Research Report 2012

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH
MEMBER OF THE HELMHOLTZ ASSOCIATION

Research Report 2012

(covers the period 2010-2011)

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Endocytic uptake of injected β–lactoglobulin (red) in proximal tubular cells which express ClC-5 (green) and are labeled with the brush border marker villin (blue). (this report page 148)

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Novarino G., Weinert S., Rickheit G., Jentsch T.J. et al. Chloride Conductance Is Crucial for Renal Endosomal Chloride-Proton Exchange Rather Than Endocytosis.

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Director's Introduction

Vorwort des Direktors

This report appears on the eve of the 20th anniversary of the MDC and finds us in an important period of transition. The mission upon which our institute was founded two decades ago was almost unique at the time, but the idea of translating findings from basic biological research into the field of medicine has now become a central theme in research. Translational research is evolving quickly in an environment of equally rapid changes. Our mission, however, stays the same: bringing science from bench to bedside. To achieve this, we have to keep pace with the changes.

Take, for instance, our long-term relationship to the Charité Universitätsmedizin. This collaboration has been very fruitful and we are now poised to take it to a new level. Research activities at the MDC and Charité will soon become closely intertwined in a new structure. We are still working out the details, but the goal is to develop a deeper, new type of partnership. It will promote clinical research and should allow us to take better advantage of our increasing understanding of basic disease processes.

We benefit from our neighbor on campus, the Leibniz-Institut für Molekulare Pharmakologie (FMP), in the form of common technology platforms, expertise in structural and chemical biology, and some very productive research collaborations, for example, a project from Gary Lewin and Thomas Jentsch on mechanisms of pain perception which resulted in a *Science* paper in December 2011.

Our partnership with universities – above all the Charité – is multifaceted, be it through joint appointments of faculty, PhD programs or through our participation in special research projects (Sonderforschungsbereiche) and Clusters of Excellence such as Neurocure (see p. 265 of this report). And a new, very direct form of contact will occur when our Berlin Institute for Medical Systems Biology (BIMSB) moves to a new building on the North Campus of the Humboldt University, in the city center.

Systems biology is a promising field of research, especially for the MDC. The constellation of groups and

Dieser Zwei-Jahresbericht erscheint im 20. Jahr unseres Bestehens. Wir blicken auf eine bewegte Zeit zurück und sehen, so viel ist gewiss, spannenden Zeiten entgegen. Die Gründer des MDC haben uns 1992 den Auftrag gegeben, Erkenntnisse aus der biomedizinischen Grundlagenforschung zu gewinnen und sie möglichst rasch in die Anwendung zu überführen: Was wir heute als „translationale Medizin" kennen, war seinerzeit ein Novum. Dieses Forschungsfeld und seine Rahmenbedingungen ändern sich rasant. Der Auftrag aber, Forschung aus dem Labor ans Krankenbett zu bringen („from bench to bedside") ist aktueller denn je, und um ihn zu erfüllen, müssen wir mit den Veränderungen Schritt halten.

So sind wir dabei, unsere langjährige und ohnehin schon sehr enge Partnerschaft mit der Charité – Universitätsmedizin Berlin in den nächsten Jahren auf eine neue Ebene zu heben. Eine gemeinsame institutionelle Verbindung soll sicherstellen, dass biomedizinische Grundlagenforschung und klinische Forschung voneinander noch mehr als bisher profitieren.

Wir werden auch unsere fruchtbare Kooperation mit dem Leibniz-Institut für Molekulare Pharmakologie (FMP) auf dem Campus fortführen, gerade auf dem Gebiet der Chemischen Biologie und Wirkstoffforschung. Es gibt bereits eine ganze Reihe von sehr produktiven gemeinsamen Projekten, die zum Beispiel zu einer Publikation von Thomas Jentsch und Gary Lewin zur Schmerzwahrnehmung führten, welche im Dezember 2011 in *Science* erschien.

Unsere Zusammenarbeit mit den Berliner Universitäten und hier natürlich vor allem mit der Charité ist sehr vielfältig. Sie reicht von der Doktorandenausbildung über viele gemeinsame Berufungen bis hin zu unseren Beteiligungen an Sonderforschungsbereichen und Exzellenzclustern wie beispielsweise Neurocure (siehe S. 265 in diesem Bericht). Das „Berlin Institute for Medical Systems Biology" (BIMSB) wird diese Kooperation noch vertiefen, wenn es sein neues Gebäude auf dem Nordcampus der Humboldt-Universität in Berlin-Mitte bezieht.

Das BIMSB steht für ein wichtiges Zukunftsfeld am MDC, die Systembiologie. Hier gibt es bereits bemerkenswerte

Photographer David Ausserhofer, Copyright MDC

platforms in BIMSB has already had some remarkable successes. The groups of Nikolaus Rajewsky, scientific head of the BIMSB, and Wei Chen found a new way to assemble a high-quality version of the genome of planaria. One recent project from the groups of Matthias Selbach, Wei Chen and Jana Wolf managed to follow the molecular expression of an entire genome in quantitative terms. And most recently, Nikolaus Rajewsky was awarded the Gottfried Wilhelm Leibniz Prize 2012 in November 2011, the highest scientific award in Germany. Systems biology projects integrate the latest technologies and approaches in genomics, proteomics, and mathematical modeling.

The overall success of our structure and approach can be demonstrated in several ways. First and foremost, the MDC has produced excellent science, exposing mechanisms that underlie many types of disease. In spite of our relatively short history, our work has gained a high reputation in the research community. This is demonstrated by our global ranking in terms of scientific impact and also by our ability to attract excellent young scientists from all over the world. Francesca Spagnoli, for instance, who is working on the mechanisms that underlie liver and pancreas development, established her group with the help of a major European Research Council Starting Grant, providing a million Euros over five years. All in all, currently nine ERC Grant holders

Erfolge. Der Arbeitsgruppe von Nikolaus Rajewsky, dem wissenschaftlichen Leiter des BIMSB, gelang es gemeinsam mit dem Team um Wei Chen, das Genom von Plattwürmern (Planaria) hochaufgelöst darzustellen. Und den Arbeitsgruppen von Matthias Selbach, Wei Chen und Jana Wolf gelang es, die molekulare Expression eines ganzen Genoms quantitativ zu beschreiben. Nikolaus Rajewsky wurde übrigens im November 2011 einer der Gottfried-Wilhelm-Leibniz-Preise für 2012 zuerkannt, das ist der höchste deutsche Wissenschaftspreis. Die Projekte der Systembiologie basierten auf dem Einsatz modernster Technologien und auf neuen Forschungsansätzen aus den Bereichen Genomik, Proteomik und mathematischer Modellierung.

Die wichtigste Voraussetzung für solche Erfolge ist Exzellenz in der Forschung. Das MDC braucht hier Vergleiche nicht zu scheuen, im Gegenteil: in internationalen Ranglisten liegen wir regelmäßig in den Spitzengruppen. Das, gepaart mit unseren erstklassigen Arbeitsbedingungen, macht uns attraktiv für ausgezeichnete Nachwuchsforscherinnen und -forscher aus aller Welt. Francesca Spagnoli etwa, die die Mechanismen der Gewebsentstehung von Leber und Bauchspeicheldrüse erforscht, erhielt einen prestigeträchtigen ERC Starting Grant. Mit ihr arbeiten derzeit neun Forscher am MDC, die alle einen ERC Grant erhielten (vier davon Advanced Grants). Außerdem sind zwei unserer Wissenschaftler, Matthias Selbach und

work at the MDC, four of them with Advanced Grants. In addition, two of our researchers are in the prestigious EMBO Young Investigator Programme (YIP), i.e. Matthias Selbach and Oliver Daumke. Matthias Selbach is working in the field of proteomics and Oliver Daumke is doing important work on the structure and interaction of G-proteins. These molecules are central to all biological processes and play a crucial role in disease.

The MDC is equally attractive to established scientists. Mathias Treier, formerly of the EMBL and the University of Cologne, arrived in 2011 to lead a new group focused on the Genetics of Metabolic and Reproductive Disorders. And the final months of 2011 are witnessing the establishment of a new lab by Klaus Rajewsky, who is highly recognized for his study of B cells, hematopoeitic stem cells and their role in cancer. His work on conditional mutagenesis has helped make the Cre/lox system one of the most important genetic technologies in modern biomedical research.

In the last two years, Tobias Pischon added epidemiology as a field of expertise at the MDC. We will move further into this field in the years to come, e.g. by working with the national cohort which is supported by the Helmholtz Association.

As a publicly funded institute, we are well aware of our obligation to keep the public informed of our research. We are currently drawing our activities in this area into an integrated communications department that will find new ways of reaching the public, thus inspiring the next generation of scientists.

Oliver Daumke, Mitglieder des sehr angesehenen EMBO Young Investigator Programme (YIP). Matthias Selbach arbeitet auf dem Gebiet der Proteomik und Oliver Daumke hat wichtige Arbeiten zur Struktur und Interaktion von G-Proteinen veröffentlicht. Diese Moleküle spielen eine zentrale Rolle bei allen biologischen Prozessen und bei Krankheiten.

Aber auch für bereits etablierte Wissenschaftlerinnen und Wissenschaftler ist das MDC attraktiv. So leitet Mathias Treier, zuvor am EMBL und an der Universität Köln, seit 2011 eine neue Gruppe, die sich mit der Genetik von Stoffwechsel- und Reproduktionsstörungen beschäftigt. Und Ende 2011 hat Klaus Rajewsky sein neues Labor bezogen. Er ist höchst anerkannt für seine Untersuchung von B-Zellen, hämatopoietischen Stammzellen und deren Rolle bei der Krebsentstehung. Mit seinen Arbeiten zur konditionellen Mutagenese in Mäusen hat er dazu beigetragen, dass das Cre/lox-System inzwischen zu einer der wichtigsten gentechnologischen Methoden der modernen biomedizinischen Forschung geworden ist.

In den zurückliegenden zwei Jahren hat Tobias Pischon am MDC die Epidemiologie aufgebaut. Wir werden uns in diesem Bereich künftig noch stärker engagieren, beispielsweise durch die Mitarbeit an der Nationalen Kohorte unter Beteiligung der Helmholtz-Gemeinschaft.

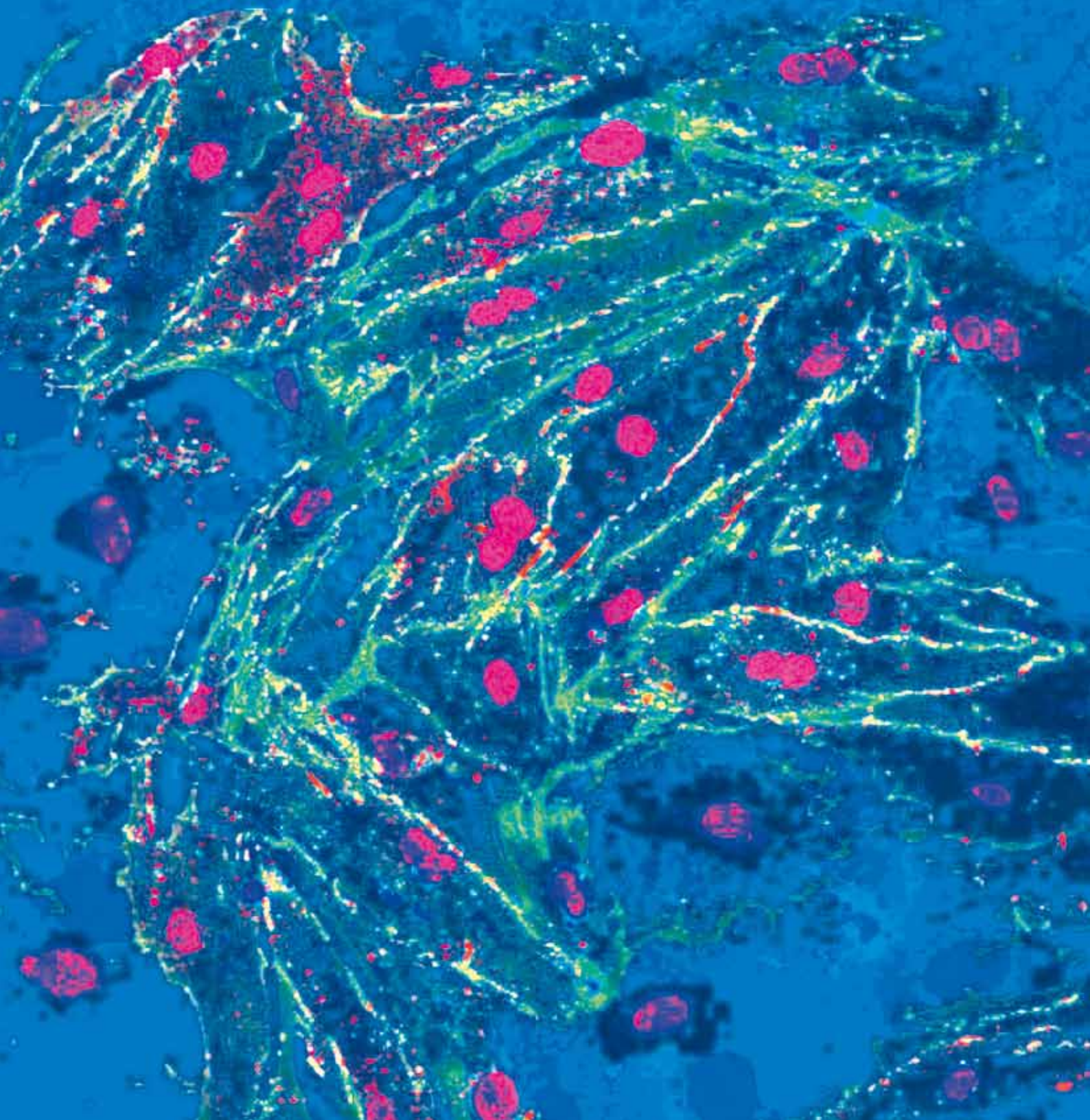
Als öffentlich gefördertes Institut sind wir uns unserer Verpflichtung sehr bewusst, die Öffentlichkeit kontinuierlich über unsere Forschung zu informieren. Hierzu arbeiten wir gegenwärtig an einem integrierten Kommunikationskonzept. Wir wollen neue Wege zum Austausch mit der Öffentlichkeit gehen und dazu beitragen, die nächste Generation von Wissenschaftlerinnen und Wissenschaftlern für unser Gebiet zu begeistern.

