cells and blockage of tumor formation and growth. A signaling cascade was identified that acts downstream of Shp2 to counter senescence: Src, focal adhesion kinase, and Map kinase inhibit senescence by activating the expression of S-phase kinase-associated protein 2 (Skp2), Aurora kinase A (Aurka), and the Notch ligand Delta-like 1 (Dll1), which block p27 and p53. Remarkably, the expression of Shp2 and of selected target genes predicts human breast cancer outcome. We conclude that therapies, which rely on senescence induction by inhibiting Shp2 or controlling its target gene products, may be useful in blocking breast cancer (published in EMBO J. 34, 1493-1508, 2015).

Gab1 and Mapk signaling are essential in the hair cycle and hair follicle stem cell quiescence

Ö. Akilli Öztürk, H. Pakula, J. Chmielowiec, J. Qi, S. Stein, L. Lan, Y. Sasaki, K. Rajewsky, and W. Birchmeier

Gab1 is a scaffold protein that acts downstream of receptor tyrosine kinases. Here, we produced conditional Gab1 mutant mice (by K14- and Krox20-cre) and show that Gab1 mediates crucial signals in the control of both the hair cycle and the self-renewal of hair follicle stem cells. Remarkably, mutant hair follicles do not enter catagen, the destructive phase of the hair cycle. Instead, hair follicle stem cells lose quiescence and become exhausted, and thus no stem cell niches are established in the bulges. Moreover, conditional sustained activation of Mapk signaling by expression of a gain-of-function Mek1(DD) allele (by Krox20-cre) rescues hair cycle deficits and restores quiescence of the stem cells. Our data thus demonstrate an essential role of Gab1 downstream of receptor tyrosine kinases and upstream of Shp2 and Mapk in the regulation of the hair cycle and the self-renewal of hair follicle stem cells (published in Cell Reports 13, 561-572, 2015).



A,B. *The beta-catenin/TCF4 interaction inhibitor LF3 was identified by AlphaScreen-based HTS.* **C.** *LF3 reduced the association of beta-catenin to the Axin2 and c-Myc promoters.* **D.** *SW480 cells contain Wnt^{low} and Wnt^{high} populations.* **E.** *LF3 reduced sphere formation only from Wnt^{high} cells.* **F.** *Tumor-free survival of mice injected with different cell populations.* **G,H.** *LF3 reduced tumor growth from Wnt^{high} cells and induced differentiation shown by IF (Cancer Res. 2015).*

A small-molecule antagonist of the beta-catenin/TCF4 interaction blocks the self-renewal of cancer stem cells and suppresses tumorigenesis.

L. Fang, Q. Zhu, M. Neuenschwander, E. Specker, A. Wulf-Goldenberg, W. I. Weis, J. P. von Kries, and W. Birchmeier

Wnt/beta-catenin signaling is essential for embryogenesis and tissue homeostasis, which has been highly conserved through evolution. Deregulation of Wnt/beta-catenin signals can initiate and promote human cancers, specifically of colon and head and neck. As such, Wnt/beta-catenin signaling represents an attractive target for cancer therapy. The interaction between beta-catenin and the transcription factor TCF4 is necessary in the transduction of the signal. We performed High Throughput Screening (HTS) using AlphaScreen and ELISA techniques to identify small molecules that disrupt this interaction. Compound LF3, a 4-thioureido-benzenesulfonamide derivative, was found as a robust inhibitor of beta-catenin/TCF4 interaction and thus a good starting point for drug discovery, as suggested by Lipinski's "rule of five". Medicinal chemistry identified an essential core structure and residues that could not be modified in LF3. Compound LF3 inhibited Wnt/beta-catenin signals in cells with exogenous reporters and in colon cancer cells with endogenously high Wnt activity. Compound LF3 inhibited features of cancer cells related to Wnt signaling, including high cell motility, cell cycle progression, and the overexpression of Wnt target genes. LF3 does not cause cell death or

interfere with cadherin-mediated cell-cell adhesion. Remarkably, the self-renewal capacity of cancer stem cells was blocked by LF3 in concentration-dependent manners, as examined by sphere formation of colon and head and neck cancer stem cells in non-attached conditions. LF3 reduced tumor growth and induced differentiation in colon tumors in mouse xenografts. LF3 is thus a specific inhibitor of canonical Wnt signaling with the potential for further development for preclinical and clinical studies in cancer treatment (published in Cancer Res. 76, 891-901, 2016).

Cancer stem cells regulate cancer-associated fibroblasts via activation of Hedgehog signaling in mammary gland tumors

Giovanni Valenti, Hazel M. Quinn, Guus J.J.E. Heynen, Linxiang Lan, Jane D. Holland, Regina Vogel, Annika Wulf-Goldenberg, and Walter Birchmeier

Many tumors display intracellular heterogeneity, with subsets of cancer stem cells (CSC) that sustain tumor growth, recurrence, and therapy resistance. Cancer associated fibroblasts (CAF) have been shown to support and regulate CSC function. Here we investigated the interactions between CSCs and CAFs in mammary gland tumors driven by combined activation of Wnt/β-catenin and Hgf/Met signaling in mouse mammary epithelial cells. In this setting, CSCs secreted the hedgehog ligand SHH, which regulated CAFs via paracrine activation of Hedgehog signaling. CAFs subsequently secreted factors that promoted expansion and self-renewal of CSCs. In vivo treatment of tumors with the Hedgehog inhibitor vismodegib reduced CAF and CSC expansion, resulting in an overall delay of tumor formation. Our results identify a novel intracellular signaling module that synergistically regulates CAFs and CSCs. Targeting CAFs with Hedgehog inhibitors may offer a novel therapeutic strategy against breast cancer (published in Cancer Research 2017 Feb 15).

New projects of the laboratory: in cooperation with the Leibniz Institute of Molecular Pharmacology in Berlin-Buch, we develop further small molecule inhibitors of Shp2 (Maria Pascual and Marc Nazare). In cooperation with the Department of Urology of the Charité Berlin, we characterise human kidney cancer stem cells and identify new treatment strategies (Annika Fendler, together with Klaus Jung and Jonas Busch). We also identify histone modifiers in their role in Wnt-dependent cancer stem cells in head and neck and colorectal cancers (in cooperation with Francis Stewart, Sylvie Robine and Daniel Louvard).

Selected Publications

Heuberger J, Kosel F; Qi J, Grossmann K, Rajewsky K, and Birchmeier W. 2014. Shp2/MAPK signaling controls goblet/paneth cell fate decisions in the intestine. Proc. Natl. Acad. Sci. USA, 111: 3472-3477.

Akilli Öztürk Ö, Pakula H, Chmielowiec J, Qi J, Stein S, Lan L, Sasaki,Y, Rajewsky K, and Birchmeier W. 2015. Gab1 and Mapk signaling are essential in the hair cycle and hair follicle stem cell quiescence. Cell Reports 13: 561-572.

Lan L, Holland JD, Qi J, Grosskopf S, Rademann J, Vogel R, Györffy B, Wulf-Goldenberg A, and Birchmeier W. 2015. Shp2 signaling suppresses senescence in PyMT-induced mammary gland cancer in mice. EMBO J. 34:1493-1508.

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Thomas Blankenstein

Molecular Immunology and Gene Therapy

We focus on three areas in cancer immunology: 1. Developing novel cancer models that better reflect human disease. 2. Employing the mechanisms of rejection of established tumors by adoptively transferred T cells with specific emphasis of the tumor stroma as target. 3. Employing T cell therapy of cancer in the clinic. Therefore, we use transgenic mice that allow the isolation of human TCRs from mice to redirect T cells towards superior antitumor activity.

IFNγ targeting of tumor stroma is required for rejection of large established tumors by chimeric antigen receptor-redirected T cells

T cells can be redirected with new antigen specificity and used for adoptive T cell therapy (ATT) by introducing either a T cell receptor (TCR), or chimeric antigen receptor (CARs). The CAR consists of an antigen (Ag)-binding single chain variable fragment (scFv) antibody domain and a signaling domain, most often the CD3ζ endodomain Although adoptive T cell therapy using chimeric antigen receptor (CAR) modified T cells (CAR-Ts) showed efficacy in treating lymphomas, complete eradication of large established solid tumors has not yet been reported. Destruction of stroma cells cross-presenting the tumor antigen of interest is essential for rejection of large established tumors by T cells in a MHC class I-restricted fashion. However, CAR-Ts recognize the antigen in an MHC-independent manner exclusively on the cancer, but not stroma, cells. Here, we report that HER-2 specific CAR-Ts eradicated large established HER-2$^+$ tumors when provided with a CD28 costimulatory signal, which could not be substituted by higher CAR affinity. Successful tumor rejection was associated with sustained influx and proliferation of transferred T cells. The absence of tumor recurrence raised the question of whether stroma targeting was involved in tumor rejection. Tumor rejection by CAR-Ts was independent of NK cell contribution and required the expression of IFNγ receptor on tumor stroma. Taken together, our results indicate that CAR-Ts can eradicate large established solid tumors by antigen-independent direct stroma destruction, whereby a cancer-driving antigen, such as HER-2, is targeted.

Mis-initiation of intrathymic MART-1 transcription and biased TCR usage explain the high frequency of MART-1 specific T cells

The immune response against melanoma-associated tumor antigens has been intensely studied in part owing to the immunogenicity of melanomas, the molecular identification of numerous melanoma-associated tumor antigens, and the ease to access tumor infiltrating lymphocytes. One such antigen is the Melanoma Antigen Recognized by T-cells 1 (MART1). Intriguingly, CD8 T cells specific for the MART-1 epitope 26-35 in the context of HLA-A*0201 are about 100 times more abundant compared to T cells specific for other tumor-associated antigens. Moreover, these antigen-specific CD8 T cells show a highly biased usage of the Va-region gene TRAV12-2. Here, we provide independent support for this notion, by showing that the combinatorial pairing of different TCRα and TCRβ chains derived from HLA-A2/MART-1_{26-35} specific T cell clones is unusually permissive in conferring this particular specificity, provided the CDR1α TRAV12-2 region is used.

Whether this TCR bias by itself accounts for the unusual abundance of these T cells has remained conjectural. Here, we provide an alternative explanation: mis-iniated transcription of the MART-1 gene resulting in truncated mRNA isoforms leads to lack of promiscuous transcription of the MART-1_{26-35} epitope in human medullary thymic epithelial cells and consequently evasion of central self-tolerance towards this epitope. The co-incidence of these two features – biased TCR usage and leaky central tolerance – might act in an independent and additive manner resulting in the high frequency of MART-1_{26-35} specific CD8 T cells.

Optimal-affinity human T cell receptors against the cancer-testis antigens MAGE-A1 and NY-ESO from antigen-negative hosts

TCR engineering can redirect patients' T cells to specifically target tumor antigens, for which TCRs are available. TCRs should be of sufficient functional affinity (for brevity "affinity" in the following) for tumor rejection but are rarely found against tumor-associated (self-) antigens (TAAs), because tolerance mechanisms select against high-affinity TCRs. Technologies to obtain high-affinity TCRs often have pitfalls. In vitro mutagenesis and selection of affinity-matured TCRs bears the risk of losing specificity or, if TCR-affinity is too high, T cell efficacy in vivo, raising the question what the optimal TCR-affinity is. In vitro priming of HLA-mismatched donor T cells can yield high-affinity TCRs against TAAs, but these must be clearly distinguished from general allo-reactive TCRs. HLA-transgenic mice might allow isolation of suitable TCRs due to missing tolerance for many human TAAs, yet immunogenicity of mouse TCR variable regions can impede T cell therapy. Surprisingly, TCRs isolated from HLA-transgenic mice had low functional affinity and usually required in vitro mutagenesis to achieve efficient cancer cell recognition, raising the question whether the repertoire of antigen-negative mice actually yields better TCRs. To overcome these limitations, we generated human TCR-αβ gene loci and chimeric HLA-A2 transgenic (ABabDII) mice with a diverse T cell repertoire to enable isolation of human TCRs against human TAAs from antigen-negative hosts.

Antigen choice is also critical in TCR gene therapy. Cancer testis (CT) antigens, like MAGE-A1, are expressed in a variety of tumors, but, with the exception of testis, were not detected in healthy adult tissues. Tolerance to CT antigens remains a matter of debate. On one hand, T cells recognizing CT antigens have been found among tumor-infiltrating lymphocytes and in the blood of tumor patients and healthy individuals. On the other hand, low levels of mRNA encoding various CT antigens, including MAGE-A1, were detected in human medullary thymic epithelial cells that play a role in deletional tolerance. Here, we describe the generation and characterization of MAGE-A1-specific TCRs from antigen-negative mice with a diverse human TCR repertoire restricted to HLA-A2. By comparison, human-derived TCRs are likely skewed towards low-affinity, compatible with tolerance. We also identified optimal-affinity TCRs specific for the CT antigen NY-ESO. The humanized mice provide a powerful tool to generate optimal-affinity TCRs, which are difficult, if at all, accessible in humans.

Targeting cancer-specific mutations by T cell receptor gene therapy

The ease of sequencing the cancer genome, identifying all somatic mutations and grafting mutation-specific T cell receptor (TCR) genes into T cells for adoptive transfer allow, for the first time, a truly tumor-specific and effective therapy. Mutation-specific TCR gene therapy might achieve optimal efficacy with least possible toxicity. Recent clinical data confirm the long-standing evidence from experimental cancer models that antigens encoded by the tumor-specific somatic mutations are potentially the best targets for adoptive T cell therapy. Open questions are, how many somatic mutations create suitable epitopes, whether only individual-specific or also recurrent somatic mutations qualify as suitable epitopes and how neoantigen-specific TCRs are most efficiently obtained. Tumor heterogeneity needs to be considered; therefore, it will be important to identify immunogenic driver mutations that occurred early, are essential for cancer cell survival and present in all cancer cells. First TCRs against recurrent somatic mutations, e.g. RAC1 frequently mutated in melanoma, have been isolated and are currently been analyzed.

Targeting human melanoma neoantigens by T cell receptor gene therapy

T cell responses in successful cancer immunotherapy seem directed towards neoantigens created by somatic mutations, but direct evidence that neoantigen-specific T cells caused regression of established cancer is lacking. We generated T cells expressing a mutation-specific transgenic T cell receptor (TCR) to target different immunogenic mutations in cyclin-dependent kinase 4 (CDK4) that naturally occur in human melanoma. Two CDK4 mutations (R24C, R24L) similarly stimulated T cell responses in vitro and were analyzed as therapeutic targets for TCR gene therapy. In a syngeneic HLA-A2-transgenic model of large established tumors, we found that both mutations differed dramatically as targets for TCR-modified T cells in vivo. While T cells expanded efficiently and produced IFN-γ in response to R24L, R24C failed to induce an effective anti-tumor response. Such differences in neoantigen quality might explain why cancer immunotherapy induces tumor regression in some individuals, while others do not respond, despite similar mutational load. We confirmed the validity of the in vivo model by showing that the MART-1-specific TCR DMF5 induces rejection of tumors expressing analog but not native MART-1 epitopes. The described model allows identification of those (neo)antigens in human cancer that serve as suitable T cell targets and may help to predict clinical efficacy.

Preventing tumor escape by targeting a post-proteasomal trimming independent epitope

Adoptive T cell therapy (ATT) can achieve regression of large tumors in mice and humans, however tumors frequently recur. High target peptide-MHC-I (pMHC) affinity and T cell receptor (TCR)-pMHC affinity are thought critical to prevent relapse. Here we show that targeting two epitopes of the same antigen in the same cancer cells by monospecific T cells, which have similar pMHC and pMHC-TCR affinity, results in eradication of large established tumors when targeting the sub-dominant but not the dominant epitope. Only the “escape” but not the “rejection epitope” required post-proteasomal trimming, which was regulated by IFNγ, allowing IFNγ-unresponsive cancer variants to evade. The data describe a novel immune escape mechanism and better define suitable target epitopes for ATT.

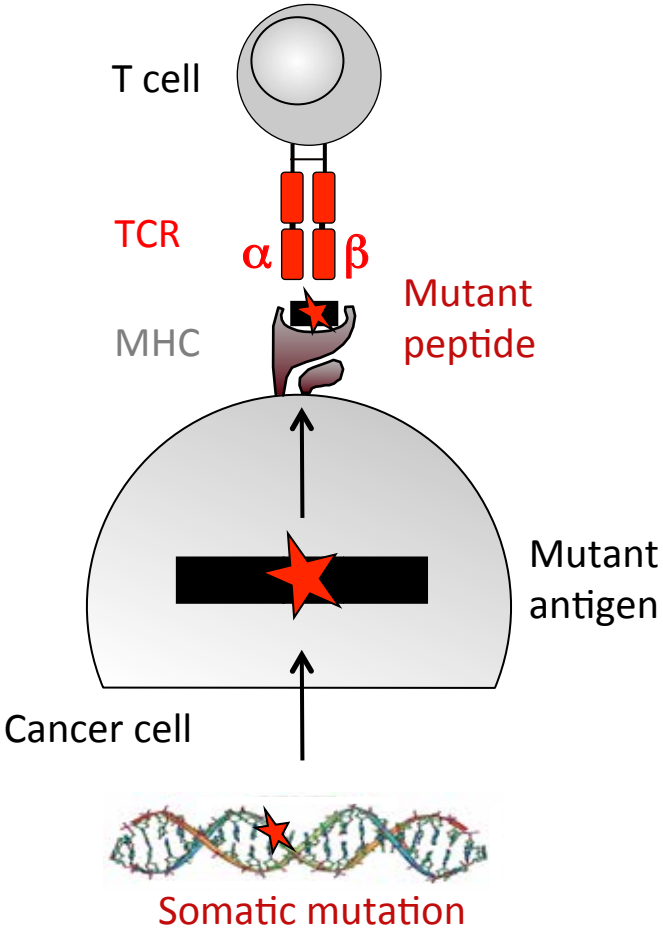

Figure 1: *Mutation-specific T cells. Somatic mutations in cancer may create a neoepitope that can be recognized by T cells.*

Selected Publications

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Oliver Daumke

Structure, function and mechanism of membrane-associated GTPases

GTPases of the dynamin and septin superfamilies are molecular machines that assemble at the surface of cellular membranes. Some of these proteins act as dynamic scaffolds that remodel the underlying membrane in a GTPase-dependent fashion; others orchestrate the recruitment of interaction partner in a temporally and spatially defined manner. We are interested to understand principles of assembly, function and regulation of these GTPases. To this end, structural studies are combined with biochemical and cell-based approaches.

Structure and antiviral function of MxA GTPases

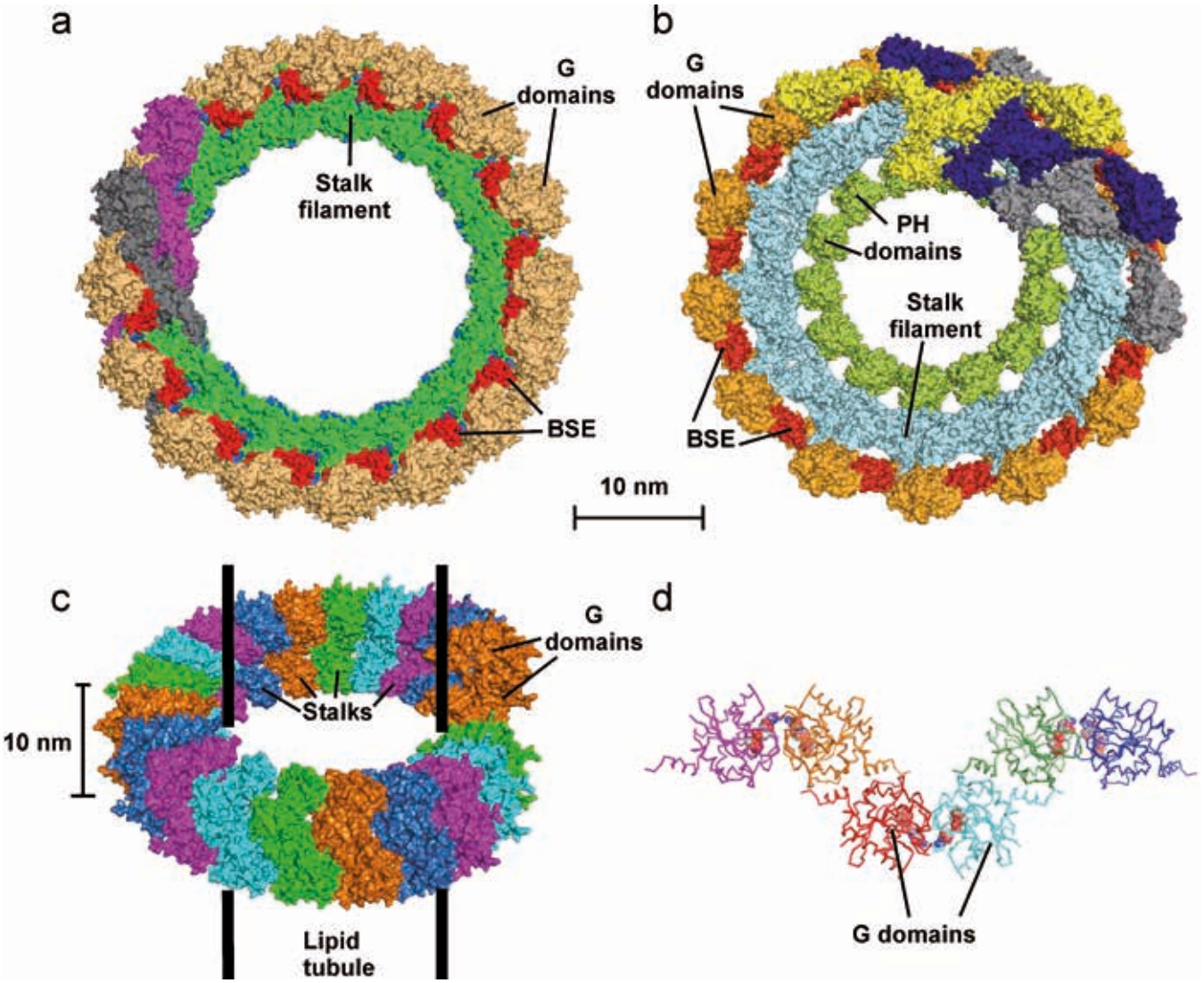
The interferon-induced Myxovirus-resistance (Mx) GTPases are central players of innate immunity. They mediate resistance against a wide range of pathogens, including influenza viruses and many other negative-strand viruses. As typical members of the dynamin superfamily, they can oligomerize in ring-like structures around tubular membranes.

Using X-ray crystallography, we found that the virus binding domain, the stalk, of human MxA has a four-helix bundle fold. In the crystals, the stalks assembled via three interfaces in a zigzag fashion. We showed that all three interfaces are required for the assembly of MxA at membrane surfaces and for its antiviral activity. Based on these results, we suggested a model how MxA assembles into ring-like oligomers and how these rings mediate the antiviral activity (Fig. 1a).

We also determined the structure of the full-length MxA molecule. We identified the bundle signalling element (BSE), a three-helix bundle, which bridges GTPase (G) domain and stalk. Based on functional experiments, we suggested a molecular pathway how nucleotide-driven rearrangements in the MxA oligomer are transmitted from the GTPase domain to the stalk. More recently, we determined crystal structures of putative targets of MxA, the nucleoproteins of Toscana and Hantavirus, and studied their RNA binding mode and assembly.

Structural insights into dynamin-mediated endocytosis

Dynamin is the founding member of the dynamin superfamily. The multi-domain protein oligomerizes around the neck of clathrin-coated vesicles and induces membrane scission in a GTPase-dependent fashion. My group is interested to understand the molecular mechanisms how dynamin mediates membrane scission. To this end, we determined X-ray structures of nearly full-length dynamin and, more recently, the structure of the dynamin tetramer. The protein comprises a GTPase domain, a BSE, a stalk and a lipid-binding pleckstrin homology (PH) domain. The interaction site between the stalk and the PH domain is often mutated in patients suffering from centronuclear myopathy, a congenital disease leading to progressive muscle weakness. Supported by biochemical and cell-based functional experiments, we proposed a molecular model for helical dynamin oligomers (Fig. 1b). Furthermore, we showed how the domain



Oligomerization of dynamin and septin-like GTPases.
a) Models of ring-like oligomers of (a) the MxA GTPase or (b) the dynamin GTPase. c) Oligomeric ring model of the EHD2 ATPase. Oligomerization is solely mediated via the GTPase domains, but not the helical domains. d) Linear GTP-dependent oligomer of GIMAP2 which assembles via two interfaces in the GTPase domain into a linear scaffold.

interplay contributes to the assembly of an auto-inhibited dynamin tetramer which is recruited to membrane surfaces of defined curvature. Finally, we introduced a dynamic model how GTP binding and hydrolysis induces scission of the vesicle neck.

Dynamin-related GTPases involved in mitochondrial remodelling

A new focus of our group has been the structural and functional characterization of mitochondrial membrane remodelling events. Mitochondria are dynamic membranous structures which continuously undergo scission and fusion. These processes are crucial for the maintenance of respiratory and metabolic activity and for the preservation of mitochondrial DNA. Malfunctioning of this process has been linked to numerous pathologies, such as neurodegenerative diseases, cardiomyopathy and cancer. Three dynamin-like proteins, dynamin-like-protein 1 (DNM1L), OPA1 (optical atrophy 1) and mitofusin are implicated in mitochondrial dynamics. Unlike in endocytosis, mitchondrial membrane fusion and division involves the double-membrane system of mitochondria. Consequently, mitochondrial dynamin-related GTPase act on distinct mitochondrial membranes, e.g. mitofusin mediates fusion of the outer mitochondrial membrane whereas OPA1 catalyzes fusion of the inner mitochondrial membrane.

We determined the crystal structure of the mitochondrial membrane scission protein DNM1L. Based on a range of biochemical and cell-based experiments, we deduced an alternative assembly mode of DNM1L at the outer mitochondrial membrane for the constriction of mitochondria. These results may have implications for mitochondria-based diseases in which DNM1L function is compromised.

Oligomerization and cellular function of Eps15 homology domain-containing ATPases (EHDs)

EHDs are ubiquitously expressed dynamin-related ATPases which are built of an amino-terminal GTPase domain, followed by a helical domain and a carboxy-terminal regulatory Eps15 homology domain. EHDs are found at the plasma membrane and at vesicular and tubular endocytic membrane structures. They are implicated in several membrane trafficking pathways, most notably during the recycling of receptor proteins to the plasma membrane.

We previously showed that EHD2 oligomerizes in ring-like structures around tubulated liposomes. By solving the crystal structure of an EHD2 dimer, we found that stable dimerization of EHD2 is mediated via the GTPase domains. We identified the lipid-binding site at the tip of the helical domains and showed that also the N-terminus of EHD2 can insert into the membrane, thereby regulating membrane recruitment and/or oligomerization of EHD2. Based on functional experiments, we suggested a model for the EHD2 oligomeric rings (Fig. 1c). These rings have a remarkably different architecture from those formed by MxA and dynamin. Eventually, we identified EHD2 as a novel integral component of caveolae opening up a wide range of cell-based approaches to study the physiological function of this protein.

GIMAPs – cellular scaffolds controlling lymphocyte survival

GTPase of the Immunity Associated Proteins (GIMAPs) comprise a septin-related GTPase family in vertebrates. The seven human GIMAP members are predominantly expressed in cells of the immune system. Some GIMAPs were proposed to regulate apoptosis by controlling the activity of Bcl2 proteins.

We determined four crystal structures of a representative member, GIMAP2, in different nucleotide-loading states. In combination with biochemical experiments, this work elucidated the molecular basis of GTP-dependent oligomerization via the GTPase domains (Fig. 1d). We also showed that GIMAP2 in Jurkat T cells localizes to

the surface of lipid droplets which are cellular storage and signalling compartments. Interestingly, GIMAP2 bound GTP with high affinity but did not hydrolyze it.

Using co-localization experiments, we recently identified GIMAP7 at the surface of lipid droplets. In contrast to GIMAP2, GIMAP7 efficiently hydrolyzed GTP. It also stimulated the GTPase activity of GIMAP2. The structure of a GTP-bound GIMAP7 dimer elucidated the mechanism of dimerization-induced GTPase activation. These data suggested that GIMAP7 acts as GTPase activating protein for GIMAP2. Furthermore, we found GIMAPs differentially down-regulated in a variety of different anaplastic large cell lymphoma lines compared to their T-cell progenitors, implicating a function of certain GIMAPs as tumor suppressors.

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Reubold,T.F., Faelber,K.*, Plattner,N., Posor,Y., Ketel,K., Curth,U., Schlegel,J., Anand,R., Manstein,D.J., Noe,F., Haucke,V., Daumke,O.*, and Eschenburg,S.* (2015). Crystal structure of the dynamin tetramer. Nature 525, 404-408.

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Photo: David Ausserhofer/MDC

Michela Di Virgilio

DNA Repair and Maintenance of Genome Stability

DNA double-strand breaks (DSBs) occur as a by-product of DNA replication and in response to ionizing radiation but are also generated as obligate intermediates of class switch recombination (CSR) in mature B lymphocytes. CSR is a deletional recombination reaction that changes the constant region of the antibody molecule, altering its effector function. This process has the function of generating highly efficient antibodies against pathogens, and it occurs through the regulated formation and repair of DSBs. Mammalian cells employ two major options to repair DSBs. In one case, DNA ends are protected from extensive processing and directly re-ligated. This pathway is known as non-homologous end-joining (NHEJ), and it is the pathway of choice in G1 phase of the cell cycle. Alternatively, the cell can profit from the availability of a homologous DNA molecule generated by DNA replication, and repair the broken DNA by copying the information in the duplicate DNA template. This process is called homologous recombination (HR), and requires extensive resection of DNA ends to occur. NHEJ and HR cannot always be used interchangeably. Repair by end-joining is essential for CSR in mature B lymphocytes, whereas homologous recombination is crucial to faithfully repair DSBs generated during DNA replication, and therefore it ensures preservation of genome integrity in this context.

DSBs become committed to a specific repair pathway shortly after their formation as a consequence of DNA end processing. A major regulatory point of DSB repair pathway choice is the initiation of 5'-3' resection of DNA ends, which interferes with direct ligation by NHEJ, and predisposes cells to homology-dependent repair (Fig. 1). The key regulator of DNA end processing is the DNA repair factor 53BP1, whose association with DSBs in G1 promotes NHEJ by preventing DNA end resection (Fig. 1). B lymphocytes lacking a functional 53BP1 protein are unable to protect DSBs from processing, and as a consequence, they cannot engage into NHEJ-mediated repair of programmed CSR breaks. This results in impaired class switching and immunodeficiency. Therefore, DNA end protection by 53BP1 is beneficial in switching B lymphocytes. On the contrary, 53BP1 ability to protect DNA ends against resection represents a barrier to homologous repair during DNA replication. Under physiological conditions, this barrier is removed by the HR protein BRCA1, which displaces 53BP1 and allows for resection and appropriate repair of DSBs by HR (Fig. 1). BRCA1 gene is frequently mutated in hereditary breast and ovarian cancers, and the inability to inactivate DNA end protection by 53BP1 in the absence of a functional BRCA1 prevents DNA end processing and physiological repair of DNA replication-associated breaks. As a consequence, DSBs are channeled into alternative NHEJ reactions

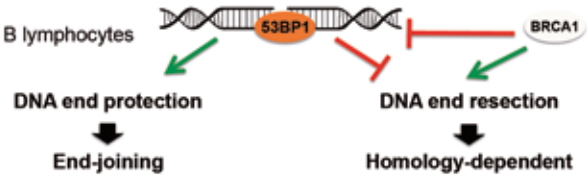

Figure 1. *53BP1 role in the regulation of DNA end processing.*

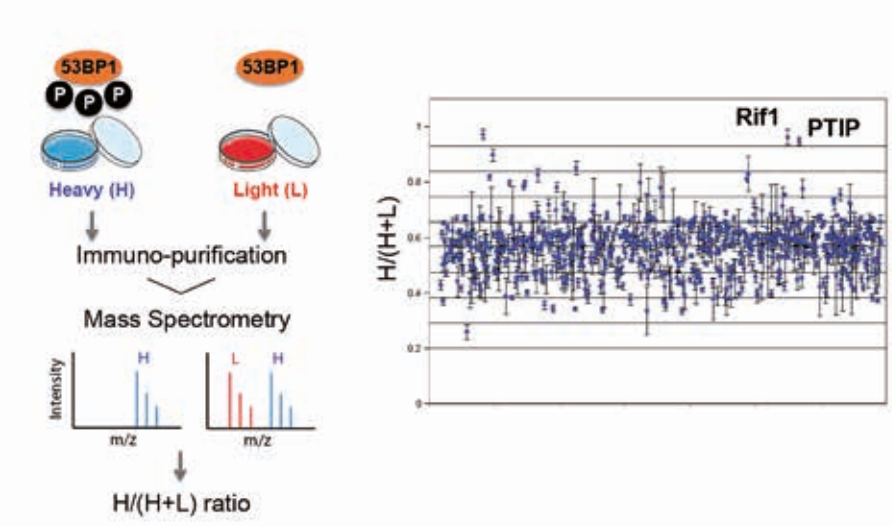

Figure 2. *Identification of phospho-dependent 53BP1 interactors: H/(H+L) ratio distribution of proteins identified by wild-type (heavy, H) versus phosphomutant (Light, L) 53BP1 SILAC pull-down. Error bars represent the standard deviation of the H/(H+L) mean value for all the peptides identified for each individual protein. Adapted from Di Virgilio et al., Science 2013.*

leading to aberrant chromosomal rearrangements. Accumulation of unrepaired breaks and chromosomal aberrations is known as genomic instability, which is a predisposing factor to carcinogenesis. Under these conditions, DNA end protection by 53BP1 has pathological consequences.

The molecular mechanisms underlying 53BP1 function in the regulation of DNA end processing remain elusive. We have previously determined that in addition to its recruitment to the chromatin surrounding the break sites, DNA damage-induced phosphorylation of 53BP1 is required for its ability to both protect DNA ends during CSR in B cells, and to promote genomic instability in BRCA1-deficient cells (Bothmer et al., Mol Cell 2011). This finding suggested that DSB-induced phosphorylation of 53BP1 was crucial for the recruitment of yet-to-be-identified DNA end protection factors. By using a SILAC-based proteomic approach for phospho-dependent 53BP1 interactors, we have subsequently identified several potential candidates as 53BP1 effectors in DNA end protection, including the DNA damage response proteins Rif1, PTIP, and its stable partner Pa1 as the top candidates (Fig. 2 and Di Virgilio et al., Science 2013). We showed that Rif1 and PTIP are indeed recruited to DSBs via interaction with phosphorylated 53BP1, and mediate 53BP1-dependent end protection during physiological (CSR) and pathological (genomic instability) DNA repair, respectively (Di Virgilio et al., Science 2013; Callen et al., Cell 2013). These findings indicate that the molecular events that are required for 53BP1 to promote ligation of DNA ends during CSR and the aberrant chromosomal rearrangements in BRCA1-mutant cells are, to some extent, distinct, and that multiple 53BP1 effectors are required to mediate DNA end protection in these different repair contexts.

Our lab employs a wide variety of techniques from classic biochemistry, molecular and cellular biology approaches to state-of-the-art proteomics and genomics methodologies to dissect the pathways and mechanisms that determine the balance between DNA end resection and protection, and that ensure appropriate DSB repair outcomes.

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Sander S, Chu VT, Yasuda T, Franklin A, Graf R, Calado DP, Li S, Imami K, Selbach M, **Di Virgilio M**, Bullinger L, Rajewsky K. PI3 Kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. **Immunity** 2015 Dec 15;43(6):1075-86. Epub 2015 Nov 24.

Callen E., **Di Virgilio M.**, Kruhlak M.J., Nieto-Soler M., Wong N., Chen H.T., Faryabi R.B., Polato F., Santos M., Starnes L.M., Wesemann D.R., Lee J.E., Tubbs A., Sleckman B.P., Daniel J.A., Ge K., Alt F.W., Fernandez-Capetillo O., Nussenzweig M.C., and Nussenzweig A. 53BP1 mediates productive and mutagenic DNA repair through distinct phospho-protein interactions. **Cell** 2013 Jun 6;153(6):1266-80. Epub 2013 May 30.

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Bothmer A., Robbiani D.F., **Di Virgilio M.**, Bunting S.F., Klein I.A., Feldhahn N., Barlow J., Chen H.T., Bosque D., Callen E., Nussenzweig A., and Nussenzweig M.C. Regulation of DNA end joining, resection, and immunoglobulin class switch recombination by 53BP1. **Mol Cell** 2011 May 6;42(3):319-29.

Start of the Group: September 2014

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Photo: David Ausserhofer/MDC

Gaetano Gargiulo

Molecular Oncology

Our research focuses on understanding the molecular mechanisms regulating tumor homeostasis and response to anti-cancer therapy. We do so by modeling human cancers in laboratory animals using genetic alterations described in patients. We wish to exploit animal models as surrogate or "targeted" patients to ultimately identify novel anti-cancer approaches and biomarkers for response.
Our group has been very recently established here at MDC. Supported by a Helmholtz Young Investigator Grant, we started recruiting scientists and students between June and December 2016. Before my appointment at MDC, I was trained at the European Institute of Oncology in Milan (EIO, Italy) and at the Netherlands Cancer Institute in Amsterdam (NKI, The Netherlands). In my most recent work, I contributed to develop an in vivo screening procedure to functionally characterize cancer genes during gliomagenesis. Alongside, I was also involved in understanding the role of tumor cell identity in lung cancer progression. Currently, we are translating the concept that changing the tumor cell identity in vivo using epigenetic drugs can introduce specific vulnerabilities into cancer cells that can be therapeutically exploited. In parallel, we have started a long-term endeavor in which we are using a novel entry point into combating Glioblastoma, the most aggressive form of brain tumors. In the fall, the group mission was reinforced by an European Research Council Starting Grant, which will further boost our activities in that direction.

Group summary

Cancer arises in normal cells by means of genetic and epigenetic alterations. We want to understand how tumor tissues balance self-growth and interactions with the host. Both processes can ensure cancer survival and govern critical cell decisions such as to whether self-renew, differentiate or die (i.e. homeostasis). Ultimately, tumor homeostasis can be viewed as the dark side of the normal tissue self-regulation.
We also want to know how do cancer cells deal with the therapy-induced stress.
Learning about these mechanisms will enable us to identify cancer-specific vulnerabilities, which in turn may pave the way to identifying more effective treatments.

Approach

One of the approaches we take is to model human cancers in laboratory animals using genetic alterations found in patients (see example in Figure 1). In turn, these models are used to study:

- genotype-to-molecular phenotype connections (Fig. 1b)
- molecular mechanisms of tumor growth and response to therapy (Fig. 1c)
- target discovery and validation (Fig. 1d)

In the long run, we aim to exploit animal models as "surrogate" or "targeted" patients to ultimately identify novel anti-cancer treatments and biomarkers for response.
Mechanistically, **we focus on genetic and epigenetic control of gene expression in cancer cells**. We make use of a combination of experimental and computational approaches among which: adult stem cells genetic engineering, *in vivo* tumor modeling, *in vivo* genetic screens and genome-

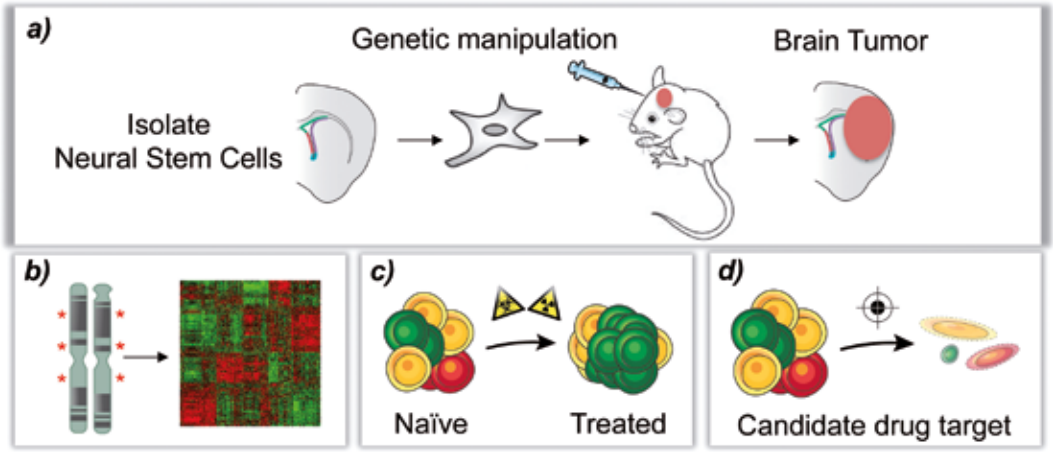

Figure 1: ***a)*** Primary neural stem cells are isolated from brain sections and then *in vitro* transformed by deleting or mutating the relevant genes according to the most recurrent aberrations discovered in patients' oncogenotypes. Subsequently, these cells are intracranially transplanted into immunocompromised rodent, which leads to the formation of a brain tumor. These models are used to investigate: ***b)*** genotype-to-molecular phenotype connections, ***c)*** mechanisms underlying cancer cell response to therapy and ***d)*** candidate drug targets and biomarkers for response.

wide binding, occupancy & expression profiling by high throughput sequencing. As we thrive to achieve a conceivably rapid translation of our experimental efforts into clinical oncology, we leverage our results against large publicly available repositories containing patients' molecular and clinical information.

Focus on solid tumors

Currently, we will focus our work on solid tumors such as brain and lung cancers. In particular, the lab established a long-term research program dealing with the Glioblastoma Multiforme (GBM). The GBM is the most common primary brain tumor, and is currently incurable. It is urgent to devise treatments best fitting individual patients (precision medicine) and be able to predict the patients' response to the chosen therapy. Both tumor heterogeneity and resistance to available treatments significantly affect GBM clinical management. As mentioned above, we approach these problems by creating and characterizing "humanized" animal models of GBM accurately reflecting patients at molecular level and exploiting these models in state-of-the-art genetic screens in vivo. In this case, we aim to identify molecular biomarkers for response to standard-of-care for patients with this disease as well as to uncover mechanisms of intrinsic and acquired resistance.
For lung cancer, we are interested in those tumor subtypes driven by the Kras oncogene, for which effective treatments are currently lacking.

Technologies

To characterize our animal models and their response to treatment as well as to perform functional genetics, we aim at exploiting the so-called 'omics' technologies, notably at the single cell and single molecule level. These technologies include – but are not limited to – RNA-seq, ATAC-seq as well as Perturb-seq like approaches. We are in a good position to engage in this endeavor as the MDC is well equipped with all of the required state-of-art technologies.

Recent Publications

Ferone, G., Song, J.-Y., Sutherland, K. D., Bhaskaran, R., Monkhorst, K., Lambooij, J.-P., et al. (2016). SOX2 Is the Determining Oncogenic Switch in Promoting Lung Squamous Cell Carcinoma from Different Cells of Origin. *Cancer Cell*, 30(4), 519–532. http://doi.org/10.1016/j.ccell.2016.09.001

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Serresi, M., Gargiulo, G., Proost, N., Siteur, B., Cesaroni, M., Koppens, M., et al. (2016). Polycomb Repressive Complex 2 Is a Barrier to KRAS-Driven Inflammation and Epithelial-Mesenchymal Transition in Non-Small-Cell Lung Cancer. *Cancer Cell*, 29(1), 17–31. http://doi.org/10.1016/j.ccell.2015.12.006

Gargiulo, G., Serresi, M., Cesaroni, M., Hulsman, D., & Van Lohuizen, M. (2014). In vivo shRNA screens in solid tumors. *Nature Protocols*, 9(12), 2880–2902. http://doi.org/10.1038/nprot.2014.185

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Photo: David Ausserhofer/MDC

Udo Heinemann

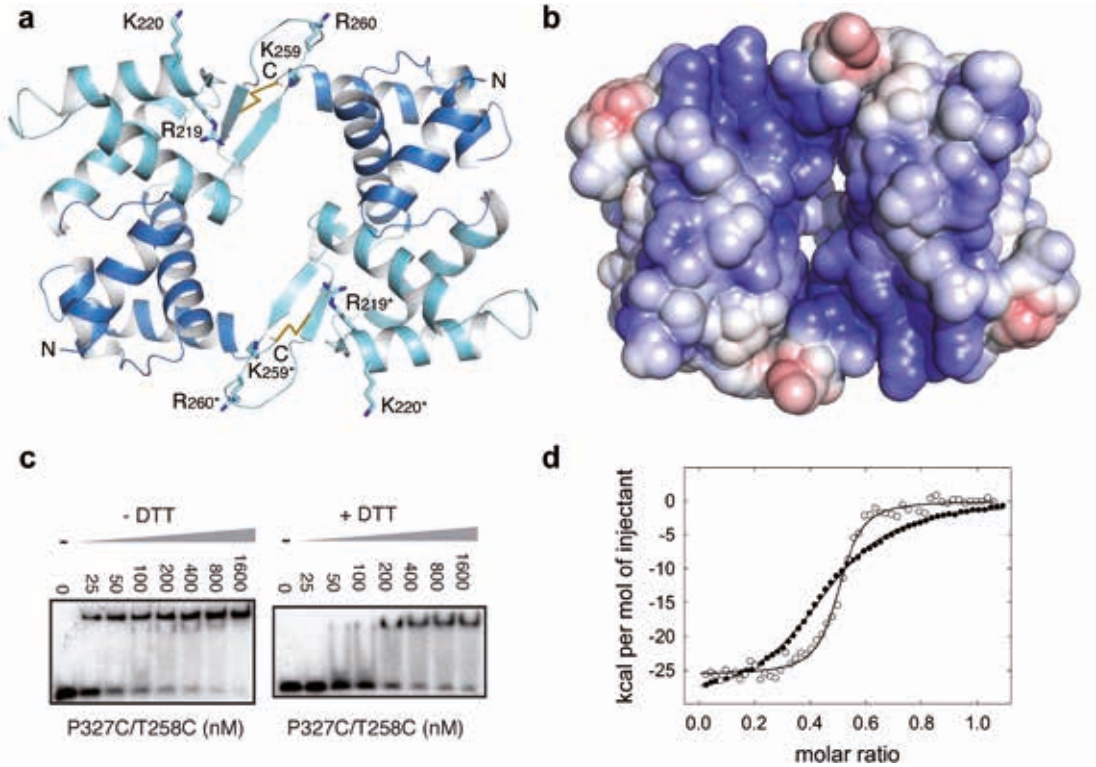
Macromolecular Structure and Interaction

The Macromolecular Structure and Interaction Laboratory combines macromolecular crystallography with biochemical and biophysical approaches to elucidate the molecular basis of biological processes at atomic detail. One major area of research is concerned with the regulation of gene expression at the transcriptional as well as translational level. Post-transcriptionally, mRNA homeostasis is ensured by RNA-binding proteins that regulate mRNA maturation, transport, translation and degradation. Among these, the human protein ROQUIN1/RC3H1 regulates the expression of genes in the immune system by binding to conserved sequence/structure motifs in the 3' untranslated region of their mRNAs. The crystal structure of the ROQUIN1/RC3H1 ROQ domain reveals a winged helix-turn-helix fold common in DNA-binding proteins and a dimeric ROQ arrangement presenting a basic dimer surface. Mutational analysis supports the hypothesis that this ROQUIN1/RC3H1 surface is involved in RNA binding. In a second major area of research, we address problems of intracellular protein transport and sorting. In all eukaryotes, the highly abundant AAA+ ATPase p97/CDC48 is involved in multiple cellular processes including the transfer of polyubiquitinated protein chains from the endoplasmic reticulum to cytosolic proteasomes for their degradation. We have determined crystal structures of human p97 bound to the extended UBX domain of the adapter protein ASPL and reveal that ASPL remodels functional p97 hexamers to form heterotetrameric p97:ASPL complexes. The p97-ASPL interaction observed in crystals and validated by biochemical and cellular assays serves as a paradigm for a new type of AAA+ ATPase regulation by UBX-domain proteins. Finally, the Helmholtz Protein Sample Production Facility produces proteins, protein crystals and crystal structures for collaborating laboratories. Our crystallographic research is greatly facilitated by privileged access to the synchrotron storage ring BESSY in Berlin.

Molecular basis of gene-expression control

In eukaryotic cells, gene expression is regulated at the levels of transcription initiation and mRNA processing, transport, translation and degradation. We are studying the molecular basis of gene-expression control by crystallographic and biochemical analyses of classical transcription factors as well as proteins and microRNAs involved in maintaining mRNA homeostasis.

ROQUIN1, also known as RC3H1 (RING finger and CCCH-type zinc finger domains 1) was reported to control the lifespan of mRNAs by binding to constitutive decay elements (CDEs) within their 3' untranslated region (3'UTR). Among the >50 vertebrate mRNAs found to contain CDEs are those encoding tumor necrosis factor-α (TNF-α) and ICOS, the inducible T-cell co-stimulator. ROQUIN1/RC3H1 therefore has important functions in inflammation, immunity and auto-immunity. In addition to the RING and zinc-finger domains and to extended, natively unfolded peptide regions, ROQUIN1/RC3H1 contains a novel ROQ domain known to mediate CDE bind-



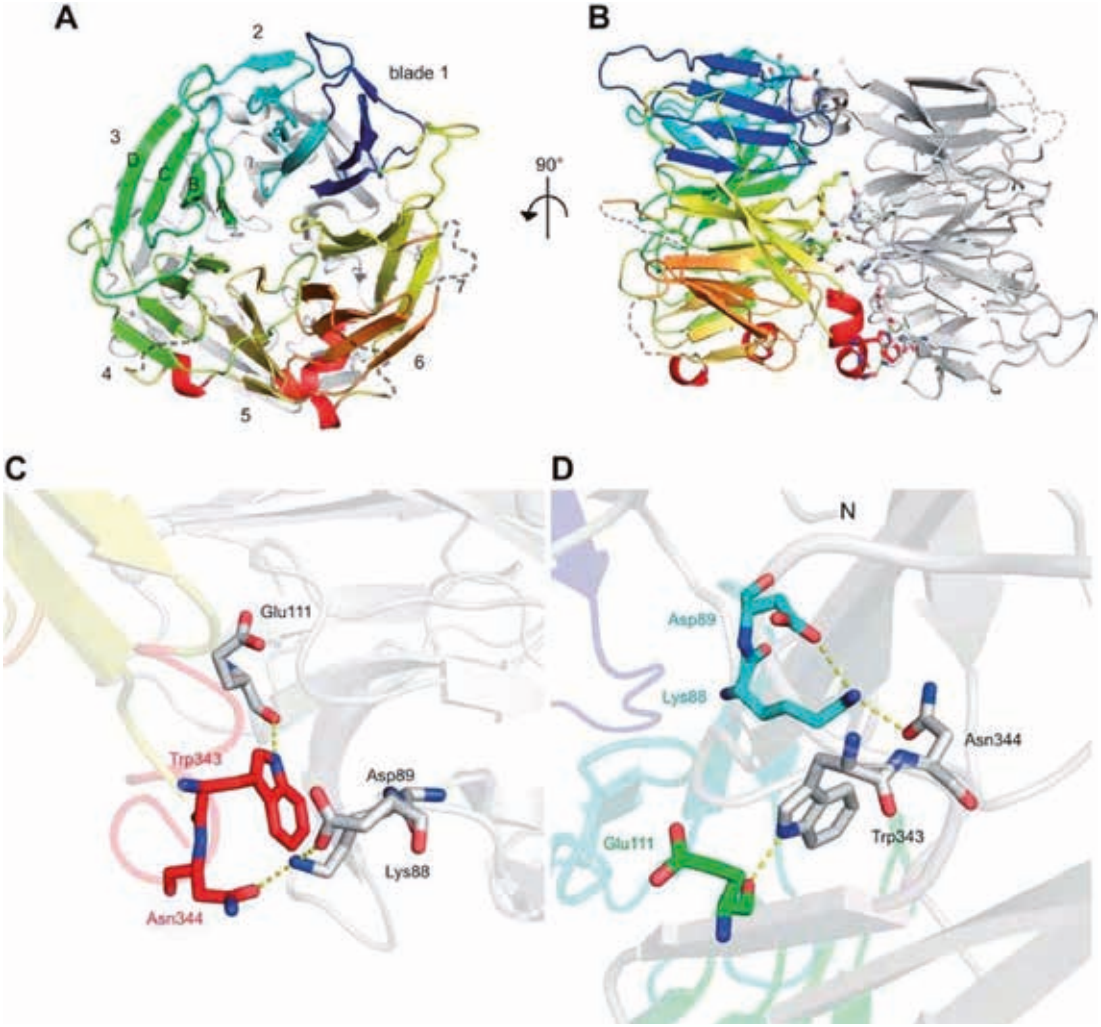
A dimeric ROQ domain of ROQUIN1/RC3H1 is involved in target RNA binding. (a) Dimeric arrangement of the ROQ domain as observed in the crystal structure. (b) The ROQ dimer interface is highly positively charged (blue surface) and is predicted to be involved in RNA binding. (c, d) An engineered, disulfide-stabilized ROQ domain dimer shows enhanced ICOS mRNA binding under oxidizing conditions (-DTT), but not under reducing conditions (+DTT). From Schuetz et al. (2014)

ing. Crystal structure analysis reveals a winged helix-turn-helix (wHTH) fold of the ROQ domain, which is decorated by four additional α-helices. wHTH domains are commonly found in proteins binding double-stranded DNA. In crystals, the ROQUIN1/RC3H1 ROQ domains are present in a dimeric arrangement, which is stabilized by polar interactions and creates a highly basic surface suited for RNA binding. Mutational analysis demonstrated that basic amino-acid residues present in this surface region are, indeed, required for ICOS mRNA CDE binding. Binding to the stem-loop structure of the CDE could be enhanced by an engineered disulfide bond, that stabilizes the ROQUIN1/RC3H1 dimer, lending support to a model where proteins associate in dimeric fashion on CDEs of target mRNAs.

On ROQUIN1/RC3H1 and other RNA-binding proteins, we collaborate closely with the laboratory of Markus Landthaler of BIMSB/MDC. In a wider collaboration, also involving the MDC groups of Stefan Kempa, Jana Wolf and Claus Scheidereit, ROQUIN1/RC3H1 binding sites were mapped in a large number of mRNA targets, and a novel mode of binding in the 3'UTR of the A20 mRNA was revealed. This work demonstrates that ROQUIN1/RC3H1 can modulate the activity of the NF-κB pathway through its regulator A20.

Molecular basis of intracellular transport

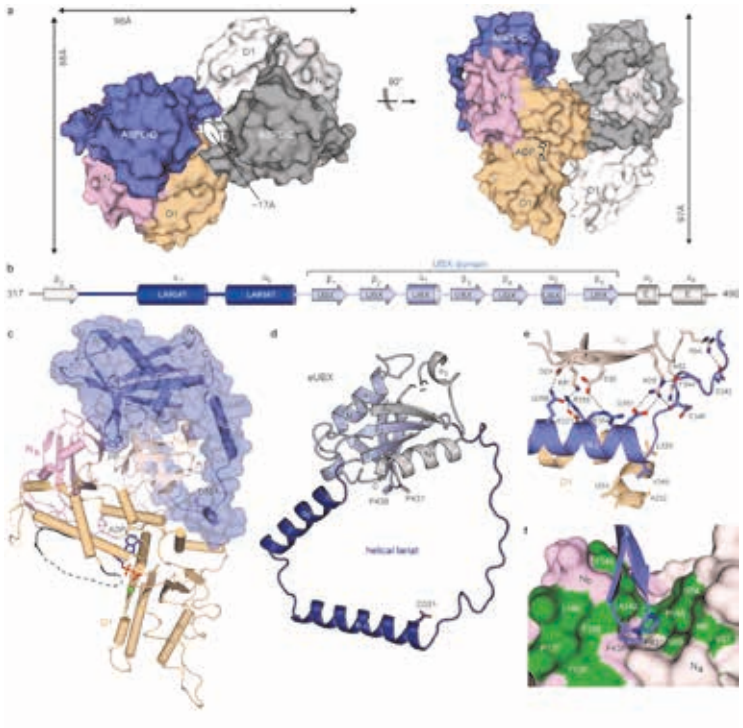
In eukaryotic cells, membrane bilayers enclose functionally distinct compartments such as the nucleus, the endoplasmic reticulum (ER), the Golgi apparatus, and lysosomes. Many proteins and other molecules travel to their destination within cellular compartments or outside the cell by crossing membrane bilayers. For proteins, these transport steps are associated to maturation and quality control processes, which ensure that defective macromolecules are recognized and degraded along their path. We study structural aspects of two different transport processes in yeast and human cells, the tethering of lipid vesicles at their target membranes and the retro-translocation of folding incompetent protein chains from the ER lumen into the cytoplasm.



The conserved N-terminal domain of Vps18 (Vps18$_{NTD}$) folds into a seven-bladed β-propeller. (A) Schematic of Vps18$_{NTD}$ showing the seven propeller blades in rainbow colors. (B) In the crystal lattice, two Vps18$_{NTD}$ propellers face each other in an arrangement stabilized by polar interactions. (C, D) Close-ups of the hydrogen-bonded contacts within the Vps18$_{NTD}$ interface. From Behrmann et al. (2014)

HOPS, the homotypic fusion and vacuole protein sorting complex of yeast, is a canonical tethering complex. HOPS shares four subunits with CORVET (class C core vacuole/endosome tethering), the other hexameric tethering complex of the endolysosomal pathway. Both HOPS and CORVET contain five subunits that are structurally similar to subunits of the vesicle coat COP II with predicted amino-terminal β-propellers and carboxy-terminal α-helical domains. A low-resolution cryo-electron microscopy structure of HOPS has been published, but high-resolution structural data for any part of HOPS or CORVET were not available until recently. The crystal structure of the amino-terminal domain of the HOPS subunit Vps18, determined in collaboration with the laboratory of Christian Ungermann (Osnabrück) has now revealed its structure as a seven-bladed β-propeller, which was shown to be critical for HOPS stability and function. The structure and interactions of the Vps18 β-propeller in the crystal lattice provide a framework for the architecture and assembly of the HOPS and CORVET vesicle tethering complexes.

AAA+ ATPases are abundant proteins in eukaryotic cells, which are recruited to their cellular site of activity by adaptor proteins and convert the energy from ATP hydrolysis into mechanical work. A proteomics study in the laboratory of Erich Wanker (MDC) identified the UBX-domain protein ASPL as the most tightly binding adaptor of the human AAA+ ATPase p97 (CDC48 in yeast). Crystal structure analysis revealed an unexpected remodeling of functional p97 hexamers by ASPL and the formation of p97:ASPL hetero-tetramers with reduced ATPase activity and



Crystal structure of a p97:ASPL heterotetramer. (a) The C-terminal domain of ASPL (ASPL-C) bound in 2:2 stoichiometry to the N-D1 fragment of human p97. (b) ASPL-C consists of an extended UBX domain (eUBX) containing strand β0 and several α-helices in addition to the canonical UBX domain. (c) ASPL-C wraps around the p97 N-domain in a geometry that is incompatible with the p97 hexamer structure. (d) An extended α-helical lariat is a key structural element for p97 remodeling by ASPL. Hotspots of the ASPL-p97 interaction include residues D351 (e), P437 and P438 (f) of ASPL-C. Modified after Arumughan et al., 2016.

impaired function in endoplasmic reticulum-associated protein degradation (ERAD). We have therefore discovered a novel inactivation mechanism for p97, which may serve as starting point for the development of new anti-cancer compounds.

Protein Sample Production Facility

The Helmholtz Protein Sample Production Facility (Helmholtz PSPF) is a joint activity between the MDC and the Helmholtz-Zentrum für Infektionsforschung (HZI) in Braunschweig. The PSPF offers expertise in protein production and biophysical characterization for structural biology to external and internal partners, using various host systems tailored to specific experimental requirements.

The Helmholtz PSPF offers a platform for initiating collaborative projects on topics that are initially outside the area of expertise of the Heinemann laboratory. In the past, these co-operations have frequently yielded crystal structures and molecular insight on proteins linked to human disease or target proteins for pharmacological intervention with small molecules. Recently, a series of structure analyses has helped to identify inhibitors of the protein tyrosine phosphatase SHP2 in collaboration with the laboratories of Jörg Rademann and Gerhard Wolber (both FU Berlin), Jens von Kries (MDC/FMP Screening Facility) and Walter Birchmeier (MDC). These inhibitors block cell motility and growth of cancer cells *in vitro* and *in vivo*.

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Behrmann, H., Lürick, A., Kuhlee, A., Kleine Balderhaar, H., Bröcker, C., Kümmel, D., Engelbrecht-Vandré, S., Gohlke, U., Raunser, S., Heinemann, U.# & Ungermann, C.# (2014) Structural identification of the Vps18 β-propeller reveals a critical role in the HOPS complex stability and function. *J. Biol. Chem.* **289**, 33503-33512.

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Uta E. Höpken

Microenvironmental Regulation in Autoimmunity and Cancer

Our group dissects cellular requirements and molecular pathways which contribute to the transformation of secondary lymphoid organs (SLOs) toward lymphoma/leukemia-permissive niches in preclinical mouse models. We aim to identify stromal elements in SLOs that collaborate with lymphoma/leukemia B cells and study lymphoma-induced cellular and molecular remodeling. Secondly, we study chronic autoimmune or pathogen-induced immune reactions that lead to lymphoid neogenesis and development of associated lymphomas. Based on the identification of pivotal stromal cell types and signaling pathways, we will probe pharmacological interventions targeted at the crosstalk between lymphoma cells and the microenvironment.

Immunosurveillance and interactions between tumor cells and its microenvironment

Cellular interactions and molecular communication pathways in the lymphoma survival niche

F. Scholz, A. Graband (collaboration with A. Rehm, A. Leutz, MDC)

Survival and progression of lymphoma cells is dependent on nodal access and microanatomical localization We showed that the chemokine receptor CCR7 is crucial for Eμ-Myc B cell lymphoma nodal dissemination. Malignant cells lodged adjacent to fibroblastic reticular cells and dendritic cells (DCs) within the T cell zone. According to their differentiation status, DCs trigger immunity or tolerance in T lymphocytes. DC-mediated B cell lymphomagenesis remained enigmatic. In DC-depleted mice we observed that progression of B cell lymphomas was delayed. Lymphoma-exposed DCs upregulated immunomodulatory cytokines, growth factors and the CCAAT/enhancer binding protein β (C/EBPβ). C/EBPβ-deleted DCs were unresponsive to lymphoma imposed cytokine changes and unable to promote lymphoma cell survival. We conclude that DCs have lymphoma-promoting functions and act directly by the provision of growth factors, provided that they express the transcription factor C/EBPβ.

Cellular stromal elements in B cell follicles that collaborate with leukemia B cells in an indolent B-CLL mouse model

V. Stache, F. Brand, T. Börding (collaboration with A. Rehm, MDC)

We characterized non-hematopoetic stromal cells in the B cell zone of SLOs that collaborate with leukemia B cells in an indolent Eμ-Tcl1 leukemia mouse model. This relationship involved chemokine-mediated guidance of leukemic cells into promoting niches, followed by a nurse-like function of the stromal follicular dendritic cell (FDC)-network (Figure 1). Crosstalk inhibition of lymphotoxin(LT)/LTRβ signaling or inhibition of the CXCL13/CXCR5 signaling axis retarded lymphoma progression. In addition, we identified gene expression signatures indicative of a skewed polarization of monocytes and neutrophils. Depletion of macrophages or neutrophils resulted in tumor retardation. Leukemia cells secreted IL-10 and by that rendered splenic neutrophils and macrophages into a tumor supportive phenotype. Also, neutrophils revealed increased expression of B-cell activating factors which supports leukemia cell survival. Hence, immune cells can also be primed towards a lymphoma-supporting milieu.

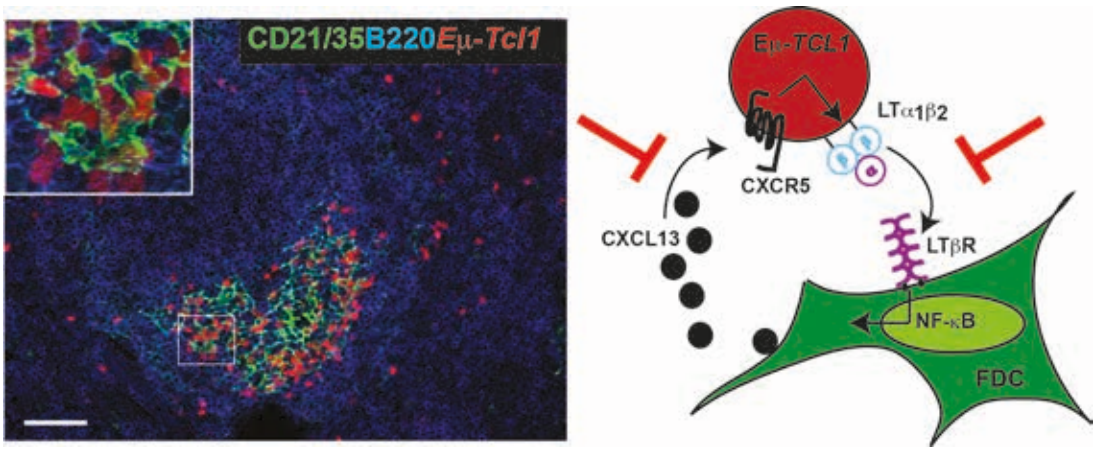

Figure 1. Leukemia cell homing and cellular interactions in the lymphoma niche.
Red–labeled Eμ-Tcl1 leukemia cells intermingle with the FDC-network. Spleen sections were stained for CD21+CD35+ FDCs (green) and B220+ B cells (blue), scale bar, 100 μm. The interference with CXCL13/CXCR5-governed leukemia cell homing and lymphotoxin-mediated tumor-stroma crosstalk provides new targets in lymphoma therapy.

CAR-T cell immunotherapy for multiple myeloma and B cell lymphoma

(collaboration with A. Rehm, J. Bluhm, O. Daumke, MDC, and W. Uckert, Humboldt-University)

Chimeric Antigen Receptors (CARs) are single-chain antibodies, anchored on the T cell surface and containing intracellular signal domains. The whole construct confers T cells with antigen-specific cytolytic capacity. We generated CAR-T cells directed against the B cell maturation antigen (BCMA) for the treatment of B cell disorders, in particular multiple myeloma (MM), mature Non-Hodgkin Lymphoma (B-NHL), and autoimmune diseases. Our anti-BCMA CAR-T cell constructs confers human T cells with antigen-specific cytolytic capacity *in vitro* and their *in vivo* efficacy has been validated in pre-clinical xeno-transplantation models of MM and B-NHLs.

The tissue-specific micromilieu regulates plasticity of T helper cell subpopulations

K. Wichner

Autoimmunity is associated with a strong genetic component, but onset and persistence of autoimmune diseases requires an additional environmental trigger. The balance between immunity and tolerance is regulated by molecular factors including the nuclear hormone receptor RORγt which is involved in functional lymphoid organogenesis. RORγt-/- mice exhibited accumulation of follicular T helper (Tfh) cells, spontaneous germinal center formation, and increased autoantibody production. Deregulated B cell responses were linked to enhanced production of IL-17. Because RORγt has been described as a key transcription factor for the generation of inflammatory Th17 cells, we revealed an unknown transcriptional flexibility in their development. Th cell transcription factors other than RORγt may induce differentiation of Il-17-producing Th cells under tissue-specific conditions.

Selected Publications:

Gätjen M, Brand, F, Grau, M, Gerlach, K, Kettritz, R, Westermann J, Anagnostopoulos I, Lenz, P, Lenz G, **Höpken UE***, Rehm, A* (2016). Splenic marginal zone granulocytes acquire an accentuated neutrophil B cell-helper phenotype in chronic lymphocytic leukemia. *Cancer Research* 76: 5253-65; *equal contribution

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Heinig K, Gätjen M, Grau, M, Stache, V, Anagnostopoulos I, Gerlach, K, Niesner R, Cseresnyes Z, Hauser A, Lenz P, Hehlgans T, Brink R, Westermann J, Dörken B, Lipp M, Lenz G, Rehm A, Höpken UE (2014). Access to follicular dendritic cells is a pivotal step in murine chronic lymphocytic leukemia B cell activation and proliferation. *Cancer Discovery* 4: 1449-1465

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Patents:

PCT-Anmeldung beim Europäischen Patentamt: PCT/EP2013/072857 "Antibody against CD269 (BCMA).

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Photo: David Ausserhofer/MDC

Ralf Kühn

IPS cell based disease modelling

Understanding how genetic variation and mutations lead to the onset of human diseases is an important question in biomedical research and the basis for developing therapeutic interventions. We are using the CRISPR/Cas nuclease technology in human induced pluripotent stem cells (hiPSCs) as central technology to investigate human genetic disease mechanisms. hiPSCs are cell lines can be indefinitely propagated but also differentiated in vitro into many cell types of the human body. The research group on hiPSC -based disease modeling is setting up a technology platform enabling the engineering, differentiation and phenotyping of hiPSCs. The lab is presently focusing on the optimisation of CRISPR/Cas induced mutagenesis in hiPSCs and their directed differentiation into dopaminergic neurons. The first topic requires the efficient delivery and action of Cas9 nuclease, gene specific sgRNAs and customized DNA repair templates molecules in hiPSCs. In the second working area we are defining the optimal combination of external and internal cues specifying the differentiation of hiPSCs into dopaminergic neurons to study genetic lesions leading to the loss of these cells in Parkinson`s disease.

Gene editing using the CRISPR/Cas nuclease system

Programmable nucleases are used for editing the sequence of target genes by breakup of the DNA at preselected positions. The latest generation of nucleases is provided by the CRISPR/Cas9 bacterial defense system that uses short, single guide (sg) RNAs for DNA sequence recognition and can be programmed towards new targets by adaption of the sgRNA first 20 nucleotides. sgRNAs are bound by the generic nuclease Cas9 and guide the complex to the complementary DNA sequence, followed by the induction of a double-strand break (DSB). DSBs are fixed in mammalian cells mainly by the NHEJ repair pathway which reconnects open DNA ends imprecisely and frequently leads to the loss of multiple base pairs at the target site. Such small, randomly sized deletions are often used for the creation of frame shift mutations within coding regions leading to gene inactivation (knockout). The generation of precise modifications such as nucleotide replacements by gene targeting requires the alternative pathway of homology directed repair (HDR), able to read new sequences from externally delivered, DNA

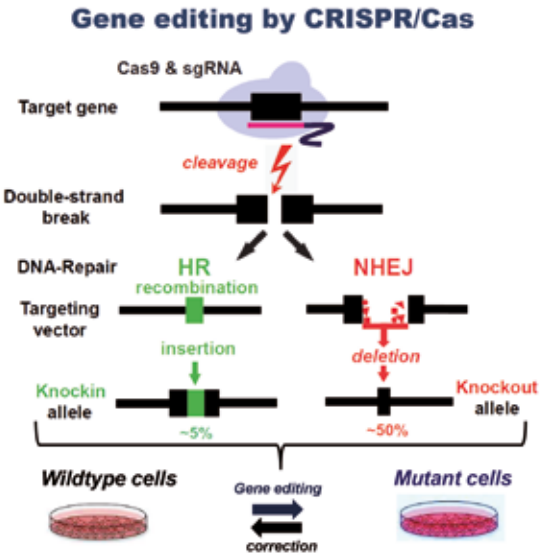

Figure 1: Gene editing in mammalians cells using the CRISPR/Cas nuclease system.
Cas9 nuclease together with sgRNA create a double-strand break (DSB) in the target gene. DSBs are either fixed by non-homologous end joining (NHEJ) repair which reconnects DNA ends imprecisely and deletes multiple base pairs (knockout) or by homology directed repair (HR), reading new sequences from a targeting vector into the DSB site (knockin).

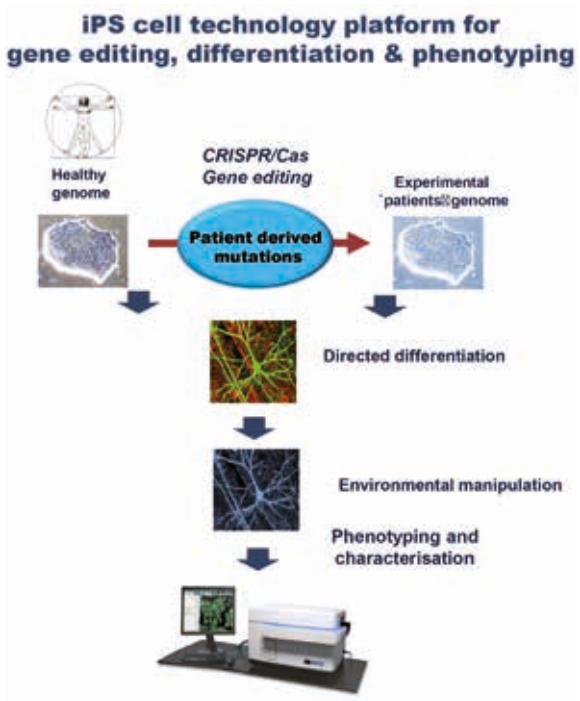

Figure 2: Technology platform for gene editing and differentiation of human IPS cells.
Using CRISPR/Cas gene editing, disease related mutations can be introduced into the genome of human induced pluripotent stem (iPS) cells. Upon the directed differentiation of iPS cells into e.g. neurons, cultures can be studied by live cell imaging for disease associated phenotypes, ultimately building a relational map of genotypes, envirotypes and phenotypes.

templates into the DSB site (knockin) (Fig. 1). Only a small fraction of DSBs in mammalian cells is repaired by HDR, making precise gene editing inefficient. Using reporter constructs indicating the ratio of NHEJ or HDR for the repair of DSBs we found that the suppression of the NHEJ enzyme DNA ligase IV by a small molecule inhibitor or by adenoviral proteins is sufficient for stimulating DSB repair by HDR. This intervention facilitates the introduction of knockin sequence modifications in cell lines and mice, as required for the precise modelling of patient derived mutations. Furthermore, this and ongoing work on the active promotion of HDR provides a basis for the future application of the CRISPR/Cas system in somatic gene therapy.

Studying disease pathways by gene editing in human IPS cells

Human induced pluripotent stem cells (hiPSCs) are early embryonic cell lines which can be established by the redifferentation of skin fibroblasts through the expression of reprogramming factors. hiPSCs can be indefinitely propagated but also differentiated in vitro into major cell types such as neurons or cardiomyocytes. By combining the hiPS cell and CRISPR/Cas nuclease technologies biomedical research can investigate human genotype/phenotype relations. The nuclease-assisted assembly of genotypes enables for example to introduce Parkinson`s disease risk alleles into `healthy´ genetic backgrounds or the correction of such alleles in patient-derived hiPS cells. For phenotypic analysis mutant and control cells are converted using lineage-specific transcription factors into differentiated cells, such as dopaminergic neurons. The differentiated cultures can be studied by live cell imaging for disease associated phenotypes, ultimately building a relational map of genotypes, envirotypes and phenotypes (Fig. 2). Since fall 2014 we focus on the set up of this hiPS cell technology platform for the simultaneous modification of up to three genes (multiplex engineering) by optimizing the delivery of Cas9, sgRNAs and HDR templates into hiPSCs and for the differentiation of hiPSCs into dopaminergic neurons. Presently we achieve in up to 30% of transfected hiPSCs the mutagenesis of two genes and can differentiate 1/3 of the cells into dopaminergic neurons. To support the analysis of cultures by live cell imaging we use fluorescent reporters by knockin into marker genes. In addition, we aim for extending the utility of the hIPSC technology platform by establishing CRISPR/Cas based conditional, i.e. inducible and cell type-specific gene knockout in differentiated cells and the three dimensional differentiation of hiPSCs into organoids which recapitulate aspects of fetal brain development.

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Photo: David Ausserhofer/MDC

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Cell Differentiation and Tumorigenesis

The inherent and extensive self-renewal property of somatic stem cells vanishes upon asymmetric division into differentiating progeny, but may recur in tumor stem cells and cause cancer. Central issues in tumor stem cell biology and in the application of somatic stem cell based therapy are the disclosure of molecular mechanisms involved in the generation of somatic stem cells, processes involved in self-renewal and cues to differentiation into functional cells. All these processes are regulated by transcription factors that integrate signals into epigenetic programs. CCAAT Enhancer Binding Proteins (C/EBP) represent an interesting family of pioneering transcriptional key regulators of cell lineage commitment, self-renewal, and cell differentiation in many organs and cell types. We study how signal dependent alterations of the C/EBP structure determine C/EBP functions in proliferation, differentiation and tumorigenesis.

Synopsis: Structure & Function of C/EBP

The C/EBP family of transcription factors harbor cell lineage instructive and trans-differentiation potential. They regulate stem cell self renewal, lineage commitment and cell maturation. C/EBPα,β,δ,ε, are highly related family members. Targeted mouse genetics revealed that the combined deletion of C/EBPα and β causes early embryonic lethality, whereas other compound C/EBP deletions displayed more subtle effects. These data suggest that C/EBPα,β are the most essential factors of the C/EBP family. The structure of C/EBPs is highly modular and adapts to signal dependent regulatory multitasking. The C-terminal basic DNA binding leucine zipper domains (bZip) can dimerize and bind to cis-regulatory palindromic ATTGC•GCAAT DNA sequence motifs. The C/EBP N-termini harbor strong trans-activation domains (TAD), whereas the center sequences represent regulatory domains (RD). Both, TAD and RD consists of several conserved short peptide regions (CR) that are intervened by less conserved intrinsically disordered regions (IDR). The overall C/EBP structure suggests structural flexibility and adaptability to interactions with co-factors and gene regulatory protein complexes. Interestingly, the pioneering transcription factors C/EBPα and C/EBPβ are single exon genes that are located on different chromosomes. Nevertheless, various C/EBPα,β isoforms can be generated by regulated alternative translation initiation. Dysregulation of C/EBPα,β isoforms expression interferes with cell differentiation and may cause tumorigenic cell transformation. In addition to alternative translation, C/EBPα,β can be decorated by a multitude of post-translational amino acid side chain modifications (PTM). Signaling events that

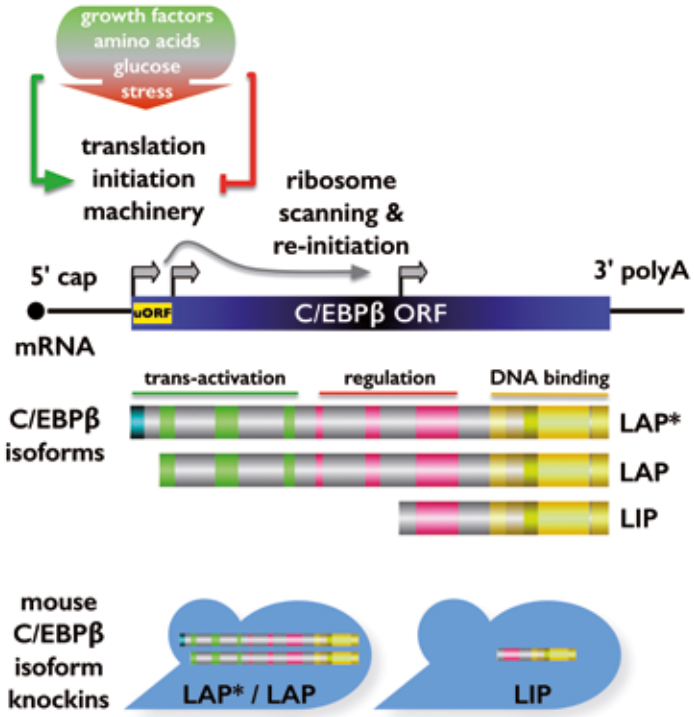
sense growth factor stimulation, nutrition, or stress regulate both isoform translation and post-translational modifications of C/EBPα,β. The combination of alternative translation and amino acid side chain modifications orchestrate diverse C/EBP functions by controlling the C/EBP interactome. Hence, how signal dependent C/EBP modifications are established and how modifications determine C/EBP functions are essential for the understanding of C/EBP biology. Our research focuses on the dynamic regulation of C/EBPs, their ability to balance cell proliferation and cell differentiation and the function of C/EBPs in disease.

Defective C/EBPβ isoform switching causes many diseases

Isoforms of C/EBPα,β are generated by alternative translation initiation at consecutive "in frame" AUG-start codons. Small upstream open reading frames (uORF) in the C/EBPβ mRNA translation leader sequence mediate appropriate start codon selection of individual isoforms. We have used targeted mouse genetics to explore the importance and vital function of individual isoforms of C/EBPβ and the consequences of defective C/EBPβ isoform switching. Murine gene replacement "knockin" mutants that can not switch between C/EBPβ isoforms express either only the long (LAP*, LAP) or the truncated C/EBPβ isoform (LIP), as shown in Figure 1. These mouse strains develop many diseases and display defects in bone homeostasis, liver regeneration, mammary gland development, innate immune response, and metabolism. Expression of the C/EBPβ LIP isoform, even from a single allele causes cancer in many tissues and suggests LIP as a dominant onco-protein. In summary, switching between C/EBPβ isoforms is important in tissue regeneration, cell differentiation, metabolic adaptation, and prevention of cancer.

Importance of protein expression control by upstream open reading frames (uORF)

Bioinformatic analysis of transcript leader sequence regions of the genome revealed that evolutionary conserved uORFs occur in many transcripts. Ribosome profiling confirmed that uORF are indeed translated. Mechanistically, uORFs consume functional pre-initiation complexes and thus curb the production of critical proteins encoded downstream on the same mRNA. Accordingly, loss-of-function uORF mutations in proto-oncogenes may cause enhanced expression of potentially harmful proto-

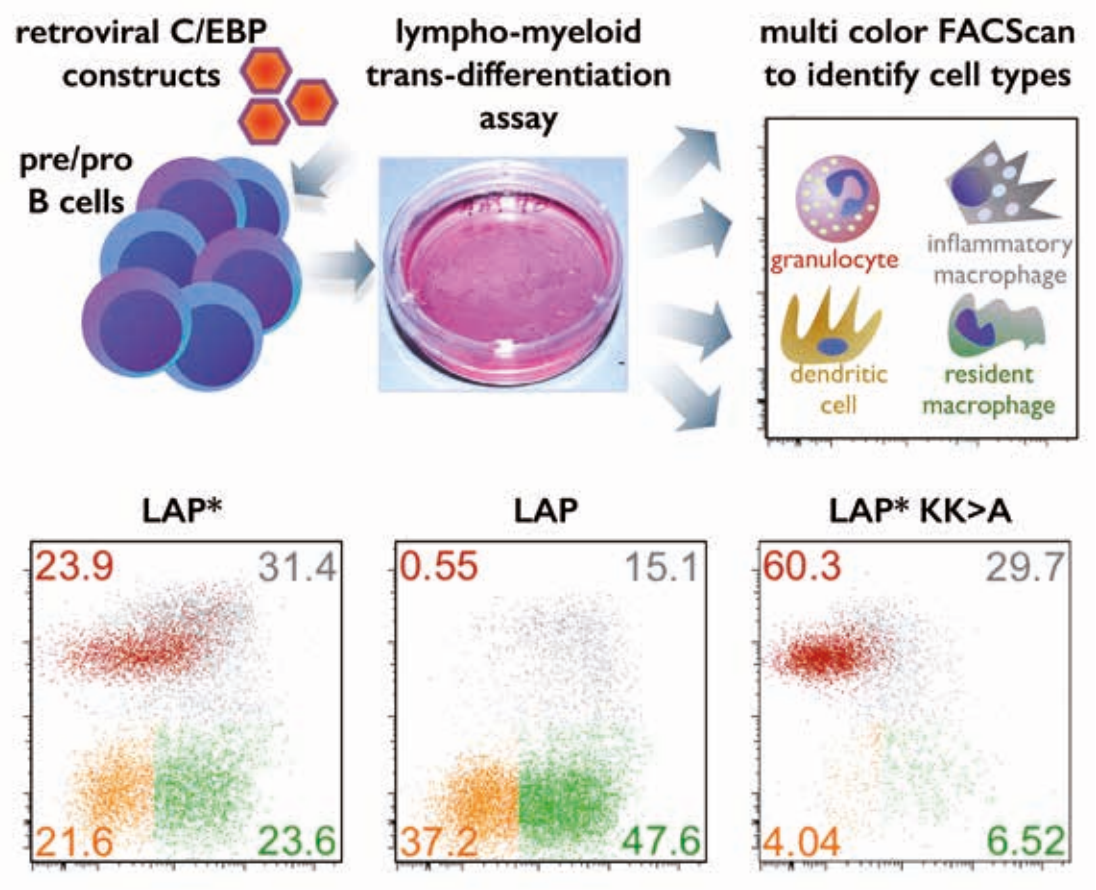


Translation of the single exon C/EBPβ transcript is regulated by an upstream open reading frame (uORF). The uORF senses the activity of the translational machinery and directs translation initiation to alternative start sites. The resulting LAP, LAP, and LIP protein isoforms have distinct gene regulatory and epigenetic potential. Mouse strains, as shown below, that cannot switch between isoform have been constructed and develop many diseases.*

oncoproteins. We explored the importance of uORF mediated protein expression control in the onco-developmentally important group of tyrosine kinases (TK) that are important regulators of cell fate and proliferation control and are frequently overexpressed in tumors. Almost 64% of all TK transcripts harbor uORFs. Experimental exploration of the impact of TK-uORFs then showed that removal of their uORFs indeed enhanced TK protein expression and may relate to increased cancer susceptibility. These results suggest driver functions for uORF loss-of-function mutations in proto-oncogenes and disclose a novel mechanism for TK overexpression in cancer. Interference with translational initiation and uORF regulation may thus represent a potential target for treatment.

Post-translational C/EBP modifications provide an index of epigenetic functions

Seeking for an explanation for the multitasking C/EBP functionality, it came to our attention that many amino acid residues in



Schematic representation of lympho-myeloid trans-differentiation assay. Various myeloid cell types can be distinguished by flow cytometry (FACScan), reflecting the epigenetic potential of the C/EBPβ structure. Underneath: Plots show that lineage assignment during trans-differentiation depends on modifications of C/EBPβ. LAP and LAP as in Figure 1, KK>A indicates a C/EBPβ point mutant with previously identified lysine (K) PTM targets mutated into alanine (A).*

C/EBPα,β represent targets of post-translational modifications (PTM). Systematic analysis by mass spectrometry (in collaboration with Gunnar Dittmar, MDC) identified dozens of novel PTM on C/EBPα and β, including K-acetylation and K-, R-methylation, S-, T-, and Y-phosphorylation. Biochemical and molecular genetic analysis revealed that alteration of individual C/EBPβ PTM sites modifies interaction with gene regulatory, chromatin modifying and chromatin remodeling complexes. For example, methylation of distinct K- and R-residues in the N-terminal C/EBPβ TAD changes the interaction with Mediator, SWI/SNF, and G9a complexes. We also observed signal dependent cross-talk between various C/EBPβ PTM that regulate global gene regulatory functions, such as an activator or repressor. We think that C/EBP may act as a meeting platform or assembly spot to coordinate interactions and diverse functions of protein complexes involved in shaping chromatin and regulating genes.

C/EBPβ structure and PTM in trans-differentiation

C/EBPs are downstream of signaling cascades and play important roles in hematopoietic lineage commitment and in establishing the innate immune system. Initially we found that ectopic expression of C/EBPs in many cell types may activate myeloid genes. Accordingly, C/EBPs entail the epigenetic competence to orchestrate gene expression and instruction of the myeloid cell fate in a signal dependent fashion. The trans-differentiation capacity of C/EBP to convert B lymphoid progenitors into myeloid cells was employed to examine how the structure of C/EBPβ directs alternative cell fates. The results of structural-functional analysis showed that C/EBPβ isoforms and PTMs pre-define the outcome of trans-differentiation into various cell types, such as monocytes/macrophages, dendritic cells, and granulocytes. Some of these results are shown in Figure 2. Moreover, point mutations of distinct PTM-sites may

alter the ability of C/EBPβ to recruit distinct chromatin remodeling complexes and to induce different cell types (Stoilova et al., 2013, PLoS ONE, 8:e65169). These findings suggest that the PTM pattern and the structural flexibility of C/EBPβ are adaptable modular properties to integrate and rewire epigenetic functions during differentiation into diverse innate immune cells (Schönheit et al. 2015, J Mol Biol. 427:670-87). What are the implications of these findings? Observations of trans-differentiation were initially regarded merely as the result of artificial manipulation, with little physiological correspondence. However, incoherent cell lineages and differentiation states were reported to emerge in pre-neoplastic tissues. Such alterations may occur in epithelia, known as 'metaplasia' or in leukemia/lymphoma known as 'lineage infidelity' and may echo results from experimental cell reprogramming. Metaplasia and lineage infidelity are examples of dysregulated cell differentiation that reflect a history of trans-differentiation and epigenetic reprogramming. We hypothesize that the remarkable trans-differentiation potential of C/EBPs plays a role in leukemic lineage infidelity. Studies are underway to determine the C/EBP interactome in conjunction with its trans-differentiation capacity. These studies will address the question whether reprogramming is involved in tumorigenic transformation (Regalo & Leutz, 2013, EMBO Mol Med, 5:1154-64).