Cardiovascular and Metabolic Diseases

Coordinator: Thomas Willnow

Basic Cardiovascular Function

Genetics and Pathophysiology of Cardiovascular Diseases



Cardiovascular and Metabolic Disease Research

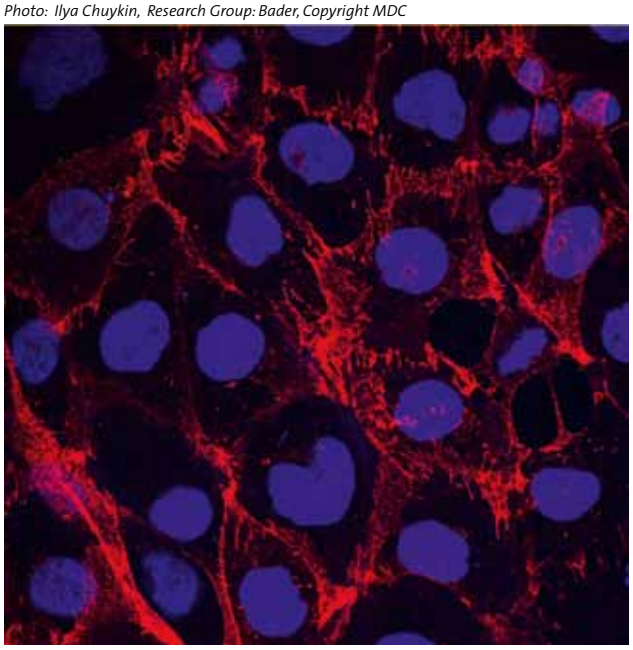
Thomas Willnow and Friedrich C. Luft

Cardiovascular disease calls to mind myocardial infarction, stroke, ruptured aneurysms, end-stage kidney disease, and small-vessel complications of diabetes mellitus. Indeed, the end products of cardiovascular disease are the most common causes of death worldwide. Cardiovascular disease has recently become global. For instance, almost half the disease burden in low-and-middle-income countries of Europe and Central Asia and further East now comes from cardiovascular diseases. In the United States, 60 million people have known cardiovascular disease, 50 million have hypertension, 13 million have coronary disease, and 5 million have had a stroke. These figures comprise 1 in 5 of the US population. Those who have cardiovascular disease but do not know it probably number about the same as those who do. Figures for the European Union are no different. Five percent of the US and EU population are known to have type 2 diabetes mellitus. This "adult-onset" diabetes is now the most common cause of diabetes in children. Kidney disease that leads to end-stage renal failure is increasing exponentially and diabetes, coupled with hypertension, is the most common cause. The number of people with a body mass index (BMI) >30 is approaching 30% of the population and half the population is overweight (BMI >25%). These figures are alarming, since they could mean a reversal of the previous trend toward ever-increasing life expectancy in our societies.

Our primary aim is to prevent cardiovascular disease and we are also necessarily concerned with its treatment. At the MDC, we primarily focus on basic research to achieve these goals. Progress will require understanding the underlying genetic and pathophysiological mechanisms and developing utilitarian models in diverse cell and animal systems. Only after these goals are well underway can we offer a passport to in vivo translational approaches and ultimately alter outcomes in patients. Our mission is broad in scope and necessarily involves molecular genetic, cell, and systems biology approaches that are similar to those used by our colleagues and collaborators focusing on cancer research or neurosciences. Medical research has evolved so that categorization in terms of diseases or target organs is no longer the center of attention. The following reports come from scientists working on a diverse spectrum ranging from mathematical modeling, bioinformatics, chromatin-based epigenetics, broad-based to narrowly-focused molecular genetics, proteomics, protein structural biology, to cell and animal systems extending from yeast, zebrafish, the mouse, rat, and larger mammals to humans. Although they are eclectic and flexible, our investigators have focal points of interest, including developmental biology and the development of organ and vascular systems; they focus on specific protein receptor families, classical cell, organ, and organismal metabolism, and vessels and organs as targets of inflammation, atherosclerosis, and hypertension-induced injury. Although we no longer have clinical departments adjacent to the campus, we have integrated the Experimental and Clinical Research Center (ECRC) into our efforts. As a result, direct collaboration, translation to other model systems, and transfer to patient-oriented research is not only possible but also mandated. The MDC has been selected to organize and conduct a major epidemiological cohort investigation, which has cardiovascular disease as a central focus. The establishment of the German Center for Cardiovascular Research, in which the MDC and the Charité Medical Faculty are prominently represented, will expand our Cardiovascular and Metabolic Disease research efforts. The investigators will present themselves in the following pages. We will comment only on a few highlights.

Matthias Selbach, Wei Chen, Jana Wolf and colleagues tracked the global protein synthetic output of a mammalian cell for the first time by measuring quantities and lifetimes and predicting rates of synthesis for the cell's RNAs and the resultant proteins. The group tracked the output of more than 5,000 genes produced by a single murine cell line. An accurate census of the cell requires counting the number of mRNAs synthesized from each gene, the number of proteins made from the template of each mRNA, and the rate at which each type of mol-

Photo: Ilya Chuykin, Research Group: Bader, Copyright MDC



ecule is degraded. The investigators found that genes with similar combinations of mRNA and protein stability shared functional properties, indicating that protein half-lives evolved under energetic and dynamic constraints. Quantitative information on all the stages of gene expression is a rich resource and helps to provide a greater understanding of underlying design principles. This basic research and the P-SILAC methodology are immediately applicable to cardiovascular cells. Friedrich Luft's group has already picked up this gauntlet in collaboration with the Selbach laboratory.

"Treasure your exceptions" – sage advice from William Bateson a century ago. He implied that rare genetic diseases could lead to very generalizable findings. Dominant mutations in NOTCH3 cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a genetic archetype of cerebral ischemic small vessel disease, leading to stroke and dementia. Now, Norbert Hübner and his laboratory reports on the development of a mouse model involving a large P1-derived artificial chromosome bearing a NOTCH3 point mutation that reproduces the main pathological features of CADASIL. The model permits the direct study of cerebrovascular dysfunction and microcirculatory failure.

Zsuzsanna Izsvák and Zoltán Ivics are largely responsible for the transposon becoming a "molecule of the year." Transposons, termed "jumping genes" by Barbara McClintock, are bits of DNA that make natural copies of themselves. The structures can move around to new positions on chromosomes as cells divide. Transposons arose probably >500,000 million years ago in ancient organisms, possibly through infections by viruses that inserted genes into cells. Gradually the transposons spread to create millions of copies that have left their footprints throughout the genomes of humans and other organisms. The two investigators have patiently developed transposon technology for gene transport, helpful not only in the production of animal models, but also in gene therapy applications for humans.

The giant muscle protein titin is an essential structural component of the sarcomere. Titin forms a continuous periodic backbone along the myofiber that provides resistance to mechanical strain. Michael Gotthardt's laboratory developed a novel titin-eGFP "knock-in" mouse and demonstrated that sarcomeric titin is more dynamic than previously appreciated. Whereas protein synthesis and developmental stage did not alter titin dynamics in this model, there was a strong, inhibitory effect of calcium on titin mobility. Their results suggest that the largely unrestricted movement of titin within and between sarcomeres primarily depends on calcium. Thus, the fortification of the titin filament system is an active rather than a passive process. The findings have direct bearing on our understanding of heart muscle diseases that make up a large percentage of heart failure patients.

Patient-oriented research also falls within the spectrum of our activities. Clinical Research Center (CRC) investigators observed that simply drinking 500 ml of tap water within 30 min can have profound effects on blood pressure, up to 50 mm Hg increase, particularly in persons ingesting sympathomimetic drugs such as phenylpropanolamine. The findings can explain how "street drugs" lead to stroke. In collaboration with Gary Lewin's laboratory, the TrpV4 ion channel was identified as the osmosensor in the portal circulation that responds to the water. In studies led by Kai Schmidt-Ott, neutropnil gelatinase-associated lipocalin (NGAL) was shown to be a reliable marker for persons developing acute kidney injury, a major harbinger of death in hospitals. Thus, preventative measures could be applied in a more timely fashion.

Thomas E. Willnow

Molecular Cardiovascular Research

VPS10P domain receptors such as SORLA and sortilin comprise a recently identified class of intracellular sorting proteins that are predominantly expressed in neurons but also in non-neuronal cell types. VPS10P domain receptors were previously considered to be orphan receptors with activities in neuronal protein trafficking that were poorly understood. However, new findings revealed unexpected roles for these receptors as essential regulators of neuronal viability and function. Recent work from our laboratory now has uncovered the molecular mechanisms of regulated protein transport and signaling through VPS10P domain receptors. Loss of this regulation contributes to devastating disorders of the nervous system including Alzheimer disease and other dementias, but also stroke or spinal cord injury. Remarkably, we also identified critical roles for SORLA and sortilin in renal ion homeostasis and systemic lipoprotein metabolism, highlighting the importance of VPS10P domain receptors for both neurological and cardiovascular (patho)physiologies.

Introduction

The VPS10P domain is a protein module that was first recognized in the vacuolar protein sorting 10 protein (VPS10P) in *Saccharomyces cerevisiae*. VPS10P is a sorting receptor that directs the trafficking of lysosomal enzymes from the Golgi to the vacuole (the lysosome in Yeast). Subsequently, this protein domain was found to constitute the unifying structural feature of a new group of type 1-membrane receptors that are conserved throughout evolution from baker's yeast to man. The members of this gene family are now known as VPS10P domain receptors. Five receptors are found in vertebrates: sortilin, SORLA, SORCS1, SORCS2, and SORCS3 (Fig. 1).

VSP10P domain receptors were initially considered a rather peculiar group of sorting proteins with unknown function. However, the mammalian receptors of the gene family surfaced as potential disease genes in a number of association studies in patients. These diseases encompass Alzheimer's disease (AD) and other types of age-related dementias (in which SORLA and SORCS1 have been implicated), bipolar disorders (in which SORCS2 has been implicated), as well as senescence of the nervous system (in which sortilin has been implicated). In addition, several common cardiovascular and metabolic disorders involving VPS10P domain receptors were identified including type 2 diabetes (which has been linked to SORCS1 and SORCS3), atherosclerosis (linked to SORLA), as well as dyslipidemia, and myocardial infarction (linked to sortilin).

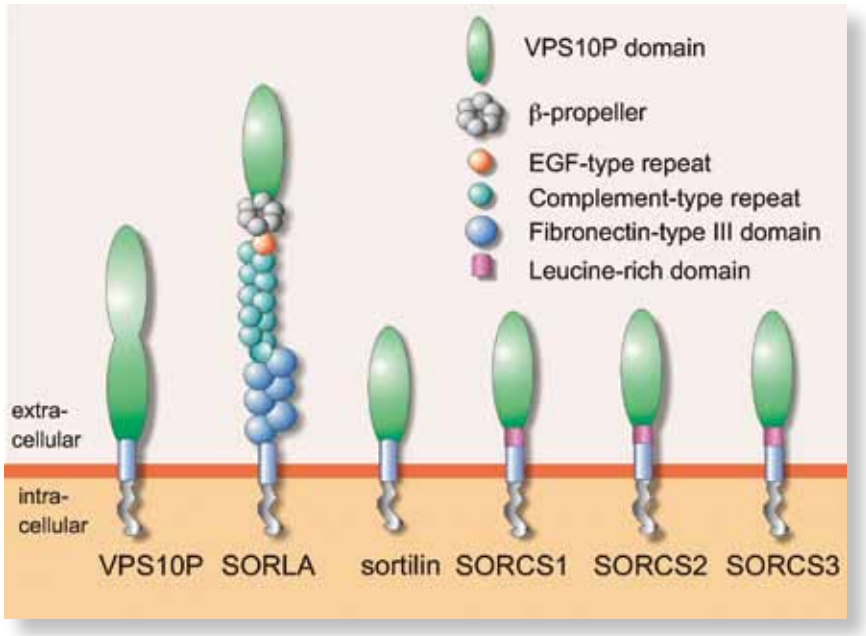


Figure 1. VPS10P domain receptors. Structural organization of VPS10P domain receptors from yeast (VPS10P) and humans (sortilin, SORLA, SORCS-1, -2, -3). The extracellular domains of the receptors are either composed of one (sortilin, SORLA, SORCS1, -2, -3) or two VPS10P domains (VPS10P), and may carry additional modules involved in protein-protein interaction (leucine-rich domains, complement-type repeats, EGF-type repeats and fibronectin-type III domains) or regulation of ligand binding (β-propeller).

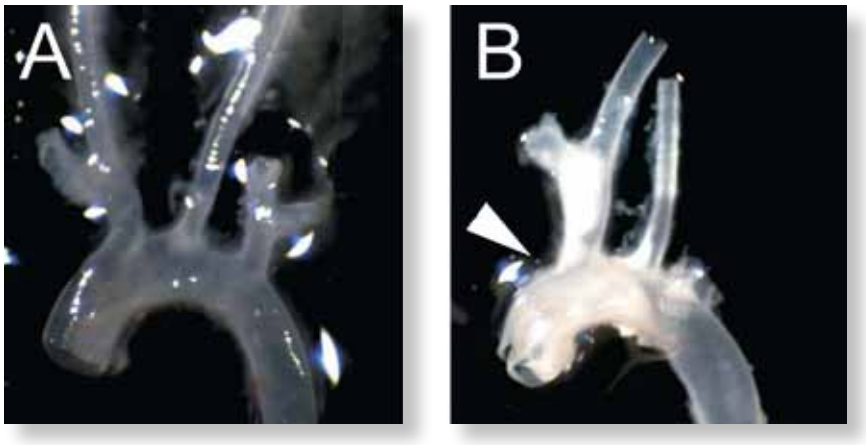


Figure 2. Sortilin deficiency in mice protects from atherosclerotic plaque formation. Atherosclerotic lesions (arrowhead) are seen in the aortas of mice expressing wild-type sortilin (B) but not in animals genetically deficient for the receptor (A).

Identification of Alzheimer disease risk genotype that predicts efficiency of SORLA expression in the brain

Safak Caglayan, Vanessa Schmidt, Anne-Sophie Carlo

SORLA is an intracellular sorting receptor for the amyloid precursor protein (APP) that modulates processing of this precursor into amyloidogenic and non-amyloidogenic products. Loss of receptor expression in neurons of cortex and hippocampus has been documented in individuals suffering from sporadic Alzheimer's disease (AD). Also, genetic variants in *SORL1* (the gene encoding SORLA) have been associated with late-onset AD in the human population. However, a direct link between distinct *SORL1* gene variants and receptor protein expression was lacking so far.

To identify *SORL1* risk genotypes that may determine receptor protein expression in the human brain, we collected brain autopsy material from 88 confirmed cases of sporadic AD. DNA, RNA, and proteins were extracted from these specimens and used for genotyping, RNA profiling, and SORLA protein quantification by ELISA, respectively. Our studies identified a distinct *SORL1* haplotype in the 3' gene region consisting of SNPs rs1699102 and rs2070045 that is associated with poor receptor expression in the brain of AD patients. These gene variations alter the *SORL1* transcript sequence, resulting in a change from frequent to rare codon usage in the risk genotype. Studies in cultured cells confirm less efficient translation of the minor receptor transcripts into protein as compared to the major transcript variant.