A C/EBPβ 'Indexing Code' in partner choice

Our results suggest that an "Indexing Code" of post-translational modifications on C/EBPs may regulate and direct protein-protein interactions. The functional consequences of individual C/EBP-PTM sites can be examined in trans-differentiation tests (Figure 2). Discerning the underlying mechanisms remains difficult and requires the knowledge of differentially interacting partner proteins. We therefore employ various methods of systematic differential proteomics to discover PTM-dependent interactors of C/EBP (together with Gunnar Dittmar, Mass Spectrometry Core Unit). To date, our screens have revealed many transcription factors and epigenetically important proteins that differentially interact with distinct C/EBP isoforms or C/EBP-PTMs. Now, we explore how the modifications on C/EBPs orchestrate interactions with other transcription factors and co-factors in order to direct gene regulatory and epigenetic machineries in a context specific fashion. The multitude of C/EBP modifications comprises an extraordinarily high number of combinatorial interactions that implicate an amplification of C/EBP dependent functional and evolutionary plasticity. We anticipate that unraveling the PTM-dependent C/EBP interaction network will help to elucidate basic and complex C/EBP functions such as in hematopoietic cell lineage choice, cell differentiation, regeneration, innate immune regulation, and tumorigenesis.

Selected Publications

Cirovic B, Schonheit J, Kowenz-Leutz E, Ivanovska J, Klement C, Pronina N, Begay V, Leutz A (2017) C/EBP-Induced Transdifferentiation Reveals Granulocyte-Macrophage Precursor-like Plasticity of B Cells. Stem Cell Reports 8: 346-359. DOI 10.1016/j.stemcr.2016.12.015

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Scheller M, Schonheit J, Zimmermann K, Leser U, Rosenbauer F, Leutz A (2013) Cross talk between Wnt/beta-catenin and Irf8 in leukemia progression and drug resistance. The Journal of experimental medicine 210: 2239-2256. DOI 10.1084/jem.20130706

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Photo: David Ausserhofer/MDC

Antonio Pezzutto

Molecular Immunotherapy

Goal of our work is to translate results of basic immunological research into preclinical models and clinical trials. For this purpose, we have a close cooperation with the MDC research groups working on tumor immunology, T.Blankenstein and W. Uckert: several projects were developed in joint efforts up to clinical trials. Our strategies cover passive and active immunotherapy. We have performed several clinical studies with dendritic cells in chronic myeloid leukemia and renal cancer, gene-modified tumor cell vaccines for treatment of advanced metastatic renal cancer, lymphoma radioimmunotherapy. In the field of DNA-vaccination we are optimizing immunogenicity of a vaccine directed against the HER-2 protein overexpressed in breast cancer. We will soon start a clinical trial with genetic engineered T cells expressing a MAGE-A1 specific T cell receptor (developed by the MDC Blankenstein group), and are further developing adoptive T cell therapy with TCR-engineered to recognize B-cell specific antigens and mutated antigens for leukemia and lymphoma therapy.

Dendritic cell-based vaccination in minimal residual CML under therapy with tyrosine kinase inhibitors

J.Westermann and J.Kopp, in cooperation with Alexander Scheffold (DRFZ).

Minimal residual disease (MRD) is the only clinical setting in which active immunotherapy using vaccines may hold promise for improving (molecular) remission and/or preventing relapse. Chronic Myeloid Leukemia (CML) is a paradigm of „targeted therapy“ in which standard treatment with tyrosine kinase inhibitors is able to achieve deep molecular remissions in most patients. Furthermore, in CML qPCR for bcr/abl is a well standardized method to quantify MRD, even at very low levels. Therefore, minimal residual CML is an ideal clinical setting for a) vaccination against leukemia-specific and leukemia-associated antigens in order to eradicate residual leukemia cells and b) to monitor vaccine efficacy even at a subclinical level. Following on a successfully concluded clinical vaccination trial using in vitro-generated, bcr-abl positive dendritic cells (DC) in patients with chronic myeloid leukemia (CML) we have started a clinical DC-vaccination trial in CML patients with minimal residual cytogenetic or molecular disease upon treatment with tyrosine-kinase inhibitors. The aim of the study is to improve cytogenetic/molecular remission by the induction of CD4+ and CD8+ T cell responses against bcr/abl, proteinase-3 and WT-1. In CML, long lasting „complete“ molecular remissions have become an attractive goal of therapy since up to 40% of these patients may remain bcr/abl-negative even after cessation of TKI treatment.

DNA Vaccination: preclinical models

Jörg Westermann, Tam Nguyen-Hoai

DNA vaccination offers several advantages over the use of peptides as vaccines (DNA covers several MHC-I and MHC-II epitopes, directly targets the endogenous presentation pathway and contains immunostimulatory CpG sequences). Furthermore, DNA vaccines can be easily produced on large-scale for the use in clinical trials across HLA barriers. In cooperation with the group of M. Lipp (Molecular Tumor Genetics) we have explored the possibility of recruiting immune cells by using plasmid DNA coding for the chemokines CCL19, CCL21 and CCL4 as possible adjuvants. In particular, coexpression of CCL19 and CCL21 with tumor antigens results in enhancement of a Th1-polarized immune response with substantial improvement of the protective effect of the vaccine. Furthermore, in cooperation with S. Mathas we are studying

the adjuvant effect of STATs and IRFs in our preclinical model. Further improvement of the vaccine potency has been achieved using a gene-gun for intradermal vaccine application (cooperation with O.Hohn/S. Norley, the Robert-Koch Institute). These encouraging results have been extended to a preclinical model using the human breast-cancer associated antigen Her-2/neu as a target antigen, with the aim of developing a vaccine for Her2/neu+ tumors (particularly breast cancer) in the clinical situation of minimal residual disease after successful standard therapy.

Identification of tumor-reactive T-cells for adoptive T-cell transfer in solid tumors, lymphoma and leukemia

Simone Rhein, Antonia Busse, Sara Boiani, Neşe Çakmak, Corinna Grunert and Özcan Cinar in cooperation with T.Blankenstein, H.Schreiber, G.Willimsky and W.Uckert (MDC, Charité)

We focus on the treatment of different malignancies by establishing adoptive therapy with T cell receptor gene transfer. In different projects, we focus on B-cell lineage-specific antigens such as CD22 (S.Rhein), CD79a and CD79b (S.Boiani), kappa and lambda immunoglobulin light chains (A.Busse, Ö.Cinar), and also explore possible targets for myeloid leukemias such as FLT3 (N.Cakmack). So far, these antigens (CD22 and CD79a/b) have been targeted mainly by antibodies but not by T cell therapy: we believe that the efficacy of natural T cell receptors against intracellular antigens might be superior as compared to the CAR-recognized surface antigens which might be modulated in vivo giving origin to surface-antigen-negative escape variants. In cooperation with T.Blankenstein (MDC), we use a humanized mouse expressing the human TCR gene repertoire. This mouse can generate high-affinity T cells against human antigens (which have not undergone thymic selection) upon immunization with peptides, protein, DNA or RNA. The mouse is transgenic for the human HLA-A2 antigen. Cloning of reactive T cells allows TCR identification that can be transduced in patients T cells for generation of large amounts of lineage-specific T cells with high therapeutic potential. In the context of a cooperative project of the Berlin Institute of Health (cooperation with T.Blankenstein, MP Klötzel, M.Hummel, H.Schreiber, W.Uckert) we also evaluate mutation-specific epitopes of key regulators of lymphocyte pathways such as MYD88, CARD11, EZH2, A20, as possible target for TCR recognition (Ö.Cinar). In a different project, A.Busse and C.Grunert are targeting tumor specific antigens expressed in squamous cell carcinoma of cervix and head and neck and in ovarian cancer, with a particularly focus on patients responding to treatment with check-point inhibitors. Tumor specific T cells are directly isolated from the fresh tumor samples/Ascites/pleural effusions for single-cell TCR sequencing and cloning. Moreover, isolated T cells are tested for recognition of mutated tumor specific epitopes identified in the context of the BIH Cooperative Research Grant. In case of HPV associated squamous cell carcinomas, isolated T cells are also tested for recognition of HPV associated antigens. Finally, a clinical study using TCR-gene modified T cells specifically recognizing the tumor-associated antigen MAGE-A1 (generated in T.Blankenstein group) is in advanced preparation. GMP-expansion of T cells for treatment of MAGE-A1 positive multiple myeloma is done in the GMP laboratory of the ERC by J.Kopp in cooperation with E.Kieback and M.Obenaus (MDC Blankenstein group).

Patents / Patent applications

PCT-Anmeldung PCT/EP2013/069569 "CD22-BINDING PEPTIDES" (O. Schmetzer and A. Pezzutto)

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Nguyen-Hoai T, Kobelt D, Hohn O, Vu MD, Schlag PM, Dörken B, Norley S, Lipp M, Walther W, Pezzutto A, Westermann J. (2012). HER2/neu DNA vaccination by intradermal gene delivery in a mouse tumor model: Gene gun is superior to jet injector in inducing CTL responses and protective immunity. Oncoimmunology. 1:1537-1545.

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Nguyen-Hoai, T., Baldenhofer, G., Sayed Ahmed, M.S., Pham-Duc, M., Vu, M.D., Lipp, M., Doerken, B., Pezzutto, A., and Westermann, J. (2012). CCL21 (SLC) improves tumor protection by a DNA vaccine in a Her2/neu mouse tumor model. Cancer Gene Therapy 19: 69-76.

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Immune Regulation and Cancer

Human B cell malignancies represent a major medical problem because of their abundance and limitations of available therapies. We study B cell lymphomagenesis in comparison to normal B cell physiology, through genetic mouse models generated by conditional gene targeting. Recent achievements include the identification of antagonizing PI3 Kinase and FOXO1 activities as key elements of germinal center B cell differentiation, and their cooperativity in B cell lymphomagenesis. Additional new insights relate to the mechanism of immune surveillance of Epstein-Barr-Virus (EBV) infected and transformed B cells, as well as various aspects of normal B cell development including its control by miRNAs. A major new direction of our work comes from the availability of CRISPR/Cas-mediated targeted mutagenesis. We have improved this tool to introduce tailored mutations and insertions, and used it to generate genetically modified mice by zygote mutagenesis. A newly generated mouse strain allowing conditional expression of the Cas9 endonuclease is being used for genetic screens in primary cells, and we have added gene editing in human cells to our agenda.

The interplay between B cell antigen receptor and Toll-like receptors in the B cell proliferative response

K. Otipoby, A. Waisman*, E. Derudder, L. Srinivasan*, A. Franklin *Former group members*

We had earlier shown that the B cell antigen receptor (BCR) is a key survival determinant for mature B cells, depending on signals of the phosphoinositide 3-kinase (PI3K) pathway. We now demonstrated that when BCR-deficient B cells are kept alive by a Bcl2 transgene, they fail to initiate a proliferative response to Toll-like receptor (TLR) engagement (Otipoby et al. 2015). As in the case of BCR-dependent B cell survival, the proliferative response of the BCR-deficient cells could be rescued by PI3K signaling. Altogether a model emerges in which B cells integrate signals from the BCR (recognizing specific antigenic determinants) and co-receptors (responding to T cell help or pathogen-associated molecular patterns) in the control of their proliferative response. In Myc-transformed mouse B cells the BCR controls the competitive fitness of the tumor cells (collaboration with S. Casola, IFOM, Milan; Varano et al. Nature, in press 2017).

The germinal center reaction and B cell lymphomagenesis

S. Sander, V.T. Chu, T. Yasuda, A. Franklin, R. Graf, D. Calado, K. Imami+, M. Selbach+, M. Di Virgilio+, L. Bullinger+, E. Kabrani *Former group members +Collaborators*

In T cell-dependent immune responses antigen-specific B cells undergo successive rounds of proliferation and selection in histological structures called germinal centers (GCs). Proliferation is accompanied by somatic hypermutation (SHM) and class-switching (CSR) of the antibodies expressed by the cells, and cells expressing high-affinity antibodies are selected into the pool of memory B cells and long-lived plasma cells. Because of their extensive proliferation accompanied by DNA breaks introduced by SHM and CSR, GC B cells are at the origin of most B cell lymphomas. We had earlier modeled a typical GC-derived malignancy, Burkitt lymphoma, in mice, by targeted induction of c-MYC and PI3K activity in GCs (Sander et al. Cancer Cell 2012). The resulting lymphomas closely resembled human Burkitt, including tertiary mutations selected during tumor progression. Among the latter we discov-

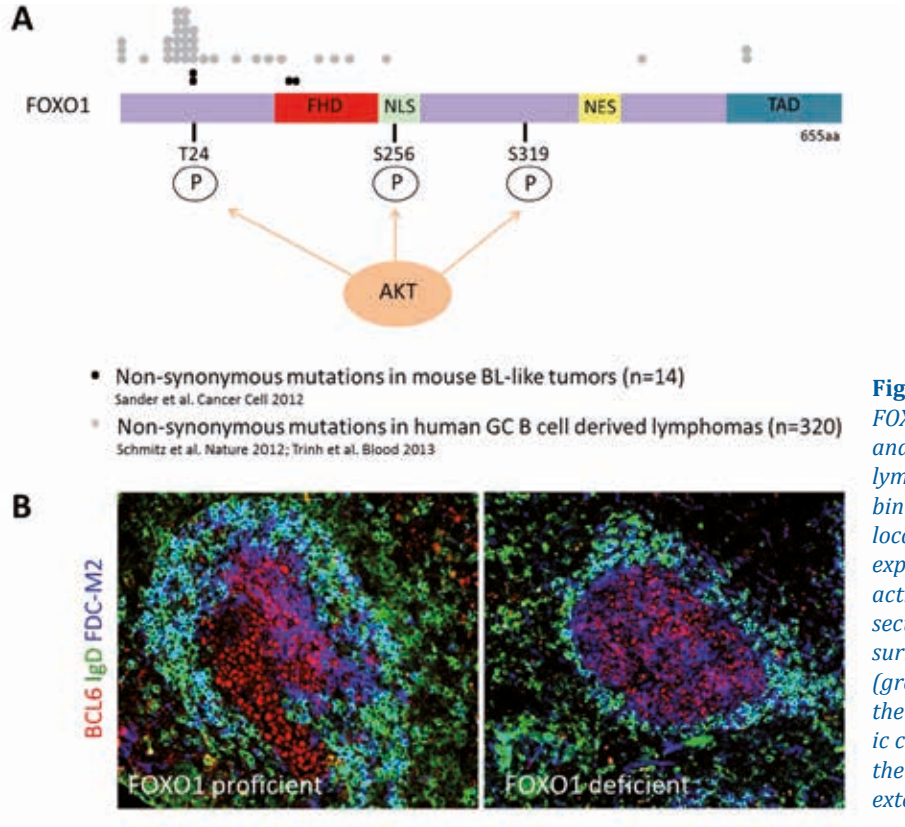

Figure 1. *(A) Non-synonymous FOXO1 mutations in murine and human GC B cell derived lymphomas (FHD, forkhead DNA-binding domain; NLS, nuclear localization signal; NES, nuclear export sequence; TAD, trans-activation domain). (B) Spleen sections showing GC B cells (red) surrounded by follicular B cells (green). In FOXO1 deficient GCs the network of follicular dendritic cells (blue) which demarcates the light zone in the wild type extends throughout the GC.*

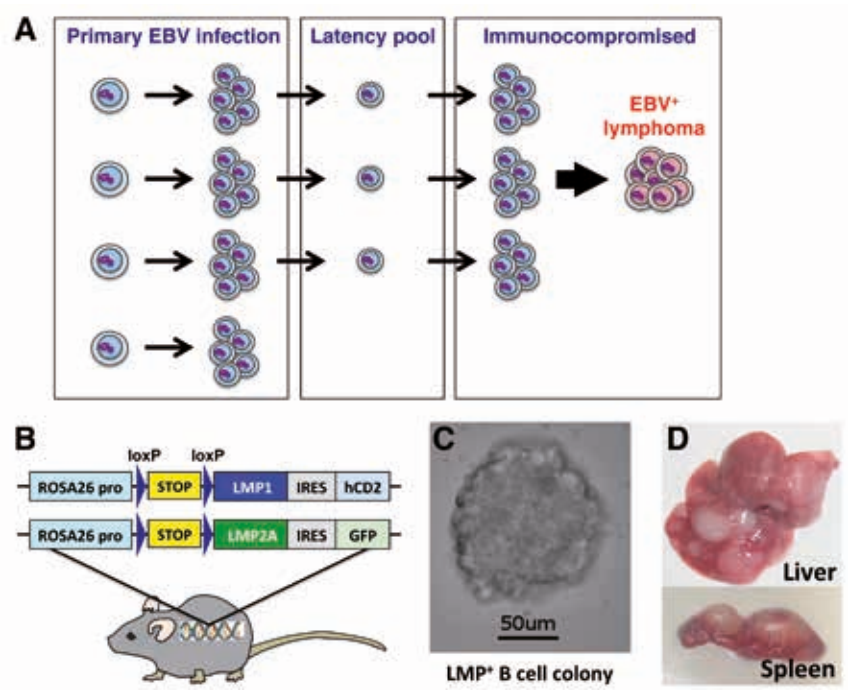

Figure 2. *(A) After primary EBV infection in humans, some latently infected B cells persist for life. In immunocompromised patients EBV+ B cells expand and lymphomas develop. (B) Mouse model for the conditional activation of the oncogenic EBV proteins LMP1 and LMP2A. Mouse B cells which have turned on LMPs form characteristic colonies similar to colonies of human EBV infected cells (C) and develop monoclonal lymphomas in immunocompromised mice (D).*

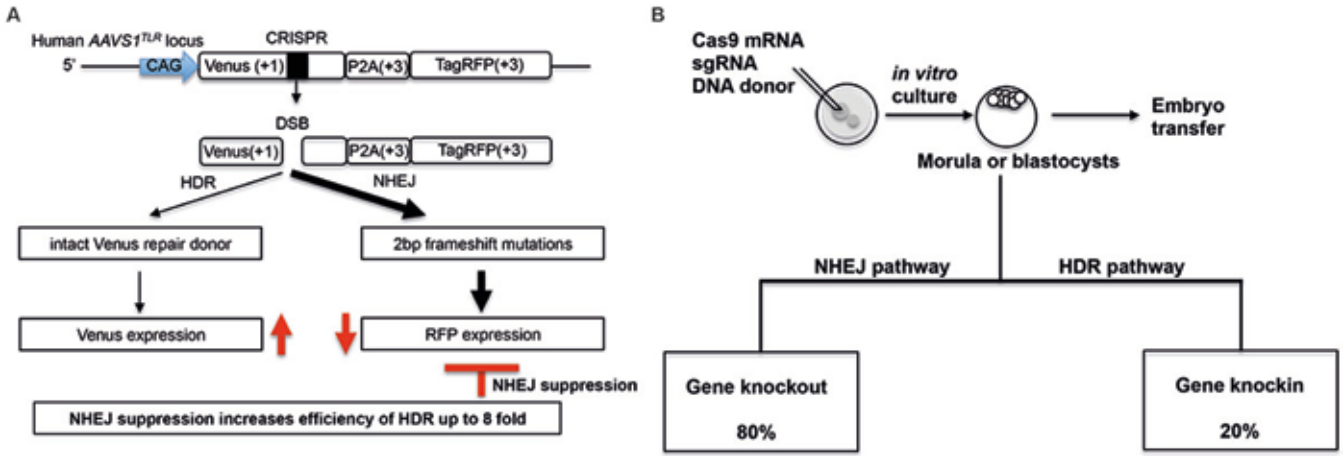

Figure 3. *CRISPR/Cas9-mediated mutagenesis. (A) A transgenic „traffic light" reporter (TLR) system to monitor DNA double strand break repair through NHEJ or HDR. NHEJ leads to RFP reporter expression, HDR in the presence of a Venus template to that of the Venus reporter. Suppression of NHEJ results in upregulation of HDR (red). (B) Scheme of C57BL/6 zygote mutagenesis. HDR observed in ~20% of mutagenic events. For details see text and Chu et al 2015; 2016.*

ered recurrent activating mutations of the FOXO1 transcription factor (TF; Fig. 1A). As this resulted in co-expression of FOXO1 and PI3K signaling, mutually exclusive in normal physiology where PI3K inactivates FOXO1, we investigated the role of FOXO1 and PI3K signaling in the GC reaction. We found a clear division of labor, with PI3K activity limited to the so-called GC Light Zone (LZ), where mutant B cells are selected, and FOXO1 nuclear expression largely restricted to the Dark Zone (DZ), where GC B cells proliferate and undergo SHM. FOXO1 turned out to be a master regulator of the GC DZ, controlling more than 60% of DZ-specific genes; in its absence, GC DZs were not formed (Fig. 1B), and, although B cell proliferation and SHM were unaffected, cellular selection and CSR in GCs were profoundly disturbed. We concluded that proper sequential proliferation/mutation and selection of GC cells is controlled by the PI3K-FOXO1 antagonism and essential for efficient antibody affinity maturation in GCs (Sander et al. 2015). Interestingly, the Burkitt-like lymphoma cells carrying activating FOXO1 mutations are dependent on concomitant PI3K and FOXO1 activity, as shown by CRISPR/Cas9 mediated targeted mutagenesis (unpublished data). Present studies explore the mechanism of this unusual cooperation and its role in lymphoma pathogenesis.

Mouse models of Diffuse Large B Cell Lymphoma and Multiple Myeloma

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Various attempts have been and are being made to model DLBCL and MM in mice. In a recent study we showed that a fraction of human DLBCLs exhibit activation of the alternative NF-kB pathway, and that activation of this pathway plays indeed an oncogenic role in a mouse model of DLBCL (Zhang et al. 2015). This complements previous evidence that activation of the canonical NF-kB pathway, together with other oncogenic events, drives DLBCL of the ABC subtype. Ongoing work explores the role of other recurrent mutations in DLBCL in tumor pathogenesis, such as activating mutations in signaling pathways emanating from the BCR and TLRs. We are also developing mouse models of MM and other tumor entities through sequential activation of oncogenic events recurrently identified in the human.

Epstein-Barr-Virus driven pathologies and immune surveillance; Hodgkin lymphoma (collaboration with the Janz/Mathas/Dörken group)

T. Yasuda, T. Sommermann, T. Weber, T. Wirtz, S. Li, M. Janz+

Since our discovery many years ago that the tumor cells in Hodgkin Lymphoma (HL), the so-called Hodgkin & Reed-Sternberg (HRS) cells, originate from pro-apoptotic GC B cells which, in EBV+ cases, have been rescued by expression of the EBV proteins LMP1 and LMP2A, we develop mouse models of EBV infection and the immune surveillance of EBV-infected B cells, as well as EBV pathologies such as X-linked Lymphoproliferative Syndrome (XLP), Post-Transplant Immunoproliferative Disorder (PTLD), and EBV+ B cell lymphomas including HL. Human B cells infected and transformed by EBV rapidly expand, but are efficiently eliminated by T and NK cells, leaving behind a small pool of latently infected cells. When immune sur-

veillance is compromised, the EBV infected cells cannot be controlled and lethal pathologies ensue (Fig. 2A). In earlier work we described that both EBV immune surveillance and pathologies can be modeled in mice by B cell-specific expression of the EBV proteins LMP1 and -2A (Zhang et al. Cell 2012; Yasuda et al. Cold Spring Harb Symp Quant Biol 2013). Fig. 2B-D shows our conditional, Cre recombinase dependent LMP alleles and the expansion of LMP$^+$ B cells and tumor formation in the absence of immune surveillance. Ongoing work explores the immunogenicity and T cell recognition of LMP$^+$ B cells and tumors, as well as the molecular mechanisms by which LMP$^+$ B cells develop into monoclonal lymphomas in vivo. We have also developed mouse models of acute EBV infection and a monogenic inherited fatal EBV-driven lymphoproliferative human disease. With respect to HL we refer the reader to the report of M. Janz and colleagues.

Gene editing in somatic cells and mouse zygotes

V.T. Chu, T. Weber, R. Graf, S. Sander, T. Sommermann, R. Kühn$^+$

Realizing the profound impact of the CRISPR/Cas9 revolution on our work, we have, in collaboration with R. Kühn, attempted to enhance homology-directed DNA repair (HDR) at the expense of non-homologous end joining (NHEJ) in CRISPR/Cas9-mediated mutagenesis, in order to optimize this approach towards the introduction of precise modifications into genomic sequences. Chemical or genetic inhibition of the NHEJ pathway indeed led to a strong increase of HDR and almost complete suppression of NHEJ (Fig. 3A; Chu et al. 2015, published together with a similar paper from the Ploegh lab at MIT). We also succeeded in the rapid generation of mice carrying knock-out or conditional alleles through CRISPR/Cas9 mutagenesis of C57BL/6 zygotes (Fig. 3B). Particularly useful for our future work is a strain carrying a conditional Cas9 allele. We have shown that this strain allows efficient targeted mutagenesis and genetic screens in primary cells through transfection of guide-RNAs (Chu, Graf et al. 2016). We are in the process of extending our work to gene editing in human cells.

Other work

MDC lab members involved: E. Derudder, A. Franklin, R. Graf, V. Labi, T. Yasuda

Due to space limitations, we refer the reader to the literature and only mention a few projects, which are close to completion. These relate to NF-kB signaling and BCR specificity in B cell homeostasis and B1/B2 subset determination, the role and functional impact of Tet enzymes in lineage-specific DNA demethylation (with Y. Bergmann and H. Ceder, Hebrew University), and an unexpected, vital role of miRNA seed matches in the ubiquitously expressed pro-apoptotic Bim gene (with C. Birchmeier, M. Landthaler and N. Rajewsky). An AID-related project was initiated by A. Franklin with O. Daumke and M. Di Virgilio.

Selected Publications

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Photo: David Ausserhofer/MDC

Armin Rehm

Translational Tumorimmunology

Studying cell biological mechanisms that define the capacity of T cells to mediate protection from infections or tumors, we have identified gene functions that amplify the secretion of effector molecules. Because a high local effector function of adoptively transferred T cells (ATT) is key for tumor eradication, this approach can complement current efforts to select for tumor-antigen specific T cells. Our strategies to boost T cell avidity and thus, improve ATT converge at the final steps of effector functions, as mediated by the secretion of cytotoxic molecules. This principle can be applied to either T cell receptor (TCR) or chimeric antigen receptor (CAR) -engineered T cells. Moreover, our work is devoted to the elucidation of tumor-stroma interactions in hematological diseases. We combine animal models, cellular and molecular biology techniques as well as gene expression profiling to gain insight into the complex network of tumor cells and a benign infrastructure within secondary lymphoid organs. These approaches are instrumental in gaining a profound understanding of mechanisms that cause lymphoma to be addicted to the local microenvironment.

Adoptive Immunotherapy: exploiting cell biological enhancers of the secretory pathway

In collaboration with Uta E. Höpken (MDC), Gerald Willimsky and Dana Hoser (Charité), Wolfgang Uckert and Mario Bunse (Humboldt-University, MDC), Thomas Willnow (MDC)

Enhancing the release of granzymes to target leukemias

Anthea Wirges, Julia Bluhm

Central to the efficiency of ATT is the amplification of functional avidity. Related to our previous results on the immune-tempering effects of EBAG9, we are analyzing whether its targeted deletion stimulates the cytolytic activity of CTLs against leukemias. We postulate that EBAG9-loss confers $CD8^+$ T cells with an enhanced cytolytic efficiency, allowing us to combine EBAG9-targeted deletion with TCR-engineering. Thus, EBAG9-deficiency provides T cells with improved sensitivity toward weak tumor antigens. The relationship between a T cell's heighthened cytolytic capacity and its predisposition to enter the memory T cell pool is under investigation.

Chimeric antigen receptor (CAR) T cells directed against target antigens expressed in hematologic malignancies

In collaboration with Uta E. Höpken (MDC) and Wolfgang Uckert (Humboldt-University)

Julia Bluhm

The advent of genetically modified T cells that express CARs has proven tremendous success in B cell leukemia treatment, despite the fact that patients were multiresistant against several lines of chemo- and antibody therapies. In these cases, ATT using CAR-T cells was administered successfully for salvage therapy. We have identified several types of B cell neoplasia which are currently incurable, and thus are suitable targets for the development of CAR-dependent immunotherapy. Based on antibody sequences, we designed CARs with an efficient in vitro and in vivo activity against Multiple Myeloma and other B-cell non-Hodgkin's lymphoma. This project is aimed at a clinical translation phase.

Tumor and microenvironment: Cellular and molecular prerequisites for onset and progression of leukemia and lymphoma

In collaboration with Uta E. Höpken, Achim Leutz (all MDC), Georg Lenz (University Hospital Münster), Holger Gerhardt and Anne-Clemence Vion (MDC)

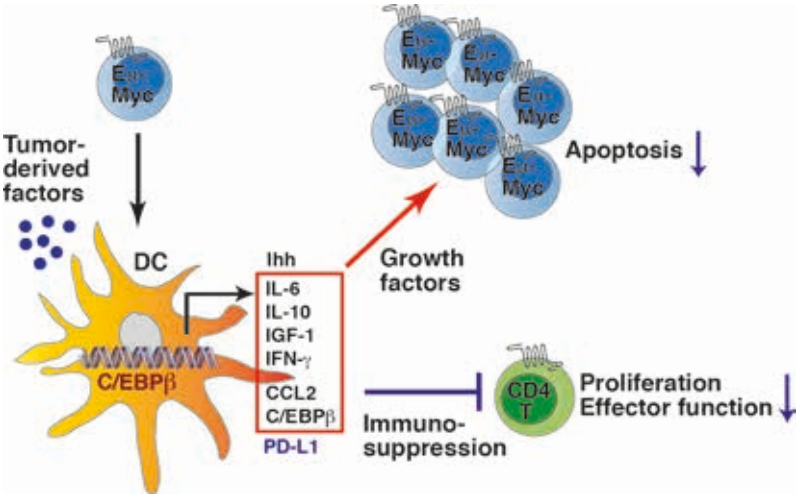

Figure 1. The transcription factor C/EBPβ controls the capacity of DCs to maintain Eμ-Myc B cell lymphoma survival. Tumor-derived factors induce a phenotyping skewing of DCs, characterized by C/EBPβ up-regulation and followed by cytokine and growth factor secretion. In addition, lymphoma-induced immunosuppression depends on the expression of C/EBPβ in context of DCs (for details, see Rehm et al. (2014), Nature Communications 5, 5057-5070)

Involvement of DCs and their transcriptional regulation in shaping a lymphoma survival niche

In an aggressive Myc-driven B cell lymphoma model tumor cells lodged within the T cell zone and intermingled with fibroblastic reticular cells (FRCs), but also with DCs. Functionally, deletion of DCs delayed progression of these lymphomas. Lymphoma-exposed DCs upregulated immunomodulatory cytokines, growth factors and the transcription factor C/EBPβ. Moreover, Eμ-Myc lymphomas induced the preferential translation of the LAP/LAP* isoforms of C/EBPβ. C/EBPβ-deficient DCs were unresponsive to lymphoma-associated cytokine changes and unable to improve Eμ-Myc lymphoma cell survival. T cell proliferation in lymphoma-bearing mice was impaired. This immune suppression was reverted by the DC-restricted deletion of C/EBPβ. Thus, we have identified the C/EBPβ-controlled DC functions as pivotal steps for the creation of a lymphoma growth promoting and immunosuppressive niche. More recently, we analyzed the chronic lymphocytic leukemia (CLL) B cell-imposed stromal alterations along their trafficking route including the splenic marginal zone, B cell follicle and FDC network. We performed gene expression profiling on laser capture microdissected spleens obtained from leukemic mice. A splenic tissue remodeling predominated by the expansion and polarized differentiation of monocytes and neutrophils was identified. This innate immune cell infiltration was not associated with a systemic inflammation. Instead, in established CLL disease macrophages exhibited an M2 polarization, whereas neutrophils were skewed toward a neutrophil B cell-helper-like phenotype. Myeloid cells provided additional growth factors, but were also involved in the structural integrity of the spleen necessary for leukemia B cell homing.

Remodeling of the lymph node vasculature during lymphomagenesis

Marleen Gloger, Lutz Menzel

Anti-angiogenic strategies have become an important therapeutic modality for solid tumors. However, attempts to combine multimodal chemo-/immunotherapies with VEGF inhibitors showed no benefit in non-Hodgkin's lymphoma. Lymph nodes are equipped with specialized endothelial cell subsets that line the blood and lymphatic vasculature. To dissect lymphoma-induced alterations, we applied gene expression profiling of endothelial cells and FRCs. Selected gene functions have been validated in mouse models and on human lymphoma specimen. To elucidate lymph node-specific peculiarities, we are exploring whether vascular growth pattern in lymphoma are different from solid tumors. In addition, we aim to identify metabolic adaptations of normally quiescent endothelial cells during lymphomagenesis.

Selected Publications

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Herda, S., Raczkowski, F., Mittrücker, H.-W., Willimsky, G., Gerlach, K., Kühl, A.A., Breiderhoff, T. Willnow, T.E., Dörken, B., Höpken, U.E., Rehm, A. (2012). The sorting receptor Sortilin exhibits a dual function in exocytic trafficking of interferon-γ and granzyme A in T cells. Immunity 37, 854-866

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Wichner, K., Stauss, D., Kampfrath, B., Krüger, K., Müller, G., Rehm, A., Lipp, M., Höpken, U.E. (2015). Dysregulated development of IL-17 and IL-21 expressing follicular helper T cells and increased germinal center formation in the absence of RORγt. FASEB J. 30, 761-774

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Oliver Rocks

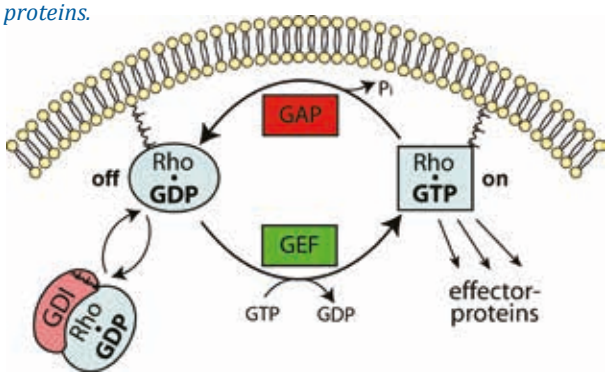
Spatio-temporal control of Rho GTPase signaling

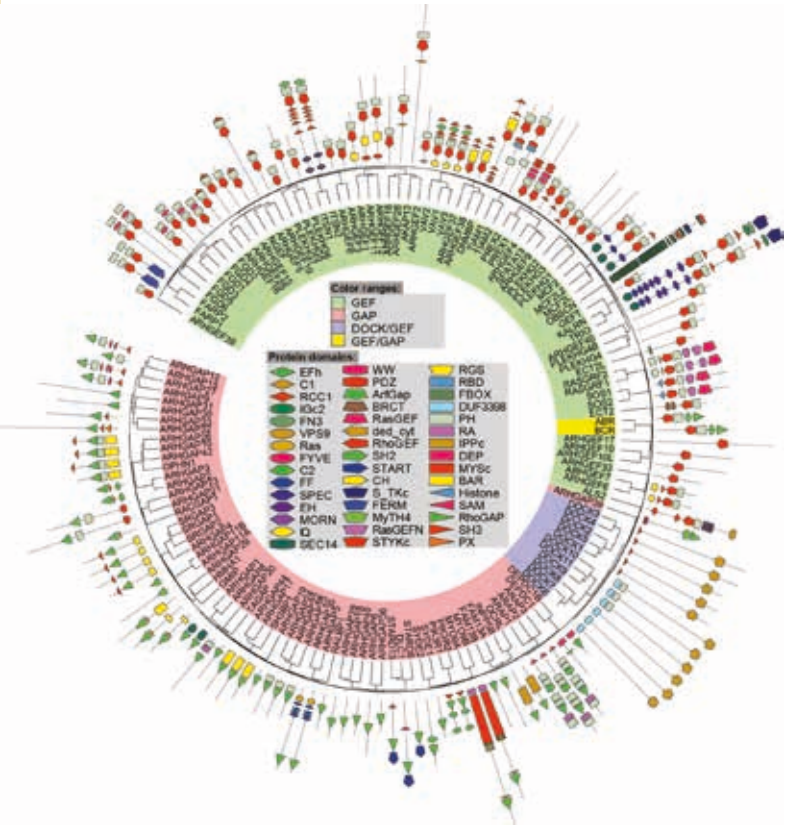
Cells frequently change their shape and their attachment to the environment in order to move or to move or to adopt to specialized tissue structures. These events are driven by coordinated changes of the cytoskeleton and are fundamental during embryonic development, immune surveillance or wound repair. Our lab is interested in the family of Rho GTPase proteins which have emerged as master regulators of the cytoskeleton. We combine advanced microscopy with cell biological and genetic approaches to study the precision control that ensures proper Rho signaling. Specificity of Rho GTPase function is achieved by the huge family of RhoGEF and RhoGAP regulatory proteins. We aim to study in detail how these proteins couple Rho activity to specific environmental cues and functional contexts in a cell. Our lab also investigates the dynamics of local Rho signaling complex formation at the membrane at the single molecule level. Insights into this spatio-temporal regulation of Rho signaling will provide a deeper understanding of morphodynamic processes in cells and organisms, both in normal and disease settings.

Signaling Specificity of Rho GTPases

Rho GTPases are molecular switches that cycle between an 'on' and an 'off' state. Only in the activated state they bind effector proteins and thereby relay incoming signals further downstream of a signaling pathway. This cycle is controlled by three classes of regulatory proteins: the large families of activating RhoGEFs (Rho guanine nucleotide exchange factors) and inactivating RhoGAPs (Rho GTPase activating proteins), and by RhoGDI proteins (Rho guanine nucleotide dissociation inhibitors), which sequester the membrane anchored Rho GTPases into the cytosol (Fig. 1). The Rho family comprises about 20 genes, with the most prominent members RhoA, Rac1 and Cdc42. Numerous Rho-regulated signaling pathways have been outlined over the past years, many of which drive central aspects of cellular morphodynamics. The different members of the Rho family thereby have distinct functions and can crosstalk with each other, resulting in a complex interplay of pathways. A precise spatio-temporal control of their activities is thus essential. The major challenge in the field is to understand how this signaling specificity is achieved by the RhoGEF/GAP/GDI regulatory proteins. These mechanisms are of great clinical relevance since deregulated Rho signaling is a key contributor to cancer metastasis and many other diseases.

Schematic representation of the molecular switch function of Rho GTPases and the function of their regulatory proteins.





Modular domain architecture of the human RhoGEF and RhoGAP proteins.

RhoGEFs and RhoGAPs

The genome encodes over 145 different RhoGEFs and GAPs which encode a diverse spectrum of subcellular targeting and protein interaction domains, besides their catalytic domains (Fig. 2). This multidomain architecture enables them to assemble specific protein complexes to target Rho activity to critical sites of cytoskeletal rearrangement, such as focal adhesions, cell junctions, endocytic vesicles or mitotic spindles. Likewise, the regulators can also connect Rho GTPases to other signal transduction pathways. However, the majority of RhoGEFs and GAPs is only poorly characterized and the cellular repertoire of spatio-temporal control of Rho GTPases not fully explored. Therefore, we have recently carried out a first systematic characterization of all mammalian RhoGEFs and GAPs, yielding a complete cDNA library of all regulators and datasets of their interactome, subcellular localization, overexpression and knockdown phenotypes and substrate specificities. We now exploit this unique resource 'one the one hand to study the control of Rho signaling at the systems level and on the other hand to characterize individual RhoGEFs and GAPs implicated in different signaling contexts. These are currently cell adhesion, guided cell migration and G protein coupled receptor/cAMP signaling'. To this end, we use live cell microscopy and biosensors to obtain information on appropriate spatial and temporal microscales and employ model organisms that closely reflect the underlying biological processes in vivo. We aim for a broader understanding of the function of the context-specific signaling scaffolds, signaling scaffolds, 'how they dynamically assemble, how they relay signals to the cytoskeleton, and how they communicate with other cellular signaling pathways'.

Temporal framework of Rho GTPase signaling

Very little is known also about the temporal control of Rho signaling. Most Rho family GTPases are lipid modified and need to be membrane associated to properly exert their signaling function. Exactly how on membranes the formation of Rho signaling complexes is initiated, maintained and terminated and whether a precise temporal control is important for proper signaling is unclear and technically difficult to be studied in cells. We employ state-of-the-art imaging techniques at the single molecule level to analyze the dynamics of membrane interaction of Rho GTPases and its modulation by regulatory and effector proteins. We thereby aim to elucidate the temporal framework in which Rho signaling complexes operate and to find novel modes of signal regulation.

Selected Publications

Eccles, RL., Czajkowski, MT., Barth, C., Müller, PM., McShane, E., Grunwald, S., Beaudette, P., Mecklenburg, N., Volkmer, R., Zühlke, K., Dittmar, G., Selbach, M., Hammes, A., Daumke, O., Klussmann, E., Urbé, S., **Rocks, O.** (2016) Bimodal Antagonism of PKA signaling by ARHGAP36. **Nat Commun.** Oct 7;7:12963. doi: 10.1038/ncomms12963.

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Rocks, O., Peyker, A. & Bastiaens, PI. (2006) Spatio-temporal segregation of Ras signals: one ship, three anchors, many harbors. **Curr Op Cell Biol** 18(4):351-7

Rocks, O., Peyker, A., Kahms, M., Verveer, PJ., Koerner, C., Lumbierres, M., Kuhlmann, J., Waldmann, H., Wittinghofer, A. & Bastiaens, PI. (2005) An Acylation Cycle Regulates Localization and Activity of Palmitoylated Ras Isoforms. **Science** 307:1746-1752

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Photo: David Ausserhofer/MDC

Claus Scheidereit

Signal Transduction in Tumor Cells and Development

Our laboratory focuses on gene expression and signal transduction processes, which are involved in tumorigenesis and development. The nuclear factor-κB (NF-κB) transcription factors are critical regulators of inflammation, immunity, stress response, cell proliferation, differentiation and survival. NF-κB activity is controlled by inhibitory IκB proteins and the IκB kinase (IKK) complex. The NF-κB/IκB/IKK signaling system is of relevance for the pathogenesis of a wide spectrum of human diseases.

The molecular constituents of IKK and NF-κB signaling cascades

The NF-κB family comprises NFKB1 (p50/p105), NFKB2 (p52/p100), RelA (p65), c-Rel and RelB, which form homo- and heterodimers and regulate numerous physiological processes by controlling inducible gene expression programs. NF-κB activity is regulated by IκB proteins, e.g. the cytoplasmic inhibitor IκBα or the nuclear co-activators Bcl-3 and IκB-ζ. Two NF-κB subunits, p50 and p52, are generated by proteasomal processing of their precursor proteins, p105 and p100, respectively.

NF-κB activation requires the IκB kinase (IKK) complex, composed of two serine-threonine kinases (IKKα and IKKβ) and the regulatory subunit IKKγ (also known as NEMO). The IKK complex integrates a wide variety of extra- and intracellular signals to catalyze phosphorylation of various IκB and NF-κB proteins as well as of other substrates. NF-κB signaling can be divided into distinct sub-pathways, which activate specific family members. Canonical signaling cascades are activated by various cell-surface and intracellular receptors with rapid kinetics. Signal transmission involves the formation of K63-linked and linear polyubiquitin chains at TRAF or RIP proteins, which mediate recruitment of regulatory protein components, including the IKK complex. The latter is activated via phosphorylation of activation loop serines. Catalytically active IKK triggers degradation of IκBs and liberation of prototypic p50-p65. Negative feedback regulation of canonical signaling pathways is provided by NF-κB dependent expression of IκBα and deubiquitinating enzymes, such as A20 or CYLD.

Non-canonical NF-κB signaling is induced by a specific set of receptors, classically triggering C-terminal processing of NF-κB2/p100 (see below). The non-canonical pathway proceeds with a slow kinetics and requires NF-κB-inducing kinase (NIK) and IKKα. In unstimulated cells NIK expression levels are kept low by a destruction complex containing TRAF and cIAP proteins.
A third IKK pathway is activated by DNA double strand breaks and depends on nuclear shuttling and SUMO-modification of IKKγ, as well as on the kinase ATM.

Processing of NF-κB precursors p105 and p100

(Patrick Beaudette, Inbal Ipenberg, Buket Yilmaz)

NF-κB p100 and p105 are the precursors of their processing products p52 and p50, but can also act as cytoplasmic IκBs, blocking nuclear translocation and DNA-binding of other Rel/NF-κB family members. The mechanisms that control processing or complete destruction of the precursor

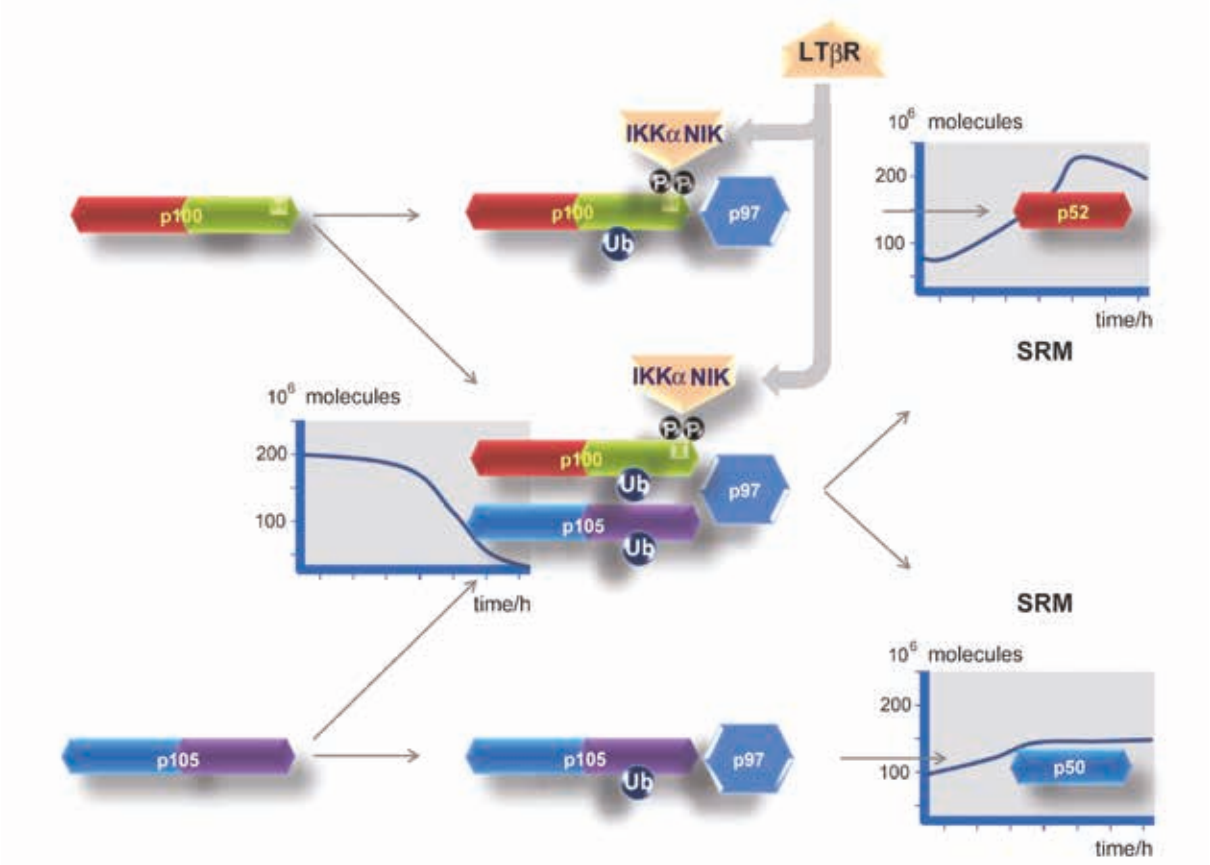

Figure 1: *The LTβR pathway triggers interdependent proteolysis of the NF-κB precursor proteins p100 and p105. NIK and IKKα regulate p105 proteolysis through C-terminal degron serines in p100. The co-generation of p50 and p52 depends on VCP/p97 ATPase activity. Quantitative mathematical models predict direct signal responsiveness of p100-p105 complexes.*

proteins by the ubiquitin-proteasome system have been considered as independent events; however, they remain poorly understood. Using mass-spectrometry-based quantitative analyses (cooperation with Gunnar Dittmar) we could demonstrate that activation of non-canonical NF-κB signaling by LTβR stimulates simultaneous processing of both p100 and p105. The precursors form hetero-complexes. Stimulus-dependent proteolysis strictly depends on the signal response domain (SRD) of p100, suggesting that the SRD-targeting proteolytic machinery acts in cis and in trans. Separation of cellular pools by isotope labeling revealed synchronous dynamics of p105 and p100 proteolysis – predominant degradation of preexisting precursors and processing of newly synthesized molecules (Yilmaz et al. 2014). In cooperation with Jana Wolf and colleagues, quantitative mathematical models were developed that describe the dynamics of the system and predict that p100-p105 complexes are signal responsive (Yilmaz et al. 2014; Figure 1).

It is still puzzling how partial proteasomal proteolysis of the NF-κB precursors can be achieved. We could demonstrate that both precursors interact with the ATPase VCP/p97, a well-known modulator of various protein degradation and processing processes. We could show that VCP/p97 ATPase activity is required to promote LTβR-triggered processing of p100 and p105 (Yilmaz et al. 2014; Figure 1). Simultaneous processing of p100 and p105 might have important implications for the regulation of target genes and for the physiological and pathological functions of precursor-derived NF-κB subunits (see below).

Canonical and non-canonical NF-κB/IKK gene networks in lymphomagenesis

(Linda von Hoff, Eva Kärgel, Kívia A. Pontes de Oliveira)

Deregulation of NF-κB and IκB family members contributes to various lymphoid malignancies via promoting, amongst others, proliferation and survival. However, it remained unknown whether and how different NF-κB dimers and nuclear IκBs regulate distinct sets of target genes and control selective oncogenic functions.

We could demonstrate that Hodgkin's lymphoma (HL) cell lines as well as primary Hodgkin and Reed-Sternberg cells display high constitutive activity of IKK/NF-κB, which triggers cell survival and growth by

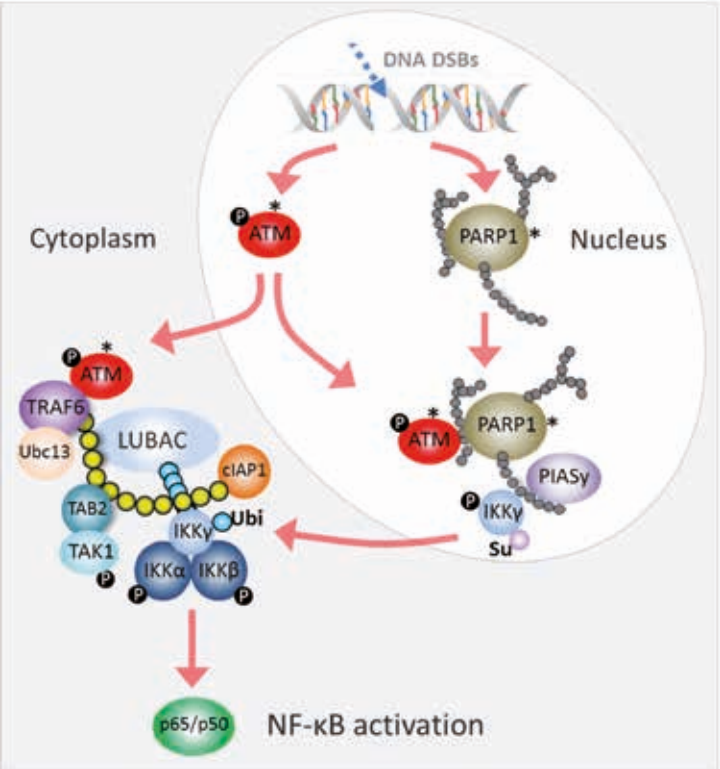

Figure 2: *DNA damage activates NF-κB through ATM and PARP-1 signaling. PARP-1 is activated and sequesters PIASy, ATM and IKKγ, which shuttles between nucleus and cytoplasm, resulting in IKKγ SUMOylation. After cytoplasmic export, ATM binds to TRAF6 to induce K63-linked poly-Ub formation. Subsequent steps are TAK1 activation, IKKγ mono-ubiquitination and IKK activation. DNA damage induced NF-κB regulates cellular apoptosis and senescence and may cause chemotherapy resistance.*

inducing an anti-apoptotic and pro-proliferative gene program. HL cells display constitutive canonical as well as non-canonical IKK/NF-κB signaling and overexpress Bcl-3. We found that processing of NF-κB precursors p100 and p105 occurs in a simultaneous, NIK-dependent manner, as observed for LTβR signaling in mouse embryo fibroblasts (see above). To determine cistromes and target gene signatures of canonical as well as non-canonical NF-κB species and of Bcl-3, we performed ChIP-sequencing and microarray analyses. We found that p50-RelA and p52-RelB activate or repress transcription of distinct genes and have selective as well as overlapping oncogenic functions. The type of gene regulation is determined by the distance and subunit occupancies of NF-κB binding regions. The gene regulation patterns distinguish Hodgkin lymphoma from other NF-κB-associated malignancies and may contribute to the understanding of lymphomagenesis (de Oliveira et al. 2016).

Mechanism of IKK and NF-κB activation by genotoxic stress

(Cristina Brischetto, Daniel Heinze, Michael Hinz, Marina Kolesnichenko, Nadine Mikuda, Michael Willenbrock)

DNA lesions evoke multiple cellular responses including DNA repair, cell cycle checkpoint control as well as activation of the transcription factors NF-κB and p53, which are believed to mediate largely opposing cellular reactions. While p53 induces cell-cycle arrest or cell death, NF-κB promotes cell survival. To analyze the functional impact of both transcription factors in more detail, we performed global gene expression studies and observed an unexpected interdependence between NF-κB and p53 mediated gene regulation. The mechanistic details of this cross-talk are currently under investigation.

Recent findings from our group and others have led to an improved understanding of the DNA damage induced NF-κB signaling pathway (Hinz et al. 2010). We showed that activation of IKK and NF-κB in response to DNA double strand breaks requires a bifurcated pathway triggered by poly(ADP-ribose)-polymerase-1 (PARP-1) and ataxia telangiectasia mutated (ATM) as DNA strand break reading heads (Figure 2). The genotoxic IKK pathway depends on unique modifications, transport processes and protein complex formations that are not involved in "classical" IKK pathways, including NEMO-SUMOylation, nuclear export of ATM, and PAR-dependent protein interactions. These specific requirements of the DNA damage induced NF-κB signaling cascade offer the possibility to find genotoxic pathway-selective inhibitors, which might have the potential to be used to overcome therapy resistance in cancer treatments. In cooperation with the screening unit of the Leibniz-Institute for Molecular Pharmacol-

ogy (FMP) we could identify small molecular weight compounds, which interfere selectively with DNA damage induced NF-κB activation. Further preclinical studies such as target validation and testing candidate inhibitors are ongoing (collaboration with Jens von Kries and Marc Nazaré).
Yet, several steps of the genotoxic signaling cascades that activate IKK are yet poorly understood and unknown essential components and activating as well as negative feedback regulators are likely involved. We utilized SILAC-based mass spectrometry strategies to identify novel pathway regulators. In a parallel study, based on a cooperation with the groups of Udo Heinemann, Markus Landthaler and Jana Wolf, it could be demonstrated that the RNA decay-promoting protein RC3H1 (also known as Roquin) binds preferentially to short-lived and DNA damage-induced mRNAs, indicating a role of this RNA-binding protein in the post-transcriptional regulation of the DNA damage response. Notably, RC3H1 affects expression of the NF-κB pathway regulators IκBα and A20. Knockdown of RC3H1 resulted in increased A20 protein expression, thereby interfering with IκB kinase and NF-κB activities, demonstrating that RC3H1 can modulate the activity of the IKK/NF-κB pathway (Murakawa et al. 2015).

Role of Eda-A1/Edar/NF-κB signaling in development and organ regeneration

(Karsten Krieger, Ruth Schmidt-Ullrich, Philip Tomann) *Principal Scientist*

To elucidate the in vivo function of NF-κB in embryonic development and organ regeneration, various loss-of-function, gain-of-function and reporter mouse models were generated in our laboratory. We previously demonstrated a key role for NF-κB in the induction of hair follicle and secondary lymph node development and in the morphogenesis of ectodermal organs such as teeth or mammary glands. Further studies mainly used the hair follicle as a model organ. Essential signals that regulate early hair follicle development include WNT/β-catenin, as well as TNF family member ectodysplasin A1 (EDA-A1) and its receptor EDAR. We demonstrated an interdependence of WNT/β-catenin and EDA-A1/EDAR/NF-κB signaling which is required for focal hair bud formation and patterning, as well as hair bud down-growth.
To unravel further functions of NF-κB in ectodermal organogenesis, we have investigated NF-κB-dependent target gene signatures during hair follicle development, which revealed that NF-κB has a multifunctional role in hair follicle induction, including ECM remodeling, orchestration of WNT, SHH and TGFβ2 signaling and regulation of the stem cell marker LHX2. We showed that the NF-κB-LHX2-TGFβ2 signaling axis controls processes essential for placode down-growth, such as E-cadherin down-regulation (Toman et al. 2016). Furthermore, we were able to define a key function for NF-κB in hair follicle regeneration (hair cycle) and in regeneration of the intestinal epithelium.

Selected Publications

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Photo: private

Clemens A. Schmitt

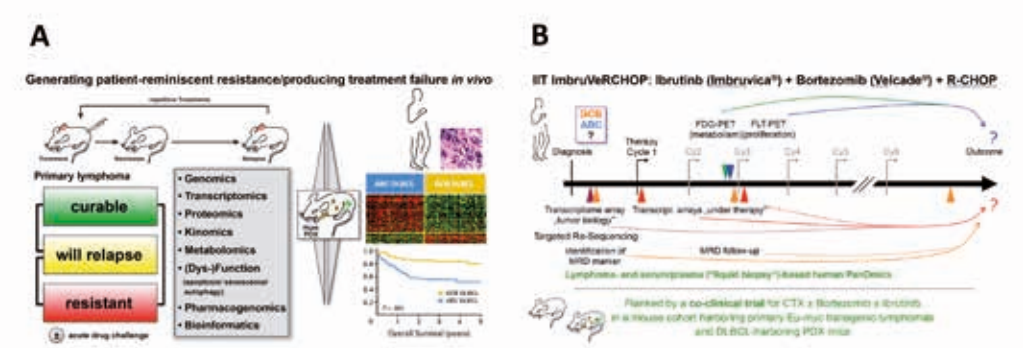
Cancer Genetics and Cellular Stress Responses in pathogenesis and treatment of lymphatic malignancies

The increasingly faster and detailed molecular dissection of cancer on one side and the rapidly growing list of lesion-based therapeutic agents on the other side are about to make the vision of "personalized cancer precision medicine" a clinical reality, although many obstacles need to be overcome before. In particular, tumor heterogeneity, bioinformatics processing of multiple omics data layers and their functional interpretation remain major challenges. We not only employ genetically tractable mouse models of cancer and apply integrative "cross-species" bioinformatics, but focus on conceptually novel – synthetic lethal and "restore & target" – therapies whose target principles are cell states with their associated vulnerabilities (such as cellular senescence) rather than single molecular lesions.

Mechanistic dissection, functional modeling, and clinical exploitation of DLBCL-derived pathogenetic components (e.g. the BCR/NF-κB signaling network) in lymphoma development, CNS tropism, and therapy

Soyoung Lee, Maurice Reimann, Aitomi Bittner, Animesh Battacharya and collaboration partners (Claus Scheidereit, Jana Wolf, Nikolaus Rajewsky and others)

Hyperactivating mutations in the B-cell receptor (BCR)/NF-κB cascade are frequently found in human diffuse large B-cell lymphoma (DLBCL), and particularly enriched for in those primarily or secondarily localizing to the CNS. NF-κB may be oncogenic with respect to cell survival and inflammation, but also tumor-suppressive regarding its role in mediating cellular senescence. We modeled distinct DLBCL-derived NF-κB mutations *in vivo*, where they accelerated Eμ-*myc* transgenic lymphoma development. Moreover, we found lymphomas with associated CNS manifestation to overexpress an NF-κB target gene signature, and, *vice versa*, distinct NF-κB mutants to promote lymphoma CNS tropism. Next, we will functionally dissect NF-κB-governed effector mechanisms, and seek to identify novel vulnerabilities (as therapeutic "Achilles' Heels") in reverse genetics models, as well as in our "PanOmics"-dissected forward omics-based drug (in)sensitivity model and currently expanded repository of DLBCL material-based patient-derived xenograft (PDX) mouse models (Fig. A). Importantly, "co-clinical trial-like" functional and multi-omics-based analyses in mouse models accompany a new investigator-initiated trial headed by this group leader, in which previously untreated DLBCL patients receive the Bruton's tyrosine kinase inhibitor Ibrutinib (Imbruvic®) and the proteasome blocker Bortezomib (Velcade®) as a proximal/distal BCR/NF-κB double-targeting "novel/novel" small compound expansion of the anti-CD20 Rituximab plus CHOP standard immunochemotherapy backbone (termed the "ImbruVeRCHOP" study), and undergo an intense multi-omics-based molecular exploration prior to and during exposure to the trial medication (Fig. B).



Data-dense "PanOmics" investigation of drug (in)sensitivity in a transgenic mouse lymphoma model followed by cross-species exploitation in human data sets and humanized PDX mouse models (A). Data-dense multiple-omics-based analyses of DLBCL patients in the ImbruVeRCHOP IIT, flanked by mouse model (transgenic and PDX)-based "co-clinical trials" that clinically and molecularly dissect components of the poly-agent regimen in order to identify determinants of sensitivity and resistance (B).

The repressive H3K9me3 mark as essential senescence relay and therapeutic target in lymphoma and melanoma

Yong Yu, Bin Yue, Maja Milanovic Jan R. Dörr, Soyoung Lee, and collaboration partners

Oncogene-induced senescence (OIS) is a terminal cell-cycle arrest that precludes pre-malignant cells from further expansion *via* transcriptional silencing of proliferation-related genes in senescence-associated heterochromatin foci (SAHF), which are characterized by the repressive trimethylated histone H3 lysine 9 (H3K9me3) modification. We now found H3K9-active demethylases – namely the Jumonji domain containing 2C (JMJD2C) and the lysine-specific demethylase-1 (LSD1) – to disable senescence and permit direct transformation under oncogenic Ras or Braf. Accordingly, melanoma cell lines and primary melanoma samples present with high-level H3K9 demethylase activity, and respond to targeted inhibitor therapy *in vitro* and *in vivo* with senescence restoration. Similarly, H3K9-active demethylase expression countered therapy-induced senescence (TIS) in lymphoma. However, detrimental features of senescent cells, such as associated reprogramming into cancer stemness, ask for their secondary co-therapeutic elimination, a conceptually novel strategy we are currently investigating ("Enforce & Eliminate").

Pharmacological restoration and therapeutic targeting of the B-cell phenotype in classical Hodgkin's lymphoma

Yong Yu, Kolja Schleich, Soyoung Lee, and collaboration partners (Jens Peter von Kries, Stephan Mathas)

Classical Hodgkin's lymphoma (cHL), although originating from B-cells, is characterized by the virtual lack of gene products whose expression constitutes the B-cell phenotype. Epigenetic repression of B-cell-specific genes was previously reported to contribute to the lost B-cell phenotype in cHL. Restoring the B-cell phenotype may not only correct a central malignant property, but also render cHL susceptible to clinically established therapies tailored for B-cell Non-Hodgkin's lymphoma (B-NHL), thereby broadening (chemo-free) therapeutic options for cHL. In collaboration with the FMP, we successfully conducted a high-throughput pharmacological screening to identify drugs that promote re-expression of the B-cell phenotype in cHL cells and sensitized them to clinically approved antibodies (such as anti-CD20 Rituximab) and small compound inhibitors (such as Ibrutinib and Idelalisib) – thereby providing proof-of-efficacy for this conceptually novel "Restore & Target" strategy.

Selected Publications

Milanovic, M., J.H.M. Däbritz, Z. Zhao, J.R. Dörr, Y. Yu, L. Dimitrova, D. Lenze, M. Metzner, K. Pardon, B. Dörken, M. Hummel, A. Trumpp, and C.A. Schmitt. 2016. Senescence-associated reprogramming promotes cancer stemness. *In revision.*

Yu, Y., B. Yue, S. Ji, P. Lohneis, L. Li, D. Dhawan, N. Qutob, M. Werner-Klein, M. Reimann, A. Elkahloun, S. Treitschke, B. Dörken, F. Mallette, Y. Samuelis, C.A. Schmitt, and S. Lee. H3K9 demethylases disable oncogene-induced senescence and serve as therapeutic targets for senescence restoration in melanoma. *In revision.*

Du, J., M. Neuenschwander, Y. Yu, J.H.M. Däbritz, N.-R. Neuendorff, K. Schleich, A. Bittner, M. Milanovic, G. Beuster, S. Radetzki, E. Specker, M. Reimann, F. Rosenbauer, S. Mathas, P. Lohneis, M. Hummel, B. Dörken, J.P. von Kries, S. Lee, and C.A. Schmitt. 2016. Pharmacological restoration and therapeutic targeting of the B-cell phenotype in classical Hodgkin's lymphoma. **Blood**. *Epub ahead of print.*

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Photo: J.-M. Huron

Michael Sieweke

Stem cell and macrophage biology

Our research is located at the interface of immunology and stem cell research, with the overarching question of how cell identity and cell numbers are controlled. In many organs of the body cells are frequently lost and need to be replaced as part of normal tissue maintenance or in response to injury. In most cases new functional cells are generated from tissue-specific stem cells that are themselves un-specified but can self-renew. As stem cells differentiate into functionally mature cells, this self-renewal capacity is typically lost. Macrophages, a specific cell type of the immune system, represent a rare exception to this pathway, since they can be maintained independently of blood stem cells by local proliferation of mature cells. We investigate the mechanisms that enable such self-renewal without loss of differentiated function. The manipulation of macrophage self-renewal might have therapeutic benefit in cancer, inflammatory disease and tissue regeneration. Insight into the underlying mechanisms could provide methods for the generation of large numbers of macrophages, as source of defined cellular therapies for these applications. Finally, the understanding of the difference of normal self-renewal to tumorigenic transformation is also of critical importance for cancer research.

Macrophages as tissue guardians

Macrophages have recently entered the limelight of immunology and beyond (Geissman et al., Science 2010, Sieweke and Allen, Science 2013), because they are involved in many physiological and pathological processes of major interest beyond their immune functions, such as regeneration, cancer, inflammatory and metabolic diseases. Macrophages are present in essentially every tissue of the body, where they can play trophic roles in tissue homeostasis, metabolism and repair. Macrophages cannot only originate from hematopoietic stem cells via blood monocyte intermediates but can also be derived from embryonic progenitors and be maintained long term in tissues by local proliferation (reviewed in Sieweke and Allen, Science 2013). Macrophages could thus be considered systemic tissue guardians and changes in their self-renewal capacity could have major impact on health and disease of parenchymal cells. In this framework we investigate the development and maintainance of tissue macrophages by differentiation from hematopoietic stem cells or local self-renewal and the role of macrophage activation states in inflammation and tissue regeneration.

Stimulation of macrophage differentiation from hematopoietic stem cells

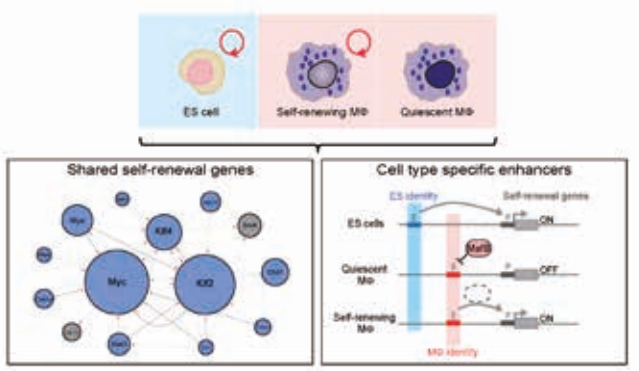
We could show that M-CSF, a myeloid cytokine important for macrophage proliferation and polarization can also act directly on hematopoietic stem cells to stimulate the myeloid differentiation pathway that gives rise to macrophages. Previously it had been widely assumed that cell fate choice in stem cells is largely determined by stochastic mechanisms. Using multiple methods of single cell analysis we could demonstrate that M-CSF can directly instruct hematopoietic stem cells (HSC) to adopt a myeloid fate (Mossadegh,Sarrazin et al., Nature 2013) in an integrated circuit with the transcription factor MafB (Sarrazin et al., Cell 2009). These mechanisms appear to be active in emergency hematopoiesis when physiological levels of M-CSF are high, such as during infections. We are now developing projects to investigate how this influences mature macrophage populations and could show that the mechanism can be used to treat neutropenic conditions after hematopoietic stem cell transplantation (Kandalla, JEM 2016).

Macrophage self-renewal activates gene network shared with stem cells

We previously discovered that deficiency for the macrophage transcription factors MafB and cMaf enables self-renewal of differentiated functional macrophages without malignant transformation or stem cell intermediates (Aziz et al, Science 2009). We have now investigated the gene regulatory mechanisms that enable macrophage self-renewal capacity to be integrated into the overall program of epigenetic macrophage identity by ChiPseq analysis of gene regulator enhancer elements. Our results demonstrate that self-renewal in macrophages involves MafB/cMaf down-regulation and concomitant activation of a self-renewal gene network that is centered on Myc and Klf2. We observed that the network is shared with embryonic stem cells but controlled from a completely distinct set of cell type specific enhancers (Soucie et al. Science 2016, fig.1). Macrophage enhancers associated with self-renewal genes are already present in quiescent cells but can become activated when direct repression by Maf transcription factors is relieved either by genetic inactivation or in resident macrophage populations that express constitutively or transiently low levels of MafB and cMaf. Our findings provide a general molecular rationale for the compatibility of self-renewal and differentiated cell functions. We also observed that these processes are relevant for maturation of resident macrophages, such as microglia in vivo (Matkovitch et al. Science 2016).

Macrophages in tissue regeneration

It has been demonstrated recently in several model organisms and organs that macrophages are required for tissue regeneration but the relative contribution of self-renewing tissue macrophages and macrophages derived from infiltrating inflammatory monocytes has remained unknown.Investigating the heart as a highly relevant in vivo system for understanding the regulation of tissue regeneration, we have shown that embryo-derived cardiac macrophages show declining self-renewal with age and are progressively substituted by monocyte-derived macrophages. Replacement of embryo-derived cells by less proliferative monocyte-derived macrophages coincides with the loss of functional regeneration capacity of the heart and might therefore be an important factor in this process (Molawi et al., JEM 2014). We are currently developing projects investigating this question and extending it to other organs such as the lung.



The self-renewal potential of both embryonic stem cells and differentiated macrophages is dependent on a shared network of self-renewal genes (left) that are controlled by distinct, lineage-specific enhancers (right). In quiescent macrophages, the transcription factor MafB binds and represses these enhancers. Loss of MafB expression results in enhancer activation and enables macrophage self-renewal. (Figure: Serena Billie)

Patents / Patent applications

Method for generating, maintaining and expanding monocytes, and/or macrophages and/or dendritic cells in long term culture, delivered 2014

Method for expanding monocytes, delivered 2015

A method for inducing extended self-renewal of functionally differentiated somatic cells, delivered 2015

Methods and compositions for use in preventing or treating myeloid cytopenia and related complications, filed 2013

Methods for expanding a population of Alveolar Macrophages in a long term culture, filed 2015

Selected Publications

O. Matcovitch-Natan, D. R. Winter, A. Giladi, S Vargas Aguilar, A. Spinrad, S. Sarrazin, H. Ben-Yehuda, E. David, F. Zelada Gonzalez, P. Perrin, H. Keren-Shaul, M. Gury, D. Lara-Astaiso, C. A. Thaiss, M. Cohen, K. Bahar Halpern, K. Baruch, A. Deczkowska, E. Lorenzo-Vivas, S. Itzkovitz, E. Elinav, M. Sieweke*, M. Schwartz*, Ido Amit* Microglia development follows a stepwise program to regulate brain homeostasis. *Science*,353(6301):aad8670 (2016) * joint last author

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Photo: David Ausserhofer/MDC

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Intracellular Proteolysis

Mechanisms of Protein Homeostasis

In all cells, both newly synthesized and pre-existing proteins are constantly endangered by misfolding and aggregation. The accumulation of such damaged proteins perturbs cellular homeostasis and provokes aging, pathological states, and even cell death. To avert these dangers, cells have developed protein quality control (PQC) strategies that counteract protein damage in a compartment-specific manner. PQC systems channel misfolded proteins either into re-folding pathways or initiate their proteolysis by the Ubiquitin Proteasome System (UPS). Key components of the UPS involved in cellular homeostasis are specific combinations of Ubiquitin Conjugating (E2) enzymes and Ubiquitin Ligases (E3 enzymes). The work of this group focuses on how specific complexes of E2's and E3's selectively dispose aberrant proteins without affecting correctly folded polypeptides.

One of the best investigated PQC pathways exist in the endoplasmic reticulum (ER). The ER is a cellular organelle through which a large number of proteins travel on their way to their final destination in membranes, exocytic and endocytic compartments, or the cell exterior. The ER is a major folding compartment of eukaryotic cells. It hosts an array of molecular chaperones, which assist in protein folding and maturation. Still, protein biogenesis is an error-prone process. A considerable fraction of all newly synthesized polypeptides fail to attain their native conformation due to mutations, transcriptional and translational errors, folding defects, or imbalanced subunit synthesis. Mature proteins can be damaged by environmental stress conditions, such as high-energy radiation, chemical insults, or metabolic by-products. Specific components of the UPS eliminate such misfolded proteins in a process termed ER associated protein degradation (ERAD). The ERAD pathway cooperates with the Unfolded Protein Response (UPR) in maintaining homeostasis in the ER. The UPR measures the degree of misfolding in the ER and regulates folding and proteolytic capacities according to the load with aberrant proteins (Fig. 1). ERAD appears to be conserved from yeast to mammals. Thus, many basic principles and components have been discovered in the model organism Saccharomyces cerevisiae. Key components of the ERAD pathway are specific E2/E3 combinations or Ubiquitin Ligase complexes such as the HRD Ligase, the Doa10 Ligase or the Asi-Complex. These membrane-bound E3 complexes recognize signals contained in misfolded proteins of the ER-lumen and the ER-membrane. Proteins committed for degradation are exported from the ER in a process termed protein dislocation and ubiquitylated (Fig. 2). However, substrates of these ligases are not limited to the ER, but also include proteins of the cytosol and the nucleoplasma.

In addition to that, further specific Ubiquitin Ligases exist in other compartments or cell types, which are involved in maintaining protein homeostasis as well. An example for that are skeletal muscle cells. Skeletal muscle atrophy, a combination of reduced muscle mass and strength, accompanies many diseases and negatively affects the course of these illnesses. Muscle atrophy is caused by a disturbed protein homeostasis with an increase in protein degradation, which is predominantly mediated by the UPS. The muscle RING-finger (MuRF) ubiquitin ligase family, which consists of three highly homologues proteins (MuRF1, 2 and 3) are important factors to maintain mus-

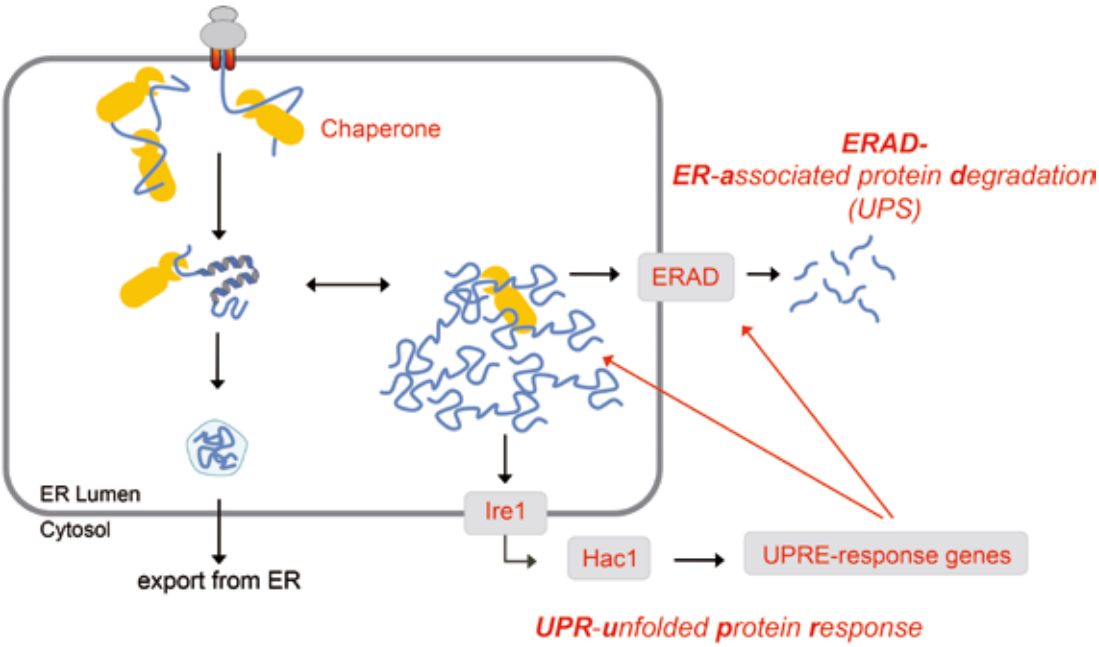

Figure 1: *Protein homeostasis in the ER. Proteins transported into the ER associate with molecular chaperones, only correctly folded proteins can leave the ER for their final destinations. Misfolded proteins are retained in the ER and are either subjected to re-folding or dislocated from the ER for destruction by the UPS (ERAD pathway). The UPR measures the content of misfolding in the ER, signals it to the nucleus, and up-regulates chaperones and UPS components of the ERAD branch.*

cular protein homeostasis. MuRF1, is highly up-regulated during atrophy, ubiquitylates muscular proteins such as myosin heavy chain (MyHC) and actin and directs them to proteasomal degradation.

In the last decade, the group has identified and characterized components of these Ubiquitin Ligase complexes using genetics, molecular biology, and protein purification strategies. However, this laboratory has now established powerful in vitro approaches and quantitative methods using purified components to understand the biochemical principles of the action of Ubiquitin Ligases.

Projects in the laboratory:

The CUE domain of Cue1 aligns growing ubiquitin chains with Ubc7 for rapid elongation

Maximilian von Delbrück and Lukas Pluska in cooperation with the laboratory of Volker Dötsch (Goethe University Frankfurt)

Ubiquitination mostly produces polymeric signals. Amazingly, little is known how the progressive assembly of ubiquitin chains is managed by the responsible Ubiquitin Ligases. Only recently ubiquitin binding activity has emerged as an important factor in Ub-chain formation. The Ubc7 activator Cue1 carries a ubiquitin binding CUE domain, which substantially stimulates K48-linked polyubiquitination mediated by the E2 enzyme Ubc7. Our results from NMR-based analysis in combination with interaction studies and in vitro ubiquitination reactions point out that two parameters accelerate ubiquitin chain assembly: the increasing number of CUE binding sites and the position of CUE binding within a growing chain. In particular interactions with a ubiquitin moiety adjacent to the acceptor ubiquitin facilitate chain elongation. These data indicate a novel mechanism for ubiquitin binding in which Cue1 positions Ubc7 and the distal acceptor ubiquitin for rapid polyubiquitination. Disrupting this mechanism results in dysfunction of the ERAD pathway by a delayed turnover of some protein quality control substrates (von Delbrück et al., 2016, MOL CELL).

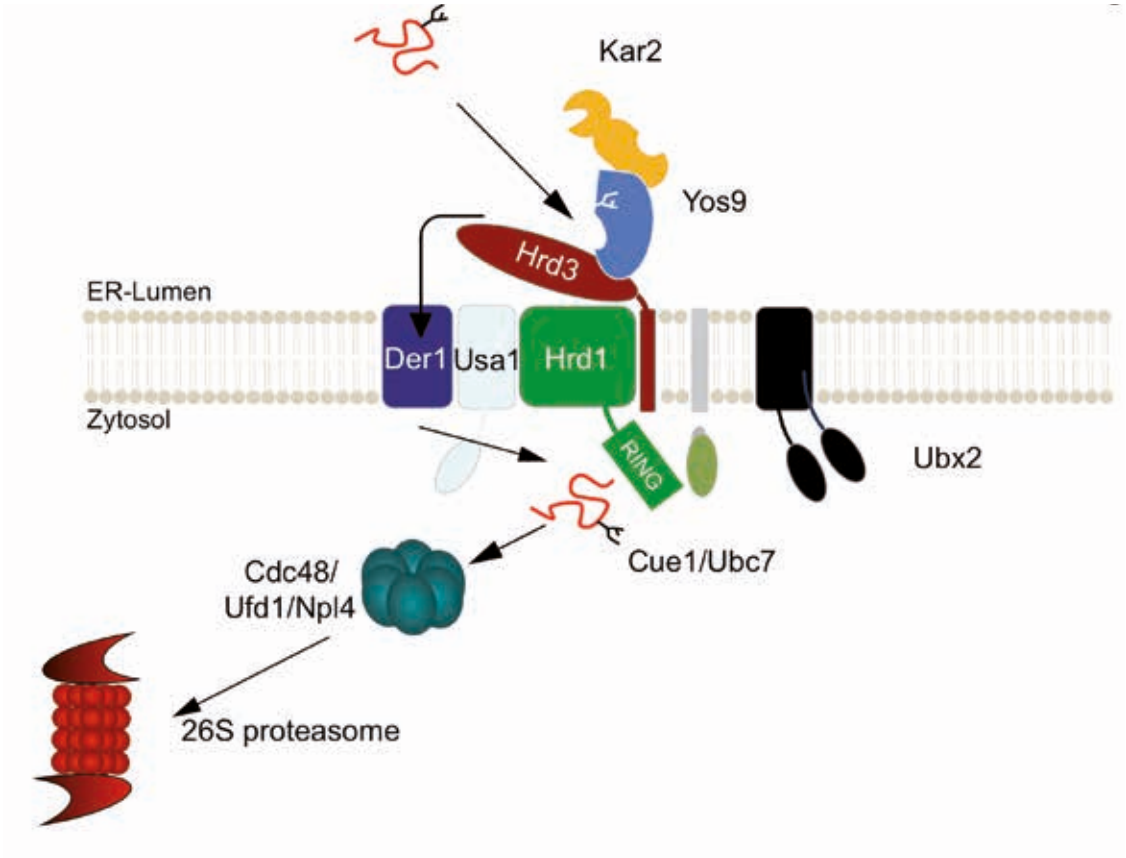

Figure 2: *Hrd ubiquitin ligase as an example of a quality control element. ER-luminal modul of the ligase (Hrd3, Yos9) associates with the chaperone Kar2. Der1, Usa1, and Hrd1 collectively build the membrane module of the ligase, which most likely forms a dislocation channel. The cytosolic module consisting of Hrd1 and Cue1/Ubc7 decorates dislocated proteins with poly-ubiquitin, which leads to recruitment Ubx2 and Cdc48/Ufd1/Npl4. This AAA-ATPase most likely provides energy for dislocation and transfers substrates to the 26S proteasome.*

Sequential poly-ubiquitylation by different E2 enzymes expands the range of a quality control E3 ligase.

Annika Weber and Ernst Jarosch in cooperation with the laboratories of Tommer Ravid (Hebrew University) and Gunnar Dittmar (MDC)

Quality control ubiquitin ligases label malfolded polypeptides with ubiquitin (Ub) chains for proteasomal degradation. These enzymes must on the one hand facilitate the attachment of Ub to a large set of highly diverse clients and on the other hand mediate accurate formation of uniform lysine 48-linked poly- Ub, which is a prerequisite for the recognition by the proteolytic machinery. Studying the yeast Doa10 Ub ligase pathway in vitro and in vivo we show that different ubiquitin conjugating enzymes take over distinct functions during substrate processing: Ubc6 mounts Ub on Doa10 client proteins, which primes them for lysine 48-linked poly-ubiquitylation by Ubc7. Importantly, the propensity of Ubc6 to conjugate Ub not only to lysine but also to other residues increases the target range of the Doa10 quality control Ub ligase. This work provides new insights on how the combined activity of specialised Ub conjugating enzymes contributes to both the versatility and specificity of protein ubiquitylation (Weber et al., 2016, MOL CELL).

The WD repeat-containing protein WDR42A is a new co-factor of the MuRF1 ubiquitin ligase in skeletal muscle atrophy.

Marcel Nowak in cooperation with the laboratories of Jens Fielitz (ECRC), Erich Wanker (MDC) and Gunnar Dittmar (MDC)

The MuRF proteins are suggested to function as indivudual ubiquitin ligases implicated in degradation of Myosin heavy chain (MyHC) or actin by the proteasome. MuRF1 is highly up-regulated under skeletal muscle atrophy and mice that lack MuRF1 are resistant to atrophy. The molecular function of the MuRF proteins and

the regulation of their activity is still only poorly understood. Using high throughput Yeast-2-Hybrid and SILAC-AP-MS screens we found several known and also new MuRF associated proteins. Among those we identified DDB1- and CUL4-associated factor 8 (DCAF8), a tryptophan-aspartic acid (WD) repeat-containing protein that specifically binds and co-localizes with MuRF1 and MuRF3 in cells. This association made it highly likely that MuRF1 may not function as an individual E3 enzyme but function as integral parts of larger cullin-type ubiquitin ligase complex. Cullin-type E3 enzymes play a role in cell cycle control and other essential cellular processes and are characterized by a central 'cullin' subunit. Using co-immunoprecipiation we found that MuRF1 is complexed with DCAF8, DDB1, Cul4A, and the RING finger protein Rbx1. Our results demonstrates that MuRF1 is part of a specific cullin-type ubiquitin ligase complex in differentiated C2C12 skeletal muscle myotubes. Furthermore we could show that MuRF1 and DCAF8 are highly up-regulated during denervation-induced muscle atrophy. Finally, we showed that siRNA-mediated down-regulation of DCAF8 in C2C12 skeletal muscle myotubes prevented dexamethasone-induced atrophy and MyHC degradation. These results establish a cullin-type ubiquitin ligase comprising DCAF8 and MuRF1 as a vital participant in skeletal muscle atrophy. This specific ligase complex could represent a target for the development of new anti-atrophic drugs. These results imply that WDR42A functions as an important co-factor of the MuRF1 ubiquitin ligase in skeletal muscle atrophy, which makes it a highly interesting target for the development of new anti-atrophic drugs.

A complex of Htm1 and the oxidoreductase Pdi1 accelerates degradation of misfolded glycoproteins

Anett Köhler, Christian Hirsch, and Ernst Jarosch in cooperation with the laboratory of Eberhard Krause (FMP)

In the ER, an enzymatic cascade generates a specific N-glycan structure of seven mannosyl and two N-acetylglucosamine residues ($Man_7GlcNAc_2$) on misfolded glycoproteins to facilitate their disposal. We show that a complex encompassing the yeast lectin-like protein Htm1 and the oxidoreductase Pdi1 converts $Man_8GlcNAc_2$ on glycoproteins into the $Man_7GlcNAc_2$ signal. In vitro, the Htm1/Pdi1 complex processes both unfolded and native proteins albeit with a preference for the former. In vivo, elevated expression of HTM1 causes glycan trimming on misfolded and folded proteins, but only degradation of the non-native species is accelerated. Thus, modification with a $Man_7GlcNAc_2$ structure does not inevitably commit a protein for ERAD. The function of Htm1 in ERAD relies on its association with Pdi1, which appears to regulate the access to substrates. Our data support a model in which the balanced activities of Pdi1 and Htm1 are crucial determinants for the efficient removal of misfolded secretory glycoproteins (Köhler et al., 2016, JBC).

Selected Publications

Mehnert, M., Sommer, T., and Jarosch, E. (2014) Der1 promotes movement of malfolded proteins through the endoplasmic reticulum membrane. Nature Cell Biol., 16, 77-86

Denzel, M.S., Storm, N.J., Gutschmidt, A., Baddi, R., Hinze, Y., Jarosch, E., Sommer, T., Hoppe, T., and Antebi, A. (2014) Hexosamine pathway metabolites enhance protein quality control and prolong life. Cell, 156, 1167-1178

Pfeiffer, A., Stephanowitz, H., Krause, E., Volkwein, C., Hirsch, C., Jarosch, E., and Sommer, T. (2016) A complex of Htm1 and the oxidoreductase Pdi1 accelerates degradation of misfolded glycoproteins J. Biol. Chem. 29, 12195-12207.

von Delbrück, M., Kniss, A., Rogov, V.V., Pluska, L., Bagola, K., Löhr, F., Güntert, P., Sommer, T. and Dötsch, V. (2016) The CUE domain of Cue1 aligns growing ubiquitin chains with Ubc7 for rapid elongation. Mol Cell 62, 918-928

Weber, A., Cohen, I., Popp, O., Dittmar, G., Reiss, Y., Sommer, T., Ravid, T., and Jarosch, E., (2016) Sequential poly-ubiquitylation by specialized conjugating enzymes expands the versatility of a quality control ubiquitin ligase. Mol. Cell 63, 827-839.

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Photo: David Ausserhofer/MDC

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Molecular Cell Biology and Gene Therapy

Adoptive T cell therapy (ATT) of cancer requires the preparation of antigen-specific T cells that recognize and eradicate tumor cells. While the use of tumor-infiltrating lymphocytes showed some efficacy of ATT for some tumor entities, the use of T cells engineered with T cell receptors (TCR) or chimeric antigen receptors (CAR) paves the way for a broader clinical application. Our group focuses on several aspects of TCR/CAR gene therapy (Fig. 1) including the identification of suitable target antigens, the generation and isolation of antigen-specific TCRs, the improvement of vector systems for the generation of gene-engineered T cells, the development of safety measures to limit side effects of TCR gene therapy, and the evaluation of ATT in pre-clinical models.

Identification and isolation of antigen-specific TCRs for ATT

F. Lorenz, E. Hilgenberg, K. Dudaniec in collaboration with D. J. Schendel (Munich)

A bottleneck of TCR gene therapy is the availability of potent TCRs, which target therapeutically relevant cancer antigens. We developed an experimental approach that enables the rapid detection of immunogenic T cell epitopes of target antigens and the isolation of antigen-specific TCRs. To do so, donor T cells were stimulated with antigen-expressing dendritic cells, which process and present only crucial peptides on MHC complexes at the cell surface. Donor T cells specific for a peptide:MHC complex receive proliferation signals and expand. To identify these T cells without knowing the exact epitope and MHC restriction, we screened the T cells for specificity by using a MHC library (artificial antigen presenting cells expressing the donor's MHC class I alleles). To identify pre-dominant TCR chains of T cells responding to a specific peptide:MHC combination, we performed next generation sequencing. Subsequent generation of TCR-engineered T cells enabled us to characterize TCR properties. Our approach led to the identification of novel TCRs specific for viral antigens associated with various diseases and cancer.

Non-Viral Sleeping Beauty transposon gene transfer for ATT

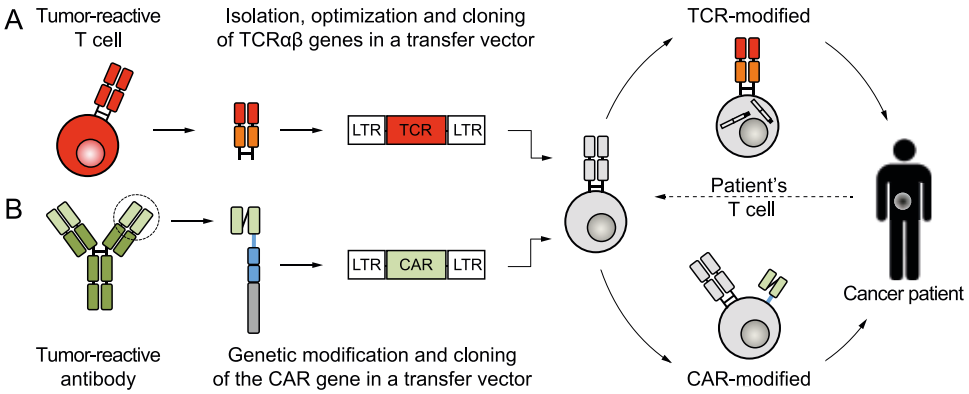
J. Clauß, M. Bunse in collaboration with Z. Iszvak (MDC) and Z. Ivics (PEI)

Transposon-based vectors are an alternative to viral vectors for fast and convenient genetic engineering of T cells. The use of transposons requires transfection of DNA into T cells as they lack the ability of viral vectors to pass genetic material through the cell membrane. We found that DNA transfection by electroporation affected the cell viability in a dose-dependent manner and the responsiveness of T cells towards CD3/CD28 stimulation. The molecular mechanisms behind these observations were investigated and an improved protocol was developed requiring only a limited amount of transfected DNA. Stable expression of TCRs and CARs in human T cells (up to 50%) was achieved by applying a size-reduced transposon vector in combination with the hyperactive SB100X Sleeping Beauty transposase. Upon antigen-specific stimulation, the engineered T cells mediated cytokine secretion and tumor cell killing. Currently, we are working on the development of an improved transposon-based expression vector encoding genetically optimized TCR or CAR genes in combination with miRNAs targeting the endogenous TCR chains.

RNAi-mediated TCR knockdown prevents autoimmunity in TCR gene therapy

M. Bunse in collaboration with T. Schumacher (Amsterdam)

In TCR-engineered T cells the transduced TCR heterodimer can form potentially self-reactive mixed TCR dimers, composed of en-



Schematic representation of TCR (A) and CAR gene therapy (B). T cells are isolated from a cancer patient and modified ex vivo using transfer vectors encoding antigen-specific TCR or CAR genes. Engineered T cells are then expanded and reinfused into the patient. CAR: chimeric antigen receptor; LTR: long terminal repeat; TCR: T cell receptor.

dogenous and transferred TCR chains. To prevent the formation of mixed TCR dimers, we developed a retrovirus vector that employs RNAi to silence the endogenous TCR and to facilitate expression of an RNAi-resistant, therapeutic TCR. By using mouse and human TCR-engineered T cells we showed that TCR expression without RNAi results in heterodimerization of transduced and endogenous TCR chains while the amount of mixed TCR dimers is significantly reduced when using the RNAi-TCR replacement vector. In a mouse model of TCR gene transfer-induced graft-versus-host-disease (TI-GVHD) all mice that received T cells transduced with a standard retrovirus vector developed lethal TI-GVHD due to formation of mixed TCR dimers. In contrast, TI-GVHD was almost completely prevented when expression of the endogenous TCR was abrogated by RNAi. Therefore, the use of the RNAi-TCR replacement vector instead of standard transfer vectors liberates TCR gene therapy from potential autoimmune side effects.

Targeting tumor neoantigens by TCR gene therapy

M. Leisegang in collaboration with H. Schreiber (Chicago) and T. Blankenstein (MDC)

T cell responses in successful cancer immunotherapy seem directed towards neoantigens created by somatic mutations. Targeting neoantigens by TCR gene therapy requires a complex and personalized approach that we explore in pre-clinical models. In a melanoma mouse model we analyzed the efficacy of ATT using TCR-engineered T cells with specificity for a somatic mutation natively expressed in an established tumor. Although the targeted antigen and the therapeutic TCR provided excellent conditions for ATT, therapy was foiled by escape of antigen-negative tumor variants. Our analysis showed that successful mutation-specific TCR gene therapy requires high levels of the targeted neoantigen or ATT has to be combined with radiotherapy to increase antigen levels.

In another model we analyzed different immunogenic mutations in cyclin-dependent kinase 4 (CDK4) that naturally occur in human melanoma. We used a syngeneic HLA-A2-transgenic mouse model of established tumors to evaluate two CDK4 mutations (R24C, R24L) as targets for TCR-gene therapy. T cells, which were modified with a patient-derived TCR, showed similar reactivity to R24C and R24L in vitro, but expanded and rejected tumors only in response to R24L in vivo. Such differences in neoantigen quality might explain why cancer immunotherapy induces tumor regression in some individuals, while others do not respond, despite similar mutational load.

Patents / Patent applications

Europäische Patentanmeldung Anmeldenummer: EP 15159212.8, Titel: Method of detecting new immunogenic T cell epitopes and isolating new antigen-specific T cell receptors by means of an MHC cell library

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Photo: David Ausserhofer/MDC

Jana Wolf

Mathematical Modelling of Cellular Processes

Complex diseases are often characterized by an accumulation of multiple perturbations, such as mutations or over-expression of proteins, in rather large and complex cellular networks. The consequences of these perturbations can hardly be analyzed by pure reasoning. Here, mathematical modeling and computational analysis contribute to a deeper understanding of the regulatory systems and provide a better basis for the interpretation of high-throughput data and identification of effective drug targets.

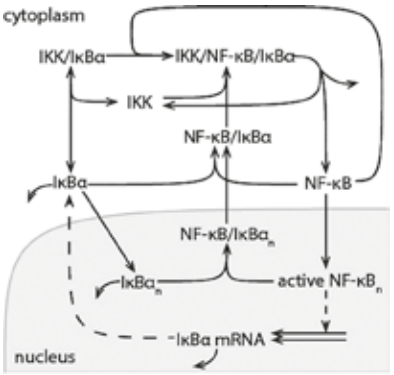
Our group develops and analyses mathematical models of mammalian signaling pathways and gene-regulatory networks in normal and disease states. For our investigations we use tools such as simulations, bifurcation analyses and sensitivity analyses. These give insights into the dynamical properties of systems and help to identify most sensitive processes and critical regulations. Another important aspect is the investigation of cell-type specific differences in signaling and gene-regulatory networks since these are critically involved in the prediction of the efficiency and possible side-effects of drugs.
Main projects in the last years have focused on signal transduction, in particular on the NF-κB/IKK pathway and the Wnt/β-catenin pathway, and gene expression. In many of our projects we closely collaborate with experimental partners in- and outside of the MDC.

Modeling the NF-κB/IKK pathway

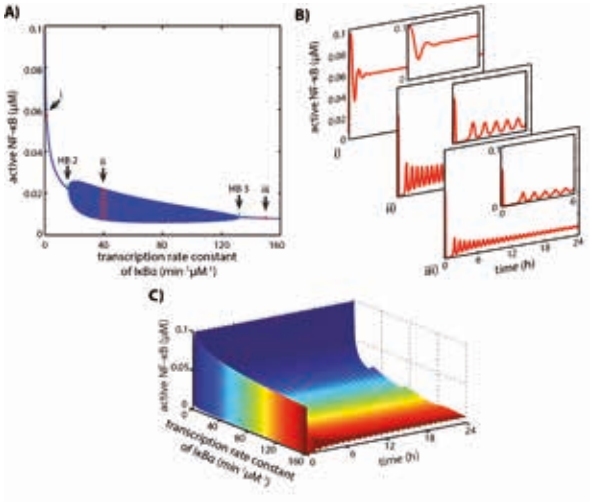
In a collaborative effort with the group of Claus Scheidereit at the MDC we aim for a systems level understanding of the IKK/NF-κB signaling pathway. This pathway is implicated in several human diseases, such as inflammatory and autoimmune diseases as well as cancer. It consists of a canonical and a non-canonical branch. Both signaling branches can be activated by a range of stimuli and lead to the activation of different members of the NF-κB transcription factor family. Interestingly, both branches act on different time scales but are linked via shared pathway components and target genes. We are interested in the regulation of the overall system and the cross-talk between the two branches.
Homeostasis and activation of the overall NF-κB/IKK signaling pathway critically depend on the processing of the precursor proteins p100 and p105. We used a mass-spectrometry based quantitative analysis and modeling approach to dissect the processes of precursor processing (cooperation with the groups of Claus Scheidereit and Gunnar Dittmar, now Luxembourg Institute of Health). Our investigations showed the dependence of canonical precursor p105 levels on non-canonical stimulation, establishing a new link between the two signaling branches.
We developed a core model of the canonical NF-κB/IKK signaling branch (Fig. 1). This core model reproduces the dynamical and stationary behavior of NF-κB very well. Due to the reduced model complexity a bifurcation analysis could be performed, demonstrating which intracellular parameters can influence the the NF-κB dynamics. We showed that NF-κB is capable of exhibiting distinct types of dynamics in response to the same stimulus. Of particular interest are oscillatory and non-oscillatory modes of behavior (Fig. 2). We identified the total NF-κB concentration and the IκBα transcription rate as two critical parameters that modulate the type of NF-κB dynamics (Fig. 2).

Investigation of oscillating systems

Oscillatory dynamics, as found in NF-κB signalling, occur in a wide variety of cellular processes, e.g. in calcium and p53 signaling responses, in metabolic pathways or within



Core model of the signal transduction in the canonical NFκB pathway.



Analysis of the possible modes of dynamical behaviour of the NF-κB core model shown in Fig. 1. (A) The bifurcation analysis shows the ranges of the IκBα transcription rate for steady states and oscillations. Examples of the dynamics are shown in (B) for specific values: i) resulting in strongly damped behaviour, ii) showing sustained oscillations and iii) weakly damped oscillations. (C) Dynamics for the range of IκBα transcription rate values.

gene-regulatory networks, such as the circadian system. Since it is of central importance to understand the influence of perturbations on the behaviour of these systems a number of studies have been undertaken to examine their robustness. The period of circadian oscillations seems to be very robust to provide reliable timing; the period of intracellular calcium oscillations is on the other hand very sensitive to allow for frequency-encoded transduction of signals. In cooperation with Antonio Politi (EMBL) and Ralf Steuer (Humboldt University Berlin) we examined the robustness of the oscillatory period towards perturbations of kinetic parameters in diverse oscillatory systems and how design principles, such as type of feedback and formalism of reaction kinetics, impact these findings. Our work highlights the importance of specific design principles for the characteristics of oscillatory systems.

Analysis of target gene expression

The Wnt/β-catenin signaling pathway regulates many physiological processes by controlling the differential expression of target genes. The deregulation of the pathway is associated with various types of cancer. We developed mathematical models of the Wnt/β-catenin pathway that include regulatory processes of target gene expression under normal and oncogenic conditions. The target gene MYC is a well-known proto-oncogenic transcription factor that regulates the expression of hundreds of genes influencing cell fate and driving cell proliferation. In collaboration with Martin Eilers at Biocenter University of Würzburg we found evidence that cancer type specific gene expression patterns can be explained by distinct MYC levels and different affinities of MYC to the promoters of target genes.

Some target genes of signaling pathways are themselves regulators of the pathway establishing interlocked negative and positive feedback loops. Important regulators of the canonical NF-κB/IKK signaling branch are IκBα and A20 that both negatively regulate the pathway. Together with the groups of Claus Scheidereit and Markus Landthaler (BIMSB/MDC) we studied the effect of post-transcriptional regulation by the mRNA binding protein RC3H1 on the system. Model simulations and experiments show that the NF-κB/IKK system is affected by post-transcriptional regulation, opening new possibilities of manipulating NF-κB/IKK system. An important regulator of the Wnt/β-catenin pathway is the E3 ubiquitin ligase F-box-protein, which is differentially expressed in wild-type and cancer cells. We investigated the consequences of the complex regulation by β-TrCP and discussed the suitability of β-TrCP as drug targets in the case of oncogenic pathway mutations.

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F. Lorenzin, U. Benary, A. Baluapuri, S. Walz, L. A. Jung, B. von Eyß, C. Kisker, J. Wolf, M. Eilers & E. Wolf (2016), Different promoter affinities account for specificity in MYC-dependent gene regulation, eLife. 5, e15161.

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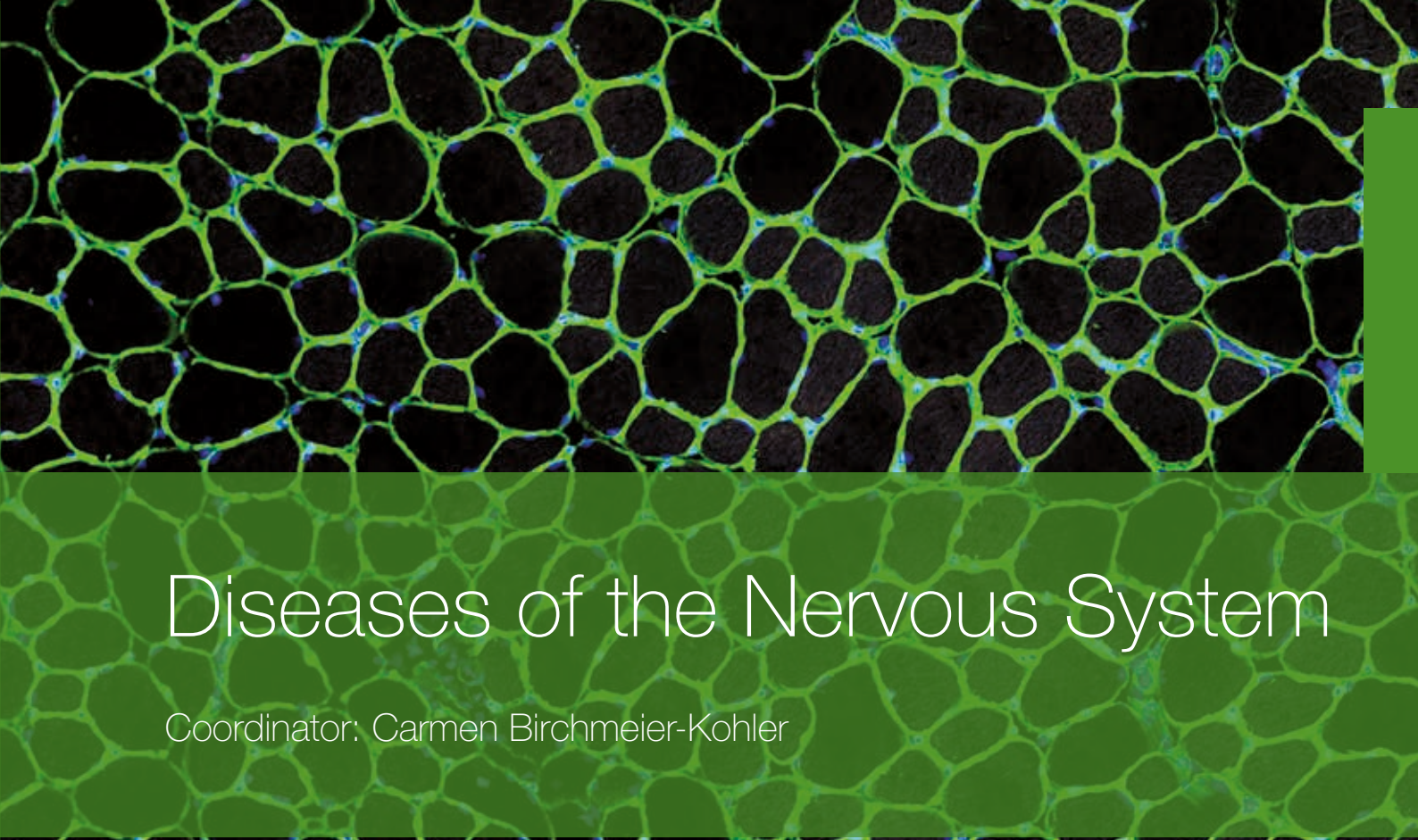
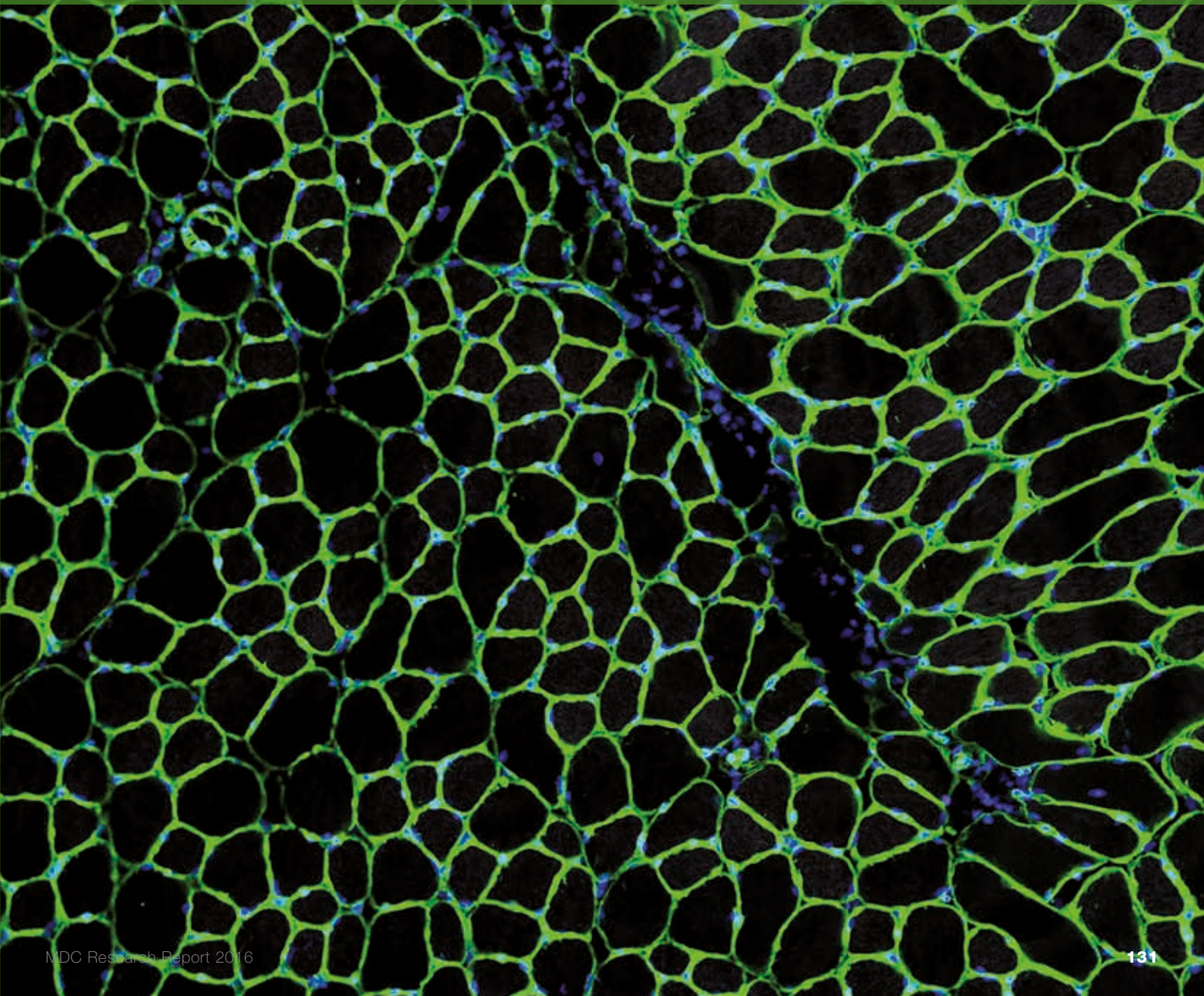


Diseases of the Nervous System

Coordinator: Carmen Birchmeier-Kohler



Diseases of the Nervous System

Coordinator: Carmen Birchmeier-Kohler

The nervous system is fundamental to the body's ability to maintain itself, to sense the environment, to move and react to stimuli, and to generate and control behavior. Hence, disorders of the nervous system that manifest themselves as neurological and psychiatric disease constitute a major challenge for affected individuals, their families, and for society. Determining the molecular basis of normal nervous system function, and discovering the changes responsible for inherited or acquired defects are important priorities in the fight against nervous system disease.

Research in the Neuroscience Department of the MDC focuses on molecular and cellular analysis of the central and peripheral nervous system. Themes and expertise covered by the department are broad and interdisciplinary, and comprise genetics, neurophysiology, proteomics and system biology, biochemistry, cell biology and stem cell research. This provides many opportunities for interactions and collaborations, and ensures an optimal environment for young researchers and group leaders. We are delighted that one of them, James Poulet, has now received his second ERC grant in 2015, a consolidator grant in addition to the starting grant in 2010. He was awarded tenure in 2016 and will continue his work at the MDC.

We were able to recruit young scientists to the department during the report period. Niccolo Zampieri joined the Neuroscience Department in 2014. He trained as a postdoc in the laboratory of Tom Jessell at Columbia University in New York, where he explored the functions of cadherins in the nervous systemused and used rabies virus to map sensory and motor circuits. Mina Gouti joined the MDC in the beginning of 2016. She trained in the laboratory of James Briscoe at the Crick Institute in London and uses mouse embryonic stem cells to generate neurons and muscle cells. She developed new protocols to produce spinal neuron types. In addition, Alessandro Prigione joined the program as Delbrück Fellow. Alessandro is a specialist on embryonic stem cells and induced pluripotent stem cells, which he uses to model mitochondrial DNA disorders. The success of the Neuroscience Program is also reflected in the fact that young researchers received attractive offers for positions from outside and left the MDC. Jochen Meier became professor at the Institut für Zoologie of the TU Braunschweig where he moved in 2015. Kathrine Poole has an offer to return to her native Australia as a Senior Lecturer at the University of New South Wales in Sidney, and will move in March 2016. Björn Schroeder will take a position in industry.

The Berlin Institute of Health was founded by 2013 and aims to support research in systems medicine at the Charité and MDC. It provides infrastructure and support for the use of high throughput genomic technologies for translational research. Currently, seven group leaders in the Neuroscience Program participate in cooperative networks funded by the BIH. For instance, a consortium on Alzheimer's disease (Speakers: E. Wanker and F. Heppner/Charité) uses system biology approaches to model proteostasis networks and aims to find new strategies for dementia treatment. A network on congenital disease (Speakers: C. Rosenmund/Charité and C. Birchmeier) aims to define novel mechanisms that cause congenital disorders by analyzing regulatory i.e. non-coding sequences. In addition, smaller research networks were established, one to analyze immune infiltration in glioblastoma (H. Kettenmann in collaboration with C. Harms/Charité) and a second that investigates core mechanisms in the pathophysiology of psychotic disorders (J. Poulet and S. Jacob/Charité). These BIH activities have strengthened translational research and collaborations between Charité and MDC researchers. In addition,

an initiative to facilitate translation of academic discoveries into therapy (SPARK BERLIN) was recently established at the BIH that provides mentoring and funding for translational projects. Two projects by Gary Lewin were selected in the first SPARK BERLIN call.

Scientists of the Neuroscience Program participate in research and graduate schools, among them the Helmholtz International Research School MolNeuro, the Charité International Medical Neuroscience program, the MyoGrad school and the newly established Einstein-Center for Neuroscience. The curriculum of these graduate schools encompasses lectures, journal clubs and courses. Furthermore, the schools organize retreats and symposia for graduate students, and they provide 'soft skills' training, for instance courses on communication and presentation skills.

Scientific highlights of the program

During the report period, several important findings were published by memebrs of the Neuroscience Program. Regulation of cell volume is critical for many cellular and organismal functions but the molecular identity of the volume-regulated anion channel VRAC had remained unknown. Thomas Jentsch and his group identified LRRC8A as indispensable component of VRAC (Voss et al., Science 2014), and their subsequent work characterized the subunit composition of this channel (Planells-Cases et al., Embo J 2015). The groups of Gary Lewin and James Poulet were able to identify a somatosensory circuit in the cortex, which is required for cooling perception in mice (Milenkovic et al., Nat. Neuroscience 2014). Gliomas are complex tumors composed of neoplastic and non-neoplastic cells, which each individually contribute to cancer formation, progression and response to treatment. The group of Helmut Kettenmann defined the function of microglia, the macrophages of the brain, in glioma progression and identified molecules that modulate microglia activity (reviewed in Nat. Neuroscience 2015). The group of Carmen Birchmeier systematically analyzed the network that controls the gene expression program in mature pancreatic beta cells and showed that combinatorial binding of three transcription factors (Insm1, Neurod1 and Foxa2) maintains the mature gene expression program (in collaboration with the groups of N. Rajewsky and W. Chen, Jia et al., Embo J 2015). Huntingtin protein with expanded polyglutamine tracts causes Huntington's disease, and its aggregates are a pathological hallmark of the disorder. The group of Erich Wanker used a network filtering approach to identify novel huntingtin interaction partners. One of those proteins, CRMP1, was found to be a suppressor of huntingtin misfolding and neurotoxicity (Stroedicke et al., Genome Research 2015).

Photo: Thomas Müller

Carmen Birchmeier-Kohler

Developmental Biology / Signal Transduction

Our lab focuses on functional analysis of genes important in development and stem cells, concentrating on muscle, the nervous system and endocrine organs. We observe that many genes control both, developmental and regenerative processes in the adult. For our work, we use mouse genetics and stem cells culture, and define molecular mechanisms by genomic and proteomic technologies.

Muscle stem cells and their niche

D. Bröhl, I. Lahmann, M. T. Czajkowski (former member of the laboratory), J. Griger, T. Zyrianova, in collaboration with H.P. Rahn (Preparative Flow Cytometry Facility, MDC) and B. Purfürst (EM Facility, MDC).

Skeletal muscle growth and regeneration rely on myogenic progenitor cells. Satellite cells are progenitor/stem cells of the postnatal muscle and reside between basal lamina and plasma membrane of myofibers. This anatomical position is called the stem cell niche.
We have previously shown that generation and maintenance of satellite cells depends on Notch signals. For instance, elimination of Rbpj, the transcriptional mediator of Notch signals, results in premature differentiation of myogenic progenitors and the formation of tiny muscle groups that lack satellite cells (Vasyutina et al., PNAS 2007). This drastic effect of a loss of Notch signals is rescued by the additional mutation of the muscle differentiation factor MyoD. Thus, a major role of the Notch pathway is to suppress MyoD in myogenic progenitors (Bröhl et al., 2012; Czajkowski et al., 2014).

Craniofacial and trunk skeletal muscles are evolutionarily distinct and derive from cranial and somitic mesoderm, respectively. Different regulatory hierarchies act upstream of myogenic regulatory factors in cranial and somitic mesoderm, but the same core regulatory network - MyoD, Myf5 and Mrf4 - executes the differentiation program. We compared the role of Notch signals in head and trunk myogenesis, and observed that the major Notch function, the suppression of MyoD and premature myogenic differentiation, is conserved during muscle development in the trunk and head.

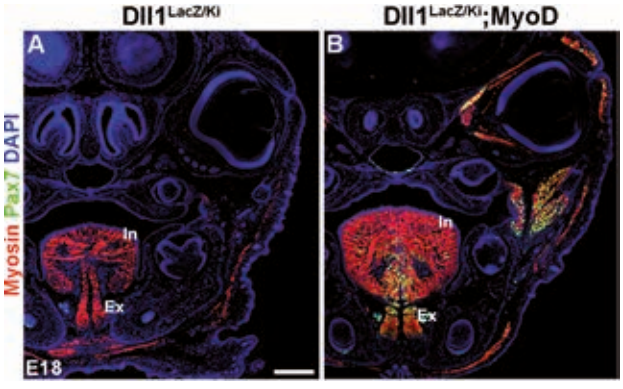


Figure 1: Notch signals suppress premature differentiation of muscle progenitor cells in cranial muscle
*Section of a head of a mouse which shows skeletal muscle (myosin, red), myogenic progenitor cells (Pax7, green) and nuclei (DAPI, blue). (**A**) Dll1 mutant mice show small muscle groups that no longer contain progenitor cells. (**B**) In the Dll1/MyoD double mutant, muscle groups are larger and full of progenitor cells.*

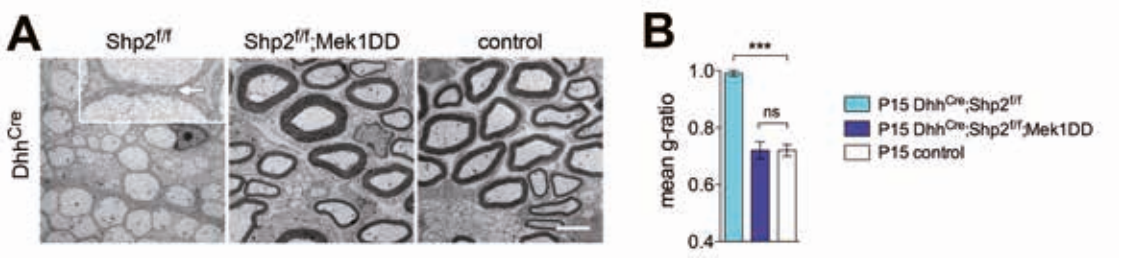


Figure 2: MAPK activity suffices to replace Shp2 signaling during peripheral myelination
*(**A**) Analysis of nerves from control and mutant mice by EM. When Shp2 is mutated (Dhhcre;Shp2flox/flox) in Schwann cells, Schwann cells are present and wrap the nerves but do not form myelin. Myelination occurs when MAPK is activated in mutant Schwann cells (Dhhcre;Shp2flox/flox; Mek1DD). (**B**) Quantification of myelin thickness (G-ratio).*

Notch signaling has a second role and is needed for colonization of the satellite cell niche in the trunk. In particular, myogenic progenitors of double mutants (mutant for Notch signaling components like Rbpj or Dll1 and MyoD) do not take up a satellite cell position under the lamina, and locate instead in the interstitial space where they poorly contribute to muscle growth. The colonization deficit is caused by insufficient generation of extracellular matrix around satellite cells. Satellite cells do thus contribute to the formation of their own niche in trunk muscle. In contrast, cranial muscle progenitors do not require Notch signals to colonize their niche. Thus, the major Notch function - suppression of premature myogenesis - is conserved in head and trunk muscle progenitor cells, but other functions like satellite cell homing diverge in cranial- and somite-derived muscle (Bröhl et al., 2012; Czajkowski et al., 2014).

Neuregulin/ErbB signaling in developmental myelin formation and nerve repair

M. Sheean (former member of the laboratory), C. Cheret, T. Müller, in collaboration with E. McShane and M. Selbach (Cell Signaling/ Mass Spectrometry, MDC), J. Walcher and G. R. Lewin (Molecular Physiology, MDC), S. Hoelper and M. Krüger (MPI Bad Nauheim), A. N. Garratt (Charité, Berlin), D. Meijer (Erasmus University, Rotterdam), K. Rajewsky and W. Birchmeier (Cancer Program, MDC), D. Bennett and F. Fricker (University of Oxford, UK).

Myelin is essential for rapid and accurate conduction of electrical impulses by axons in the central and peripheral nervous system. Myelin is formed in the early postnatal period and myelin formation in the peripheral nervous system depends on axonal signals provided by Nrg1 (an EGF-like growth factor). This signal is mediated in Schwann cells by ErbB tyrosine kinase receptors and the tyrosine phosphatase Shp2.
Developmental myelination depends on type III Nrg1 that is produced by peripheral sensory and motoneurons and presented in the axonal membrane. Myelination deficits that arise during development due to a lack or dampened Nrg1/ErbB2 signals cannot be compensated at later time points. Nrg1 is also required for effective nerve repair in adulthood and acts during an early phase of remyelination. Remyelination and nerve repair are severely delayed in the absence of Nrg1 but ultimately occur even when the signal is lacking. Thus, myelination and remyelination are controlled by similar signals, but differences exist (cf. Birchmeier and Bennett, 2016).
Erk/MAPK transmit signals provided by tyrosine kinase receptors, but other pathways like PI3kinase/Akt, Cdc42 or PLC-gamma also act downstream. All of these pathways are known to be activated by Nrg1/ErbB and were suggested to control specific aspects of the Nrg1/ErbB-dependent myelination program like myelin gene expression or morphogenic movements during myelination. Our previous work using mouse genetics demonstrated that loss of ErbB3/Shp2 severely impairs Schwann cell development and disrupts the myelination program. We recently found that activated MAPK compensates for the absence of ErbB3 or Shp2 during Schwann cell development and myelination (Sheean et al., 2014). Thus, MAPK activity suffices to replace Nrg1/ErbB singaling during myelination.

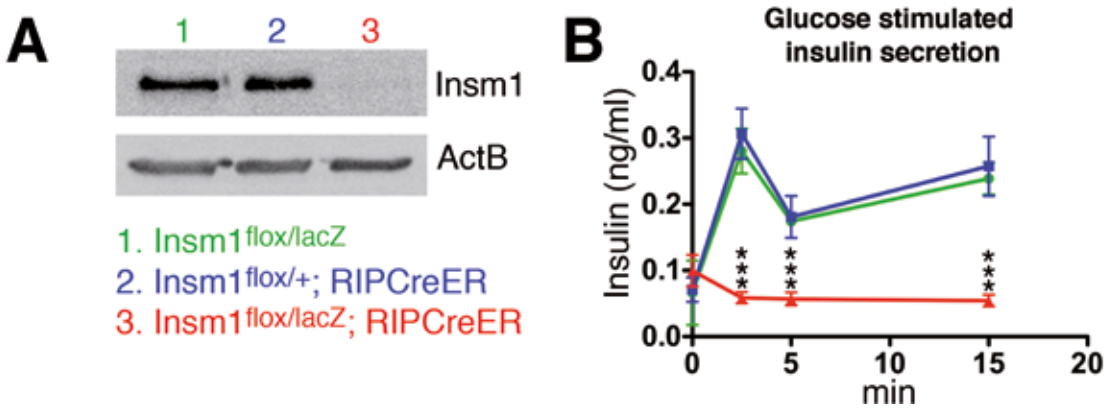


Figure 3: Insm1 maintains mature pancreatic beta-cell function
*(**A**) Western blot analysis after introduction of a Insm1 mutation in mature beta-cells; low Insm1 protein in mutant (RIP-creERT2;Insm1flox/flox) compared to control cells (Insm1flox/flox or RIP-creERT2;Insm1flox/+). (**B**) Conditional mutant mice showed a severe deficit in glucose-stimulated insulin secretion. Animals received a glucose injection that results in insulin release in control but not in mutant mice.*

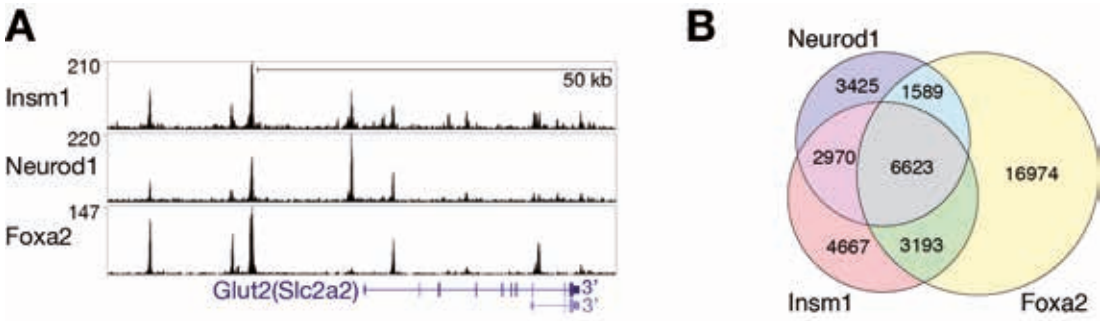


Figure 4: Insm1 cooperates with Neurod1 and Foxa2 to control gene expression
*Insm1, Neurod1 and Foxa2 binding sites in chromatin from a beta-cell line were defined by ChIP-seq and bioinformatics. (**A**) Binding sites on the glucose transporter Glut2 (Slc2a2) gene that is needed for glucose-stimulated insulin secretion. (**B**) Analysis of all Insm1 binding sites in the genome demonstrated large degrees of overlap with Neurod1 and Foxa2 binding sites.*

Insm1 cooperates with Neurod1 and Foxa2 to maintain mature pancreatic β-cell function

S. Jia, T. Müller, D. Blasevic (former member of the laboratory), in collaboration with A. Ivanov and N. Rajewsky (Systems Biology, MDC), W. Sun and W. Chen (Scientific Genomics Platform, MDC), and M. Poy (Molecular Mechanisms of Metabolic Disease, MDC).

Key transcription factors control the gene expression program in mature pancreatic beta-cells, but their integration into regulatory networks is little understood. Insm1 encodes a zinc finger factor and we found

that Insm1 controls differentiation of beta-cells and other endocrine cell types in the pancreas, intestine, pituitary, lung and adrenal medulla. We recently showed that Insm1, Neurod1 and Foxa2 directly interact and together bind regulatory sequences in the genome of mature pancreatic beta-cells. Insm1 ablation in mature beta-cells in mice resulted in pronounced deficits in insulin secretion and gene expression. Insm1-dependent genes in developing and adult beta-cells markedly differed. In particular, when the mutation was introduced in adult, mature beta-cells assumed an immature beta-cell character in respect to gene expression and function.

We defined Insm1 binding sites in beta-cells, and discovered that many binding sites are cooperatively bound by Insm1, Neurod1 and Foxa2. Binding sites co-occupied by Insm1/Neurod1/Foxa2 were mainly located in intergenic and intronic sequences and comparison with histone modification data indicated that many correspond to enhancer sequences. Furthermore, sites co-occupied by all three factors represent high affinity binding sites. Remarkably, combinatorial binding sites of Insm1, Neurod1 and Foxa2 but not binding sites of Insm1 alone correlated and explained gene expression changes observed in Insm1 mutant beta-cells. Moreover, human genomic sequences corresponding to murine sites co-occupied by Insm1/Neurod1/Foxa2 were enriched in single nucleotide polymorphisms associated with glycolytic traits. Thus, combinatorial Insm1/Neurod1/Foxa2 binding identifies regulatory sequences that maintain the mature gene expression program in beta-cells, and disruption of this network results in functional failure.

Selected Publications

Insm1 cooperates with Neurod1 and Foxa2 to maintain mature pancreatic β-cell function. Jia S, Ivanov A, Blasevic D, Müller T, Purfürst B, Sun W, Chen W, Poy MN, Rajewsky N, Birchmeier C. EMBO J. 2015 May 12;34(10):1417-33.

Divergent and conserved roles of Dll1 signaling in development of craniofacial and trunk muscle. Czajkowski MT, Rassek C, Lenhard DC, Bröhl D, Birchmeier C. Dev Biol. 2014 Nov 15;395(2):307-16.

Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals. Bröhl D, Vasyutina E, Czajkowski MT, Griger J, Rassek C, Rahn HP, Purfürst B, Wende H, Birchmeier C. Dev Cell. 2012 Sep 11;23(3):469-81. doi: 10.1016/j.devcel.2012.07.014.

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Research Focus

We use human and mouse pluripotent stem cells (PSCs) to model embryo development in vitro and understand the mechanisms that regulate cell fate decisions during neuromuscular system development.

During embryonic development spinal cord motor neurons are generated with high precision along the anterior – posterior (AP) axis and establish connections with skeletal muscles to control movement. Previous studies have shown that development and survival of motor neurons and muscles depends on each other but until recently their generation were considered as independent events. Striking evidence coming from clonal lineage analysis experiments in the mouse embryo suggested that a common bipotent progenitor, that can give descendants to both the spinal cord and paraxial mesoderm, exists in vivo. These cells, called neuromesodermal progenitors (NMPs), reside in the caudal lateral epiblast region of the embryo during embryonic development. NMP cells are important for axis elongation and correct tissue growth but have been largely ignored in the stem cell field. Following the cues from mouse embryonic development we have recently succeeded in generating NMP cells *in vitro* from mouse and human pluripotent stem cells (Figure 1). The *in vitro* generation of these cells opens up new opportunities for the study and treatment of neuromuscular diseases as it gives unprecedented access to the simultaneous development of both neural and mesodermal cell types in the "dish". Our focus is to understand how these two tissues are generated and interact in space and time during human development. This will allow us to unravel the mechanisms of human embryo development and evaluate how defects in early development of these tissues may predispose to disease in adult life. To address these questions we are using gain and loss of gene function approaches (CRISPR-Cas9), next generation sequencing technologies (single cell RNA-seq) as well as live cell imaging techniques.

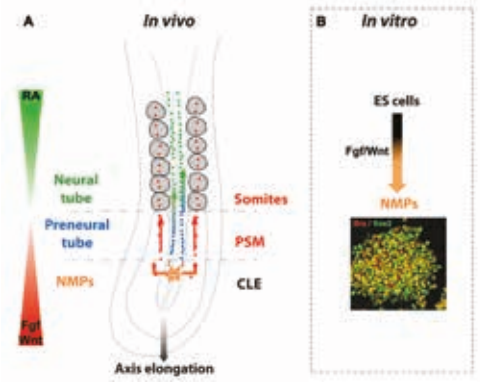

Figure 1: Progressive differentiation of NMPs during development of the Central Nervous System. *(A) Schematic of a wild type embryo, looking down onto the surface of the posterior end of the embryo. The posterior gradients of Wnt and Fgf signals (red), emanating from the caudal lateral epiblast (CLE), oppose the activity of retinoic acid (RA, green), which is produced by the developing somites. Neuromesodermal progenitor (NMP) cells (orange dots) located in the CLE transit either through a preneural tube (PNT) stage (blue dots) to spinal cord progenitors (green dots), or through a presomitic mesoderm stage (PSM) to form the somites (red cells). Green and red arrows show the distinct developmental paths of NMP cells. (B) Timely activation of Wnt/Fgf signaling in mouse and human differentiating ES cells results in the generation of NMP cells expressing Brachyury (T/Bra) and Sox2 protein, equivalent to those found in the CLE region of the embryo.*

Neuromesodermal Progenitor Cells (NMPs)

During normal embryonic development part of the posterior spinal cord, muscles and bones are generated from a neuromesodermal progenitor population (NMP). NMPs are a transient population, arising early in gastrulation, which can be identified *in vivo* by the co-expression of the transcription factors *Sox2* and *Brachyury (T/Bra)*. During development, FGF and Wnt signalling emanating from the posterior part of the embryo are instrumental for the induction and maintenance of NMP cells

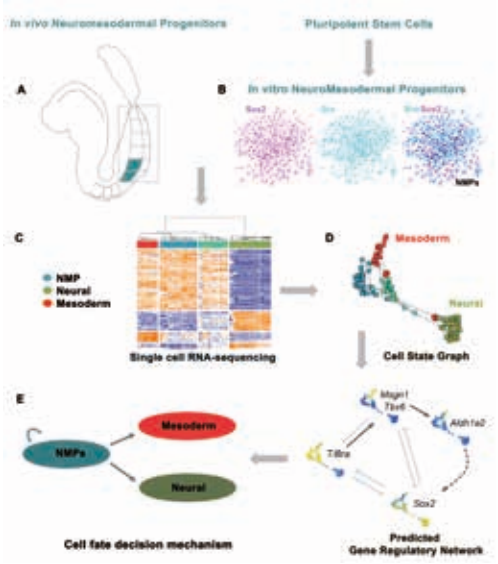

Figure 2: Single cell analysis reveals the differentiation route of NMPs towards the neural and mesodermal lineage. *Single-cell RNA-sequencing analysis of NMP cells isolated from mouse embryos (A) and in vitro NMP cells (B) defines their molecular signature and resemblance. (C) Hierarchical clustering of single NMP cells reveals four distinct clusters. One associated with NMP identity (cerulean), a transitioning NMP cluster (light green), a neural progenitor (green) and a mesodermal cluster (red). (D) Pseudoteporal ordering of single cells depicts the two different developmental trajectories towards the neural and mesodermal lineages. (E) Analysis of single cell RNA-sequencing data predicts a gene regulatory network that balances the generation of neural and mesodermal tissue to facilitate orderly axis extension during embryonic development.*

(Figure1). Using the same signals, we have recently succeeded in generating NMP cells *in vitro* from mouse and human embryonic stem cells (ESCs) (Gouti et al., 2015; Gouti et al., 2014). Single cell RNA-sequencing analysis revealed that *in vitro* generated NMPs closely resemble the *in vivo* counterparts (Gouti, 2017). Furthermore, *in vitro* NMP cells can then be coaxed into either spinal cord neurons and / or paraxial mesoderm cells by exposure to specific signals at the correct time windows. We are using this system to gain a better understanding of how NMP cells decide between the spinal cord or paraxial mesodermal lineage (Figure 2). Overall, our results illustrate the importance of mimicking normal embryonic development for the efficient generation of specific cell types from PSCs.

Modeling Human Neuromuscular Diseases Using Patient iPS Cells.

The ability to culture induced pluripotent stem (iPS) cells and edit their genome offers advantages for regenerative medicine applications. In conjunction with our *in vitro* system, it provides a unique opportunity to understand the pathology of neuromuscular diseases. The last years, remarkable progress has been made in the generation of spinal cord motor neurons (MNs) from human PSCs. However, the ability of these MNs to connect and control human skeletal muscle remains uncertain. We use the NMP differentiation system to generate in parallel nerve and muscle cells in the dish and understand the origins of disease and the contribution of either lineage (neural – muscle) to its pathology (Figure 3). Apart from implications in disease modeling and drug screening, our novel culture system enables new promises for regenerative medicine.

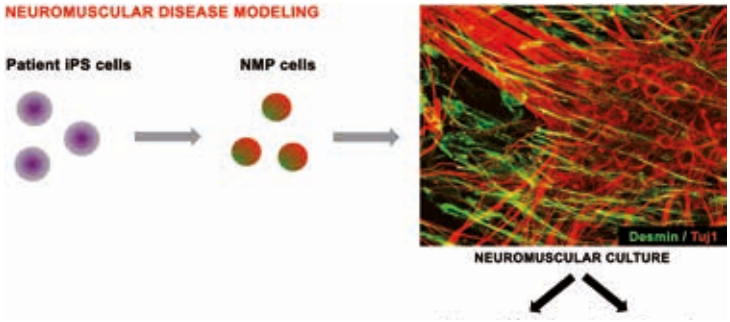

Figure 3: NMPs as a tool for disease modeling and drug screening. *Patient derived somatic cells can be reprogrammed to generate induced pluripotent stem cells (iPS cells) carrying the disease related phenotype. Directed differentiation of iPS cells through an NMP intermediate stage results in the generation of neurons (Tuj1+) and muscle cells (Desmin+). Neuromuscular cultures generated from patient specific iPS cells can be used for disease modeling and high-throughput drug screening.*

Selected Publications

Gouti, M., Delile, J., Stamataki, D., Wymeersch, F. J., Huang, Y., Kleinjung, J., Wilson, V., Briscoe, J. (2017). A gene regulatory network balances neural and mesoderm specification during vertebrate trunk development. Developmental Cell *In Press*.

Gouti, M., Metzis, V., and Briscoe, J. (2015). The route to spinal cord cell types: a tale of signals and switches. Trends Genet *31*, 282-289.

Gouti, M., Tsakiridis, A., Wymeersch, F.J., Huang, Y., Kleinjung, J., Wilson, V., and Briscoe, J. (2014). In vitro generation of neuromesodermal progenitors reveals distinct roles for wnt signalling in the specification of spinal cord and paraxial mesoderm identity. PLoS Biol *12*, e1001937.

Start of the Group: April 2016

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Photo: David Ausserhofer/MDC

Annette Hammes

Molecular Pathways in Cortical Development

We study the regulation of morphogen activity during neural development and in the adult cortical stem cell niche. Deficits in morphogen signaling lead to severe malformations of the central nervous system (CNS), including holoprosencephaly and neural tube closure defects. Another current project in our lab is addressing the question whether migratory neuronal precursors need mechanical cues for proper differentiation and pathfinding. We hypothesize that besides the well established chemical signaling, migrating neurons need mechanosensitive protein complexes to transduce motion and interaction with their environment into electrical signals.

Sophisticated regulation of morphogen activity in the embryonic and adult brain

in collaboration with AG Prof. Thomas Willnow

LRP2 (low density lipoprotein receptor related protein 2) is a multifunctional endocytic receptor. We previously clarified the molecular mechanism underlying the holoprosencephaly (HPE) phenotype of mouse embryos deficient for this receptor. LRP2 promotes morphogen signaling as a SHH co-receptor to patched 1 in the ventral forebrain neuroepithelium (Christ et al., 2012). The receptor is also important for neuronal progenitor proliferation and neurogenic output in the adult cortical stem cell niche by maintaining balanced morphogen activity (Gajera et al., 2010). In the retinal stem cell niche, the ciliary marginal zone (CMZ), LRP2 antagonizes SHH activity by mediating endocytic clearance of the morphogen to protect quiescent progenitor cells from mitogenic stimuli. Loss of LRP2 in mice leads to ectopic proliferation in the CMZ progenitor niche (Christ et al., 2015). Our findings document the ability of LRP2 to act in a context dependent manner as activator or inhibitor of the SHH pathway. Overall the data substantiate the emerging concept that auxiliary receptors are critical modulators of morphogen delivery and signal reception in target tissues.

New insights into receptor mediated uptake of folic acid during neurulation

Dr. Nora Mecklenburg, Dr. Esther Kur

LRP2 deficient embryos suffer from impaired closure of the cranial neural tube. This defect is unrelated to the etiology of holoprosencephaly and hinted at an additional role for LRP2 in the dorsal domain of the developing brain. Neural tube closure defects (NTDs) have been attributed to impaired folic acid (vitamin B_9) metabolism in mice and humans. We therefore asked whether LRP2 might be required for delivery of folic acid to neuroepithelial cells during neurulation. Our results showed that LRP2 mediates endocytic uptake of folic acid and its binding protein FOLR1 (folate receptor 1), a crucial step for proper neural tube closure (Figure 1; Kur et al., 2014). These findings substantiate our hypothesis that LRP2 teams up with other receptors forming co-receptor complexes to ensure not only ligand specificity in a tissue dependent manner but also to ensure precisely timed and titrated uptake of signaling molecules. Moreover, to better understand the molecular mechanisms of LRP2 dependent NTDs, we will further investigate whether other factors such as altered morphogen signaling or disturbed cilia function in addition to impaired folic acid uptake modulate the NTD phenotype in LRP2 deficient mouse embryos.

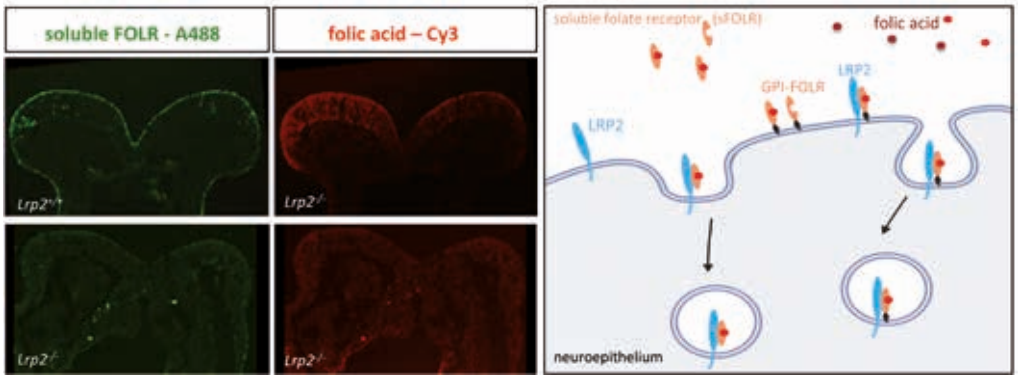

Figure 1. *Immunohistological detection of sFOLR1-A488 and folic acid-Cy3 on coronal head sections of E8.5 whole-embryo cultures. Uptake of these added ligands was only seen in wild-type but not in Lrp2$^{-/-}$ rostral neural folds. The model depicts LRP2 dependent endocytic uptake of folic acid into neuroepithelial cells. Soluble FOLR1 and GPI-anchored FOLR1 bind folic acid. Internalization of these complexes relies on the interaction with the co-receptor LRP2.*

Understanding the genetic causes of neural tube defects (NTDs)

Fabian Paul, Deborah Kohler, Dr. Nora Mecklenburg

Identifying novel pathways contributing to NTDs

LRP2 deficient mice are ideal to study the multifactorial etiology of human CNS anomalies since the severity of the *Lrp2*$^{-/-}$ NTD phenotype varies strongly depending on the genetic background. This suggests a strong impact of mouse strain associated genetic modifiers. We generated RNAseq data from *Lrp2* mutant embryos on C57BL/6N background suffering from severe CNS anomalies and from mutants on FVB/N background with a mild phenotype. The aim is to identify differentially regulated pathways and new modifier genes modulating the severity of NTDs. The genetic modifiers identified in our studies will be also used to evaluate analogous gene-gene interactions in NTD patient data in collaboration with Dr. Angela Kaindl and Dr. Gregory Wulczyn (Charité, Berlin). The goal is to improve genetic risk assessment and to gain deeper insights in the pathogenesis of human NTDs.

The role of LRPs in WNT-signaling dependent development of the CNS

Another LRP family member, the WNT receptor LRP6, has been implicated in neural tube defects in mice and NTD patients. To test for gene-gene interactions among *Lrp* gene family members, linked to the WNT pathway, we are currently analyzing *Lrp4*$^{-/-}$; *Lrp5*$^{-/-}$ and *Lrp4*$^{-/-}$; *Lrp6*$^{-/-}$ compound mutant embryos. Initial data show more severe and highly penetrant CNS anomalies in these compound mutant mouse embryos compared to single mutants, suggesting important new functions for LRP4 and LRP5 in the developing brain that may be synergistic with LRP6 activity. We will test the hypothesis that the phenotypes seen in *Lrp4*; *Lrp6* and *Lrp4*; *Lrp5* compound mutants are linked to disturbed canonical WNT/ß-catenin dependent and/or non-canonical WNT planar cell polarity pathways.

The role of mechanotransduction in the motility and migration of neuronal precursor cells

Carina Fürst in collaboration with AG Prof Gary Lewin

Cell migration is a fundamental process in the developing brain and strongly influenced by chemical signals. However there are still many open questions concerning the mechanisms underlying neuronal migration. We hypothesize that neuronal precursors use mechanotransduction to probe their physical environment during migration. First recordings of mechanically gated currents in neuroepithelial cells provide evidence that neuronal precursors are indeed mechanosensitive. We will further characterize mechanical signaling in neuronal development using mouse genetics, electrophysiology, and high-resolution imaging.

Selected Publications

Eccles RL, Czajkowski MT, Barth C, Müller PM, McShane E, Grunwald S, Beaudette P, Mecklenburg N, Volkmer R, Zühlke K, Dittmar G, Selbach M, Hammes A, Daumke O, Klussmann E, Urbé S, Rocks O. Bimodal antagonism of PKA signaling by ARHGAP36. *Nature Commun.* 2016 Oct 7;7:12963.

Nasrallah R, Fast EM, Solaimani P, Knezevic K, Eliades A, Patel R, Thambyrajah R, Unnikrishnan A, Thoms J, Beck D, Vink CS, Smith A, Wong J, Shepherd M, Kent D, Roychoudhuri R, Paul F, Klippert J, Hammes A, Willnow T, Göttgens B, Dzierzak E, Zon LI, Lacaud G, Kouskoff V, Pimanda JE. Identification of novel regulators of developmental hematopoiesis using endoglin regulatory elements as molecular probes. *Blood.* 2016 Aug 23.

Christ A, Christa A, Eule JC, Bachmann S, Wallace V, Hammes A, Willnow TE. LRP2 acts as SHH clearance receptor to protect the retinal margin from mitogenic stimuli. *Dev Cell.* 2015 Oct 12;35(1):36-48.2015

Kur E, Mecklenburg N, Cabrera RM, Willnow TE, Hammes A. LRP2 mediates folate uptake in the developing neural tube. *J Cell Sci.* 2014 May 15;127(Pt 10):2261-8.

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Photo: Stefan Jentsch

Thomas Jentsch

Physiology and Pathology of Ion Transport

Ion transport across cellular membranes is crucial for cellular homeostasis and has integrative functions such as transepithelial transport or signal transduction. We study ion transport at various levels: from biophysical and structure-function analysis of transport proteins to their role in the cell and the organism. We have discovered several human 'channelopathies' and have generated and analyzed many mouse models.

Until recently, we have focused on CLC Cl^- channels and transporters, Anoctamin Ca^{2+}-activated Cl^- channels, KCNQ K^+ channels, and KCC K^+-Cl^- cotransporters. Their disruption led to pathologies ranging from epilepsy, deafness, and neurodegeneration to osteopetrosis and kidney stones. We are particularly interested in the control of neuronal excitability, sensory physiology and ion homeostasis in endosomes and lysosomes.

After >3 years of intense effort, we achieved in 2014 another ambitious goal and identified LRRC8 heteromers as constituting the volume-regulated anion channel VRAC. This ubiquitously expressed channel with many proposed functions has been known biophysically for >30 years, but the underlying proteins have remained unknown. This breakthrough opens up an entire new field. We are now focusing much of our effort on VRAC. We will continue to investigate CLCs, but will stop working on Anoctamins, KCNQs and KCCs after successfully concluding ongoing projects.

Fig. 1: The Journal of Physiology commemorated the discovery of CLC channels and transporters by Thomas Jentsch 25 years ago *with a special issue. The cover image shows the CLC chloride channel of E. coli (PDB ID: 1KPK) embedded in a lipid bilayer membrane (J Physiol, 593, 2015).*

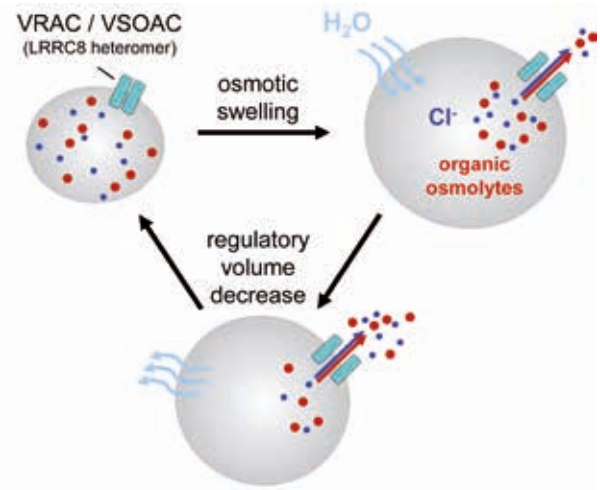

Fig. 2: Schematic diagram of VRAC's function in regulatory volume decrease. *VRAC is closed under resting conditions (top left) and opens after osmotic swelling. The resulting efflux of chloride and organic osmolytes decreases cellular osmolarity, resulting in water efflux and regulatory volume decrease.*

CLC Cl^- channels and transporters

Anja Blessing, Tony Daubitz, Corinna Göppner, Sabrina Jabs, Maja Hoegg-Beiler, Carmen Ludwig, Ian Orozco, Sonali Saha, Tobias Stauber, Till Stuhlmann, Florian Ullrich, Janis Vogt, Stefanie Weinert

The CLC gene family, discovered by us 25 years ago (Fig. 1), encodes plasma membrane Cl^- channels and Cl^-/H^+ exchangers of intracellular membranes. We generated mouse models for most CLCs, identified ancillary β-subunits, discovered that vesicular CLCs are Cl^-/H^+-exchangers, performed structure-function analysis, and uncovered several pathologies resulting from their dysfunction. Vesicular CLCs were believed to be Cl^- channels that facilitate vesicular acidification by shunting proton pump currents, but surprisingly they are Cl^-/H^+-exchangers. Mice in which we converted ClC-5 and ClC-7 to uncoupled Cl^- channels unexpectedly displayed roughly the same phenotype as the respective KOs, suggesting a role of these CLCs in vesicular Cl^- accumulation. However, some phenotypes of the 'uncoupled' *Clcn7*$^{unc/unc}$ mice were less severe than in *Clcn7*$^{-/-}$ mice, indicating either a rescue of Cl^-/H^+-exchange by the ClC-7^{unc} Cl^- conductance, or an ion transport-independent function of the ClC-7 protein (that is present in *Clcn7*$^{unc/unc}$, but not in *Clcn7*$^{-/-}$ mice). To distinguish between these possibilities, we created mice with a point mutation that totally abolishes ClC-7 ion transport. These mice showed that some phenotypes of *Clcn7*$^{-/-}$ mice depend on the absence of the ClC-7 protein rather than the loss of ion transport. We characterized functionally ClC-7 mutants identified in patients and osteopetrotic cattle and titrated the amount of osteoclast-expressed ClC-7 with respect to osteopetrosis in transgenic mice. We are currently analyzing *Clcn3*$^{unc/unc}$ mice to investigate whether the endosomal ClC-3 needs, like ClC-5 and ClC-7, exchange activity for its biological role. We showed that ClC-2 disruption leads to leukodystrophy in mice and others identified *CLCN2* mutations in a form of human leukodystrophy. Human MLC-type leukoencephalopathy can be caused by mutations in the membrane protein MLC1 and in GlialCAM, a cell adhesion molecule that physically interacts with MLC1. GlialCAM also binds to ClC-2 and changes its current properties. GlialCAM directs both MLC1 and ClC-2 to cell-cell contacts of transfected cells. We have generated *Glialcam*$^{-/-}$ and *Glialcam*$^{dn/dn}$ mice, which carry a mutation found in human leukodystrophy. Comparison with *Mlc1*$^{-/-}$ and *Clcn2*$^{-/-}$ mice showed that GlialCAM is important for the localization of ClC-2 and MLC1 also *in vivo*. ClC-2 currents were reduced in oligodendrocytes of all three mouse models, suggesting that the lack of glial Cl^- channels contributes to the pathology of all three forms of leukodystrophy. However, the loss of GlialCAM has additional pathogenic effects. We are now studying cell-type specific ClC-2 KOs to pinpoint the cell type in which loss of ClC-2 is pathogenic.

K^+-Cl^- cotransporters

Kathrin Gödde

Previously, we constitutively disrupted all KCl-cotransporter isoforms (KCC1-4) in mice. We now focused on KCC2 which lowers the cytoplasmic Cl^- concentration in neurons, a prerequisite for the inhibitory action of GABA- and glycine-receptor Cl^- channels. After having published our work on mice with specific KCC2 elimination in cerebellar granule and Purkinje cells, we now disrupted Kcc2 specifically in mitral cells of the olfactory bulb, the first relay

Fig. 3: VRAC: dual role in drug resistance. *VRAC regulates cell volume by releasing Cl^- and organic osmolytes like taurine, and also mediates uptake of cancer drugs like cisplatin. Cisplatin enters cells by passive diffusion and through VRACs containing subunit LRRC8D (red). It kills cells by modifying nuclear DNA, and opens VRACs possibly by generating reactive oxygen species in mitochondria, thus further stimulating cisplatin uptake and apoptotic volume decrease (EMBO J, 34: 24, 2015).*

station in olfactory information processing. Synaptic inhibition of mitral cells was markedly reduced and behavioural experiments showed that these mice could not distinguish closely similar odours. These studies are complemented with in vivo electrophysiology.

Anoctamin (TMEM16) Ca^{2+}-activated Cl^- channels

Jonas Münch, Sebastian Albrecht

After having shown that Ano2 is the long-sought Ca^{2+}-activated Cl^- channel of olfactory sensory neurons (OSN) in the main olfactory epithelium (MOE), but that it is dispensable for olfaction, we now focus on the vomeronasal organ (VNO) that is e.g. responsible for the detection of pheromones. The signal transduction in the VNO is different from the MOE and it expresses both Ano1 and Ano2. We generated mice lacking both Ano1 and -2 in OSNs and cross them to mice lacking the upstream Trpc2 cation channel to genetically and functionally dissect the poorly understood signal transduction cascade in the VNO.

(4) KCNQ K^+ channels

Pawel Fidzinski, Matthias Heidenreich, Ian Orozco, Sebastian Schütze

Of the five different isoforms of KCNQ (Kv7) K^+ channels, KCNQ2-5 mediate 'M-currents' that regulate neuronal excitability. We previously showed that KCNQ2 and -3 underlie a form of human epilepsy, and that KCNQ4 mutations can cause human deafness which we then analysed with mouse models. We also demonstrated a role of KCNQ4 and -5 in the vestibular organ. We now focused on the very sensitive D-hair skin mechanoreceptors. In contrast to rapidly adapting mechanosensors, where we had demonstrated a role of KCNQ4 in mice and men, we found KCNQ3 in the very sensitive skin D-hair nerve endings where it dampens excitation. Our recent work on the CNS function of KCNQ5, which combines immunohistochemistry, slice electrophysiology, in vivo registrations and behavioural studies, indicates a role of KCNQ5 in dampening synaptic inhibition, whereas KCNQ2 and -3 dampen excitation.

VRAC volume-regulated anion channels

Andreia Cruz e Silva, Tony Daubitz, Deborah Elger, Maja Hoegg-Beiler, Jennifer Lück, Darius Lutter, Jonas Münch, Rosa Planells-Cases, Mayya Polovitskaya, Momsen Reincke, Tobias Stauber, Till Stuhlmann, Florian Ullrich, Felizia Voss, Pingzheng Zhou, Joanna Ziomkowska

Swelling-activated Cl^- currents mediated by a postulated channel named VRAC (volume-regulated anion channel) (a.k.a. VSOR or VSOAC) have first been described in the 1980s and hundreds of papers described its properties and suggested physiological roles. This channel was (controversially) believed to also conduct organic osmolytes like taurine and was postulated to not only play a role in cell volume regulation (Fig.

2), cell division and migration, but also in apoptosis, cancer drug resistance, gliotransmitter release, and stroke. Progress in this area, however, had stalled because despite many attempts the molecular identity of VRAC remained unknown until our identification of LRRC8 heteromers in 2014 and the parallel identification of the LRRC8A subunit by Patapoutian and colleagues. We used a genome-wide siRNA screen in a functional assay that relied on swelling-induced iodide influx and subsequent YFP fluorescence quenching. We identified LRRC8A as an essential subunit. It displays 4 transmembrane domains and 17 cytoplasmic Leucine-Rich Repeats (hence its name LRR Containing 8A). Expression studies and KO cells generated by CRISPR-Cas9 showed that LRRC8A is not sufficient for VRAC activity but needs at least one other member (LRRC8B-E). We showed that LRRC8 heteromers are crucial for regulatory volume decrease and that they also mediate swelling-activated taurine efflux. Our work (Science 2014) opened an entire field. Our next paper (EMBO J. 2015) confirmed the hypothesis that downregulation of VRAC, which mediates apoptotic volume decrease, impairs the progression of drug-induced apoptosis. With cisplatin, a widely used anticancer drug, there was an unforeseen twist: We discovered that it enters cells through VRAC (Fig. 3). Uptake of cisplatin, as well as taurine efflux, was greatly stimulated by the LRRC8D subunit. Analysis of databases revealed a clinically important correlation of reduced tumor expression of LRRC8D with poor survival in cis/carboplatin treated patients with ovarian cancer. The observed dependence of substrate selectivity on LRRC8 subunit composition shows that these proteins form the pore of VRAC. Our lab is now tackling many exciting aspects of VRAC structure/function, physiology, and role in disease.

Pending Patents

LRRC8-Comprising protein complexes and methods for identification of VRAC modulators
Inventors: Jentsch, Voss, Stauber; Applicants: MDC/FVB
EP2919009 (A1), filed on 2014-06-17; US2015253303 (A1) filed on 2015-03-10

Chloride transporter ClC-7 and cell-based screening method
Inventors: Jentsch, Stauber; Applicants: MDC/FVB
EP2420830 (A1), filed on 2011-08-16

Selected Publications

Gödde K., Gschwend O., Puchkov D., Pfeffer C.K., Carleton A., **Jentsch** T.J. (2016). Disruption of Kcc2-dependent inhibition of olfactory bulb output neurons suggests its importance in odour discrimination. Nature Comm. 7, 12043

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Weinert S., Jabs S., Hohensee S., Chan W.L., Kornak U., **Jentsch** T.J. (2014). Transport activity and presence of ClC-7/Ostm1 complex account for different cellular functions. EMBO Rep. 15, 784-791.

Voss F.K., Ullrich F., Münch J., Lazarow K., Lutter D., Mah N., Andrade-Navarro M.A., von Kries J.P., Stauber T., **Jentsch** T.J. (2014). Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. Science 244, 634-638.

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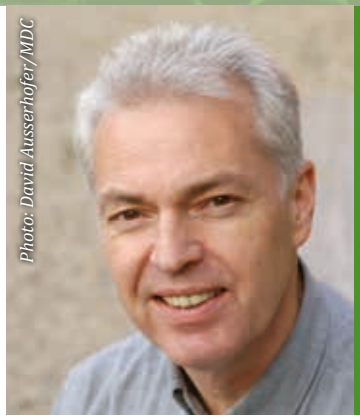


Photo: David Ausserhofer/MDC

Helmut Kettenmann

Cellular Neurosciences

Our goal is to understand the role of glial cells in physiology and pathology. We analyze how glial cells communicate among each other and with neurons. We determine how astrocytes and oligodendrocytes are connected via gap junctions and how the panglial network influences neuronal function. Another focus of our research is on microglial cells, the immune mediators and pathologic sensors of the central nervous system. We study the expression of transmitter receptors in microglial cells and how activation of these receptors influences microglial functions. Within the context of pathology we are currently studying the importance of microglial cells in stroke, Alzheimer's disease and schizophrenia. Another line of research addresses the question how microglia interact with glioma cells. We aim to understand this interaction on a molecular level for the purpose of identifying therapeutic targets.

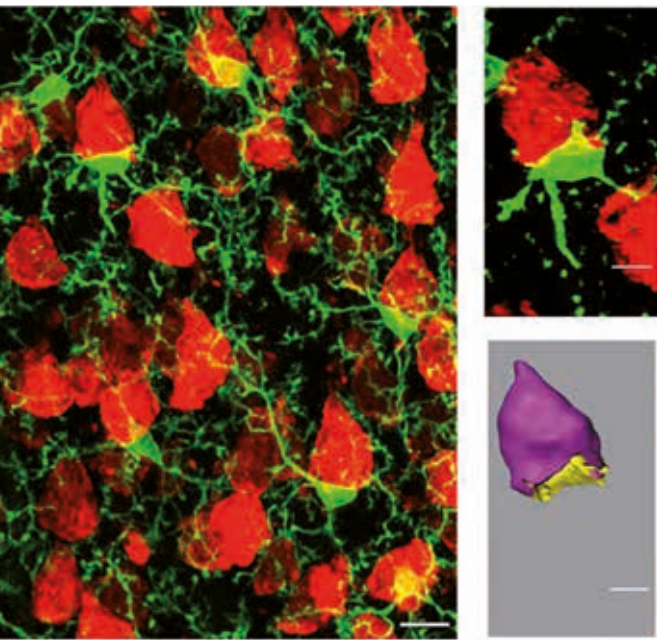
Introduction

The central nervous system contains two major cell populations, neurons and glial cells. It has become evident that glial cells are essential for the proper functioning of the brain. The different types of glial cells fulfil distinct tasks. Oligodendrocytes are the myelin-forming cells of the central nervous system and ensure rapid signal conduction in the white matter. The role of astrocytes is less well defined; they provide guiding structures during development and represent important elements for controlling the composition of the extracellular space mediating signals between the brain endothelium and neurons. They form intimate contact with synapses and neuronal activity results in astrocyte responses. Microglial cells are immune-competent cells in the brain and their functional role is best defined as the first responsive elements during pathologic events. The present research program is focused on four topics: (1) the extent of gap junction coupling within the glial network and its impact on brain function (2) the functional expression of transmitter receptors in microglia (3) the response of microglial cells to brain injury and (4) the interaction of gliomas with microglia and astrocytes.

Structural and functional analyses of panglial coupling networks and its impact on brain signaling

Niklas Meyer, Christiane Nolte (in collaboration with Christian Steinhäuser, University of Bonn)

Gap junction coupling among glial cells plays an important role for brain homeostasis, brain communication and brain energy supply. Most previous studies have focused on the network formed by astrocytes. We now characterize communication between all types of glial cells, in particular astrocytes and oligodendrocytes. We found that microglial cells are not part of the panglial network. We now address the question whether these panglial networks are heterogeneous with respect to brain region, comparing different white and gray matter regions. We are characterizing junctional properties and their modulation by neurotransmitters. Moreover, we now address the question whether the coupled network has functional implications for energy supply to neurons. We aim to present a more comprehensive view of the panglial net-



Satellite microglial cells are closely associated with neurons. On the left neurons are shown in red, microglia in green. The right upper image shows such a satellite microglia/neuron pair. Below the contact zone between microglia and neuron is illustrated.

work and its heterogeneity and function in the brain. (Funded by DFG).

Microglial express functional receptors for neurotransmitter/ neurohormones

Amanda Costa, Meron Maricos, Martin Mersch, Susanne A. Wolf

We have addressed the question whether microglia express neurotransmitter receptors to sense neuronal activity. We have previously identified receptors for the neurotransmitters/neurohormones GABA, adrenaline, dopamine, bradykinin, serotonin, endothelin-1, substance P and histamine. We are characterizing these responses not only in microglia in culture, but also in situ and in freshly isolated cells.

A subpopulation (11%) of freshly isolated adult microglia also respond to the muscarinic acetylcholine receptor agonist carbachol with a Ca^{2+} increase, indicating the expression of functional receptors. The carbachol-sensitive population increased in microglia/brain macrophages isolated from the tissue of mouse models for stroke and Alzheimer's disease, but not for glioma and multiple sclerosis. As an example, microglia cultured from adult and neonatal brain contained a small carbachol-sensitive subpopulation which was increased in microglia isolated from stroke tissue. This increase correlated with an upregulation of the muscarinic receptor subtype 3 (M3). Carbachol is a chemoattractant for microglia and down-regulates their phagocytic activity. (Funded by DFG and BIH).

Microglial cells show an intrinsic spontaneous electrical activity uncorrelated to neuronal responses

Laura Maria Korvers, Marina Matyash, Marcus Semtner, Stefan Wendt

A subpopulation of microglia, called satellite microglia is defined by a close morphological soma-to-soma association with a neuron, indicative of a direct functional interaction. Indeed, ultrastructural analysis revealed closely attached plasma membranes of satellite microglia and neuron. Evoked local field potentials or action potentials and postsynaptic potentials of the associated neuron did not lead to any correlated membrane potential change in the satellite microglia as revealed by double patch-clamp recordings in cortex and hippocampus. Microglia, however, show spontaneous transient membrane depolarizations which were not correlated with neuronal activity. These events could be divided into fast and slow rising depolarisations which exhibited different characteristics in satellite and non-satellite microglia. A second form of spontaneous activity are transient increases in intracellular Ca^{2+} which we study in cultured microglial cells. These Ca^{2+} increases are due to release from internal stores.

The response of microglial cells to brain injury

Masataka Ifuku, Philipp Jordan, Susanne A. Wolf, Stefan Wendt

During any kind of disease or any pathological event such as after trauma, stroke or in multiple sclerosis, the resting microglial cell transforms into an activated form characterized by an ameboid morphology. Activated microglia can proliferate, migrate to the site of injury, phagocytose, and release a variety of factors like cytokines, chemokines, nitric oxide (NO) and growth factors. We are studying microglial properties in different mouse models of disease including Alzheimer's disease and stroke (in collaboration with Matthias Endres and Christoph Harms, Neurology, Charité). In collaboration with the FMP we identified drug like compounds that change specific microglial function such as NO production. We will test these novel compounds in the above mentioned mouse models for their therapeutic potential.(Funded by DFG)

Microglia as therapeutic target in psychiatric disorders

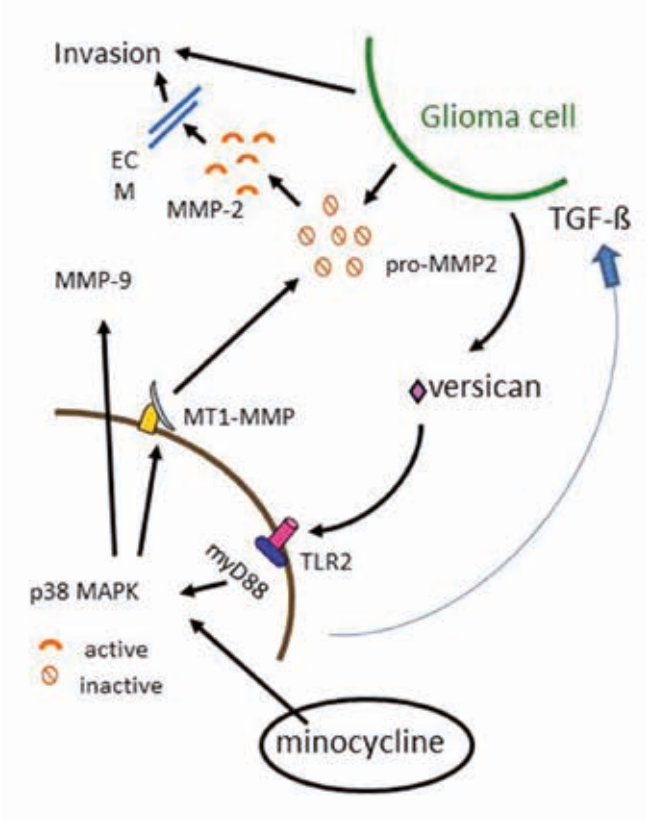
Dilansu Gueneykaya, Daniele Mattei, Susanne A. Wolf

Many studies discuss an immunological component in the onset and progression of psychiatric disorders. While the peripheral immune system has been intensively investigated, little is known about the role of microglia. We thus aim to study the microglia phenotype derived from rodent models of schizophrenia including epigenetic factors. To model a schizophrenia-like endophenotype, we used maternal immune stimulation by injecting polyinosinic-polycytidilic acid (Poly I:C) into pregnant rats or mice to mimic an anti-viral immune response. We identified microglia IL-1β and TNF-α increase constituting the factors correlating best with decreases in net-neurogenesis and impairment in pre-pulse inhibition of a startle response in the Poly I:C model. Treatment with the antibiotic minocycline normalized microglial cytokine production in the hippocampus and rescued neurogenesis and behavior. Moreover genome wide analysis of microglia by RNAseq isolated from PolyI:C animals revealed a significant change in the transciptome that was normalized by minocycline treatment. These findings strongly support the idea to use anti-inflammatory drugs to target microglia dysregulation as an adjunctive therapy in schizophrenic patients.

What are the properties of glioma associated microglia/brain macrophages

Alice Buonfiglioli, Jan-Frederik Fischer, Felipe de Almeida Sassi, Susanne A. Wolf (In collaboration with Michael Synowitz, University of Kiel, Dolores Hambardzumyan, Emory University, Atlanta, Eric Holland, Fred Hutch Cancer Center, Seattle and David Gutmann, Washington University, St. Louis)

Gliomas comprise the majority of cerebral tumors and patients have a poor prognosis since there is essentially no concept for successful treatment. A large fraction of the cells within a glioma are brain macrophages/microglial cells (GAMs) which can amount up to 30%. These cells are a mix of intrinsic microglia and monocytes invaded from the periphery. We have established a procedure to isolate, purify and culture human and mouse GAMs. Corresponding control (non-glioma–associated microglial cells) human cells are obtained from patients with epileptic seizures that underwent temporal lobe surgery or from post-mortem isolated microglia. We have determined the genome wide expression profile of the GAMs from mouse and human and found that these cells acquire a distinct phenotype which does not fit into the classical scheme of the M1 or M2 phenotype, a definition originating from the macrophage field. Genetic profiling of the different microglial populations will indicate new pathways of glioma interactions with GAMs. We also determine how bulk glioma cells and glioma stem cells differentially affect the GAM phenotype and found that GAMs promote glioma stem cells via IL-6 signaling (Funded by DFG, BIH and NIH).



Scheme of interaction between glioma and microglia/brain macrophages. Glioma release the inactive pro-MMP2 which is cleaved into active MMP2 by microglial MT1-MMP. Its expressionn is stimulated by versicans released from glioma cells. Versican activates TLR2 signaling in microglial cells which leads to the up regulation of MT1-MMP. TLR2 signaling in microglia also triggers MMP9 release. This cascade promotes glioma growth (modified after Hambardzumyan et al., 2016).

Microglia/brain macrophages promote glioma growth

Alice Buonfiglioli, Jan-Frederik Fischer, Verena Haage, Felipe de Almeida Sassi, Susanne A. Wolf (In collaboration with Michael Synowitz, University of Kiel, and Dolores Hambardzumyan, Emory University)

We have found that microglial cells strongly promote glioma growth and invasion. There is an interesting interplay between microglial and glioma cells. Glioma cells release the metalloprotease MMP2 which is important for degradation of extracellular matrix and promotes invasion. This metalloprotease is, however, released in an inactive, larger form and it needs to be cleaved to acquire its activity. This cleavage is accomplished by the ectoenzyme MT1-MMP. An endogenous ligand for Toll-like receptors (TLR), versican, is released from glioma and activates TLR2 toll-like receptors in the surrounding GAMs. TLR2 signaling triggers the expression of MT1-MMP in GAMs. The activation of the TLR2 pathway also induces the release of MMP-9 from GAMs. Thus, glioma cells exploit GAMs to promote their invasion. Thus interfering with TLR receptors or their intracellular pathways reduced the rapid expansion of glioma cells as we could show with TLR2-interfering antibodies and microglia cells have become a new target for glioma research and for potential therapy. (Funded by DFG and NIH).

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Photo: David Ausserhofer/MDC

Gary Lewin

Molecular Physiology of Somatic Sensation

Somatic sensation includes all those sensations that we consciously feel after stimulation of the body, e.g. touch, warmth, cooling, or even limb movement. We experience these sensations as a direct result of the activation of sensory neurons that are located in the dorsal root ganglia (DRG). In our group we are interested in the molecular mechanisms that allow these neurons to transduce these varied stimuli. Sensory neurons can, for example, detect changes in temperature of the skin in the non-noxious (not painful) as well as the noxious range (painful heat, or cold). They can also detect gentle movement of the skin as well as intense mechanical stimulation of the skin that is normally harmful. The nature of the transduction molecules involved in transforming mechanical and thermal stimuli into electrical signals that lead to perception is a major focus in the lab. Mechanosensitive channels required for touch sensation have recently been identified in sensory neurons in work from our group. It is increasingly clear that mechanosensitive channels serve as important initiators of critical signalling events in cell types outside of sensory systems, a new area being explored in the lab.

Molecular Basis of Mechanotransduction

Caglar Gök, Carina Fürst, Raluca Fleischer, Regina Herget, Mirko Moroni, Kate Poole, Christiane Wetzel, Martha Rocio Servin-Vences

Mechanotransduction is the process whereby receptor proteins present in the endings of sensory neurons are able to detect mechanical stimulation of the tissue they innervate. We have used information from genetic experiments with the nematode worm C. elegans to identify possible vertebrate candidate proteins that might detect mechanical stimuli. Genetic screens for touch insensitive worms have turned up around 15 genes whose function is necessary to confer touch sensitivity. These genes were named mec for mechanically insensitive and we have focused on identifying the role mammalian orthologs of these genes play in touch sensation. The mec genes in C. elegans have been proposed to work together in a mechanotransduction complex. An essential component of this complex is the membrane protein MEC-2 that forms a hairpin in the membrane and might regulate the activity of the mechanotransduction channels. We have cloned and characterized vertebrate homologues of mec genes and have created mouse mutant alleles to characterize the in vivo function of these genes. MEC-2 is a member of a large family of proteins that contain a stomatin-like domain. A member of this family called STOML3 (stomatin like protein-3) was cloned by our group, and we generated a mouse model with a null mutation of the STOML3 locus. In STOML3 mutant mice many mechanoreceptors (or touch receptors) in the skin do not work in the absence of the STOML3 protein.

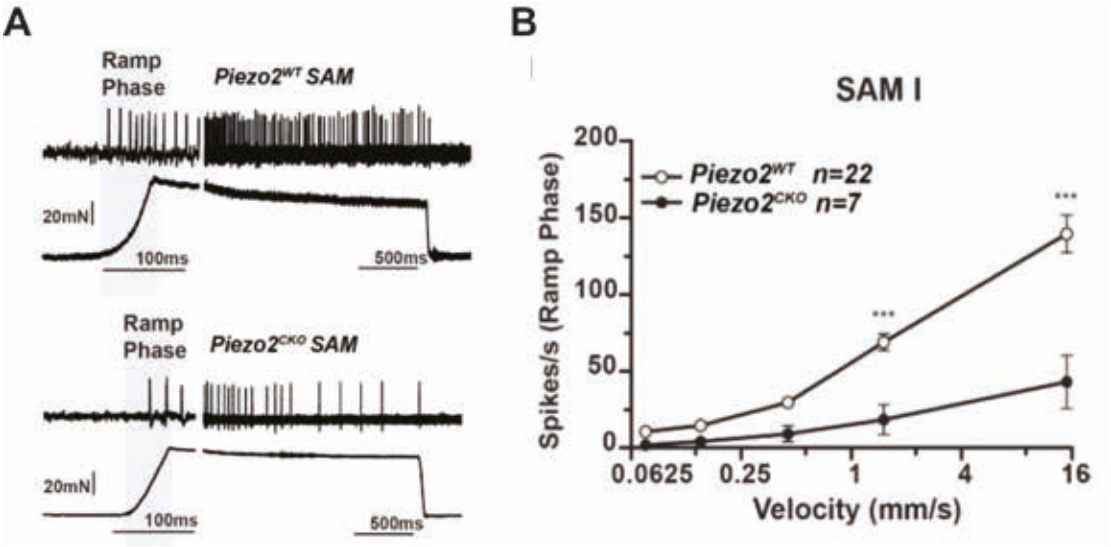

Figure 1 *Dramatic loss of mechanosensitivity in slowly adapting mechanoreceptors (SAM are type I Merkel cell receptors) after conditional deletion of the Piezo2 gene in sensory neurons (A). A cardinal feature of all SAMs is high sensitivity to moving stimuli. (B) is a quantitatve measure of the receptors velocity sensitvity which is virtually absent after Piezo2 gene deletion. (B) data reproduced from Ranade et al 2014 Nature.*

The central core of any mechanotransducer is an ion channel or channels(s), unfortunately the molecular nature of this channel has not yet been definitively identified in sensory neurons. There is some evidence that acid sensing ion channels (ASICs) which belong to the Deg/ENaC family of ion channels have an influence on the transduction of mechanical stimuli by sensory neurons. Indeed we have shown that there are genetic interactions between stomatin-domain proteins and acid sensing ion channels. Recently, two new mechanosensitive ion channels have been identified in mammals called Piezo1 and Piezo2. Piezo 2 is a huge trimeric membrane protein that is expressed in touch receptors of the dorsal root ganglia. In collaboration with researchers at the Scripps Research Institute in California we recently showed that Piezo2 is absolutely required for touch receptor function (Ranade et al 2014). Indeed the loss of function in mice with conditional deletion of the Piezo2 gene in touch receptors has remarkable similarities to the sensory phenotype of STOML3 mutant mice generated in our lab.