In conclusion, our findings have identified the first functional *SORL1* gene variant that affects expression of this AD risk gene in the human brain and they suggest impaired translation as the underlying cause of poor receptor expression in affected individuals.

Quantitative modeling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease

Vanessa Schmidt

The extent of proteolytic processing of APP into neurotoxic Aβ peptides is central to the pathology of AD. Accordingly, modifiers that increase Aβ production rates are risk factors in the sporadic form of AD.

In a novel systems biology approach we combined quantitative biochemical studies with mathematical modeling to establish a kinetic model of amyloidogenic processing, and to evaluate the influence by SORLA, an inhibitor of APP processing and important genetic AD risk factor. Contrary to previous hypotheses, our studies demonstrate that secretases represent allosteric enzymes that require cooperativity by APP oligomerization for efficient processing. Cooperativity enables swift adaptive changes in secretase activity with even small alterations in APP concentration. We also show that SORLA prevents APP oligomerization both in cultured cells and in the brain *in vivo*, eliminating the preferred form of the substrate and causing secretases to switch to a less efficient non-allosteric mode of action.

Our data represent the first mathematical description of the contribution of genetic risk factors to AD substantiating the relevance of subtle changes in SORLA levels for amyloidogenic processing as proposed for patients carrying *SORL1* risk alleles.

Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression

Michael Rohe

SORLA acts as sorting receptor for APP that regulates intracellular trafficking and processing into amyloidogenic Aβ. Overexpression of SORLA in neurons reduces while inactivation of gene expression (as in knockout mouse models) accelerates amyloidogenic processing and senile plaque formation.

The current study aimed at identifying molecular pathways that control SORLA gene transcription *in vivo* and that may contribute to low levels of receptor expression in the brain of patients with AD. Using screening approaches in primary neurons, we identified brain-derived neurotrophic factor (BDNF) as a major inducer of *Sorla* that activates receptor gene transcription through the extracellular regulated kinase (ERK) pathway. In line with a physiological role as regulator of *Sorla*, expression of the receptor is significantly impaired in mouse models with genetic *(Bdnf -/-)* or disease-related loss of BDNF activity in the brain (Huntington's disease). Intriguingly, exogenous application of BDNF reduced Aβ production in primary neurons and in the brain of wild-type mice *in vivo*, but not in animals genetically deficient for *Sorla*.

These findings demonstrate that the beneficial effects ascribed to BDNF in APP metabolism act through induction of Sorla that encodes a negative regulator of neuronal APP processing

SORLA/*SORL1* functionally interacts with SPAK to control renal activation of Na^+-K^+-Cl^- cotransporter 2

Juliane Reiche, Anne-Sophie Carlo

Proper control of NaCl excretion in the kidney is central to bodily functions. Yet, many mechanisms that regulate reabsorption of sodium and chloride in the kidney remain incompletely understood.

Here, we identified an important role played by SORLA in functional activation of renal ion transporters. We demonstrate that SORLA is expressed in epithelial cells of the thick ascending limb (TAL) of Henle's loop and that lack of receptor expression in this cell type in SORLA-deficient mice results in the inability to properly reabsorb sodium and chloride during osmotic stress. The underlying cellular defect was correlated with an inability of the TAL to phosphorylate Na^+-K^+-Cl^- cotransporter (NKCC) 2, the major sodium transporter in the distal nephron. SORLA functionally interacts with Ste-20-related proline-alanine-rich kinase (SPAK), an activator of NKCC2, and receptor deficiency is associated with mis-sorting of SPAK.

Our data suggest a novel regulatory pathway whereby intracellular trafficking of SPAK by the sorting receptor SORLA is crucial for proper NKCC2 activation, and for maintenance of renal ion balance.

Sort1, encoded by the cardiovascular risk locus 1p13.3, is a novel regulator of hepatic lipoprotein export

Tilman Breiderhoff

Recent genome-wide association studies (GWAS) have revealed strong association of hypercholesterolemia and myocardial infarction with SNPs on human chromosome 1p13.3. This locus covers three genes: *SORT1, CELSR2* and *PSRC1*. However, which of the candidates

represents the cardiovascular disease gene remained unclear.

Here, we demonstrated that sortilin, encoded by *SORT1*, is a novel intracellular sorting receptor for apolipoprotein (apo) B100. It interacts with apoB100 in the Golgi and facilitates the formation and hepatic export of apoB100-containing lipoproteins, thereby regulating plasma low-density lipoprotein (LDL) cholesterol. Absence of sortilin in gene-targeted mice reduces secretion of lipoproteins from the liver and ameliorates hypercholesterolemia and atherosclerotic lesion formation in LDL receptor-deficient animals (Fig. 2). In contrast, sortilin overexpression stimulates hepatic release of lipoproteins and increases plasma LDL levels.

Our data have uncovered a novel regulatory pathway in hepatic lipoprotein export and suggest a molecular explanation for the cardiovascular risk being associated with 1p13.3.

Perspective

Dysregulation of vesicular protein transport is emerging as a molecular mechanism of major importance underlying many disease processes. Obviously, intracellular sorting receptors of the VPS10P domain receptor gene family play key roles in these processes. Our future work has yet to refine the molecular details how sortilin and SORLA affect trafficking and functional expression of neuronal target proteins including APP. Furthermore, novel activities of the neuronal protein transport machinery may be uncovered as we learn more about the orphan receptors SORCS1, SORCS2, and SORCS3. As well as in the nervous system, SORLA and sortilin are also distinctly expressed in non-neuronal cell types in kidney and liver. Our studies have uncovered important roles played by these receptors in renal ion transport and blood pressure regulation (SORLA) or in control of systemic cholesterol homeostasis (sortilin). In the future, we expect to gain novel insights into protein sorting pathways that may be central to the development of renal and metabolic disorders.

Selected Publications

Kjolby, M, Andersen, OM, Breiderhoff, T, Fjorback, AW, Pedersen, KM, Madsen, P, Jansen, P, Heeren, J, Willnow, TE*, Nykjaer, A*. (2010). Sort1, encoded by the cardiovascular risk locus 1p13.3, is a novel regulator of hepatic lipoprotein export. Cell Metab. 12, 213-223. * joined corresponding authorship

Reiche, R, Theilig, F, Rafiqi, FH, Militz, D, Mutig, K, Todiras, M, Christensen, EI, Ellison, DH, Bader, M, Nykjaer, A, Bachmann, S, Alessi, D, Willnow, TE. (2010). SORLA/SORL1 functionally interacts with SPAK to control renal activation of Na^+-K^+-Cl^- cotransporter 2. Mol. Cell. Biol. 30, 3027-37.

Rohe, M, Synowitz, M, Glass, R, Paul, SM, Nykjaer, A, Willnow, TE. (2009). Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. J. Neurosci. 29, 15472-8.

Jansen, P, Giehl, K, Nyengaard, JR, Teng, K, Lioubinski, O, Sjoegaard, SS, Breiderhoff, T, Gotthardt, M, Lin, F, Eilers, A, Petersen, CM, Lewin, GR, Hempstead, BL, Willnow, TE*, Nykjaer, A*. (2007). Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat. Neurosci. 10, 1449-57. * joined corresponding authorship

Rogaeva, E, Meng, Y, Lee, JH, Gu,Y, Kawarai, T, Zou, F, Katayama,T, Baldwin, CT, Cheng, R, Hasegawa, H, Chen, F, Shibata, N, Lunetta, KL, Pardossi-Piquard, R, Bohm, C, Wakutani, Y, Cupples, LA, Cuenco, KT, Green, RC, Pinessi, L, Rainero, I, Sorbi, S, Bruni, A, Duara, R, Friedland, RP, Inzelberg, R, Hampe, W, Bujo, H, Song, YQ, Andersen, OM, Willnow, TE, Graff-Radford, N, Petersen, R, Dickson, D, Der, SD, Fraser, PE, Schmitt-Ulms, G, Younkin, S, Mayeux, R, Farrer, LA, St George-Hyslop, P. (2007). The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer's Disease. Nat. Genet. 39, 168-177.

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Developmental Neurobiology

Sonic hedgehog (SHH) is a regulator of forebrain development that acts through its receptor patched 1. However, little is known about the cellular mechanisms whereby SHH governs specification of the rostral diencephalon ventral midline (RDVM), a major forebrain organizer, during neurulation. We identified LRP2, a member of the LDL receptor gene family, as component of the SHH signaling machinery in the RDVM. LRP2 acts as an apical SHH binding protein that sequesters SHH in its target field and controls internalization and cellular trafficking of SHH/patched 1 complexes. Lack of LRP2 in mice and in cephalic explants results in failure to respond to SHH despite functional expression of patched 1 and smoothened, whereas overexpression of LRP2 variants in cells increases SHH signaling capacity. Our data identify a critical role for LRP2 in SHH signaling and reveal the molecular mechanism underlying forebrain anomalies in mice and patients with LRP2 defects.

LRP2 is an auxiliary SHH receptor required to specify the embryonic ventral forebrain

(Annabel Christ, Anna Christa, Esther Kur)

Impaired development of the ventral forebrain of LRP2 mutants is caused by defects in SHH distribution

In recent studies we could identify the SHH pathway as the primary target of LRP2 function. In LRP2 deficient embryos SHH protein fails to localize to the apical surface of the rostral diencephalon ventral midline (RDVM) neuroepithelium. Consequently the SHH downstream signaling cascade is severely disturbed, resulting in a misspecification of the forebrain organizer region ultimately causing holoprosencephaly.
Two mechanisms might explain the SHH signaling defects in LRP2 mutants: LRP2 could act as a SHH receptor mediating proper binding of the morphogen to the apical surface of the neuroepithelium. Alternatively, lack of receptor expression might impact on downstream signaling events in the SHH pathway.

Cell intrinsic SHH downstream signaling pathway is functional in LRP2 deficient neuroepithelial cells

SAG, a small molecule agonist, can actually activate smoothened and therefore the downstream signaling cascade in the absence of SHH.
SAG treatment could rescue SHH downstream target expression in the LRP2 mutants, suggesting that the cell intrinsic SHH downstream signaling machinery in LRP2 mutants is intact. Is therefore the proper targeting and binding of SHH to the apical surface of the neuroepithelium disturbed despite the presence of the essential components of the SHH signaling machinery like primary cilia, patched 1 and smoothened in *Lrp2*$^{-/-}$ embryos?

LRP2 is the initial binding site for SHH at the apical surface of the neuroepithelium

To address the issue of SHH binding properties in LRP2 deficient embryos we kept neurulation stage embryos in culture and incubated them with labeled SHH. Wild type embryos showed binding of the added SHH, whereas mutants lacking LRP2 could not bind SHH. These results suggest that LRP2 is an essential component of a SHH receptor complex in the neuroepithelium crucial for SHH binding to patched 1 and indispensable for SHH signaling to occur. This concept was also strengthened by further results from our lab showing that proper intracel-

lular trafficking of SHH with its receptor patched is also dependent on the presence of LRP2. We could demonstrate that in the forebrain neuroepithelium of wild type embryos SHH as well as LRP2 are sorted to early endosomes and then to the recycling, rather than to the lysosomal compartment. LRP2 deficient embryos don't show this trafficking to endosomes at all.

LRP2 increases cellular SHH signaling capacity

To finally elucidate the molecular mechanism of LRP2 in SHH signaling, we performed additional studies in cell culture models. By expressing *Lrp2* constructs in SHH responsive fibroblasts we could show a significant increase in the SHH signaling capacity of these cells after SHH stimulation compared to cells without LRP2 expression.

Conclusion

We propose a model whereby LRP2 forms a co-receptor complex with patched 1 for SHH on the apical surface of the neuroepithelium in a time window critical for forebrain specification. SHH internalized by the patched 1/LRP2 complex is recycled to the apical surface of the neuroepithelium, presumably to further increase local concentration of inductive signals in this forebrain organizer center.

Altogether these studies present a new concept in SHH signaling in the ventral forebrain and also reveal the molecular mechanisms underlying the developmental disturbances in the CNS in mice and in patients with LRP2 deficiencies, suffering from Donnai-Barrow syndrome associated with forebrain anomalies.

A role for LRP2 in adult neurogenesis

(Chandresh Gajera, Helena Emich)

In the adult brain, *Lrp2* is expressed in ependymal cells, a specialized epithelial layer lining the brain ventricles. Intriguingly, expression of the receptor is restricted to the stem cell niche in the lateral ventricle. The subependymal zone (SEZ) is one of two regions of ongoing adult neurogenesis in the mouse forebrain. Neuronal precursors generated in this region migrate to the olfactory bulb where they differentiate into mature neurons. SHH promotes neurogenesis in the adult SEZ, and this effect requires repression of BMP activity.

Recently, we obtained exciting new results from the analysis of a new LRP2 deficient mouse model, providing evidence that LRP2 has a direct role in adult neurogenesis. We showed that LRP2 deficiency in adult mice causes misspecification of stem cells and impaired proliferation of neural precursor cells in the SEZ, resulting in decreased numbers of neuroblasts reaching the olfactory bulb. Reduced neurogenesis coincides with increased BMP4 expression and enhanced activation of downstream mediators phospho-SMAD1/5/8 and ID3 in the stem cell niche.

Our findings suggest a novel mechanism whereby LRP2-mediated downregulation of BMP4 signaling in the ependyma modulates the microenvironment of the SEZ. We hypothesize that LRP2 enables adult neurogenesis to proceed by controlling the critical balance between the competing morphogens BMP4 and SHH in the stem cell niche.

We are currently testing whether LRP2 function in the ependyma directly modulates SHH concentrations in the stem cell niche, similar to the mechanism detected in the neuroepithelium of *Lrp2*$^{-/-}$ embryos.

Perspectives

SHH binding, involving several auxiliary receptors to patched 1, like LRP2, as well as the intracellular sorting of SHH to recycling endosomes are new concepts in SHH signaling, which are of major importance. Regulation of the SHH pathway is central for specification of the embryonic neuroepithelium, for adult neurogenesis in the cortical stem cell niche, and in other SHH responsive tissues during development and in disease. Our future work will focus on refining studies of the LRP2 and SHH pathway also in the context of primary cilia function in the embryonic and adult forebrain. Insights from our analyses of the LRP2 and SHH pathway and interacting signaling networks will lead to improved understanding of the molecular and pathophysiological mechanisms underlying embryonic forebrain and adult neurogenesis defects in animals and humans.

Selected Publications

Willnow, TE, Hammes, A, Eaton, S. (2007) Lipoproteins and their receptors in embryonic development – more than cholesterol clearance. Development, 134: 3239-3249

Gajera CR, Emich H, Lioubinski O, Christ A, Beckervordersandforth-Bonk R, Yoshikawa K, Bachmann S, Christensen EI, Götz M, Kempermann G, Peterson AS, Willnow TE, Hammes A (2010) LRP2 in ependymal cells regulates BMP signaling in the adult neurogenic niche. J Cell Sci.1;123 (Pt 11): 1922-1930.

Christ A, Terryn S, Schmidt V, Christensen EI, Huska MR, Andrade-Navarro MA, Hübner N, Devuyst O, Hammes A, Willnow TE. (2010) The soluble intracellular domain of megalin does not affect renal proximal tubular function in vivo. Kidney Int. 78(5): 473-477

Kur E, Christa A, Veth KN, Gajera CR, Andrade-Navarro MA, Zhang J, Willer JR, Gregg RG, Abdelilah-Seyfried S, Bachmann S, Link BA, Hammes A, Willnow TE. (2011) Loss of Lrp2 in zebrafish disrupts pronephric tubular clearance but not forebrain development. Dev Dyn. 240(6): 1567-1577

Christ A, Christa A, Kur E, Lioubinski O, Bachmann S, Willnow TE, Hammes A. (2011) LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline for inductive signals. Dev Cell. in press.

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Anchored Signalling

A-kinase anchoring proteins (AKAPs) are scaffolding proteins necessary for the formation of multi-protein complexes that spatially and timely co-ordinate the propagation and integration of cellular signalling. AKAPs sequester protein kinase A (PKA) and further signalling molecules including other kinases, phosphodiesterases and phosphatases to various cellular compartments. We have shown that interactions between AKAPs and PKA in renal collecting duct principal cells are crucial for vasopressin (AVP)-mediated water reabsorption. In addition, we have identified several other proteins involved in the process, including myosin Vb, Rab11 and p38 MAPK. In cardiac myocytes, we identified a protein complex comprising AKAP18δ, PKA and phospholamban that participates in the control of the myocyte relaxation. A major focus of our group is the identification and characterisation of new AKAPs and other proteins and their interactions to gain insight into the molecular mechanisms underlying AVP-mediated water reabsorption and cardiac myocyte contractility. Dysregulation of AKAP-based signalling causes or is associated with diseases including chronic heart failure. A second focus of the group is the development of small molecule disruptors of AKAP-dependent protein-protein interactions for validation of AKAPs as potential drug targets for the treatment of chronic heart failure.

Identification and characterisation of new AKAPs controlling AVP-mediated water reabsorption and cardiac myocyte contractility

(Philipp Skroblin, Marie Christine Moutty, Dörte Faust, Andrea Geelhaar)

AKAP-dependent protein-protein interactions are crucially involved in the control of cardiac myocyte contractility and arginine-vasopressin (AVP)-mediated water reabsorption in the kidney. Several AKAPs involved the two processes have been identified. For example, we identified AKAP18δ and observed that its direct interaction with PKA and phospholamban in cardiac myocytes enhances reuptake of Ca^{2+} into the sarcoplasmic reticulum. Thereby, AKAP18δ participates in the control of cardiac myocyte relaxation. In renal principal cells, AKAP18δ directly interacts with PKA and phosphodiesterase 4 (PDE4). This signalling module is involved in the control of the localisation of the water channel aquaporin-2 (AQP2) and thereby in the regulation of AVP-mediated water reabsorption. Several lines of evidence indicate that there are AKAPs, and in particular AKAPs and their interactions with PKA, involved in the two processes that have not been identified, yet. Therefore, our group has initiated the identification of novel AKAPs.

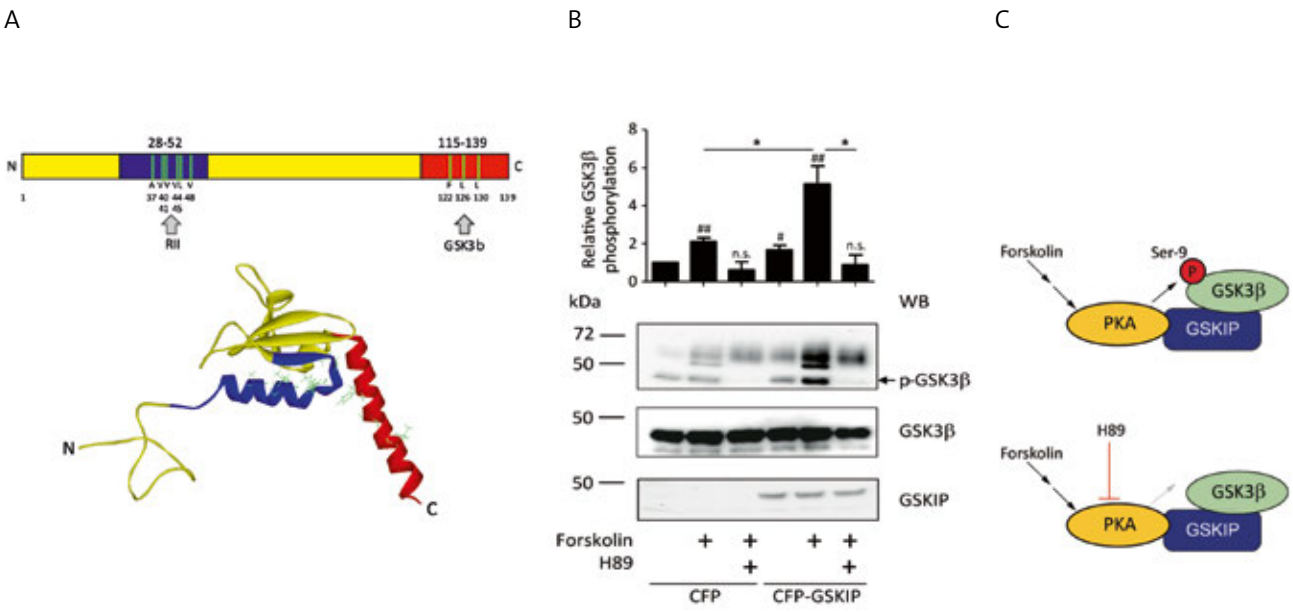

Figure 1. The AKAP GSKIP binds PKA and GSK3β, and controls the PKA-mediated phosphorylation of GSK3β. **A**. Heteronuclear single quantum coherence (HSQC) NMR experiments were carried out to determine amino acids of GSKIP involved in the interaction with regulatory RII subunits of PKA. Upper panel, schematic representation of GSKIP; indicated are the PKA (amino acids 28-52) and GSK3β binding (amino acids 115-139) domains; lower panel, NMR structure (PDB ID: 1SGO) of GSKIP with the PKA binding domain indicated. **B**. and **C**. GSKIP forms a complex with PKA and GSK3β, thus mediating the phosphorylation of GSK3β by PKA. **B**. HEK293 cells were transfected to express CFP (control) or CFP-GSKIP and the phosphorylation of GSK3β was analysed by Western Blotting. CFP-GSKIP enhanced the phosphorylation of GSK3β after activation of PKA (*via* forksolin). This effect was blocked with the PKA inhibitor (H89). **C**. Schematic illustrations of the PKA-GSKIP-GSK3β complex. B. and C., adapted from Hundsrucker et al., 2010.

PKA anchoring domains of AKAPs are structurally conserved amphipathic helices, 14-18 amino acid residues in length. Based on 3D structures and single amino acid substitution analyses of PKA-binding domains, we developed an AKAP signature motif. This motif was used for homology screening of protein databases to retrieve putative PKA-binding proteins. The PKA-binding domains of identified proteins were spot synthesized as arrays of 25mer overlapping peptides. An interaction with PKA was elucidated by incubation of the peptide arrays with radioactively labelled PKA. This approach identified several new AKAPs, amongst them GSKIP (GSK3β interaction protein). Further characterization of GSKIP confirmed its AKAP function and showed that it forms a ternary complex consisting of GSKIP, PKA and GSK3β. GSKIP facilitates the PKA-catalysed phosphorylation and thus the inactivation of GSK3β (Fig. 1). Hence GSKIP provides the means for integrating PKA and GSK3β signalling. The binding of GSKIP to GSK3β is evolutionarily conserved, whereas the ability to interact with PKA is restricted to vertebrates.

In the future we will analyse a potential involvement of GSKIP and its interactions in biological processes, in particular in the regulation of cardiac myocytes contractility and AVP-mediated water reabsorption. For this we have generated a knockout mouse model.

Pharmacological interference with AKAP-PKA interactions – implication for the treatment of heart failure

Cindy Büssow, Adeeb El-Dahshan, Beate Eisermann, Frank Götz, Jelena Milic, Gesa Schäfer, Silvia Niquet, Kerstin Zühlke, Marie Christine Moutty, Jessica Tröger

AVP binds to vasopressin V2 receptors (V2R) on the surface of renal collecting duct principal cells and thereby stimulates activation of PKA. PKA, in turn, phosphorylates AQP2 eliciting the redistribution of the water channel from intracellular vesicles into the plasma membrane. This facilitates water reabsorption from primary urine and thereby fine-tuning of body water homeostasis. Defects of the process cause nephrogenic diabetes

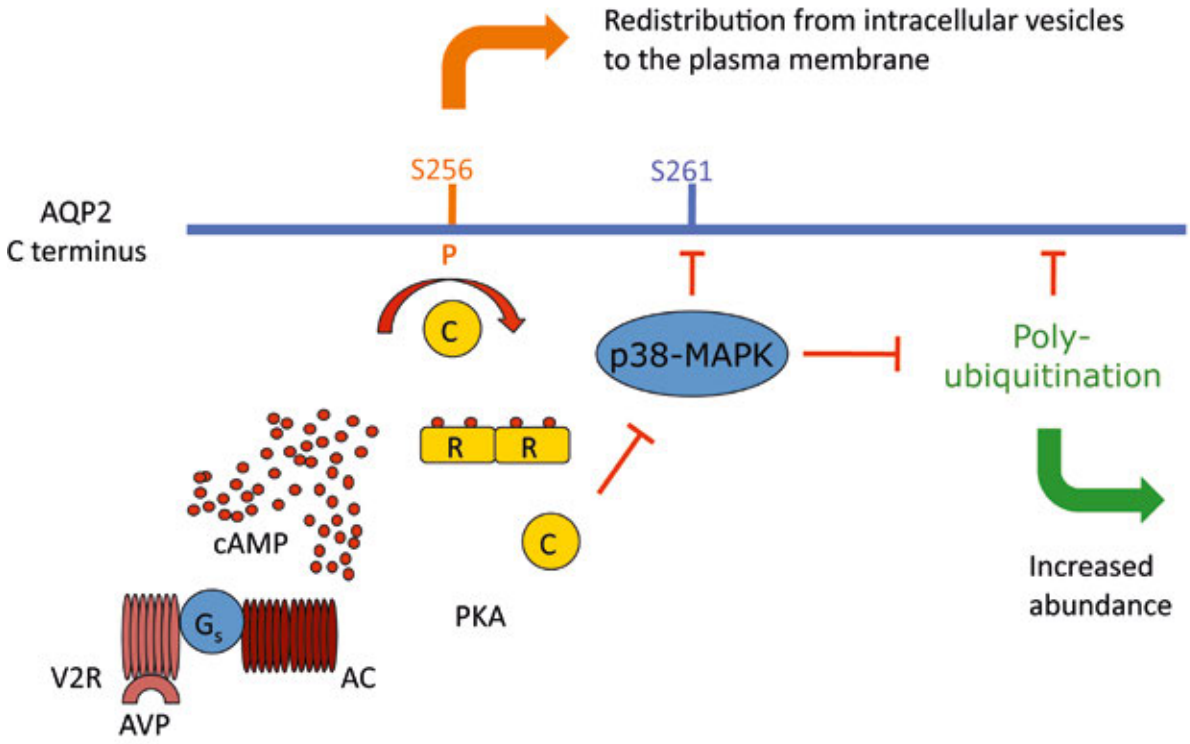

Figure 2. PKA and p38-MAP kinase crosstalk contributes to regulation of aquaporin-2 abundance. Under resting conditions, when the water channel AQP2 mainly localizes in the perinuclear region of renal principal cells, it is phosphorylated at S261. Activation of PKA through AVP leads to inhibition of p38-MAPK, which, in turn, is associated with dephosphorylation of AQP2 at S261 and de-polyubiquitination of AQP2, preventing proteasomal degradation

insipidus (NDI). On the other hand, up-regulation of the process in response to elevated levels of AVP such as in chronic heart failure (CHF) or the syndrome of inappropriate antidiuretic hormone secretion (SIADH) can cause excessive water retention and hyponatremia, the most common electrolyte disorder. Our data indicated that disruption of AKAP-PKA interactions in renal principal cell prevents the AVP-mediated water reabsorption. In addition, disruption of AKAP-PKA interactions in cardiac myocytes, isolated hearts and rodent models increases contractility. Thus disruption of AKAP-PKA interactions may beneficially influence development and/or progression of chronic heart failure. Therefore, we initiated the identification of small molecule disruptors of AKAP-PKA interaction and aim to commercialise this approach.

We established a technology platform, which allows the identification of disruptors by high-throughput screening of small molecule libraries. Identified hits are optimised with regard to affinity and selectivity by chemical modification. Through this we identified a small molecule, FMP-API-1, which disrupts AKAP-PKA interactions in cardiac myocytes by binding to PKA and, at the same time, activates PKA. This dual effect increases the contractility of cultured cardiac myocytes and isolated rat hearts. Further molecules, which apparently disrupt AKAP-PKA interactions without activating PKA are currently being characterised. Their effects on the development of chronic heart failure are tested in two mouse models; one is the thoracic aortic constriction (TAC) model, the second is a model for excessive water retention, SIADH. One of the molecules prevents the AVP-induced redistribution of AQP2 from intracellular vesicles into the plasma membrane of renal principal cells and thus excessive water retention in the SIADH model. In the TAC model, it decreases cardiac hypertrophy and improves contractility parameters of the failing heart. Therefore, small molecule disruptors of AKAP-PKA interactions may pave the way to a novel concept for the treatment of chronic heart failure.

Identification of proteins controlling AVP-mediated water reabsorption

Jana Bogum, Dörte Faust, Andrey C.C. Goncalves, Aline Kirschner

Only a few proteins and stimuli are known to regulate the localisation of AQP2. A major goal of our group is to gain mechanistic insight into the molecular mechanisms controlling AVP-mediated water reabsorption by identifying new players that participate in the control of AQP2. Such new players may also be involved in other cAMP/PKA-controlled exocytic processes such as renin secretion from juxtaglomerular cells in the kidney.

The C-terminal tail of AQP2 contains a phosphorylation site at S261 (pS261), which is phosphorylated under resting conditions (Fig. 2). The phosphorylation decreases in response to V2R stimulation in cultured principal cells. Recently, we have shown that p38 mitogen-activated protein kinase (MAPK) is one of the kinases controlling the phosphorylation of S261. The AVP-dependent decrease of pS261 is associated with a reduction in p38 MAPK activity, a decrease in poly-ubiquitination of AQP2 and reduced proteasomal degradation. This increases AQP2 abundance. This novel regulatory mechanism of AQP2 abundance is likely to play a role in rapidly increasing water reabsorption by the renal collecting duct in response to AVP.