Using a newly developed assay based on elastomeric pili we could also show that the sensitivity of Piezo1 and Piezo2 ion channels to mechanical displacement is exquisitely tuned by the STOML3 protein (Poole et al 2014). The use of this newly developed pili technique allows us to measure with extremely high precision fast mechanotransduction in almost any cell type. The lab is now investigating the physiological role of "new and old" mechanically gated channels in a variety of non-sensory cell types.

Stomatin domain proteins, molecular membrane scaffolds with multiple roles in physiology and pathophysiology

Caglar Gök, James Hall, Kate Poole, Jane Reznick

Stomatin-domain proteins can regulate both ion channels and transporter proteins. The structural and molecular basis of such regulation remained a mystery until recently. Together with the Daumke group we have used structural biology techniques and structure function studies to examine the molecular mechanisms of stomatin-domain protein function. By using X-ray crystallography techniques the structure of the mammalian stomatin-protein was solved: We showed that the basic building block of this protein is a banana-shaped dimer that can form the basis of higher order oligomeric structures in the membrane. The oligomerization of stomatin-domains appears to be necessary for their ability to modulate both

ASIC proteins as well as mechanosensitive ion channels like Piezo1 and Piezo2 (Poole et al 2014). Ongoing work in the lab seeks to examine the precise in vivo function of other poorly understood stomatin-domain containing proteins such as STOML1. The use of mouse models and advanced cellular imaging techniques has revealed unexpected roles for stomatin-like proteins in metabolic diseases.

The activity of mechanosensitive ion channels expressed by sensory neurons can be measured using high-resolution electrophysiology techniques. We have recently shown that such ion channels in the membranes of cultured DRG neurons can be activated by stimuli in the nanometer range (Poole et al 2014). We have also gathered considerable evidence that the mechanosensitive channels are actually opened via a protein tether that attaches to laminin-containing extracellular matrices. We have recently also shown that STOML3 is required to maintain the high sensitivity of endogenous Piezo2 currents in primary sensory neurons. We also developed small molecules that inhibit the oligomerization of STOML3 and these drugs work in vivo and in vitro to decrease the sensitivity of Piezo ion channels. Indeed these small molecules can reversibly silence touch receptors in the mouse and can be used to treat painful mechanical hyper sensitivity in mice that follows nerve lesions (Wetzel et al 2016).

Touch, hearing, and thermal sensation

Regina Herget, Johannes Kühnemund, Rabih Moshourab and Jan Walcher

Hereditary deafness is a relatively common phenomenon and a large number of genes have been identified that when mutated lead to deafness in mouse and man. We are working with several deaf mutant mice to examine whether genes required for normal mechanotransduction in the inner ear may also be required for normal cutaneous sensation. Our data indicate that members of the unconventional myosin protein family have a common function in sensory neurons and in hair cells, mechanotransducing cells of the inner ear. In both cell types these proteins may function to regulate the adaptation of the mechanotransduction channels. We are currently working on further hearing genes that may also affect cutaneous mechanosensation. The same genes as we study in the mouse are also mutated in humans and it is possible that the perception of cutaneous touch stimuli is altered in such patients. We have measured psychometric functions in healthy and hearing impaired people in order to describe quantitatively differences in the perception of touch. We carried out a large twin study which showed that touch and also thermosensation are both heritable in humans. The results of these studies have also revealed that there appears to be a strong link between the sense of touch and the sense of hearing. We could demonstrate that one gene, mutations in which cause severe hearing loss and blindness is also associated with poor touch performance in humans. This gene USH2A encodes a large extracellular protein that is thought to form a structural link within the sterocillia of inner ear hair cells. The role of this protein in touch receptors remains to be investigated. Our ability to sense temperature changes in the skin is actually quite remarkable. Humans can easily detect skin cooling of just 1°C or less. In collaboration with James Poulet we have now shown that mice also have such an exquisite temperature sense and have started to identify the sensory afferents that relay this information to the cortex (Milenkovic et al 2014). We are now using optogenetic and optochemical tools to determine which sensory afferents contribute to the perception of warming and cooling (Frank et al., Nat. Comm.6, 2015, doi: 10.1038/hcomms8118). We constantly try to apply our basic findings in mouse to humans. We have established a battery of psychometric testing procedures that enable us to measure very precisely touch performance in healthy volunteers as well as defined patient cohorts. We identified the c-kit receptor as an essential regulator of the heat transduction mechanism in mouse nociceptive sensory neurons. As a follow up to these studies we could recently show that humans being treated with drugs that

interfere with c-kit signaling actually modify noxious heat perception (Ceko et al 2014 Pain DOI: 10.1016/j.pain.2014.03.010).

The Naked Mole-Rat an extremeophile mammal

Damir Omerbasic, Tania Kosten, Jane Reznick, Valerie Begay-Müller

The naked mole rat is an unusual subterranean rodent in many respects. It is the only known poikilothermic mammal (ie. cold blooded), it lives in colonies with an insect-like social structure, and it is also the longest-lived rodent species known (lifetimes in excess of 32 yrs). Interestingly, although this animal has normal acute pain responses it displays no hypersensitivity (so called hyperalgesia) to a variety of inflammatory and chemical stimuli. What is particularly striking in the naked mole-rat is that the animals completely lack a neuronal or behavioral response to acid. We suspect that at the heart of this specialized adaptation lies in distinct gene variants encoding ion channels and associated channels that are required for the transduction of painful stimuli. We are at present cloning and characterizing genes coding ion channels from the naked mole rat to address this issue. We have cloned and started to characterize the naked mole rat capsaicin receptor, an ion channel called TRPV1, as well as the tyrosine kinase receptor trkA the activation of which by NGF can potently potentiate TRPV1 (Omerbasic et al 2016). We were very interested in understanding how naked mole-rats are completely insensitive to acid. We could show that this insensitivity can be explained by a unique variant of the nociceptor-expressed voltage gated sodium channel $Na_v1.7$ which is potently blocked by protons in this species leading to behavioral insensitivity to acid (Smith et al 2011 Science DOI: 0.1126/science.1213760).

However, our interest in naked mole-rat physiology goes beyond the study of sensory systems. We are studying molecular changes in naked mole rats that may account for their complete lack of thermogenesis, and uniquely low metabolic rates. We have recently discovered that naked-mole-rats have evolved a remarkably unique carbohydrate metabolism that may help them survive severe hypoxic stress. The metabolic basis of mole-rat resistance to hypoxic stress is a major new research direction in the lab.

Selected Publications

Wetzel C, Pifferi S, Picci C, Gök C, Hoffmann D, Bali KK, Lampe A, Lapatsina L, Fleischer R, St. John Smith E, Bégay V, Moroni M, Estebanez L, Kühnemund J, Walcher J, Specker E, Neuenschwander M, von Kries JP, Haucke V, Kuner R, Poulet JFA, Schmoranzer J, Poole K, Lewin GR. Small molecule inhibition of mechanotransduction modulates touch perception and can control neuropathic pain. Nat. Neurosci. 2017 Feb 20(2): 209-218. doi: 10.1038/nn.4454. (epub ahead of print)

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see also Chapter Molecular Pathways in Cortical Development

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Photo: David Ausserhofer/MDC

James Poulet

Neural Circuits and Behaviour

Our goal is to understand neural mechanisms of sensory processing and perception. We examine brain regions associated with sensing and movement of the mouse forepaw, with a special focus on neocortical circuits. To investigate the link between neural activity and sensory perception we record and manipulate genetically identified neurons in mice performing trained behavioural tasks with their forepaw.

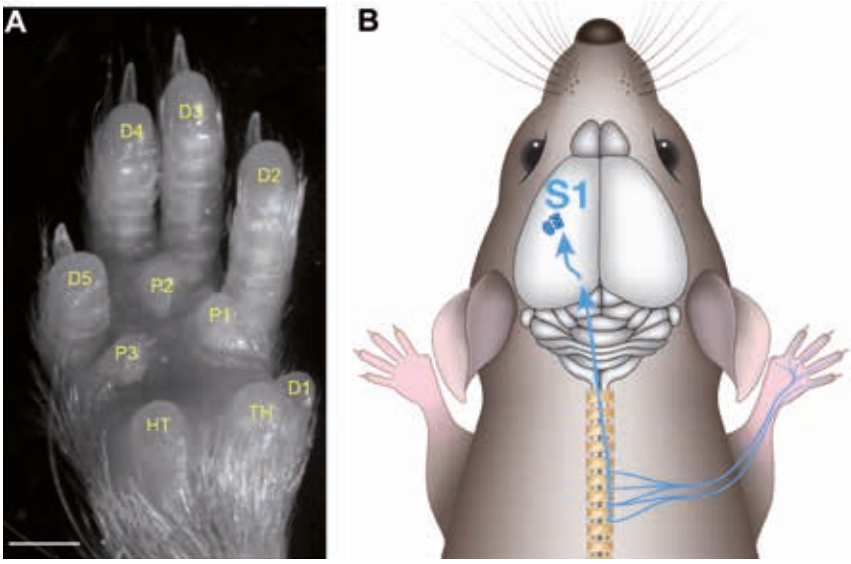

Figure 1. The mouse forepaw system. A. *Image of the glabrous skin of the right forepaw, mice have 5 digits (D1-D5) and 5 pads (P1-3, Thenar pad, TH, and the HypoThenar pad, HT), scale bar 1 mm.* **B.** *Cartoon of the mouse forepaw system and the sensory pathway in blue from forepaw to somatosensory cortex (S1) via spinal cord, brainstem and thalamus.*

The mouse forepaw system

The mouse forepaw has a similar structure and function to the human hand (Figure 1). Mice use their forepaws not only for locomotion but also for manipulating objects and sensing their immediate tactile environment. The mouse forepaw has 5 digits, albeit the first more of a stump than a thumb. The glabrous skin is innervated with a full complement of primary sensory afferent neurons processing the multiple modalities of somatosensation including light touch, proprioception, deep touch, temperature and pain.

Peripheral and central circuits of thermal perception

The temperature of an object provides important information for animals performing tactile tasks. Temperature and touch input are thought to closely interact in the brain but the primary sensory afferents in the skin and central circuits they engage to enable the perception of surface temperature were unclear. In this project (Milenkovic et al., 2014), we delivered high-resolution thermal and tactile stimuli to the forepaw digits (Figure 2). We showed that mice are able to detect small amplitude (~ 2ºC) cooling stimuli, making their thermosensory performance equivalent to that of humans. We went on to show that primary somatosensory cortical neurons respond both to tactile and thermal stimulation of the forepaw and are required for non-noxious cooling perception. Using mutant mice, we showed that the transient receptor potential melastatin 8 (TRPM8) protein is necessary for mild cold temperature perception. Finally, in collaboration with the lab of Gary Lewin at the MDC, we performed primary sensory nerve recordings. It was thought that sensory afferent C fibers were only in-

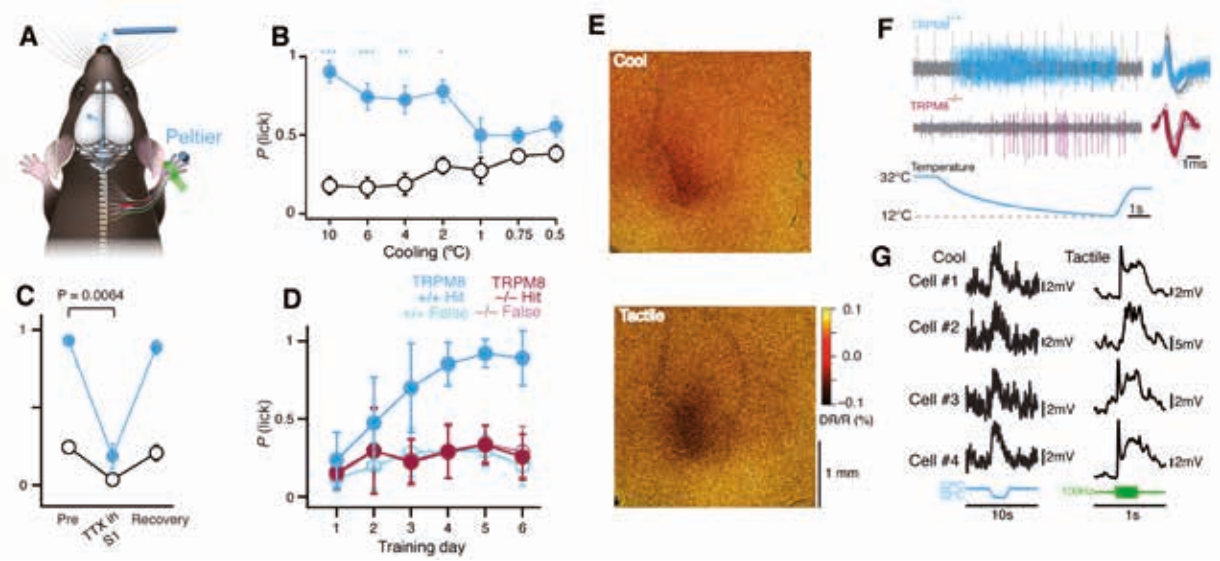

Figure 2. Mild cold thermal perception in mice is mediated by primary somatosensory cortex. A. *Setup.* **B.** *Mice lick in response to forepaw cooling* **C.** *Pharmacological silencing of S1 abolishes cooling perception.* **D.** *TRPM8 mutant mice cannot perceive cooling.* **E.** *Intrinsic optical imaging of S1 shows overlapping response to cooling and touch.* **F.** *Sensory afferent responses to cooling.* **G.** *Averaged membrane potential responses to cooling and touch in 4 example cortical neurons.*

volved in pain processing, but we show that they are the likely drivers for non-noxious cooling perception and cortical thermal responses in mice.

In vivo monosynaptic transmission between sensory cortex neurons

Monosynaptic connections between cortical neurons underpin cortical sensory processing but little is known about monosynaptic transmission in vivo. Recently we developed a method to make some of the first recordings of synaptically connected neurons in vivo (Jouhanneau et al., 2015). We show that layer 2 cortical pyramidal neurons form a sparsely connected network with an overrepresentation of bidirectional connections. The majority of unitary excitatory postsynaptic potentials were small in amplitude (< 0.5 mV), with a small minority > 1 mV. In comparison to prior in vitro work, synaptic connections in "Downstates" were less reliable and showed little short-term synaptic depression. Our approach will help bridge the gap between monosynaptic connectivity and function and allow investigations into the impact of network activity on synaptic transmission.

A sensory-triggered goal-directed reaching task for mice

To monitor sensory perception we have developed a new behavioural platform. Multiple mice are trained simultaneously to report a tactile stimulus delivered to the forepaw by reaching and pressing a target sensor using the same paw. Mice perform the task with short latency, high accuracy and low spontaneous false alarms rates, giving us a novel, highly sensitive psychophysical testing platform. We currently examine the activity of subsets of cortical neurons during this behaviour and their impact on perception and forepaw movement.

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Mitochondria and cell fate reprogramming

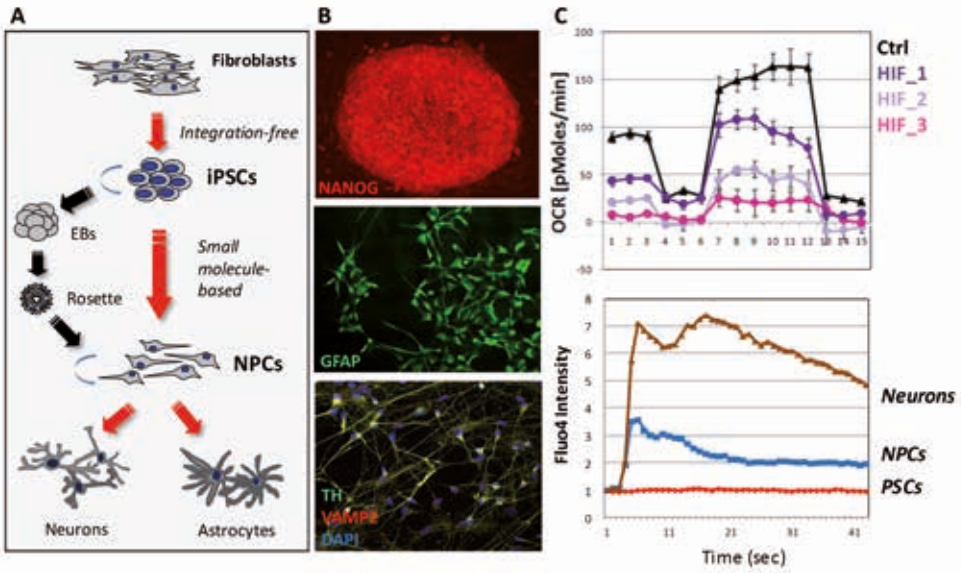
Induced pluripotent stem cells (iPSCs), generated through reprogramming of somatic cells back into an embryonic-like pluripotent state, hold great promises for biomedical research.

We are interested in dissecting the contributing role of mitochondria and energy metabolism in enabling cellular conversion. By building a mitochondria-centered model of cell fate reprogramming and differentiation with a targeted neuronal focus, we wish to unveil the relevance of mitochondrial modulation for cell identity, neuronal commitment, reprogramming, and rejuvenation.

Additionally, we seek to apply the iPSC technology for modeling neurological diseases affecting the mitochondria either directly, such as mitochondrial DNA (mtDNA) disorders, or indirectly, like Huntington's disease (HD). The development of alternative modeling approaches is highly needed for conditions affecting the nervous system, whose understanding has been hindered by the inability to sample live neuronal cells. This is particularly important for mtDNA disorders, which lack viable modeling tools due to the hurdles associated with engineering mtDNA. Reprogramming-derived neurons can be eventually employed as disease-relevant cell type-specific cellular systems for the discovery of novel disease-modifying therapeutic strategies for these untreatable brain disorders.

Mitochondrial reprogramming in cell fate conversion and neural specification

Recent advances in stem cell technologies reveal the plasticity of the mammalian epigenome. We have previously demonstrated that mitochondria are also drastically reconfigured upon reprogramming of somatic cells to induced pluripotent stem cells (iPSCs), a process that is paralleled by a metabolic switch towards glycolysis. This glycolytic shift represents an early reprogramming event, preceding the expression of genes controlling pluripotency and self-renewal. Thus, metabolic transformation might control cell fate identity. Modulation of bioenergetics is particular important for the development of the nervous systems, which consumes almost half of the whole-organism basal metabolic rate. However, the regulatory role of mitochondria and energy metabolism in dictating neural commitment and orchestrating its epigenetic rewiring remains to be investigated.In this project, we seek to dissect the potentially instructive role of bioenergetic restructuring for cell fate conversion and neural specification in particular. We initially focus on defined cellular states (fibroblasts, iPSCs, neural progenitors, neurons, and astrocytes) to map their mitochondrial/metabolic signature and the downstream effects on the epigenetic landscape. We then analyze the mechanistic function of mitochondria and energy metabolism during reprogramming to iPSCs and upon glial and neuronal differentiation, by altering nutrient availability and by chemical and genetic manipulation of critical mitochondrial and metabolic regulators. Finally, we wish to integrate the obtained data to computationally build a metabolic map of cell identities. This will be coupled to mitochondrial-related high throughput assays to possibly identify enablers of metabolic-driven cellular conversions. Overall, these studies may lead



(A) Schematic cartoon of our disease modeling approach.(B) Following integration-free iPSC generation (upper image), patient and control cells are coaxed into astrocytes (middle image) and neurons (lower image). (C) Assessment of mitochondria-related functions include bioenergetic profiling and calcium imaging.

to a better understanding of the importance of mitochondria and metabolism for brain development and for the control of cell fate plasticity and specification.

An iPSC-systems biology approach for modeling and discovery of therapeutic strategies of inherited basal ganglia disorders

The project aims to generate patient-derived neuronal cell models of two genetic disorders affecting the basal ganglia and lacking effective treatments. Maternally inherited Leigh syndrome (MILS) is an infantile encephalopathy due to point mutations within the MT-ATP6 gene of the mitochondrial DNA (mtDNA). Huntington's disease (HD) is a neurodegenerative disorder caused by the CAG triplet repeat expansion within the gene encoding the protein Huntingtin. Basal ganglia are highly dependent on mitochondrial-based energy production. Hence, the two diseases represent in a way two paradigmatic examples, one exhibiting a direct mitochondrial implication and one where mitochondrial dysfunctions are considered a secondary insult. Human basal ganglia neurons (GABAergic and dopaminergic) and glia cells (astrocytes) will be obtained from induced pluripotent stem cells (iPSCs) generated from patient and control fibroblasts using integration-free Sendai-based reprogramming. The chance to identify a disease phenotype within the reprogramming-derived basal ganglia cells and to develop potential therapeutic strategies might be diminished when using only a conventional reductionist molecular biology approach. In this study, we therefore broaden the levels of investigations by combining standard functional and biochemical assays with global OMICS-driven analyses (systematic transcriptomics, proteomics, and metabolomics) to unravel the disease mechanisms without an a priori knowledge. This would allow the generation of computational models for the two neuronal disease states and to in silico predict the targets of potential interventional strategies. The computational predictions will be validated in follow-up cellular experiments. Finally, based on the identified disease pathways, we seek to establish scalable assays amenable to high-throughput and focused compound screenings. If successful, this iPSC-driven systems biology approach may represent an innovative platform for drug discovery of complex genetic brain diseases.

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Fritz G. Rathjen

Developmental Neurobiology

The correct wiring of the brain is of critical importance for its function. It is orchestrated by multiple molecular factors and cellular mechanisms during developmental periods. The research of Fritz G. Rathjen's group focuses on several topics of the "wiring" problem. For example, we investigate the role of cGMP signaling on axon branching, of signaling systems implicated in the regulation of interneuronal synapses and the function of Ig cell adhesion proteins on calcium waves in the fine tuning of circuits. Moreover, we study neuronal mechanisms implicated in ultrasonic vocalizations of mice.

The impact of cGMP signaling on axon branching, sensory perception and vocalization

Npr2-mediated cGMP signaling is implicated in axonal bifurcation of sensory axons

During extension of axons to their target fields at embryonic or early postnatal stages most, if not all, axons generate different types of branches. These arbors enable an individual neuron to innervate several distinct neurons which might be located in different parts of the brain. Therefore, axon branching enormously contributes to the complexity of neuronal circuits and is essential for an integrated processing of information. Consequently, impairment of axon branching causes severe deficits in brain function. It is therefore of essential interest to unravel molecular mechanisms implicated in the regulation of axon branching.

Our previous investigations showed that cGMP signaling is essential for axon branching of dorsal root ganglia (DRG) and cranial sensory ganglia (CSG) in vivo. The cGMP signaling cascade is composed of the ligand CNP, the receptor guanylyl cyclase Npr2 and the serine/threonine kinase cGKI. In the absence of any of these components, axons of sensory neurons no longer bifurcate and instead individual axons only turn in a descending and ascending direction of the spinal cord or hindbrain (Figure 1).

A major open question is to what extent the mechanism underlying axon bifurcation of DRG or CSG neurons can be generalized. Our current investigation concentrate on the branching of axons from mesencephalic trigeminal neurons (MTN) which also express Npr2 and cGKI. MTNs are a very specialized group of cells that simultaneously innervate motor neurons of the hindbrain and jaw closing muscles and therefore coordinate the movement of maxilla and mandible (Figure 1).

Npr2-mediated signaling affects palmitoylation of specific proteins in growth cones

To get mechanistic insights into the process of axonal bifurcation we have begun to study downstream events of Npr2-mediated signaling. Biochemical as well as immu-

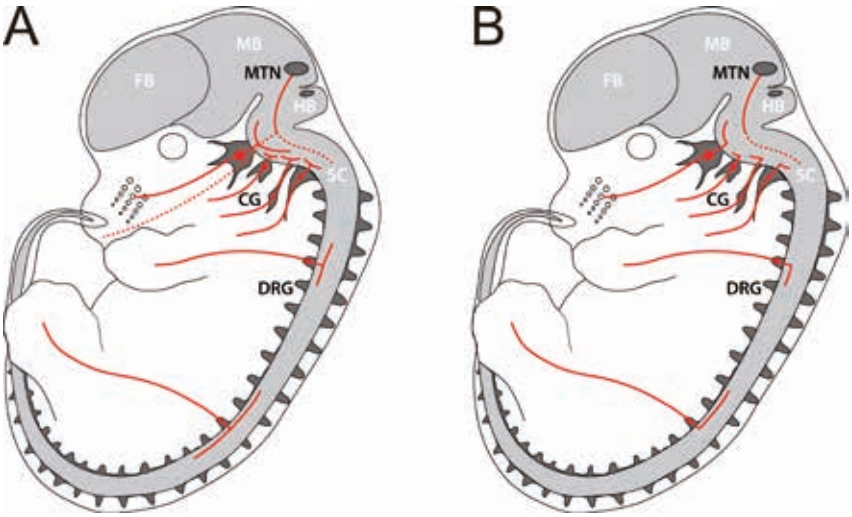

Figure1: Axon bifurcation of three types of sensory neurons is controlled by the CNP-Npr2-cGKI signaling cascade: DRG, CSG and MTN neurons.
A) Wildtype, B) Mutant. In the absence of CNP, Npr2 or cGKI sensory axons are unable to bifurcate when entering the spinal cord or hindbrain. CG – cranial ganglia; DRG – dorsal root ganglia; MTN – mesencephalic trigeminal neurons; FB – forebrain; MB – midbrain; HB – hindbrain; SC – spinal cord.

nocytological studies indicated that the kinase cGKI is associated with palmitoyl-rich membranes in the vesicular compartment suggesting that cGKI might regulate intracellular protein trafficking by protein S-palmitoylation - a reversible post-translational modification involved in the shuttling of proteins from intracellular membranes to the plasma membrane. Indeed, we demonstrated by a biochemical approach that cGMP signaling regulated S-palmitoylation of a narrowed number of proteins. Candidates are currently tested by genetic methods whether they control axonal bifurcation.

Degradation of cGMP by PDE2A (phosphodiesterase 2A)

To elucidate the signaling components essential for the regulation of cGMP levels we investigated the localization of PDE in embryonic DRGs. PDE2A was found in a pattern overlapping with that of Npr2- and cGKI. Measurements of cGMP levels in DRGs from wild-type and PDE2A-deficient mice after CNP stimulation resulted in a strong increase of cGMP in the PDE2A knockout that was not compensated by other PDEs. However, this increase in cGMP levels did not result in altered branching pattern of DRG axons indicating that axon bifurcation is stable to a rise in cGMP levels.

Axon bifurcation is essential for a correct representation of the periphery within the CNS

We have also begun to examine the impact of disturbed axon bifurcation on sensory perception. Painful stimuli are detected by sensory neurons which reveal the above described disturbed axon bifurcation. However, Npr2 has also a critical function for longitudinal bone growth in that its absence causes a disproportionate growth accompanied by a dwarfism. To overcome these obstacles we have generated a floxed allele of Npr2 which was then selectively inactivated in sensory neurons by the Wnt1-Cre driver. This approach resulted in animals with normal-sized bones but with impaired axon bifurcation. Initial studies on these mice revealed a decrease in acute nociceptive pain sensation suggesting that the skin is incompletely represented in the spinal cord in the absence of Npr2.

Npr2 mediated cGMP signaling is implicated in ultrasonic vocalization

Since Npr2-mediated cGMP signaling is implicated in a broad variety of physiological functions and since the guanylyl cyclase Npr2 in addition to its localization in sensory neurons is expressed by groups of motor neurons in the hindbrain at early embry-

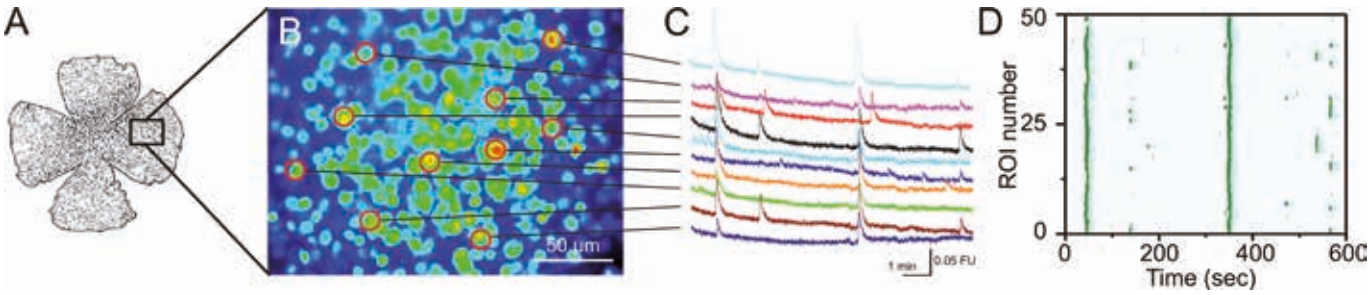

Figure 2: Analysis of calcium waves in the developing retina of the mouse. *A) A flat-mounted mouse retina (P2) is loaded with the calcium-sensitive dye Fura-2 and fluorescent intensities were analyzed in regions encircled as indicated (ROI – regions of interest) in B). C) Example fluorescence traces from representative retinal ganglion cells are shown. D) Fluorescence intensities were converted to heat maps to indicate the number of waves over time.*

onic and postnatal stages we explored its role in ultrasonic vocalization of mice. Mice are able to generate an array of ultrasonic vocalizations in a variety of behavioral contexts, for example infants produce so-called isolation-induced calls when separated from their mothers. These isolation calls can be elicited very reliably and quantified. Coordinating movements of the larynx, the vocal tract and the respiratory system are required for vocalization.

Our studies reveal that in Npr2- or CNP-deficient mice the number and frequency of isolation ultrasonic calls are significantly reduced suggesting that cGMP signaling either interferes with the differentiation of specific neurons or modulates an acute function of neurons. Our current investigations concentrate on the identification of the neuronal populations that are essential to produce ultrasounds. These genetic studies might contribute to characterize neuronal circuits underlying call usage and structure.

Synapse differentiation by the Lrp4-agrin complex and by CALEB

Development and plastic changes of synapses are of critical importance for the connectivity and functionality of the brain. Despite the fundamental role of synaptogenesis in neuronal circuit formation, the molecular components orchestrating synapse formation in the brain are not fully understood in contrast to the neuromuscular junction. Here Lrp4 acts as agrin receptor resulting in the activation of the protein tyrosine kinase receptor MuSK. We now demonstrate that Lrp4 is also expressed in the cortex with high levels at developing periods. Analysis of synapse formation in microisland cultures by patch clamp recordings indicated that Lrp4 also mediates synapse formation on cortical neurons upon application of soluble agrin suggesting that Lrp4 might have a function on cortical neurons similar to that one at the neuromuscular junction.

CALEB is a transmembrane protein with an EGF-like domain which is selectively localized in the postsynaptic compartment of central neurons. Previous investigations demonstrated an impaired presynaptic function in CALEB-deficient mice by patch clamp recordings. In addition, the elimination process of supernumerary climbing fiber synapses on Purkinje cells was found to be distorted in the absence of CALEB. These deficits are restricted to early postnatal stages suggesting a transient role for CALEB in synapse maturation.

Role of Ig cell adhesion proteins of the CAR subfamily

The group of Dr Rathjen has also a long standing interest in the functional characterization of cell adhesion proteins of the immunoglobulin (Ig) superfamily. Currently, we are concentrating on the CAR (coxsackievirus and adenovirus receptor) subgroup of IgCAMs which is composed of CAR itself, BT-IgSF (brain and testes Ig superfamily) and CLMP (CAR-like membrane protein). Our genetic studies reveal that these components control a variety of cellular and organelle functions ranging from the peristaltic of the intestine and of the ureter to the embryonic development of the heart and male fertility.

Regulation of calcium waves in developing retinae in the absence of IgCAMs

During early phases of development a coarse pattern of connectivity is established in the brain and with the help of neuronal activity the coarse connections are then fine-tuned. For example, in the developing visual system correlated bursts of action potentials detected as calcium waves propagate among neighboring retinal cells. These calcium waves are believed to provide cues within their activity important for the refinement of eye-specific segregation. Similar spatio-temporal patterns of calcium waves have been reported in other regions of the nervous system as well suggesting that this type of neuronal activity is important for the refinement of neuronal circuits in target areas. We hypothesize that some IgCAMs which mediate intimate cell-cell contacts modulate the frequency and pattern of calcium waves. We have therefore recorded spontaneous calcium waves in the developing retina in the absence of different cell adhesion molecules of the IgSF such as NCAM, L1, CAR, TAG1, DSCAML1 and others (Figure 2).

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Photo: NeuroCure

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Role of GPRIN1 on kainate-receptors function

Our mental capacities, the possibility of making coherent decisions, solve complex problems, and display elaborated behaviors, rely on the specialized way in which the brain treats information. Brain cells use a wide range of neurotransmitters to communicate.

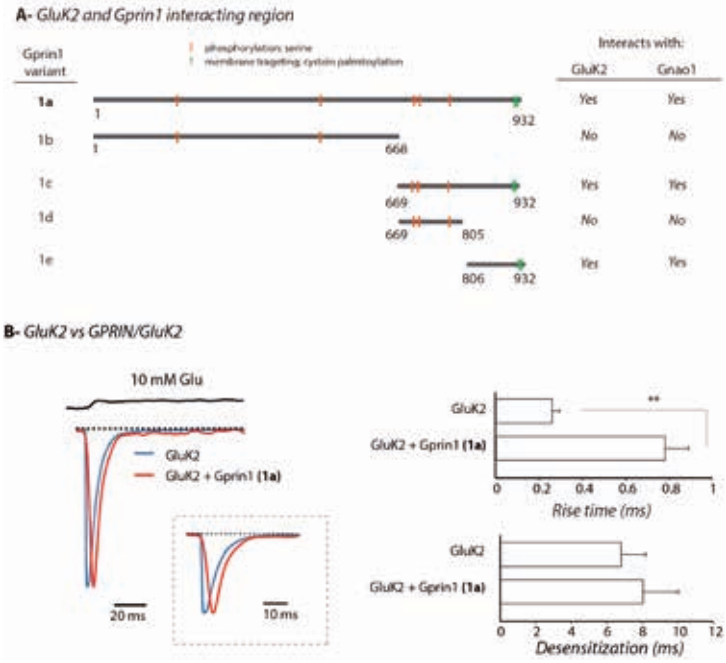

Figure1- *Schematic structure of Gprin1 protein, showing membrane targeting region, and main phosphorylation regions. Variants that interact with different proteins Gao/i or GluK2 are shown* **(A).** *Currents elicited by application fast of glutamate (10mM for 200 ms) to excised outside-out patches of Hek298 cells, transfected with GluK2 (blue) or GluK2 and Gprin1 (red)* **(B)** *(t-test- ** p< 0.01).*

These neurotransmitters have a large spectrum of cellular actions, which are achieved by activation of specific receptors. Interestingly, one neurotransmitter can activate diverse sets of receptors that may contribute differently to neuronal communication. Glutamate, for example, activates ionotropic receptors (leading fast excitation) as well as metabotropic receptors (act as modulators). There are three main types of ionotropic glutamate receptor, NMDA, AMPA and kainate receptors (KARs). While AMPA and NMDARs mediate most excitatory postsynaptic currents, KAR contribution to excitability is localized to discrete synapses, and they serve other functions. These range from neuronal development to excitability and modulation of neurotransmitter release. They are found prepostand perisynaptically. Many of KARs functions are mediated by activation of heterotrimeric G proteins. The complexity of functions and polarized localization of KARs are dominated by intricate protein associations. We want to identify KAR-interacting proteins that are of key importance to understand their physiology in the healthy and diseased brain, and to develop targeted pharmacology.

One salient characteristic of KARs is their slow kinetics. These receptors share structural conformation and sensitivity to many drugs with AMPARs, but their currents differ in fundamental ways. KARs yields excitatory postsynaptic currents (EPSCs) with smaller amplitude, and slower rise and decay time than AMPARs. To understand difference in kinetics of these receptors studies of recombinant DNA expressed in heterologous systems have been of great help. However, currents from recombinant KAR DNA do not recapitulate endogenous currents. Indeed, in heterologous systems,

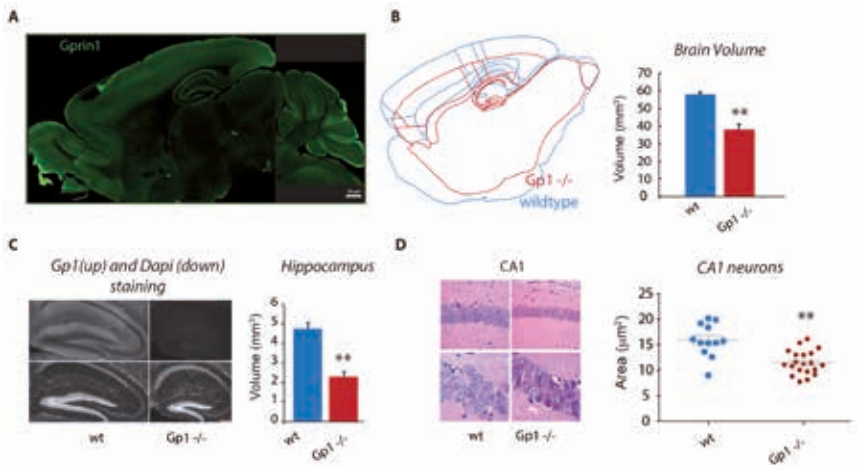

Figure 2- *Photography showing that Gprin1 is enriched in the cerebral cortex, the hippocampus, the cerebellum and the olfactory bulb in mice* **(A).** *Drawing showing that obliteration of Gprin1 leads to brain volume reduction of about 30% compared to wildtype* **(B).** *The hippocampal formation is rounder in Gprin1 mutants and the volume is reduced* **(C).** *This can be reflected in a thinner CA1 layer, with CA1 cells smaller and unhealthy* **(D).** *(t-test- **p<0.01 or *p<0.05)*

AMPARs and KARs currents are comparable. This suggests that KARs synaptic proteins confer them particular properties. Extensive elucidation of these proteins is needed. We found a KAR-interacting protein, the G protein-regulated inducer of neurite outgrowth 1 (Gprin1) that is associated with neurite development and interacts with GTP bounded Gao/i, the inhibitory G alpha subunit. We used immunoprecipitation and found the region that binds to GluK2 (Fig.1A). We use a system to determine the kinetics of GluK2 in response to glutamate. We use a fast perfusion tool, installed on an electric piezo, allowing fast application of glutamate to patches of cells that express GluK2 recombinant DNA. We found that Gprin1 slows down the rise time of GluK2 currents (Fig.1B). This effect may account in part for the slow kinetics observed in hippocampal GluK2.

To extend these findings to the native situation we generated a transgenic mouse, which lacks Gprin1 (Gp1-/-). We found that Gprin1 is expressed mainly in the hippocampus, cortex, cerebellum and olfactory bulb (Fig.2A). Importantly, obliteration of Gprin1 leads to a decrease in brain volume, with all regions measured (hippocampus, cortex and cerebellum) reduced in the same proportion (Fig. 2B). The changes for the hippocampus are shown in detail (Fig.2C). Albeit the size reduction, all layers of the hippocampus are present. Finally, there are less cells in the CA1 and their cell body is smaller (Fig.2D). Gprin1 accumulates in the membrane of cultured hippocampal neurons(Fig.3A). We analyzed the morphology of neurons of the hippocampus in CA1 area of Gp1-/- mice(Fig.3B). There is no difference in neurite length but an increased angle of bifurcation between sister branches in the apical dendrites(Fig.3C). This is interesting because we know that there are less neurons in CA1 and the brain is smaller. But it may indicate that the neurons that manage to survive grow normal neurites, as shown by Sholl analysis(Fig.3D). These results are important since they validate the Gprin1 mutants as a model to study KARs physiology in brain slices and *in vivo*.

In summary, the diversity of functions of KARs suggests that they associated with a set of synaptic proteins that confer them specific properties and modes of actions. Extensive identification of these proteins is essential to generate advances in this field. Our data support a role for Gprin1 as a partner of GluK2. Association with Gprin1 changes the kinetics of the receptors and Gprin1 obliteration affects postnatal brain development, possibly by orchestrating the function of neurotransmitter receptors, including KARs. Next, we will elucidate the role of Gprin1 and endogenous KARs function and its potential role in neuronal development.

Selected Publications

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Beed P, Gundlfinger A, Schneiderbauer S, Song J, Bohm C, Burgalossi A, Brecht M, Vida I, Schmitz D. Inhibitory gradient along the dorsoventral axis in the medial entorhinal cortex. Neuron 2013; 79:1197-1207.

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Photo: Sigrid Schnögl/MDC

Erich Wanker

Proteomics and Molecular Mechanisms of Neurodegenerative Diseases

My group's research focusses on 'Neuroproteomics', the protein-based investigation of neurodegenerative diseases. In detailed, hypothesis-driven studies we address mechanisms of protein misfolding and aggregation, with the aim of understanding the molecular mechanisms of neurodegeneration in Huntington's, Alzheimer's and further diseases causally related to the misfolding of proteins. In particular, we aim to elucidate the molecular principles by which abnormally folded proteins, their complexes and aggregates cause cellular toxicity and neuronal dysfunction.

In our efforts to promote translation of basic research into benefits for patients, we identify and characterize modulators of protein misfolding cascades in disease (Ehrnhoefer et al., Nat Struct Mol Biol, 2008; Bieschke et al., Nat Chem Biol, 2011). We have previously demonstrated that expanded polyglutamine (polyQ) sequences trigger misfolding and aggregation of N-terminal huntingtin fragments *in vitro* and *in vivo* (Scherzinger et al., Cell, 1997; Davis et al., Cell, 1997).

Our second field of activity is systems biology, in particular using interactomics approaches, which are also mainly applied to neurodegenerative disease processes. Previously, we developed an automated yeast two-hybrid (Y2H) system, which we used to generate a focused protein-protein interaction network for the huntingtin protein relevant to Huntington's disease (Goehler et al., Mol. Cell, 2004), as well as a large interaction map of the human proteome (Stelzl et al., Cell, 2005). Recently, we identified highly relevant interactions between the triple A ATPase VCP/p97 and an adaptor protein that effects a fundamental structural change in VCP from a homohexamer to a heterodimer with far-reaching functional implications (Arumugham et al. 2016). We are constantly developing new methods for the identification and validation of protein-protein interactions, most recently DULIP, a dual luminescence-based co-immunoprecipitation assay for interactome mapping in mammalian cells, (Trepte et al., J Mol Biol, 2015.)

As interactomics core facility of the Max Delbrück Center, we offer screenings on our platform to interested colleagues.

More recently, we have started new lines of translational research establishing methods to detect disease-relevant misfolded protein species in patient biosamples. These investigations are directed at the development of predictive disease markers which are a prerequisite for the clinical investigation of new disease-modifying therapies targeting neurodegeneration before symptoms of irreversible neuronal damage arise.

IntegraMent – Interactome networks and perturbed cellular functions in schizophrenia

An "integrated understanding of causes and mechanisms of mental disorders" is sought in the collaborative project IntegraMent, funded by the Federal Ministry of Education and Research under its systems medicine initiative, e:med. Our role in this national consortium is to map interactions of proteins involved in this family of diseases. Such efforts are crucial to the understanding of these underresearched diseases, since the cellular functions of

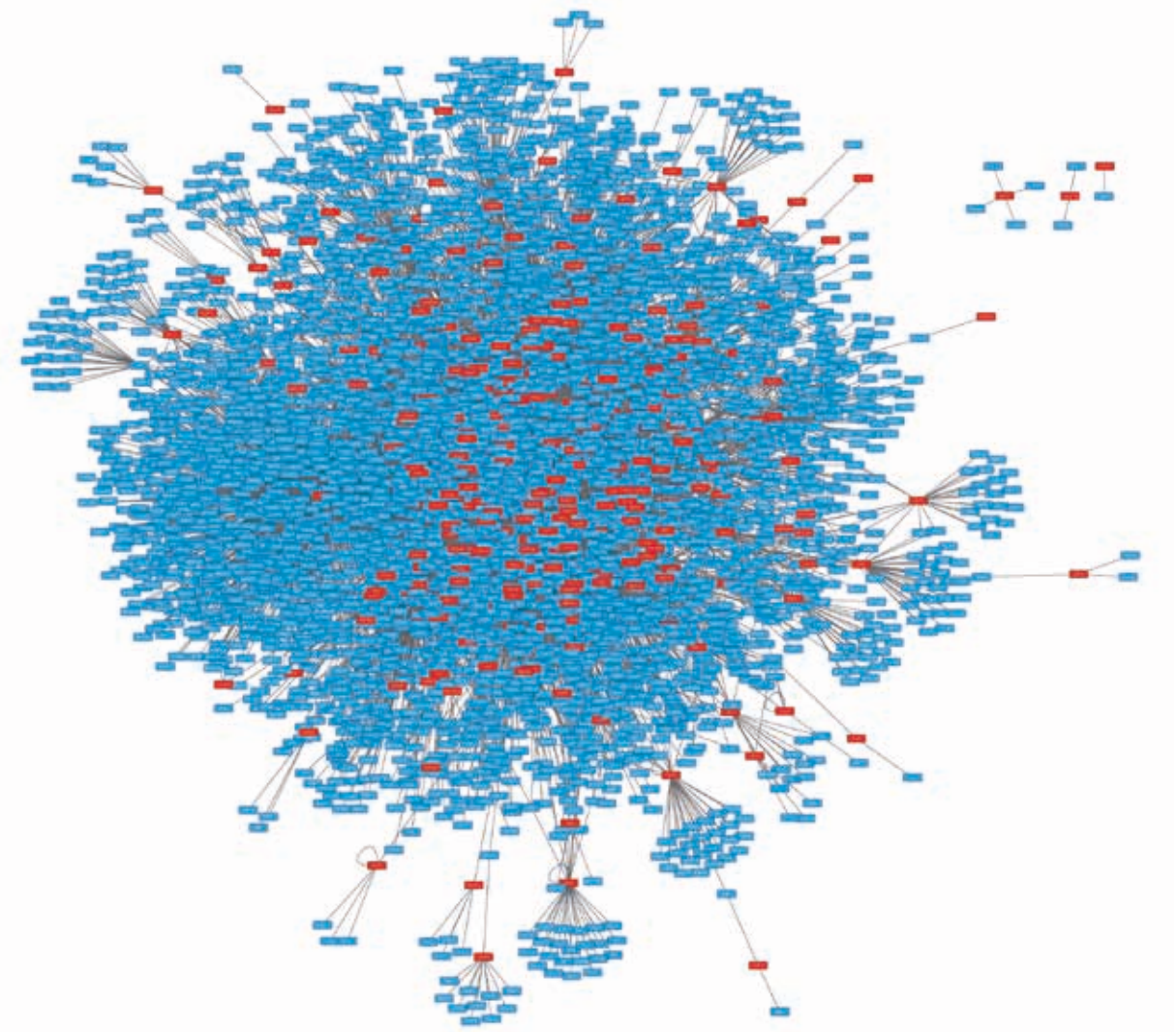

Figure 1: An interaction network of proteins involved in schizophrenia. *The network is based on a list of 286 protein coding genes implicated in the pathogenesis of the disease and contains 4,302 proteins forming nearly 8,000 connections. The network is significantly more connected than randomly generated networks. Red: Protein coding genes primarily associated with schizophrenia. Blue: First degree interacting partners of proteins encoded by schizophrenia-associated genes.*

the involved proteins, their relationships, and their roles in disease mechanisms are largely unknown. Three specific goals have been pursued: First, the computational prediction of interaction networks for proteins with known relevance to disease. Second, the systematic validation of the involved protein-protein interactions. Third, the investigation of selected interactions in disease models and patient biosamples. This systems-biology driven approach will allow the consortium to define – for the first time – functional protein interaction networks and may also identify novel biomarkers for the molecular diagnosis of mental diseases that currently can only be diagnosed via assessment of psychiatric symptoms. We first compiled a list of 286 protein-coding genes which are implicated in the pathogenesis of schizophrenia, from which we generated a schizophrenia-specific interaction network connecting 4,302 proteins via 7,991 interactions (Figure 1). We integrated gene expression datasets and applied computational methods to identify disease-relevant clusters in the network as the basis for in-depth validation studies that are to follow.

ERA-NET Neuron and Berlin Institute of Health: Collaborative translational research to fight Alzheimer's disease

In 2013-14 we launched, and have coordinated since, two translational consortia: The ERA-NET Neuron programme funds efforts to establish new methods for the discovery of novel drug candidates or diagnostic approaches for Alzheimer's disease. Together with researchers in France, Belgium and Italy, we study aggregated amyloid-beta, one of the polypeptides suspected of triggering neurodegeneration in Alzheimer's. On the one hand, we are interested to target it effectively with chemical substances to create therapeutic effects; on the other, we develop approaches to detect the molecular changes it causes before patients show symptoms of dementia. This is one of the most important

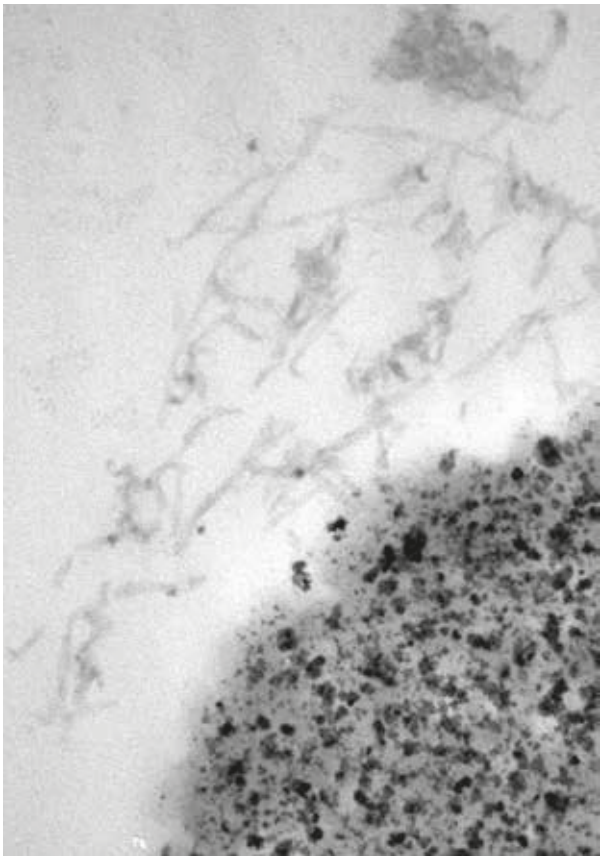

Figure 2: Electron microscopy image of amyloid-beta aggregates extracted from postmortem brain. *Magnetic beads in combination with special antibodies are used to extract aggregates of the amyloid-beta polypeptide from biological samples. The large sphere is one of the beads in the reaction, floating above it, as it were, we see thin fibrils of amyloid-beta.*

goals pursued by Alzheimer researchers in academia and industry at the moment: Finding prognostic disease markers is hoped to facilitate pre-symptomatic treatment of the disease. We have laid groundwork with regard to the amyloid-beta peptide by finding a way to extract aggregated peptide from postmortem brain samples in order to determine those of its properties that are relevant to pathogenesis (Figure 2).
More systematic, large scale experimentation has been started in the framework of the newly founded Berlin Institute of Health: Together with six other basic and clinical research groups at the Max Delbrück Center and Charité University Medicine, we have started systems biology efforts to define changes in protein networks in Alzheimer's. Systematic approaches are flanked by in-depth studies of potential new disease markers or chemical modulators of disturbed molecular profiles in the disease.

Investigating protein aggregation and neurodegeneration related to Huntington's disease in fruit flies

Huntington's disease is a neuromuscular disease. This means neurological disturbances adversely affect movement abilities of patients while also causing progressive psychiatric and cognitive symptoms. Underlying these symptoms is neurodegeneration, which in turn is caused by a gene mutation in the huntingtin protein. Mutated huntingtin contains an elongated stretch of over 40 repeats of the amino acid glutamine, whereas

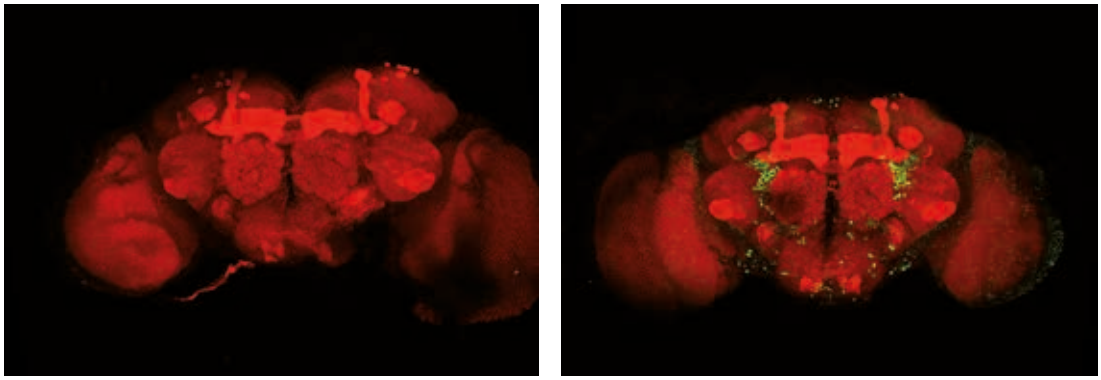

Figure 3: Immunofluorescence images of Drosophila melanogaster brains. *Brains of 9-days-old flies without (left) and with induction of mutated huntingtin expression for 6 days (right). Mutated huntingtin aggregates visualised as green dots.*

the unmutated gene has under 40. This extended polyglutamine stretch causes the protein to misfold and aggregate. A useful system to study the disease mechanism of huntingtin aggregation and neurodegeneration is the fruitfly Drosophila melanogaster. Expression of mutant huntingtin with 96 polyglutamine repeats using the conventional UAS-GAL4 expression system results in an early expression in the Drosophila lifecycle. This early pan-neuronal expression leads to formation of aggregates in the larvae and pupae stage which results in neurodegeneration even in freshly hatched flies. To investigate the molecular sequence of events that leads to the onset of neurodegeneration, we employed an inducible expression system called GeneSwitch. Induced expression of mutated huntingtin for six days in adult flies led to the formation of aggregates in the fly brain. To visualise huntingtin aggregates in fly heads, immunofluorescence microscopy was used, showing them as green spots (Figures 3). We also investigated the relation between the quantity of aggregates and their toxicity in a life span assay. Healthy fruit flies live to about 100 days, while flies undergoing six days of mutant Huntingtin expression die at an age of about 38 days.

A platform for large-scale investigation of protein-protein interactions facilitates regional and inter/national collaboration

Interactomics approaches are highly powerful to enable new discoveries of molecular connections in pathways, cellular compartments or disease mechanisms. New cutting-edge assays are constantly being established in our group and offered as service or in the framework of collaborative projects to researchers in and outside the Max Delbrück Center. Recently, we reported DULIP: A Dual Luminescence-based Co-Immunoprecipitation Assay for Interactome Mapping in Mammalian Cells (Trepte et al., J Mol Biol, 2015). DULIP is a second-generation protein-protein interaction screening method of high utility, e.g., of detecting the effects of point mutations on interaction strength.
We can run yeast two-hybrid, DULIP and FRET-based assays for researchers interested to use network biology approaches. We put great effort in validation and benchmarking of our systems to provide high-confidence, highly reliable interaction datasets. Please approach us if you are interested in screening your protein of interest for interaction partners. So far we have performed screenings for over 20 collaborating labs in Berlin, Germany, Europe and the USA.

Patents / Patent applications

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Wanker, E.; Wiglenda, T.; Babila, J.; Boeddrich, A.; Schmidt, M.; Schiele, F. (2011) Azo compounds reduce formation and toxicity of amyloid beta aggregation intermediates, WO2011117305

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A. Arumughan, Y. Roske, C. Barth, L. L. Forero, K. Bravo-Rodriguez, A. Redel, S. Kostova, E. McShane, R. Opitz, K. Faelber, K. Rau, T. Mielke, O. Daumke, M. Selbach, E. Sanchez-Garcia, O. Rocks, D. Panáková, U. Heinemann, and E. E. Wanker, "Quantitative interaction mapping reveals an extended UBX domain in ASPL that disrupts functional p97 hexamers.," Nat. Commun., vol. 7, p. 13047, Oct. 2016.

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P. Trepte, A. Buntru, K. Klockmeier, L. Willmore, A. Arumughan, C. Secker, M. Zenkner, L. Brusendorf, K. Rau, A. Redel, and E. E. Wanker, "DULIP : A Dual Luminescence-Based Co-Immunoprecipitation Assay for Interactome Mapping in Mammalian Cells," J. Mol. Biol., 2015.

M. Stroedicke, Y. Bounab, N. Strempel, K. Klockmeier, S. Yigit, R. P. Friedrich, G. Chaurasia, S. Li, F. Hesse, S.-P. Riechers, J. Russ, C. Nicoletti, A. Boeddrich, T. Wiglenda, C. Haenig, S. Schnoegl, D. Fournier, R. K. Graham, M. R. Hayden, S. Sigrist, G. P. Bates, J. Priller, M. A. Andrade-Navarro, M. E. Futschik, and E. E. Wanker, "Systematic interaction network filtering identifies CRMP1 as a novel suppressor of huntingtin misfolding and neurotoxicity.," Genome Res., vol. 25, no. 5, pp. 701–13, 2015.

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Photo: David Ausserhofer/MDC

Niccolò Zampieri

Development and Function of Neural Circuits

A fundamental question in neuroscience regards the mechanisms used during development to wire the nervous system. Just by peeking at the anatomy of the nervous system it is evident that neuronal subtypes are assigned highly stereotyped positions, where neurons grouped in the same structure not only share positional coordinates but typically receive connections from similar inputs and send projections to common targets. Precise spatial organization appears to represent a major strategy to simplify the problem of connectivity. In addition, specific molecular cues, or labels, have a prominent role in wiring neural circuits and indeed several families of molecules involved in axonal guidance and synapse formation have been identified. However, the contributions and interplay of these two strategies - neuronal location and label - are still not well defined. Understanding the mechanisms that control neuronal positioning is therefore an important step in defining the logic that drives neural circuit assembly.

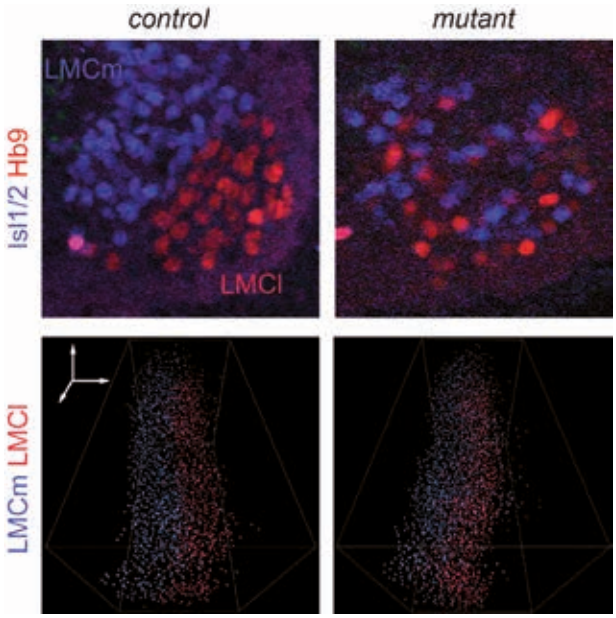

Figure 1: Digital reconstruction of motor neuron organization. *Upper panels, immunohistochemical analysis a single section of a mouse lumbar spinal cord showing motor neuron subtypes identified by expression of the transcription factors Isl1/2 (LMCm) and Hb9 (LMCl). Lower panels, 3D reconstructions of the organization of LMCm and LMCl neurons of control and mutant mice with defect in motor neuron organization.*

In order to tackle this question we use as a model system the mouse spinal cord where motor neurons controlling the activity of limb muscles are clustered into defined structures, termed pools, which occupy stereotyped and invariant positions across individuals. In order to generate coordinated movements, muscle groups with different biomechanical functions have to be activated in precise patterns. Thus, the selectivity with which sensory neurons and interneurons form connections with different motor pools is critical for the timely activation of muscle contraction. The mechanisms that

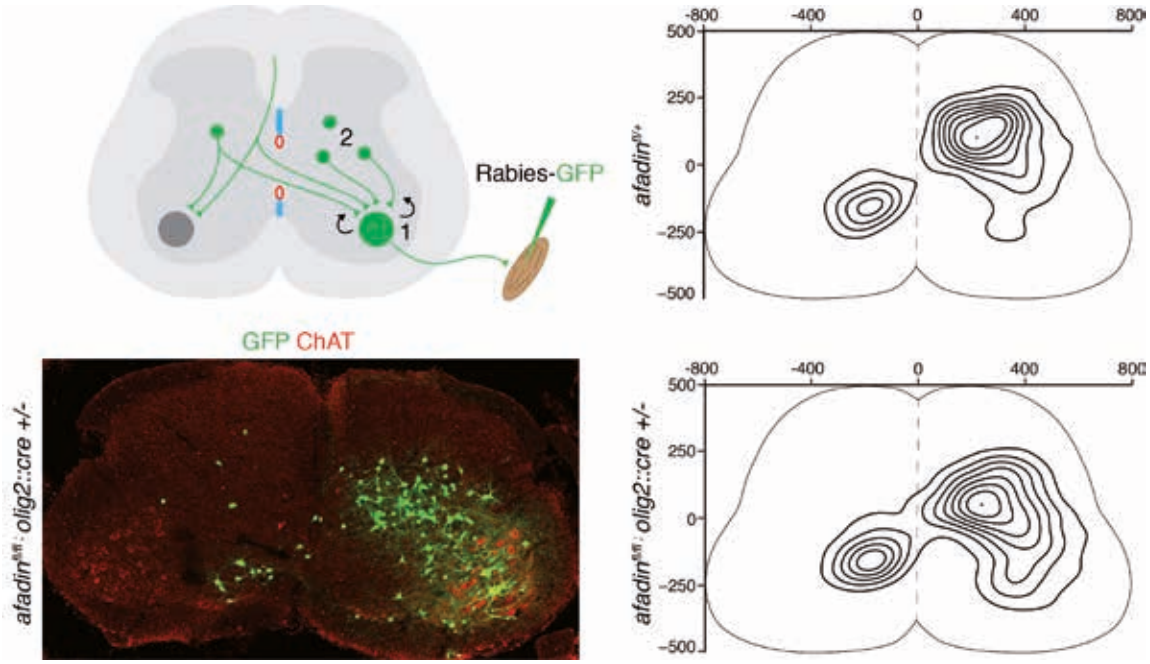

Figure 2: Rabies mapping of motor circuits organization. *Strategy, representative image and mapping of pre-motor interneurons in the spinal cord labelled via rabies virus transsynaptic tracing.*

dictate these patterns of connectivity remain largely obscure. Recent studies have shown that the specificity of sensory neuron to motor neuron connectivity is achieved, in part, through the ability of sensory axons to project to precise areas within the spinal cord. These results suggest that the spatial organization of motor neurons subtypes might be indeed a critical constraint for sensory input specificity. The influence of motor neuron settling position in determining sensory input specificity provides an indication that the elaborate positional organization of motor neuron cell bodies has critical roles for the formation of selective connections in motor circuits.

In previous work we identified the cadherin/catenin adhesive signaling as a key player in the control of motor neuron positioning. Classical cadherin expression distinguishes motor neurons at a molecular level and perturbation of cadherin adhesive function prevents segregation and clustering of motor pools. These findings document a key role for cadherin/catenin in orchestrating motor pools organization. However, it is still not clear how cadherin signaling controls motor neuron positioning and whether other signaling systems are involved. By combining molecular and genetic approaches, ongoing experiments are defining the molecular mechanisms and contributions of different adhesion systems focusing on classical cadherins and more recently extending the analysis on members of immunoglobulin superfamily of cell-cell adhesion molecules. In order to quantitatively study motor neuron organization, we are developing novel methods to generate three-dimensional maps of motor neuron positions in the whole spinal cord (Figure 1). In a parallel approach, we are analyzing assembly of motor circuits by taking advantage of viral and anatomical tracing techniques to highlight connectivity patterns between motor neurons and their synaptic partners in control and mutants mice to define the role of precise cell body position for the assembly of neural circuits (Figure 2).

Selected Publications

Zampieri N., Jessell TM., Murray A. (2014). "Mapping sensory circuits by anterograde transsynaptic transfer of recombinant rabies virus." Neuron, Feb 19; 81(4):766-778.

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Ciatto C, Bahna F, Zampieri N, VanSteenhouse HC, Ranscht B, Katsamba P, Ahlsen G, Posy S, Vendome J, Jessell TM, Honig B, Shapiro L. (2010). "T-cadherin structures reveal a novel cadherin adhesive binding mechanism." Nat. Struct. Mol. Biol. Mar;17(3):339-47.

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Berlin Institute for Medical Systems Biology

Nikolaus Rajewsky

Prevalent and devastating diseases such as cancer, cardiovascular disease, metabolic diseases and neurodegenerative disorders are usually multifactorial diseases involving many genes. To extend our understanding of the interplay of functions involving different genes in health and disease, an important goal of systems biology is to analyze gene regulatory networks. To this end we collect genome-wide quantitative data to integrate them into predictive models. This challenge is at the heart of Medical Systems Biology and is by nature highly interdisciplinary, combining molecular and cellular biology, biochemistry, mathematics, physics and engineering. The past ten years have particularly highlighted that cells employ multiple sophisticated levels of gene regulation, which include organization of chromosomes in the nucleus, epigenetic gene regulation, transcription, post-transcriptional gene regulation and post-translational modifications. Importantly, all of these levels interact and regulate each other and are involved in disease development. At the MDC, the grand challenge and Scientific Mission of the "Berlin Institute for Medical Systems Biology" (BIMSB) is to integrate these levels of gene regulatory control into comprehensive and predictive models of how gene regulatory changes are linked to phenotypes.

Scientific Technology Platforms

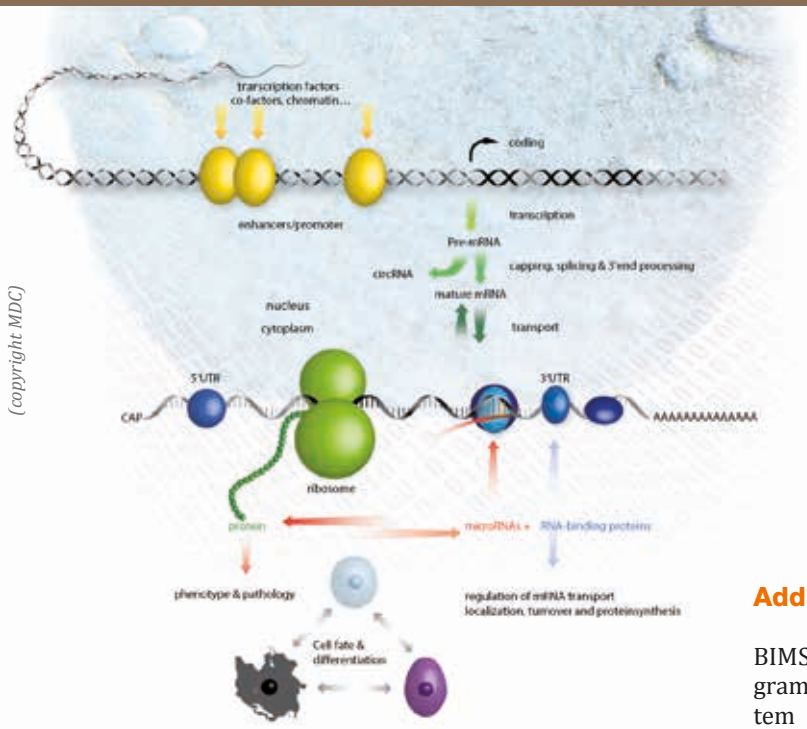
High throughput technologies are key for many research aspects at BIMSB. We have therefore established essential Scientific Technology Platforms for 'omics'-technologies. The newly recruited PIs have installed and integrated these technologies in scientific projects, often in collaboration with other labs at the BIMSB/MDC. Sascha Sauer directs the Genomics platform which offers diverse applications, including genome and transcriptome sequencing and assembly, and ChIP-Seq. Stefan Kempa develops cutting edge methods to quantify the metabolome as well as the proteome, with a special focus on aspects of the metabolism of tumors and stem cells. The Bioinformatics Platform is headed by Altuna Akalin who started in spring 2014. In addition, a new Light Mircoscopy Platform was established by Andrew Woehler in 2016.

Scientific group leaders at BIMSB

BIMSB was fortunate to recruit several outstanding new Junior and Senior group leaders since 2009. First to join was Markus Landthaler ‚RNA Biology and post-transcriptional regulation'. Junior groups investigate aspects of gene regulatory mechanisms and use model systems which were not established on campus before: Marina Chekulaeva 'Non coding RNA's and mechanisms of cytoplasmic regulation', Baris Tursun, 'Gene regulation and cell fate decision in C. elegans', Robert Zinzen 'Systems biology of neural tissue differentiation in Drosophila', Jan Philipp Junker 'Quantitative Developmental Biology', Stephan Preibisch 'Microscopy, Image Analysis and Modeling of Developing Organisms' and Roland Schwarz 'Evolutionary and Cancer Genomics'. In 2017, two new Junior groups will join BIMSB: Dario Lupianez 'Epigenetics and Sex Development' and Christoph Lippert 'Statistical Genomics'. In addition to Senior Group leaders Uwe Ohler 'Computational regulatory genomics', and Ana Pombo 'Epigenetic regulation and chromatin architecture', Irmtraud Meyer joined in 2015. She is a computational biologist studying RNA structure. Benedikt Obermayer 'Computation and Theory' and Scott Lacadie 'Functions of enhancer during zebrafish development' are Delbrück Fellows in BIMSB groups.

Publications

BIMSB scientists have published outstanding and highly-cited papers. 256 publications/cited more than 13500 times. Often, BIMSB publications feature two or more BIMSB authors, demonstrating the collaborative spirit. For example, the Landthaler and Rajewsky labs published a study in Cell (Rybak-Wolf et al. 2014) where they discov-



(copyright MDC)

ered a new class of binding sites for a key enzyme in small RNA biology (Dicer). Other recent highlights: The Ohler lab discovered that human promoters are intrinsically bi-directional (Duttke et al. Mol Cell 2015). The Pombo lab found that methylation of RNA POLII non-consensus lysine residues marks early transcription in mammalian cells (Dias et al eLIFE 2015). Reviews in leading journals were published by the Pombo lab (Nat Rev Mol Cell Biol 2015) and the Rajewsky lab (Nat Rev Genetics 2015). Other key publications originate from the Landthaler lab (Murakawa et al Nature Comm. 2015), Rajewsky lab (Rybak-Wolf et al. Mol. Cell 2015), Delbrück Fellow Obermayer with several BIMSB labs (Mackowiak et al. Genome Biol 2015) and the Ohler lab (Calviello et al. Nat Methods 2015). In addition to publications, BIMSB groups filed already 6 patent applications with focus on new technologies and diagnostic principles of molecular components of gene regulatory networks.

The new BIMSB building

The new building for BIMSB, located on the Campus of the HU Life Sciences in the center of Berlin, will accommodate up to 25 research groups, approximately half experimental and half computational. The building is currently in construction and will support high-tech labs, flexible work spaces and communication areas. BIMSB groups will be moving in 2018. The architectural and scientific concept will be complemented by competitively selected art works for the inside and outside of the building. The overall theme for the art projects is to ‚break walls' between disciplines and people.

Additional Activities

BIMSB has established an Exchange program to train the next generation of System Biologists. The program allows PhD students to work on collaborative research project be tween BIMSB labs and partners at the Center for Genomics and Systems Biology at New York University. In addition to seminar and lecture series, BIMSB organizes the annual Berlin Summer Meeting and Summer Schools, as well as joint retreats with the MRC-Clinical Sciences Centre (UK). In spring and fall 2015, BIMSB PIs and all BIMSB teams, respectively, held 2 day retreats to discuss science, technologies, collaborations and strategies.

Grants, Awards & Peer reviews

In addition to the start-up funding of BIMSB, the labs have successfully competed for extra- mural funding. Since its start, BIMSB labs have acquired a total of 32 Mio € additional funds from the DFG, BMBF, EC, NIH, GIF, and others. Some of the highlights are: ERC starting grants (Tursun, Junker), the Gottfried Wilhelm Leibniz Prize (Rajewsky), Human Frontiers Program grant (Rajewsky), the German Epigenome Consortium 'DEEP', and de.NBI (BMBF funded bioinformatics infrastructure in Germany).

Model of the new BIMSB building in the center of Berlin

Photo: David Ausserhofer/MDC

Altuna Akalin

BIMSB Bioinformatics / Mathematical Modelling

Recently, high-throughput methods enable researchers to do a much more thorough characterization of genomes and epigenomes. Complex diseases like cancer can only be understood by computational analysis of such high-throughput data. The amount of data generated calls for application of statistics and machine-learning in a broad scale. In the light of this, our research interests are in direction of understanding the genetic and epigenetic control mechanisms of cellular differentiation and complex diseases through computational analysis and integration of high-throughput datasets, such as microarrays and next generation sequencing. Ultimately, we want to discover genomic and epigenomic anomalies and interaction between them that lead to disease. In addition, we will strive to keep most up-to-date expertise in computational biology and data analysis, and develop tools that provide analysis techniques that are relevant, reproducible and well-documented.

(Epi-)genomics and disease

Katarzyna Wreczycka, Jonathan Ronen, Alexander Gosdschan

Discovering which abnormalities lead to complex diseases such as cancer remains as a challenge. To complicate the matters, most cases are unique to their selves and do not allow for simple all-embracing modeling of the disease. However, our previous work has shown that certain types of cancers share mutations on pathways relating to DNA modifications. These new class of cancers are distinguished by certain abnormalities in their epigenomic profiles, which paves way for different approaches in patient stratification and drug design. We are developing computational methods to analyze epigenomic profiles of samples to identify regions with abnormality and classify samples according to those abnormalities. We are using statistical and machine-learning methods to that end, and have developed and have been maintaining the popular software, "methylKit". In addition, we are also focusing other genomics profiling data frequently generated for Cancer samples (SNPs, CNVs and gene expression) to develop computational methods to classify tumor subtypes. In summary, we analyze high-throughput genomics data sets and develop methods to investigate epigenomic and genomic abnormalities and differences in various normal and cancer cell types.

Computational analysis of long-range gene regulation

Inga Patarcic

Gene regulation is a complex event where multiple partners interact with each other to achieve gene regulation. The level of expression of a gene is initially controlled by transcriptional regulatory mechanisms, such as enhancers and insulators which can act upon their target genes from large distances. Mutations on enhancers or structural variations that disrupt enhancer target pairing are associated with various diseases. In order to have full picture on long-range gene regulation, one must take into account all the different whole-genome data sets that provide information on the location and effect of the long-range regulators. We are working on to develop methods that take into account multi-level gene regulation information (CAGE, RNA-seq, Histone modifications, DNA methylation) to more precisely define the location and activity of the long-range regulatory elements.

Omics data integration and machine learning approaches

Vedran Franke, Katarzyna Wreczycka, Brendan Osberg

Most successful approaches in modeling complex phenomena, such as complex diseases or

gene regulation, make use of different types of data. We believe that only through integration of multi-layered data, complex biological processes can be understood in a system-wide level. This requires efficient preprocessing, integration, analysis and visualization of diverse data sets. We have built a comprehensive tool, "genomation", for integrating and visualizing genomics. With the help of this tool diverse genomics data sets (ChIP-seq, BS-seq, RNA-seq, CAGE, etc.) can be integrated and visualized over the regions of interest in the genome. The tool can achieve data set specific normalization and clustering of regions based on genomics data to uncover genome-wide patterns. In the last couple of years, we added more statistics functionality, parallelized data processing and optimized visualization functions for faster graphics generation. In principal, integration and analysis problem of big datasets can be solved by translating computer science and statistics research to biology, and developing those methods further where necessary. In collaboration with other platforms and groups at MDC, we are aiming to use statistics and machine-learning techniques to uncover relationships in high-dimensional biological data from diverse sources.

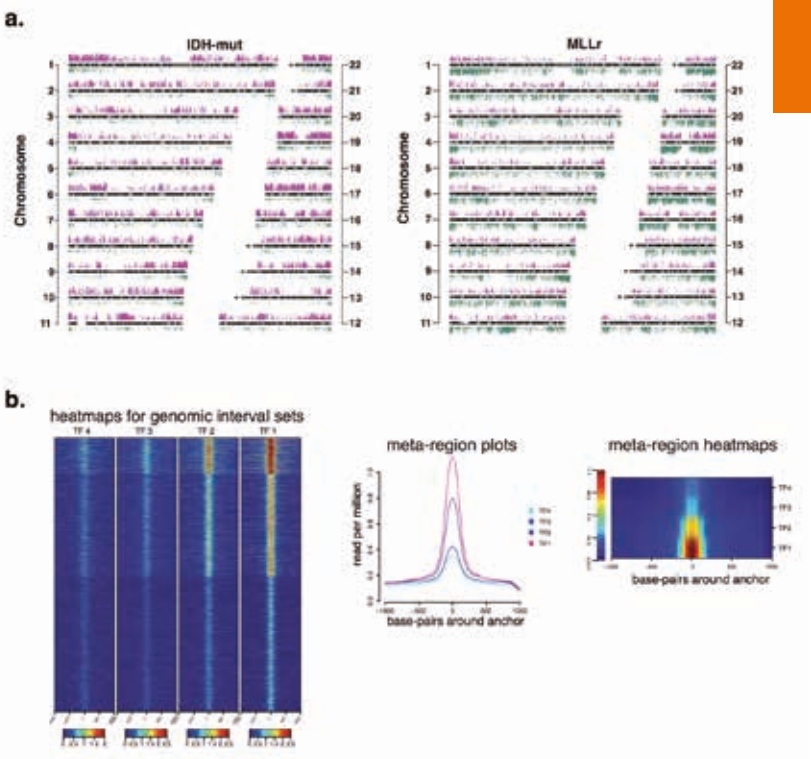
Infrastructure for RNA Bioinformatics

Bora Uyar, Dilmurat Yusuf, Dan Muntenau, Ricardo Wurmus (in Collaboration with Ohler & Rajewsky Labs)

We are part of the "RNA Bioinformatics Center" (RBC), which is an initiative supported by "German Network for Bioinformatics Infrastructure – de.NBI".

The center deals with developing methods for all RNA-related data sets not limited to transcriptome analysis but also RNA structure analysis, prediction of ncRNA targets, definition and classification of RNA transcripts and the analysis of protein-RNA interactions. Within this framework, we are developing bioinformatics tools and maintaining local infrastructure (such as online bioinformatics workbench, Galaxy) for RNA biology. We have also developed and currently maintaining the version 2 of DoRiNA database. This database includes RNA binding protein interactions from CLIP-seq experiments as wells as miRNA predictions from multiple experimental and computational resources. We also recently developed a toolkit, RCAS, for annotation of regions of interest on the transcripome. RCAS is an automated system that provides dynamic genome annotations for custom input files that contain transcriptomic regions. On top of these, we are integrating tools developed at Ohler and Rajewsky labs to galaxy workbench. This will enable wet-lab researchers to analyze their own data without the need for programming knowledge.

a) Examples of DNA methylation abnormalities in two different subtypes of acute myeloid leukemia. b) Examples of data integration and visualization techniques using in-house software.



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Uyar B, Yusuf D, Wurmus R, Rajewsky N, Ohler U and **Akalin A** (2017). "RCAS: an RNA centric annotation system for transcriptome-wide regions of interest." Nucleic Acids Research. doi: 10.1093/nar/gkx120.

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Akalin A * # , Franke V *, Vlahoviček K, Mason CE, Schübeler D. genomation: a toolkit to summarize, annotate and visualize genomic intervals. Bioinformatics , 2014 (# Co-corresponding author, * Equal contribution) **Akalin A*,** Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome Biology, 2012 Oct 3;13(10):R87

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Non-coding RNAs and mechanisms of cytoplasmic gene regulation

Our broad interest is in understanding the molecular mechanisms that regulate intracellular RNA localization, translation, and stability and roles of RNA-binding proteins and non-coding RNAs in these processes. We are particularly interested in the subcellular aspect of these processes. The subcellular localization and translation of mRNAs are fundamental to the establishment of the body axis, cell migration, and synaptic plasticity, which serves as a foundation of learning and memory. In mRNAs, they are mediated by specific cis-regulatory elements, so called zip-codes. These elements are bound by trans-acting factors (RBPs, miRNAs), which regulate transport, stability or translation. So far, our knowledge is restricted to only a few examples of zip-codes and regulatory factors. We aim to identify these elements on a genome-wide scale and dissect molecular mechanisms underlying their function. To identify proteins and RNAs that are differentially localized and translated between neurites and soma of neuronal cells, we developed a neurite/soma fractionation scheme in combination with mass spectrometry, RNAseq, Riboseq and bioinformatic analyses. Using this approach, we quantified 7323 proteins and the entire transcriptome and identified hundreds of neurite-localized proteins and locally translated mRNAs. Our results demonstrate that mRNA localization is the primary mechanism for protein localization in neurites and may account for more than a half of the neurite-localized proteome.

Background and overall aims

Identification of the neurite-localized proteome, transcriptome and translatome
Targeting proteins to specific cellular compartments is a fundamental biological process. It underlies formation of body axis, cell growth and migration, synaptic plasticity, and a vast range of other biological processes. **Proper subcellular localization of proteins can be achieved (a) by transporting proteins with molecular motors as parts of ribonucleoprotein complexes (RNPs) or vesicular organelles, (b) through mRNA localization and local translation or (c) via local translation of equally distributed mRNAs.** Specific examples for each mechanism have been described in the literature, but it is unclear to what extent each contributes to the overall protein distribution asymmetry. One reason for this is that most genome-wide studies focused on a particular level of gene expression (transcriptome, proteome, or translated transcriptome) or a single cellular compartment (e.g. axon without comparison to the cell bodies). Therefore, **we aim to determine the extent by which each of these separate localization mechanisms contributes to the overall asymmetry of neuronal protein distribution.** For that, we developed an approach allowing us to separate neurons on subcellular compartments – cell bodies (soma) and neurites (axons and dendrites) – and analyzed them by RNAseq, Riboseq and mass spectrometry (MS, **Fig. 1A**).

Identification of neurite-localized RBPs and dissecting their roles in local RNA metabolism
RNA localization and local translation are mediated by *R*NA-*b*inding *p*roteins (RBPs), interacting with specific *cis*-regulatory ele-

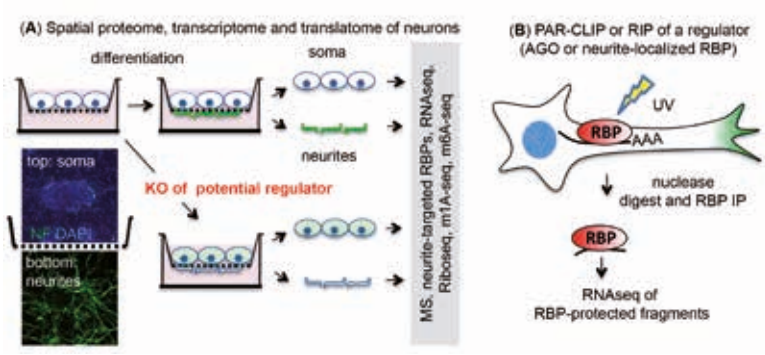

Fig. 1. An outline of the workflow, including accomplished and future objectives: Identification of local proteome, transcriptome, translatome and deciphering roles of RBPs and miRNAs. (A) *Scheme illustrating neurite/soma separation using porous membrane and fluorescent images of the induced neurons taken above and below the membrane. Separation is followed by omics analyses to identify local proteome, transcriptome, translatome, epitranscriptome and RBPs. Roles of potential regulators of local RNA metabolism (local RBPs, AGO, miRNAs) are analyzed via CRISPR/Cas-mediated KOs followed by spatial omics analyses to define the regulated step of metabolism and* **(B)** *PAR-CLIP and RIP to identify the targets of investigated RBPs.*

ments in RNAs. RBPs have the potential to bind and regulate hundreds to thousands of targets. So far, our knowledge is restricted to only a few examples of such RBPs, so our next aim is **to identify neurite-targeted RBPs with potential to regulate local RNA metabolism and dissect their mechanisms of function.** We used a neurite/soma fractionation scheme combined with MS for identification of RBPs enriched in neurites and a combination of PAR-CLIP, RNA-IP and CRISPR/CAS-mediated RBP knockouts (KO) for dissection of selected RBPs functions (**Fig. 1B**).

Deciphering the roles of miRNAs in mRNA localization and local translation

The miRNA pathway provides an alternative way to recruit a complex of proteins to a specific group of mRNAs. miRNAs are targeting half of all eukaryotic genes and function as guides, by pairing with partially complementary sites in their target mRNAs, thereby recruiting a complex of proteins which represses mRNA function. Thus, our next aim is **to dissect the roles of miRNAs in subcellular localization and translation of mRNAs in neurons on a genome-wide level.** For that, we knocked down the miRNA pathway in neurons and are testing how RNA localization and local translation are affected, using a neurite/soma fractionation scheme.

Mechanisms and conservation of miRNA-mediated silencing

Our current knowledge about the mechanisms of miRNA silencing is restricted to higher animals (vertebrates, arthropods, nematodes) and plants. In higher animals, miRNAs mediate translational repression and decay of their target mRNAs. We and others have shown that this mechanism relies on the proteins of GW182/TNRC6 family that function downstream of Argonaute (AGO) to recruit the CCR4-NOT deadenylation complex via conserved tryptophane-containing motifs (W-motifs). Interestingly, in cnidarian species *Nematostella*, miRNAs bind to their targets via nearly-perfect matches and AGO cleaves the target, similarly to plants. At the same time, it possesses GW182 protein, leaving the question about the primary mechanisms of the miRNA pathway open. Using a combination of pulldowns, MS, mutational analysis and reporter assays in cultured human cells, we have shown that *Nematostella* GW182 recruits the CCR4-NOT deadenylation complexes via its W-motifs, thereby inhibiting translation and promoting mRNA decay. Our finding suggests that the mechanism of miRNA silencing via the CCR4-NOT complex is conserved for at least 600 million years and was already present in ancient animals (*Mauri et al.*, 2016).

Selected Publications

Pamudurti NR*, Bartok O*, Jens M*, Ashwal-Fluss R*, Stottmeister C, **Ruhe L**, Hanan M, Wyler M, Perez-Hernandez D, Ramberger E, Shenzis S, Samson M, Dittmar G, Landthaler M, **Chekulaeva M**, Rajewsky N and Kadener S (2017). Translation of circRNAs. *Molecular Cell*, 66(1):9-21.e7

Mauri M, Kirchner M, Aharoni R, **Ciolli Mattioli C**, **van den Bruck D**, Gutkovitch N, Modepalli V, Selbach M, Moran Y, and **Chekulaeva M#** (2016). Conservation of miRNA-mediated silencing mechanisms across 600 million years of animal evolution. *Nucleic Acids Research*, 45 (2), 938-950, doi:10.1093/nar/gkw792.

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Pamudurti NR*, Bartok O*, Jens M*, Ashwal-Fluss R*, Stottmeister C, **Ruhe L**, Hanan M, Wyler M, Perez-Hernandez D, Ramberger E, Shenzis S, Samson M, Dittmar G, Landthaler M, **Chekulaeva M**, Rajewsky N and Kadener S (2017). Translation of circRNAs. *Molecular Cell*, 66(1):9-21.e7

Chekulaeva M*, Mathys H*, Zipprich J, Attig J, Colic M, Parker R, and Filipowicz W (2011). miRNA repression involves GW182-mediated recruitment of CCR4–NOT through conserved W-containing motifs. Nature Structural and Molecular Biology 8(11): 1218-26. *Recommended by Faculty1000.*

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Jan Philipp Junker

Quantitative developmental biology

Biology is a study in contrasts between variation and stability. On the one hand, generation of complex spatial patterns during development proceeds with striking precision: the different organs are generally formed at the correct position, at the right time, and with a defined size. On the other hand, regulation should also not be too rigid, since embryos need to flexibly adjust to environmental perturbations and correct errors caused by noisy gene expression. Focusing on the zebrafish as a model system, we study the interplay between variation and stability in embryonic and adult tissues. Using single-cell transcriptomics, we aim to understand how cell type diversity in vertebrate animals is generated and regulated, and which compensatory mechanisms are triggered upon perturbation. To this end, we develop novel experimental and computational approaches for spatially-resolved transcriptomics and massively parallel lineage analysis on the single cell level. We use these approaches in combination with light microscopy and tools from zebrafish developmental biology.

Spatially-resolved transcriptomics

We recently developed a strategy for spatially-resolved transcriptomics in 3D (Junker et al., Cell, 2014). This method, called 'tomo-seq', combines traditional histological techniques, novel approaches for low-input RNA-seq, and mathematical image reconstruction to generate transcriptome-wide 3D maps of gene expression (Figure 1). Tomo-seq has three important advantages compared to traditional techniques such as *in situ* hybridization: It is genome-wide, it can be readily applied to mutants or other rare samples, and it is suitable for comparing individual embryos. Tomo-seq is highly complementary to microscopy-based techniques like single-molecule FISH (Junker et al., Dev Cell, 2014; Soh, Junker et al., PLoS Genet, 2015) that offer absolute quantification and sub-single-cell spatial resolution for selected genes. In ongoing work (Karoline Holler), we have successfully expanded tomo-seq to sub-single-cell resolution by identifying maternally deposited transcripts that are localized within the zebrafish oocyte. Our aim is to use this genome-wide sub-single-cell spatial dataset to identify the 3'UTR-mediated mechanisms that are responsible for intracellular mRNA localization. In other ongoing work we aim to combine single-cell RNA-seq with mechanisms that retain information about the immediate spatial proximity of cells in a tissue, enabling us to systematically identify the effect that the local niche has on cell fate decisions.