Selected Publications

Christian, F, Szaszák, M, Drewianka, S, Friedl, S, Lorenz, D, Furkert, J, Vargas, C, Schmieder, P, Götz, F, Göttert, H, Joshi, M, Reif, B, Haase, H, Morano, I, Kass, R, Hampel, K, Kashin, D, Genieser, H-G, Herberg, FW, Willoughby, D, Cooper, DMF, Baillie, GS, Houslay, MD, v. Kries, JP, Zimmermann, B, Rosenthal, W, Klussmann, E. (2011). Small molecule AKAP/PKA interaction disruptors that activate PKA increase cardiac contractility. J. Biol. Chem. 286, 9079–9096.

Nedvetsky, PI, Tabor, V, Tamma, G, Beulshausen, S, Mutig, K, Boltzen, M, Petrucci, O, Vossenkämper, A, Wiesner, B, Bachmann, S, Rosenthal, W, Klussmann, E. (2010). Protein kinase A and p38 MAP Kinase crosstalk regulates the abundance of aquaporin-2 in renal principal cells at the post-translational level. J. Am. Soc. Nephrol. 21, 1645-56.

Hundsrucker, C, Skroblin, P, Christian, F, Zenn, M, Popara, V, Joshi, M, Herberg, FW, Reif, B, Rosenthal, W, Klussmann, E (2010). The domain of unknown function (DUF) 727 of glycogen synthase kinase interaction protein (GSKIP) facilitates anchoring of protein kinase A. J. Biol. Chem. 285, 5507–5521.

Lygren, B, Carlson, C, Santamaria, K, Lissandron, V, McSorley, T, Litzenberg, J, Lorenz, D, Wiesner, B, Rosenthal, W, Zaccolo, M, Tasken, K, Klussmann, E. (2007) AKAP complex regulates Ca2+ re-uptake into heart sarcoplasmic reticulum. EMBO. Rep. 8, 1061-1067.

Stefan, E, Wiesner, B, Baillie, GS, Mollajew, R, Henn, V, Lorenz, D, Furkert, J, Santamaria, K, Nedvetsky, P, Hundsrucker, C, Beyermann, M, Krause, E, Pohl, P, Gall, I, MacIntyre, AN, Bachmann, S, Houslay, MD, Rosenthal, W, and Klussmann, E. (2007) Compartmentalization of cAMP-dependent signaling by phosphodiesterase-4D is involved in the regulation of vasopressin-mediated water reabsorption in renal principal cells. J. Am. Soc. Nephrol. 1, 199-212.

Patent applications

Klußmann E, Schäfer, G. Milic, J., Rosenthal, W. Schillinger, C., Krause, G., Rademann, J. Peptidomimetika zur Hemmung von AKAP-PKA-Interaktionen als Arzneistoffe. EP11168008.8.

Klußmann E, Milic, J., Bergmann, M., Rosenthal, W. Arylaminomethylenbenzothiophenone als Arzneimittel. 2011011818380200DE.

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Molecular Muscle Physiology

Contraction of all muscle types is elicited by increasing myoplasmic Ca^{2+} and interaction of Type II myosins with thin (actin) filaments. In striated muscle, Ca^{2+} bind to troponin C, which turn the thin filament "on", allowing myosin force-generating actin interactions. We are studying the functional roles in particular of subunits of key proteins of Ca^{2+} handling and force generation, i.e. the L-type Ca^{2+} channel and type II myosins in striated and smooth muscle. Any change of these proteins by mutation, differential gene expression, alternative splicing of the transcripts, or post-translational modification modulate striated and smooth muscle function. Understanding muscle contraction regulation at the molecular and functional levels provide the opportunity to develop new therapeutic strategies for the treatment of cardiovascular and skeletal muscle dysfunction.

N- and C-terminal domains of essential myosin light chains (ELC) modulate cardiac functions

Daria Petzhold, Janine Lossie, Ralf Meißner, Petra Sakel, Petra Domaing

We modelled the missing 46 N-terminal amino acid of the ELC to the contemporary actin-myosin-S1 complex (Figure 1). The N-terminus of ELC showed a rod-like 91 Å structure being long enough to bridge the gap between the ELC core of myosin-S1 and the appropriate binding site of the ELC on the actin filament. ELC/actin interaction could be inhibited by a peptide-competition approach with synthetic petides comprising the most N-terminal amino acids 1-15, which bind to the appropriate ELC binding site of actin. Recordings of cardiac function during peptide competition could then direct into a possible physiological role of ELC/actin interaction.

To test the hypothesis that expression of N-terminal ELC peptides could modulate intrinsic contractility of the whole heart, we generated transgenic rats (TGR) that overexpressed minigenes encoding the N-terminal 15 amino acids of human atrial ELC (TGR/hALC-1/1-15) or human ventricular ELC (TGR/hVLC-1/1-15) isoforms in cardiomyocytes. Synthetic N-terminal peptides revealed specific actin binding, with a significantly ($P<0.01$) lower dissociation constant (K_D)) for the hVLC-1/1-15-actin complex compared with the K_D value of the hALC-1/1-15-actin complex. For the first time we showed that the expression of N-terminal human ELC peptides in TGR (3-6 µM) correlated with significant ($p<0.001$) improvements of the intrinsic contractile state of the isolated perfused heart (Langendorff mode). The positive inotropic effect of ELC peptides occurred in the absence of a hypertrophic response. Thus, N-terminal ELC peptides may represent a valuable tool for the treatment of the failing heart.

We specifically monitored the functional effects of a cell-permeable peptide containing the 15 amino acid N-terminal peptide from human ventricular light chain-1 (hVLC-1) on contraction and intracellular Ca^{2+} signals after electrical stimulation in primary adult cardiomyocytes. Synthetic hVLC-1/1-15 as a TAT fusion peptide was taken up by cardiomyocytes within 5 min with more than 95% efficiency. Analysis of the functional effects of

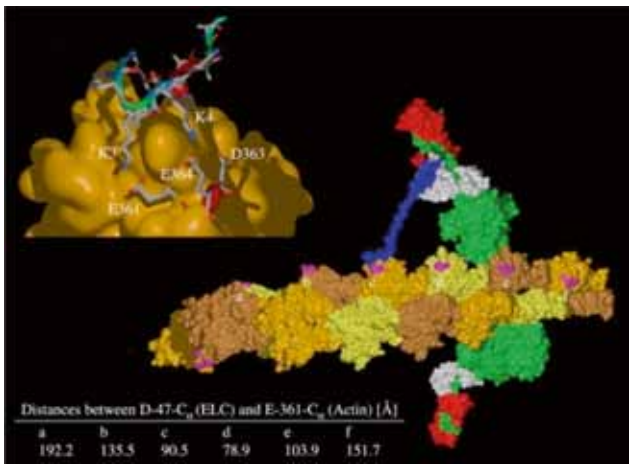

Figure 1. 3D-model of the actomyosin complex. (A) Gauss–Connolly surfaces are used to visualize the molecular complex. Actin units are coloured orange, brown, and yellow. The myosin S1 head (green), the regulatory light chain (red), the shortened essential light chain (white), the 46 N-terminal residues of A1 (blue), and clusters of acidic residues on actin (pink) are shown. (B) More detailed view on the potential interaction of N-terminal APKK of A1 with acidic residues on actin. Ionic interactions between lysine residues (K3 and K4) of APKK and acidic residues (E361 and E364) on actin were assumed.

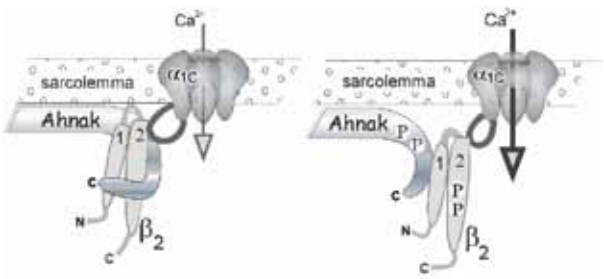

Figure 2. Proposed model for sympathetic control of I_{CaL} by ahnak1. Under basal conditions, I_{CaL} carried by the a1C-subunit is repressed by strong ahnak1/β2-subunit binding (left panel). Upon sympathetic stimulation, PKA sites in ahnak1 and/or in β2 become phosphorylated. This releases the β2-subunit from ahnak1 inhibition resulting in increased I_{CaL}.

the cell-permeable hVLC-1 revealed an enhancement of the intrinsic contractility of cardiomyocytes without affecting the intracellular Ca^{2+}. Furthermore, we analysed structural requirements for the non-endocytotic uptake mode of cell-penetrating peptides, We demonstrated that the transduction efficiency of arginine-rich peptides increases with higher peptide structural rigidity. Consequently, cyclic arginine-rich cell-penetrating peptides showed enhanced cellular uptake kinetics relative to their linear and more flexible counterpart.

In addition, we tested the hypothesis that different binding affinities of the C-terminus of human cardiac ELC isoforms to the IQ1 motif of the myosin lever arm provide a molecular basis for distinct sarcomeric sorting and inotropic activity. We employed circular dichroism and surface plasmon resonance spectroscopy to investigate structural properties, secondary structures, and protein-protein interactions of a recombinant head-rod fragment of rat cardiac β-MYH amino acids 664-915 (rβ-$MYH_{664-915}$) with hALC1 and hVLC-1. Double epitope-tagging competition was used to monitor the intracellular localization of exogenously introduced hALC-1 and hVLC-1 constructs in neonatal rat cardiomyocytes. Contractile functions of A1 isoforms were investigated by monitoring shortening and intracellular-free Ca^{2+} (Fura-2) of adult rat cardiomyocytes infected with adenoviral (Ad) vectors using hALC-1 or β-galactosidase as expression cassettes. hALC-1 bound more strongly (three-fold lower K_D) to rβ-$MYH_{664-915}$ than did hVLC-1. Sorting specificity of both ELC isoforms to sarcomeres of cardiomyocytes rose in the order hVLC-1 to hALC-1. Replacement of endogenous hVLC-1 by hALC-1 in adult rat cardiomyocytes increased contractility while the systolic Ca^{2+} signal remained unchanged. Thus, intense myosin binding of hALC-1 provides a mechanism for preferential sarcomeric sorting and Ca^{2+}-independent positive inotropic activity.

Hypertrophic cardiomyopathy (HCM) is caused by mutations in genes encoding proteins of the cardiac sarcomere. Thus, HCM associates with five missense mutations in the essential ventricular myosin light chain gene (M149V, E143K, A57G, E56G, R154H). We employed circular dichroism and surface plasmon resonance spectroscopy to investigate structural properties and protein-protein interactions of a recombinant head-rod fragment of rat cardiac β-myosin heavy chain (rβ-$MYH_{664-915}$) and normal or five mutated (M149V, E143K, A57G, E56G, R154H) hVLC-1 forms. Double epitope tagging competition was used to monitor the intracellular localization of exogenously introduced normal and E56G mutated (hVLC-1^{E56G}) hVLC-1 constructs in neonatal rat cardiomyocytes. Fluorescence lifetime imaging microscopy (FLIM) was applied to map the microenvironment of normal and E56G mutated hVLC-1 in permeabilized muscle fibers. Affinity of M149V, E143K, A57G, and R154H mutated hVLC-1/ rβ-$MYH_{664-915}$ complexes were significantly lower compared with the normal hVLC-1/ rβ-$MYH_{664-915}$ complex interaction. In particular the E56G mutation induced an about 30fold lower MYH affinity. Sorting specificity of E56G-mutated hVLC-1 was negligible compared with normal hVLC-1. Fluorescence lifetime of fibers replaced with hVLC-1^{E56G} increased significantly compared with hVLC-1 replaced fibers. Disturbed myosin binding of mutated hVLC-1 may provide a pathomechanism for the development of hypertrophic cardiomyopathy.

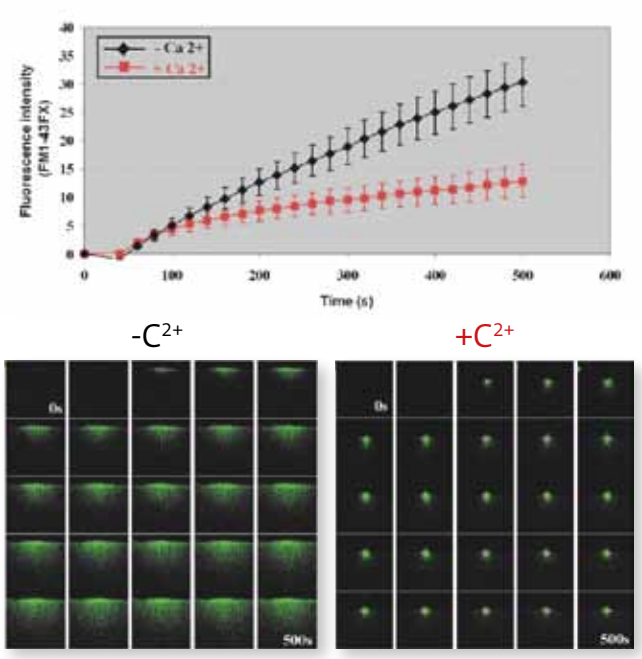

Figure 3. Membrane resealing assay performed on wild-type mouse single skeletal muscle fibres. Membrane damage (5x5µm) was induced with a two-photon confocal laser-scanning microscope (LSM 510 META, Zeiss) coupled to a 488-nm Argon Ti:Sapphire Laser in the presence of FM 1-43FX fluorescence dye. **Top**: Plot of fluorescence intensity (n=10) against time in the presence (red line) and absence (black line) of Ca^{2+}. Data are means ±SEM. **Bottom**: fluorescence obtained in the presence (right) and absence (left) of Ca^{2+}.

The role of myomesin missense mutations on the genesis of hypertrophic cardiomyopathy

Romy Siegert (In collaboration with Cemil Öczelik, University Medicine Charité, Berlin)

Myomesin plays an important structural and functional role in the M-band of striated muscles. Three missense mutations in the myomesin gene have recently been detected in patients with HCM. We studied the molecular pathomechanisms causing the development of HCM by the myomesin mutation V1490I. Analytical ultracentrifugation experiments, circular dichroism spectra, and SPR spectroscopy of myomesin fragments were carried out to investigate the effects of the mutation V1490I on structure and function of myomesin domains 11-13 and 12-13. Both the wild type and mutated myomesin domains My11-13 revealed similar secondary structures and formed stable dimers. Mutated myomesin domains My11-13 and My12-13 dimers revealed a reduced thermal stability and a significantly decreased dimerisation affinity, showing disturbed functional properties of V1490I mutated myomesin. However, monomeric myomesin domains My11-12, i.e. without dimerisation domain 13 showed no difference in thermal stability between wild type and V1490I mutated myomesin. In

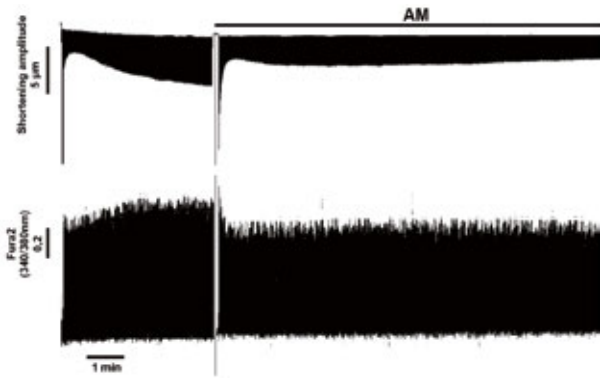

Figure 4. Effect of adipocyte-conditioned medium (AM) on shortening amplitude (top trace) and Fura-2 signal (bottom trace) of an adult rat cardiomyocyte. A, B) Representative chart recordings of shortening amplitudes.

conclusion, the V1490I mutation associated with HCM lead to myomesin proteins with abnormal functional properties which affect dimerisation properties of myomesin domain 13. These effects may contribute to the pathogenesis of HCM.

Ahnak1 – a novel, prominent modulator of cardiac L-type Ca^{2+} channels

Hannelore Haase, Ines Pankonien, Karin Karczewski, Steffen Lutter (in collaboration with Nathan Dascal, University Tel Aviv, Israel)

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-sub- unit. Previous work suggested that the interaction between ahnak1 and β2-subunit plays a role in L-type Ca^{2+} current (ICaL) regulation. We performed structure-function studies with the most C-terminal domain of ahnak1 (188 amino acids) containing a PxxP consensus motif (designated as 188-PSTP) using ventricular cardiomyocytes isolated from rats, wild-type (WT) mice and ahnak1-deficient mice. In vitro binding studies revealed that 188-PSTP conferred high-affinity binding to β2 (K_D approximately 60 nM). Replacement of proline residues by alanines (188-ASTA) decreased β2 affinity about 20-fold. Both 188-PSTP and 188-ASTA were functional in ahnak1-expressing rat and mouse cardiomyocytes during whole-cell patch clamp. Upon intracellular application, they increased the net Ca^{2+} influx by enhancing ICaL density and/or increasing ICaL inactivation time course without altering voltage dependency. Specifically, 188-ASTA, which failed to affect ICaL density, markedly slowed ICaL inactivation resulting in a 50-70% increase in transported Ca2+ during a 0 mV depolarising pulse. Both ahnak1 fragments also slowed current inac-

tivation with Ba^{2+} as charge carrier. By contrast, neither 188-PSTP nor 188-ASTA affected any ICaL characteristics in ahnak1-deficient mouse cardiomyocytes. Our results indicate that the presence of endogenous ahnak1 is required for tuning the voltage-dependent component of ICaL inactivation by ahnak1 fragments. We suggest that ahnak1 modulates the accessibility of molecular determinants in β2 and/or scaffolds selectively different β-subunit isoforms in the heart.

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

Andreas Marg, Hannelore Haase, Petra Domaing

AHNAK function and subcellular localization in skeletal muscle are unclear. The aim of the project is the elucidation of the role of ahnak protein family in skeletal muscle fibers. The transmembrane protein dysferlin seems to anchor ahnak1 and ahnak2 to the sarcolemma, thus providing a membrane-stabilizing dysferlin-ahnak-actin complex. We investigate whether the ahnak protein family is important for membrane stability and Ca2+ handling of skeletal muscle fibers. A laser-assisted membrane resealing and a Fura2-based fluorescence assay of electrically stimulated enzymatically isolated single skeletal muscle fibers from mouse Flexor digitorum brevis will be applied.

We used specific AHNAK1 and AHNAK2 antibodies to analyzed the detailed localization of both proteins in mouse skeletal muscle. 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 (Figure 3). 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.

Identification of new adipocyte-derived cardiodepressant factors

Christiane Look (in collaboration with Valeria Lamounier-Zepter, University Dresden)

The causal relationship between obesity and heart failure is broadly acknowledged; however, the pathophysiological mechanisms involved remain unclear. We investigated whether human adipocytes secrete cardioactive substances that may affect cardiomyocyte contractility. We cultivated adipocytes obtained from human white adipose tissue and incubated isolated rat adult cardiomyocytes with adipocyte-conditioned or control medium. Human adipocytes exhibited cardiodepressant activity with a direct and acute effect on cardiomyocyte contraction. This adipocyte-derived negative inotropic activity directly depressed shortening amplitude as well as intracellular systolic peak Ca^{2+} in cardiomyocytes within a few minutes (Figure 4).

Through mass spectrometry and immunoblotting, we have identified the cardiodepressant factor as "fatty acid binding protein 4" (FABP4). FABP4 acutely depressed shortening amplitude as well as intracellular systolic peak Ca^{2+} in a dose-dependent manner in isolated rat cardiomyocytes. Heart-specific FABP isoform (FABP3) revealed a similar cardiodepressant effect. The N-terminal amino acids 1 to 20 of FABP4 could be identified as the most effective cardiodepressive domain. We could exclude any effect of FABP4 on action potential duration and L-type Ca^{2+} current, suggesting a reduced excitation-contraction gain caused by FABP4 as the main inhibitory mechanism. We conclude that the release of FABP4 from adipocytes may be involved in the development of cardiac contractile dysfunction of obese subjects.

Selected Publications

Lättig-Tünnemann G, Prinz M, Hoffmann D, Behlke J, Palm-Apergi C, Morano I, Herce HD, Cardoso MC. (2011) Backbone rigidity and static presentation of guanidinium groups increases cellular uptake of arginine-rich cell-penetrating peptides. Nat. Commun. 2:453.

Lamounier-Zepter V, Look C, Alvarez J, Christ T, Ravens U, Schunck W-H, Ehrhart-Bornstein M, Bornstein SR, Morano I (2009) Adipocyte fatty acid-binding protein suppresses cardiomyocyte contraction: a new link between obesity and heart disease. Circ. Res.,105:326-34.

Tünnemann, G, Behlke, J, Karczewski, P, Haase, H. Cardoso, MCh, Morano, I. (2007). Modulation of muscle contraction by a cell permeable peptide. J Mol Med.12:1405-12

Aydt EM, Wolff G, Morano I (2007) Molecular modelling of the myosin-S1(A1) isoform. J. Struct. Biol., 159:158-63.

Haase H, Dobbernack G, Tünnemann G, Karczewski P, Cardoso C, Petzhold D, Schlegel WP, Lutter S, Pierschalek P, Behlke J, Morano I. (2006) Minigenes encoding N-terminal domains of human cardiac myosin light chain-1 improve heart function of transgenic rats. FASEB J. 20:865-73.

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Neuromuscular and Cardiovascular Cell Biology

Our long-term goal is to establish how changes in biomechanics are translated into molecular signals and vice versa. 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 exercise or immobilization change skeletal muscle growth. We focus on titin, the largest protein in the human body and the multifunctional coxsackie-adenovirus receptor (CAR).

To lay the groundwork for the *in vivo* analysis of titin's multiple signaling, elastic, and adaptor domains, we have generated various titin deficient mice (knock-in and conditional knockout animals) and established a tissue culture system to study titin's muscle and non-muscle functions. We utilize a combination of cell-biological, biochemical, and genetic tools to establish titin as a stretch sensor converting mechanical into biochemical signals. Using a comparable loss of function approach we have created a conditional knockout of the coxsackie-adenovirus receptor to demonstrate that CAR is crucial for embryonic development and determines the electrical properties of the heart.

Cardiac alternative splicing

Michael Radke, Thirupugal Govindarajan, Martin Liss, Padmanabhan Vakeel, Vita Dauksaite

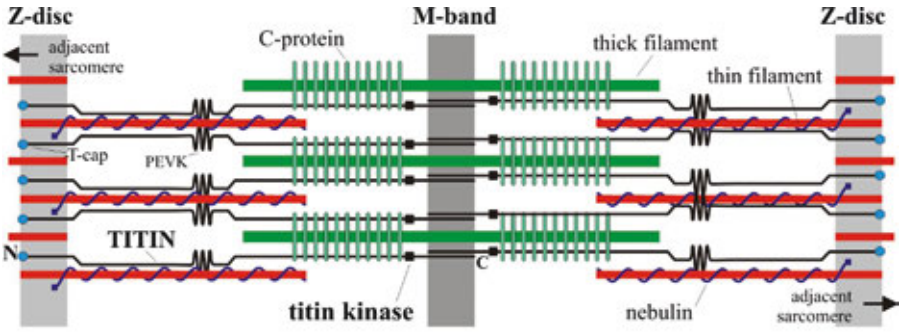
Alternative splicing is a major mechanism to adjust the cardiac proteome to the differential requirements of embryonic and postnatal physiology. We use a naturally occurring splice deficient rat strain, high throughput technology, and *in silico* analysis to identify factors that regulate cardiac alternative splicing and their target profiles. This approach has led us to identify various regulatory, metabolic, and structural proteins that determine cardiovascular physiology and adaptation. In addition to improving our knowledge of splicing as a prerequisite for cardiac adaptation, we will translate our findings towards developing novel therapeutic options for patients with cardiovascular disease emphasizing diastolic dysfunction with titin as a main contributor to the elastic properties of the heart.

Titin based mechanotransduction

Michael Radke, Thirupugal Govindarajan, Christopher Polack, Douaa Megahed

Titin is a unique molecule that contains elastic spring elements and a kinase domain, as well as multiple phosphorylation sites. Therefore, it has been frequently speculated that titin could act as a stretch sensor in muscle. So far it has remained unknown how the stretch signal is processed, i.e. how the mechanical stimulus stretch is converted into a biochemical signal.

To investigate the stretch signaling pathway, we apply mechanical strain *in vivo* (plaster cast for skeletal muscle; aortic banding for the heart) and in tissue culture (cultivation of primary cells on elastic membranes). The resulting changes in the proteome of our titin kinase and spring element deficient animals are used to map the mechanotransduction pathway.



Schematic diagram of the sarcomere. Titin forms a continuous filament system along the muscle fiber overlapping in the M-band (titin C-terminus) and in the Z-disc (N-terminus). The titin kinase is found near the edge of the M-band region, while the elastic PEVK resides in the I-band. Titin interacts with a plethora of sarcomeric proteins, such as T-cap and C-protein.

Sarcomeric and non-sarcomeric functions of titin

Nora Bergmann, Katharina da Silva Lopes, Thirupugal Govindarajan, Fabian Freiberg, Franziska Rudolph

Overlapping titin molecules form a continuous filament along the muscle fiber. Together with the multiple binding sites for sarcomeric proteins, this makes titin a suitable blueprint for sarcomere assembly. The use of transgenic techniques does not only allow us to address the function of titin's individual domains in sarcomere assembly, but also to follow sarcomere assembly and disassembly using fluorescently tagged proteins. Understanding the structural and biomechanical functions of titin will help elucidate the pathomechanisms of various cardiovascular diseases and ultimately aid the development of suitable therapeutic strategies.

Recently titin has been proposed to perform non-muscle functions following its localization to mitotic chromosomes and cleavage furrows as well as the D-titin related chromosome abnormalities and aneupoidity found in *Drosophila melanogaster*. Our preliminary data indicate that titin is present in virtually every cell-type tested and our knockout of titin's M-band region displays a defect in implantation. Currently we are extending our analysis to the titin dependent regulation of the cell-cycle.

Functional analysis of the Coxsackie-Adenovirus Receptor

Chen Chen, Uta Wrackmeyer, Ulrike Lisewski, Fabian Freiberg, Joanna Kaldrack

CAR was cloned as a receptor used by adeno- and coxsackievirus to enter cells but its physiological role has remained obscure. We have generated both tissue culture and animal models to study CAR's function in cardiac remodeling, inflammatory cardiomyopathy, and basic cellular processes such as endocytosis and cell-cell contact formation. Our data suggest a critical role of CAR in the conduction of electrical signals from the atria to the cardiac ventricle. The inducible heart-specific knockout of CAR has enabled us to completely block the entry of coxsackievirus into cardiomyocytes and prevent all signs of inflammatory cardiomyopathy.

Selected Publications

da Silva Lopes K, Pietas A, Radke MH, Gotthardt M. (2011) Titin visualization in real time reveals an unexpected level of mobility within and between sarcomeres. J Cell Biol. 193(4):785-98

Shi, Y., Chen, C., Lisewski, U., Wrackmeyer, U., Radke, M., Westermann, D., Sauter, M., Tschöpe, C., Poller, W., Klingel, K., Gotthardt, M. (2009) Cardiac deletion of the Coxsackievirus-adenovirus-receptor abolishes CVB3 infection and prevents myocarditis in vivo. JACC 7;53(14):1219-26

Lisewski, U., Shi, Y., Wrackmeyer, U., Chen, C., Fischer, R., Schirdewan, A., Juettner, R., Rathjen, F., Poller, W., Radke, M., Gotthardt, M. (2008) The tight junction protein CAR regulates cardiac conduction and cell-cell communication. JExMed 205(10):2369-79

Radke, M., Peng, J., Wu, Y., McNabb, M., Nelson, O.L., Granzier, H., Gotthardt, M. (2007) Targeted deletion of Titin's N2B region leads to diastolic dysfunction and cardiac atrophy. Proc. Natl. Acad. Sci. USA. 104(9), 3444-3449

Peng J., Raddatz, K., Molkentin, J.D., Wu, Y., Labeit, S., Granzier, H., Gotthardt, M. (2007) Cardiac hypertrophy and reduced contractility in titin kinase deficient hearts. Circulation 13;115(6):743-5

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Salim Seyfried

Zebrafish Cardiovascular Developmental Genetics

Vertebrate organs are derived from epithelial or endothelial sheets of cells that undergo complex morphogenetic transformations. We are studying the zebrafish heart, a relatively simple organ compared with its mammalian counterpart, to better understand the signaling events that instruct the assembly of the early heart tube.

Initially this organ consists of only the outer myocardial and inner endocardial cell layers. We would like to understand: What are the signals that regulate the morphogenesis of myocardium and endocardium? To what extent do these two tissues communicate during cardiac looping, cushion formation, and trabeculation? What determines the differentiation of endocardium into its different subpopulations such as cushion cells? In collaboration with clinical researchers, we are using developmental genetics combined with cell biological and pharmacological approaches to develop animal models for human cardiovascular diseases. Our long-term interest is to understand how the cellular mechanisms controlling zebrafish cardiogenesis shape our own heart and its associated blood vessels.