Massively parallel lineage tracing

Besides spatial position, lineage history constitutes another main determinant of cell identity. Single-cell RNA-seq can systematically identify all different cell types in a tissue or organ. However, it does not yield direct information about lineage relationships and differentiation trajectories. We have developed the first method that allows simultaneous transcriptome profiling and lineage tracing in thousands of single cells from the same animal (Bo Hu, Nina Mitic, Bastiaan Spanjaard). Our approach, called 'scartrace', uses the short insertions or deletions ("genetic scars") that are created at the target site of Cas9 as heritable cellular barcodes for lineage tracing (Figure 2). In successful pilot experiments we

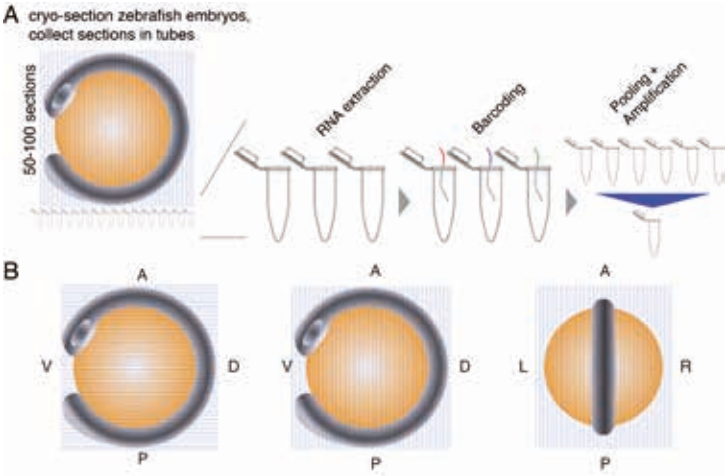

Figure 1. The tomo-seq method for spatially resolved transcriptomics. *A. The sample of interest (here: zebrafish embryo at somitogenesis stages) is cryosectioned, and RNA from individual sections is extracted, reverse transcribed, and linearly amplified. B. The approach can be extended to 3D by generating datasets for embryos sectioned along 3 different axes followed by computational image reconstruction.*

marked individual cells with genetic scars in a GFP transgene at early developmental stages by injecting Cas9 and sgRNA into 1-cell stage zebrafish embryos. We found that the resulting scars have very high complexity, suggesting that we can use the CRISPR/Cas9 system for efficient barcoding of individual cells. (Junker et al., BioRxiv, 2016). We are currently expanding this system to achieve greater control of scarring dynamics, to add mechanistic information about cumulative signaling intensity on top of lineage information, and to place lineage trees in 3D space.

Variability and robustness

We have recently received an ERC starting grant (start date: January 2017) to use the methods described above for studying variability and robustness during vertebrate development and in adult life. In an ongoing project (Roberto Moreno), we study cause and consequence of stochastic heart inversion along the left/right axis, a phenomenon that happens with a surprisingly high frequency of around 5% in wildtype zebrafish. Our preliminary data links this phenomenon to variable assignment of cells to the three germ layers during gastrulation. As a next step, we will use the scartrace method to reveal how lineage trees adapt to developmental perturbations such as reduction of specific progenitor pool sizes in the developing heart. Furthermore, we will determine how adult organs regenerate after injury. The zebrafish is an ideal model system for this line of research, as many organs recover fully even after massive injury. We will focus on the heart and brain, two organs that regenerate efficiently in the zebrafish, but through different mechanisms (the brain has adult stem cells, while the heart has not).

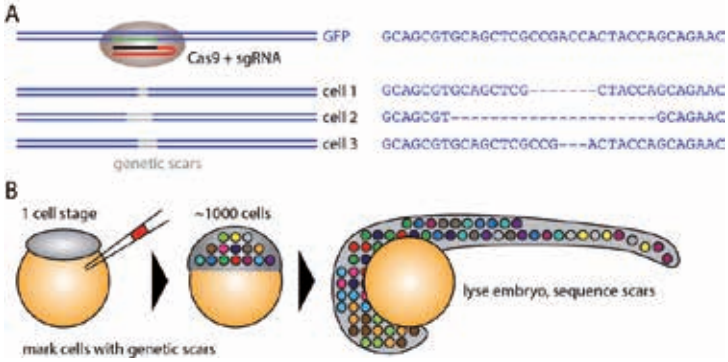

Figure 2. Massively parallel single-cell lineage tracing. *A. Cells are marked with indels ("genetic scars"). Each cell will carry a different scar as nonhomologous end joining of Cas9-induced double-strand breaks is a highly variable process. B. We inject Cas9 and sgRNA into 1-cell stage zebrafish embryos, which leads to cell labeling at early developmental stages. The scars are transmitted to daughter cells and serve as lineage readouts.*

Selected Publications

Junker, J.P., Spanjaard, B., Peterson-Maduro, J., Alemany, A., Hu, B., Florescu, M., and van Oudenaarden, A. (2016). Massively parallel whole-organism lineage tracing using CRISPR/Cas9 induced genetic scars. Biorxiv, doi: 10.1101/056499

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Junker, J.P., Peterson, K.A., Nishi, Y., Mao, J., McMahon, A.P., and van Oudenaarden, A. (2014). A Predictive Model of Bifunctional Transcription Factor Signaling during Embryonic Tissue Patterning. Developmental Cell 31, 448–460.

Junker, J.P., Noël, E.S., Guryev, V., Peterson, K.A., Shah, G., Huisken, J., McMahon, A.P., Berezikov, E., Bakkers, J., and van Oudenaarden, A. (2014). Genome-wide RNA Tomography in the Zebrafish Embryo. Cell 159, 662–675.

Junker, J.P., and van Oudenaarden, A. (2014). Every cell is special: genome-wide studies add a new dimension to single-cell biology. Cell 157, 8–11.

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Photo: David Ausserhofer/MDC

Scott Lacadie

Dynamics and functions of the enhancer landscape during zebrafish development and cardiogenesis

The temporal and spatial regulation of gene expression are key determinants of cellular identity within multicellular eukaryotes. Such fine spatiotemporal control occurs via the binding of transcription factors within accessible, non-protein-coding regions of chromatinized DNA. Studies suggesting that the majority of human disease-associated genetic variants fall in such regions of the genome highlight the importance of gene regulation for proper tissue formation and function. Pressing challenges are to understand how gene regulatory regions function and how they are impared in disease. These challenges must be met in the context of dynamic, multicellular systems that reflect the true complexity of biology. The zebrafish as a vertebrate genetic model provides an ideal system for in vivo testing of transcriptional regulatory regions wherein many tissues are highly parallel to their human counterparts and can be easily manipulated with medium to high throughput. Our goal is to identify, classify, and functionally characterize the temporal and spatial dynamics of transcriptional regulatory regions during zebrafish embryonic development and to use the zebrafish to mechanistically dissect human regulatory regions harbouring disease variants.

Transcriptional regulation during zebrafish development

Regulation of gene expression at the transcriptional level centers around regions of open chromatin where transcription factors bind to DNA and post-translational

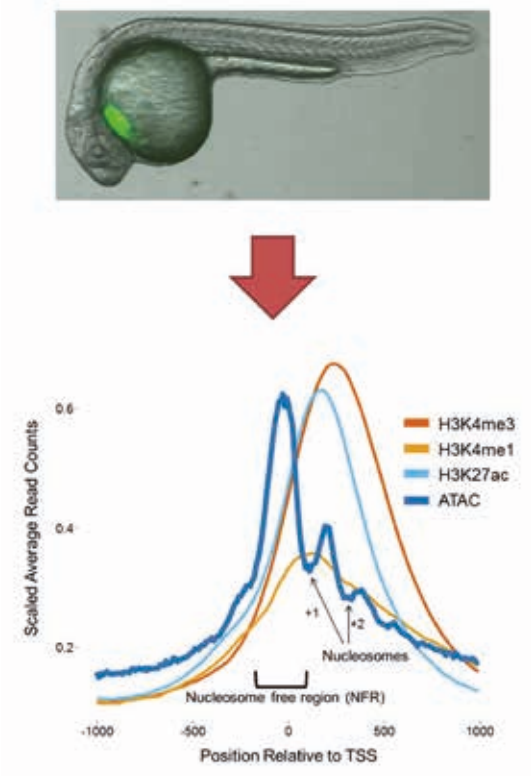

Figure 1: *Transgenic zebrafish are being used to define cell-specific open chromatin regions and the post-translational modification state of their associated nucleosomes. Plotted are ATAC-seq data we generated on cells from the embryo body and whole embryo ChIP-seq data for H3K4me1, H3K4me3, and H3K27ac taken from Bogdanovic et al., 2012.*

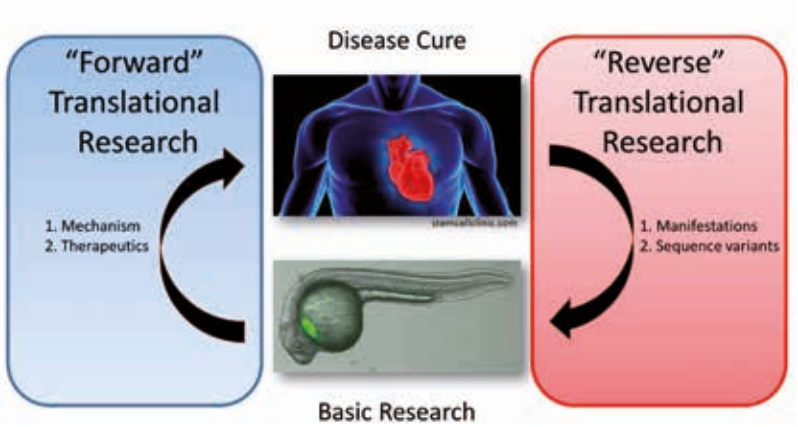

Figure 2

modifications of histones within nucleosomes reflect the activity state of the regulatory element. Since much of our understanding about transcriptional regulation comes from homogenous cancer cell cultures, it is imperative that future studies are carried out in models where cellular make-up and signaling activity accuratley capture the intricate variation of biological systems. Genome-wide assessment of regulatory architecture within specific cell populations isolated from animals has recently become more feasible with the advent of techniques such as ATAC-seq for the measurement of DNA accessibility (Buenrostro et al., 2013) and BiTS-ChIP for genome-wide occupancy profiling (Bonn et al., 2012). Applying these techniques to vertebrate biology will greatly deepen our understanding of tissue specific gene regulation.

We perform genome-wide assays for chromatin architecture to define and classify the activity of regulatory regions within specific cell populations during zebrafish development (Figure 1). We use the DNA sequences from these regions to predict transcription factor activity and to shed light on the combinatorial code of transcriptional regulation. Cutting edge CRISPR/Cas9 technology is used to assess the in vivo function of entire regulatory regions, predicted binding sites, and candidate trans factors. The identification of zebrafish regulatory regions orthologous to human promoters and enhancers will reveal fundamental aspects of how such regions must be organized to maintain function throughout evolution. Using these orthology relationships together with zebrafish transgenesis methods, we test human disease-associated regulatory genetic variants to dissect their mechanism in disease formation. Our systems biology approach combines the latest sequencing-based technologies with elegant computation and in vivo vertebrate developmental genetics to produce mechanistic insights into both basic biology and human disease (Figure 2).

Directionality of human promoters

One frequent and intriguing characteristic of human promoters is divergent transcription, in which unstable, reverse-oriented transcripts occur upstream of gene transcription start sites. We used DNaseI hypersensitivity to define boundaries of promoter regions and determined that a sizeable fraction of human promoters indeed lack divergent transcription. From our studies it is clear that promoters showing divergent trancription contain two core promoters, one at each edge of the open chromatin region arranged in opposite direction, while unidirectional promoters contain only one core promoter at the forward edge. Reverse core promoters drive the production of these unstable transcripts, but are not necessary for positioning of the upstream nucleosome, where transcriptional activity is associated with a unique combination of histone post-translational modifications. We envision reverse directed core promoters as specific regulators of forward transcription through the competition with forward core promoters for basal RNA polymerase machinery.

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Duttke, S.H.C., **Lacadie, S.A.*****, Ibrahim, M.M., Glass, C.K., Corcoran, D.L., Benner, C., Heinz, S., Kadonaga, J.T., Ohler, U. (2015). Perspectives on Unidirectional versus Divergent Transcription. Mol. Cell 60, 348–349.

Duttke, S.H.C., **Lacadie, S.A.*****, Ibrahim, M.M., Glass, C.K., Corcoran, D.L., Benner, C., Heinz, S., Kadonaga, J.T., Ohler, U. (2015). Human promoters are intrinsically directional. Molecular Cell, 57, 674–684.

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Lacadie S.A., Ibrahim M.M., Gokhale S.A., Ohler U.(2016). Divergent transcription and epigenetic directionality of human promoters. FEBS J, 283(23):4214-4222.

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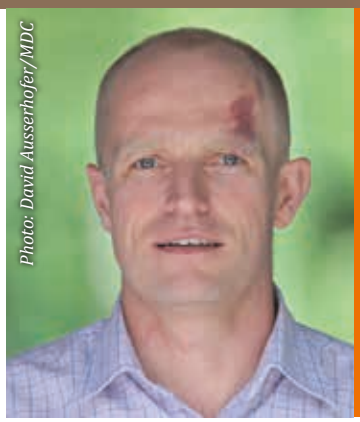

Photo: David Ausserhofer/MDC

Markus Landthaler

RNA Biology and Posttranscriptional Regulation

Our main interest is the understanding of posttranscriptional regulatory networks controlling gene expression in humans. Nascent mRNA stably associates with a large number of mRNA-binding proteins to form ribonucleoprotein (RNP) complexes. These complexes play a key role in the regulation of spatial and temporal changes in protein synthesis by controlling transport, storage, decay and translation of mRNAs. Deregulation and failed coordination of these mechanisms contribute to pathophysiologicial development and conditions. A prerequisite for a systems level understanding of post-transcriptional regulation is a transcriptome-wide high-resolution map of mRNA-protein contacts that allow us to study how these interactions control the fate of mRNA transcripts. To achieve this goal we use photo-crosslinking approaches (PAR-CLIP [Hafner & Landthaler et al. Cell 2010]) and protein occupancy profiling on mRNA [Schueler et al. Genome Biol. 2014] in combination with next-generation sequencing to identify functional RNA-protein interactions at single nucleotide resolution. In addition we employ mRNA interactome capture in combination with mass spectrometry [Baltz et al. Mol Cell 2012] to characterize changes in the composition of mRNP complexes in response to intra- and extracellular signals.

Post-transcriptional regulation by RNA-binding proteins

Roberto Arsie, Karol Rogowski, Ulrike Zinnall

Mammalian genomes encode several hundred RNA-binding proteins, each containing one or multiple domains able to recognize target mRNA in a sequence and/or structure dependent manner. The association of these proteins with transcripts regulates the biogenesis and translation of mRNA. For the majority of mRNA-binding proteins the target transcripts and their function in RNA metabolism are unknown, limiting our understanding of post-transcriptional regulatory processes. In particular, we are interested in the characterization of known and novel mRNA-binding proteins. By combining maps of functional RNA-protein interactions with cell-based and biochemical assays, we determine the dynamic assembly of RNA-binding proteins on their target mRNAs as well as the elements and mechanisms guiding mRNA maturation, localization, turnover and protein synthesis (Figure 1).

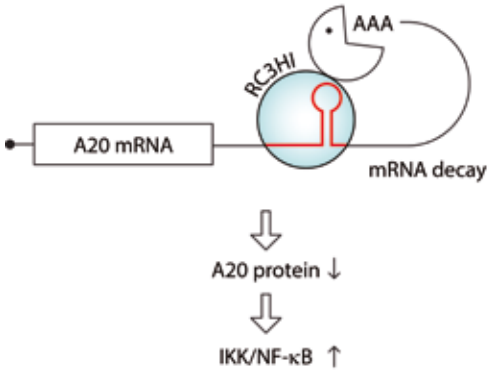

Figure 1: *Posttranscriptional regulation of A20/TNFAIP3 mRNA by the RNA-binding protein RC3H1*

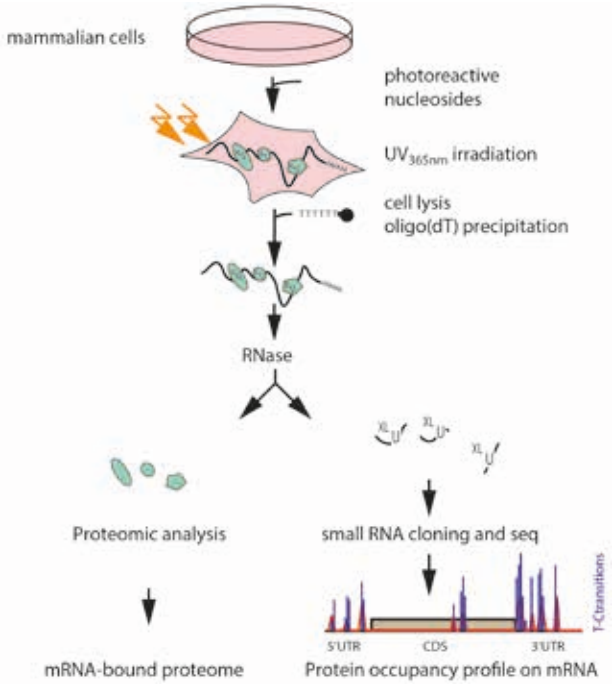

Figure 2: *Identification of the mRNA-bound proteome and protein occupancy profiling on mRNA.*

Specificity and function of RNA helicases

Nicolai Kastelic

RNA helicases are a family of highly conserved proteins that utilize NTP hydrolysis to unwind RNA structures and/or remodeling of ribonucleoprotein complexes. RNA helicases participate in all biological processes that involve RNA metabolism, including transcription, splicing and translation and have been implicated in disease states such as tumorigenesis and the replication of viruses. We are using our PAR-CLIP approach to define functional interactions of helicases, their helicase-inactive variants and RNA that are typically transient in nature. The identification of RNA target sites provides the foundation for biochemical and reverse genetic approaches to investigate the remodeling mechanism of ribonucleoprotein complexes by helicases. These studies give insights into the determinants of target RNA selection, functional interactions with other RNA-interacting proteins and the physiological role of RNA helicases.

Dynamics of the mRNA-bound proteome in response to intra- and extracellular signals

Nicolai Kastelic , Marcin Kolinski, Miha Milek, Emanuel Wyler

We developed a photoreactive nucleotide-enhanced UV crosslinking and oligo(dT) purification approach to identify the mRNA-bound proteome using quantitative proteomics (mRNA interactome capture) and to display the protein occupancy on mRNA transcripts by next-generation sequencing. Application to a human embryonic kidney cell line identified close to 800 proteins with more than 240 novel mRNA interactors.

Our observations indicate the presence of a large number of mRNA binders with diverse molecular functions participating in combinatorial posttranscriptional gene-expression networks. More recently we described a global comparison of protein occupancy profiles on mRNA transcriptomes from different cell types, and provided evidence for altered mRNA metabolism as a result of differential protein-mRNA contacts. Our work demonstrated the value of protein occupancy profiling on protein coding transcripts for assessing the cis-regulatory mRNA sequence space and its dynamics in growth, development and disease.

We use the described methods to monitor the dynamic changes of the mRNA-protein interactome to capture differentially binding proteins and occupied cis-regulatory mRNA regions as a consequence of intra- and extracellular signals. These data provide insights into the mechanisms that lead to changes in mRNA turnover and protein synthesis in response to genotoxic stress, viral infection and during embryonic stem cell differentiation (Figure 2).

Selected Publications

Wessels HH, Imami K, Baltz A, Small S, Selbach M, Ohler U#, **Landthaler M#**. (2016) The early fly embryo mRNA-bound proteome. *Genome Research* 26, 1000-1009.

Murakawa Y, Hinz M, Mothes J, Schuetz A, Yasuda T, Mastrobuoni G, Friedel CC, Dölken L, Rajewsky K, Kempa S, Schmidt-Supprian M, Heinemann U, Wolf J, Scheidereit C, **Landthaler M**. (2015) RC3H1 represses the IKK/NF-κB negative feedback regulator A20 by binding to a 3'UTR composite structure-sequence element. Nature Communications 6, 7367

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Schueler M, Munschauer M, Gregersen LH, Finzel A, Loewer A, Chen W, **Landthaler M#**, Dieterich C# (2014) Differential protein occupancy profiling of the mRNA transcriptome. Genome Biology 15:R15

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Irmtraud M. Meyer

RNA Structure and Transcriptome Regulation

My group develops dedicated computational methods for investigating how RNA structural features and RNA-RNA interactions regulate the expression of protein-coding and non-coding genes. Our primary focus are the transcriptomes of eukaryotic model organisms such as the fruit fly (Drosophila melanogaster), the mouse (Mus musculus) and the human (Homo sapiens). For this, we aim to combine computational analyses developed by us with large scale data sets generated using state-of-the-art high-throughput sequencing and investigation protocols.

When the human genome sequence was released more than a decade ago, it came as a surprise to many that the number of protein-coding genes was not radically different from the corresponding gene count for the more humble organism Caenorhabditis elegans (C. elegans). The current gene counts (20313 for human (GRCh38.p5) versus 20447 for C. elegans (WBcel235)) are stunningly similar. The gene count itself is thus only a poor measure for the complexity of the corresponding organism.

The genome in any cell of a living organism is not a static entity, but generates diverse functional products as function of time. These define the status of that cell and also respond to internal and external changes. The primary products of the genome are transcripts (RNA sequences). These are typically not the final functional products, but need to be processed before they yield the final products (proteins and RNAs). From all we know, complex organisms like ours have developed more refined ways for processing their primary transcripts into different, functional products according to the status of the corresponding cell.

Another surprise in the wake of the human genome sequencing project was the realisation that only a small fraction (<2%) encodes protein information. Moreover, many genes do not encode a protein product (25180 so-called RNA genes (GRCh38.p5)) and even the primary transcripts of protein-coding genes contain a seemingly disproportionate fraction of non-coding nucleotides (introns, untranslated regions). This is surprising, given that introns are excised before the remaining, shortened RNA sequence is translated into the corresponding protein.

My group develops dedicated computational methods and algorithms to understand how the transcriptome is regulated. Our main goal is to understand the role that RNA structure and interactions between different transcripts play in regulating transcripts in the living cell. For this, we study transcriptome data from model organisms such as the fruit fly, the mouse and the human.

Beyond the one-dimensional view of transcripts:

More often than not, figures in textbooks illustrate the Central Dogma of Biology by depicting transcripts as linear molecules inside a eukaryotic cell, with transcription and splicing seemingly happening consecutively. What we know from many dedicated experiments, however, is that processes such as splicing, RNA editing and

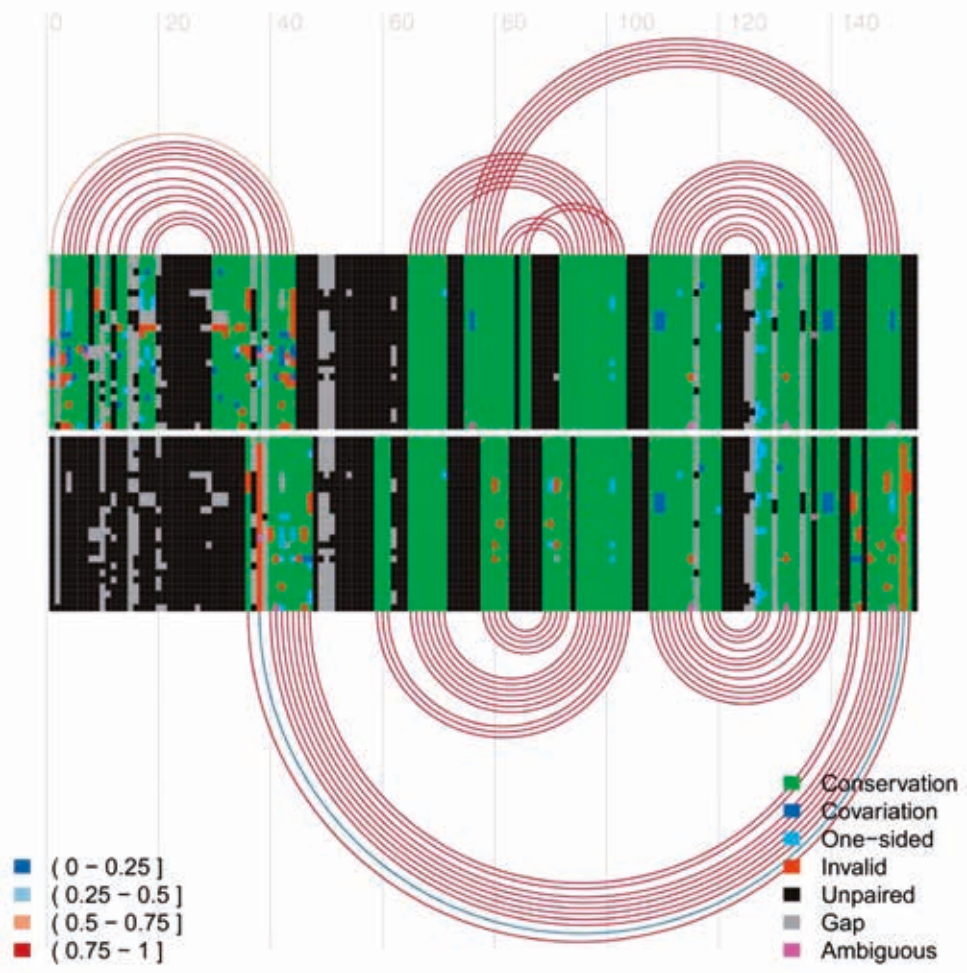

Figure 1: Arc-plot for the HDV ribozyme made using R-Chie (www.e-rna.org). Each arc represents one pair of base-paired alignment columns. Arcs and the alignment at the top show the alternative structure and the active structure; those at the bottom the inhibitory alternative structure. The left legend specifies the percentage of canonical base-pairs for each arc. The right legend colour-codes the nucleotides and specifies the evolutionary evidence supporting each arc.

RNA structure formation can happen co-transcriptionally, i.e. while the transcript emerges from the genome. Similarly to protein information, information on RNA structure can be directly encoded in the transcript itself. We thus expect that RNA structural features and RNA-RNA interactions are widely used for regulating gene expression on transcript level.

Modelling RNA structures in vivo:

In order to devise computational methods for detecting the RNA structural features that are functionally relevant in vivo, it is worth acknowledging the complexity of the cellular environment and the impact this may have on the structure formation process (Lai, Proctor and Meyer, RNA 2013). By devising the new RNA secondary structure prediction program CoFold (Proctor and Meyer, Nucl. Acids Res. 2013), we showed that it is possible to capture the overall effects of the speed and directionality of transcription in vivo. Our method yields significantly improved predictions, especially for long transcripts (> 200 nt) such as ribosomal RNAs. We know already that the sequences of structured RNAs not only encode information on their final RNA structure, but also on transient structural features of their co-transcriptional folding pathway (Meyer and Miklós, BMC Mol Biol 2004). It turns out that orthologous transcripts from related organisms also have similar co-transcriptional folding pathways and that distinct transient RNA structure features can be as conserved and functionally relevant as those of the final RNA structure (see Figure 1; Steif and Meyer, RNA Biology 2012; Zhu, Steif, Proctor and Meyer, Nucl. Acids Res. 2013; Zhu and Meyer, RNA Biology 2015). This has obvious implications for many state-of-the-art methods in RNA secondary structure prediction as these typically assume that any given transcript folds into exactly one (but not more) functional RNA structure. A probabilistic method developed earlier by us (Transat, see Wiebe and Meyer, PLoS Compbio. 2010) aims to address this problem and has allowed us to detect individual, conserved RNA secondary structure features of pseudo-knotted structures, ribo-switches and transient structures which are otherwise notoriously difficult to predict.

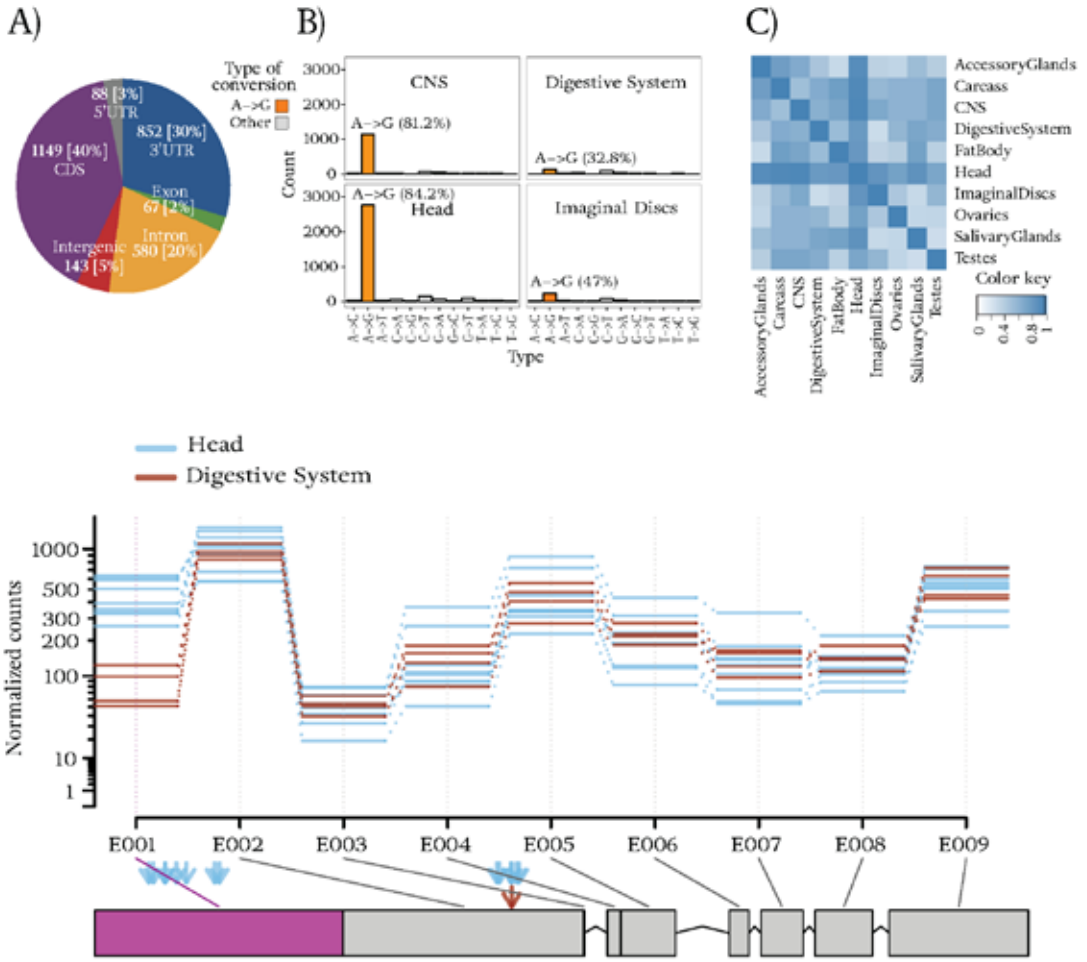

Figure 2: *(A) Genomic context of identified editing sites. (B) Distribution of conversion types for four tissue types. (C) Percentage of common editing sites between pairs of tissues. (Bottom) Gene CG5850 is differentially expressed between head (blue) and digestive system (red) and editing and splicing may affect each other. X-axis: exons of the gene, y-axis: number of reads normalized by library size. Arrows show editing sites. The purple box is predicted to be alternatively expressed.*

RNA structure features involved in splicing regulation:

Viral genomes such as Hepatis-C and HIV-1 are known to encode functional RNA structure in protein-coding regions as one major constraint for their genomes it to remain short. We contributed early on to these studies by showing that these RNA structures can be reliably predicted provided the know protein context is explicitly taken into account (Pedersen, Meyer, Forsberg, Simmonds and Hein, Nucl. Acids Res. 2004; Pedersen, Forsberg, Meyer and Hein, Mol. Biol. Evol. 2004; Watts et al., Nature 2009). Functional RNA structures overlapping protein-coding regions, however, are not the preserve of viral genomes, but can also regulate the alternative splicing and translation of eukaryotic protein-coding genes e.g. in Arabidopsis thaliana, mouse and human (Meyer and Mikl√≥s, Nucl. Acids Res. 2005; Sch√∂ning et al., Nucl. Acids Res. 2008). In order to explore the link between RNA structure and alternative splicing on a transcriptome-wide scale, we recently analysed tissue-specific high-throughput transcriptome data from the fruit fly. Using a new analysis pipeline that explicitly captures the requirement for double-stranded regions, we identified around 2000 novel editing sites as well as numerous (244) regions where RNA editing and alternative splicing are likely to influence each other (see Figure 2). The detected RNA editing sites are approximately three times more likely to occur in exons with multiple splice sites than unique ones and conserved RNA structure features overlap 39% (96/244) of these regions (see Figure 3). We therefore conclude that local RNA structure features have the potential to regulate alternative splicing via structural changes induced by RNA editing (Mazloomian and Meyer, RNA Biology 2015).

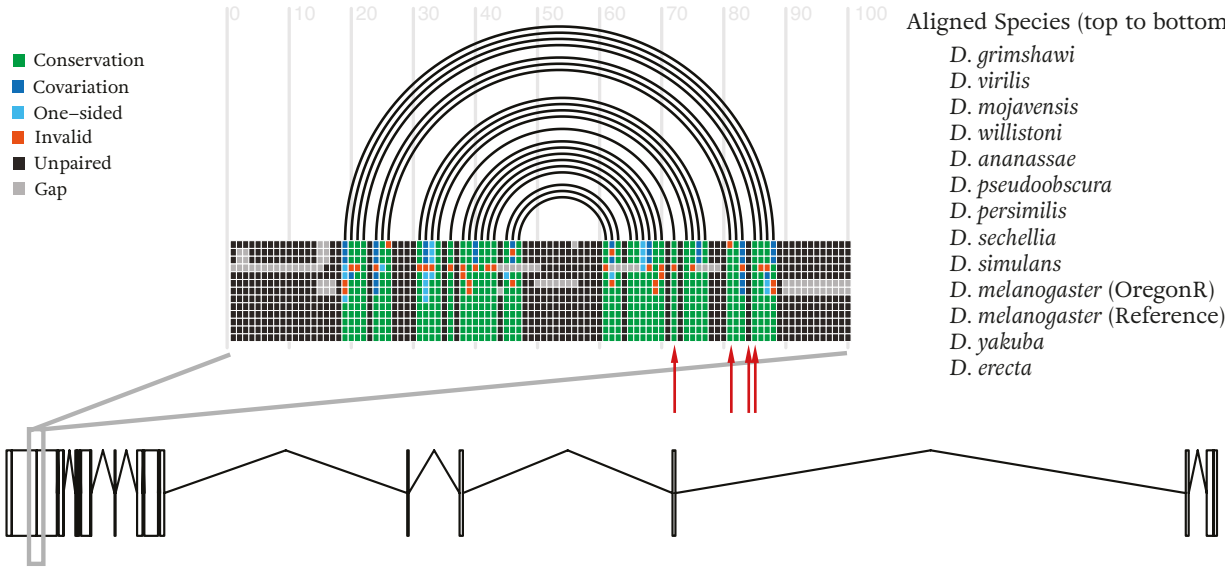

Figure 3: *(Top) Arc-plot for the highlighted region of the Cip4 gene containing a predicted, conserved RNA secondary structure overlapping RNA editing sites (red arrows) that could influence alternative splicing via structural changes. The left legend colour-codes the nucleotides according to the evidence supporting each arc, see also Figure 1. Figure made using R-Chie (www.e-rna.org). (Bottom) Gene structure of the Cip4 gene with grey box highlighting the structure-containing part at the top.*

Trans RNA-RNA interactions regulating the transcriptome:

RNAs not only have the potential to form RNA structure, but can also interact with other RNAs in trans. This involves the same simple structural building blocks, namely hydrogen bonds between complementary nucleotides ({G,C}, {A,U} and {G, U}). It is thus much more straightforward to evolve or design a desired RNA structure or trans RNA interaction using an RNA than a protein. We thus expect many trans RNA-RNA interactions beyond those already known (miRNA-mRNA, snoRNA-rRNA, etc) to be discovered. We have shown how the power of the comparative approach can be harnessed in the context of coding gene prediction, RNA gene prediction and RNA structure prediction and the prediction of trans RNA-RNA interactions. Based on our recent survey of methods for predicting de novo trans RNA-RNA interactions (Lai and Meyer, 2016), we intend to focus on computational methods that can better handle full-length transcripts and that can differentiate between different kinds of evolutionary constraints.

In the last few years, transcriptomics has made dramatic advances through the invention of new transcriptome-wide investigation protocols (e.g. CLASH, SeqZip) as well as a new generation of sequencing techniques (e.g. nanopore and SMRT sequencing) that allow for significantly longer read-lengths. We want to combine dedicated computational analyses and methods developed by us with transcriptome-wide data sets generated via state-of-the-art sequencing and experimental protocols to gain a global and detailed understanding of how RNA structure and RNA-RNA interactions regulate eukaryotic gene expression on transcript level.

I will be joining the MDC early 2016 from the University of British Columbia in Vancouver, Canada, and look forward to setting up collaborations with new colleagues at the BIMSB and MDC and the wider scientific community in Berlin, Germany and Europe (Contact: irmtraud.meyer@mdc-berlin.de).

Selected Publications:

Lai D, Meyer IM (2016) A comprehensive comparison of general RNA-RNA interaction prediction methods. Nucleic Acids Research 44(7):e61.

Mazloomian A, Meyer IM (2015). Genome-wide identification and characterization of tissue-specific RNA editing events in D. melanogaster and their potential role in regulating alternative splicing. RNA Biology 12(12):1391-1401

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Start of the Group: January 2016

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Evolutionary Modeling and Inference in Computational Systems Biology

As more animal genomes get sequenced, computational biology gains new power to understand biological function from an evolutionary perspective. Developing methods for network inference from heterogeneous and noisy data, we use evolutionary correlations to understand collective functions of post-transcriptional gene regulation and simplify protein folding. Further, we use evolutionary modeling and inference to study small open reading frames (ORFs). So-called micropeptides encoded by these ORFs have recently emerged as important regulators of development and physiology, but computational identification is challenging due to their small size. We predict hundreds of novel conserved small ORFs from their characteristic evolutionary signatures. In several ongoing collaborations, we now investigate coding or regulatory functions of small ORFs in mammalian cell lines and in the model organisms D. rerio, D. melanogaster, and C. elegans.

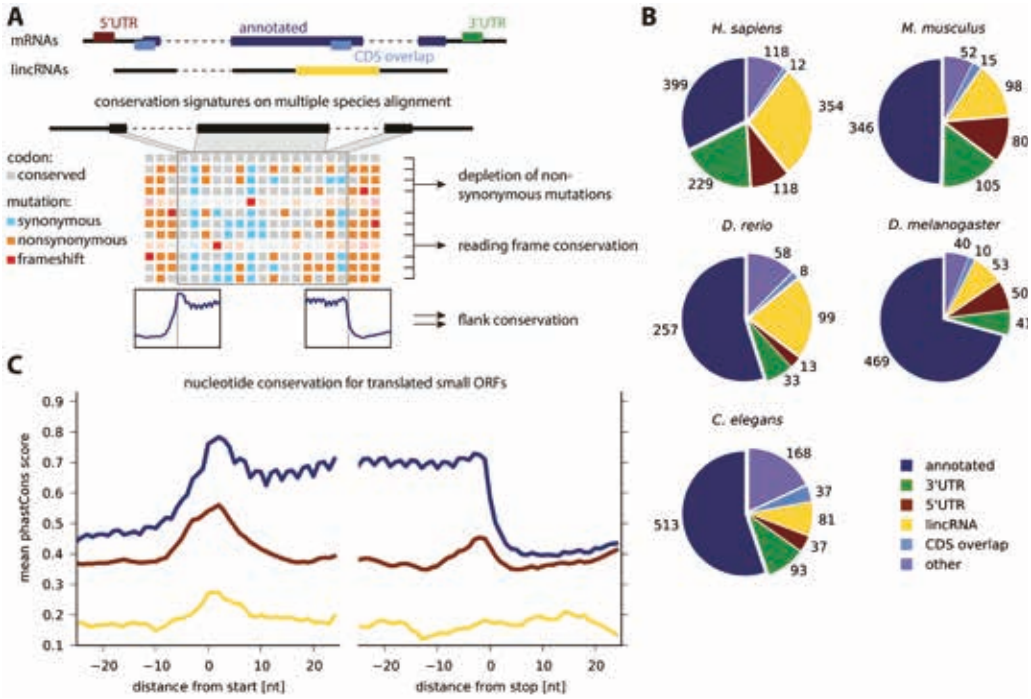
Inference of regulatory networks

Gene regulatory networks are densely interconnected systems of trans-acting regulators and cis-acting sequence elements. This interdependency creates informative correlations in gene expression levels or conservation patterns. We develop methods for network inference using observed correlations, with a particular focus on techniques to reduce bias from confounding factors such as stochastic noise, indirect correlations due to covariates, or heterogeneity in the underlying samples. We applied these methods to understand features of post-transcriptional regulation by microRNAs (miRNAs). This regulation appears to depend less on individual links between a miRNA and its target site, but rather to be conveyed by the collective regulation by multiple miRNAs on entire target sets. With the function of the network conserved throughout evolution, this implies that individual target sites do not evolve independently of each other. Indeed, using evolutionary correlations between vertebrate miRNA target sites, we corroborate the conservation of combinatorial regulation and identify different regulatory strategies for the targeting of different gene sets. We also developed and analyzed methods to distinguish direct from indirect correlations in amino acid substitution patterns given alignments from phylogenetically closely related species, which can be used to simplify and aid computational protein folding.

Small open reading frames

Genome-wide expression profiling shows that a large fraction of the genome is transcribed, partly into canonical coding mRNAs, and partly into RNAs without long ORFs. The functions of most of these long non-coding RNAs (lincRNAs) are poorly understood. However, recent observations suggest that small ORFs on lincRNAs are often translated and produce peptides detectable via mass spectrometry. In fact, some of the encoded micropeptides have essential biological roles in physiology or development. Yet, translation of small ORFs could also exert regulatory functions, for instance via nonsense-mediated decay. Despite numerous publications on this subject, there is still little consensus about the abundance of functional sORFs in animals, although complete gene catalogs are essential to explain the molecular basis of organismal phenotypes.

In a collaboration between the Rajewsky and Giraldez labs, we identified hundreds of small ORFs encoded by human and zebrafish lincRNAs using complementary methods: ribosome profiling was used to detect translated



Prediction of conserved small ORFs in animals. A: *searching the transcriptome for small ORFs, we evaluate conservation signatures from multiple species alignments and use machine learning to predict conserved small ORFs.* **B:** *hundreds of small ORFs are predicted in different transcriptome regions of 5 animal species.* **C**: *nucleotide-level conservation is different for translated ORFs in 5'UTRs or lincRNAs than for annotated small protein-coding genes.*

small ORFs, and evolutionary conservation was used to predict putatively functional coding small ORFs. Building on these findings, we performed a much more comprehensive study and identified conserved small ORFs in the transcriptomes of human, mouse, zebrafish, fruit fly and the nematode *C. elegans*. About 2,000 putatively functional animal small ORFs are predicted by this rigorous computational screen. Systematic homology analysis identified a number of widely conserved candidates of high interest for functional follow-up studies. Further, we leveraged a large amount of published experimental data to confirm and characterize expression of conserved small ORFs on multiple levels, such as RNA-seq, ribosome profiling and mass spectrometry.

Together with the Ohler and Landthaler labs, we investigated translated small ORFs in noncoding transcriptome regions. While we found highly conserved and translated micropeptides encoded in 5'UTRs or lincRNAs, there was generally only limited overlap between conserved and translated small ORFs. In contrast to known small protein-coding genes, translated ORFs in 5'UTRs or lincRNAs are actually often only conserved in their positions but not their encoded peptide sequences, suggesting that only some have coding, but many others have regulatory functions. We will combine these computational findings with high-throughput data from RNA expression and translation profiling in a cell line model system for neuronal differentiation. Developing computational models for translational regulation by small ORFs and other cis-acting sequence elements, we aim to obtain a quantitative understanding of post-transcriptional gene regulation in this system.

In collaboration with Ferdinand Le Noble at KIT, we use CRISPR/Cas to create a zebrafish knock-out model for a 25aa peptide conserved across vertebrates. With in-situ hybridization data and morpholino knockdowns indicating functions in the brain, we also use mammalian cell lines to study molecular functions in neuronal development and differentiation by means of RNA-seq and CRISPR/Cas genome editing. Together with the Selbach lab, we use computational protein docking and affinity purification followed by mass spectrometry to find protein interaction partners of selected micropeptides. In further collaborations, we investigate micropeptides with possible roles in heart disease using animal models and clinical samples, we study functions of small ORFs in immunity, and we screen small ORF knockout phenotypes in D. melanogaster using CRISPR/Cas.

Selected Publications

L. Calviello, N. Muhkerjee, E. Wyler, H. Zauber, A. Hirsekorn, M. Selbach, M. Landthaler, B. Obermayer, and U. Ohler, "Detecting actively translated open reading frames in ribosome profiling data", Nat. Meth., 13:165 (2016)

S.D. Mackowiak, H. Zauber, C. Bielow, D. Thiel, K. Kutz, L. Calviello, G. Mastrobuoni, N. Rajewsky, S. Kempa, M. Selbach, and B. Obermayer*, "Extensive identification and analysis of conserved small ORFs in animals", Genome Biol., 16:799 (2015)

B. Obermayer and E. Levine*, "Inverse Ising inference with correlated samples", New J. Phys., 16:123017 (2014)

B. Obermayer and E. Levine*, "Exploring the miRNA regulatory network using evolutionary correlations", PLoS Comput. Biol., 10:e1003860 (2014)

A.A. Bazzini*, T.G. Johnstone, R. Christiano, S.D. Mackowiak, B. Obermayer, E.S. Fleming, C.E. Vejnar, M.T. Lee, N. Rajewsky*, T.C. Walther, and A.J. Giraldez*, "Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation", EMBO J. 33:981 (2014)

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Photo: David Ausserhofer/MDC

Uwe Ohler

Computational Regulatory Genomics

According to current understanding, complexity in higher organisms is not achieved by a more complex repertoire of parts, i. e. genes, but instead by the more complex regulation of the parts. The expression of genes is tightly controlled on several levels — a large number of protein and RNA factors and DNA and RNA sequence elements enable the precise regulation of interacting gene products. It is a key challenge to decipher these complex networks of players and interactions, and to move biology from case studies to an integrated, global approach.

Our lab develops and applies genomics and computational approaches to understand mechanisms of gene regulation in eukaryotic organisms. Computational biology has become indispensable to analyze and ultimately make sense of large-scale data sets that look at the phenomenon of gene regulation from different angles. Our long term goal is to investigate how regulatory networks enable the correct development of complex organisms, with their multitude of cell types that carry out different functions despite the same genome.

Computational biology and gene regulation

Understanding gene regulation emerged as a crucial challenge after the initial sequencing of the human genome, as misregulation of gene expression is at the heart of many developmental defects and diseases. In this context, the particular strength of a multidisciplinary group like ours lies in the combination of genomics-oriented data generation and analysis with computational modeling. We frequently frame questions as classification problems and use machine learning approaches to make testable predictions. These predictions are often validated experimentally, either by us or our friends and collaborators. At the same time, inspection of model parameters may allow us to draw conclusions about the importance of particular biological features.

Quantifying gene expression at different layers of gene regulation

The rise of deep sequencing technologies has changed the world of genomics research in ways we are only beginning to appreciate. One striking aspect is its versatility; in addition to determining the DNA sequence of a genome, many biological processes can be quantified by means of sequencing.

One popular application is the quantification of mRNA steady-state levels via (paired-end) RNA sequencing (RNA-seq). A single RNA-seq data set contains millions of small (~100 nucleotide) reads, and it is a well-known challenge to assemble these into the complete set of all alternative splice isoforms of expressed genes. To guide and improve this process, complementary information can be used. We developed an isoform annotation algorithm that is informed by genomic features (including start/stop codon, splice sites, coding potential) as well transcript features from RNA-seq (Majoros et al, Bioinformatics 2014). In this way, we restricted the annotation of splice isoforms to those that contain a consistent open reading frame (ORF). While we cannot annotate non-coding transcripts in this manner, the annotations of protein-coding genes improved significantly, particularly for lowly expressed genes where RNA-seq data alone does not provide enough coverage.

Using clever modifications and extensions, RNA-sequencing protocols go beyond the

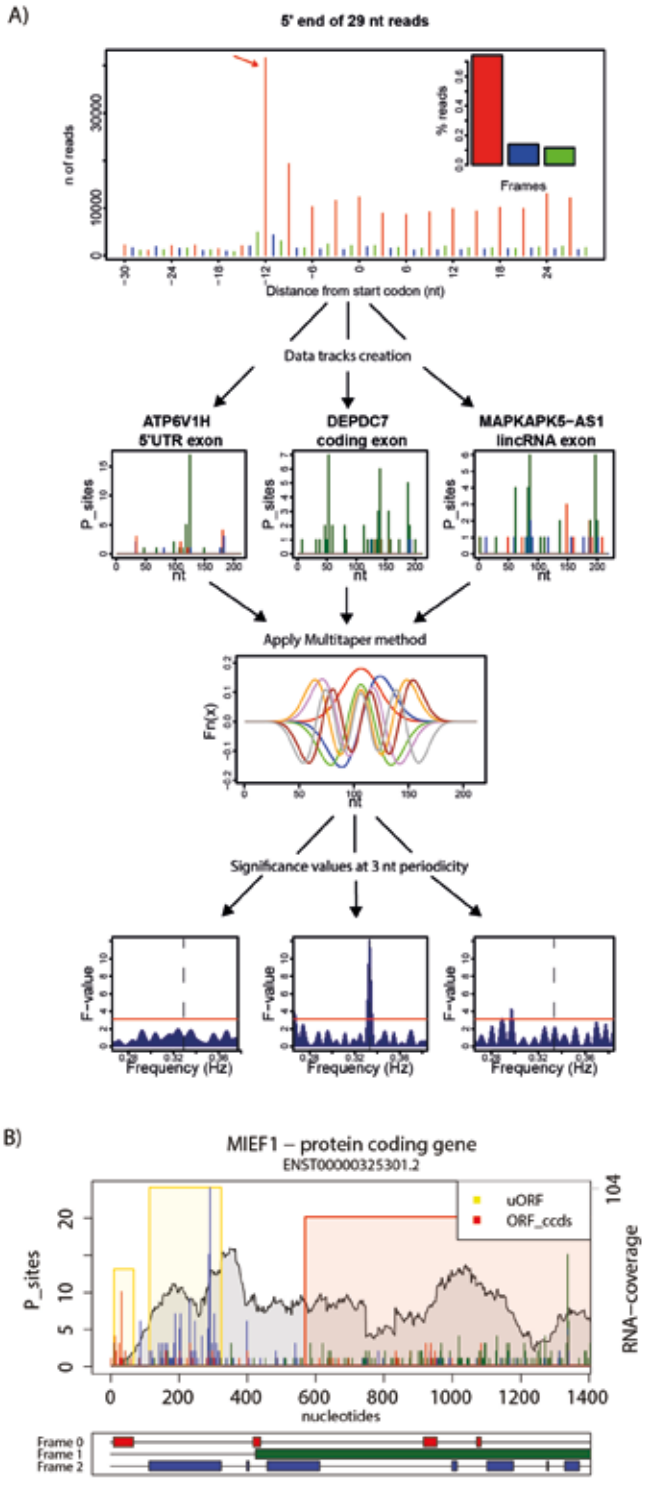

Figure 1. *RiboTaper, a computational method to detect actively translated open reading frames (ORFs) from RiboSeq data. (A) The workflow. Top: Using known ORFs, the optimal read size and offset from the read start can be determined, which indicates the precise P-site of the translated codon. Middle: Example P-site profiles for three exons. Bottom: Only the coding exon exhibits a significant 3-nucleotide periodicity. (B) ORF annotations include both coding and upstream ORFs.*

steady-state transcriptome and quantify gene expression regulation from nascent transcription to protein synthesis. For instance, to determine which, and how strongly, ORFs are translated, ribosome profiling maps the positions of translating ribosomes. As the ribosomes move one codon at a time, the resulting reads exhibit characteristic, three-nucleotide periodic footprints along the transcriptome. We have developed RiboTaper, a rigorous statistical approach that identifies translated regions on the basis of this periodicity (Calviello et al, Nature Methods, in press; Figure 1). We used RiboTaper to derive an extensive map of cell-type specific translation that covered ORFs for >11,000 protein-coding genes from a single experiment. Mass spectrometry data confirmed that RiboTaper achieved excellent coverage of the cellular proteome. Although they frequently contain short ORFs, our analysis also suggested that only relatively few of the currently annotated long non-coding RNAs appear to encode stable polypeptides.

Transcriptional control: from proximal initiation to distal regulation

In eukaryotes, transcriptional regulatory elements are located in different regions relative to the gene they control. The core promoter, a ~100 nucleotide long region centered on the transcription start site (TSS), is contained within the proximal promoter region directly upstream of a gene. It has been traditionally assumed to perform the function to recruit the basal transcription complex to the correct genomic location, and thus to contain similar sequence patterns across all genes. In contrast, distal non-coding regulatory regions such as enhancers contain different combinations of sequence patterns recognized by specific transcription factors (TFs) and thus convey condition-specific activity (Ohler and Wassarman, Development 2010).

The application of deep sequencing has shown transcriptional activity at many places in the genome, going substantially beyond annotated protein-coding genes. In ongoing work, we examine transcription initiation for different types of pervasive

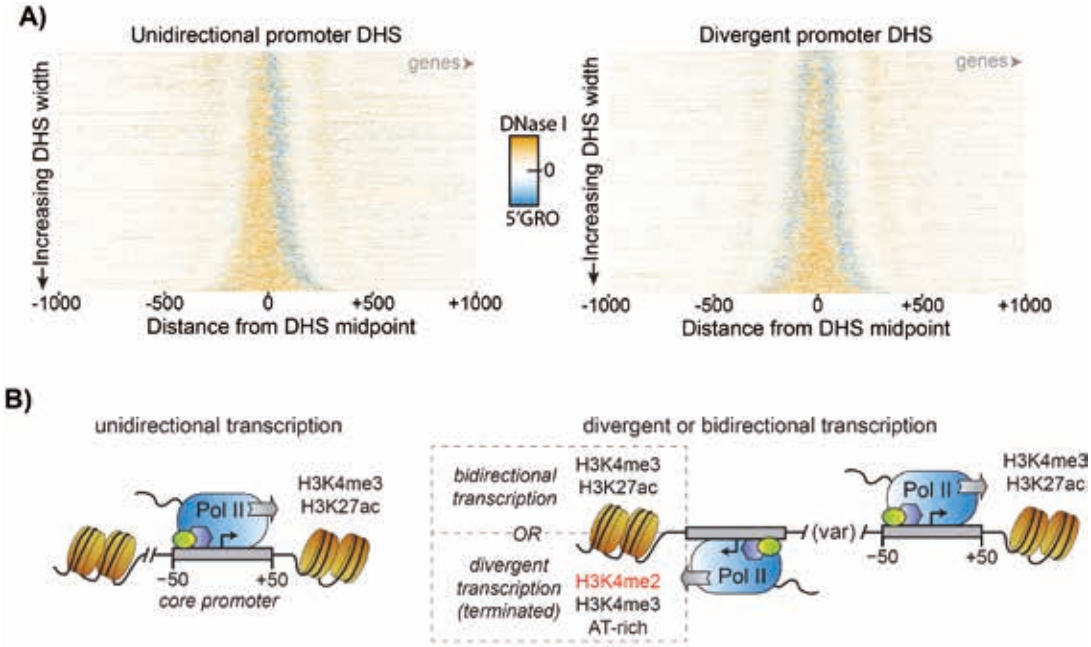

Figure 2. *Many human promoters are divergently transcribed. (A) By mapping transcription start sites (TSS) of nascent RNA transcripts (5'GRO; blue) to regions of open chromatin (DNase I; red), TSS locations could be precisely mapped to the edges of proximal promoters. In this way, promoters showing transcription in forward-only or both directions were identified. (B) Divergent and unidirectional promoters exhibit significant differences in chromatin and sequence composition.*

transcription. For instance, many mammalian promoters show divergent transcription in both directions, i.e. in the forward direction towards the annotated gene and in the reverse direction away from the gene. Notably, this phenomenon is not conserved across all eukaryotes (cf. Core et al, Cell Rep 2012). We could show that human core promoters are inherently unidirectional and that reverse-oriented transcripts originate from distinct reverse-directed core promoters (Duttke, Lacadie et al, Mol Cell 2015; Figure 2). A new algorithm enabled us to use DNase I accessibility to precisely define proximal promoter borders (Ibrahim et al, Bioinformatics 2015), which revealed that for expressed genes across different cell types, about 25-45% of promoters are unidirectional. These unidirectional promoters are depleted at their upstream edges of the distinct reverse core promoters. Divergent transcription is thus not an inherent property of the transcription process but rather the consequence of the presence of two core promoters. We are currently examining whether reverse directed transcripts have an influence on the expression of the forward counterpart.

For a long time, the large non-coding genomic sequence space of higher eukaryotes made it difficult to pinpoint distal regulatory regions that are targeted by TFs. Several recent genomic approaches provide "global regulatory profiles", which reflect where regulatory factors target specific regions andsites in DNA. Specifically, DNase-seq provides coarse-resolution maps of nucleosome-free sites or "open chromatin", which are universal hallmarks of all types of transcriptional regulatory elements including proximal promoters. We are now investigating DNase-seq data at nucleotide resolution, to investigate whether it is possible to pinpointing individual TF binding events (protected "footprints" in the DNase profiles) within open chromatin (Yardimci et al, Nucleic Acids Res 2014). To this end, we built simple baseline models that deconvolved DNase experimental bias from genuine binding signal at putative footprints. In this manner, we found that available data is frequently not deep enough to reliably identify genuine binding, and that some TF binding events are inherently hard to identify, for instance when the TF recognition sequence overlaps with the experimental bias.

RNA regulation: identifying target sites of regulatory proteins

The last decade has seen an outstanding increase in appreciation of the role of post-transcriptional gene regulation. RNA-binding proteins (RBPs) and small, regulatory RNAs such as microRNAs (miRNAs) recognize specific sites in target RNA transcripts and influence their fate. It is now feasible to map the precise target sites of post-transcriptional regulators via cross-linking followed by deep sequencing (CLIP-seq). In

previous work, we have implemented specific approaches for reliable, high-resolution identification of binding sites, as well as for follow-up prediction of regulatory sequence motifs (Ascano et al, Nature 2012). While we are developing the next generation of algorithms to deal with a wider array of experimental methods and improved protocols, we have continued to apply existing methods in several collaborative studies, for instance to determine the target sites of Regnase-1/Roquin (Mino et al, Cell 2015) and RBPMS (Farazi et al, RNA 2014).

Putting the pieces together: networks involving different regulatory factors or mechanisms

Our work on DNA and RNA-based regulation converges in projects where we aim to dissect quantitative contributions of multiple regulatory factors or mechanisms, and how they work together to define correct expression. For instance, several RBPs target so-called AU-rich sequence elements (AREs) in the 3' untranslated region of mRNAs. ZFP36, also known as tristetraprolin or TTP, and ELAVL1, also known as HuR, both interact with AREs, but they have antagonistic roles. We determined ZFP36 binding preferences using CLIP, integrated it with existing data for ELAVL1, and examined the combinatorial regulation of AU-rich elements by ZFP36 and ELAVL1 (Mukhkerjee et al, Genome Biol 2014).
Using partial correlation analysis, we were able to quantify the association between ZFP36 binding and differential target RNA abundance upon ZFP36 overexpression, independent of effects from confounding features. We identified thousands of overlapping ZFP36 and ELAVL1 binding sites; careful investigation showed that ZFP36 degrades transcripts through specific ARE sequence variants, representing a subset of the sequences ELAVL1 interacts with to stabilize transcripts. Thus, ZFP36 and ELAVL1 bind an overlapping spectrum of RNA sequences, yet with differential relative preferences that dictate combinatorial regulatory potential. Motivated by these results, we are currently expanding our integrative models to include larger numbers of proteins, as well as accounting for regulatory effects at different layers of gene expression.
The described studies frequently resulted from joint work and had the support from a number of excellent colleagues, including James Kadonaga (UC San Diego); Tom Tuschl (Rockefeller University); Philip Benfey, Greg Crawford, (Duke University); Markus Landthaler, Benedikt Obermayer and Matthias Selbach (MDC).

Selected Publications

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Epigenetic regulation and chromatin architecture

Our laboratory aims to understand mechanisms of gene expression which act at multiple levels, from the local action of transcription factors, to long-range chromatin looping events that connect regulatory DNA sequences with the genes they regulate, to how whole chromosomes are positioned within cell nuclei. Binding of transcription factors at gene promoters helps recruit chromatin remodelers and the transcription machinery to transcription units. Upon recruitment, RNA polymerases either remain primed for subsequent activation (e.g. after environmental stress, during differentiation or disease), or directly transcribe the DNA template into RNA molecules, which code for proteins or for structural RNAs that constitute many enzymatic complexes.

An increasingly important aspect of gene regulation are the mechanisms by which distant regulatory DNA sequences physically interact with gene promoters to recruit or activate RNA polymerase II (RNAPII), the enzyme responsible for transcribing protein-coding genes, and many structural and regulatory RNAs. Much is known about the transcription cycle of protein-coding genes by RNAPII, which entails a fascinating and highly coordinated sequence of processes that promote nascent transcript maturation concomitant with transcription. As the nascent RNA emerges from the polymerase, it is capped on its 5' end. The polymerase progresses through the coding region and introns are spliced out from the nascent transcript. Upon termination, the addition of a protective polyA tail to the transcript 3' end also occurs on chromatin. These co-transcriptional events are mediated by exquisite post-translational modifications of the RNAPII enzyme itself, which coordinate the timely recruitment of appropriate machineries at each stage of the transcription cycle.

RNAPII regulation in pluripotent and differentiated cells

Robert A. Beagrie, Giulia Caglio, João Dias, Ana Miguel Fernandes, Carmelo Ferrai, Izabela Harabula, Alexander Kukalev, Kelly J. Morris, Marta Oliveira, Tiago Rito, Marisa Saponaro, Markus Schueler, Konstantina Skourti-Stathaki, Elena Torlai Triglia

Appropriate recruitment of the RNA processing machinery and chromatin remodelers to chromatin is mediated by post-translational modification of the C-terminal domain (CTD) of the largest RNAPII subunit, RPB1 (Brookes & Pombo 2009, EMBO Rep. 10, 1213). The CTD is an unusual disordered domain composed of an heptapeptide consensus sequence, Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7, repeated 52 times in mammals. All aminoacids are targets for post-translational modifications, of which we know most about Serine phosphorylation.

After recruitment of unphosphorylated RNAPII to promoters, the CTD becomes phosphorylated on Ser5 residues (S5p), followed by sequential phosphorylation on Ser7 (S7p) and Ser2 (S2p) in the transition to elongation. S5p recruits the capping enzyme and H3K4 histone methyltransferases (HMTs), which mark active promoters. S7p is thought to mark the transition to elongation, whereas S2p recruits H3K36 HMT, to mark coding regions, and the splicing and polyadenylation machineries. The regulation of RNAPII at the transition between initiation and elongation is also influenced by CTD modifications at non-canonical aminoacids, most often found at position 7 of the most C-terminal heptapeptide repeats (Figure 1). In 2015, we showed that non-canonical repeats of the CTD with Ser7-to-Lys7 substitutions (i.e. Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-**Lys7**) are both mono- and di-methylated, and that this modification

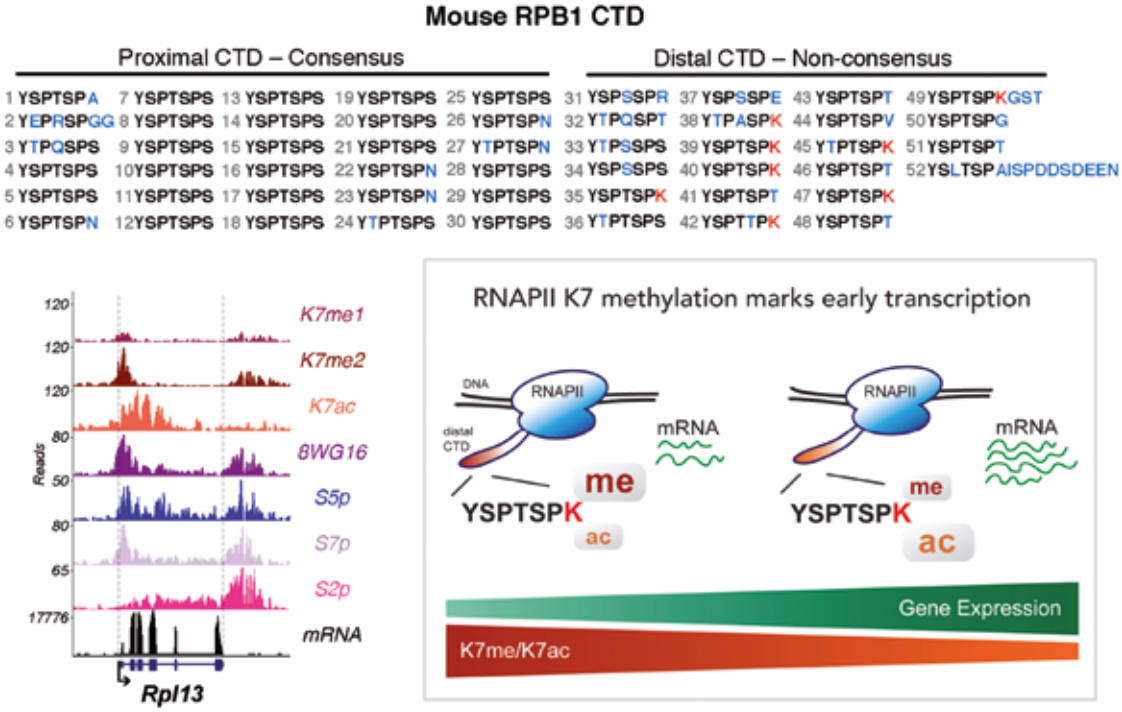

Figure 1: *Two novel post-translational modifications of RNA polymerase II (RNAPII) mark the earliest stages of the transcription cycle. Mouse RPB1 C-terminal domain is composed of 52 heptapeptide repeats; non-consensus amino acids are enriched for at the distal region. Chromatin occupancy of RNAPII-K7me1, K7me2, K7ac, 8WG16, S5p, S7p and S2p, and mRNA-seq profiles are shown for active gene Rpl13. A balance between K7me and K7ac fine-tunes gene expression. Adapted from Dias et al. (2015).*

occurs at the earliest stages of the transcription cycle at active genes in mammalian cells (Dias et al. 2015 eLIFE).

Regulation of RNAPII by post-translational modifications extends beyond the changes associated with active transcription cycles. Phosphorylated RNAPII has been reported at inducible genes, before activation, such as heat-shock. Our group has identified a novel poised form of RNAPII at silent developmental regulator genes in mouse ES cells, which are repressed by Polycomb complexes, but become activated through early differentiation and cell commitment (Stock et al. 2007, Nature Cell Biol. 9, 1428). Two major Polycomb Repressor Complexes, PRC1 and PRC2, are important for maintaining the poised state of primed developmental regulator genes. Polycomb complexes modify histone tails; PRC2 contains Ezh2, which methylates H3K27, and PRC1 contains Ring1A and Ring1B, which monoubiquitinylate H2AK119. In 2012, we showed that Polycomb and RNAPII are found co-associated on chromatin at developmental regulator genes (Brookes et al. 2012). We used chromatin immunoprecipitation (ChIP) coupled with next generation sequencing, to map the phosphorylation state of RNAPII and Polycomb occupancy across the genome. Surprisingly, not only did we find RNAPII at developmental regulator genes under Polycomb repression, but its presence was positively correlated with the amount and occupancy of Polycomb. To characterize the extent of transcriptional activity at poised genes, which are targets of Polycomb repression, we are analyzing chromatin-bound RNAs using genome-wide approaches, and CAGE-seq data.

Our unbiased genome-wide analyses of RNAPII and Polycomb occupancy also identified novel gene targets of Polycomb regulation in mouse ES cells, which were

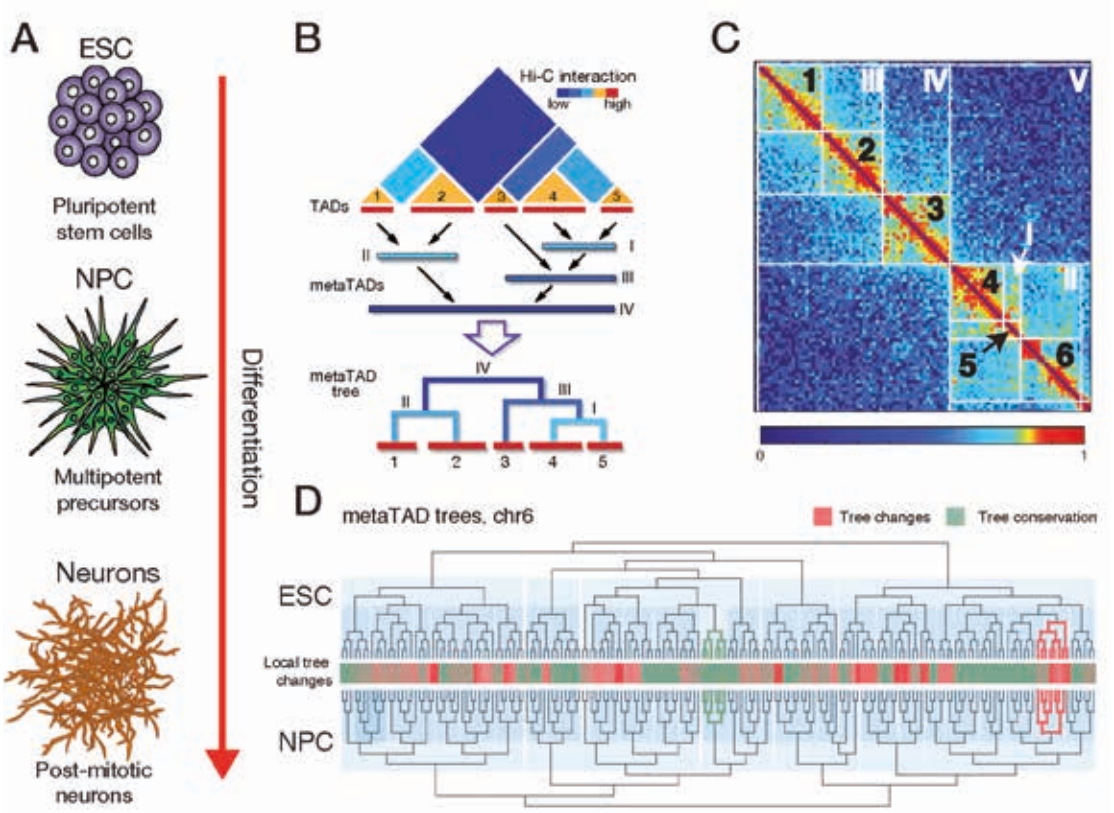

Figure 2: *(A) Scheme of mouse embryonic stem cells (ESC), neuronal progenitor cells (NPC) and differentiated neurons. (B) A hierarchical topology of meta-domains (metaTADs) are found by clustering individual topological domains (TADs). (C) Contact map of a sub-chromosomal regions (Hi-C data). (D) Meta-domain trees for chr6 in ESC and NPC. Two examples of highly similar (green) and dissimilar regions (red) are indicated (Adapted from Fraser et al., 2015.).*

more unexpected. In particular, we found a cohort of genes which are active in ES cells but whose expression is dampened by Polycomb repression (Brookes et al. 2012 Cell Stem Cell). This group of genes includes many genes important in cell cycle, cancer, signaling and energy metabolism. Our current studies aim to better understand their regulation in the transition from pluripotency to differentiated cellular states.

To probe for the dynamic changes in polymerase and chromatin conformation that accompany re-programming of gene expression during cell commitment, we have extended our analyses to neural percursor cells and differentiated neurons and to cardiac lineages. Our current bionformatics analyses aim to understand how Polycomb repression acts to establish poised states of RNAPII occupancy at genes in pluripotency and differentiation.

Genome architecture: mechanisms of 3D chromatin folding

Mariano Barbieri, Robert A. Beagrie, Giulia Caglio, Carmelo Ferrai, Rieke Kempfer, Dorothee Kraemer, Alexander Kukalev, Gesa Loof, Tiago Rito, Julieta Ramirez Cuellar, Markus Schueler, Leo Serebreni, Marta Slimak-Mastrobuoni, Christoph Thieme, Elena Torlai Triglia

Long-range gene regulation works through chromatin looping mechanisms that bring together the promoters of genes with distant regulatory regions (e.g. enhancers; reviewed in Beagrie & Pombo 2016 Bioessays). This contact, mediated by transcription factors and other chromatin associated components, promotes the recruitment of the transcriptional machinery to the gene promoter, acting as the starting point for the synthesis of transcripts. Genes that

regulate early development are often expressed at different stages of differentiation and in cell types, and are accordingly regulated through specific interactions with different regulatory regions. In addition to contacts with their respective enhancers (which have been described up to 1 Mbp from gene promoters), genes also co-associate with other genes in 3D space. Our group has shown that Active and Polycomb-repressed genes associate, respectively, with 'active transcription factories' (containing RNAPII-S5p, S2p) and with 'Poised transcription factories' (containing RNAPII-S5p and Polycomb; Ferrai et al. 2010 PLoS Biol.; Brookes et al. 2012 Cell Stem Cell), and suggested that such associations establish specific chromatin topologies that are important for silencing in the primed state, or for robust activation upon induction.

To explore the changes in chromatin contacts that accompany the transcriptional reprograming of mouse ES cells during neuronal differentiation, we mapped chromatin contacts genome wide by Hi-C, and matched CAGE data (Fraser et al. 2015 Molecular Systems Biology). Our analyses have shown that chromatin contacts extend across the length of each chromosome (across tens of megabases), and that the hierarchical folding and reorganization of chromosomes are linked to transcriptional changes during cellular differentiation (Figure 2). Polymer physics modeling demonstrated that hierarchical folding of chromatin promotes efficient packaging without loss of contact specificity.

We apply different approaches to study the mechanisms underlying complex chromatin folding patterns associated with long-range regulation, and how they might be established through different chromatin epigenetic states. With Prof. Mario Nicodemi (Univ. Naples, Italy), we have shown that available data on chromatin contacts, both originated by 3C approaches and fluorescence in situ hybridization (FISH), can be explained with the Strings & Binders Switch (SBS) model (Nicodemi & Pombo 2014 Curr. Op. Cell Biol.). Currently, we are using the SBS model to explore the 3D topology of specific genomic loci affected in congenital disease in mouse ES cells and neurons by simulating chromatin occupancy maps of epigenetic features as interaction sites. We also apply single cell fluorescence imaging to study the cell-to-cell variability and physical distances that characterize chromatin contacts. We currently develop novel approaches to map chromatin contacts at the single cell level, which can be applied in tissues (such as biopsies) to help investigate the close relationship between chromatin folding and genome function.

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Stephan Preibisch

Microscopy, Image Analysis & Modeling of Developing Organisms

Our lab aims at understanding more about how gene regulatory networks define the development of organisms. We approach the understanding of these complex systems by developing computational and experimental solutions for in-toto visualization, statistical analysis, and perturbation of developing specimen. We combine light & electron microscopy, transcription imaging, genetics and deep sequencing with computer vision and software development. While our lab is focused on C. elegans as a model organism we additionally collaborate with different labs on various model organisms providing our computational and imaging expertise.

High-resolution atlases of *C. elegans*

We combine different types of microscopic acquisitions into one common computational representation of C. elegans exploiting its high degree of stereotypicity. The basis for the integration of live and fixed acquisitions of C. elegans are high-resolution spatial atlases acquired using Serial Section Transmission Electron Microscopy (Fig. 1). Following the reconstruction of the datasets by aligning thousands of two-dimensional image tiles in three-dimensional space (Cardona et al.), we segment and annotate the complete volume to create computational representations of entire C. elegans at specific developmental stages. The fixed lineage of C. elegans subsequently allows direct mapping of lower resolution light microscopy data onto these 'virtual nematodes' using the nuclear locations.

Lightsheet microscopy data

The relatively small size of C. elegans and the fact that cells are densely packed in nervous system requires high-resolution light microscopy acquisitions in order to unambiguously detect all nuclei for mapping. We employ lightsheet microscopy (Huisken et. al.) to maximize optical resolution while minimizing photo damage. In order to exploit the full potential of lightsheet microscopy we develop multiview image reconstruction, image analysis and data management software (Fig. 2 and publications). All resulting image data can be integrated into the 'virtual nematodes' thereby creating powerful, descriptive in-toto representations.

Single-molecule RNA imaging

We study gene regulation in C. elegans combining deep sequencing and ChIP-seq data with single-molecule RNA imaging (Fig. 3). In collaboration with the lab of Sevinc Ercan (NYU) we currently focus on uncovering molecular mechanisms underlying

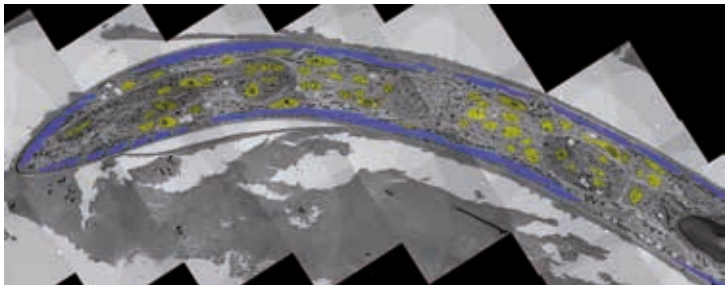

Figure 1: *Acquisition of an entire C. elegans larvae in dauer stage using transmission electron microscopy (acquired at HHMI Janelia). The imaged specimen has a length of around 500um and a diameter of 25um. The dataset consists of 25.000 individual images covering 561 physical sections totaling a size of approximately 1 terabyte. The atlas of cellular location and function resulting from the ongoing reconstruction will serve as basis for the mapping of light microscopy acquisitions.*

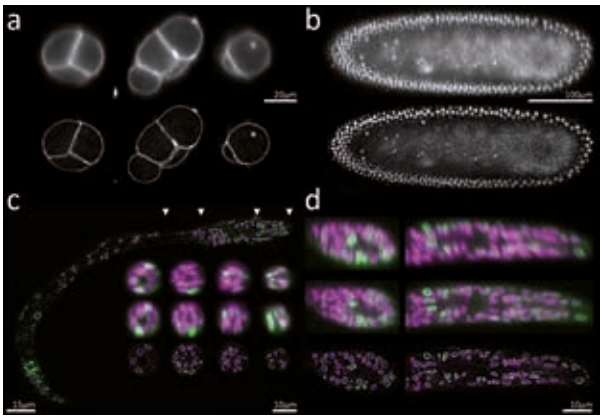

Figure 2: *Multiview (MV) deconvolution of lightsheet microscopy data. (a) C. elegans embryo in 4 cell stage, (b) Drosophila embryo in blastoderm stage. The upper row shows normal fusion, the lower row the result of MV deconvolution. (c,d) MV Deconvolution of a C. elegans in L1 stage, arrows mark the cross-sections shown as insets. The upper two rows shows orthogonally acquired raw data, the lower row the result of MV deconvolution capable of identifying all 558 nuclei unambiguously.*

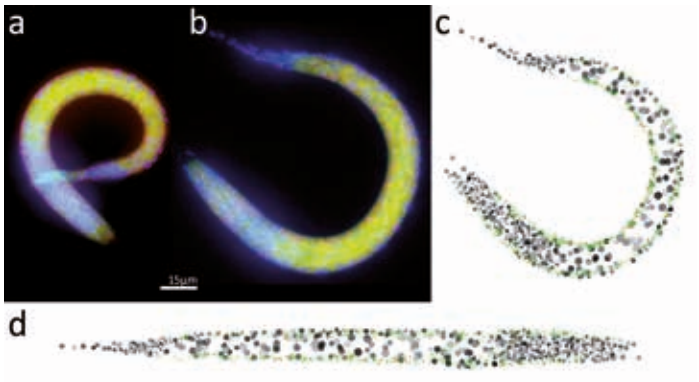

Figure 3: *Single molecule FISH experiments in C. elegans. (a,b) co-staining for two different isoforms of a gene highlighting the spatial expression pattern in different developmental stages. (c) computational representation of individual mRNA isoforms and nuclear locations from (b). (d) straightened C. elegans larva used for computational comparison and statistical pattern analysis.*

transcriptional repression of the C. elegans X-chromosome, which is an important process compensating the presence of a second X-chromosome in hermaphrodites. Following candidate selection based on existing genome-wide datasets, we use single-molecule fluorescent in-situ hybridization (smFISH) to visualize individual mRNAs to measure fine spatio-temporal differences in expression between wildtype and mutant animals at single-cell level. The smFISH experiments performed on fixed specimen are mapped to a four-dimensional model of C. elegans development acquired by lightsheet microscopy using the location of nuclei as reference. By studying specific mutants we are aiming at dissecting the mechanism of transcriptional repression at the molecular level.

Software development

We develop software solutions for image processing based on ImgLib2 and BigDataViewer (publications) that we provide as open-source to the community. Most software is made available as user-friendly plugins in Fiji (Schindelin et al.), capable of utilizing cluster infrastructure and GPU hardware. We develop versatile solutions applicable to many different types of image data and biological questions.

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Carlas Smith*, Stephan Preibisch*, Aviva Joseph, Bernd Rieger, Sjoerd Stallinga, Eugene Myers, Robert H. Singer and David Grunwald (2015), "Nuclear accessibility of β-actin mRNA measured by 3D single-molecule real time (3D-SMRT) microscopy", Journal of Cell Biology 209(4), 609-619.

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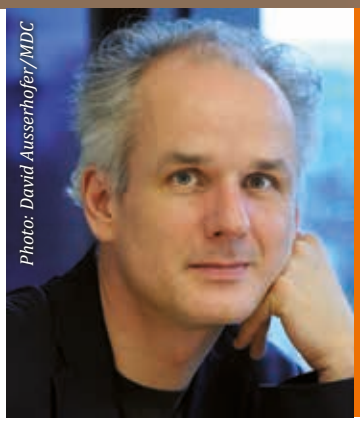

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Nikolaus Rajewsky

Systems Biology of Gene Regulatory Elements

My lab uses experimental (molecular biology, biochemistry) and computational methods (bioinformatics, computational biology) to dissect, systems-wide, function of gene regulation in metazoans. One major focus is to understand more about post transcriptional gene regulation. We investigate general mechanisms and principles of gene regulation by microRNAs and RNA binding proteins in cell lines and in vivo. For example, we are studying function and mechanisms of post-transcriptional gene regulation during early development in C. elegans. We use CrispX-Cas to mutate individual regulatory sites in vivo. Furthermore, we have established planaria as a model system in our lab. Planaria are famous for their almost unlimited ability to regenerate any tissue via pluripotent adult stem cells. We investigate molecular mechanisms of pluripotency and the role of post-transcriptional gene regulation in planarian stem cell biology and regeneration. More recently, we have started to quantify RNAs in single cells and implemented state-of-the-art technology in our lab (microfluidics). We routinely capture RNA of thousands of individual cells in 15 minutes. We develop computational methods to analyze these data and start to apply the entire approach to understand more about gene expression in neuronal systems and tumors, which are both well known to harbor important heterogeneity at the single cell level.

Introduction

A major lesson from recent genomics is that metazoans share to a large degree the same repertoire of protein-encoding genes. It is thought that differences between cells within a species, between species, or between healthy and diseased animals are in many cases due to differences in when, where and how genes are turned on or off. Gene regulatory information is to a large degree hardwired into the non-coding parts of the genome. Our lab focuses on decoding transcriptional regulation (identification and characterization of targets of transcription factors in non-coding DNA) and post-transcriptional control mediated by RNA binding proteins and small, non-coding RNAs, in particular microRNAs. microRNAs are a recently discovered large class of regulatory genes, present in virtually all metazoans. They have been shown to bind to specific cis-regulatory sites in 3' untranslated regions (3' UTRs) of protein-encoding mRNAs and, by unknown mechanisms, to repress protein production of their target mRNAs. Our understanding of the biological function of animal microRNAs is just beginning to emerge, but it is clear that microRNAs are regulating or involved in a large variety of biological processes and human diseases, such as developmental timing, differentiation, signalling, homeostasis of key metabolic gene products such as cholesterol, cardiovascular diseases and cancer. Overall, however, it is clear that miRNAs are only a small part of the entire post transcriptional gene regulation apparatus used by cells, and we are beginning to systematically explore the largely unknown function of RNA binding proteins.

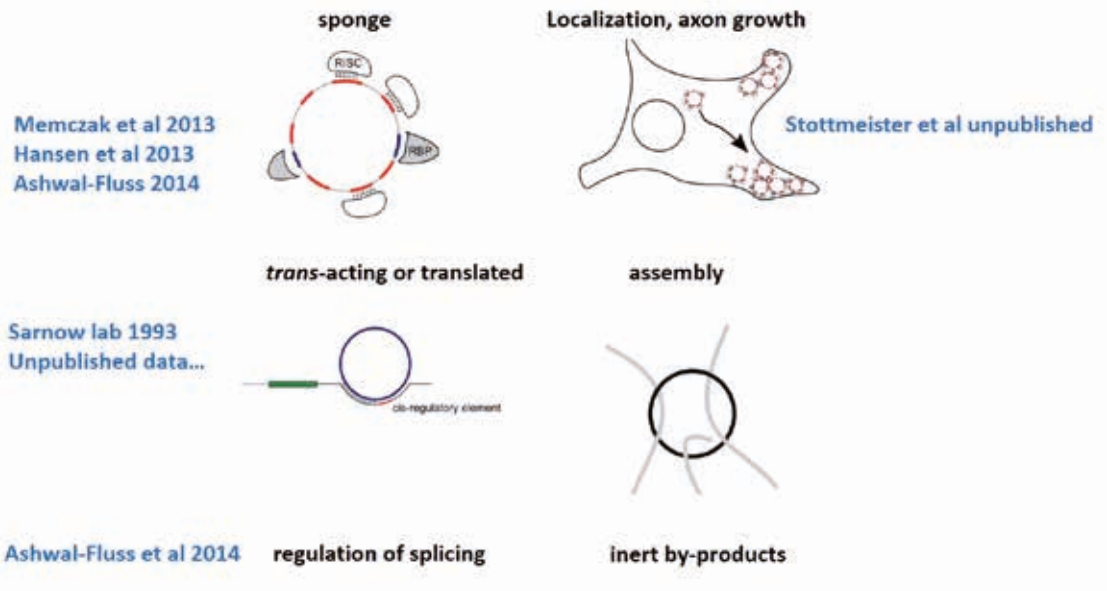

Figure 1: *Possible functions of circRNAs: (a) binding miRNAs or RNA binding proteins (RBPs) to change their local free concentration (b) transport of miRNAs/RBPs inside the cell (c) acting in trans (d) involved in assembly of other molecules (e) involved in regulating splicing (f) no function.*

It is clear that a better understanding of gene regulation and in particular of the just emerging universe of non-coding RNAs can only come by integrating various data sources (comparative sequence analysis, mRNA expression data, protein-protein interactions, mutant phenotypes from RNAi screens, polymorphism data, experimentally defined gene regulatory networks, ChIP-chip data, etc) since each data source alone is only a partial description of how cells function. For example, to understand microRNA function, we not only need to identify their targets but also to decode how microRNAs are transcriptionally regulated. A major focus of the lab is therefore in developing methods that integrate different data sources and methods to produce global and yet specific predictions about how, when, and where genes are regulated. This will ultimately lead to the identification and functional description of gene regulatory networks. We will continue to test, develop and "translate" these methods and their predictions using specific biological systems, such regeneration in planaria and early embryogenesis in C. elegans.

Specifically, we have developed one of the first microRNA target finding algorithms and could later on show that microRNAs very likely regulate (in a functionally important way) thousands of genes within vertebrates, flies, and nematodes (Krek et al., Nature Genetics 2005; Lall et al., Current Biology 2006; Gruen et al., PloS Computational Biology 2006). We have further helped to elucidate the function of microRNAs in pancreatic beta cells (insulin secretion), in liver (cholesterol level), and other systems. We have shown that microRNAs leave cell type specific mRNA expression signatures on hundreds of genes (Sood et al., PNAS 2006), and that human genotyped SNP data can be used to explicitly demonstrate and quantify the contribution of microRNA targets to human fitness (Chen and Rajewsky, Nature Genetics 2007). We have further developed computational methods (miRDeep) to predict miRNAs from high throughput sequencing data (Friedlaender et al., Nature Biotechnology 2008). We have also pioneered approaches that allowed to experimentally assay, genome-wide, the impact of miRNAs on protein synthesis (Selbach et al., Nature 2008). A major ongoing effort is currently to use and develop several key high- throughput technologies for in vivo studies in C. elegans and planaria: high-throughput proteomics (SILAC), RNA sequencing, and new methods that allow the genome-wide identification of binding sites of RNA binding proteins. Very recently published new biochemical and computational methods to obtain context-dependent, in-vivo and precise maps of genome-wide miRNA:target interactions (Grosswendt et al. Molecular Cell 2014).