Asymmetric behaviors of myocardial cells drive zebrafish heart tube formation

Stefan Rohr, Cécile Otten

Many vertebrate organs are derived from monolayered epithelia that undergo morphogenetic changes to acquire their final shapes. Little was known about the tissue movements or cellular dynamics underlying early cardiac morphogenesis. In particular, the process by which the flat heart field is transformed into a linear tube was largely unexplored in vertebrates. In a recent study, we described a completely unexpected tissue morphogenetic process by which the nascent heart tube is generated in the zebrafish embryo. We discovered that asymmetric involution of the myocardial epithelium from the right side of the heart field initiates a complex tissue inversion which creates the ventral floor of the primary heart tube whereas myocardial cells derived from the left side of the heart field contribute exclusively to the future dorsal roof of this organ. Intriguingly, asymmetric left-right gene expression within the myocardium correlates with asymmetric tissue morphogenesis and disruption of left-right gene expression causes randomized myocardial tissue involution. Failure to generate a heart tube did not affect the acquisition of atrial versus ventricular cardiac cell shapes. Therefore, establishment of basic cardiac cell shapes precedes cardiac function. Together, these results provided a framework for characterizing single cell behaviors during the formation of the zebrafish primary heart tube.

Control of cardiac laterality by combinatorial TGF-β network signaling

Justus Veerkamp, Florian Priller, Franziska Rudolph, Zoltan Cseresnyes, Marc Renz

Defective L/R patterning has been associated with a plethora of congenital cardiac and other organ malformations which underscores the great medical relevance of this process. It was known that signaling by two TGF-β signaling pathways, Nodals and BMPs, is essential for the correct establishment of cardiac laterality. However, it was largely unknown, how these two TGF-β signaling pathways crosstalk and which effector genes are

required for cardiac morphogenesis. In an extension of our previous study on the cell biology of cardiac tube formation, we could show that endocardial and myocardial progenitor cell motility is critically dependent on BMP signaling dosage. Transcriptome profiling of BMP-dependent cardiac gene expression combined with functional studies revealed the importance of extracellular matrix composition for cardiac cell motility. We found that Nodal signaling antagonizes BMP signaling within the cardiac field, in part by regulating the extracellular matrix gene *hyaluronan synthase 2*, and that the asymmetric modulation of extracellular matrix composition weakens BMP signaling activity on the left. Our findings imply that Nodal initiates cardiac laterality by dampening BMP activity, which increases motility of cardiac progenitor cells towards the left (Fig. 1).

Control of myocardial morphogenesis by the endocardial cerebral cavernous malformations complex

Marc Renz, Cécile Otten, Franziska Rudolph, Johan Duchene (in collaboration with Yuan Zhu, Ulrich Sure, University of Duisburg-Essen)

Familial cerebral cavernous malformations (CCM) are inherited vascular abnormalities with a poorly understood etiology that are caused by mutations in *CCM1/KRIT1, CCM2,* or *CCM3/PDCD10.* While the CCM complex has been mainly implicated in endothelial vessel formation and maintenance of vascular integrity, little was known about the putative cardiac function of these proteins. In zebrafish and mouse, loss of CCM components causes massive cardiac dilation phenotypes that have not been further characterized. In functional studies, we found that loss of the CCM complex affects myocardial morphogenesis including formation of the atrioventricular cushions at the boundary between both chambers. As an entry point to the molecular description of the CCM complex during cardiac development, we performed whole transcriptome gene expression profiling on highly purified embryonic heart tissue containing endocardial and myocardial cells of zebrafish. These analyses revealed that zebrafish endothelial CCM complex proteins act as inhibitors of the shear-stress responsive transcription factors of the Krüppel-like factor 2 (Klf2) subfamily, which are induced by oscillatory, blood flow during cardiac cushion formation. Loss of Klf2a or Klf2b ameliorates *ccm2* mutant cardiac dilation defects. Therefore, the CCM complex controls endocardial cell biology by restricting expression of the flow-responsive transcriptional regulators Klf2a/b to the cardiac cushions, thereby ensuring proper myocardial morphogenesis.

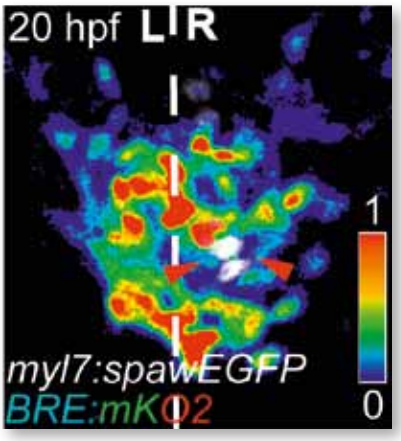

Figure 1. Misexpression of the Nodal ligand Spaw within myocardium antagonizes local BMP signaling activity. Shown is the cardiac field with clones of cells that misexpress the Nodal ligand Spaw from a Tg[*myl7:spaw_IRES_CAAX-eGFP*] expression construct on the right side of the cardiac cone (white cells). The transgenic reporter line Tg[*BRE-AAVmlp:dmKO2*]^{mw40} drives expression of destabilized monomeric Kusabira-Orange 2 (dmKO2) and indicates the activity of BMP signaling. Misexpression of Spaw on the right side of the cardiac cone (see red arrowheads) antagonizes BMP signaling. BMP signaling intensities are indicated by color range. L, left; R, right.

Selected Publications

Zhang, J, Piontek, J, Wolburg, H, Piehl, C, Liss, M, Otten, C, Christ, A, Willnow, TE, Blasig, IE, Abdelilah-Seyfried, S. (2010). Establishment of a neuroepithelial barrier by Claudin5a is essential for zebrafish brain ventricular lumen expansion. Proc. Natl. Acad. Sci USA, 107:1425-30.

Rohr, S, Otten, C, Abdelilah-Seyfried, S. (2008). Asymmetric involution of the myocardial field drives heart tube formation in zebrafish, Circ. Res. 102, e12-19.

Bit-Avragim, N, Hellwig, N, Rudolph, F, Munson, C, Stainier, D, and Abdelilah-Seyfried, S. (2008). Divergent polarization mechanisms during vertebrate epithelial development mediated by the Crumbs complex protein Nagie oko. J. Cell Sci. 121, 2503-2510.

Cibrián-Uhalte, E, Langenbacher, A, Shu, X, Chen, JN, Abdelilah-Seyfried, S. (2007). Involvement of Na,K ATPase in myocardial cell junction maintenance. J. Cell Biol. 176, 223-230.

Rohr, S, Bit-Avragim, N, Abdelilah-Seyfried, S. (2006). Heart and soul/PRKCi and Nagie oko/Mpp5 regulate myocardial coherence and remodeling during cardiac morphogenesis. Development 133, 107-115.

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Angiogenesis and Cardiovascular Pathology

Vascular network remodeling and the formation of new blood vessels (angiogenesis and arteriogenesis) plays an important role in the pathophysiology of ischemic cardiovascular disease, hypertension, and cancer, which are the most common causes of mortality in western society. Our goal is to generate novel genetic insights in the regulation of vascular development that can translate into therapeutic strategies to treat these diseases. Our research projects therefore aim at understanding the molecular regulation of angiogenesis and arteriogenesis. We focus on three crucial aspects: differentiation and guidance of angiogenic vessel sprouts by endothelial tip cells, imprinting of arterial-venous identity in blood vessels by hemodynamic factors, and the formation and adaptation of native collaterals in the context of ischemic diseases. We address vascular development using an integrative molecular and physiological approach in zebrafish, and mouse models. Our signalling pathways of interest include Dll4-Notch, VEGF-receptor-1, neuron navigators, and mechanosensing of hemodynamic factors.

Tip Cell biology and Vessel Guidance

Recent studies of vascular network development in the embryo identified several novel aspects of angiogenesis crucial to generate a functional, and stable branched vascular network. These aspects include: the differentiation and guidance of endothelial tip cells in angiogenic vessels, and the formation of functional branches. We addressed tip cell formation and angiogenic sprout guidance using a genetic approach and in vivo imaging techniques in mouse, and zebrafish. We discovered that neural guidance genes expressed in the vascular system control vessel branching morphogenesis by regulating the movement of endothelial tip cells at the leading edge of angiogenic vessel sprouts. We demonstrated that Delta-like 4 (Dll4)-Notch signalling plays a critical role in the differentiation of endothelial cells into tip cells in response to VEGF gradients. Recently, we defined the role of VEGF receptor-1, Flt1, in tip cell formation using zebrafish. We showed that soluble Flt1 produced by sprouting vessels acts, in a Notch-dependent manner, as a negative regulator of tip cell formation and sprout guidance. Soluble Flt1 mediated sprout guidance is a process that involved interaction with the nervous system. This interaction determines the distribution of soluble Flt1 surrounding the sprout, and controls the direction of sprout expansion. We are currently investigating the role of Flt1 at the neuro-vascular interface in the context of vessel guidance and neurogenesis. We are furthermore interested in how attractive and repulsive guidance cues are conveyed into physical movement of tip cells towards or away from the gradient. While it is established that during evolution the vascular system has co-opted growth control mechanisms from the nervous system, we recently observed that neural guidance molecules can also shape endodermal organs. In zebraf-

In vivo imaging of vessel branching in control (left panel) and Flt1 morphants (right panel) using transgenic zebrafish embryos expressing GFP in the vasculature. Note the stereotype vessel pattern in control, and the hyperbranched vessels in Flt1 morphants. (From Janna Krueger et. al., Development, 2011).

ish, neuron navigator 3 (Nav3) controls embryonic liver development involving coordination of hepatoblast migration, in a process highly reminiscent of tip cell guidance. We are currently characterizing the physiological role of other neuron navigator family members, in the context of angioblast migration and sprout differentiation.

Arteriogenesis and Ischemic Disease

Arteriogenesis, the outward remodeling of pre-existing small collateral arterial networks, occurs as a response to vascular occlusion or stenosis and importantly determines the clinical outcome of ischemic cardiovascular disease. Release of vasodilators and activation of inflammatory pathways allowing influx of monocytes may result in revascularization and restoration of blood flow into the hypoperfused ischemic area. Therapeutic arteriogenesis is considered of major clinical importance to treat the increasing population with complex occlusive artery diseases. Distinct differences exist between animal strains and patients with regard to collateral development and response to angiogenic growth factors. We aim at understanding the molecular mechanism accounting for such differences. In particular we focus on the formation of native collaterals and efficiency of collateral recruitment and maintenance. In mice, the native collaterals are already detectable at time of birth suggesting that the critical time-window for native collateral formation is in the embryo. Using a loss and gain of function approach in mice, we obtained evidence showing that Dll4-Notch acts as a negative regulator of native collateral development. We are now testing if modulating native collateral number via interference with Notch signalling can improve the clinical outcome in stroke and peripheral hindlimb ischemia models. The adaptive vascular recovery upon such pathological insults, are evaluated using laser-doppler flow, and standard angiography techniques, MRI and microCT.

Hemodynamic forces exerted by flowing blood play a critical role in initiation and maintenance of arteriogenic responses. We are interested which biophysical signals exerted by flowing blood can activate specific genetic programs essential for arterial differentiation and maturation. We recently observed that only flow related pulsatile shear forces induced and maintained arterial identity genes. Exposure to constant shear forces induced venous specification pathways. The morphological processes underlying flow induced arterial patterning included shear stress induced lumen size adaptation, and intussusception also called splitting angiogenesis. Intussusception resulted in molding out expanding arterioles from the capillary plexus. Arterial flow induced expression of the gap junction protein Gja5. Genetic ablation of Gja5 in mice resulted in reduced arteriogenesis. In particular we noted reduced native collateral formation, and impaired flow induced outward remodelling in arteries from Gja5 mutant mice. Our work suggests that Gja5 is involved in mechanotransduction, and thereby mediates shear signals in arterial structure. Using a systems biology approach we also measured flow regulated microRNA's in endothelial cells. We characterized and validated a subset of these microRNA's and found that they play a unique role in regulating arterial specification during vascular network development. The therapeutic potential of these findings will be explored in CABG models.

Selected Publications

Krueger J, Liu D, Scholz K, Zimmer A, Shi Y, Klein C, Siekmann A, Schulte-Merker S, Cudmore M, Ahmed A, le Noble F (2011). Flt1 acts as a negative regulator of tip cell formation and branching morphogenesis in the zebrafish embryo. Development. 138 (10): 2111-20.

Klein C, Mikutta J, Krueger J, Scholz K, Brinkmann J, Liu D, Veerkamp J, Siegel D, Abdelilah-Seyfried S, le Noble F (2011). Neuron navigator 3a regulates liver organogenesis during zebrafish embryogenesis. Development. 138 (10): 1935-45.

Buschmann I, Pries A, Styp-Rekowska B, Hillmeister P, Loufrani L, Henrion D, Shi Y, Duelsner A, Hoefer I, Gatzke N, Wang H, Lehmann K, Ulm L, Ritter Z, Hauff P, Hlushchuk R, Djonov V, van Veen T, le Noble F (2010). Pulsatile shear and Gja5 modulate arterial identity and remodeling events during flow-driven arteriogenesis. Development. 137 (13): 2187-96.

Pries AR, Hoepfner M, le Noble F, Dewhirst MW, Secomb TW (2010). The shunt problem: control of functional shunting in normal and tumour vasculature. Nature Rev Cancer. 10 (8): 587-93.

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Francesca M. Spagnoli

Molecular and Cellular Basis of Embryonic Development

Understanding how distinct cell types arise from common multipotent progenitor cells is a major quest in developmental biology.

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 central aim of our research is to understand the mechanisms that pattern and establish competence within the embryonic endoderm in order to progressively specify the pancreatic organ domain. In particular, we focus on the spatio-temporal mechanisms that restrict specification of the pancreas versus neighboring tissues, such as the liver. Additionally, we are interested in the interplay between tissue-architecture and cell fate specification in the developing pancreas. A comprehensive analysis of pancreas formation will serve as a paradigm for understanding fundamental mechanisms of organogenesis. In addition, the knowledge of these key developmental steps will be fundamental for advances in therapeutic approaches for incurable diseases, which target the pancreas, such as diabetes.

In vivo lineage analysis of pancreatic and hepatic precursor cells

Elisa Rodríguez Seguel, Francesco Boccellato, Heike Naumann

During embryogenesis, the pancreas originates from distinct outgrowths of the dorsal and ventral foregut endoderm. Both outgrowths give rise to endocrine and exocrine cells and, subsequently, fuse to form one single organ. The ventral pancreas arises next to the hepatic endoderm and they possibly originate from a common bipotent precursor. However, a single cell having the dual potential to differentiate along the hepatic and pancreatic lineages has never been isolated neither *in vivo* nor *in vitro*. One main focus of my laboratory is to investigate how pancreatic versus hepatic fate decision occurs in the endoderm at both the cellular and molecular level.