Circular RNAs

We have very recently shown that single-stranded circular RNAs (circRNAs) are a widespread class of animal RNAs with tis-

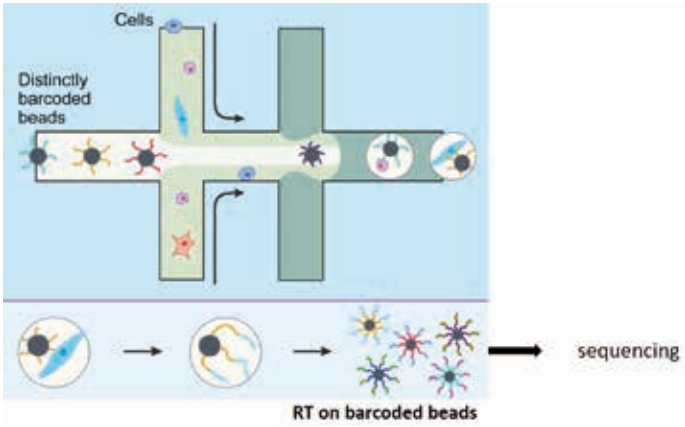

Figure 2: *Single Cell Sequencing via "Drop-Seq". We have implemented a recent advance in single-cell sequencing technology (adapted from Macosko et al. Cell 2015). Each bead carries oligos which can bind the polyA tail of mRNAs/lncRNAs. Single cells enter in suspension, beads in lysis buffer. Each oligo contains a bead-specific barcode. Therefore, the contents of all droplets can be amplified, sequenced as one library, and de-convolved computationally later on. The approach is the fastest and most inexpensive method to date. We capture 4,000 "transcriptomes' in 15 minutes runtime (Alles et al. unpublished).*

sue and developmental stage specific expression (Memczak et al. Nature 2013). circRNAs are usually expressed from exons, are found in the cytoplasm, and are unusually stable. For a particular circRNA (CDR1as) we could show that this circRNA can function as a sponge to sequester miR-7, a neuronal specific miRNA. We are investigating possible functions of circRNAs and mechanisms of circRNA biosynthesis (Ashwal-Fluss et al. Molecular Cell 2014, Ivanov et al. Cell Reports 2015, Rybak-Wolf et al. Molecular Cell 2015). We collaborate with the Kadener lab, Hebrew University, Jerusalem). However, it is also possible that many circRNAs are inert by-products of splicing (Figure 1). Even in this case, because of their unusual stability and differential expression, it will be extremely interesting to study circRNAs as possible biomarkers for processes (Memczak et al PloS One 2015). Generally we have found that hundreds of circRNAs are highly abundant and often specifically expressed in each neural tissue that we have examined (Rybak-Wolf et al. Molecular Cell 2015. We have also developed assays to pull down circRNAs after RNA:protein crosslinking with subsequent mass-spec to identify proteins which are specifically bound to circRNAs (using the non-crosslinked pull down as a control, Stottmeister et al. unpublished). These assays allow the mechanistic understanding of knock-down phenotypes that we observes for circRNAs.

Development in *C. elegans*

Although C. elegans is one of the most famous model systems for developmental biology, it has been impossible to use most high-throughput technologies to study differential gene expression and networks during very early embryogenesis (for example the oocyte to one-cell embryo transition upon fertilization). To overcome this problem, we have developed a novel method ("eFACS") that allows us to sort embryos at precise stages during embryogenesis via FACS sorting (Stoeckius, Maaskola et al., Nature Methods 2009). For example, we can now routinely obtain ~60,000 one-cell stage embryos (at a purity of >98%) in one FACS run, enough to apply virtually any high-throughput method of interest. We have used eFACS to assay the dynamics of small RNA expression during embryogenesis. We discovered a wealth of orchestrated, specific changes between and within virtually all classes of small RNAs. These findings open the door for many computational and functional follow up studies. For example, we have shown that sperm transmits RNA into the zygote, and that hundreds of paternal mRNAs/small RNAs are present in the one-cell embryo (Stoeckius et al., submitted). Very recently we have established CrisR-Cas in C. elegans (Froehlich et al. unpublished) and are currently probing the function and regulatory mechanisms of binding sites in 3' untranslated regions.

Stem cell biology

We used massive next generation sequencing to identify miRNAs and piRNAs in S. mediterranea. We also identified miRNAs that seem specifically linked to stem cell biology. A number of these miRNAs are conserved in humans (Friedlaender & Adamidi et al., PNAS 2009). We have further developed experimental and computational methods that allowed us to assemble the planarian transcriptome via next generation sequencing of mRNAs (Adamidi et al., Genome Research 2011). We have used FACS and subsequent RNA-seq and shotgun proteomics to obtain planarian stem cells and to define which genes are specifically expressed in them. Comparison to mammalian embryonic stem cells revealed that molecular mechanisms for pluripotency are deeply conserved throughout life and that planarian stem cells are indeed informative for mammalian stem cell biology (Oenal et al. EMBO J 2012). Part of this work involved

collaborations with the Sanchez lab (Utah), Chen, Dieterich, and Kempa labs (MDC). Currently we are studying the role of alternative splicing in pluripotency. We have discovered that two alternative splice regulators bind proximal to the same set of exons to antagonistically regulate alternative splicing. One of these factors is expressed in stem cells, the other one in differentiated cells. In this way both factors together control functionally important and cell type specific inclusion or exclusion of exons (Solana et al submitted).

Towards systematic decoding of the "post-transcriptional regulatory code"

We have started to use biochemical methods such as PAR-CLIP (Hafner et al., Cell 2010) to investigate mechanisms and function of post-transcriptional gene regulation. For example, we have studied the human RNA binding protein HuR. We identified >4000 functionally relevant targets of HuR and could show that HuR regulates mRNA processing and alternative splicing (Lebedeva et al., Molecular Cell 2011), previously unknown functions of HuR. Very interestingly, we also found that HuR directly and strongly controls the expression of one conserved human microRNA (miR-7). Ultimately we need to study regulatory relationships not in cell lines but in vivo. In a proof of principle experiment, we have shown that it is possible to biochemically identify targets of RBPs in C. elegans and at nucleotide resolution (Jungkamp et al., Molecular Cell 2011). This new method ("iPAR-CLIP") allows us to systematically identify the targets and to study the function of any RBP in C. elegans One immediate goal was to identify the targets of DICER and other central components of small RNA pathways in vivo (Rybak-Wolf et al Cell 2014). Together with a DICER PAR-CLIP in human cell lines performed by the Landthaler lab, we discovered that DICER has not only "active" target sites which are cleaved. In fact, many mRNAs and lncRNAs harbor small hairpin structures which are specifically bound by DICER but not cleaved ("passive" sites). We could show that passive sites are biologically active and that they are enriched in mRNAs which encode proteins which interact with DICER, for example proteins found in P-bodies (or granules). These molecular networks are conserved from C. elegans to man. We also found that a lncRNA is highly covered with passive DICER binding sites and indeed in vivo bound passively by DICER- expression of this transcripts therefore inhibits DICER free concentration. We have during the past years developed simple quantitative models which can be used to study systematically and quantitatively such competition effects- expression of a RNA with binding sites can lead, under certain stoichiometric conditions, to reduced availability and therefore binding of the regulator to other binding sites in the transcriptome (Jens and Rajewsky Nature Reviews Genetics 2015).

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Rybak-Wolf A, Jens M, Murakawa Y, Herzog M, Landthaler M (co-corresponding), Rajewsky N* (2014). A variety of Dicer substrates in Human and C. elegans. Cell 1153-67.

Ivanov A, Memczak S, Wyler E, Torti F, Porath HT, Orejuela MR, Piechotta M, Levanon EY, Landthaler M, Dieterich C, Rajewsky N (2015). Analysis of intron sequences reveals hallmarks of circRNAs biogenesis in animals. Cell Reports 170-7.

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Jens M and Rajewsky N (2015). Competition between target sites or regulators shapes post-transcriptional gene regulation. Nature Reviews Genetics 113-26 (Analysis Paper).

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Evolutionary and Cancer Genomics

Our group was founded in October 2016 and investigates the aetiology and functional implications of intra-tumour heterogeneity using computational approaches, with a focus on chromosomal rearrangements, somatic copy-number alterations and changes in chromatin structure. To this end, we develop and apply machine learning and statistical methods to reconstruct the evolutionary history of cancer in the patient and associate germline and somatic variation with changes in gene regulation. Our group currently consists of two PhD students and one visiting PhD student from London. We collaborate with a wide range of experimental labs within and outside the MDC to tackle the most pressing questions in cancer genomics.

Previous work – reconstructing the evolutionary history of cancer

Cancer is characterised by ubiquitous somatic alterations, such as single nucleotide variants (SNVs), somatic copy-number alterations (SCNAs) and genomic rearrangements, that shape the genetic landscape of cancer and contribute to intra-tumour heterogeneity (ITH) and genome plasticity. To understand the aetiology of ITH in cancers with substantial SCNAs, we developed MEDICC (Minimum Event Distance for Intra-tumour Copy-number Comparisons), an algorithm for the reconstruction of the evolutionary history of cancer in the patient from allele-specific copy-number profiles (Schwarz et al. 2014). The algorithm is based on finite-state transducers (FSTs), an algorithmic framework heavily used in natural language processing, that allows to define edit operations on sequences and to construct all possible paths of converting one such sequence into another. In MEDICC, FSTs define biologically meaningful events of amplification and deletion of arbitrary length on the genome, which are then used to compute the minimum number of such events to transform one genome into another as an estimate of evolutionary divergence.

We applied this method to a new cohort of patients under treatment for high-grade serous ovarian cancer (HGSOC). We demonstrated ongoing somatic copy-number evolution in HGSOC, identified potential mutator phenotypes and demonstrated associations between different degrees of ITH and patient progression and survival (Schwarz et al. 2015).

Establishing between-genome phasing for copy-number variation

The accuracy of tree inference from genomic rearrangements depends on the quality of the phasing of copy-numbers: the assignment of major and minor copy-numbers to the two physical parental alleles. So far phasing has been done using evolutionary criteria alone, a heuristic and computationally expensive procedure which impedes probe-level resolution tree reconstruction (Schwarz et al. 2014).

We are currently developing and applying a novel phasing algorithm for SCNAs from multi-region tumour profiles (Figure 1). Using the shared genetic background of multiple samples from the same patient we assign copy-numbers to physical alleles based on the bi-allelic frequency (BAF) distribution of heterozygous single-nucleotide polymorphisms (SNPs). In combination with our previously established evolutionary phasing algorithm this provides a new, accurate and fast phasing method which leverages the available SNP data effectively. This is a

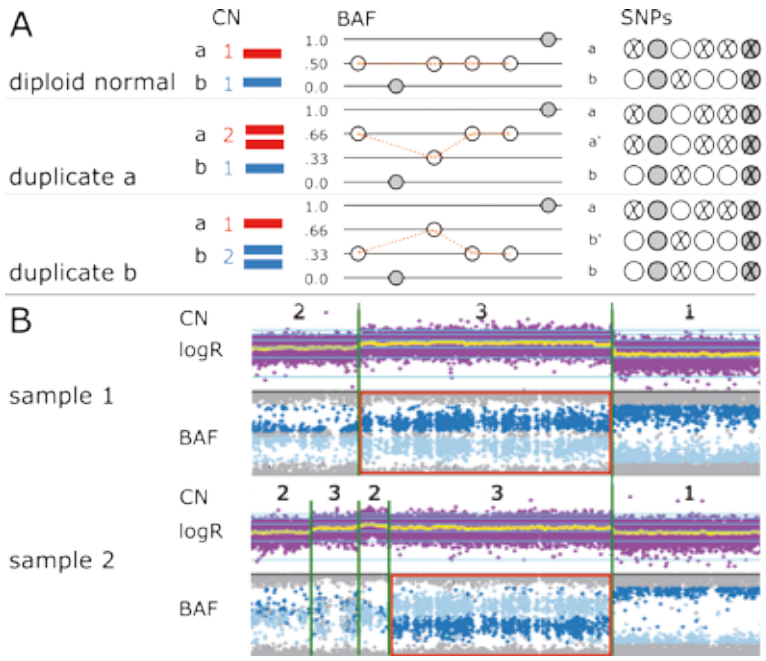

Figure 1 – Reference phasing for allele-specific copy-number profiles
A: Schematic of the reference phasing: Using a common genetic background, amplifications on the two parental alleles can be distinguished based on the BAF patterns. Only heterozygous probes (white) are informative. **B:** Example of an inverted BAF pattern in HGSOC: Within a segmental duplication of CN 3 (red square), sample 2 shows an inverted BAF pattern compared to the reference, indicating the amplification happened on the opposite allele. Breakpoints are indicated as green vertical lines, average total DNA content (logR) is shown in yellow.

crucial step towards probe-level resolution tree inference on genomic rearrangement events in cancer and exact quantification of genetic heterogeneity for routine applications in translational cancer research.

Assessing the effect of somatic variation on the cancer regulatory landscape using deep learning

With the tools in hand to accurately reconstruct tumour evolution, the question remains which somatic events are functional, i.e. contribute to cancer fitness and progression, and which do not. As part of the TCGA/ICGC Pan-Cancer Analysis of Whole Genomes (PCAWG) working group, we are currently investigating the effect of different types of somatic variation on the regulatory landscape across different cancer types. Using 1200 whole-genome sequenced cases with tumour and matched normal samples and associated RNAseq profiles, we map somatic and germline variants to allele-specific expression (ASE) readouts and quantify the effects of different mutation types.

To be able to assess the directionality of the change in allele-specific expression, phasing of both germline as well as somatic variants is essential. In segments of chromosomal loss or gain, the phase of germline variants is directly evident from the BAF, where within a SCNA segment SNPs on the same haplotype co-cluster in the corresponding BAF bands. We combine this phasing with statistical phasing on regions lacking SCNAs to achieve near-to-complete long-range phasing for germline and SCNAs. This allows for the accurate aggregation of allelic expression to quantify gene-level ASE. We then phase individual somatic variants to the nearest germline heterozygous site, in instances were both co-occur on the same NGS read. Our preliminary results demonstrate a strong effect of SCNAs on ASE, accounting for about 80% of total ASE variability, with germline and somatic points mutations both contributing roughly equally to the remaining variability.

It is so far largely unclear to which extent non-coding SNVs contribute to cancer gene regulation through disruption of transcription factor binding sites or modification of chromatin states. To estimate such functional effects of individual SNVs de-novo, we train a convolutional neural network, to predict regulatory effects on 918 epigenetic features, including transcription factor binding sites, DNase I sensitivity and histone modifications. We use this model to predict the regulatory effect of ~10M phased SNVs from PCAWG, using randomised null variants to identify SNVs that were significant positively and negatively associated with specific regulatory marks.

Selected Publications

Spatial and temporal heterogeneity in high-grade serous ovarian cancer: a phylogenetic analysis.
Schwarz RF, Ng CK, Cooke SL, Newman S, Temple J, Piskorz AM, Gale D, Sayal K, Murtaza M, Baldwin PJ, Rosenfeld N, Earl HM, Sala E, Jimenez-Linan M, Parkinson CA, Markowetz F, Brenton JD. – PLoS Med. 2015 Feb 24;12(2)

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Cancer evolution: mathematical models and computational inference.
Beerenwinkel N, Schwarz RF, Gerstung M, Markowetz F. – Syst Biol. 2015 Jan;64(1)

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Photo: David Ausserhofer/MDC

Baris Tursun

Gene regulation and cell fate decision in *C. elegans*

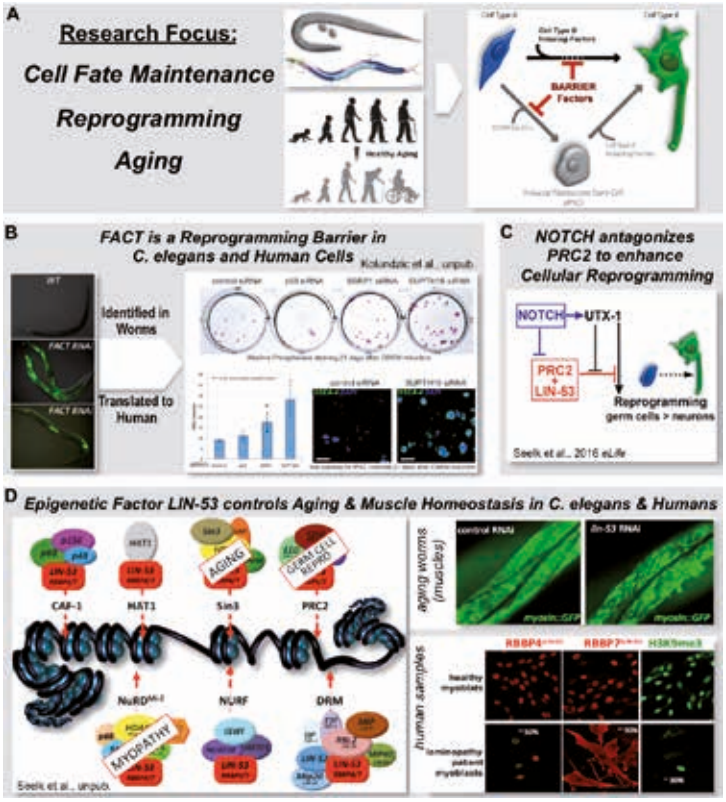
Understanding mechanisms which safeguard cellular identities is key to improving the reprogramming of differentiated cells. We use Caenorhabditis elegans as a genetic model organism to systematically study the molecular basis of cell fate maintenance and protection. C. elegans provides genetic tractability and visualization of cell conversions in vivo. We utilized large-scale genetic screens to identify factors safeguarding cell fates and acting as a barrier for direct conversion of cells to a different identity (Transdifferentiation / Direct Reprogramming). Additionally, we are studying the interconnection of Aging and Cellular Reprogramming (Figure A). Our findings could help overcome the limitations of Direct Reprogramming in order to generate the cellular material for prospective tissue replacement therapies.

Genetic screens to identify barriers for direct reprogramming

A straightforward strategy to identify factors counteracting direct reprogramming (barrier factors) is to perform genetic screens, which can easily be applied to C. elegans. Using mutagenesis and RNAi screens we systematically interrogate the entire genome of animals for 'barrier factors' in vivo. We use transgenic worms that allow mis-expression of cell fate-inducing master transcription factors combined with fluorescent fate reporters, to monitor cell conversions in vivo. Our mutagenesis and whole-genome RNAi screens (knock-down of 20K individual genes) identified around 160 novel factors, whose depletion allows the ectopic induction of neuronal fates in non-neuronal tissues such as germ, epidermal, and intestinal cells.

The FACT complex safeguards cell fates and counteracts reprogramming

We identified subunits of the histone chaperone complex FACT and the associated histone chaperone SPT6 to act as barriers for induced cellular reprogramming. Further characterization of these factors revealed that reprogramming upon loss of FACT or SPT6 depends on the activity of the Aurora-Kinase AIR-2. AIR-2 is required for the phosphorylation of Histone H3, thereby triggering activation of silenced chromatin domains. Our findings suggest that the FACT complex protects repressed chromatin to prevent activation of gene expression, triggered by AIR-2. This new type of gene regulation links FACT and the Aurora-Kinase B to safeguarding of cellular identities and, importantly, may be conserved because these factors have more than 90% amino acid homology with human counterparts. Moreover, in collaboration with S. Diecke (Stem Cell Facility) we showed that FACT acts as a barrier for iPSC reprogramming also in human fibroblasts (Fig. B)." Notably, we previously identified that the histone chaperone LIN-53 protects germ cells against reprogramming (Tursun et al., 2011 Science). In an analogous mouse study, the CAF-1 complex, which consists of the LIN-53 homolog CAF-1p48/RBBP4/7, was recently identified by another group as a barrier for iPSC reprogramming (Cheloufi et al., Nature 2015). The marked conservation of function for the same factor, from worm to mouse, gives us confidence that more C. elegans findings, with regard to cellular reprogramming, can be translated to higher organisms and, importantly, human tissues.



The histone chaperone LIN-53 is a barrier for cellular reprogramming and regulates Aging and muscle maintenance

As mentioned above, the histone chaperone LIN-53 (CAF-1p48/RBBP4/7) acts as a barrier for reprogramming in worms and mammals. Furthermore, our ongoing characterization of LIN-53 in the context of cellular reprogramming revealed that Notch signaling promotes reprogramming by counteracting the chromatin-regulating Polycomb Repressive Complex 2 (PRC2), with which LIN-53 associates in order to safeguard germ cell identity (Figure C; Seelk et al., in revision). Yet in different tissues, LIN-53 appears to play distinct roles from counteracting reprogramming. Further characterization of lin-53 mutants revealed that LIN-53 is crucial for muscle maintenance and normal aging. Mutants show strong motility defects in addition to a shortened lifespan. Genetic analysis of the muscle and aging phenotypes revealed that loss of LIN-53 connects myopathy and premature aging by affecting the chromatin remodeling complex NuRD and the histone deacetylase complex SIN3. Intriguingly, the muscle and aging phenotypes can be uncoupled by specifically depleting NuRD or SIN3, respectively. Moreover, by examining human samples of laminopathy patients (collaboration with Simone Spuler's group), we discovered that human LIN-53 proteins are diminished or mis-localized to the cytoplasm in primary human myoblasts with Lamin mutations (Figure D). Many laminopathy patients suffer from myopathy and some show symptoms of premature aging that parallel the phenotypes of worms lacking LIN-53. Hence, it is conceivable that affecting LIN-53 levels and functionality causes the symptoms of laminopathy patients. Currently, we are collecting more evidence that LIN-53 plays a role in muscle homeostasis and aging regulation in both worms and humans.Our aim is to better understand the mechanisms which safeguard cell fates in differentiated cells and in the context of Aging. Our investigations may lead to the identification of conserved mechanisms due to the fact that the C. elegans genome is ~60% homologous to the human genome.

Selected Publications

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Tursun B#, Patel T, Kratsios P, Hobert O#. Direct conversion of C. elegans germ cells into specific neuron types. Science 2011; 331: 304-308

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Photo: David Ausserhofer/MDC

Robert P. Zinzen

Systems biology of neural tissue differentiation

How do cells acquire and maintain their identity? This question remains a central issue in developmental and stem cell biology. Our lab aims to understand the molecular mechanisms undifferentiated embryonic cells employ to organize into distinct cell types and tissues. With the advent of modern '-omics' approaches, it is becoming possible to investigate embryonic development on genome wide scales. No longer limited to asking how individual genes are regulated, we can now ask how the genome is regulated to coordinate development. Furthermore, new methodologies allow increased resolution of these approaches – from tissue specificity to even individual cells.

Because of its genetic tractability and experimental accessibility, we approach this question in embryonic development of the fruit fly *Drosophila melanogaster*. Using nervous system (NS) development as a model, we focus on how cell populations subdivide and give rise to specific neuroblasts (NBs) and NS components. Our aim is to understand the molecular mechanisms underlying NS specification, the regulated delamination of NBs, and their differentiation.

Transcription factor & chromatin landscapes

A. Guimaraes, A. Glahs

Coordinated specification and differentiation in development is driven largely via spatiotemporal regulation of gene expression. Whether a cell expresses a particular gene is directed by how that gene's cis-regulatory elements (CRMs) interpret the cellular environment, e.g. which transcription factors (TFs) are recruited to CRMs. We are utilizing chromatin immunoprecipitation (ChIP) followed by next generation sequencing (SEQ) to identify the genome-wide binding profiles of key neurogenic TFs. We aim to identify target genes and the CRMs through which TFs act. This is crucial for understanding the regulatory network that shape the NS.

A more encompassing view of genome regulation can be gained by investigating chromatin state dynamics. Specific histone modifications are not only predictive of enhancers, promoters, etc., but also of their regulatory state. However, since chromatin features are employed differentially throughout the embryo, identification of the tissue specific signatures requires isolation of the cell populations of interest. BiTS (Batch isolation of Tissue Specific chromatin) allows the isolation of defined cell populations by FACS, which are then used for ChIP-SEQ. We are uncovering the chromatin signatures in the NS and its components with exquisite resolution in space and time. The functional integration of TF binding and chromatin state data remains an important goal to assess how TFs shape their chromatin environment and how the chromatin environment regulates the interaction of TFs with the genome.

Transcriptomes resolved in space & time

P. Wahle, A. McCorkindale

A caveat of ChIP-SEQ studies is that binding data cannot definitively identify target genes or predict transcriptional responses. To assess the effect individual TFs have on global transcription in vivo, we have established genotypes to isolate homozygous mutant embryos and comprehensively assess the effect of key neurogenic TFs on developmental transcriptomes and lineage decisions in the NS. By combining cell type specific transcriptomic and TF binding, we aim to identify the regulatory effects of neurogenic TFs. A

regulatory level that remains understudied are noncoding RNAs (ncRNAs). We are systematically assessing both the spatial and the temporal expression dynamics of various types of ncRNAs (i.e. micro-, circular-, and long ncRNAs) in order to investigate their functional roles in neurogenesis.

Toward single cell resolution

Philipp Wahle, with the Rajewsky lab, esp. Nikos Karaiskos

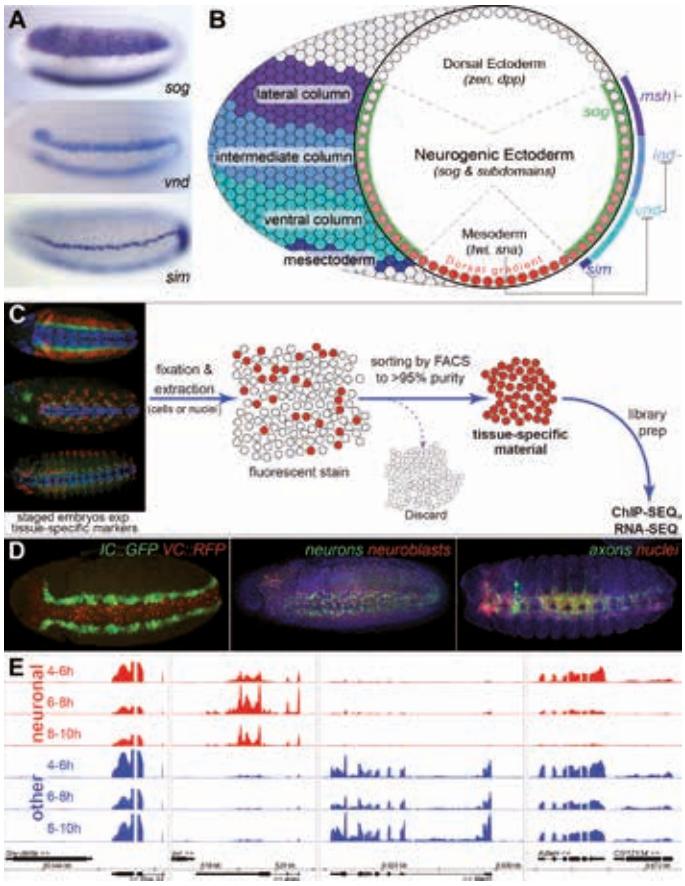
New methodologies make it possible to extract transcriptome information from single cells. In a pilot project with Nikolaus Rajewsky's lab, we have deconstructed the early 6000-cell embryo, sequenced its individual cells, and mapped them back to their original embryonic positions. What emerges is a virtual embryo – a single-cell transcriptome-resolved blueprint of development: individual cells and positions can be queried for their global gene expression programs (www.dvex.org). This yields unprecedented insight into the combinatorial gene expression programs that drive development, cellular heterogeneity and even reveals evolutionary gene expression divergence. Applying this proof-of-principle to nervous system development promises new insights into the regulation of neurogenic fate decisions.

Network extension

S. Krüger, A. Guimaraes

A central aim of the lab is to gain a mechanistic understanding of how individual CRMs translate their cellular environment into regulatory (in)activity; however, it is clear that our provisional networks of NS development are still very incomplete. To extend the NS networks, we have created flies that allow for tissue-specific biochemical isolation of individual CRMs together with the proteins that bind to them in vivo. Using mass spectrometry to identify the bound proteins, we are particularly interested in how CRM activity is affected by both, the temporal dynamics of CRM-protein interactions across development, as well as in the tissue specificity of CRM-protein complexes. With this method, we are not only approaching a mechanistic understanding of individual CRMs, but once identified, we are able to probe novel interactors for their global roles.

Overall, the lab's aim is to utilize a systems biology approach to uncover the interdependencies among connected regulatory levels (e.g. TF binding dynamics, chromatin landscape, transcriptional output, and the regulatory networks involved) to establish a predictive model of NS development: we want to understand the regulatory interactions driving tissue development in the developing embryo to a degree that allows modeling the global effects of systematic perturbations.



Dissection of the Drosophila NS. *A) Early signs of NS subdivision in the cellularizing embryo. B) Schematic representation. C) BiTS workflow. D) Embryos stained for tissues and cell types to be sorted as indicated at (L->R) st.9, st.11, and st.13. E) Sample tracks of developmental transcriptomic data from neuronal (red) and non-neuronal cells (blue) for (L->R) a ubiquitous, neuronal, non-neuronal, and a gene showing tissue-specific temporal regulation.*

Selected Publications

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Zinzen R.P.; Bonn S.; Girardot C.; Gustafson E.H.; Perez-Gonzalez A.; Delhomme N.; Ghavi-Helm Y.; Wilczynski B.; Riddell A.; Furlong E.E. Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. Nature Genetics 44 (2): 148-156 (2012-02)

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Zinzen R.P.; Senger K.; Levine M.; Papatsenko D. Computational models for neurogenic gene expression in the Drosophila embryo. Current Biology 16 (13): 1358- 1365 (2006-07-11)

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The Experimental and Clinical Research Center (ECRC)

Friedrich C. Luft

The Experimental and Clinical Research Center (ECRC)

Friedrich C. Luft

The ECRC is a joint project of the MDC and the Charité Medical Faculty. The mission of the ECRC is to pursue basic science directly relevant to clinical medicine. The mission statement could be: *"We bring together the courage and imagination of basic researchers and clinician/scientists"*. The idea of bringing novel ideas to patient care more quickly is termed "translational" research. The concept is not new. For instance, insulin was discovered in 1921 and given to a diabetic patient in 1922. The Nobel Prize was awarded for this work in 1923. The ECRC is there to keep that goal alive, but we are not naïve. The 2005 international review panel founding the ECRC recognized that the ECRC needed dedicated well-trained clinicians heading research groups. These clinicians deserved a state-of-the-art infrastructure. Equally important, the clinicians required world-class basic scientists, who are committed to working on clinical problems with clinicians that are generally less knowledgeable in the science. These marriages are seldom made in heaven. Who remembers Bertram Collip, the biochemist who purified insulin so that the compound could actually be given to a human? Without him, no treatment, no prize, and more importantly no cure.

The ECRC was designed as an interface between MDC scientists and Charité clinician/scientists. We are located in a previous hospital building named after Robert Rössle, a Charité pathologist in the Virchow line of succession. ECRC groups acquire laboratory space and infrastructure through competition. The space is provided for five years and the contract can be renewed, again on a competitive basis. Scientific productivity is measured in publications and grant support. Not all, but most ECRC groups are led by clinicians who also have patient-care responsibility at one of the Charité campuses and/or in our outpatient facility. Two of our group leaders are clinicians at the HELIOS-Klinikum-Berlin, a large private hospital adjacent to the campus.

Recruitment, mentoring, and interactions are our main tools at the ECRC. To entice clinicians into a scientific career, we initiated a research fellowship for clinicians at the MDC years ago. This fellowship for two years with extension enables clinicians to accrue scientific expertise and possibly an additional degree. The program has been very popular and was thereafter adapted institution-wide by the Charité. Subsequent career development is a problem; however, two possibilities are attachment to an existing ECRC group or establishing a new independent group. Another challenge is bringing MDC scientists and clinician/scientists together. We have developed a partnership granting system to that end to foster such collaborations.

The ECRC has a facility that exists nowhere else in Berlin, although that state-of-affairs may change in the future. We have a designated clinical research unit (CRU) to study humans, outside of the health-care system and solely for the purposes of science. This unit is operative since 2000 and has been extremely productive in fostering investigator-initiated mechanistic research. Much very valuable clinical research can be performed in a patient-care setting. Most clinical research viewed by the public fits this venue. For instance, comparing tablet A with tablet B, or new cancer treatment X compared to old cancer treatment Y, or coated stent Q versus bare-metal stent R needs no special unit because extra resources to test these questions are minimal. More difficult are studies involving "new ideas". Just suppose we develop a new mesenchymal stem-cell-derived therapy for fragile-X syndrome. The commercial application is not obvious, the condition is not that widespread, and the health-care system is not enamored with the idea of supporting this

Fig. 1. Philipp Du Bois and Jens Fielitz explain their project to Angela Merkel, while Friedrich Luft looks on.

endeavor. That is where the CRU steps in.
The uniqueness of the ECRC is the mission. We are at the source of medically related basic science. The MDC is the best broad-based institute that Germany has to offer. Ideas outside the MDC could be channeled via that source. Our medical school has the entire city of Berlin at its disposal. Thus, patient recruitment (from 4 million inhabitants) is possible as is nation-wide recruitment. We view "translational research" as our job. We admit that we cannot guarantee beating the diabetes team of Banting, Best, McLeod, and Collip. But this goal is a worthwhile aim.

Research on human subjects in the Clinical Research Unit (CRU)

Patient/proband-oriented research is the final step in bringing medical innovations to humans or in revealing physiological or pathological mechanisms in man. This bench-to-bedside and bedside-to-bench process is today conducted under the trendy catchword "translational" research. This idea was not lost on the reviewers of the Berlin-Institute of Health reviewers of 2012. These reviewers specifically recommended more financial outlays for patient-oriented research in the form of CRUs; our design was the prototype. Our CRU is modeled after the US general clinical research centers and it has functioned well since 2000. We have succeeded in making the CRU independent of any patient-care endeavors that concern third-party payers (cross financing). We have recruited physicians, nurses, scientists, technical experts, and students.
Our CRU is located in an earlier hospital building and relies on excellent facilities included the former new bone-marrow transplant unit. We are well equipped for cardiovascular and metabolic studies with resources not available elsewhere, including a normobaric hypoxia chamber, a

total-body metabolic chamber, and a large gamut of techniques including microdialysis, spiro-ergometry, muscle-nerve recordings, lower-body negative pressure, insulin and glucose-clamp studies, and indirect calorimetry. We also conduct detailed body composition measurements. We adapt our capabilities to the investigator's needs. Any clinical investigator at the Charité is eligible to use our facility and its infrastructure after applying to and receiving approval by our Institutional Advisory Committee. We can now add biobanking and data-management strategies that have been added to the program.

We have not focused on inpatient studies but could do so if required. As a result, for pharmacological investigations we currently are not doing phase 1 studies but conduct phase 2 and phase 3 clinical trials, although our focus has been primarily on physician-investigator initiated mechanistic research. We have cooperated actively with the MDC magnetic resonance imaging center, particularly with Jeanette Schulz-Menger. Our mission is also directed at supporting our outpatient clinics covering various disciplines. In the outpatient clinics, we have excellent ultrasound capabilities including state-of-the-art echocardiography and a laboratory aimed at biobanking samples appropriately. We have added a genetic screening and mutation-detection capability that meets German patient-care standards and have a physician with the required additional board certification for such investigations.

Human Data Management

For data management of all human data, we use REDCap, a clinical data management system (also called electronic case report form) developed at Vanderbilt University in 2004. The REDCap Consortium is composed of 873 active institutional partners in 71 countries and is currently used in more than 89,000 projects with over 116,000 users. Thus, REDCap is one of the most frequently used clinical data management systems worldwide. Our group has joined the REDCap consortium. The entire program can be inspected at: www.project-

Fig. 2. Angela Merkel PhD, a physicist, tackles the atomic force microscope at the ECRC.

Photo: David Ausserhofer/MDC

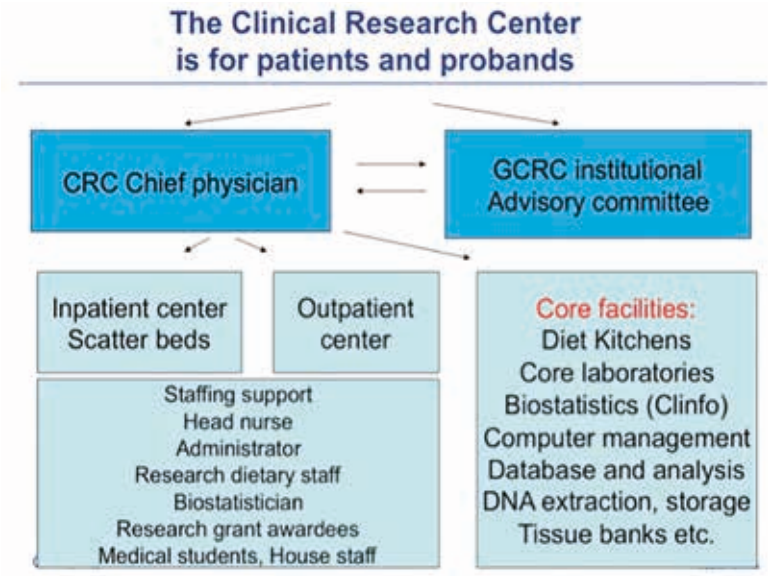

Fig. 3. Our CRU organizational concept is adapted from the general clinical research center idea formulated by NIH. We supply infrastructure and various services for any investigator approved by the Institutional Advisory Committee.

redcap.org. We now manage all our outpatient contacts with an electronic medical records system that we installed in 2014. The Regenstrief Institute is an internationally respected informatics and healthcare research organization, recognized for its role in improving quality of care, increasing efficiency of healthcare delivery, preventing medical errors and enhancing patient safety. The Regenstrief Institute, Inc., a renowned informatics and healthcare research organization, is dedicated to the improvement of health through research that enhances the quality and cost-effectiveness of health care, by conducting health-services research (HSR). HSR works to improve healthcare delivery and health outcomes of patients through research on patient safety, quality of care, patient-provider communication strategies, applied health information technologies, system redesign, and medical education research. This healthcare information technology can be inspected at: *www.regenstrief.org*.

Education

ECRC members have not only access to all MDC facilities but also are welcome at all MDC seminars that happen daily. In addition to internal laboratory meetings, there is a monthly ECRC seminar to introduce members to what other researchers in the building are doing. Finally, we have a weekly *Clinical Journal Club*. At this function, Friedrich C. Luft presents *every* paper published in *N Engl J Med and Lancet* from the following week. The material is announced in a brief summary ahead of time over email portals of the MDC and ECRC. The presentation can be accessed as well. The written material is in English, while the presentation (medical student and house-staff level) is held in German. Outside visitors are welcome and the material is available weekly via Webex or YouTube (load the file down weekly at *http://www.charite-buch.de/*).

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Outpatient clinics

Skeletal muscle disturbances (congenital and acquired)
Psychiatry (Memory)
Psychiatry (Cognitive assessments)
Medical Genetics (Multidisciplinary)
Neurology (Skeletal muscle disorders)
Neurology (Inflammatory CNS disorders)
Cardiology (Cardiomyopathy)
Nephrology (Diabetes registry)
Metabolism (Diabetes and lipids)
Pediatric allergy (Atopic dermatitis)


Photo: David Ausserhofer / MDC

Dominik N. Müller


Photo: David Ausserhofer / MDC

Ralf Dechend

Hypertension-induced Target-Organ Damage

The group's major research interests are the renin-angiotensin system[2-4] the immune system[1-5] and how both systems cause hypertension-induced target-organ damage. Recent work has extended the concept analyzing how environmental factors such as a high salt affect the microbiome, immune cells, target organs, and autoimmunity. In a translational approach, the Dechend/Müller laboratory focuses primarily on the placenta, heart, and kidneys. The group also cooperates closely with MDC scientists and Charité clinician scientists and has also been a resource for young clinicians and doctoral students beginning their careers in experimental and clinical cardiovascular research.

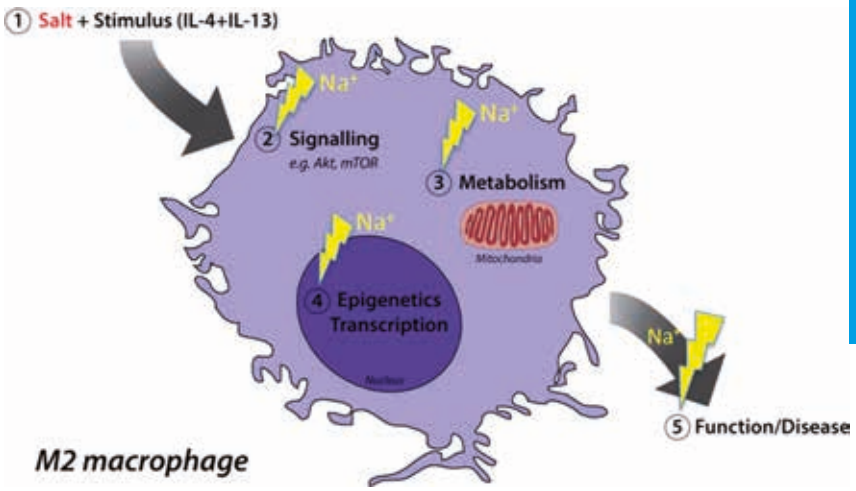
The immune system, salt, and hypertension-induced target organ damage

Hypertension induces target-organ damage; however, the mechanisms are unclear. In general, the immune system is traditionally considered to be a complex biological system that wards off disease, typically by fighting the invasion of foreign microorganisms, such as bacteria and viruses. We and others found that the immune system plays a pivotal role in the pathogenesis of Angiotensin (Ang) II-induce target-organ damage.

Together with Jens Titze (Vanderbilt University), we address the regulatory interaction between immune cells, lymph vessels, and interstitial matrix components for maintenance of internal environmental composition, blood-pressure regulation, cardiovascular target-organ damage, and immunity. We provided evidence that sodium, a known risk factor for cardiovascular disease, interacts with the immune system.[1,5] Increased NaCl concentrations induced an increase in the cytokine-driven TH17 activation in naïve T cells and alter M1 and M2 macrophage action.[1,5] The outcome of this increased TH17 activation resulted in an accelerated and more severe autoimmune disease (multiple sclerosis) in mice on a high-salt diet.[5]

Macrophages are an important innate immune cell type, which fulfills a plethora of homeostatic functions beyond host defense. Functional diversity is reflected by a continuous spectrum of different activation states and M(LPS) also referred to as "M1" and M(IL-4+IL-13) also referred to as "M2" may be viewed as the extreme pro- and anti-inflammatory poles of macrophage activation, respectively. Similar to T cells, physiologically increased environmental sodium concentrations affected pro- and anti-inflammatory activation of macrophages differentially. Pro-inflammatory activation of macrophages with LPS was significantly boosted in the presence of high NaCl concentrations and enhanced inflammatory mediator and effector molecule expression.

In contrast, for murine M2 macrophages activated with IL-4+IL-13 recently found that activation was blunted *in vitro* and *in vivo*.[1] For instance, M(IL-4+IL-13) cells activated in the presence of additional NaCl had a significantly reduced ability to suppress $CD4^+$ T cell proliferation *in vitro* and a high-salt diet delayed cutaneous wound healing. Salt effect on M(IL-4+IL-13) activation was independent of tonicity.[1] Instead, we found evidence that salt affected M2 macrophages *via* an Akt-mTOR metabolic signaling pathway and changes in cellular metabolism (Figure 1). It is now acknowledged that adaptions of cellular metabolic programs are crucial for proper immune cell activation. Metabolic "rewiring" is not simply adopted to meet the energetic needs of blasting lymphocytes; instead, it also controls effector functions and fate decisions of adaptive immune cells. One future research goal of our laboratory is to elucidate the role of salt on immuneometabolism and immune cell function.



Putative mechanisms how sodium affects IL-4 and IL-13-induced M2 macrophage activation.

Pathogenesis of preeclampsia

Preeclampsia is a leading cause of maternal death, and perinatal morbidity and mortality resulting from premature delivery and intrauterine growth restriction. New onset of hypertension and proteinuria arises secondary to maternal endothelial and inflammatory and immunological dysfunction. We induced Treg in our established transgenic rat model for preeclampsia by application of superagonistic monoclonal antibody for CD28. Induction of Treg had no influence on maternal hypertension and proteinuria, but it substantially improved fetal outcome by ameliorating intra uterine growth restriction.

By whole genome expression analysis of placental tissue using microarrays, we found CD74 (Cluster of differentiation 74) in placental macrophages to be one of the most down-regulated genes in placentas from preeclamptic women. Naïve and activated macrophages lacking CD74 showed a shift towards a pro-inflammatory signature and reduced macrophage adhesion to trophoblasts. CD74-knockout mice showed disturbed placental morphology, reduced junctional zone and smaller placentas with fetal growth restriction. These data show that altered macrophage-trophoblast interaction is involved in preeclampsia.

We found altered genomic imprinting in gene expression in preeclampsia. Statistical analyses of dependence between imprinting to probe distance showed that the difference was driven by altered expression of the imprinted transcription factor Distal-less homeobox 5 (DLX5). We identified loss of imprinting as one underlying mechanism for its dysregulation. Overexpression of DLX-5 into trophoblasts reduced proliferation and increased metabolic activity. Transcriptome analysis of trophoblasts with DLX5 overexpression showed several dysregulated pathways, which suggests that DLX5 is involved in stress responses. DLX5 transcriptome strongly clusters with that of preeclamptic placentas, and are separated from the trophoblast wild-type cells suggesting that DLX5 can model the differential gene expression seen in preeclampsia and might be a potent transcription factor involved in pathogenesis of the disease. We perform these experiments in close collaboration with Prof. Zsuzsanna Izsvak (MDC) and Prof. Ann-Cathrine Staff from Oslo.

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Patents

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2. Patent application, 2015. DE 102015115192.8 'Short-chain fatty acids for the treatment of cardiovascular disease' Inventors: Muller DN, Wilck N, Dechend R, Marko L, Bartholomaeus H, Balogh A.

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Photo: David Ausserhofer/MDC

Maik Gollasch

Blood Vessel Function and Target-Organ Damage / Registry Diabetic Nephropathy

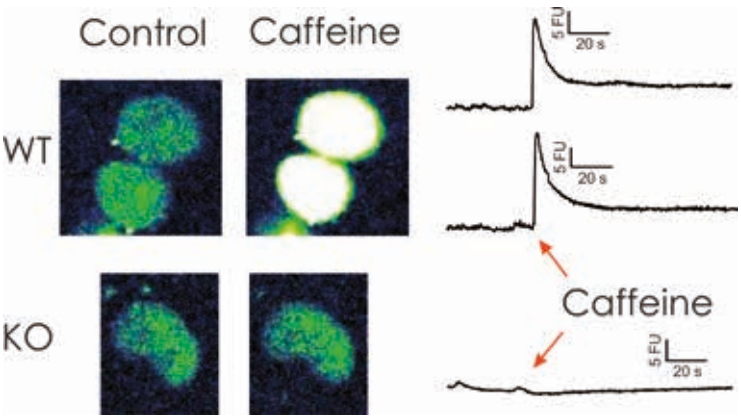
Our group focuses on ion channels, primarily in vascular smooth muscle cells (VSMC), to clarify mechanisms contributing to hypertension and cardiovascular disease. Potassium channels, chloride channels and transient receptor potential (TRP) channels have received special attention. In collaboration with Björn Schroeder, we investigated the role of smooth muscle TMEM16a chloride channels. We showed that VSMC TMEM16 channels function as regulator of agonist-dependent arterial constriction and systemic blood pressure. In collaboration with Thomas Jentsch and Michael Bader, we provided definitive evidence that myogenic responses of mouse mesenteric and renal arteries rely on ligand-independent, mechanoactivation of AT1R subtype a. We are currently studying biased agonism and ryanodine receptor isoforms (Figure 1) in VSMC potassium channels gating and myogenic tone. A particular attention is paid to various KCNQ potassium channel isoforms in periadventitial vasoregulation. Thomas Jentsch is helping us here. We also have a project focusing on the perivascular adipose tissue (PVAT) as a source for relaxing factors. Here, we collaborate with Wolf-Hagen Schunck, who has peaked our interests in eicosanoids. We also collaborate with Huang Yu, Hong Kong, China. Together, we developed the novel concept that perivascular adipose tissue (PVAT) function requires considering heterogeneous PVAT as a specialized organ that can differentially regulate vascular function depending on its anatomical location. Finally, in collaboration with the German Institute of Human Nutrition (DifE), we are studying human diabetic nephropathy with a focus on genetics.

TMEM16a and TRP channels

TMEM16a, TRPC6, and TRPV1 channels are expressed in the vasculature. We used smooth muscle specific TMEM16a deficient mice and found that TMEM16a downregulates agonist-induced vasoconstrictions and thereby contributes to blood pressure regulation. Our current research in this area is directed towards identifying role of TMEM16a and ryanodine receptor isoforms in collateral arterial networks. In collaboration with Thomas Willnow, we have examined the role of the choroid plexus in the pathogenesis of multiple sclerosis, and found that claudin 3 (CLDN3) may be regarded as a crucial and novel determinant of blood-cerebrospinal fluid barrier integrity. We have also found that TRPV1 and TRPV4 channels play a role in regulating renal blood flow. We found that TRPV1 channels can contribute to ischemia/reperfusion (I/R) -induced kidney injury.

Eicosanoids, H_2S and inflammation

EETs serve as endothelial-derived hyperpolarizing factors (EDHF), but may also affect cardiovascular function by anti-inflammatory mechanisms. Our current research in this area is directed towards identifying the role of H_2S producing enzymes as regulator of vasodilatory EETs and nitric oxide (NO). In collaboration with Michael Bader, we found that angiotensin-converting enzyme 2 (ACE2) regulates vascular function by modulating nitric oxide release and oxidative stress. In diabetes mellitus, insulin-induced relaxation of arteries is impaired and the level of orthotyrosine (o-Tyr), an oxidized amino acid is increased. We found that elevated levels of o-Tyr contribute to vasomotor dysfunction in diabetes mellitus. By an improved tag-switch method, we identified thioredoxin to act as depersulfidase. We also identified a novel role of Anti-AT1 receptor and –ETA receptor antibodies in pulmonary arterial hypertension associated with systemic sclerosis. Both



Intracellular calcium release is visualized by calcium sensitive dyes and Nipkow spinning disc microscopy. Three freshly-isolated cells are shown in this figure: The upper two cells are isolated from wild-type (WT) mice; the lower cell is isolated from a ryanodine receptor type 2 deficient (KO) mouse. The stimulator of intracellular ryanodine receptors caffeine (10 mM) induced a strong calcium release signal in WT, but not in KO cells.

antibodies may contribute to pulmonary hypertension via increased vascular endothelial reactivity and induction of pulmonary vasculopathy. In collaboration with Ralf Dechend, we also identified a novel role of vitamin D in hypertension and target-organ damage. Our data suggest that even short-term severe vitamin D deficiency may directly promote hypertension and impacts on renin-angiotensin system components that could contribute to target-organ damage.

Vasodilator signals from perivascular adipose tissue

We have identified a vasorelaxing factor produced in the perivascular adipose tissue (ADRF). Our recent work showed that KCNQ channels could represent the subtype of K_v channels involved. The "third gas", namely H_2S, could represent ADRF. However, other adipokines may also play a role. We identified alterations in the paracrine control of arterial tone by periadventitial adipose tissue in animal models of hypertension and metabolic disease. KCNQ and cystathionine gamma-lyase deficient mice are available to us to clarify the role of K_v channels and H_2S. ADRF and its putative targets (KCNQ channels) might represent exciting new targets for the development of drugs for treatment of cardiovascular and metabolic disorders. Overall, our and other data indicate that dysfunctional perivascular adipose tissue (PVAT) contributes to cardiovascular risk.

Genetic renal diseases

An outgrowth of Maik Gollasch's clinical responsibilities has been a focus on clinical genetics related to renal diseases. We have performed functional analyses of mutations causing familiar kidney diseases with specific emphasis on TRPC6 channels in focal and segmental glomerulosclerosis (FSGS). We identified a unique CD2AP mutation in a German family, supporting the overall concept that CD2AP-associated nephropathy is an autosomal dominant form of FSGS in man. We are also studying human diabetic nephropathy with a focus on genetics. For these purposes, we recently established an Outpatient Kidney Clinic at Charité Campus Buch and the Registry of Diabetic Nephropathy (http://www.charite-buch.de/rdn/). We are continuously recruiting patients for our Registry and renal/vascular disease-specific family studies. Through these studies, we hope to identify novel mechanisms leading to increased cardiovascular risk and target-organ damage and novel treatment targets.

Primary funding

The Deutsche Forschungsgemeinschaft (GO 766/13-1, GO766/15-1; GO766/17-1; GO766/18-1, FOR1368), DAAD, Alexander von Humboldt Stiftung, ERA-EDTA and cooperative grants between the MDC and ECRC have funded this work.

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Photo: David Ausserhofer/MDC

Ralph Kettritz

Nephrology and Inflammatory Vascular Diseases

Our group is interested in vascular biology with special focus on autoimmune vasculitis. Patients develop autoantibodies against neutrophil and monocyte constituents that reside in the cells' cytoplasm and are called anti-neutrophil cytoplasmic autoantibodies (ANCA). The major ANCA target antigens are myeloperoxidase (MPO) and proteinase 3 (PR3). What breaks tolerance is not clear, but once ANCA are generated they activate neutrophils and monocytes. These activated cells adhere to the endothelium and cause severe necrotizing vasculitis. This highly inflammatory process can affect small vessels of every organ; however, the kidneys and lungs are the most life-threatening manifestations. Patients develop rapidly progressing necrotizing crescentic glomerulonephritis (NCGN) and often require dialysis. Timely immunosuppressive treatment improves renal function. However, treatment, including steroids, cytotoxic drugs and more recently CD20-targeted B-cell biological, is not disease specific. Translational research is needed to develop more specific and less toxic treatment protocols. Oxidative and non-oxidative effector mechanisms participate in ANCA vasculitis and provide potential novel treatment targets.

Neutrophil serine proteases (NSPs) accelerate vascular inflammation

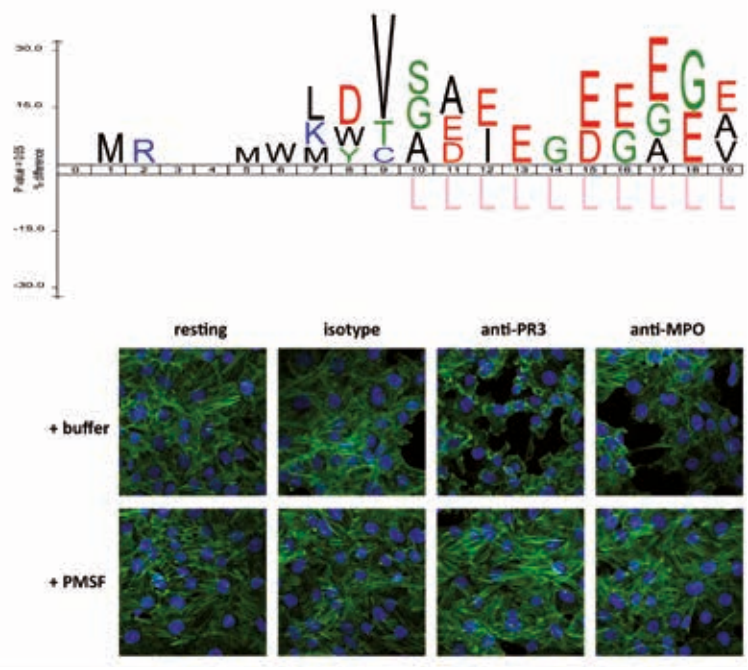
Neutrophil granules contain large amounts of NSPs that include human neutrophil elastase, PR3, and cathepsin G. Once the neutrophil is activated (e.g. by ANCA), these NSPs are released into the environment where they act as powerful proteolytic enzymes. NSPs shape the inflammatory response and have high potential for collateral tissue damage. We studied NSPs in two inflammatory settings with relevance to ANCA vasculitis: 1) NSPs as tissue-damaging enzymes that are transferred from the neutrophil to endothelial cells (EC), and 2) NSPs as regulator of proinflammatory IL-1β generation in neutrophils and monocytes.

We observed that all three NSPs are transferred to EC where they accumulate and retain their enzymatic activity. In collaboration with Gunnar Dittmar's group at the MDC, we used terminal-amine isotopic labeling of substrates (TAILS) to identify several novel endothelial NSP substrates. We characterized the extended cleavage pattern and generated corresponding IceLogos for each protease. Based on the gene ontology analysis we showed that NSPs released from ANCA-activated neutrophils entered the EC, disturbed the cytoskeletal architecture (figure), and caused endothelial permeability increase with increased albumin flux. Serine protease inhibitors abrogated these effects.

Our second project identified NSPs as important regulators of IL-1β generation in ANCA-stimulated monocytes and neutrophils in vitro and in an ANCA mouse model. Active NSPs, and particularly PR3, were efficient alternatives to the classical caspase-1 dependent inflammasome and promoted pro-IL1b processing into IL-1β. Using an ANCA mouse model we showed that inactive or lacking NSPs protected against NCGN and this effect was accompanied by a significantly reduced IL-1β generation. Treatment of mice with the specific IL-1 receptor antagonist, anakinra, protected from the disease suggesting that IL-1β is a critical inflammatory mediator and a treatment target in this model.

Phagocyte NADPH oxidase (Phox) restrains the inflammasome

Several clinical antioxidant trials in a variety of diseases have failed indicating that reac-



IceLogo for proteinase 3 (PR3) depicting amino acids that were most likely (above the x-axis) or least likely (below the x-axis) present in the substrate at the indicated P or P' site. Active neutrophil serine proteases, including PR3 are released from ANCA-stimulated neutrophils, accumulate in EC, and cause actin cytoskeleton disruption and permeability increase. Serine protease inhibition by PMSF abrogates this effect. Phalloidin (green) and DAPI (blue) staining.

tive oxygen species (ROS) have more complex functions. We tested the hypothesis that ROS produced by Phox restrain inflammation by down-regulating caspase-1, thereby reducing IL-1β generation and limiting ANCA-induced NCGN. In an MPO-ANCA disease model, mice transplanted with Phox-deficient BM, showed increased renal IL-1β levels and accelerated disease. Therapeutic IL-1β receptor blockade abrogated aggravated NCGN. IL-1β generation was strongly accelerated in Phox-deficient monocytes and neutrophils challenged with MPO-ANCA and this accelerated IL-1β generation was abrogated when caspase-1 was blocked. The causative role of caspase-1 was firmly established by showing that gp-91^{phox}/caspase-1 double-deficient mice were rescued. Our study indicated that Phox-generated ROS down-regulate caspase-1, keeping the inflammasome in check and thereby limiting ANCA-induced inflammation. IL-1 receptor blockade provided also in this study protection from the disease and is a promising treatment strategy. Importantly, our data question the benefit of antioxidants.

Genetic regulation of the PR3-presenting neutrophil surface receptor CD177 and disruption of the CD177/PR3 complex on neutrophils as a treatment strategy

PR3 is the major autoantigen in European patients with vasculitis. We identified neutrophil-specific CD177 as a PR3-presenting membrane receptor. CD177 is restricted to a variable, but in a given individual stable neutrophil subset. Only this CD177-expressing neutrophil subset presents high PR3 amounts on the neutrophil surface. The percentage of CD177^{pos}/mPR3^{high} neutrophils has clinical implications for ANCA vasculitis risk and clinical outcomes. We are working on the genetic and epigenetic regulation of this CD177/mPR3 phenotype. We have strong evidence that DNA methylation plays an important role.

We also teamed up with Oliver Daumke's group to elucidate the crystal structure of CD177 and the CD177:PR3 complex. We found previously that, on CD177^{pos}/mPR3^{high} neutrophils, PR3 and CD177 form of a larger signaling complex within lipid rafts. Recruitment of the transmembrane b2-integrin CD11b/CD18 then allows initiation of intracellular signaling pathways and neutrophil activation by PR3-ANCA. We believe that detailed structural information will help to therapeutically interfere with PR3 binding to CD177, or, alternatively, with anti-PR3 antibody-binding to CD177-presented PR3. Either approach would have the potential to prevent neutrophil activation by PR3-ANCA.

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Biology of Malignant Lymphomas

A detailed molecular understanding of the oncogenic process is fundamental for the development of new therapeutic strategies for human lymphomas. We aim to gain insight into the network of oncogenic defects in B and T lymphoid cells by using a wide range of cell and molecular biology techniques combined with high-throughput and screening approaches as well as in vivo mouse models. Our special interest is focused on the identification and characterization of deregulated signaling events and aberrant transcription factor activities in lymphoid malignancies. Using classical Hodgkin lymphoma (cHL) and anaplastic large cell lymphoma (ALCL) as model systems, we analyze the role of lineage infidelity, cellular reprogramming and activation of lineage-inappropriate survival signals in lymphomagenesis. In multiple myeloma we study the impact of tumor – microenvironment interactions as well as of intrinsic genetic and functional defects on the malignant transformation of terminally differentiated B cells by employing in vitro and in vivo strategies. Collectively, our work does not only address fundamental aspects of lymphoma pathogenesis, but also aims at providing therapeutic solutions.

Deregulated transcription factor activities as a driving force of malignant transformation and lineage infidelity in human lymphomas

Aberrant transcription factor (TF) activities are key regulators of malignant transformation of hematopoietic cells. In this regard, cHL provides a unique example among lymphoid malignancies given the exceptional diversity and strength of deregulated TFs in the malignant Hodgkin-/Reed-Sternberg (HRS) cells that target cellular differentiation as well as growth and survival pathways. In this part of our work, we aim to understand the hierarchical order and cooperative activity of such TF activities in HRS cells using a combinatorial approach that integrates the analysis of accessible chromatin studies with global gene expression and epigenetic data. This search revealed enrichment of DNA binding motifs for the inducible transcription factors AP-1, NF-kB, STAT and IRFs in the active chromatin landscape of cHL. Moreover, these motifs were specifically occupied in HRS cells, indicating a chronic activity of the corresponding factors. Apart from confirming the role of AP-1, NF-kB (both defined in HL by our lab in cooperation with the group of C. Scheidereit, MDC) and STAT factors for cHL pathogenesis at a genome-wide level, a key finding of this analysis was the identification of overexpressed IRF5, a central regulator of inflammatory gene expression, as a specific defect of HRS cells. Our work provides evidence that IRF5 in combination with NF-kB orchestrates the HL-specific gene expression program, which offers an explanation for the unique inflammatory phenotype of cHL. We are currently transferring the approach described herein to other lymphoid malignancies to identify key transcriptional regulators of the respective lymphoma entities.

Apart from the direct modulation of cellular processes like growth or apoptosis, even single TFs can enforce malignant transformation by disrupting the physiologic differentiation program. A number of lymphoid tumors display a phenotype that is in accordance with such a reprogramming process, including cHL, PEL and ALCL. Among these entities, cHL constitutes the most prominent example for lineage infidelity and reprogramming. In previous work, we have shown that the activity of the TF E2A is disrupted in the malignant cells of cHL and ALCL. This defect is directly linked to a reprogramming process that results in the

up-regulation of genes usually suppressed in lymphoid cells, including the myeloid CSF1 receptor (CSF1R) gene. Remarkably, CSF1R expression in HL cells is not mediated by the canonical myeloid promoter, but is activated by an upstream long terminal repeat (LTR) element, providing the first example of such an alternative proto-oncogene activation. In addition, our work has significant impact on the development of new treatment strategies. For example, inhibition of CSF1R activity resulted in reduced proliferation or cell death of lymphoma cells. Ongoing projects include work on the molecular mechanisms of the reprogramming processes in lymphoid cells, their therapeutic implications and, in cooperation with the group of K. Rajewsky (MDC), the generation of transgenic mouse models to study the impact of lymphoma-associated factors on cell growth, apoptosis and differentiation *in vivo*.

Identification and characterization of molecular defects in B and T cell-derived malignancies

Apart from our work on deregulated TF networks, we are interested in the identification of additional genetic and functional defects of B and T cell lymphomas. In particular, we focus on the pathogenesis of B cell-derived malignancies such as cHL and multiple myeloma as well as T cell-derived malignancies (ALCL and cutaneous lymphoproliferative disorders). It is our aim to investigate the mechanisms leading to the t(2;5)-translocation in ALCL and to evaluate the contribution of breakpoint-proximal gene deregulation to ALCL pathogenesis and for prognosis and development of new treatment strategies for ALCL.

Furthermore, to identify factors that are involved in the formation of the HL-specific tumor microenvironment, we screened gene expression profiles of HRS and B non-Hodgkin cell lines with a focus on cytokines and cytokine receptors. With this approach, we identified an up-regulation of IL-15 and the corresponding receptor components in Hodgkin cell lines. We demonstrated that IL-15 promotes growth and apoptosis resistance, stimulates JAK/STAT signaling and is involved in the regulation of HRS cell-specific gene expression. In summary, this study identified IL-15 as an important part of the complex interactions between tumor cells and the microenvironment in HL.

Finally, we postulated that a defect of the cellular degradation machinery might be involved in the unusually strong activation of transcription factors, including NF-kB, AP-1, STATs and IRFs, in cHL. By the analysis of microarray data, we identified a recurrent inactivation of the putative ubiquitin E3-ligase PDLIM2 in cHL and ALCL. Loss of PDLIM2 expression was associated with *PDLIM2* promoter methylation, altered splicing and genomic alterations, and contributes to the activation of NF-kB and AP-1 in these lymphoma cells. Thus, inactivation of PDLIM2 promotes activation of inflammatory signaling pathways, thereby contributing to lymphoma pathogenesis.

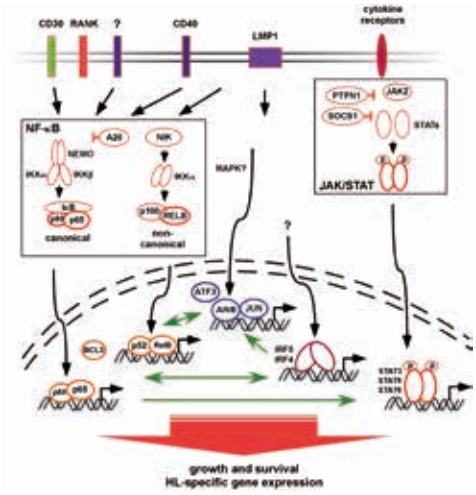

Figure 1. Key signaling pathways in cHL. *Major signaling pathways in HRS cells are canonical and non-canonical NF-kB (defined in cHL by groups Dörken and Scheidereit), and JAK/STAT signaling. Further key players are IRF5, which together with NF-kB coordinates HL-specific gene expression (defined in cHL by group Dörken), and high-level activation of AP-1/CREB members (JUN, JUNB, ATF3) (defined in cHL by groups Dörken and Scheidereit). Green arrows indicate interactions.*

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Photo: private

Friedemann Paul

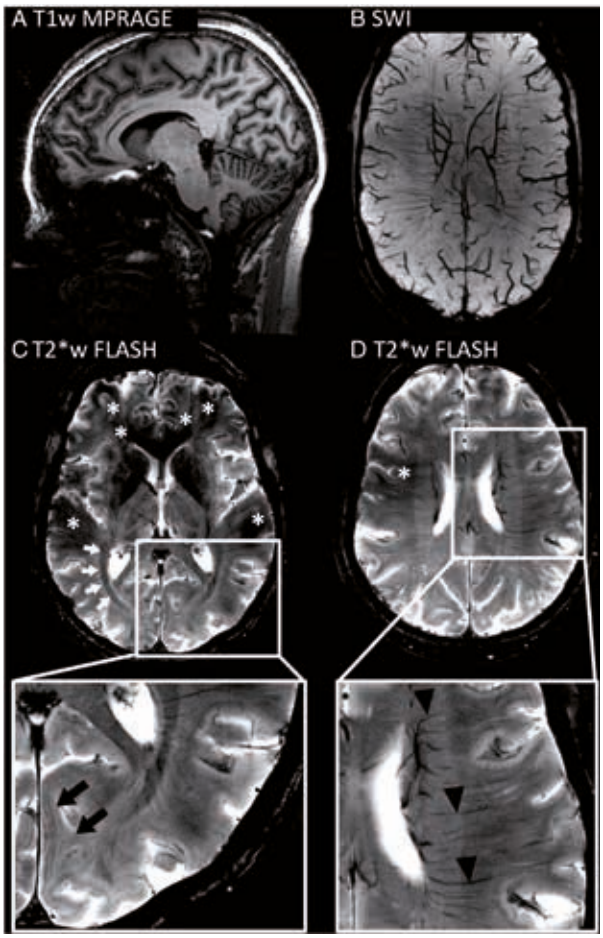
Outpatient Clinic for Neuroimmunology

Friedemann Paul is head of the Out-patient Clinic of Neuroimmunology at the ECRC caring for more than 1500 patients each year suffering from neuroimmunological disease like Multiple Sclerosis (MS), Neuromyelitis optica (NMO) Susac syndrome or Myasthenia gravis. In co-operation with the NeuroCure Clinical Research Center his research is dedicated to translational medicine running numerous trials to develop new diagnostic and therapeutic approaches in neuroimmunological disorders. The main focus is on developing so-called neuroprotective (nerve cell protecting) treatment and establishing modern examination procedures such as MRI (magnetic resonance imaging) and OCT (optical coherence tomography). He works closely with basic scientists, clinical neurologists and neuro-imaging researchers on local, national and international level.

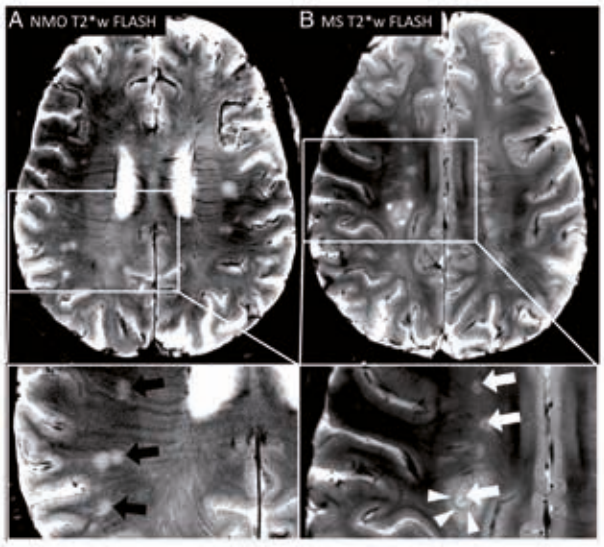
Multiple Sclerosis

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system that primarily affects young adults. It is caused by autoimmune processes, meaning the body's immune system falsely recognizes tissue of its own nervous system as "foreign" and causes an inflammatory reaction, resulting in damage to nerve cells and axons with their enveloping protective layer. A variety of neurological symptoms may occur: paralysis, visual disturbances, abnormal sensations or balance problems.

The neuroimmunology team focuses on developing and establishing new disease course and prognostic markers for MS. This includes, for example, improving possibilities in the field of magnetic resonance imaging (MRI) and ultra-high field MRI, or establishing new imaging techniques such as optical coherence tomography (OCT). We could show that despite differences in disease course and clinical characteristics primary progressive and relapsing remitting MS exhibit identical lesion morphology in ultra-high field (7 Tesla) MRI.



*7 Tesla MRI images of brain structures. A 7T T1w MPRAGE shows high-resolution anatomical images. B 7T SWI depicts tiny brain veins. C,D 7T T2*w FLASH (resolution 0.2mm x 0.2mm x 2mm) depict highly myelinated structures like the optic radiation (white arrows) or the stripe of Gennari (black arrows). Tiny veins emerge in the periventricular white matter (black arrowheads). Image quality of 7T gradient echo images may be reduced due to inhomogeneities or artifacts (asterisks).*



*Neuromyelitis optica (NMO) versus Multiple Sclerosis (MS) lesion morphology. 7T T2*w FLASH images from exemplary NMO (A) and MS (B) patients. A small central vein shows within the inner third of most MS lesions (white arrows). An acute MS lesion is characterised by a hypointense rim (white arrowheads) and a surrounding edema (asterisks). A central vessel is not visible in NMO lesions (black arrowheads) despite using high spatial resolution of 0.2mm x 0.2mm x 2mm.*

Because there is as yet no cure for MS, treatment strategies centre primarily on improving symptoms and modifying the disease course. In this context the team develops and conducts clinical trials with the aim of improving treatment options in MS and to learn more about the mechanism of action of these therapies next to new pathophysiological aspects of the disease itself.

Neuromyelitis optica

Neuromyelitis optica (NMO) has long been regarded as a variant of MS. Today we know that NMO is an independent disease, which is based on different disease mechanisms.

Typical manifestations of NMO are, on one the hand, unilateral or bilateral optic neuritis, often leading to severe vision problems and, on the other hand, long-segmented inflammation in the spinal cord, which can lead to severe paralysis, abnormal sensations, gait disorders, and bladder dysfunction.

The disease is caused by an autoimmune response to a cellular water channel known as aquaporin-4. Antibodies against this water channel can be detected in most patients with NMO. As with MS, there is currently no cure for NMO. As successor of the at present widely used immunosuppressant therapy upcoming therapeutic strategies focus on highly specific pharmacological interventions in the immune system.

The neuroimmunology group is considered one of the leading centres in NMO research both nationally and internationally. New diagnostic approaches are developed, e.g., with OCT or MRI imaging and therapeutic studies conducted. We were able to introduce a paradigm to differentiate between MS and NMO cerebral lesions in ultra-high field MRI based on the fact that NMO lesions compared to MS lesions lack a central vein and rarely show a T2*w-hypointense rim. Our growing cohort study of NMO patients offers a wide opportunity to investigate clinical as well as pathopyhsiolocial aspects of the disease.

Primary funding sources

German Research Foundation (DFG Exc 257 and other grants) and the German Ministry of Education and Research (Competence Network Multiple Sclerosis KKNMS and other grants), research grant from the Guthy Jackson Charitable Foundation/National Multiple Sclerosis Society of the USA, European Union Seventh Framework Programme (FP7/2007-2013), EU funding program ERACOSYS-MED, Walter und Ilse Rose Stiftung, Leifheit-Stiftung, Sanitätsrat Dr. Arthur Arnstein Stiftung, Federal Ministry for Economic Affairs and Technology (ZIM funding line)

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Photo: Bierderlack/Charité

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Cardiac MRI

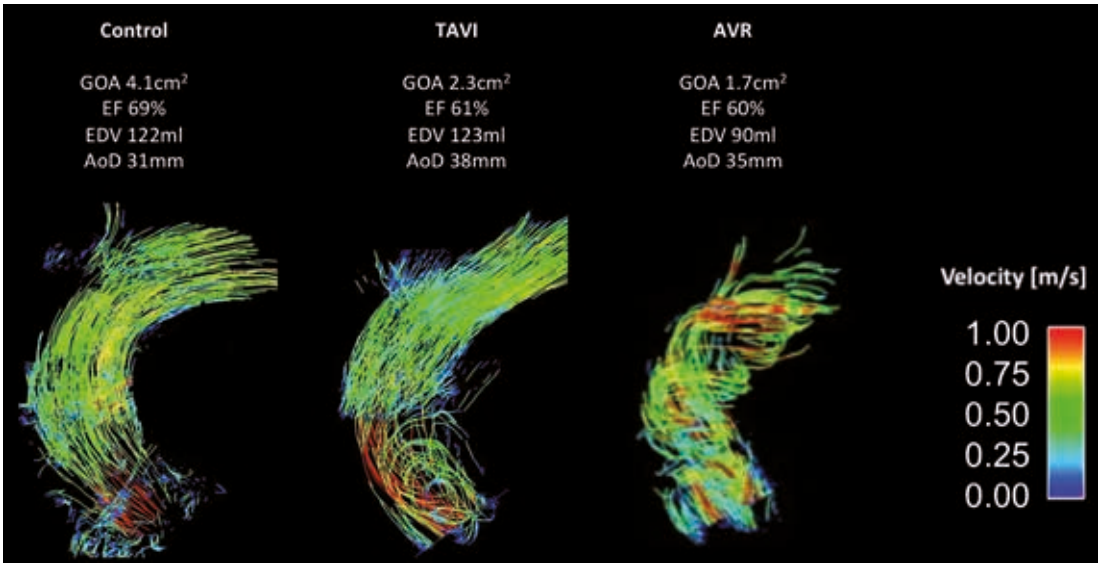
The CMR group at the Experimental Clinical Research Center (ECRC) and Clinics for Cardiology and Nephrology of the HELIOS Clinics Berlin Buch has focused research on in vivo assessment of functional and structural myocardial abnormalities related to non-ischemic diseases and ischemic heart disease. Our main hypothesis is, that the in-vivo differentiation of myocardial structural injury is possible in preserved ejection fraction and will help to explain remodeling processes. Furthermore, we understand cardiovascular disease as systemic disorders and aim to understand the mechanism and the interaction of vascular and cardiac disorders. In many projects, we are treading the path from the phantom/model including development of techniques into clinical research. Developing and establishing innovative imaging biomarker will allow for quantification of structural integrity and in-vivo evaluation of prognostic relevant processes.

As Cardiovascular Magnetic Resonance (CMR) has the unique possibility to differentiate myocardial injury, we are focusing on clinical research using a 1.5 clinical MRI-scanner with the advantage to reach out to higher field strengths at the MDC. The group was able to expand the research activities by developing translational research tools applying advanced imaging modalities in close collaboration with the Berlin Ultra-High-Field Facility (B.U.F.F.). This could be established fast, because the head of the Experimental MR-Group (Prof Niendorf) has a strong interest and experience in CMR-research. A strong collaboration with scientists from all over the world allows us to proof and use dedicated techniques for myocardial differentiation. Examples for cooperation are Peter Kellman (NIH, Bethesda, USA), Frederick Epstein (Univ. of Virginia, USA), Tobias Schaeffter (PTB, Berlin, Germany)

We have installed a post-processing lab (CMR reading and development) giving us the opportunity to speed up processes. During the last year we developed standardized procedures (SOP) for quantitative analysis to improve and introduced a systematic quality check of all data. That goes along with the introduction of staff scientist structure. In 2011 we founded an academic outpatient department of cardiology focused on myocardial disease at the ECRC and established a research database dedicated on patient oriented research. During the last year a fruitful cooperation between the Muscular group (headed by Prof Spuler, ECRC) was established. That drives the understanding of "neuro-cardiology" in muscular dystrophy. The group has several outreach activities. Jeanette Schulz-Menger is e.g. the Principal Investigators of Germany of the NIH sponsored HCMR trial (risk assessment for patients with HCM) and is co-heading the Focus Group for Cardiovascular Imaging of the German National Cohort. Furthermore, she was elected as the president of Society for Cardiovascular Magnetic Resonance. Florian von Knobelsdorff and she were members of the writing group in societal position statements.

Myocardial tissue injury and integrity

The assessment of myocardial tissue changes during follow-up is an ongoing challenging task in cardiovascular research and clinical cardiology and has been our main focus since years. The in vivo differentiation of myocardial structure, cell integrity and identification of (patho-)physiological mechanism is the driving force of our research. CMR has the capability to differentiate between the various forms of myocardial injuries (e.g. edema, hyperemia and fibrosis) already in preserved ejection fraction. During the last years we have prepared the ground to the next step towards



Quantitative assessment of focal fibrosis in hypertrophic cardiomyopathy.

quantification of structural changes including assessment of extracellular volume. The underlying approach is parametric mapping. As a first step we published recently (2014) normal values at different field strengths and are currently working on the assessment of non-ischemic heart diseases. Kind and dosage of contrast-media is another influencing factor for quantitative approaches. We have faced its potential influence as published in 2015 in a prospective randomized trial. The understanding of cardiac function is usually focused on the left ventricle. We could show, that in case of thoracic deformation the right ventricle is predominantly altered and may profit from surgical correction. There is a growing interest in the understanding diastolic dysfunction as a prognostic factor. We could show that the analysis of myocardial deformation reveals differences between different types of left ventricular hypertrophy and volunteers. These preliminary results at rest and stress are another connection to our interest in the assessment of the aorta.

Vascular MR

As mentioned, we are understanding cardiovascular diseases as systemic disorders and so far we have already published assessment of vascular changes of the aorta including quantification of wall alteration. The aortic wall itself is known to be affected by different hemodynamic factors promoting aortic remodeling. In 2015 we were able to publish the impact of interventional replacement of the aortic valve (TAVI) applying 4D-flow imaging. (see fig 2) First further results are indicating a relation between left ventricular remodeling and aortic wall changes in aortic stenosis. Another aspect is the accurate quantification of flow velocity also in patients with arrhythmias. We have performed systemic analysis from phantom experiments to patient assessment for evaluation of real-time flow. That research is intended to identify markers for a more precise and personalized therapy guiding in this patient cohort

Cardiac MR at Ultra Highfield

The close collaboration with B.U.F.F. at MDC allowed to publish several joint paper including the worldwide first paper assessing cardiac function of the right ventricle in volunteers at 7.0 Tesla in 2013. In December 2015 the worldwide first paper in cardiac patients at 7.0Tesla was accepted for publication. We were able to identify subtle myocardial changes .

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Photo: private

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Muscle Research Unit and Out-patient Clinic for Muscle Diseases

We are interested in muscle sciences. In our outpatient clinic at the ECRC we care for >1500 patients with genetic and acquired skeletal muscle diseases. Therapies for muscular dystrophies are not available. Our research is motivated and directed towards changing this by investigating the potential of muscle stem cells in regeneration and restoring muscle function and by possible pharmacologic intervention based on detailed understanding of the molecular pathophysiology of muscle wasting. Our main disease models are muscular dystrophies due to mutations in the dysferlin and the lamin A/C genes. Equally important are the recruitment of excellent young scientists to the field as well as the translation of our research into the public. MyoGrad is a graduate program dedicated solely to muscle sciences (DFG GK1631, UFA).

Muscle stem cells – Satellite cells

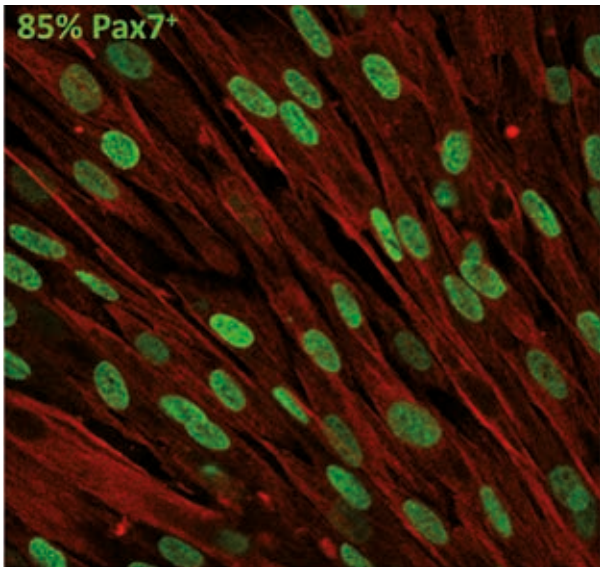
Skeletal muscle harbors its own stem cells called satellite cells located in the stem cell niche between sarcolemma and basal lamina. Satellite cells are essential for muscle regeneration. If muscle is injured or if satellite cells are removed from their microenvironment they become activated and differentiate into myoblasts and myotubes. Earlier attempts to use the therapeutic potential of satellite cells failed because differentiation interfered with their ability to regenerate muscle. We are trying to preserve the regenerative capacity of satellite cells yet expanding them. We also aim to genetically repair mutations in genes causing muscular dystrophy.

Dysferlinopathies

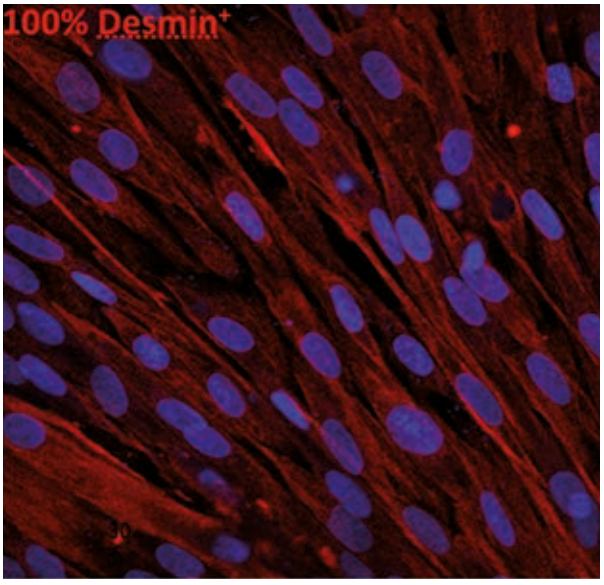
Dysferlin, a large protein located at the sarcolemma, is involved in membrane repair after microinjury, a physiological consequence of normal muscle activity. Mutations in dysferlin lead to muscular dystrophy. We found that dysferlin is essential for normal glucose metabolism in muscle (Schöwel et al. in revision).

Acquired myopathies: Critical illness myopathy and statin-induced myopathy

Inhibitors of 3-hydroxy-3-methylglutaryl-coenzym-A-reductase (statins) are prescribed to millions of patients of whom 10% develop muscle related side effects. We are exploring genetic and structural aspects of this common myopathy. Critical illness myopathy occurs as a severe side effect of intensive care unit treatment. In a large translational and interdisciplinary approach that involved several ECRC groups we have shown that the failure to activate AMPK and to translocate GLUT4 to the sarcolemma contributes significantly to muscular atrophy.



Pax7 positive human muscle precursor cells (satellite cells) after 5 weeks in culture



Funding sources
Deutsche Forschungsgemeinschaft (MyoGrad IGK1631; SP 1152/11-1), German French University UFA; Jain Foundation; Foundation Gisela Krebs

www.muskelforschung.de

Patents

PCT-Anmeldung (WO 2016/030371). Cultivating human muscle satellite cells.

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Translational Oncology of Solid Tumors

Metastatic dissemination of primary tumors is directly linked to patient survival and critically limits successful therapy in many tumor entities. Our translational concepts aim at the identification of key molecules in tumor progression and metastasis for improved prognosis and therapy of solid cancers. We discovered MACC1, S100A4 and transcriptional targets or protein binding partners thereof as new diagnostic, prognostic and predictive key players for tumor progression and metastasis. Biomarker development was done in established and patient-derived 3D cultures, cell line-derived and patient-derived xenografts (PDX) and newly generated genetically engineered mouse models. We exploited this knowledge for improved prognosis and treatment response prediction using tissue and blood of cancer patients. Novel therapeutic approaches are currently tested in clinical trials to treat patients with metastatic disease using small molecule inhibitors acting on these biomarkers.

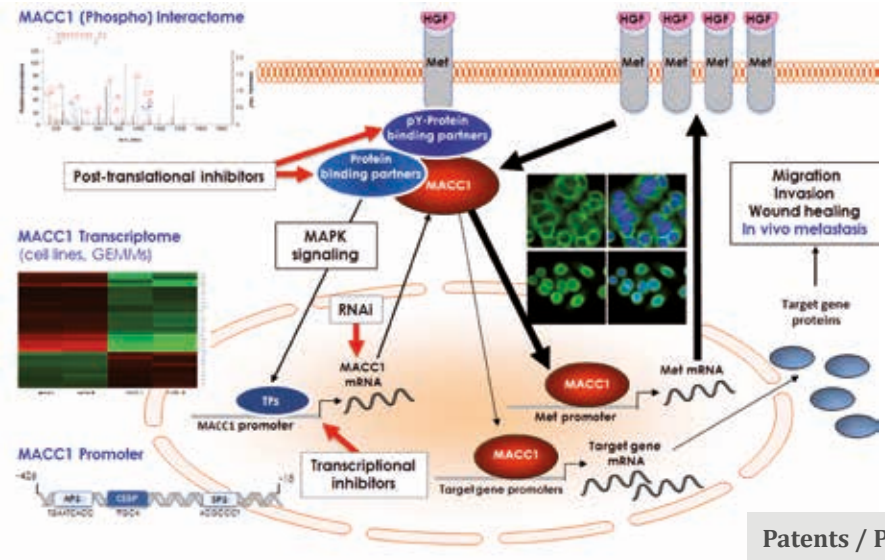
Cancer cell signaling of MACC1 and S100A4 for pathways-based interventions

MACC1 was discovered in our group acting as decisive driver for tumor progression and metastasis for a broad variety of solid cancers. By transcriptomics, we found MACC1 target genes, e.g. SPON2, which induce metastasis and are prognostic for colorectal cancer (CRC) patients. We explored the miRNA landscape for MACC1- and S100A4-induced metastasis, identified miRNAs epigenetic regulations, interplays for metastasis prognostication and patient survival. We identified the MACC1 interactome (G. Dittmar, MDC) and phospho-interactome (B. Küster, TU Munich) by mass spectrometry (MS). We found kinases essential for MACC1 Y-phosphorylation, and entire signalosomes of receptor tyrosine kinases binding pY-MACC1.We used a MACC1 promoter reporter for high throughput screenings (HTS) using the FMP-ChemBioNet (J. von Kries, FMP) and DKFZ/EMBL libraries (N. Gunkel, DKFZ, J. Lewis, EMBL). The S100A4 promoter reporter was used for HTS using the NCI/NIH-Lopac library (R.H. Shoemaker, NCI/NIH Bethesda MD). We identified the first MACC1 and S100A4 transcriptional inhibitors.

Preclinical developments for understanding and intervening in tumor progression and metastasis

We established in vivo intervention strategies targeting MACC1 and S100A4 for metastasis inhibition. shRNA acting on MACC1 or S100A4 or on transcriptional or post-translational targets thereof decreased metastasis. The small molecule transcriptional inhibitors of MACC1 or S100A4, identified by HTS, restricted MACC1- or S100A4-induced metastasis in mice. This repositioning of already FDA-approved drugs for the new indication of metastasis restriction paves the way for clinical trials.

Based on our MS data, we showed MACC1-induced metastasis restriction in mice when deleting MACC1 pY sites or inhibiting MACC1-phosphorylating kinases using drugs tested in clinical trials. We generated PDX models from tumors and metastases of CRC patients (B. Rau, Charité, J. Hoffmann, epo), and evaluated biomarkers and pathways for progression, metastasis, and response prediction towards conventional and targeted therapies. We generated the first transgenic MACC1 mouse models vil-MACC1, vil-MACC1/Apc^{Min} (B. Jerchow, MDC). vil-MACC1/Apc^{Min} mice showed increased number and size of tumors, possessed an accelerated adenoma-carcinoma-sequence, an invasive phenotype linked to reduced survival, and increased Wnt and pluripotency signaling with up-regulation of Nanog and Oct4. These pluri-



MACC1 induces cell motility, proliferation, and scattering in cell culture and metastasis in mouse models. It acts as transcription factor regulating e.g. Met expression and also interacts with proteins via e.g. its SH3 domain or proline-rich motif. By microarrays we identified the MACC1 transcriptome. By HTS using the MACC1 promoter we identified first transcriptional MACC1 inhibitors. By MS we identified the MACC1 interactome and phospho-interactome for novel interventions.

potency markers are regulated by MACC1 and strongly correlate with MACC1 levels in CRC patients. We provide first evidence that MACC1-induced tumor progression in CRC acts, at least in part, via the newly discovered MACC1/Nanog/Oct4 axis.

Clinical translation for improved prognosis, prediction and restriction of tumor progression and metastasis

We demonstrated MACC1 as metastasis inducer, prognostic biomarker for CRC and as a target for metastasis inhibition. MACC1 outperforms KRAS, BRAF, and MMR mutation, and improves prognosis combined with KRAS13 mutation. We showed highest prognostic value of MACC1 at the CRC tumor invasion front (V. Kölzer, Uni Bern). We established a novel, non-invasive liquid biopsy test demonstrating the prognostic value of circulating MACC1 transcripts in patient blood in prospective studies. We identified MACC1-regulated genes, demonstrated their metastasis-inducing potential in mice and their prognostic value for CRC patient survival. We confirmed MACC1 as prognostic biomarker and driver for progression and metastasis in tumors and blood from hepatobiliary, gastric, pancreatic, breast cancer and glioblastoma patients (A. Lederer, S. Burock, C. Denkert, K. Detjen, Charité, C. Hagemann, Uni Würzburg), and as predictive marker for patient stratification for adjuvant treatments in CRC stage II (Hoffmann La Roche Pleasanton CA, Ventana Tucson AZ). We showed the importance of S100A4 for CRC in the context of its receptor RAGE, which itself and combined with S100A4 serves as prognostic biomarker for metastasis and survival. Therapeutic translation is done in a prospective clinical phase II trial treating CRC patients for metastasis inhibition with a newly identified, FDA-approved small molecule (NCT02519582; U. Keilholz, Charité,). Therapeutic outcome is monitored by circulating S100A4 transcripts in patient blood.

Patents / Patent applications

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Scientific Infrastructures & Technology Platforms

Jutta Steinkötter

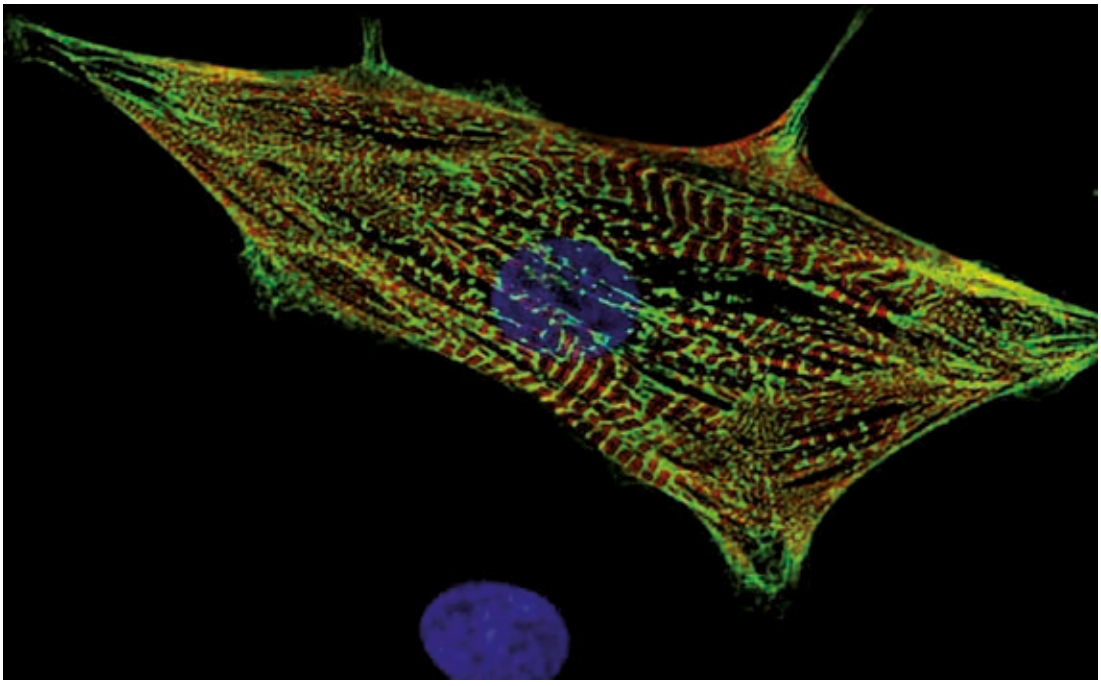
Scientific Infrastructures & Technology Platforms

Jutta Steinkötter

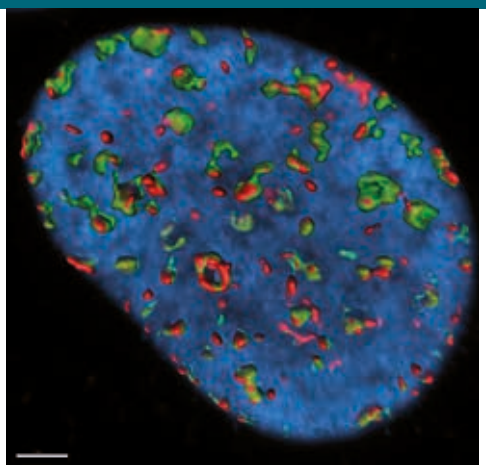
Technology Platforms at the MDC support established and front-end application of technologies, provide instrumentation and methodologies and are engaged in collaborative research projects and technology developments. Driven by the demands of the MDC's scientific topics, the portfolio of our Technology Platforms develops hand in hand with progress and innovation in research and with new developments on the technology market.
The concept of the MDC's technology platforms is to provide state-of-the-art services as well as specific tailor-made technology applications and co-development of methodologies at the forefront of scientific and technological innovation. This approach is a powerful technology support for research groups and results in high-profile publications and patentable innovation.

Strategic developments of the past years initiated the establishment and combinations of expertise, resources and services for new Technology Platforms.
BIMSB established Platforms for Genomics, Integrative Proteomics and Metabolomics, Bioinformatics and Light Microscopy. These platform concepts were blueprints for BIH Platforms, where know-how and expertise contributed to the set-up of BIH Platforms at the MDC, such as *Genomics*, *Metabolomics* and *Proteomics* serving the needs of the BIH researchers and projects. Additional technologies and infrastructures were established in collaboration with the BIH: the *Pluripotent Stem Cell Platform* and the *Biobank* are hosted with one branch at the MDC and a second branch at the Charité. And last but not least, another collaboratively and jointly operated research infrastructure is the Chemical Biology of the FMP, supported by the MDC.

Shown are human induced pluripotent stem cells derived cardiomyocytes. The structure of the sarcomeres were stained using antibodies against cardiac markers alpha actinin (red) and cardiac troponin (green). The nucleus was stained with DAPI (blue).



Graphic: Sebastian Diecke, MDC



Graphic: Anje Sporbert, MDC

3D reconstruction of the DNA (blue) replication machinery in the cell nucleus visualized by proteins (green) involved in DNA synthesis and incorporated nucleotides (red).

Most of the MDC's Platforms are expanding their portfolio of technologies and instruments and therefore increase the potential for collaborations and technology innovation. The Genomics Platform for example provides not only standard service for Genome and Transcriptome Sequencing on Illumina and PacBio Systems but is also developing applications for single cell analysis, based on cell sorting and various microfluidics systems. This major trend shapes research in translational and basic areas and many approaches are directed towards cell type identification, tracking, cell fate decisions and mechanisms of disease. Expanding capacities for animal phenotyping were planned and constructed in the past years and in 2017, the upcoming reporting period, the in vivo Pathophysiology will offer more space for sophisticated imaging, physiology, behavior and molecular studies of mice. More structural work will be supported through the EM Platform, which recently installed a new CryoEM.
As shown in the platform reports, they substantially contribute to innovation and publication output of the institute, and based on recruitments, investments and strategic expansions of the past years, they can offer state-of-the-art instrumentation, knowledge and expertise for research projects at the MDC and in collaboration with local, national and international research partners.

Executive Management

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Scientific Board

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Central Technology Platforms and Core Facilities

Advanced Light Microscopy
Anje Sporbert

Biobank (MDC and BIH)
Tobias Pischon

Bioinformatics
Altuna Akalin

Chemical Biology (MDC and FMP)
Jens Peter von Kries & Marc Nazaré

Electron Microscopy
Bettina Purfürst

Genomics (MDC and BIH)
Sascha Sauer

Magnetic Resonance
Thoralf Niendorf

Metabolomics (BIH)
Jennifer Kirwan

Light Microscopy
Andrew Woehler

Pathophysiology
Arnd Heuser

Pluripotent Stem Cells (MDC and BIH)
Sebastian Diecke

Preparative Flowcytometry
Hans-Peter Rahn

Proteomics (MDC and BIH)
Gunnar Dittmar

Proteomics / Metabolomics
Stefan Kempa

Transgenics
Ralf Kühn

Photo: David Ausserhofer/MDC

Anje Sporbert

Advanced Light Microscopy

The Advanced Light Microscopy (ALM) technology platform provides scientists with access to state-of-the-art microscopes and imaging techniques and supports research projects with customized scientific, methodological and technical expertise. ALM currently supports about 200 researchers from more than 45 groups, mainly from the MDC and BIMSB, but also from ECRC, Charite and FMP.

ALM currently hosts **14 high-end, cutting edge imaging systems** that cover confocal laser scanning, multi-photon, wide-field fluorescence, total internal reflection (TIRF), light-sheet, spinning-disk and 3D-stimulated emission depletion (STED) microscopy, fluorescence lifetime imaging (FLIM) as well as laser microdissection. This portfolio of advanced techniques permits the imaging of a wide range of fixed specimens as well as live imaging of cells, tissue preparations, organoids, small organisms and animals.

Advanced image analysis and processing tools are available on dedicated image analysis workstations for 3D reconstructions, colocalization, quantification, counting, tracking, stitching, and deconvolution.

Regular seminars covering theoretical principles and main applications of light/fluorescence microscopy are given each month. Each ALM user receives in-depth, **individualized practical training** in the safe handling of microscopes to ensure the correct acquisition of data. Regular performance tests of our instrumentation are another measure we take to ensure and maintain the high quality of scientific data acquired with ALM setups.

The ALM developed **core facility management software** to offer easy access to users and tools for efficient communication, support and troubleshooting. The software provides online user registration, microscope booking, news, reporting of incidents and mailing lists. The software can readily be adapted to suit the needs of other core facilities.

In **collaborative imaging projects** we can offer very customized scientific and methodological support covering steps including project planning, the optimization of sample preparation and image acquisition, image analysis workflows and even the establishment of new imaging methods.

Selected projects & Method development

Label-free imaging – polarised second harmonic generation (SHG), *A. Margineanu:* 2-photon microscopy can be used not only to excite fluorescence in tissues and organs at higher depths, but also to generate other effects such as SHG. SHG imaging has been applied to the study of changes in the extracellular matrix. Because SHG yields information on the orientation of collagen fibers, adding polarisation imaging offers even more insights. The ALM's intravital 2-photon microscope has been modified to permit polarised SHG measurements of cartilage samples obtained from mice (Fig.1A, collaboration AG Poole).

Fluorescence lifetime imaging (FLIM), *A. Margineanu/M. Richter:* Fluorescence microscopy measures not only the intensity and cellular distribution of dyes, but also other properties of fluorophores such as the lifetime. This method is sensitive to the fluorophore environment and can be used to detect intracellular protein-protein interactions. The energy transfer from a donor to an acceptor (labeling the proteins of interest) occurs only when the two fluorophores are at distances <10 nm. FLIM provides a more robust approach then ratiometric imaging since only the reduction of the donor lifetime must be detected. It is also more quantitative, providing donor-acceptor distances and interacting population fractions. FLIM-FRET has been applied

at ALM to identify substrates that could interact with the titin kinase catalytic domain (Fig.1B, collaboration AG Gotthardt) and to identify interacting compartments of the peptide loading complex (collaboration AG Klötzel).

FLIM can also detect biologically active molecules using dyes or genetically-encoded biosensors which change their fluorescence lifetimes upon binding the molecule of interest. One biosensor applied at ALM is Peredox, which detects changes in the cytoplasmic redox ratio of NAD(P)H/NAD+. Together with the biosensor, FLIM of the endogenous cellular fluorophores NAD(P)H and FAD gives an indication of the energy metabolism of cells.

Cleared specimens for confocal and light-sheet microscopy, *M. Richter/A. Sporbert:* Imaging entire organs or specimens greatly benefits from rendering tissues more transparent by removing scattering substances such as lipids and matching the refractive index of the sample to the optical setup. Numerous clearing techniques have been published in the last years. We have optimized the high-resolution imaging of cleared rat hearts, mouse embryos and spinal cords for further analysis by 3D reconstructions (collaborations with AG Müller, W. Birchmeier (Fig.1C), Spagnoli).

Laser-assisted membrane injuries, *A. Sporbert:* High-power lasers can be used to cut and catapult cells and tissues (through laser-microdissection microscopy) and also to create targeted cellular injuries. Uses include a range of biophysical and mechanistic studies targeting cells or their components. An assay for reversible membrane damage was implemented in skeletal muscle cells and cardiomyocytes to evaluate the repair capacity of cell membranes (collaboration AG Morano, Fig.1D).

In-vivo/intravital 2-photon microscopy *(A. Margineanu)* for small animals and intact tissue preparations is invaluable in the validation of results that have been obtained by standard techniques in physiological environments. Examples of projects we have supported are the multi-color imaging of mouse brain (AG Priller), mouse brain stem (AG Siffrin) and spleen (AG Höpken).

Quantum dot triexciton imaging *(K. Grohmann)*: Higher order excited states can be generated in some types of commercially available Quantum Dots (QD) by conventional lasers. Detection of these tri-excitons combined with deconvolution results in a up to 1.7 fold higher spatial resolution of QD labeled subcellular structures using standard confocal microscopes.

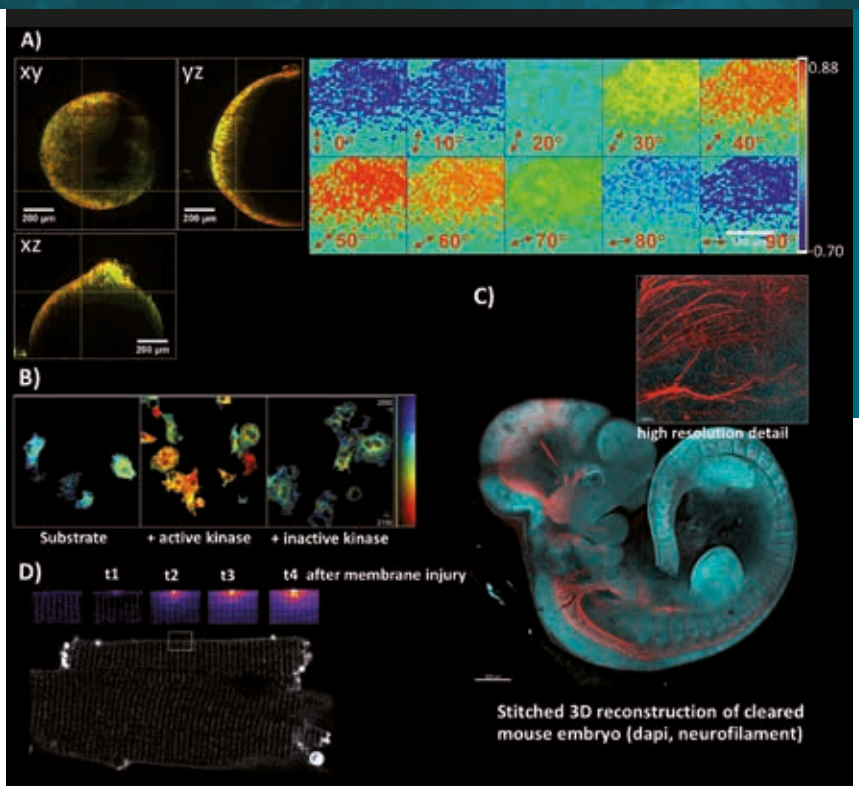

Fig1. A) Polarised SHG from cartilage *in parallel (red) and perpendicular (green) detection. Colour-coded images show the calculated polarisation images (sample M. Servin-Vinces)* **B) Protein-protein interactions in situ using FLIM imaging.** *Reduced GFP lifetime (red) indicates specific interaction (sample F. Rudolph)* **C) High resolution 3D reconstruction** *of 480 stitched images (sample F. Heuberger)* **D) Laser-assisted membrane injury** *of cardiomyocyte (sample L. vanVuuren).*

Selected Publications

Zessin P.J.M.; **Sporbert A.**; Heilemann M. PCNA appears in two populations of slow and fast diffusion with a constant ratio throughout S-phase in replicating mammalian cells, Scientific Reports 6: 18779 (2016-01-13)

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Warren S.C., **Margineanu A**, Katan M., Dunsby C., French P.M. (2015) Homo-FRET Based Biosensors and Their Application to Multiplexed Imaging of Signalling Events in Live Cells, Int. J. Mol. Sci., 16(7):14695-14716

Heinig K.; Gaetjen M.; Grau M.; Stache V.; Anagnostopoulos I.; Gerlach K.; Niesner R.A.; **Cseresnyes Z.**; Hauser A.E.; Lenz P.; Hehlgans T.; Brink R.; Westermann J.; Doerken B.; Lipp M.; Lenz G.; Rehm A.; Hoepken U.E. (2014) Access to follicular dendritic cells is a pivotal step in murine chronic lymphocytic leukemia B cell activation and proliferation , Cancer Discovery 4: 1448-1465

Sporbert A.; Cseresnyes Z.; Heidbreder M.; Domaing P.; Hauser S.; Kaltschmidt B.; Kaltschmidt C.; Heilemann M.; Widera D. (2013) Simple method for sub-diffraction resolution imaging of cellular structures on standard confocal microscopes by three-photon absorption of quantum dots PLoS ONE 8 (5)

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Photo: David Ausserhofer/MDC

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Biobank

The biobank offers scientists a platform for state of the art storage and access of liquid biosamples at ultra-low temperatures. Samples are stored at temperatures below -160°C in the vapor phase of liquid nitrogen in a fully automated system. This allows high quality and reliability for storage as well as swift identification, fast access and efficient retrieval of samples for analysis. The biobank also provides advice and consultation for projects with biospecimens regarding design, planning and implementation of studies (e.g. SOPs). As part of the Berlin Institute of Health (BIH) the biobank also stores samples of BIH research projects, cohort studies and trials. In addition, the biobank stores biological samples from 30.000 participants of the Metropolitan area Berlin-Brandenburg of the German National Cohort.

Focus

The biobank is equipped to support basically any type of research study that requires storage of liquid samples at ultra-low temperatures (below -160°C). It is particularly suitable for projects that require both, the ability that collected biosamples may be stored over longer time periods but also that they may quickly be made available for analysis if necessary. For example, this includes cohort studies, clinical trials, but also experimental studies (e.g. stem cells).

Equipment and infrastructure

The biobank primarily consists of a fully-automated liquid nitrogen storage system. The samples are stored in the vapor phase of liquid nitrogen at temperatures below -160°C. To avoid repeated freeze-thaw cycles samples are preferably stored in tubes with small volumes. The system currently holds 2 large dimension liquid nitrogen tanks that have the capacity to store 2.5 million 250 µL tubes; however, the system can also hold tubes with 700 µL, 1000 µL and 2 mL. The system guarantees that there is no interruption of the cold chain during the process of transferring the samples into the biobank. The tubes with biological samples are preferably transported to the biobank on racks in boxes on dry ice. The boxes are opened in a dedicated room at a temperature of -20°C and the racks are immediately put from dry ice into a -80°C buffer system. A fully automated robot system transfers the racks to the liquid nitrogen tanks at a temperature of -20°C, and the tracks are put into the tanks for storage at temperatures below -160°C. The system also allows to store and retrieve single tubes or sets or collection of tubes. For these instances, a fully automated tube picker is integrated into the system that works at a temperature of -80°C.

The biobank is equipped with a laboratory information management system (LIMS) that is able to provide full documentation about the samples stored within the system, including but not limited to characteristics of the samples, temperature, storage condition, as well as about research projects and analysis results.

Contribution to major research initiatives

The MDC stores biological samples from 30.000 participants of the Metropolitan area Berlin-Brandenburg of the German National Cohort (GNC). The GNC includes participants from the general population aged 20-69 years who are followed up for the incidence of major chronic diseases. Initial recruitment of participants into the study, including a detailed phenotyping and collection of biological samples, has been started in 2014 and will be completed by 2019. In addition, there will be a re-assessment of all study participants between 2018 and 2023. Every 2-3 years questionnaires on lifestyle changes and new major diseases will be

Photos: Horst Krüger/MDC

collected. Mortality follow-up and systematic linkage with disease registries will be performed. The GNC is thus optimally designed to study the association of baseline biomarkers as well as changes in biomarker concentrations over time, with risk of incident diseases.

The MDC also stores biological samples from 1200 participants of the BioCog project (Biomarker Development for Postoperative Cognitive Impairment in the Elderly), which is supported within FP7 by the European Union. The aim of BioCog is to discover, identify and develop biomarkers for the prediction of postoperative cognitive impairment (POCI) in elderly people. POCI occurs frequently after surgery among persons within the general population, and includes postoperative delirium as well as postoperative cognitive dysfunction. In BioCog, biological samples are being collected before surgery as well as on different time points after surgery, thus allowing detailed investigations for prediction of adverse outcomes.

As part of the Berlin Institute of Health (BIH) the biobank also stores samples of BIH research projects, cohort studies and trials. The BIH Biobank Core Facility is one biobank at two locations (Charité CVK and MDC) with a synchronized laboratory information system (LIMS). The BIH Biobank Core Facility will store a wide range of different types of specimens collected in BIH research project under safe and quality-controlled conditions and will make them available to BIH researchers. The BIH Biobank will also provide advice and consultation for BIH projects with biospecimens regarding design, planning and implementation of studies (e.g. SOPs), data protection issues, and ethical issues. A number of services are or will be offered, such as tissue sectioning and histology as well as immunohistochemistry, DNA or RNA extraction, etc.

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Photo: David Ausserhofer/MDC

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Bioinformatics Platform, BIMSB

Bioinformatics is the application of computational methods to the management and analysis of biological data. By using bioinformatics, high-dimensional data can be translated to biological knowledge. With the advent of high-throughput technologies in biology, computational analysis needs of the labs are on the rise. This creates a challenge in method development, application and also a demand in training new individuals.
BIMSB Bioinformatics platform creates and maintains bioinformatics tools and databases, and studies biology using those in-house or publicly available computational techniques. In addition, the platform provides collaboration opportunities, helpdesk, and training for MDC scientists. The platform also provides maintanence for hardware that hosts BIMSB web apps, group compute servers and virtual machines. The platform aims to help MDC scientists address their biological questions using and developing computational methods.

Databases and web apps

The BIMSB Bioinformatics platform maintains and develops databases and web apps for bioinformatics. With the explosion of genomics data, visualizing genomics annotation in harmony with high-throughput datasets became more and more important. We are maintaining external and internal databases and web applications that contain analytical tools for bioinformatics.

We are maintaining the DoRiNA database (*http://dorina.mdc-berlin.de*) for RNA binding protein interactions; this database is associated with our local copy of UCSC genome browser (*http://genome.mdc-berlin.de*). Our local copy of the UCSC genome browser contains annotation and genome-wide datasets for human and most popular model organisms. In the last year, we started to provide space and instructions for users to link their data to the local UCSC browser for easy visualization.

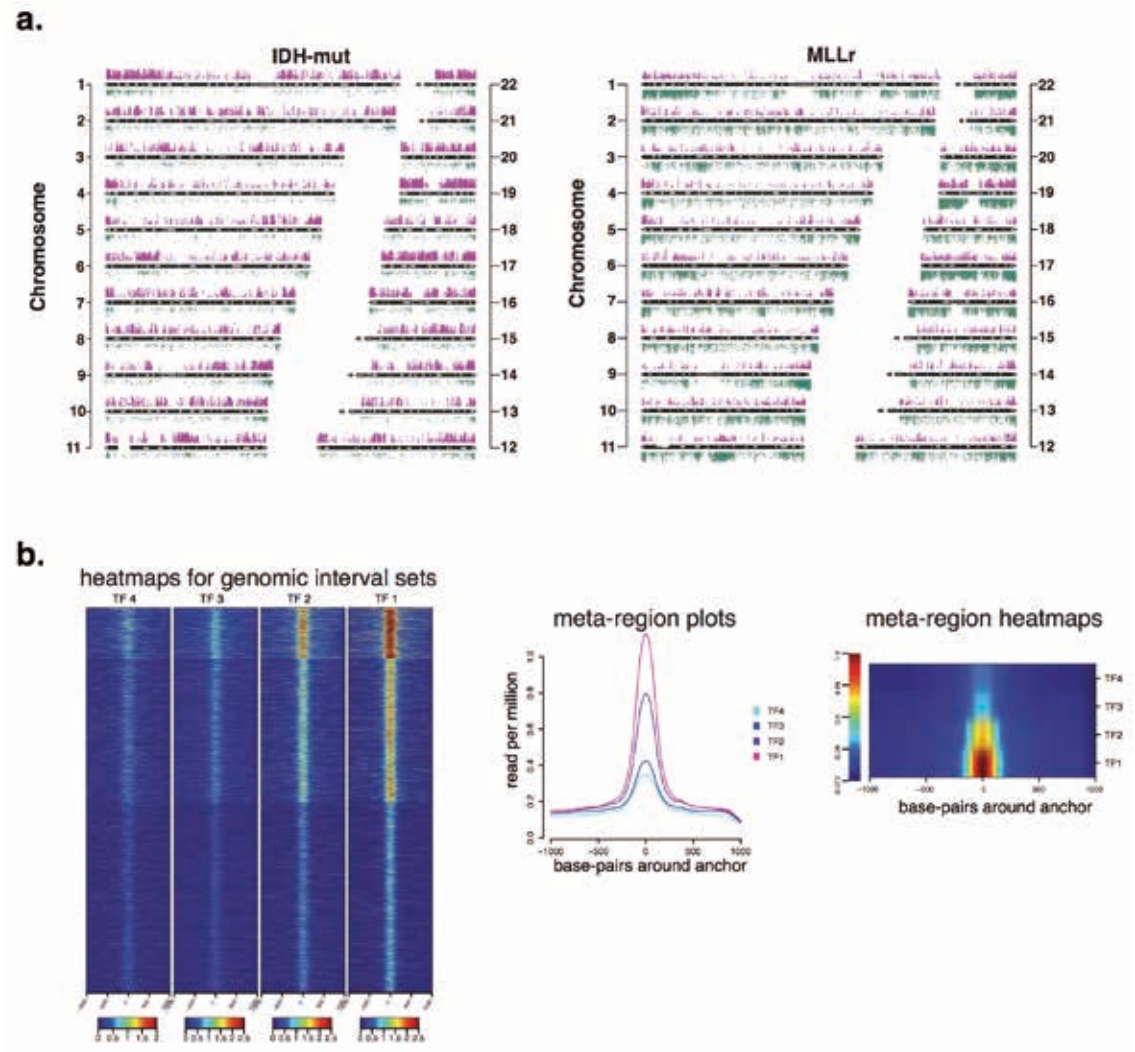
We also provide a shiny server which contains statistical web applications and interactive documents (*http://shiny.mdc-berlin.de*). The server lets users manipulate the data in real-time, they can sort, filter, or change assumptions, and customize their analysis for their specific needs.

On top of these, in 2016, we started to provide the Galaxy workbench for bioinformatics. Galaxy is a framework for doing bioinformatics without the knowledge of programming. The users simply link or upload their data to their galaxy session and select the tool and the parameters they want to run using a web browser based interface. Our Galaxy server is available at *http://galaxy.mdc-berlin.net*. Until now, we will held two tutorials on how to use the galaxy framework and these will continue in the future.

Scientific software repositories for reproducible research

Since December 2014 the Bioinformatics Platform is using GNU Guix to satisfy the requirements for scientific software environments of our HPC users. GNU Guix is an advanced functional package manager, offering support for per-user software profiles, simultaneous installation of different versions and package variants, transactional upgrades and roll-backs, and a controlled build environment to maximize reproducibility.

Over the past years, we have added hundreds of package definitions for tools and libraries used in the field of bioinformatics, statistics, and machine-learning to the quickly growing collection of scientific software in Guix. The packages available are listed at *http://guix.mdc-berlin.de*



Guix allows us to offer tailor-made bioinformatics software environments for individual users, projects, and whole groups. These environments are portable across different machines, easily reproducible, and safely isolated from changes, providing a reliable basis for reproducible research.

Consultation and training

The platform also offers knowledge-base, help-desk and training for MDC scientists. We provide a regular help-desk session, called "Bioinformatics walk-in clinics", where users are welcome to ask any bioinformatics question and would not need a prior appointment. We can direct users to right tools and methods or different groups who have the right expertise. In addition, this avenue could be used to initiate collaborations.

The platform offers regular training opportunities, such as courses on R programing, Unix and SGE, and version controls system git. Since 2015, we organize international hands-on courses on computational genomics. Most of the applicants for these courses are from other institutes abroad and in Germany. In total, we have provided 11 courses/tutorials to 188 participants.

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Photo: Silke Oßwald/FMP

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Photo: Silke Oßwald/FMP

Marc Nazaré

Chemical Biology Platform

Screening Unit – Medicinal Chemistry – Drug Design

The Chemical Biology Platform provides interdisciplinary scientific support in Screening, Medicinal Chemistry and Computational Chemistry and is jointly operated by the MDC and the Leibniz Institut für Molekulare Pharmakologie*, which is located on our joint Campus in Berlin-Buch. Jens Peter von Kries, Marc Nazaré and Ronald Kühne provide expertise in the identification and chemical optimization of drugs and the generation of chemical tools for proof-of-concept studies or of labeled biosensor molecules. A broad range of state of the art technologies for High-Throughput-Screening in small molecule libraries actually containing about 66.000 drug-like compounds or in RNAi-libraries targeting human or mouse genomes are available for screening approaches. The platform enables to identify and optimize bioactive small molecules as tools to unravel molecular mechanisms in health and disease and contributes to identify and validate novel therapeutic targets.*

Screening Compound Collections (Small Molecule Drugs & RNAi)

The screening compound collections are continuously curated and extended and provide currently more than 66.000 compounds. About 30.000 compounds have been selected for screening according to their structural similarity with drugs from the World Drug Index containing substructures of approved drugs. Additional 3.300 compounds are identical with approved drugs or experimental clinical candidates suitable for a potential drug repurposing. 9.000 compounds are donations from a network of chemists and 20.000 compounds were derived from a natural product collection from Analyticon Discovery.

The genome wide RNA-interference collections (siRNA) target mouse (four siRNA molecules per gene, pooled) or human genomes (three separate siRNA molecules). From 40.000 (mouse) to 140.000 (human) transfections are required for a genome-wide screen, if not limited to the so called “druggable genome”.

Highlight Projects

Chronic Pain and Mechano-Receptors

Mechanoreceptors in the skin allow the perception of the slightest brush by transforming movement into electrical signals controlled by the stomatin-like protein-3 (STOML3). In collaboration with the group of Gary Lewin we identified small-molecule inhibitors of STOML3 oligomerization reducing the sensitivity in sensory neurons and silencing mechanoreceptors in vivo which may allow to treat tactile-driven pain following neuropathy.

Identification of the gene for the Volume-Regulated Anion Channel VRAC

The group of Thomas Jentsch and the Screening Unit identified a long-sought gene encoding for a channel protein that regulates cell volume. For this purpose, mRNA of 21.687 human genes was targeted by ~140.000 transfections with interfering RNA using a cellular reporter system for iodide influx and intra-cellular quenching of an iodide-sensitive yellow fluorescent protein. The Volume-Regulated Anion Channel was stimulated by a change of medium containing different salt conditions. The inhibition of quenching provided a read out for high-speed kinetic imaging and the identification of LRRC8 heteromers as essential components of VRAC.

Drugs for functional dissection of different paths for NFKB activation

The laboratory of Claus Scheidereit has a major interest in understanding the molecular

mechanisms in regulation of NFKB signaling in development and disease like in cancer or inflammation. Applying High-Content-Screening with automated microscopes and the fluorescent detection of NFKB translocation to the nucleus, we identified drugs which specifically interfere only with DNA induced NFKB activation and therefore provide unique tools to functionally dissect the molecular mechanisms behind. The drugs identified were further validated by chemical synthesis and derivatization. Iterative rounds of synthesis, testing and analysis showed a well-defined structure-activity-relationship which will assist the identification of the target proteins.

Development of specific inhibitors of the tyrosine phosphatase Shp-2

In stark contrast to their validated significance in signal transduction and disease pathology, phosphatases are notoriously difficult to inhibit using small molecules. The protein tyrosine phosphatase Shp-2 plays a critical role in growth factor-mediated processes, primarily by promoting the activation of the RAS/ERK signaling pathway. Aberrant gain-of-function mutations are associated with several metastatic cancers. In collaboration with Walter Birchmeier, a re-scaffolding approach that involves replacing the former framework of a tyrosine phosphatase Shp2 inhibitor (W. Birchmeier et al., PNAS, 2008), led to the discovery of novel structural classes and eliminated several chemical liabilities, i.e. unfavorable structural features. These novel compounds are not only active in a sub-micromolar range in the Shp2-enzyme assay, but are also effective in the low micromolar range on hepatocyte growth factor (HGF)-stimulated canine MDCK-C cells, as well as human pancreatic tumor cells for epithelial-mesenchymal transition (EMT), a hallmark of cancer cell dissemination.

Tryptophan hydroxylase (TPH) inhibitors

The neurotransmitter serotonin [5-hydroxytryptamine (5-HT)] is causally involved in multiple aspects of mood control in the central nervous system, such as regulating sleep, anxiety, drug abuse, and food intake. In peripheral tissues, serotonin regulates vascular tone, gut motility, primary hemostasis, and cell-mediated immune responses, and is associated with diseases like irritable bowel syndrome and carcinoid syndrome. The biosynthesis of serotonin is a highly-regulated two-step process, starting with the essential amino acid L-tryptophan (Trp), while tryptophan hydroxylase (TPH) is the initial and rate-limiting enzyme in the biosynthesis of serotonin. In collaboration with the groups of Michael Bader and Udo Heinemann (both MDC), and Jens von Kries (FMP), we have identified and further developed highly active TPH inhibitors that are able to modulate physiological serotonin levels. The X-ray co-crystal structures obtained with our inhibitors allowed us to elucidate the binding mode and to reveal the structural determinants for the remarkably efficient protein-ligand interaction of these inhibitors. Several inhibitors are currently undergoing in vivo efficacy studies in mice.

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Team funded by FMP, third party or **MDC ***BIH.

Photo: David Ausserhofer/MDC

Bettina Purfürst

Electron Microscopy

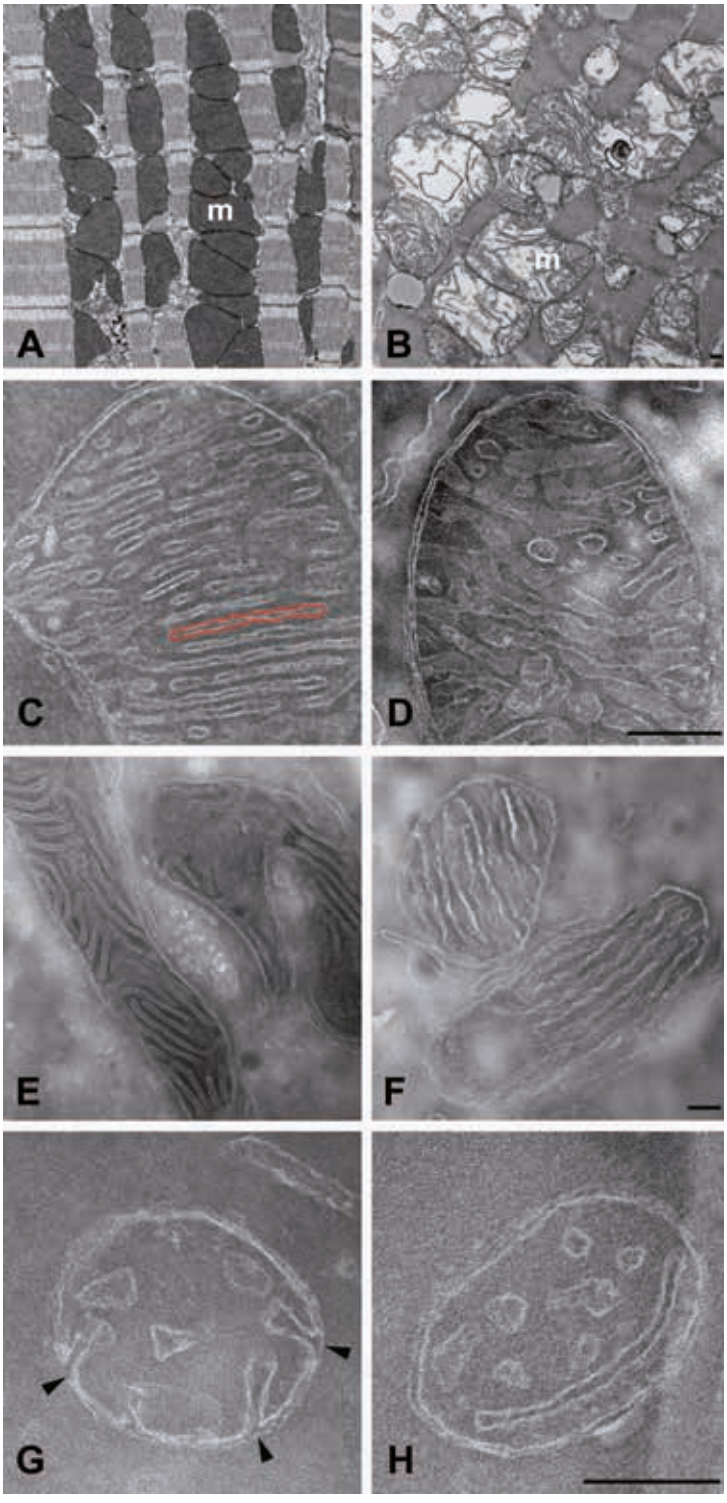
In the period reported, we realized more than 25 EM-projects from nearly all research areas of the institute and the related organizations. There is a constant high demand for phenotyping on the ultrastructural level, mainly with the mouse system and cell cultures, but also with other specimen like the naked mole rat, Drosophila and yeast. Interestingly, we had a range of projects to the ultrastructure of mitochondria over the last years, and I will highlight some of these issues and results here:

Mitochondria are rich of proteins and appear as darkly stained, compact organells following classical plastic embedding and contrast (A, heart tissue). Following even short periods (30 min !) of stress like oxygen shortage, tissue damage or insufficient fixation conditions they tend to swell or even burst, as shown in B for heart tissue after an experimentally induced cardiac infarction (collaboration with M. Gotthardt). This has to be considered carefully in phenotyping (projects on heart and skeletal muscle with the groups of G. Lewin, I. Morano, T. Röpke, A. Heuser, B. Schröder, E. Klussmann and J. Fielitz).

The inner membrane structure of mitochondria is, even in strongly fixed classical EM-preparations, not always preserved well. Cryosectioning according to Tokuyasu yield here much better results (C-H). With this method, the membranes appear white, as known from negative contrast, and show much more details. In normal, wild type tissue the arrangement of the inner mitochondrial membranes can vary considerably, as shown for heart (C) and brain cortex (D), and can be quantified in most cases (projects with G. Lewin and E. Klussmann).

In cell cultures, the inner mitochondrial structure is much more difficult to visualize, although single cells should be better accessible for the fixans than tissue. Cell cultures are always artificial systems and the cells may often suffer from suboptimal conditions concerning the medium, oxygen supply, cell density, passage number and others. In many cases, we could not confirm the “good morphology in the light microscope” on the ultrastructural level. Cells in culture often grow suboptimal and finally tend to die, as shown for the mitochondrial structure in MCF-7 cells in E and F (collaboration with W. Chen, other projects with O. Rocks, F. Wanker and O. Daumke).

Recently, we have started again to analyze ultrathin cryosections from yeast, following the protocols developed by Frank Vogel years ago. And we could immediately show clear differences in the inner membrane architecture: in some mutants, the contacts between the outer and inner membranes were lost (G and H, collaboration with O. Daumke).



EM-images from different projects showing mitochondria A,B: mouse heart, **plastic embedding**, *mitochondria (m) are compact, darkly stained organells (A), which tend to swell under stress (B, induced cardiac infarction)*
C-H: **cryosections**, *mouse heart (C) and brain (D), MCF-7 cell cultures, growing optimal (E) or too dense (F), yeast cells depicting the transitions between the outer and inner membranes in the wild type (G, arrowheads) which lack in mutants (H)*
Bar = 200 nm

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S Waiczies, S Lepore, K Sydow, S Drechsler, M-C Ku, C Martin, D Lorenz, I Schütz, HM Reimann, B Purfürst, MA Dieringer, H Waiczies, M Dathe, A Pohlmann, T Niendorf. Anchoring dipalmitoyl phosphoethanolamine to nanoparticles boosts cellular uptake and Fluorine-19 magnetic resonance signal. Sci Reports 5:8427 (2015)

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Photo: privat

Sascha Sauer

Genomics, BIMSB

Novel technologies such as next generation sequencing have revolutionized genome research. The Scientific Genomics Platform at the MDC focuses on development and implementation of new methods and their application in pilot studies to enable the provision of cutting-edge methodologies for a broad range of life science researchers. The platform consists of established units for diverse genomics applications – particularly using highly parallel sequencing and associated bioinformatic data analysis. In general, the platform has established and continuously provides support to cater a large spectrum of state-of-the-art sequencing technologies and protocols, which are relevant for systems wide approaches to the genome, the epigenome and the transcriptome. This includes methods ranging from whole genome (re) sequencing, targeted genome sequencing, various epigenomic profiling methods to the many variants of transcriptome sequencing. A particular future focus of the group lays on single cell genomics approaches. The established technology platforms are open to internal and external scientists and were very recently extended in capacity and biomedical applications through tight scientific cooperation with the Berlin Institute of Health. The platform especially supported and collaborated with numerous research groups at the MDC and the Charité, as well as with other external institutions such as the DKFZ and the IZW. Further information can be found at: http://genomics.mdc-berlin.de

NGS instruments and applications

The Scientific Genomics Platform is currently equipped with short-read next generation sequencing systems from Illumina including two HiSeq 4000, one HiSeq 2500, three NextSeq 500 (Illumina), and one MiniSeq. Moreover, for long-read sequencing SMRT instruments from Pacific Biosciences such as an RSII and a very recently purchased Sequel system are available. So far, the RSII has for example been used for specific projects such as whole genome sequencing of organisms and for novel approaches for lineage tracing using long-read sequencing of large PCR products.

Whole-genome sequencing

The Illumina Nextera whole-genome library preparation kit features the rapid creation of gDNA libraries from very low input sample concentration (> 50ng genomic DNA). During library creation, a process termed 'tagmentation' simultaneously fragments and tags DNA, making fragments amenable to direct PCR amplification. A sequencing output of ~80Gb per genome sample, which is required to cover the whole genome with >20x, can be achieved by generating ~200 millions paired-end 2x100/150nt reads.

Exome sequencing

The Agilent SureSelect exome enrichment system is routinely used to prepare exome sequencing libraries. With a sequencing output of ~10Gb, an average of ~80% of all reads fall within the targeted regions+/- 100bp and ~95% of all targeted bases are covered >= 20x. A sequencing output of ~10Gb per exome sample can be achieved on a thoroughly optimized workflow using the HiSeq systems and the SureSelect V5 enrichment system, generating ~200 million single end 100/150nt reads for each probe. Furthermore, newer methods such as as Nextera Rapid Capture Exome are being used.

RNA-seq
A large number of varying protocols are being applied to analyze RNA at large-scale. RNA samples are processed in content-defined batches, and standardized single-end or paired-end libraries are created using established protocols (e.g. the optimized Illunina Truseq Chemistry, and never protocols provided by alternative vendors). Depending on the project, various sequencing lengths and depths are being applied using the most efficient short-read sequencing devices for total or for RNA sequencing, respectively. Future development will be dedicated to decrease the amount of required RNA material, increasing throughput by appropriate robotics and reducing costs.

Small RNA-seq
Starting from usually 1 µg total RNA, a size selection is performed for RNAs between 18 and 30 nt to enrich for small RNAs, including miRNAs. Subsequent library preparation steps comprise: adapter ligation and PCR amplification. The final small RNA library is obtained by a further size selection step. Usually 1x50nt single-end is performed with a minimum of few million reads per sample, or more, depending on the research question.

ChIP-seq
Starting from about 10 ng of ChIP-DNA, the following library preparation steps are performed: end-repair, a-tailing, adapter ligation, purification and a final PCR enrichment. After quality control of the sequencing library, up samples are labeled with unique barcodes and pooled for sequencing. Usually 1x50nt single-end sequencing with a minimum output of 40 million reads for each sample is performed. Future development will be dedicated to decrease the amount of ChIP-DNA material, increasing throughput by appropriate robotics and reducing costs.

DNA methylation analysis
Methylome–wide analysis was mainly performed using Reduced Representation Bisulfite Sequencing (RRBS). The RRBS method was selected because DNA methylation pattern can be analyzed on the nucleotide level in CpG island and CpGI shores with a coverage of around 10 % of all CpG positions. Moreover this method is feasible with a very low amount of DNA for sequencing a minimum of 50 million reads for each samplewith of 100 nt lenghts. Recent developments were dedicated to apply Whole Genome Bisulfite Sequencing (WGBS).

Single cell applications
The group continuously implements new methods. Since autumn 2016 in particular various complementary single cell genomics approaches were set up including:
- Fluidigm C1
- FACS-seq *(based on BD Aria III and Melody)*
- Dolomite's Drop-seq system
- 1CellBio's inDrop system
- 10X Genomics' Chromium system

The focus was so far on single cell transcriptome analyses. In the future the group aims to extend the portfolio to DNA analyses on the level of single cells, as well as on simultaneous analysis of (epi)genomic and transcriptomics features.

In addition to providing Scientific Genomics Platform, as the predecessor of the group leader position, Prof. Wei Chen, Dr. Sauer is pursuing independent, third-party-funded research his associated Laboratory of Functional Genomics, Nutrigenomics and Systems Biology.

Selected Publications

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Photo: David Ausserhofer/MDC

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Magnetic Resonance (B.U.F.F.)

B.U.F.F. builds on collaborations with our partners from the Charité, the Leibniz Institute for Molecular Pharmacology (FMP), the German Metrology Institute (PTB), Siemens Healthcare, Bruker Biospin and other (inter)national institutions. To learn more about the mission of B.U.F.F. please visit https://www.mdc-berlin.de/BUFF.

Equipment and research infrastructure

B.U.F.F. provides state-of-the-art infrastructure for interdisciplinary imaging projects and is equipped with small animal (9.4 T) and whole-body human (7.0 T & 3.0 T) MR scanners **(Figure 1)**. B.U.F.F. runs an S1 certified laboratory and supports small scale animal housing. To foster translational research and clinical studies B.U.F.F. is equipped with reception areas and changing rooms to comfort and guide volunteers and patients **(Figure 2, left)**. All technical prerequisites needed for clinical studies are setup including emergency equipment and extra patient monitoring units. Members of B.U.F.F. have access to infrastructure for hardware and MR pulse sequence development. **(Figure 2, right)**.

Figure 1: *Picture photographs illustrating the of the whole-body human (7.0 T & 3.0 T) and small animal (9.4 T) MR scanner environments at B.U.F.F.*

Figure 2: *Overview of the volunteer/patient preparation areas* **(top left)**, *the MR hardware development laboratories* **(top right)** *and the clinical imaging setup* **(bottom left)** *available at B.U.F.F.* **bottom right**: *Members of B.U.F.F. congratulating the 1000th volunteer of the population imaging study of the national cohort (NAKO) for supporting epidemiological and medical research.*

Research focus

Research at B.U.F.F. concentrates on the development of enabling MR technology through Thoralf Niendorf's group on experimental UHF-MR (1-4). The focus is on new ways of mapping anatomy, morphology, microstructure, function, physiology and metabolism. These efforts are designed to characterize (patho)physiological and biophysical processes to promote transfer from basic research to (pre)clinical studies and vice versa. To highlight the anatomical detail and spatial resolution capabilities of MRI **Figure 3** surveys examples of *in vivo* mice phenotyping and from *in vivo* human imaging. The hard work of B.U.F.F. members resulted in 65 peer-reviewed publications during 2014-16, 70+ abstract contributions to the 2014/15/16 ISMRM meetings plus one US patent. The head of B.U.F.F. (Thoralf Niendorf) recently received an **ERC Advanced Grant** by the European Research Council.

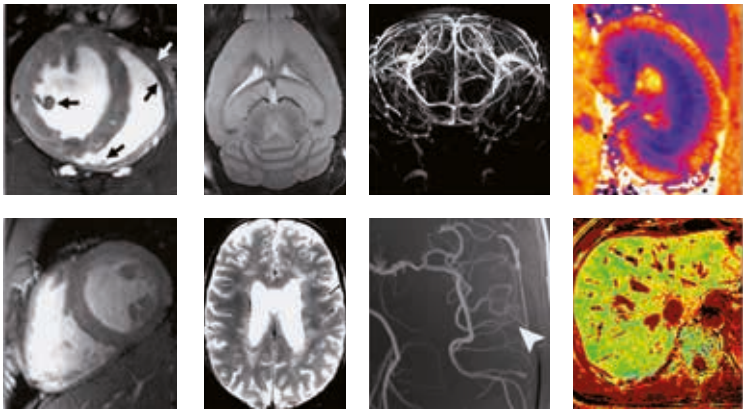

Figure 3: *Illustration of the anatomical detail provided by non-invasice MRI.* **Top:** *In vivo MRI of the mice heart (resolution 50 µm), of the mice brain (resolution 30 µm), of the vessel tree of the mice brain (resolution 30 µm) and of the mice kidney (resolution 30 µm).* **Bottom:** *In vivo MRI of the human heart (resolution 1.0 mm), of the human brain (resolution 0.5 mm), of the vessel tree of the human brain (0.5 mm) and of the human liver (1.0 mm) obtained at 7.0 T.*

Contributions to major research initiatives

Members of B.U.F.F. form a key/integral part of major research initiatives and support the Helmholtz Alliance for Imaging and Curing Environmental Diseases (ICEMED), the DFG research group FOR 1368 on *Hemodynamics of Acute Kidney Injury*, the imaging program of the *German Center for Cardiovascular Research* (DZHK), the *individualized Medicine (iMED)* initiative of the Helmholtz Association, the BMBF project on targeted radiofrequency heating (*THERAHEAT*), the BMBF funded validation project on the diagnosis of renal diseases (*renalMROXY*), the *German Ultrahigh Field Imaging* (GUFI) network, the *EU COST Action PARENCHIMA* on renal imaging, the DFG funded project on Fluorine MR technologies to study cellular therapies, the *Marie Sklodowska Curie project SODIUMMRI-4-EU* (EU project 752489), the DFG funded *graduate school on quantitative imaging* (BIOQIC) and the *European Ultrahigh-Field Imaging Network in Neurodegenerative Diseases* (EUFIND). B.U.F.F. is part of the imaging facilities of the Berlin Institute of Health (BIH).

German National Cohort at B.U.F.F.

B.U.F.F. is home of the Berlin site of the imaging program (5) of the German National Cohort (NAKO) which accommodates 30.000 volunteers to be included in a comprehensive MR examination. 6000 of these volunteers are examined at B.U.F.F. This team recently celebrated the examination of the 3000th volunteer **(Figure 3)**.

Teaching and education initiatives

Members of B.U.F.F. are chairing and organizing the *Annual Symposium of Ultrahigh Field Magnetic Resonance: Clinical Needs, Research Promises and Technical Solutions* which attracts about 250 MR technology leaders and distinguished clinical experts, all bridging disciplinary boundaries. On behalf of the European Society for Magnetic Resonance in Medicine and Biology members of B.U.F.F. chaired and organized a one week workshop *RF coil design:* Build your own which is fully (over)booked every year.

Public engagement initiatives

B.U.F.F. actively contributes to public engagement with open tours to the *'Lange Nacht der Wissenschaften' (Long Night of Science).* B.U.F.F. is also engaged into disseminating its research to the public by supporting the *Life Science Learning Lab* (German: "Gläsernes Labor"). B.U.F.F. runs a very fruitful partnership with the *Robert-Havemann Gymnasium,* Berlin which we support through school presentations, on-site visits and lab-tours. For further details about B.U.F.F.s public engagement please visit *www.mdc-berlin.de/BUFF*.

Basic research and clinical science opportunities

The B.U.F.F. team is always looking for collaborators committed to the value and power of non-invasive imaging. We are ready to enter into fair collaborative enterprises aiming at moving the needle by first class imaging sciences and by attracting extramural funding with the goal to foster multi-disciplinary and translational research at the MDC. Further details please visit www.mdc-berlin.de/BUFF or contact the head of B.U.F.F. (Thoralf Niendorf, *thoralf.niendorf@mdc-berlin.de*, +49 30 9406 4505).

Selected Publications

Niendorf T, Paul K, Oezerdem C, Graessl A, Klix S, Huelnhagen T, Hezel F, Rieger J, Waiczies H, Frahm J, Nagel A, Oberacker E, Winter L. W(h)ither Human Cardiac and Body Magnetic Resonance at Ultrahigh Fields? Technical Advances, Practical Considerations, Applications, and Clinical Opportunities NMR Biomed 2015, DOI: 10.1002/nbm.3268 [epub ahead of print].

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Niendorf T, Pohlmann A, Reimann HM, Waiczies H, Peper E, Huelnhagen T, Seeliger E, Schreiber A, Kettritz R, Strobel K, Ku MC, Waiczies S. Advancing Cardiovascular, Neurovascular, and Renal Magnetic Resonance Imaging in Small Rodents Using Cryogenic Radiofrequency Coil Technology. Front Pharmacol 2015;6:255.

Niendorf T, Pohlmann A, Arakelyan K, Flemming B, Cantow K, Hentschel J, Grosenick D, Ladwig M, Reimann H, Klix S, Waiczies S, Seeliger E. How bold is blood oxygenation level-dependent (BOLD) magnetic resonance imaging of the kidney? Opportunities, challenges and future directions. Acta Physiol (Oxf) 2015;213(1):19-38.

Bamberg F, Kauczor HU, Weckbach S, Schlett CL, Forsting M, Ladd SC, Greiser KH, Weber MA, Schulz-Menger J, Niendorf T, Pischon T, Caspers S, Amunts K, Berger K, Bulow R, Hosten N, Hegenscheid K, Kroncke T, Linseisen J, Gunther M, Hirsch JG, Kohn A, Hendel T, Wichmann HE, Schmidt B, Jockel KH, Hoffmann W, Kaaks R, Reiser MF, Volzke H. Whole-Body MR Imaging in the German National Cohort: Rationale, Design, and Technical Background. Radiology 2015:142242.

Group Leader
Prof. Dr. Thoralf Niendorf

Secretary
Rosita Knispel

Photo: Christian Kruppa/BIH

Jennifer Kirwan

Metabolomics, BIH Platform at MDC

The Berlin Institute for Health Metabolomics Facility undertakes metabolomics research with clear translational medicine potential. A dedicated and highly skilled team, we collaborate with researchers and clinicians at the Charité and MDC to advance medical knowledge, especially the interplay between heart and brain health.

We are equipped with state-of-the-art, high resolution, highly mass accurate mass spectrometry instrumentation enabling both targeted and non targeted metabolomics to be undertaken. An automated sample preparation robot and a separate derivatisation robot enables robust and reproducible epidemiological scale analyses to be undertaken.

Technology and applications

Metabolomics aims for a global analysis of all metabolites within a cell or biological system (for example, blood, urine, cells, saliva etc) and measures the perturbations that occur in disease states or other changes in conditions e.g. nutrition, exercise, different genotypes etc. A variety of mass spectrometers and related instrumentation is used to enable to enable a wide range of molecules to be measured, often at widely variable concentrations and with very different physicochemical properties.

Facilities include:

- Leco GCxGC-Electron impact-Time of Flight mass spectrometer: a gas chromatography based system that allows the measurement of small molecules and is especially useful for polar molecules. A previously validated method enabling a quantitative, targeted analysis of up to nearly one hundred defined metabolites involved in central carbon metabolism is possible on this system.
- Thermo Quantiva: a triple quadrupole mass spectrometer allows the measurement of small molecules and lipids. Its fast scanning speeds make it particularly useful for targeted analysis and it can be combined with liquid chromatography for prior separation of complex biological matrices.
- Thermo Q-Exactive (based on Orbitrap technology): a high resolution, highly mass accurate Fourier transformation mass spectrometer that is used for lipidomics and metabolomics analyses
- Agilent 1290 two-dimensional liquid chromatography system: for detection, identification and separation of molecules with similar chemical properties: especially useful for lipids
- Owlstone Field Asymmetric Ion Mobility Spectrometer: an ion mobility technology that enables separation of compounds based on cross sectional area to enable improved analysis by mass spectrometry of mixed isomeric species.
- To support epidemiological size metabolomics studies we have developed a robotic system to facilitate the preparation of the required standard mixtures for metabolomics analyses and to perform the extraction and sample preparation procedures in an automated way. This robotic system is a significant step towards lab automation, as it includes the handling of solid substances and liquids in a single system specifically designed for our workflows.

Collaborations

We are currently collaborating with several different research groups, both local and international. Examples of projects we are currently involved in include: the metabolomics changes seen in Alzheimer models as the disease develops, the metabolomics of depressive illness and the lipid changes associated with heart disease. We concentrate on projects that have relevance to improving people's health or quality of life.

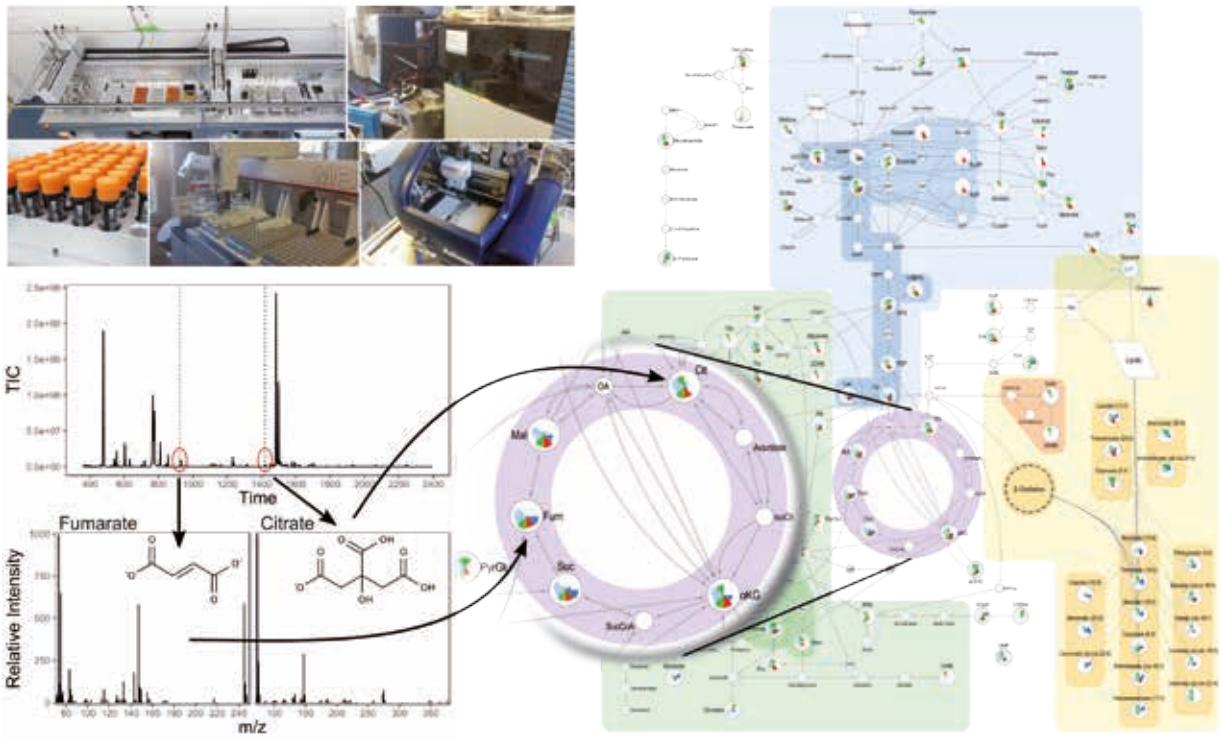


Figure 1: The Berlin Institute for Health Metabolomics Facility is composed of a range of state-of-the-art technologies which are used in combination to globally map the metabolome (the range of metabolites in a biological system) and assess how the metabolome is altered by disease states.

Recognising the importance of the "value added" approach of systems biology, we work closely with other -omic platforms to improve the biological information we gain from any individual study. This is particularly notable with our close collaboration with the Bioinformatics Core Facility where strategic cooperation to embed staff members in each group will enable the transfer of knowledge between both facilities.

External to the MDC, we support the development of precision medicine to improve medical treatment and prognosis. The Head of the Facility, Dr Jennifer Kirwan is a member of the Working Party in Precision Medicine and Pharmacogenomics for the international Metabolomics Society.

Method development

Metabolomics is still a relatively young science and methods are constantly being developed to enlarge the range and extent of the analyses we can perform. Over the next few years, we are working closely with other groups to develop and validate new methods to answer specific research problems. The majority of these methods will have applications to other projects thus enabling a rapid expansion of the abilities of the Metabolomics Facility.

Selected Publications

The Head of the Group took over only two months before the end of this reporting period, but we anticipate new publications will follow very soon!

Start of the Group: October 2016

Group Leader
Dr. Jennifer Kirwan

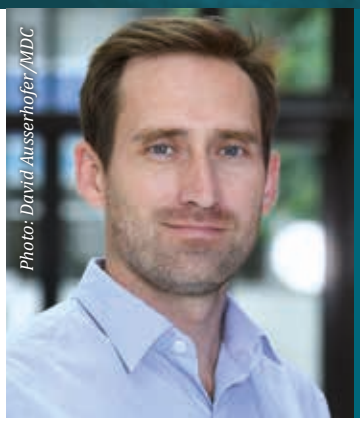


Photo: David Ausserhofer/MDC

Andrew Woehler

Light Microscopy Platform, BIMSB

Light microscopy has long been an essential technology for the progress of life sciences research. Advances in this technology have kept pace with the demands of modern investigation. We now have the ability to monitor the structure and function of living systems at scales that vary by orders of magnitude. Modern light microscopy allows us to monitor the development of whole organisms, the structure and function of single cells, as well as the localization and dynamics of single molecules.

The BIMSB Light Microscopy Platform was recently established to support research projects for MDC scientists at the new BIMSB research center in Berlin Mitte. The new facility will host several high-end instruments including confocal, TIRF, live-cell, light sheet, and super resolution microscopes. We will also provide expert support for project planning, sample preparation, data analysis and visualization as well as training for advance fluorescence methods such as FRET, FLIM, FRAP, FCS. In addition to hosting core light microscopy technology, the platform will work towards microscopy-based solutions for systems biologists that include automated high-throughput microscopy, as well as development of computational methods for the analysis and management of large biological imaging data sets.

Research Focus

In addition to providing support for MDC and BIMSB research projects, our group is continuing to development microscopy-based approaches to study the regulatory mechanisms synaptic plasticity. The ability of the neuronal synapse to weaken or strengthen over time is key to information processing and storage in the CNS. Changes in the efficiency of synaptic transmission that occur on the millisecond to second time scale, known as short term plasticity (STP), are regulated by a number of processes at the axon terminals (or presynaptic boutons). Here, upon arrival of an action potential, synaptic vesicles (SVs) fuse with the plasma membrane releasing neurotransmitters into the synaptic cleft where they bind to post synaptic receptors to propagate signal. Vesicular membrane and protein contents are then endocytosed, recycled, and refilled

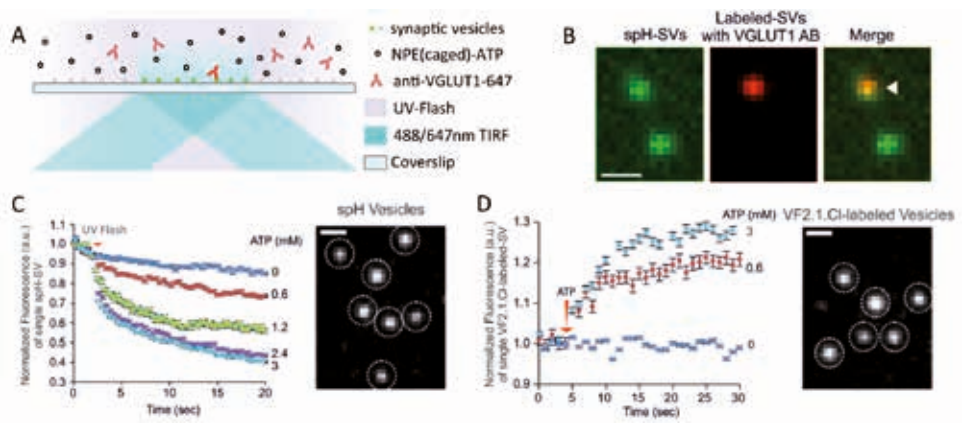


Figure 1. Electrochemical gradient measured at the single vesicle level. A) *A schematic representation of labeled SVs imaged using a custom-built wide-field UV-flash uncaging / TIRF microscope.* **B)** *SV phenotyping is performed by on-stage immuno-labeling against VGLUT1 or VGAT.* **C)** *Averaged spH response to NEP-ATP uncaging is shown with a representative image of spH-SVs.* **D)** *Averaged VF.2.1.Cl response and a representative image of VF2.1.Cl-labeled SVs are presented.*

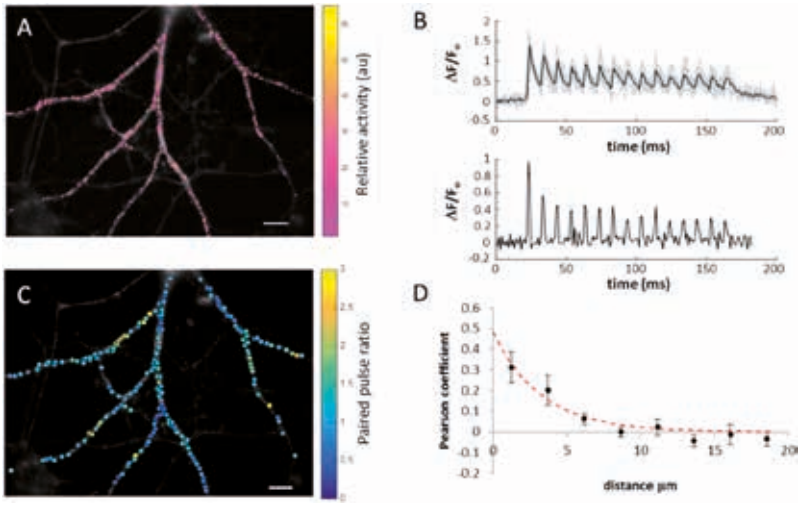


Figure 2. Imaging synaptic release at hippocampal boutons. A) *Primary neurons expressing a glutamate sensor were imaged, during which 15 APs were elicited at 10 Hz. Pseudo-color map represents synaptic release.* **B)** *Traces from repeated stimuli sampled from a single synapse are plotted in gray, the mean in black, and the deconvoled amplitudes below.* **C)** *PPR is mapped back to the respective synapse.* **D)** *Analysis of spatial correlation reveals a PPR length constant of 4.1 µm.*

with neurotransmitter before they are able to again contribute to synaptic transmission. We are currently investigating several steps in this cycle including acidification and refilling of SVs with neurotransmitters as well as the priming before fusion.

Regulation of the SV proton electrochemical gradient

In order to maintain robust synaptic function, SVs must be refilled with thousands of neurotransmitter molecules within seconds after endocytosis. This process is fueled by a proton electrochemical gradient generated across the SV membrane by the vacuolar ATPase. It is unclear how transmitter molecules that carry different net charges can be efficiently sequestered by a common mechanism while maintaining charge neutrality and osmotic balance. We developed an approach to study the generation and maintenance of proton electrochemical gradient in single SVs. By imaging purified SVs labeled with either luminal pHluorin or VF2.1.Cl under TIRF illumination we are able to reliably characterize changes in pH or membrane potential, respectively (Fig 1). By coupling in epiflorescent UV excitation we are able to flash uncage NPE(caged)-ATP and measure the fast kinetics of acidification and membrane potential generation in these diffraction limited organelles. Furthermore we perform on stage immuno-labeling to characterize the molecular composition of the SVs measured (i.e. Glutamaterigics vs GABAergic) to study how the presence and abundance of different SV constituents affect the proton electrochemical gradient that drives neurotransmitter refilling (Farsi et al. 2016).

Optical readout for synaptic plasticity

Glutamatergic synapses show large variations in strength and short-term plasticity (STP). The current state of the art approach to studying STP relies on patch clamp-based electrophysiology. In addition to recording postsynaptic response, careful investigation of STP requires precise control of single presynaptic boutons. This has limited such investigations to large synapses such as the calyx of held and hippocampal mossy fiber boutons. Optical approaches utilizing calcium and pH sensitive reporters have been developed to help address these limitations however do not provide sufficient SNR nor temporal kinetics to monitor STP. We have successfully developed an approach using a genetically encoded glutamate sensor to directly monitor the release of glutamate into the synaptic cleft (Fig 2). This approach allows us to monitor stimulus trains at 100's of synapses simultaneously to characterize properties such as paired pulse facilitation (Taschenberger et al. 2016). We are continuing the development of this and other microscopy-based approached to offer greater insight into the molecular machinery and signaling processes at play during STP at the many synapses inaccessible by electrophysiology-based techniques.

Selected Publications

Farsi Z, Woehler A. Imaging Activity-Dependent Signaling Dynamics at the Neuronal Synapse Using FRET-Based Biosensors. *Methods Mol Biol.* 2017;1538:261-275.

Taschenberger H, **Woehler A**, Neher E. Superpriming of synaptic vesicles as a common basis for intersynapse variability and modulation of synaptic strength. *Proc Natl Acad Sci U S A*. 2016 Aug 2;113(31):E4548-57.

Farsi Z, Preobraschenski J, van den Bogaart G, Riedel D, Jahn R, **Woehler A**. Single-vesicle imaging reveals different transport mechanisms between glutamatergic and GABAergic vesicles. *Science*. 2016 Feb 26;351(6276):981-4.

Woehler A, Lin KH, Neher E. Calcium-buffering effects of gluconate and nucleotides, as determined by a novel fluorimetric titration method. *J Physiol*. 2014 Nov 15;592(22):4863-75.

Hua Y, **Woehler A**, Kahms M, Haucke V, Neher E, Klingauf J. Blocking endocytosis enhances short-term synaptic depression under conditions of normal availability of vesicles. *Neuron*. 2013 Oct 16;80(2):343-9.

Start of the Group: May 2016

Group Leader
Dr. Andrew Woehler

Scientists
Dr. Zohreh Farsi
Marie Walde

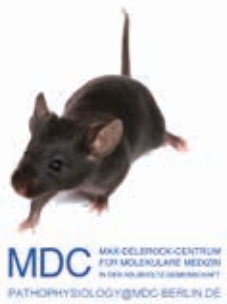
Technical Assistant
Agnieszka Klementowicz

Photo: Peter Himsel/MDC

Arnd Heuser

Pathophysiology

The Pathophysiology Platform provides MDC researchers with a central resource for the study of animal disease models. We use validated approaches and state-of-the-art instrumentation that allow for sensitive screening of phenotypic variations. Despite numerous advances in research techniques that avoid the use of animals, such as in vitro methods and advanced computer modelling, there is still the need for a whole system approach to preclinical research and for data from conscious and unconscious animals.

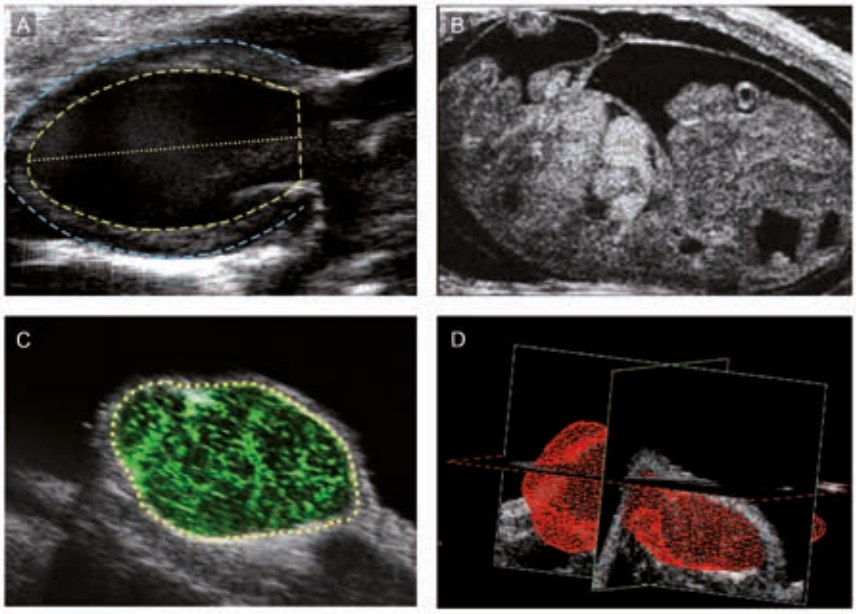


Veterinary Blood Tests
The Pathophysiology Platform can assist research investigators who would benefit from the availability of services that replicates for animals the laboratory tests that are routine for humans. Examples include blood chemistry, urinalysis, and hematology.

Understanding the complex physiological interactions between the cardiovascular, respiratory and central nervous systems is critical for the development of many human pharmaceuticals and therapies. We focus on techniques which minimize animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing future use of animals. As such, we use a variety of non-invasive in vivo test tools that allow monitoring of physiological and bioelectrical variables (e.g. blood pressure, heart rate, ECG and respiration) in conscious animals. Non-invasive, whole body imaging of small animals using techniques such as High-Frequency Ultrasound and Time-Domain-Nuclear-Magnetic-Resonance imaging is helping to reduce the number of animals used in basic research and drug development. We continuously try to improve scientific procedures and husbandry which minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable.

High-Frequency Ultrasound

Ultrasound is a powerful imaging modality that enables non-invasive, real-time visualization of organs and tissues. We perform sonographic studies of small animals using a High-Frequency Ultrasound system. Its transducers produce frequencies as high as 70MHz, which has permitted the identification of structures as small as 30µm with a maximum temporal resolution of 740fps. This makes the platform ideal for pre-clinical research because it permits a study of many organs, tissues, and the behavior of biological systems that need to be studied at this tiny scale. The non-invasive nature of ultrasound means that it can be used with no harm to animal or human subjects. We



The Methodological Spectrum of the Pathophysiology Platform. *High Frequency Ultrasound Imaging:(A) Parasternal Long Axis View of a Murine Heart,(B) Micro-Ultrasound Imaging of a Murine Embryo at E12.5,(C) Contrast Imaging of a Melanoma Tumor,(D) 3D-Color Doppler of a Murine Testicle.*

have established a wide variety of protocols to analyze the phenotypes of mice and rats: For cardiovascular studies, ultrasound imaging can be used to detect the presence of localized or generalized hypertrophy or thinning of the myocardium of the left ventricle (LV) and the presence of regional or global wall motion abnormalities associated with systolic dysfunction. The analysis of transmitral flow profiles allows the detection of abnormal filling patterns associated with LV diastolic dysfunction. Strain analysis provides a highly sensitive speckle-tracking based echocardiographic imaging technique that offers quantitation of the velocity of the walls, displacement, strain, strain rate, and time to peak analysis. Because of its non-invasive nature ultrasound can be used in longitudinal studies for tumor diagnostics and therapy monitoring. 3D-Ultrasound imaging allows the reliable delineation of tumor boundaries and the assessment of tumor heterogeneity. In addition, ultrasound imaging is ideal for studying pregnancy and development in mice and rats.

Body Composition Analysis

Time-Domain-Nuclear-Magnetic-Resonance imaging (TD-NMR) is a powerful tool for the non-invasive quantification of solids and liquids in objects to be studied. Without anesthesia or other preparations, animals such as mice and rats can be tested for body composition of fat, free body fluids and lean tissue within a few minutes. After a stimulated adjustment of nuclear particle spins within a high energetic magnetic field, specific patterns of relaxation times of spin rearrangements are recorded when the magnetic field is removed. These relaxation patterns can be correlated to the density of body compounds, i.e. lean tissue, fat and free fluids, giving concrete values of their proportional composition. The NMR-whole-body composition analyzer allows a non-invasive and reliable quantification of whole body components of small animals.

Selected Publications

Embryonic cardiomyocytes can orchestrate various cell protective mechanisms to survive mitochondrial stress. Magarin M, Pohl T, Lill A, Schulz H, Blaschke F, Heuser A, Thierfelder L, Donath S, Drenckhahn JD. **J Mol Cell Cardiol. 2016 Aug;97:1-14**

CD74-Downregulation of Placental Macrophage-Trophoblastic Interactions in Preeclampsia. Przybyl L, Haase N, Golic M, Rugor J, Solano ME, Arck PC, Gauster M, Huppertz B, Emontzpohl C, Stoppe C, Bernhagen J, Leng L, Bucala R, Schulz H, Heuser A, Weedon-Fekjær MS, Johnsen GM, Peetz D, Luft FC, Staff AC, Müller DN, Dechend R, Herse F. **Circ Res. 2016 Jun 24;119(1):55-68.**

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Impaired myocardial development resulting in neonatal cardiac hypoplasia alters postnatal growth and stress response in the heart. Drenckhahn JD, Strasen J, Heinecke K, Langner P, Yin KV, Skole F, Hennig M, Spallek B, Fischer R, Blaschke F, Heuser A, Cox TC, Black MJ, Thierfelder L. **Cardiovasc Res. 2015 Apr**

Bcl10 mediates angiotensin II-induced cardiac damage and electrical remodeling. Markó L, Henke N, Park JK, Spallek B, Qadri F, Balogh A, Apel IJ, Oravecz-Wilson KI, Choi M, Przybyl L, Binger KJ, Haase N, Wilck N, Heuser A, Fokuhl V, Ruland J, Lucas PC, McAllister-Lucas LM, Luft FC, Dechend R, Müller DN. **Hypertension. 2014 Nov;64(5):1032-9.**

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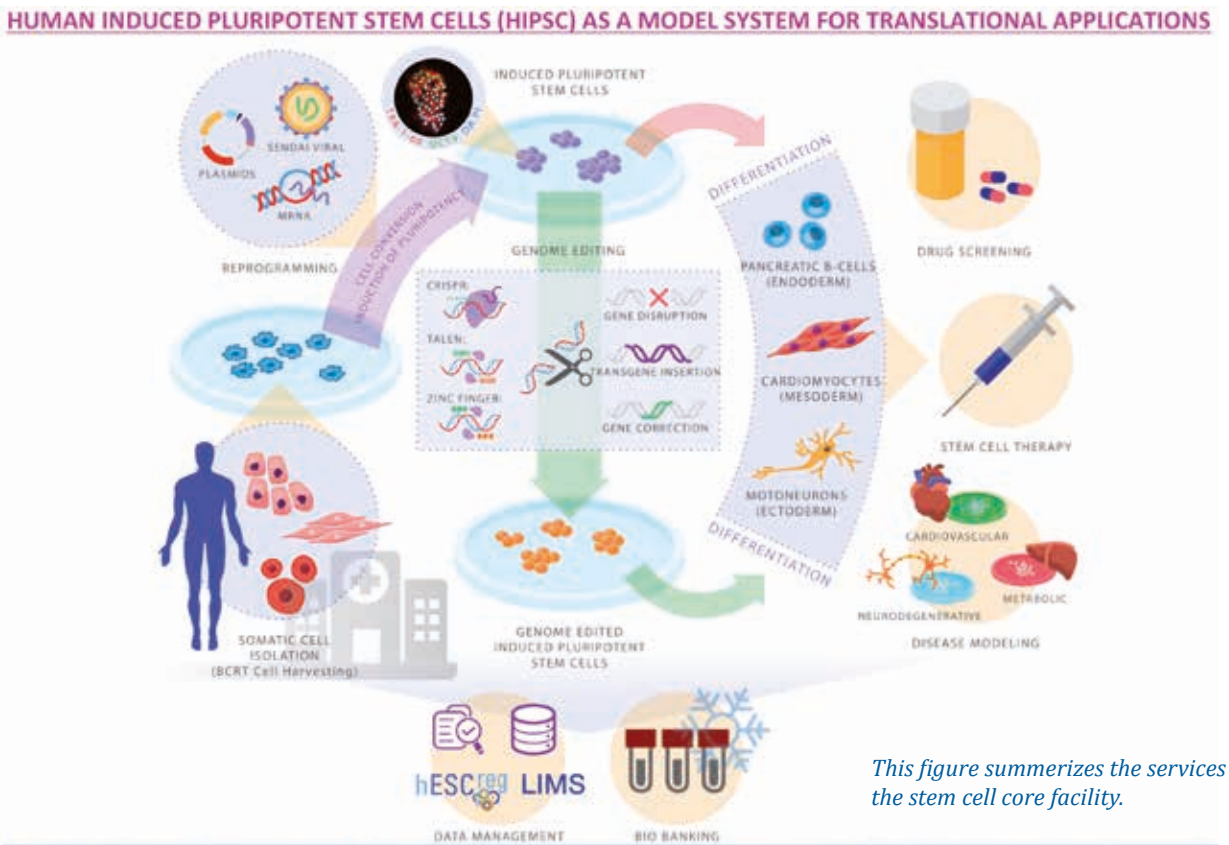
Cornelia Stärkel

Photo: David Ausserhofer/MDC

Sebastian Diecke

Pluripotent Stem Cells



This figure summerizes the services offered by the stem cell core facility.

SOMATIC CELL ISOLATION	REPROGRAMMING + CHARACTERIZATION	BANKING + PROVISION	DIFFERENTIATION	STANDARDIZED PROTOCOLS	TRAINING + PROJECT CONSULTING	GENOME EDITING
Isolation of patient specific primary cells	• Derivation of induced pluripotent stem cells • Characterization and quality control of the derived cell lines	Generation of cell banks Provision of cell lines	Differentiation of iPSCs into specialized cell types like cardiomyocytes, endothelia cells, podocytes....	Development and provision of standardized protocols	Training is offered on a regular basis	Generation of isogenic and reporter cell lines for disease modeling

SERVICES/RESOURCES OFFERED BY THE STEM CELL CORE FACILITY

Cooperation with **BCRT Cell Harvesting Core:** • Tissue sampling • Providing clinical information of the donor • Isolation of fibroblasts, PBMC, urinary cells...	• Different reprogramming techniques: Sendai virus or episomal plasmids • Characterization of iPSCs: pluripotency marker, differentiation potency, genotype... • Quality control: genomic stability, sterility identity	• Cell banking in cooperation with **BIH Biobanking Core** • Provision of reference cell lines	• Provision of protocols • Support during the establishment of new/published protocols	• Protocols for: Derivation, culture, preservation and genetic manipulation of hiPSC	• Basic Training: knowledge and hands-on expertise of maintaining and manipulating hiPSCs in tissue culture • Advaced Training: Reprogramming, genetic manipulation... • Project consulting	• Design and distribution of TALEN constructs (gene, disruption, gene correction, insertion of reporter constructs)

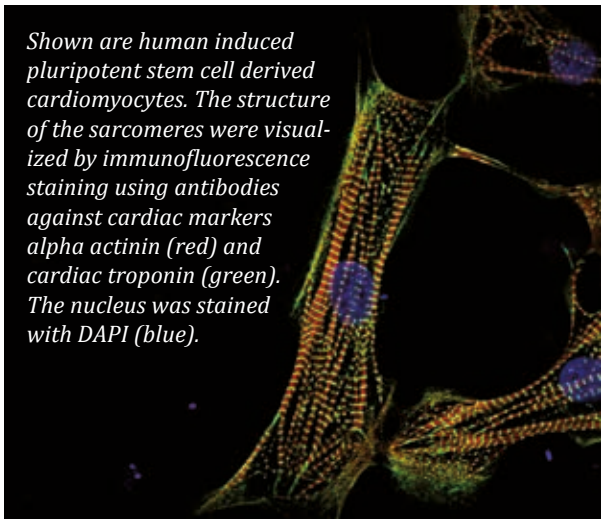
The Pluripotent Stem Cell Core Facility at the Max Delbrück Center is a joint project of the Berlin Institute of Health and the MDC in Berlin Buch. The facility was established in September 2014 and is located on the first floor of the Walter Friedrich Haus. In 2014 the laboratories, including S1 and S2 cell culture labs, were set up and specialized equipment was purchased to provide MDC/BIH scientists with the latest, cutting-edge stem cell technologies. These include a comprehensive range of methods for cell cultures and molecular biology that are required to derive, maintain, characterize and differentiate human induced pluripotent stem cells (hiPSC). For example, as a component to ensure quality control for newly derived iPSCs, an iScan System from Illumina was purchased to allow the parallel analysis of chromosome stability and a confirmation of their identity. For cell characterization, a QuantStudio 6 real-time PCR system, a digital PCR machine and fluorescence microscopes are available. Furthermore, to carry out genetic engineering on the cells, transfection devices (Neon and Amaxa, Nucleofection) were purchased. The cell culture labs of the core facility are equipped with class 2 safety cabinets and a special picking hood. This hood is essential for many of the steps involved in manual cell manipulations, such as selecting clones after reprogramming, the genetic manipulation of hiPSCs, or the removal of cells that have spontaneously differentiated.

In addition to setting up the infrastructure, the facility provides hands-on training in basic stem cell culture techniques for scientists who are planning to work with hPSCs. In 2014-2016 five stem cell courses consisting of theoretical and practical sections were offered and successfully conducted.

The services of the core facility are summarized in figure one and include the extraction of primary cells and the reprogramming, differentiation and genome editing of human pluripotent cells. Additionally we help and guide scientists who perform their own experiments under our supervision.

Recently, the core facilities established a feeder-free cultivation system of hiPSC using a range of chemically defined conditions including homemade, chemically defined media. Moreover, we have established neuronal, endothelial and cardiac differentiation protocols in close cooperation with several research groups on the campus. The core facility also successfully performed initial genome engineering experiments including specific knock-out studies of several genes and the generation of reporter cell lines using CRISPR/CAS9- or TALEN-mediated gene targeting.

With the German Stem Cell Network (GSCN) and others, we have initiated a network of stem cell core facilities for iPS derivation across Germany. Here the goal is to establish common standards through the sharing of cell lines, tools and protocols which will lead to higher efficiency and comparability across different experimental frameworks. We are convinced that this will fundamentally benefit the stem cell research community across Germany.



Shown are human induced pluripotent stem cell derived cardiomyocytes. The structure of the sarcomeres were visualized by immunofluorescence staining using antibodies against cardiac markers alpha actinin (red) and cardiac troponin (green). The nucleus was stained with DAPI (blue).

Selected Publications

Karakikes I, Termglinchan V, Cepeda DA, Lee J, **Diecke S**, Hendel A, Itzhaki I, Ameen M, Shrestha R, Wu H, Ma N, Shao NY, Seeger T, Woo NA, Wilson KD, Matsa E, Porteus MH, Sebastiano V, Wu JC. A Comprehensive TALEN-Based Knockout Library for Generating Human Induced Pluripotent Stem Cell-Based Models for Cardiovascular Diseases. Circ Res. 2017 Feb 28. pii: CIRCRESAHA.116.309948. doi: 10.1161/CIRCRESAHA.116.309948. PMID:28246128

Diecke S, Lu J, Lee J, Termglinchan V, Kooreman NG, Burridge PW, Ebert AD, Churko JM, Sharma A, Kay MA, Wu JC. Novel codon-optimized mini-intronic plasmid for efficient, inexpensive, and xeno-free induction of pluripotency. Sci Rep. 2015 Jan 28;5:8081. doi: 10.1038/srep08081.

Diecke S, Jung SM, Lee J, Ju JH. Recent technological updates and clinical applications of induced pluripotent stem cells. Korean J Intern Med. 2014 Sep;29(5):547-57. doi: 10.3904/kjim.2014.29.5.547. Epub 2014 Aug 28.

Burridge PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, **Diecke S**, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC. Chemically defined generation of human cardiomyocytes. Nat Methods. 2014 Aug;11(8):855-60. doi: 10.1038/nmeth.2999. Epub 2014 Jun 15.

Redmer T*, **Diecke S***, Grigoryan T, Quiroga-Negreira A, Birchmeier W, Besser D. (*Shared co-first authors) E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming. EMBO Rep. 2011 Jul 1;12(7):720-6. doi: 10.1038/embor.2011.88.

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Photo: David Ausserhofer/MDC

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Preparative Flowcytometry

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry. It was designed in 1971 by Len Herzenberg to provide a fast method for physical separation of heterogeneous mixtures of cells into distinct subsets based on their light scattering and fluorescent characteristics. The FACS Core Facility at the MDC mainly provides assistance in operating such cell sorters. These sorters can isolate up to 20,000 cells per second for large-scale cell isolation and allow single cells to be precisely spotted into microtiter plates or onto slides for analytical purposes. In addition, the staff of the FACS Core Facility assists in the design and setup of more specialized or sophisticated experiments that use additional state of the art analytical flow cytometers. The facility operates one 3-colour laser line analyzer (BD LSR), one 5-colour laser line analyzer (BD Fortessa), and four high-capacity digital cell sorters (BD Aria).

Sorted cells are routinely prepared for gene expression analysis by microarrays, RNA sequencing, quantitative real-time PCR, DNA sequencing, live-cell imaging or adoptive transfer experiments in animals. Numerous groups from the MDC, BIMSB, ECRC and the FMP have used the facility over the last few years for such purposes.

The greatest number of requests for all preparative cell sorting at the MDC arise from primary human and murine cells, which are characterized by their immunophenotypic parameters. For example, the group of Bernd Dörken routinely uses FACS enrichment of specific subsets of tumor and primary murine hematopoietic cells as well as of genetically modified subpopulations of various cell types in order to analyse the biological functions of candidate genes in particular with relevance to lymphomagenesis. A recent example of such a work which required cell sorting experiments of various cell types is the characterization of transcription factors IRF5 in combination with NF-kB as key regulators in classical Hodgkin lymphoma (Kreher, Bouhlel et al. 2014).

Similarly, the group of Klaus Rajewsky generated various mouse models of lymphomas and fatal lymphoproliferative disorders and uses flow cytometry to elucidate the underlying mechanisms of pathogenesis. Their work relies on genetically modified mouse strains, for instance conditional loss- or gain-of-function mutants. In addition, they enhanced genome editing using the CRISPR/Cas9 technology in mammalian cells to increases the efficiency of CRISPR/Cas9-mediated mutations or homologous recombination events (Chu, Weber et al. 2015).

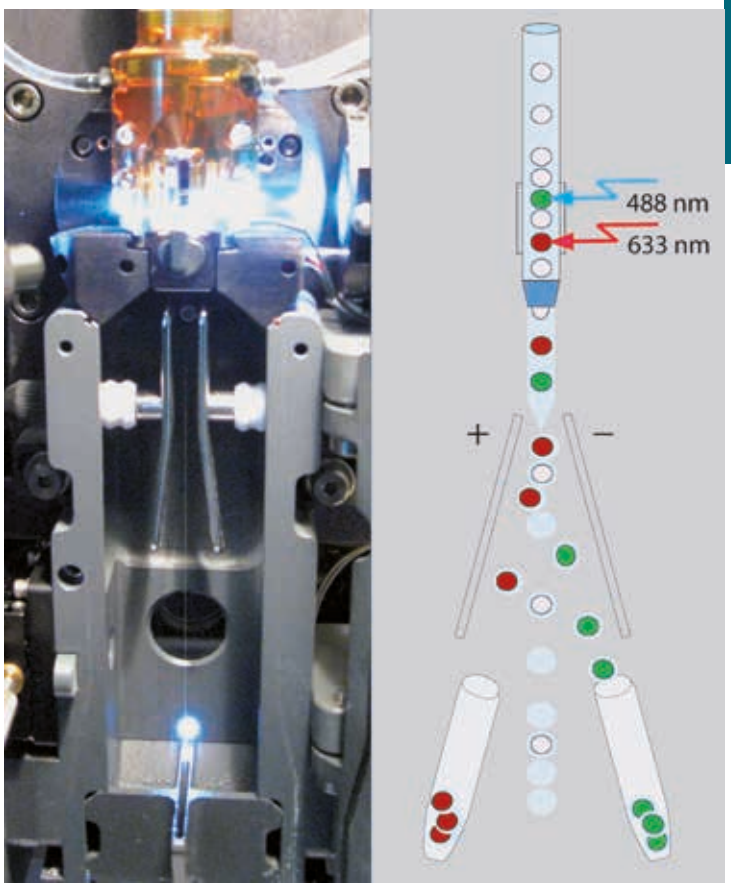
Stromal cells are pivotal for the onset and survival of lymphoma B cells, but which stromal cell subpopulations in the lymph nodes contribute to the formation of a lymphoma growth-promoting niche had remained elusive. The groups of Armin Rehm and Uta E. Höpken have applied a combination of cell enrichment using magnetic bead and FACS to isolate very rare stromal cell populations of mesenchymal, myeloid and endothelial origin and analyzed their gene expression on a genome wide scale. This was a prerequisite for the analysis of specific tumor-induced gene expression profiles in stroma-located monocytic, dendritic and other lymphoma-accessory cell types (Rehm, Gatjen et al. 2014).

FACS cannot only be used for the analysis of the haematopoietic lineage, but also gained in importance for other cells populations. Reliable cell surface biomarkers are becoming available for such cell populations, and they also can be labeled by transgenic technologies. For example, the group of Walter Birchmeier uses FACS to isolate and analyze stem cells from normal tissues and from tumors. They isolated stem cells in the skin or the mammary gland, as well as rare cell populations present during heart development, or cancer stem cells that represent rare cell types present in tumors (Holland, Gyorffy et al. 2013).

Stem cells called Satellite cells also exist in the adult muscle. Satellite cells are needed for muscle growth and for the regeneration of injured muscle tissue. Although satellite cells account for only a small fraction of the muscle tissue, they can be isolated and enriched by FACS. Thus, changes in gene expression in these stem cells can be assessed in mouse mutants, and the isolated cells can be cultured for a more detailed characterization. The group of Carmen Birchmeier sorts satellite cells to investigate how signaling pathways control their development and function (Brohl, Vasyutina et al. 2012).

Another use of FACS technology is the generation and genetic modification of induced pluripotent stem (iPS) cells. For example, the Pluripotent Stem Cell Core Facility generates human pluripotent stem cells that are modified by introducing or correcting a disease-causing mutation, and these cells are enriched using FACS. They are then used by the groups of Norbert Hübner, Thomas Willnow or Erich Wanker to define the molecular mechanisms that underlie diseases. Moreover, these cells can be tagged with various reporters encoding fluorescent proteins to enrich for cell types that are formed when these cell types differentiate. The groups of Simone Spuler and Zsuzsanna Izsvak use this approach to study the mechanisms underlying cell fate decisions.

The group of Nikolaus Rajewsky devised a method to collect large amounts of precisely staged C. elegans embryos by FACS which are then used for high-throughput genomic and biochemical analyses. This new method already contributed towards a more complete understanding of gene regulatory networks during early C. elegans development.



Left: Inside view of a FACSAria (flow cell with sort block and deflection plates), Right: Schematic view of the separation principle

Selected Publications

Brohl, D., E. Vasyutina, M. T. Czajkowski, J. Griger, C. Rassek, H. P. Rahn, B. Purfurst, H. Wende and C. Birchmeier (2012). "Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals." Dev Cell **23**(3): 469-481.

Chu, V. T., T. Weber, B. Wefers, W. Wurst, S. Sander, K. Rajewsky and R. Kuhn (2015). "Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells." Nat Biotechnol **33**(5): 543-548.

Holland, J. D., B. Gyorffy, R. Vogel, K. Eckert, G. Valenti, L. Fang, P. Lohneis, S. Elezkurtaj, U. Ziebold and W. Birchmeier (2013). "Combined Wnt/beta-catenin, Met, and CXCL12/CXCR4 signals characterize basal breast cancer and predict disease outcome." Cell Rep **5**(5): 1214-1227.

Kreher, S., M. A. Bouhlel, P. Cauchy, B. Lamprecht, S. Li, M. Grau, F. Hummel, K. Kochert, I. Anagnostopoulos, K. Johrens, M. Hummel, J. Hiscott, S. S. Wenzel, P. Lenz, M. Schneider, R. Kuppers, C. Scheidereit, M. Giefing, R. Siebert, K. Rajewsky, G. Lenz, P. N. Cockerill, M. Janz, B. Dorken, C. Bonifer and S. Mathas (2014). "Mapping of transcription factor motifs in active chromatin identifies IRF5 as key regulator in classical Hodgkin lymphoma." Proc Natl Acad Sci U S A **111**(42): E4513-4522.

Rehm, A., M. Gatjen, K. Gerlach, F. Scholz, A. Mensen, M. Gloger, K. Heinig, B. Lamprecht, S. Mathas, V. Begay, A. Leutz, M. Lipp, B. Dorken and U. E. Hopken (2014). "Dendritic cell-mediated survival signals in Emu-Myc B-cell lymphoma depend on the transcription factor C/EBPbeta." Nat Commun **5**: 5057.

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Proteomics

Cells communicate with their environment and react to different stimuli and changes in the environment. These reactions can either be based on transcriptional activity or chemical modifications of existing proteins, so called post-translational modifications. In order to gain insight into the regulation of these protein-interaction networks it is necessary to identify proteins as well as their modifications and to measure the protein expression levels within a cell. This requires the identification of several hundred or thousand proteins and their quantification in a complex mixture. Advances in modern mass-spectrometry based proteomics made it possible to match all these criteria. The technique quickly rose to be the default method for large-scale protein identification in life sciences.

The core facility mass-spectrometry offers a wide range of proteomic methods for the identification and quantification of proteins and peptides. Besides the identification of single proteins in solutions and SDS gel slices, the mass-spectrometry core facility uses a number of proteomic techniques in different collaborations with MDC groups at the MDC. In general the techniques can be divided into two groups: targeted and discovery or non-targeted proteomics.

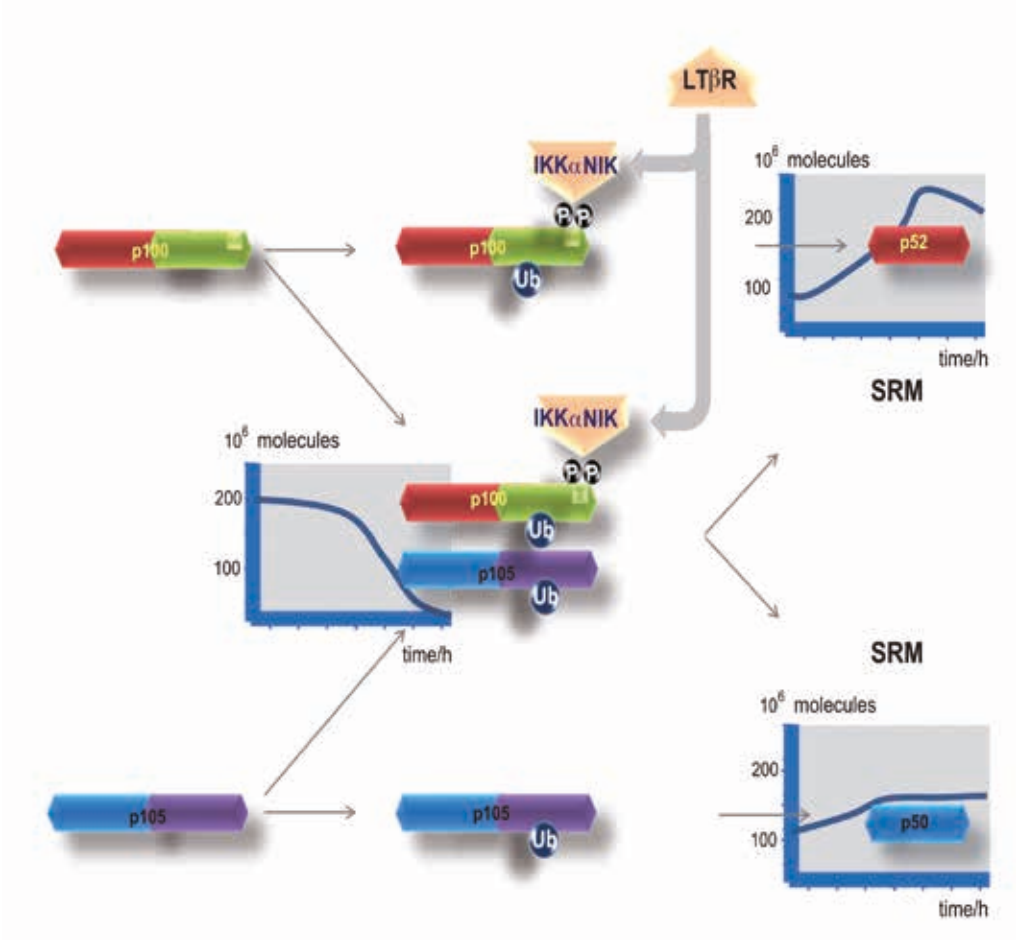
Targeted proteomics

Monitoring the regulation of proteins under different conditions can provide deep insight into regulatory networks, which underly different signal transduction cascades. For many cascades the major players of these pathways are already identified. Incorporating this knowledge into a targeted strategy allows to monitor changes in the concentration of these players, avoiding the sequencing of unrelated, not regulated proteins in the cell. A method that allows the selection of a limited number of proteins is selected reaction monitoring (SRM). This technique has the advantage of a high sensitivity combined with short run times on the liquid chromatography systems. Therefor opening the possibility of measuring large quantities of different samples in a short time period and quantifying all components of the cascade.

Several collaborations of the core facility within the MDC are now based on selected reaction monitoring experiments. The core facility used a tripple quadrupol mass-spectrometer for this type of analysis (Q-TRAP 5500). One example of this technique involves the research on the inflammatory NF-κB pathway. For this project a quantification method for the two NF-κB precursor proteins p100 and p105 and their proteolytically shorted versions p52 and p50 was developed. By using specialized SRM techniques it was possible to quantify these molecules in cell extracts. This quantitative data was used to generate mathematical models, which allowed the identification of a new signaling complex in the NF-κB system.

Non-targeted proteomic approaches

The increased sensitivity of mass-spectrometers called for different ways to analyzed the recorded data. In the core facility all measurements are done quantitatively. For this several methods for the introduction of isotopic labels have been established. Among them (is) metabolic labeling. Metabolic labeling of cells offers great advantages for the quantification of proteins. The cells of interest are therefore cultured in media, which contain isotopic labeled (non radioactive) amino acids. The additional mass of the amino acids can later be detected by mass- spectrometry. Comparison of two different samples is possible by analyzing the



Model of the processing of the two NF-kB molecules p100 and p015 to the p52 and p50, respectively. Activation by lymphotoxin stimulation leads to the induced processing of the the two precursor-proteins. Using targeted mass-spectrometry the absolute amounts of p100, p105, p52 and p50 at different time points after stimulation were determined and used in the mathematical models.

sample on a high accuracy orbitrap mass spectrometer (Q-Exactive). The results of these measurements are relative ratios for each protein detected in the two different samples. This techniques is currently used by the core facility for the quantification of immuno-precipitations and large scale protein identifications.

For samples, which cannot be cultured in media containing heavy amino acids, another technique is available. This technique relies on a highly reproducible liquid chromatography, since two different chromatographic runs have to match.

Identification of post-translational modifications

The regulation of protein activity or interactions with other proteins can be modulated by the modification of side groups of the amino-acids in the peptides sequence. For the understanding of the molecular mechanisms, which regulate these proteins, it is necessary to gain insight into the different post-translational modifications of proteins. These modifications can be detected by mass-spectrometry based proteomic analysis. The core facility actively pursues the development of new methods for the identification of modification sites by ubiquitin-like proteins. For the identification of phosphorylation sites the core facility now provides specialized methods for improved sensitivity.

Selected Publications

Beaudette, P., Popp, O., & Dittmar, G. (2015). Proteomic techniques to probe the ubiquitin landscape. Proteomics, n/a–n/a. http://doi.org/10.1002/pmic.201500290

Jerke, U., Hernandez, D. P., Beaudette, P., Korkmaz, B., Dittmar, G., & Kettritz, R. (2015). Neutrophil serine proteases exert proteolytic activity on endothelial cells. Kidney International. http://doi.org/10.1038/ki.2015.159

Kanashova, T., Popp, O., Orasche, J., Karg, E., Harndorf, H., Stengel, B., et al. (2015). Differential proteomic analysis of mouse macrophages exposed to adsorbate-loaded heavy fuel oil derived combustion particles using an automated sample-preparation workflow. Analytical and Bioanalytical Chemistry. http://doi.org/10.1007/s00216-015-8595-4

Tuorto, F., Herbst, F., Alerasool, N., Bender, S., Popp, O., Federico, G., et al. (2015). The tRNA methyltransferase Dnmt2 is required for accurate polypeptide synthesis during haematopoiesis. The EMBO Journal, 34(18), 2350–2362. http://doi.org/10.15252/embj.201591382

Yılmaz, Z. B., Kofahl, B., Beaudette, P., Baum, K., Ipenberg, I., Weih, F., et al. (2014). Quantitative dissection and modeling of the NF-κB p100-p105 module reveals interdependent precursor proteolysis. Cell Reports, 9(5), 1756–1769. http://doi.org/10.1016/j.celrep.2014.11.014

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Photo: David Ausserhofer/MDC

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Proteomics / Metabolomics, BIMSB

*The 'integrative proteomics and metabolomics' research and technology platform is an integral part of the **B**erlin **I**nstitute for **M**edical **S**ystems **B**iology (BIMSB). We are an Interdisciplinary team consisting of biologists, engineers, chemists/pharmacologists and bio-informaticians.*

Our core interest is to decode the regulation of metabolism at the molecular level. The chemical space of metabolites spans a wide range of physicochemical properties. The size of the molecule, its polarity and complexity finally determines the separation method and its detectability by the mass spectrometer. Therefore, we have installed three different mass spectrometers coupled to several chromatographic systems to achieve a high coverage of the biochemical network of cells and organs (Fig. 1).

- **GCxGC-EI-ToF, GC-Q Exactive**, gas chromatography based systems that allows the measurement of small molecules (intermediates of the metabolism)
- **QQQ-LC-MS**, a triple quadrupole mass spectrometer allows the measurement of small molecules, lipids and peptides (proteomics)
- **Q-Exactive**, a fourier transformation mass spectrometer that is used for proteomics and metabolomics analyses

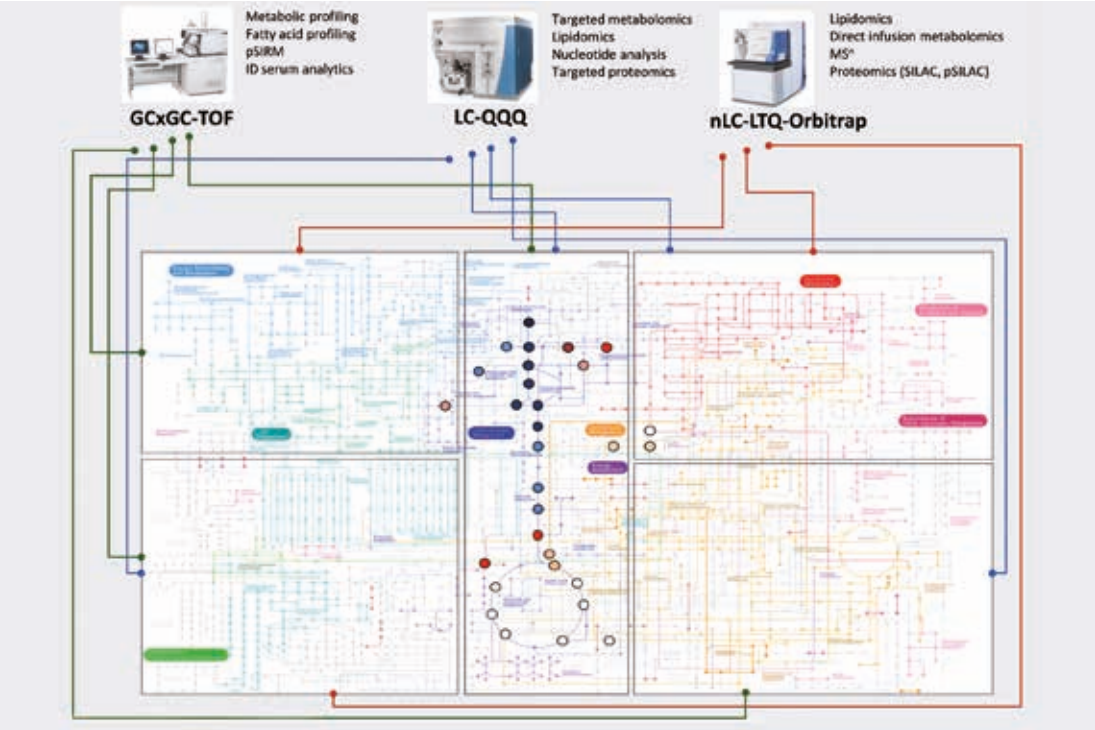

Figure 1. Overview about the mass spectrometers installed at BIMSB and related methods. *During the last years we have installed a GC-ToF-MS, a triple-quadrupole-MS and a LTQ-Orbitrap-Velos. The scheme shows the mass spectrometers, established methods and related metabolic pathways that can be analyzed.*

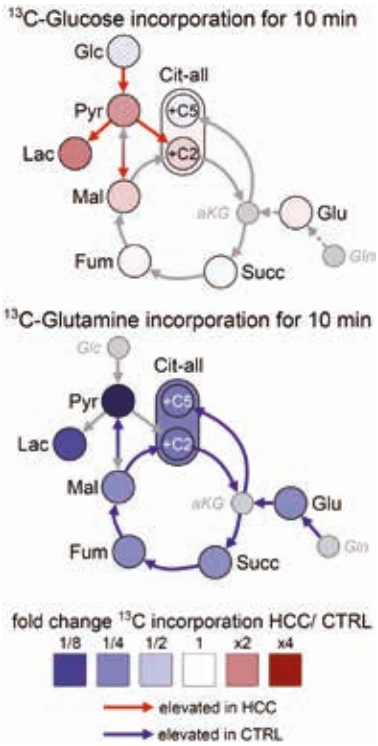

Figure 2. *Dynamic analysis of glucose and glutamine metabolism in HCC tissues of ASVB mice. Stable isotope labeled glucose (^{13}C glucose) and glutamine (^{13}C glutamine) were applied i.p. and tumor and liver tissue were taken after 10 min. ^{13}C carbon incorporation into central metabolites was analyzed using GC-MS based metabolomics (in vivo pSIRM).*

Using the established mass spectrometry based platforms we are able to quantitatively measure metabolites (small molecules) lipids and proteins from variety of sample species. In addition to our own scientific projects, we collaborate with our colleagues at the BIMSB and MDC and apply proteomics and metabolomics techniques;

- Metabolic profiling (targeted, non-targeted, lipid profiling)
- Stable isotope resolved metabolomics (in cell cultures and in vivo)
- Proteome analyses (SILAC, in vivo SILAC and targeted proteomics)

Metabolic Regulation

Central metabolism is highly flexible and continuously adjusted to the physiological program of the cell, organ and organism; and its regulation appears at the (post-) transcriptional, (post-) translational and allosteric level. Central metabolism is a metabolic super pathway including e.g. glycolysis, pentose phosphate pathway, tri carboxylic acid cycle, amino acid metabolism, glycogen and nucleotide metabolism. Our goal is to characterize the metabolic reprogramming events during cellular differentiation and transformation at the molecular level. We aim to understand **(i)** the metabolic differences of stem cells and cancer cells. **(ii)** How metabolism can serve as a target for cancer treatment.

Currently, we focus our technology development and research on decoding the crosstalk between metabolism and post-transcriptional gene regulation. RNA or DNA binding proteins as well as RNA and DNA itself can be target of a number of modifying enzymes. These metabolic modifications contribute substantially to the post-transcriptional regulatory code. In addition, the metabolic status of the cell can influence gene expression at (post-) transcriptional and (post-) translational levels. In order to analyze the crosstalk between metabolism and gene regulation we are developing new metabolomics and proteomics approaches. Using comprehensive nucleotide profiling and RNA-protein interaction studies, we identified a nucleotide responsive and protective metabolic feedback mediated by the 3'UTR of MYC.

Selected Publications

Dejure FR#, Royla N#, Herold S, Kalb J, Walz S, Ade CP, Mastrobuoni G, Vanselow J, Schlosser A, Wolf E, Kempa S* and Eilers M* 2017 The 3'-UTR of MYC couples RNA polymerase II function to ribonucleotide levels, accepted in EMBO Journal 2017 (*,#equal)

von Eyss B, Jaenicke LA, Kortlever RM, Royla N, Wiese KE, Letschert S, McDuffus LA, Sauer M, Rosenwald A, Evan GI, Kempa S, Eilers M. A MYC-Driven Change in Mitochondrial Dynamics Limits YAP/TAZ Function in Mammary EpithelialCells and Breast Cancer. Cancer Cell. 2015 Dec 14;28(6):743-57.

Bielow C, Mastrobuoni G, Kempa S. Proteomics Quality Control: Quality Control Software for MaxQuant Results. J Proteome Res. 2015 Dec 28.

Pietzke M, Zasada C, Mudrich S, Kempa S. Decoding the dynamics of cellular metabolism and the action of 3-bromopyruvate and 2-deoxyglucose using pulsed stable isotope-resolved metabolomics. Cancer Metab. 2014 Jun 30;2:9.

Liu L, Ulbrich J, Müller J, Wüstefeld T, Aeberhard L, Kress TR, Muthalagu N, Rycak L, Rudalska R, Moll R, Kempa S*, Zender L, Eilers M, Murphy DJ. Deregulated MYC expression induces dependence upon AMPK-related kinase 5. Nature. 2012 Mar 28;483(7391):608-12. (*corresponding for metabolomics)

Patents:

Kempa, Stefan; Pietzke, Matthias; Zasada, Christin;Short term isotope pulse labeling method for analysing metabolic products in biological samples, 2015, US Patent 20,150,330,969

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Photo: David Ausserhofer/MDC

Ralf Kühn

Transgenic Core Facility

Engineering of the mouse genome to create targeted mutants is a key technology for biomedical research. The Transgenic Core Facility (TCF) provides a central service for all kinds of reproductive technologies, supporting MDC research groups and their collaborators in their work with genetically modified mouse lines. One focus of the TCF is the rederivation of mouse lines by embryo transfer to exclude pathogens and and the cryopreservation of lines using embryo or sperm freezing. The second focus of the TCF is the generation of new mouse lines by microinjection of genetically modified embryonic stem cells into blastocysts or of DNA & RNA molecules into zygotes which induce genetic modifications in one-cell embryos.

Rederivation and cryopreservation of mouse lines

The Transgenic Core Facility (TCF) offers in-house service for all kinds of reproductive technologies to support MDC research groups and their collaborators which are working genetically modified mouse lines. Starting at the planning phase, we support users with the design and layout of their projects. One focus of our work is the rederivation by embryo transfer for the import of mouse lines from external facilities and for the transfer of immune deficient strains into the new pathogen-free facility, without the burden caused by opportunistic infections. Furthermore, we cryo-preserve the germ cells of precious mouse lines and bring frozen lines back to life. Cryopreservation is an important tool to prevent gene drifting, the loss of colonies in case of infection and to save cage space and resources occupied by lines which are not actively used for research. Since most groups choose to conserve mouse sperm this is the main form of germ plasm we freeze down and store in liquid nitrogen. Due to the growing number of mouse models worldwide, we see an increase in the number of organisations who offer to send frozen material instead of live mice. These lines are revitalised at the MDC follwing a variety of protocols depending on the method of cryo-preservation. There are individual projects that do not fit

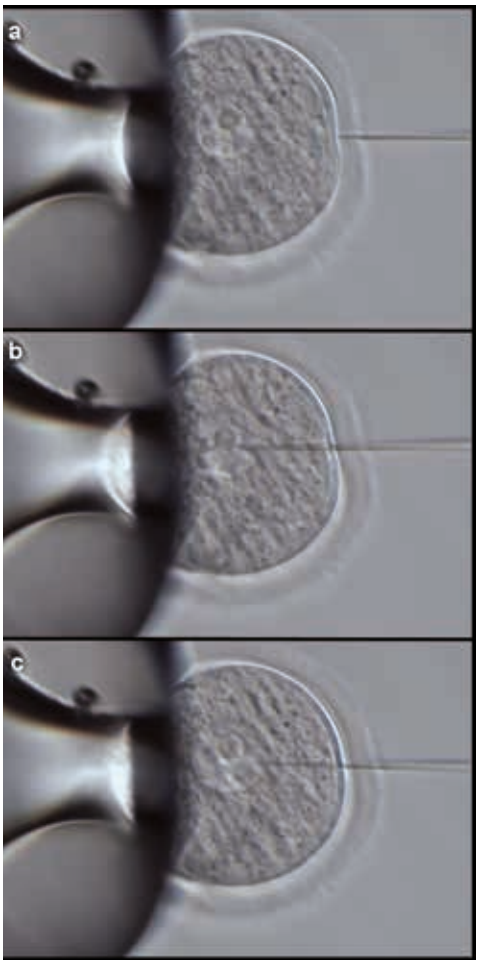

Figure 1 Pronuclear microinjection.
A zygote fixed to the holding capillary (a) is penetrated with the injection capillary (filled with DNA/RNA solution and moved further to place the capillary`s tip in middle of a pronucleus (b). When the injection pressure is applied a swelling of the injected pronucleus is observed visible (c), indicating successful microinjection.

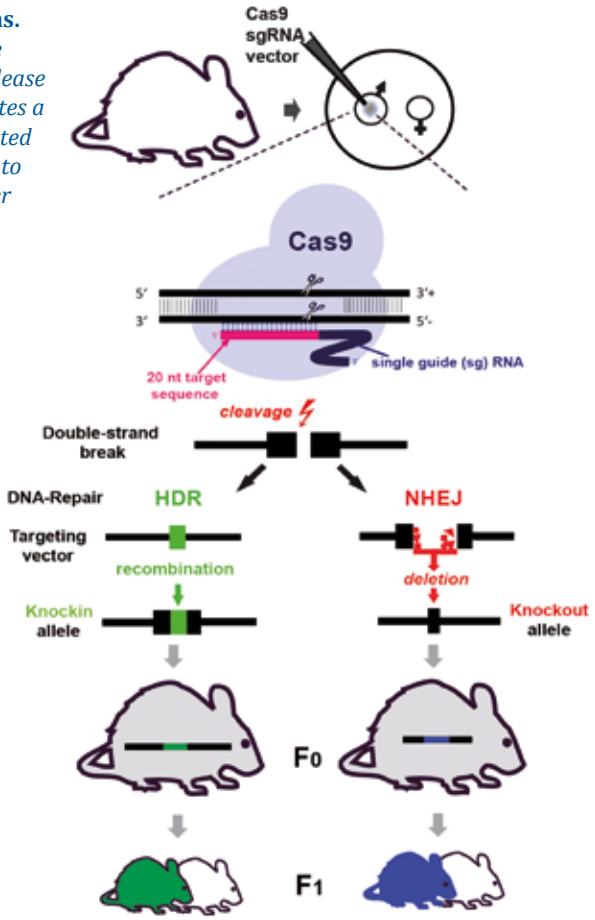

Figure 2 Gene editing in mouse zygotes using CRISPR/Cas. *Zygotes are microinjected with Cas9 mRNA, sgRNA and a gene targeting vector. Upon the production of Cas9 protein the nuclease binds together with sgRNA to the selected target site and creates a double-strand break. DSBs can be repaired by homology-directed repair (HDR) or non-homologous end-joining (NHEJ), leading to a knockin or knockout allele. Founder mutants (F0) are further mated to establish a mutant line.*

into any of the above that can be supported by the TCF and we will help whenever it comes to the production, isolation, manipulation, culture or retransfer of pre-implantation embryos. Moreover, we can give advice on cloning and targeting strategies, ES cell culture and derivation and coat color genetics.

Generation of genetically modified mouse lines

A second focus of the TCF is the generation of new genetically modified mouse lines. This is traditionally accomplished by gene targeting in embryonic stem (ES) cells and the generation of germline chimaeras obtained through the microinjection of recombinant ES cells into blastocysts. In recent years engineered nucleases, introduced into zygotes by pronuclear microinjection (Fig. 1), able to create targeted double-strand breaks (DSB) in the genome of the one-cell embryo, have emerged as powerful tool for the single step production of targeted mutants, independent of ES cells. Proof of principle was provided with zinc-finger nucleases and TALENs, both of which have been displaced by the more versatile and efficient CRISPR/Cas9 nuclease system. This system is composed of the generic Cas9 nuclease that is guided to specific target sites by short sgRNAs which include 20 nucleotides complementary to the target sequence. Gene editing is achieved by endogenous DSB repair pathways, either imprecisely by non-homologous end joining (NHEJ) causing small deletions, or by homology-directed repair (HDR) using repair template vectors for the precise insertion of new sequences (knockin) (Fig. 2). In mouse zygotes, CRISPR/Cas9 has been efficiently used for generating small deletions and knockout mutations by the NHEJ repair pathway, reaching frequencies of up to 50% in pups derived from Cas9/sgRNA microinjections, even in inbred backgrounds such as C57BL/6. In contrast, HDR events with co-injected targeting vectors occur less frequently in zygotes at rates of 5–10%. The TCF offers advice, protocols and template plasmids for the preparation of CRISPR/Cas reagents, performs the microinjection into zygotes and embryo transfer into foster females, and provides help with genotyping of the resulting founder mutants. In addition, we aim for developing and testing new protocols and reagents which increase the recovery of knockin mutants.

Patents / Patent applications

Record 2014/15/16

21 new mouse lines produced by injection of recombinant ES cell lines

35 new mouse lines produced by pronuclear microinjection (incl. 18 projects using CRISPR/Cas)

217 mouse lines preserved by sperm freezing

172 mouse lines transferred by hygienic rederivation or revitalised by in vitro fertilisation

Selected Publications

Brandl, C., Ortiz, O., Röttig, B., Wefers, B., Wurst, W., Kühn, R. (2015). Creation of targeted genomic deletions using TALEN or CRISPR/Cas nuclease pairs in one-cell mouse embryos. FEBS Open Bio 5, 26–35.

Chu, V.T., Weber, T., Wefers, B., Wurst, W., Sander, S., Rajewsky, K., and Kühn, R. (2015). Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. Nat. Biotechnol. 33, 543–548.

Chu, V.T, Weber, T., Graf, R., Sommermann, T., Petsch, K., Sack, U., Volchkov, P., Rajewsky, K., Kühn, R. (2015) Efficient generation of Rosa26 knock-in mice using CRISPR/Cas9 in C57BL/6 zygotes. HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/26772810"BMC Biotechnol. 2016 Jan 16;16(1):4.

Chu, V.T., Graf, R., Wirtz, T., Weber, T., Favret, J., Li, X., Petsch, K., Tran, N.T., Sieweke, M.H., Berek, C., et al. (2016). Efficient CRISPR-mediated mutagenesis in primary immune cells using CrispRGold and a C57BL/6 Cas9 transgenic mouse line. Proc. Natl. Acad. Sci. U.S.A. 113, 12514–12519.

Group Leader
Dr. Ralf Kühn

Technical Assistants
Rainer Kabisch
Katja Becker
Andrea Leschke
Sabine Manz
David Schreiber

Administrative Assistance
Christine Krause

Photo: Harry Schnitger /MDC

Annex

Appointments and Recruitments

Research Groups

2014

Holger GERHARDT began work at MDC, Charite, DZHK and BIH in Berlin
Several research institutions in Berlin have jointly succeeded in attracting the angiogenesis specialist Holger Gerhardt to the capital city. Since September he is research group leader at MDC and at Berlin Institute of Health (BIH) and concurrently W3 Professor of Experimental Cardiovascular Research at the Charité Berlin. Furthermore, he is integrated into the German Center for Cardiovascular Research (DZHK). After studying biology and receiving his PhD in 2000, Gerhardt worked as a postdoctoral fellow in der lab of Christer Betsholtz in Sweden until 2004. His most recent position was at the London Research Institute, UK, and concurrently at the Vesalius Research Center of the Flanders Institute of Biotechnology (VIB) at the Catholic University of Leuven, Belgium. Gerhardt investigates the formation of blood vessels in organism and disease development and is seeking ways to stop pathological vessel growth.

Young-Ae LEE appointed to W3 Professorship for Molecular Pediatric Allergology at Charité
With Young-Ae Lee, the MDC succeeded to recruit an excellent clinician scientist to its faculty. The joint appointment to the full professorship for Molecular Pediatric Allergology by the MDC and the Charité is supported through additional funding from the Impulse and Networking Fund of the Helmholtz President. Lee is a board-certified pediatrician and human molecular geneticist. Her research is concerned with the genetics and genomics of chronic inflammation, the genetic etiology of allergic disease in childhood and with disease risk prediction. Lee received her medical degree from Free University Berlin after several years of clinical and research training in molecular genetics and genetic epidemiology at distinguished academic institutions, including Harvard University and the University of California, San Francisco. She completed her postgraduate clinical training in Pediatrics and Allergology at Charité Children's Hospital, Berlin. She was appointed Assistant Professor in 2002, and promoted to Associate Professor in 2008.

Matthias SELBACH received W3 Professorship at Charité
Matthias Selbach studied biology at the University of Münster and earned his PhD in the lab of Thomas F. Meyer at the Max Planck Institute for Infection Biology, Berlin. In 2004, he proceeded to the lab of Matthias Mann in Odense, Denmark, and followed him to the Max Planck Institute of Biochemistry in Munich. As a postdoctoral fellow in a renowned proteomics lab, quantitative mass spectrometry became a cornerstone of his work. Since 2007, he headed the independent junior group "Cell signalling and mass spectrometry" at the MDC. From 2010, he was also an associate professor at the Charité. In 2014, Selbach accepted the appointment to a full professorship for Proteomics at the Charité in cooperation with the MDC. His team combines expertise in proteomics and cell biology to study biological systems at the global scale. Recent research highlights are the first systematic analysis of the impact of microRNAs on protein synthesis and the first global quantification of the entire gene expression cascade in mammalian cells.

Hans Schildbach endowed professorship for Kai SCHMIDT-OTT
The nephrologist and clinical researcher Kai Schmidt-Ott accepted the appointment to a W2 professorship for Urogenital Cancer Research at Charité. The professorship is jointly funded by the Foundation for Urological Research and the MDC. Schmidt-Ott joined the MDC in 2007 and has worked as a Junior-Professor for Molecular Medicine at Charité since 2009, having carried out postdoctoral work in the laboratory of Jonathan Barasch at Columbia University, New York, USA. By means of this joint appointment, Schmidt-Ott can continue his career pathway with focus on the development of renal and uronary tract and intensify the cooperation of MDC and Charité towards the development of new approaches in prevention, diagnostics and therapy of acute renal injury.

Altuna AKALIN joined the MDC/BIMSB
In March 2014, Altuna Akalin from Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, was recruited as head of the bioinformatics platform and independent research group leader at BIMSB. After studies in Istanbul, Turkey, Akalin moved for his PhD studies to the University of Bergen, Norway, and spent some time for a collaboration project on high-throughput sequencing data analysis as a visiting student researcher at RIKEN Yokohama Institute, Japan. From 2010, Akalin stayed at Weill Cornell Medical College, New York, first as a postdoctoral and then as a senior research associate. In 2013, he continued as a postdoctoral fellow in the lab of Dirk Schuebeler at Friedrich Miescher Institute. Akalin's research focusses on the computational analysis and integration of high-throughput datasets, such as microarrays and next generation sequencing, to understand the genetic and epigenetic control mechanisms of cancer.

Michela Di VIRGILIO started Helmholtz Young Investigators Group
Since September 2014, Michela DiVirgilio is heading an independent research group at MDC. Di Virgilio is a specialist in DNA repair and maintenance of genome stability and her group is co-funded from the Helmholtz program. Having studied Biology at the University of Milano, she completed her PhD thesis under the supervision of Jean Gautier at Columbia University, New York, USA. She then joined the lab of Michel Nussenzweig at the Rockefeller University, New York, USA, for her postdoctoral training.

Ralf KÜHN recruited to MDC and Berlin Institute of Health (BIH)
Ralf Kühn was recruited to the MDC from Helmholtz-Zentrum München where he had worked as a staff scientist and research group leader in the Institute for Developmental Genetics with focus on genome engineering and disease modeling in mice, embryonic and induced pluripotent stem cells. Kühn completed his PhD thesis in the lab of Klaus Rajewsky at the University of Cologne. After a postdoc time at the Institute for Genetics in Cologne, he held a position as Head of Mouse Genetics and Principle Scientist at Artemis Pharmaceuticals GmbH, Cologne. From May 2014, Kühn took over the Transgenic Core Facility at MDC and established in parallel his own BIH-funded research group that is using induced pluripotent stem cell lines and designer nuclease technologies to study disease mechanisms in human cells by reverse genetics.

Niccolo ZAMPIERI recruited from Columbia University
Niccolo Zampieri was jointly recruited by MDC and Charité/NeuroCure from Columbia University, New York, USA. He was offered a NeuroCure group leader position with labs at the MDC starting in September 2014. Zampieri studied Biology at the University of Milano where he also received his PhD degree. He completed his PhD thesis in Moses Chao's lab at New York University, USA, and moved to Columbia University for his postdoctoral work. Zampieri's research is aiming at understanding the mechanisms that control neuronal positioning and synaptic specificity during development of the nervous system and at defining the logic that drives neural circuit assembly.

2015

Wei CHEN received full professorship for Functional Genomics and Systems Biology at Charité
After studies in Biochemistry and Medical Genetics at Xiamen and Sichuan University in China, the Chinese scientist Wei Chen completed his PhD study at Max Planck Institute for Molecular Genetics, Berlin where he focused on the analysis of structural variations in the human genome and their roles in causing human congenital disorders. During this period, Chen became interested in the development of novel genomics technology. Right after finishing his PhD thesis, Chen established a research group in the same institute that became one of the pioneers in the field of next generation sequencing (NGS). In 2009, he moved his lab to the newly established BIMSB at MDC and set up from the scratch one of the leading NGS facilities in Europe. Chen accepted an appointment to a full professorship for Functional Genomics and Systems Biology at Charité starting in August 2015. Wei Chen accepted an offer for a full professorship at Southern University of Science and Technology of China, Shenzhen, and left the MDC/BIMSB in June, 2016.

Irmtraud MEYER appointed to full professorship at Free University Berlin
The computational scientist Irmtraud Meyer from University of British Columbia, Vancouver, Canada, received the call for the W3 professorship "Bioinformatics of RNA Structure and Transcriptome Regulation" at the Free University Berlin and a research group leader position at MDC in 2015. The joint recruitment is co-funded from the Impulse and Networking Fund of the Helmholtz President. The focus of Meyer's research is on transcriptome regulation by RNA folds, RNA-RNA interactions and RNA-protein complex formation. After studies in physics and mathematics, she did her PhD under supervision of Richard Durbin at the University of Cambridge, UK. She continued her academic career in the UK as a postdoctoral researcher at the University of Oxford and at the European Bioinformatics Institute, Cambridge. Meyer started working at MDC in January 2016.

Recruitment of Jan Philipp JUNKER for a junior group leader position at BIMSB
Jan Philipp Junker from Hubrecht Institute, Utrecht, Netherlands, began as a new junior group leader at MDC's BIMSB in November 2015. His team works on quantitative developmental biology at the interface between experimental and theoretical science. His research goal is to develop a comprehensive understanding of the spatiotemporal interplay between transcription factor binding, chromatin modifications, and gene expression for establishing and maintaining cell fates and complex body plans. Junker studied Physics at the Technical University Munich where he also received his PhD degree. Then he worked as a postdoctoral fellow in the team of Alexander van Oudenaarden at Massachusetts Institute of Technology, USA. Since 2009, he was Senior Postdoc at Hubrecht Institute.

Recruitment of Stephan PREIBISCH for a junior group leader position at BIMSB
Stephan Preibisch studied computer science at the Technical University Dresden. Then he applied for the PhD program of the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden and joined the interdisciplinary team of Pavel Tomancak. He continued there for a short postdoc term before he moved to the Howard Hughes Medical Institute Janelia Farm Research Campus in Ashburn, USA. In 2012, he became Human Frontier Science Program Fellow under supervision of Robert H. Singer, Albert Einstein College of Medicine, New York, and Eugen Myers, Max Planck Institute for Molecular Cell Biology and Genetics. In September 2015, Stefan Preibisch was recruited to the MDC/BIMSB. His team is aiming at creating a complete digital representation of the nematode *Caenorhabditis elegans* in the dauer diapause that will combine microscopy data of all scales as well as high-resolution imaging of transcription.

2016

Michael GOTTHARDT received W3 professorship at the Charité
In August 2016, the MDC research group leader Michael Gotthardt accepted an appointment to the full professorship for Experimental und Translational Cardiology at the Charité Medical Faculty Berlin. Michael Gotthardt studied medicine at the University of Heidelberg and completed his thesis at the Max Planck Institute for Molecular Genetics in Berlin. After clinical internships in Berlin and Boston, he worked as a postdoctoral fellow at the University of Texas in Dallas. Michael Gotthardt has been leading a reserach team at the MDC since 2002. Until today, he is also holding a Research Assistant Professorship at the Washington State University in Pullman and a Research Associate Professorship at the University of Tucson in Arizona. Michael Gotthardt is continuing his research group at the MDC in order to increase the understanding of the cause and progression of inherited heart and muscle diseases and aid in developing novel therapeutic strategies.

Gaetano GARGIULO started Helmholtz Young Investigators Group at MDC
Gaetano Gargiulo was recruited in September 2016 from the Netherlands Cancer Institute in Amsterdam to establish with co-funding of the Helmholtz Association a junior research group on "Molecular Oncology" at the MDC. Together with his team, Gargiulo works on the molecular characterization of the Gliobastoma, which is a brain tumor with a very bad clinical prognosis. Gaetano Gargiulo is generating so-called Glioblastoma

avatars as disease models for an improved treatment. Gargiulo did his graduate studies at the University of Milano in Italy and received his PhD degree in Molecular and Cell Biology from the University of California at Berkeley. He spent his first postdoc term in Eileen Furlong's group at the EMBL in Heidelberg, where he got additional training in genomics and epigenomics.

Mina GOUTI recruited from Francis Crick Institute
Since April 2016, Mina Gouti is heading an independent junior group at the MDC, which is also part of the Helmholtz Program "Personalized Medicine". Gouti and her team are using human and mouse pluripotent stem cells to model and understand normal and pathological development of the neuromuscular system. After studies in Molecular Biology at the University of Hertfordshire, Mina Gouti finished her master thesis in the group of Amanda Fisher at the Imperial College of Science, Technology and Medicine. For her PhD thesis, Mina Gouti moved to the Biomedical Research Foundation of the Academy of Athens. After two postdoctoral terms in Athens and London, she became Senior Investigator Scientist in the lab of James Briscoe at the Francis Crick Institute in London.

Roland SCHWARZ starts new BIMSB Junior Research Group
Roland Schwarz studied Computer Science at the University of Applied Sciences in Würzburg and became graduate student in the bioinformatics team of Tobias Müller at the University's Biocenter. He worked as a postdoctoral fellow at the Cancer Research UK Cambridge Institute in the group of Florian Markowetz and subsequently with a Marie Curie Fellowship at the European Bioinformatics Institute in Hinxton, UK, in the group of Nick Goldman. Schwarz accepted the offered position as junior group leader at BIMSB and started his group "Evolutionary and Cancer Genomics" in October 2016. He is interested in how somatic alterations and genomic rearrangements contribute to tumor fitness and progression in the clinic and to what extent somatic variation interacts with the germline genetic background to shape the regulatory landscape of cancer.

Technology Platforms

2014

Sebastian DIECKE had been Interim Director of the Stem Cell Core at the Cardiovascular Institute of Stanford School of Medicine in California before he was recruited to the BIH/MDC. In 2014, he established the subsidiary of the **BIH Core Facility Stem Cells** which is located on Campus Buch.

2016

Since mid-2016, **Jennifer KIRWAN** is heading the **BIH Metabolomics Core Facility**, which is also located on Campus Buch. Before she moved to Berlin, she had gathered experience in managing the Metabolomics Facility at the University of Birmingham in UK for over five years.

Sascha SAUER took the leadership of **Genomics Technology Platforms of BIH and BIMSB** in August 2016. Dr. Sauer, who is also principal investigator in the "European Sequencing and Genotyping Infrastructure", was recruited from the Max Planck Institute for Molecular Genetics in Berlin where he held an independent group leader position.

Andrew WOEHLER is biomedical engineer by training. He was leading a junior group within the Cluster of Excellence and DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain at the Max Planck Institute for Biophysical Chemistry in Göttingen when he was offered the leadership of the **BIMSB Light Microscopy Technology Platform** starting from May 2016.

European Research Council (ERC) Grants

Since 2007 the European Research Council (ERC) has been funding scientists in basic research with one of the most important and prestigious funding programs in Europe. In total, scientists of the MDC have been awarded 21 of the highly sought-after ERC grants, 18 of them were raised directly at the MDC. 16 grant winners are currently working at the MDC.

ERC Advanced Grants

Funding period: 5 years

Advanced Grants support established researchers who are seen as independent research leaders in their own right. To qualify for an application, researchers need an excellent scientific track record over a period of at least ten years. An Advanced Grant supports researchers for 5 years with € 2.5 million (up to € 3.5 million in exceptional cases).

Thomas Willnow
2014 | BeyOND
Metabolic basis of neurodegenerative disease

Zsuzsanna Izsvák
2011 | TRANSPOSOstress
Impact of stress-induced transposon activities on human disease

Thomas J. Jentsch
2011 | CYTOVOLION
Ion homeostasis and volume regulation of cells and organelles

Gary Lewin
2011 | EXTREMOPHILE MAMMAL
Molecular exploitation of an extremophile mammal

Klaus Rajewsky
2010 | LYMPHOMA
Modeling lymphoma pathogenesis in mice - from basic mechanisms to pre-clinical models

Martin Lohse*
2008 | TOPAS
Towards the Quantal Nature of Receptor/cAMP Signals

ERC Consolidator Grants

Funding period: 5 years

Consolidator Grants are intended for independent scientists at a stage in their career at which they are consolidating their research teams and programs. The grant supports researchers with up to € 2.75 million.

James Poulet
2016 | ACoolTouch
Neural mechanisms of multisensory perceptual binding

Oliver Daumke
2014 | MitoShape
Structural basis of mitochondrial inner membrane shape and dynamics

ERC Starting Grants

Funding period: 5 years

Starting Grants support young and aspiring researcher leaders and promote their early scientific independence. The grant supports scientists with up to € 2 million.

Gaetano Gargiulio
2016 | iGBMavatars
Glioblastoma Subtype Avatar Models for Target Discovery and Biology

Jan Philipp Junker
2016 | SPACEVAR
Quantitative analysis of variability and robustness in spatial pattern formation

Michela Di Virgilio
2014 | DNAendProtection
DNA end protection in Immunity and Cancer

Baris Tursun
2014 | EPROWORM
Safeguarding Cell Identities: Mechanisms Counteracting Cell Fate Reprogramming

Holger Gerhardt*
2012 | REshape
Reverse engineering of vascular patterning through mosaic in vivo analysis of endothelial cell shape regulation

Michael Gotthardt
2011 | CardioSplice
A systems and targeted approach to alternative splicing in the developing and diseased heart: Translating basic cell biology to improved cardiac function

Jan-Erik Siemens**
(since February 2013 Medical Faculty of Heidelberg)
2011 | ThermoReg
Peripheral and Central Mechanisms of Temperature Detection and Core Body Thermoregulation

James Poulet
2010 | BrainStates
Brain states, synapses and behaviour

Matthew Poy
2010 | IsletVasc
Molecular Mechanisms Regulating Pancreatic Islet Vascularization

Francesca Spagnoli
2009 | HEPATOPANCREATIC
Mechanisms underlying cell fate decision between pancreas and liver

ERC Proof of Concept Grant

Funding period: 18 months

Gary Lewin
2016 |

Francesca Spagnoli
2014 | TheLiRep
Exploring the Potential for Therapeutic Lineage Reprogramming of Diabetes

Martin Lohse*
2013 | FRESCA
FRET-based receptor screening assays

* ERC Grant recipients have not been at the MDC at the time they received the grant.

** ERC Grant recipients received the funding at their time at the MDC, but left the research center in the meantime.

Awards

2014

Hua YU (Beckman Research Institute of City of Hope)
Humboldt Research Award
Alexander von Humboldt Foundation
(for a collaboration with Thomas Blankenstein)

Michael SIEWEKE (CIML; CNRS)
Einstein BIH Visiting Fellow
(for a collaboration with Klaus Rajewsky)

Jane HOLLAND
Curt Meyer Memorial Price
Berlin Cancer Association

Nikolaus RAJEWSKY
Honorary PhD
Rome's Sapienza University

Michael SIEWEKE
Elected EMBO member

Clemens SCHMITT and Soyoung LEE
Johann Georg Zimmermann Research Award
MHH-plus-Stiftung (Medizinische Hochschule Hannover)

Florian HERSE
Rösslin-Preis
Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG)

Michela DI VIRGILIO
Female Independency Award
Berlin School of Integrative Oncology (BSIO)

Ulrike STEIN, Peter SCHLAG, Ulrich ROHR (Hoffmann-La Roche)
Felix Burda Award for Colon Cancer Research

Marvin JENS
Springer Award for Outstanding Theses in Systems Biology

2015

Yehudit BERGMAN (HUJI Jerusalem)
Helmholtz International Fellow Award
(for a collaboration with Klaus Rajewsky and within the SignGene Research School)

Robson Augusto SOUZA DOS SANTOS (Federal University of Minas Gerais Belo Horizonte)
Georg Forster Research Award
Alexander von Humboldt Foundation
(for a collaboration with Michael Bader)

Amanda FISHER (ICL London)
Helmholtz International Fellow Award
(for a collaboration with Nikolaus Rajewsky)

Ulrich MÜLLER (Scripps Research Institute)
Einstein BIH Visiting Fellow
(for a collaboration with Gary Lewin)

Jichang WANG and Zsuzsanna IZSVAK
GSCN Publication of the Year
German Stem Cell Network (GSCN)

Michael BADER
Gold Medal E.K. Frey – E. Werle Foundation

2016

Nir FRIEDMAN (HUJI Jerusalem)
Humboldt Research Award
Alexander von Humboldt Foundation
(for a collaboration with Nikolaus Rajewsky and within the SignGene Research School)

Jan Philipp JUNKER
ARCHES Prize
Minerva Foundation (MPG/BMBF)

Markus LANDTHALER
Science Award
GlaxoSmithKline Foundation

Roland SCHWARZ
Preis der Berlin-Brandenburgischen Akademie der Wissenschaften, gestiftet von der Monika Kutzner Stiftung zur Förderung der Krebsforschung

Matthias LEISEGANG
Curt Meyer Memorial Price
Berlin Cancer Association

Véronique GEBALA
Helmholtz Doctoral Prize
Helmholtz Association

Felix LORENZ, Elisa KIEBACK, Julian CLAUß and Inan EDES
Grand Prize
One Start Life Sciences Startup Award

Selected Highlight Publications

2014

Argonaute2 Mediates Compensatory Expansion of the Pancreatic β Cell. Tattikota, S. G., Rathjen, T., McAnulty, S. J., Wessels, H.-H., Akerman, I., van de Bunt, M., et al. (2014). *Cell Metabolism*, 19 (1), 122–134. https://doi.org/10.1016/j.cmet.2013.11.015

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Blood Flow and Bmp Signaling Control Endocardial Chamber Morphogenesis. Dietrich, A.-C., Lombardo, V. A., Veerkamp, J., Priller, F., Abdelilah-Seyfried, S. (2014). *Developmental Cell, 30* (4), 367–377. https://doi.org/10.1016/j.devcel.2014.06.020

LRP2 Acts as SHH Clearance Receptor to Protect the Retinal Margin from Mitogenic Stimuli. Christ, A., Christa, A., Klippert, J., Eule, J. C., Bachmann, S., Wallace, V. A., et al. (2015). *Developmental Cell, 35* (1), 36–48. https://doi.org/10.1016/j.devcel.2015.09.001

PI3 Kinase and FOXO1 Transcription Factor Activity Differentially Control B Cells in the Germinal Center Light and Dark Zones. Sander, S., Chu, V. T., Yasuda, T., Franklin, A., Graf, R., Calado, D. P., et al. (2015). *Immunity, 43* (6), 1075–1086. https://doi.org/10.1016/j.immuni.2015.10.021

Sequential Poly-ubiquitylation by Specialized Conjugating Enzymes Expands the Versatility of a Quality Control Ubiquitin Ligase. Weber, A., Cohen, I., Popp, O., Dittmar, G., Reiss, Y., Sommer, T., et al. (2016). *Molecular Cell, 63* (5), 827–839. https://doi.org/10.1016/j.molcel.2016.07.020

Kinetic Analysis of Protein Stability Reveals Age-Dependent Degradation. McShane, E., Sin, C., Zauber, H., Wells, J. N., Donnelly, N., Wang, X., et al. (2016). *Cell, 167* (3), 803–815.e21. https://doi.org/10.1016/j.cell.2016.09.015

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Changes in neural network homeostasis trigger neuropsychiatric symptoms. Winkelmann, A., Maggio, N., Eller, J., Caliskan, G., Semtner, M., Häussler, U., et al. (2014). *The Journal of Clinical Investigation, 124* (2), 696–711. https://doi.org/10.1172/JCI71472

Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination. Sheean, M. E., McShane, E., Cheret, C., Walcher, J., Müller, T., Wulf-Goldenberg, A., et al. (2014). *Genes & Development, 28* (3), 290–303. https://doi.org/10.1101/gad.230045.113

Tuning Piezo ion channels to detect molecular-scale movements relevant for fine touch. Poole, K., Herget, R., Lapatsina, L., Ngo, H.-*D., Lewin, G. R. (2014).* Nature Communications, 5, 3520. https://doi.org/10.1038/ncomms4520

Identification of LRRC8 Heteromers as an Essential Component of the Volume-Regulated Anion Channel VRAC. Voss, F. K., Ullrich, F., Münch, J., Lazarow, K., Lutter, D., Mah, N., et al. (2014). *Science, 344* (6184), 634–638. https://doi.org/10.1126/science.1252826

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2015

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Crystal structure of the dynamin tetramer. Reubold, T. F., Faelber, K., Plattner, N., Posor, Y., Ketel, K., Curth, U., et al. (2015). *Nature, 525* (7569), 404–408. https://doi.org/10.1038/nature14880

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2016

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Scientific Meetings and Conferences

2014

Date	Event	Host / Organizer
15.-17.05.2014	**European Worm Meeting**	Dr. Baris Tursun
12.-14.06.2014	**7th Berlin Summer Meeting „From Systems Biology to Systems Medicine“**	Prof. Nikolaus Rajewsky
20.06.2014	**5th Annual Scientific Symposium „Ultrahigh Field Magnetic Resonance Clinical Need, Research Promises, Technical Solutions”**	Prof. Thoralf Niendorf
29.09.-03.10.2014	**36th FGMR Discussion Meeting, GDCh**	Prof. Helmut Oschkinat (FMP) Prof. Thoralf Niendorf
20.-22. 11.2014	**International DZHK-Symposium “Receptors, G proteins and integration of Ca2+ signaling in the cardiovascular system**	DZHK, MDC

2015

Date	Event	Host / Organizer
28.-29.05.2015	**Brain Tumor Meeting**	Dr. Baris Tursun
04.-06.06.2015	**8th Berlin Summer Meeting Localization of cellular Processes**	Prof. Nikolaus Rajewsky
26.06.2015	**6th Annual Scientific Symposium „Ultrahigh Field Magnetic Resonance Clinical Need, Research Promises, Technical Solutions”**	Prof. Thoralf Niendorf
10.07.2015	**Frontiers in Developmental Neuroscience Symposium in Honour of Carmen Birchmeier**	Prof. Carmen Birchmeier
07.-09.10.2015	**ECBS & ICBS joint meeting 2015 “Bringing Chemistry to Life”**	Dr. Ronald Frank (FMP) Prof. Michael Bader, Prof. Gary Lewin

2016

Date	Event	Host / Organizer
01.-03.06.2016	**European Worm Meeting**	Dr. Baris Tursun
24.06.2016	**7th Annual Scientific Symposium „Ultrahigh Field Magnetic Resonance Clinical Need, Research Promises, Technical Solutions"**	Prof. Thoralf Niendorf
12.09.2016	**Frontiers in DNA Repair**	Dr. Michela di Virgilio
13.-14.10.2016	**9th Berlin Summer Meeting** **Circular RNAs and RNA Modifications**	Prof. Nikolaus Rajewsky
4.11.2016	**Mechanisms of Molecular and Cellular Immunity 1964-2016** **Symposium hosted by Klaus Rajewsky**	Prof. Klaus Rajewsky
28.11.2016	**Dechema Colloqium** **Games of tag – Letting protein modifications out of the lab**	BBB with MDC

Selected Seminars 2014 – 2016

MDC LECTURE

02.11.15 **Frederik W. ALT** *(Boston Children's Hospital, Harvard Medical School, Howard Hughes Medical Institute)*
Chromosomal Loop Domains, Genomic Rearrangements, and Cancer

18.12.15 **Tom RAPOPORT** *(Harvard Medical School, Howard Hughes Medical Institute)*
How an organelle gets into shape

03.11.16 **Michel C. NUSSENZWEIG** *(Howard Hughes Medical Institute, The Rockefeller University)*
The HIV Problem

25.11.16 **Botond ROSKA** *(Friedrich Miescher Institute for Biomedical Research (FMI)*
The first steps of vision: cell types, circuits and repair

ELENA TIMOFÉEFF-RESSOVSKY LECTURE

Notable Women in Science and Medicine

29.04.14 **Nine KNOERS** *(University Medical Center Utrecht)*
The potential of next generation sequencing for renal disorders: scientific impact and clinical utility

10.06.14 **Hua Eleanore YU** *(Cancer Immunotherapeutics Program, Comprehensive Cancer Center)*
STAT3 pathway: from fundamental discoveries to clinical translation

26.03.15 **Anne-Lise BOERRESEN-DALE** *(Oslo University Hospital)*
Towards integrated "omics" for personalized treatment of breast cancer

03.12.15 **Hannelore EHRENREICH** *(Max Planck Institute of Experimental Medicine)*
Neuropsychiatric diseases: Early steps towards biological disease definition

04.03.16 **Anne CORCORAN** *(Babraham Institute)*
Local and global epigenetic regulation of immunoglobulin V(D)J recombination

06.07.16 **Cornelia WEYAND** *(Stanford University)*
The Warburg Effect – from cancer to autoimmune disease

07.09.16 **Emmanuelle CHARPENTIER** *(Max Planck Institute for Infection Biology)*
Genome Engineering with CRISPR-Cas9 and -Cpfl: lessons learned from bacteria

LUNCHTIME SEMINAR SERIES

DISTINGUISHED SPEAKERS

28.08.14 **Amir ORIAN** *(Technion-Israel Institute of Technology)*
Identification of a genetic network that maintains the differentiated identity and tissue homeostasis

18.09.14 **Sebastian KADENER** *(Hebrew University)*
Linear splicing efficiency and trans-acting factors determine the production rate of circRNAs

18.06.15 **Yehudit BERGMAN** *(Hebrew University Medical School)*
Epigenetic programming of allelic choice

19.11.15 **Angela de PACE** *(Systems Biology Department at Harvard Medical School)*
Precision and plasticity in animal transcription

29.09.16 **Martyn GOULDING** *(Salk Institute)*
Genetic approaches for dissecting the circuitry for touch and movement

24.11.16 **Stirling CHURCHMAN** *(Harvard)*
Gene expression regulation at high resolution

BIMSB DISTINGUISHED SPEAKERS

21.04.15 **Matthias HENTZE** *(EMBL)*
RNA-binding proteins, metabolism and a new function of the genome

05.05.15 **Eric WESTHOF** *(IBMC/CNRS)*
How far can we predict RNA architectures and RNA binding of proteins

26.05.15 **Anders KROGH** *(University of Copenhagen)*
Bayesian transcriptome assembly improves detection and quantification of alternative transcripts in RNAseq

01.09.16 **Oliver HOBERT** *(Howard Hughes Medical Institute, Columbia University)*
A regulatory map of the C. elegans nervous system

06.12.16 **Wolf REIK** *(Babraham Institute)*
Epigenetic reprogramming in mammalian development

CANCER LECTURE

01.04.14 **Angelika EGGERT** *(Charité – Universitätsmedizin Berlin)*
On the way to Systems Medicine Approaches in Neuroblastoma

13.06.14 **Michael KARIN** *(UCSD School of Medicine)*
Inflammation, Immunity and Cancer

16.06.14 **Hans SCHREIBER** *(University of Chicago)*
Directing T cells to eradicate cancer with TCRs, CARs or fusion proteins

13.09.14 **Yinon BEN-NERIAH** *(The Hebrew University)*

14.08.15 **Robert G. ROEDER** *(The Rockefeller University)*
Mechanistic studies of the function of diverse transcriptional co-activators in animal cells

13.10.15 **Jerry L. WORKMAN** *(Stowers Institute for Medical Research)*
Serine and SAM responsive complex SESAME regulates histone modification crosstalk by sensing cellular metabolism

01.12.15 **Eduardo MORENO** *(University of Bern)*
Regulating the cellular composition of our bodies using fitness fingerprints

09.02.16 **Andreas STRASSER** *(The Walter and Eliza Hall Institute of Medical Research)*
Learning the mechanisms of cell death to develop novel cancer therapies

05.09.16 **Michael KARIN** *(USCD School of Medicine)*
Metabolic stress and inflammation in chronic liver disease and cancer

09.11.16 **Michael RAPE** *(UC Berkeley)*
Ubiquitin-dependent control of stem cell fate and function

NEUROSCIENCE SEMINAR SERIES

DISTINGUISHED SPEAKERS

16.04.14 **Nils BROSE** *(Max Planck Institute of Experimental Medicine)*
Neuroligins at inhibitory synapses – from synaptogenesis to autism spectrum disorders

11.06.14 **Martyn GOULDING** *(Molecular Neurobiology Laboratory, The Salk Institute)*
Making sense of the sensorimotor circuits in the spinal cord

01.08.14 **Hannah MONYER** *(Universitätsklinikum Heidelberg, DKFZ)*
Local and long-range GABAergic inhibition and its function for spatial coding in the hippocampal-entorhimal formation

29.09.15 **Alison LLOYD** *(MRC LMCB, UCL, London and visiting NeuroCure Fellow)*
Multicellular complexity of peripheral nerve regeneration

11.12.15 **Ryoichiro KAGEYAMA** *(Kyoto University)*
Oscillatory control of somitogenesis and neurogenesis

22.01.16 **Gerd KEMPERMANN** *(Center for Regenerative Therapies (CRTD) / DFG research center at TU Dresden)*
New neurons and hippocampal individuality

08.07.16 **Elena CATTANEO** *(Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases, University of Milan)*
Neurons from stem cells for Huntington's Disease research: combining genetics with cell biology and evolution

WOLLENBERGER SPECIAL

Cardiovascular and Metabolic Disease Seminar Series

20.10.14 **Luc MAROTEAUX** *(INSERM U839, UPMC, Institut du Fer à Moulin)*
Serotonin from development to pathophysiology: The 5-HT2B receptor contribution

11.03.15 **Alvin H. SCHMAIER** *(Case Western Reserve University)*
Mas agonism in thrombosis

23.03.15 **Antonio BALDINI** *(University Federico II; Institute of Genetics and Biophysics)*
Balancing gene haploinsufficiency by targeting chromatin: the case of congenital heart disease

10.06.16 **Matthias RIEF** *(Technical University Munich)*
Mechanics of single protein molecules

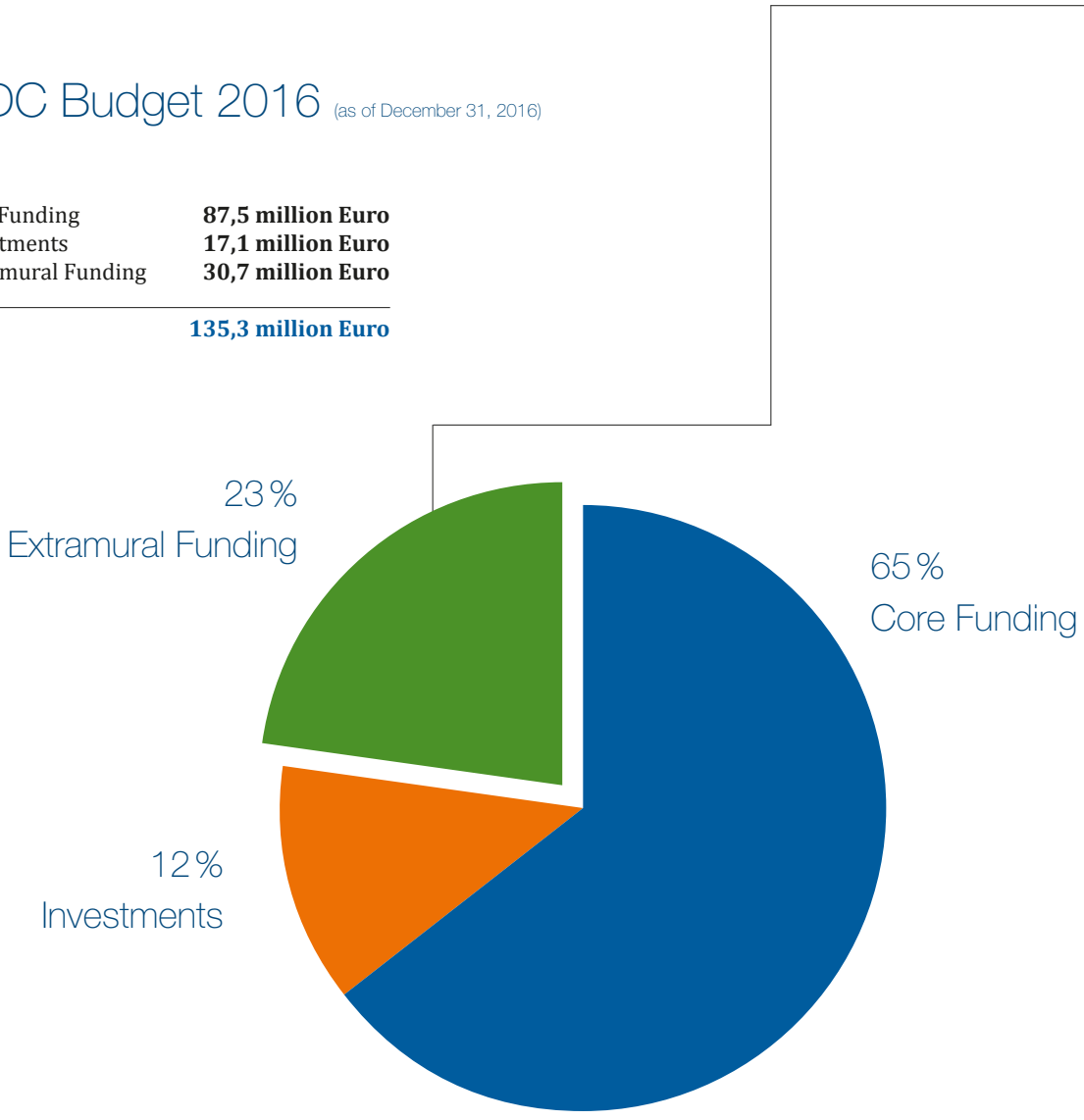
Facts and Figures

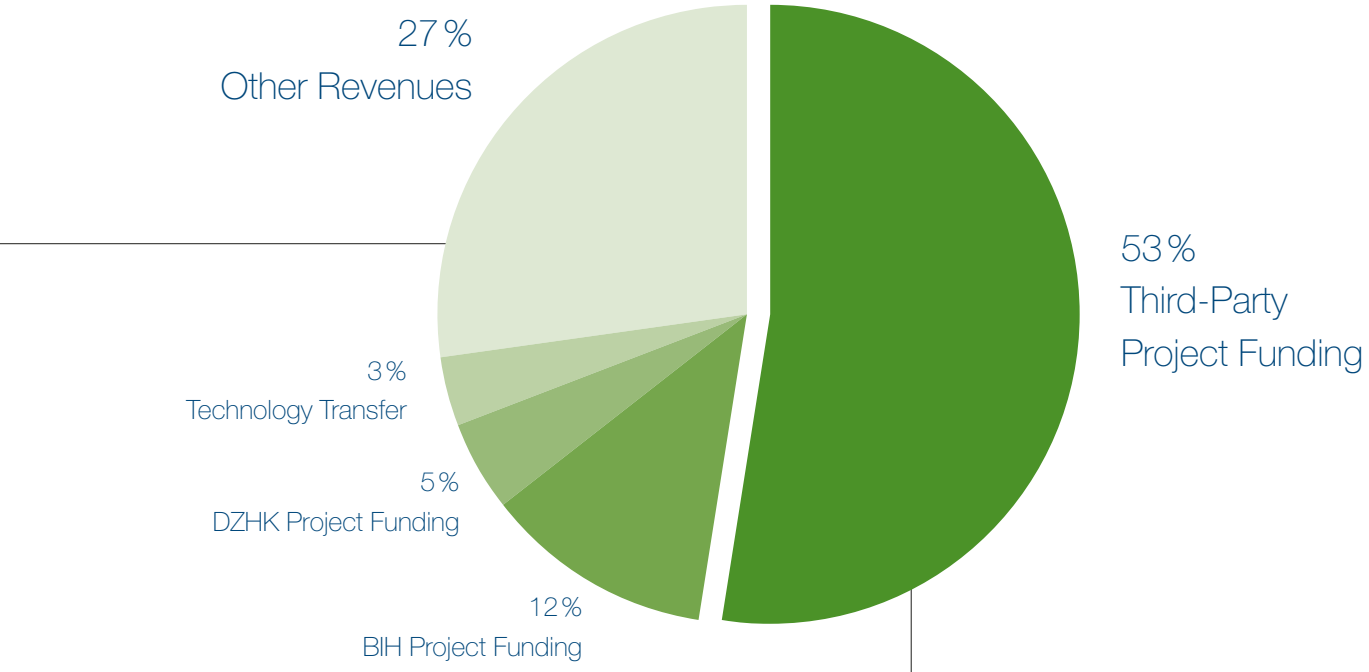
Personnel (as of December 31, 2016)

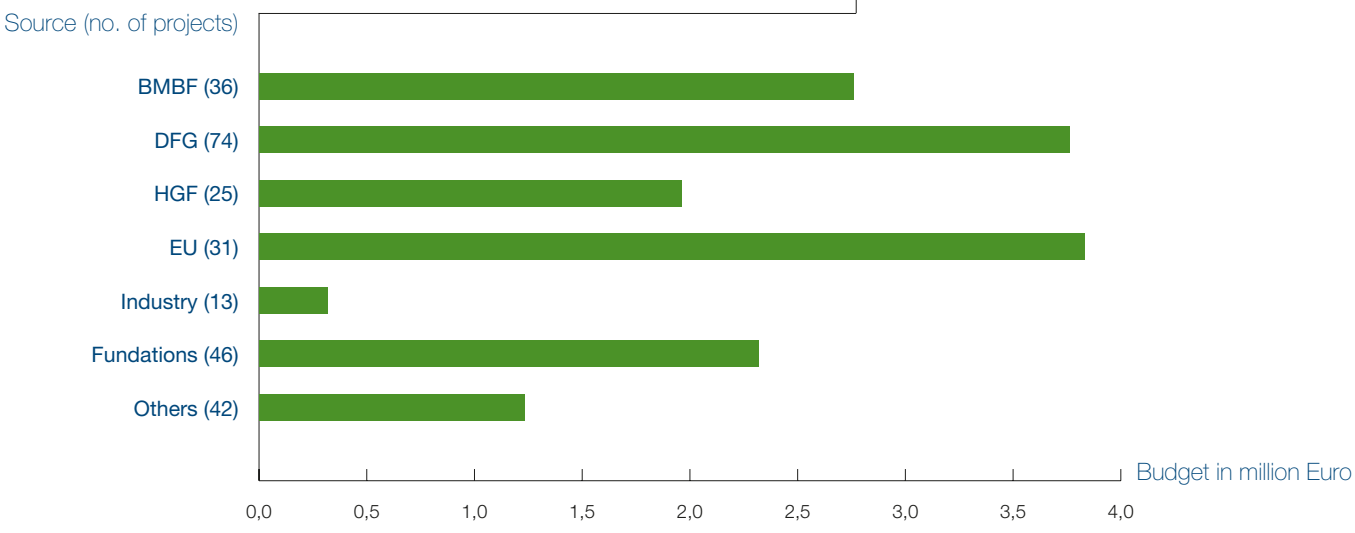
Staff and Guests	**1660**
thereof in research	**1291**
thereof PhD students	**363**

MDC Budget 2016 (as of December 31, 2016)

Core Funding	**87,5 million Euro**
Investments	**17,1 million Euro**
Extramural Funding	**30,7 million Euro**
Total	**135,3 million Euro**




27 %
Other Revenues
53 %
Third-Party
Project Funding
3 %
Technology Transfer
5 %
DZHK Project Funding
12 %
BIH Project Funding


Source (no. of projects)
BMBF (36)
DFG (74)
HGF (25)
EU (31)
Industry (13)
Fundations (46)
Others (42)
Budget in million Euro
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0

Organizational Structure

Supervisory Board

The Supervisory Board is the supervisory committee of the MDC and is responsible for monitoring the legality, effectiveness, and economic efficiency of the management of all the activities of the corporation. It determines which decisions of the Board of Directors require the prior approval of the Supervisory Board and may issue instructions about important matters involving political aspects of the research and financial affairs of the corporation.

The Supervisory Board consists of no more than twelve members. Its members include, among others: two representatives from the federal government, a representative of the State of Berlin, the chairman of the Scientific Advisory Board, the Presidents of the Humboldt Universität zu Berlin and the Freie Universität Berlin, and two MDC representatives.

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MinDir'in Bärbel Brumme-Bothe
Federal Ministry of Education and Research

Vice-Chairperson

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Senat Chancellery, Berlin

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Freie Universität Berlin

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Bayer Pharmaceuticals

Prof. Dr. Patrick Cramer
Max Planck Institute for Biophysical Chemistry

Prof. Dr. Horst Domdey
BioM GmbH

MinR Dr. Jan Grapentin
Federal Ministry of Education and Research

Prof. Dr. Annette Grüters-Kieslich
Charité-Universitätsmedizin Berlin

Prof. Dr. Sabine Kunst
Humboldt-Universität zu Berlin

Prof. Dr. Hannah Monyer
Deutsches Krebsforschungszentum

Prof. Dr. Claus Scheidereit
Max-Delbrück-Center for Molecular Medicine

Dr. Jana Wolf
Max-Delbrück-Center for Molecular Medicine

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Max Planck Institute for Biophysical Chemistry

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University of Freiburg

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University Medical Centre Utrecht

Prof. Dr. Anna Tramontano †
Sapienza University of Rome

* As of 31.12.2016

Organization Chart of the MAX DELBRÜCK CENTER FOR MOLECULA

Corporation under public

Scientific Advisory Board
Chair
Prof. Dr. Patrick Cramer
(MPI for Biophysical Chemistry)

Supervisory Board
Chair
Bärbel Brumme-Bothe (Fed. Min. Education an

Scientific Council
Chair
Prof. Dr. Gary Lewin

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Chair, Scientific Director
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Dr. Heil

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Prof. Dr. Friedrich C. Luft (cr)
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Prof. Dr. Thoralf Niendorf
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Prof. Dr. Tobias Pischon
Dr. Matthew Poy
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Prof. Dr. Matthias Treier
Prof. Dr. Thomas Willnow

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Management:
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Light Microscopy
Dr. Andrew Woehler

Electron Microscopy
Dr. Bettina Purfürst

iPS
Dr. Sebastian Diecke

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Prof. Thoralf Niendorf

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N.N.

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Dr. Arnd Heuser

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Dr. Hans-Peter Rahn

Transgenics
Dr. Ralf Kühn

Bioinformatics
Dr. Altuna Akalin

Legend
cr = cross-reference
PF = permanent fellow

Mailing address: Robert-R

R MEDICINE in the Helmholtz Association
law

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

d Research)

ive Director
ke Wolke

Staff Council
Dagmar Gerhard

Women's Representative
Dr. Christiane Nolte

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Hazardous Goods Officer:	Yvette Wefeld-Neuenfeld
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IT Security Officer:	Dr. Torsten Johr
Research Ombudsman:	Prof. Dr. Jens Reich
Ph.D. Ombudspersons:	Dr. Daniela Panáková, Prof. Dr. Matthias Selbach

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Head of administration:
Dr. Regina Jünger (Charité)

Program-Management:
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Prof. Dr. Ralph Kettritz
PD Dr. Sabine Klaassen
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Proteomics / Metabolomics
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Dr. Gesa Schäfer

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Vera Glaßer (interim)

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Graduate Office
Dr. Michaela Herzig

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Stefanie Rach

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Dr. Barbara Wiskow (interim)

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Internal Audit
Uta Brinker
Kirstin Müller

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Magdalena Milsch-Hetzig

IT
Dr. Alf Wachsmann

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Dr. Dorothea Busjahn

Technical Facility Management
Construction: Ralf Streckwall
Operation: Michael Arnold

Purchasing
Dr. Carsten Oehme

Central Services
Rüdiger Mehlhase

Legal Affairs and Technology Transfer
Dr. Claudia Thurow

össle-Strasse 10, D-13125, Berlin

Issue: December, 2016
www.mdc-berlin.de/organigram-E

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Campus Berlin-Buch
Der Gesundheit verpflichtet

Common Facilities

A 8 Gate House with Café Max and apartments

A 9 Reception gate

A 13 Gläsernes Labor / Life Science Learning Lab; Campus Info Center

A 14 Cafeteria

MDC Guesthouses

B 54 Hans Gummel House

B 61 Salvadore Luria House with kindergarden

Research

Max Delbrück Center for Molecular Medicine (MDC)

C 27 Walter Friedrich House

C 31.1 -2 Max Delbrück House

C 31.3 -5 Energy and Infrastructure Center South (EZS)

C 83 MDC.C / Max Delbrück Communications Center

C 84 Hermann von Helmholtz House

B 63, 64 Research Services

B 88 Ultrahigh Field Facility

B 89 Max Rubner House

A10 Library

Leibniz-Institut für Molekulare Pharmakologie (FMP)

C 81 FMP

Facilities shared by MDC and FMP

C87 Timoféeff-Ressovsky-Haus

Clinical Research

B 42-53 Experimental and Clinical Research Center (ECRC)

Companies

A 7 Eckert & Ziegler AG, Eckert Wagniskapital und Frühphasenfinanzierung (EWK)

A 15 Car mechanics, EZAG, Gardener

B 55 **OCVH - Oskar-und-Cécile-Vogt-Haus**
BBB-post office, 8sens.biognostic, FILT, MRI, ConGen, E.R.D.E., ART-CHEM, LIPIDOMIX, Roboklon, Myelo, Fresenius, Patent lawyers Dr. Baumbach, L.O.T., WISE, Octreopharm, Institut E & G, NIKON, Hermes-Analytik, Steinbeis-Transfer-Institut, I.M.S.M., AJ Innuscreen

B 64 epo

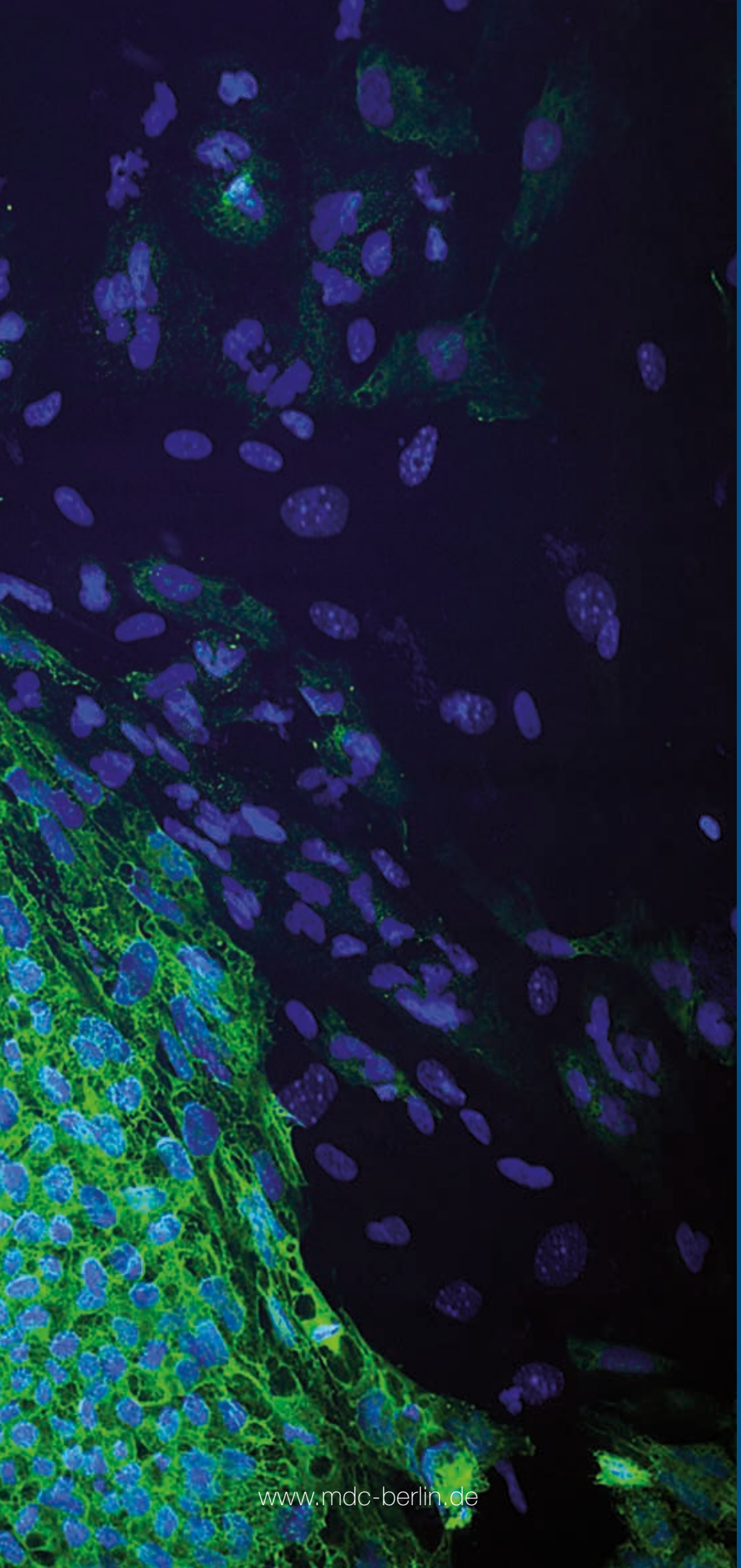
D 16/23 Eckert & Ziegler AG

D 79 **Erwin Negelein House**
Akademie der Gesundheit, BioTeZ, imaGenes, AviTop, GlycoUniverse

D 80 **Otto Warburg House**
ALRISE, Silence Therapeutics, Celares, OMEICOS Therapeutics

D82 **Karl Lohmann House**
EPO GmbH

D85 **Arnold Graffi House**
BBB, stratec, aokin, Biosyntan, emp, ascenion, MerLion, KFFB, Prof. Wanker, Nutrineu, AMAL, pi-Pharmalmage, GLYCOTOPE, S. Langmacker accountant



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ASSOCIATION