To conduct a comprehensive *in vivo* analysis of the hepatic-pancreatic lineage in the mouse embryo, we use transgenic reporter models that express EGFP or photoconvertible fluorescent proteins under the control of lineage-specific promoters. We are using these new genetic tools to: i. address *in vivo* and *in vitro* if the liver and pancreas arise from a common bipotent precursor; and ii. trace and molecularly profile the presumptive precursor cell and its descendants in the mouse embryo. All together, these experiments will determine how the hepatic-pancreatic lineage is established *in vivo,* whether a bipotent endodermal precursor exists, and provide us with its molecular signature.

Molecular mechanisms controlling pancreas versus liver fate decision

Nuria Cerdá-Esteban, Julia Kofent, Igor Pongrac

Hepatic and pancreatic endoderm share a common set of intrinsic regulatory factors, such as the FoxA and GATA transcription factors, and are exposed to the same extrinsic signals, FGF and BMP. However, it remains unclear how the same factors can activate pancreatic genes, such as Pdx1 and Ptf1a, in the future pancreas, without inducing them in hepatic progenitors; and analogously, they enable liver development, but not pancreatic development in the hepatic endoderm. Both intrinsic and extrinsic regulators of this cell fate decision are under study in my laboratory.

How and when extrinsic factors, such as BMPs, control the emergence of liver versus pancreas from the same

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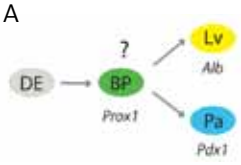
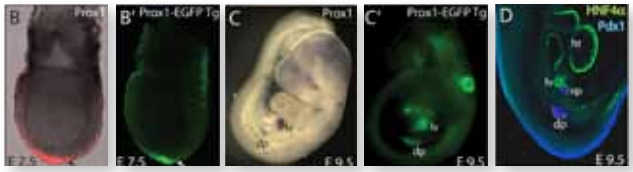


Stepwise specification of pancreatic progenitor cells within the endoderm. **(A)** Schematic model of the emergence of the pancreatic and hepatic lineage from a common endodermal precursor. **(B-C)** Prox1-EGFP transgenic (Prox1-EGFP Tg) mouse strain reproduces the endogenous pattern of Prox1 expression in the mouse embryos between embryonic stage (E) 7.5 (B, B') and 9.5 (C, C'). Arrows indicate Prox1 expression in the anterior definitive endoderm (B, B') at E7.5. **(D)** Whole-mount immunofluorescence for Pdx1, marking both dorsal and pancreatic buds, and HNF4α in the hepatic bud at E9.5. Abbreviations: Alb, albumin; BP, bipotent precursor; DE, definitive endoderm; Lv, liver; Pa, pancreas; vp, ventral pancreas; dp, dorsal pancreas; ht, heart.



embryonic region? To address these open questions, we have developed genetic approaches to perturb the signaling *in vivo* in a controlled temporal and spatial fashion. These experiments will elucidate: if BMP exerts a role *in vivo* in hepatic versus pancreatic fate decision in mammals; the window of time during which the endoderm is competent to respond to BMP; and different potential functions of BMP in the endoderm according to the embryonic stages.

Our previous studies have elucidated the molecular events downstream of the GATA factors within the anterior endoderm in *Xenopus laevis*. We identified the TALE homeodomain protein, TG-Interacting Factor 2 (TGIF2), as a downstream target of GATA factors and developmental regulator of the pancreatic versus hepatic fate decision in the endoderm. Interestingly, TGIF2 promotes pancreatic fate within the endoderm at the expense of hepatic markers. We are currently investigating whether TGIF2 is sufficient to convert liver into pancreas upon over-expression in *ex-vivo* models, such as embryonic and adult liver cells. This gene candidate study will provide insights into intrinsic regulators of the pancreatic versus hepatic decision and how a same factor can activate one fate and repress the other.

In addition to TGIF2, we have defined a suite of novel GATA putative targets involved in the formation of pancreas and/or liver. Among those, we are currently pursuing functional studies on: i. antagonists of the BMP signaling pathway, as cell-autonomous mechanism protecting the pancreatic territory from BMP; and ii. epigenetic modulators, as potential dynamic regulators of cell identity.

All together, these experiments will provide insight into the mechanisms that restrict cell fate within the anterior endoderm and define developmental regulators that are able not only to specify one fate but also antagonize the other (eg. pancreatic versus hepatic). This knowledge will be crucial for establishing lineage-reprogramming strategies of liver to pancreas toward a new cure for diabetes.

Control of morphogenesis and tissue-architecture in the developing pancreas

Kristin Petzold, Heike Naumann

In another line of research, we are investigating the mechanisms that control pancreas morphogenesis during embryonic development. The development of functional organ architectures relies on coordinated morphogenesis and growth. In the developing pancreas, the branching epithelium is organized in discrete domains, including a distal tip, which is the reservoir of multipotent progenitors, and a trunk domain of differentiated cells. How branching occurs and is coordinated with progenitor proliferation in the pancreas is largely unknown.

We have started to shed light on these questions, by investigating the role of Rho GTPase and its regulator the RhoGAP-domain-containing protein, Stard13. We found that Stard13 transcript is expressed in the pancreatic endoderm and enriched at the distal tip when branching occurs in the mouse embryo. Conditional ablation of Stard13 expression in the developing pancreas disrupts epithelial morphogenesis and tip domain organization, resulting in hampered proliferation of tip progenitor cells and, subsequent, organ hypoplasia. Finally, we have shown that Stard13 integrates these events by exerting negative control over Rho signaling during pancreas development. Our findings not only identify a function of the RhoGAP Stard13 in conferring spatio-temporal regulation on the ubiquitous Rho GTPase within the pancreas, but also offer new insights into the mechanisms by which the pancreatic epithelium shapes itself to create a mature organ, linking Rho control of epithelial remodeling to pancreas organ size determination.

Selected Publications

Spagnoli*, FM, Rosa*, A, Brivanlou, AH. (2009). Mesendodermal lineage specification in embryonic stem cells is under the control of a conserved microRNA family. Developmental Cell 16, 517-27.*co-first authors

Spagnoli, FM and Brivanlou, AH. (2008). The Gata5 target, TGIF2, defines the pancreatic region by modulating BMP signals within the endoderm. Development 135, 451-461.

Spagnoli, FM. (2007). From endoderm to pancreas: a multistep journey. The CMLS Journal 64, 2378-2390.

Sapkota, G, Alarcón, G, Spagnoli, FM, Brivanlou, AH., Massagué J. (2007). Balancing BMP signaling through integrated inputs into the Smad1 linker. Molecular Cell. 25, 441–454.

Spagnoli, FM and Brivanlou, AH. (2006). The RNA Binding Protein, Vg1RBP, is Required for Pancreatic Fate Specification. Developmental Biology. 292, 442-456.

Structure of the Group

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Kristin Petzold
Igor Pongrac

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Heike Naumann

*part of the period reported

Kai M. Schmidt-Ott

Emmy Noether Research Group

Developmental Biology and Pathophysiology of the Kidney

The kidney is a central organ in cardiovascular diseases. It excretes toxins into the urine, regulates volume and solute homeostasis in the body, and produces hormones. The kidney is not only itself a target in cardiovascular disease and but also is centrally involved in cardiovascular homeostasis. Moreover, kidney failure constitutes one of the most important risk factors for other cardiovascular diseases. The kidney is composed of structural units called nephrons, which consist of several different types of renal epithelial cells that facilitate directional transport. Our group studies the molecular mechanisms of epithelial morphogenesis and the maintenance of epithelial integrity later in life. We focus on transcription factors and their regulation of aspects of epithelial differentiation. We employ a wide spectrum of approaches, including genetic and experimental animal models of kidney disease, molecular and developmental biology techniques, and systems biology.

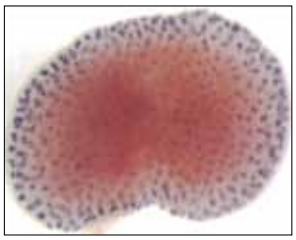
Introduction

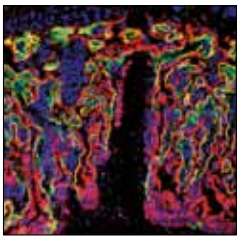
In the mammalian embryo, formation of the definitive kidney is initiated during mid-embryogenesis, when the ureteric bud, an epithelial tubule extending from the posterior Wolffian duct, interacts with an adjacent progenitor cell population, the metanephric mesenchyme. The Wolffian duct undergoes branching morphogenesis to give rise to the ureter, renal pelvis and collecting duct system, while the metanephric mesenchyme converts into epithelial cells that subsequently get patterned along the proximal-distal axis to form the different cell types of the nephron. As these cells differentiate, they obtain epithelial characteristics, including the establishment of apico-basal polarity and the formation of epithelial-specific junctions. Our laboratory investigates the molecular mechanisms underlying these events, which are intriguing for several reasons.

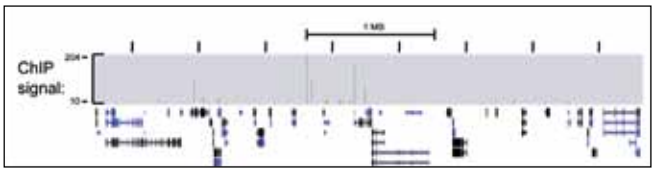
First, mammalian embryonic kidney development constitutes a classical model system in developmental biology, in which branching morphogenesis and tubulogenesis occur in parallel. The sequence of events can be closely monitored in organ cultures, in which key in vivo aspects of nephrogenesis are recapitulated. Exogenous or genetic perturbations of kidney development result in congenital kidney diseases. Furthermore, adult kidney epithelia preserve the ability to reactivate molecular pathways from earlier developmental stages in certain disease states, including tumors, kidney fibrosis, and acute tubular injury.

WNT Signaling in Kidney Development and Disease

Wnt proteins are a family of secreted molecules that are important for various aspect of embryonic development. Several WNT molecules, including Wnt4, Wnt7b, and Wnt9b, are centrally involved in kidney development. We previously found that Wnt signaling via the β-catenin/TCF/Lef pathway mediates aspects of epithelial differentiation in metanephric mesenchymal progenitor cells. In addition, this pathway provides proliferative and antiapoptotic signals that regulate the size of the progenitor pool. As part of a DFG funded project (Schm 1730/2-1) we characterize the target gene program of TCF/Lef signaling in the kidney. One crucial aspect of our future studies will be to elucidate the role of Wnt signaling in kidney disease, with an emphasis on

A 

B 

C 

Epithelial morphogenesis, differentiation and disease are studied in model systems such as the kidney (**A**) and the placenta (**B**). Systems biology approaches are used to gain global insight into molecular mechanisms. **C** shows an example of an experiment to detect genome-wide binding of a transcription factor to DNA as determined by chromatin immunoprecipitation followed by next generation sequencing. Binding intensity (green) is shown relative to the genomic position. Genes (blue) are shown on the bottom.

the canonical TCF/Lef-dependent pathway. For this purpose we are using different genetic and experimental mouse models, which we analyze using genome-wide analysis of expression and transcription factor binding. We are working in close collaboration with Thomas Willnow (MDC), Friedrich C. Luft (ECRC), and Jonathan Barasch (Columbia University, New York). We believe that a detailed elucidation of the transcriptional network controlled by TCF/Lef will yield fundamental insights into growth, remodeling, and regeneration in the kidney.

Grainyhead-type Transcription Factors in Epithelial Development and Homeostasis

While TCF/Lef-dependent signaling may account for early developmental programs in renal epithelial progenitors, it may in fact be inhibitory to terminal differentiation of tubular epithelia. Therefore, we are seeking complementary transcriptional regulators that induce the establishment of epithelial polarity and the expression of segment-specific markers. Using expression profiling, we identified two candidate transcriptional regulators of terminal differentiation, which belong to the CP2 group of transcription factors (Tcfcpl1 and Grhl2), which are highly and specifically expressed in the distal nephron. Characterization of Grhl2 is part of a DFG-funded project (Schm 1730/3-1) within FG "Epithelial mechanisms of renal volume regulation" (Speaker: Sebastian Bachmann, Charité Berlin). The projects benefit from our close collaborations with Jonathan Barasch (Columbia University, New York), Walter Birchmeier (MDC), Carmen Birchmeier (MDC), Thomas Willnow (MDC), and Michael Bader (MDC).

Molecular Diagnosis of Renal Injury

Re-expression of embryonic marker molecules is a common feature in disease states and is believed to participate in compensation and regeneration. Neutrophil gelatinase-associated lipocalin (NGAL) is a protein in the developing kidney that is sufficient to induce differentiation in embryonic renal epithelial progenitors. NGAL is also markedly reactivated in tubular injury of the kidney and its urinary excretion is closely correlated with the temporal onset and severity of tubular injury. In collaboration with Jonathan Barasch (Columbia University, New York), Friedrich C. Luft (ECRC), Ralph Kettritz (ECRC), we are conducting basic and clinical studies to study the biology and clinical applications of NGAL in kidney injury. We are aiming to optimize diagnostic algorithms that utilize NGAL measurements 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.

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

Selected Publications

Nickolas TL, Schmidt-Ott KM*, Canetta P, Forster C, Singer E, Sise M, Elger A, Maarouf O, Sola-Del Valle DA, O'Rourke M, Sherman E, Lee P, Geara A, Imus P, Guddati A, Polland A, Rahman W, Elitok S, Malik N, Giglio J, El-Sayegh S, Devarajan P, Hebbar S, Saggi SJ, Hahn B, Kettritz R, Luft FC, Barasch J. Diagnostic and Prognostic Stratification in the Emergency Department Using Urinary Biomarkers of Nephron Damage – A Multicenter Prospective Cohort Study. Journal of the American College of Cardiology. 2012. * Co-First und -Corresponding author.

Singer E, Elger A, Elitok S, Kettritz R, Nickolas TL, Barasch J, Luft FC, Schmidt-Ott KM. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. Kidney Int. 2011; 80(4):405-14.

Werth, M., Walentin, K., Aue, A., Schönheit, J., Wuebken, A., Pode-Shakked, N., Vilianovitch, L., Erdmann, B., Dekel, B., Bader, M., Barasch, J., Rosenbauer, F., Luft, F.C., and Schmidt-Ott, K.M. 2010. The transcription factor grainyhead-like 2 (Grhl2) regulates the molecular composition of the epithelial apical junctional complex. Development. 2010;137(22):3835-45.

Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, Viltard M, Yu W, Forster CS, Gong G, Liu Y, Kulkarni R, Mori K, Kalandadze A, Ratner AJ, Devarajan P, Landry DW, D'Agati V, Lin CS, Barasch J. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat Med. 2011;17(2):216-22.

Schmidt-Ott K.M., Masckauchan T.N., Chen X., Hirsh B.J., Sarkar A., Yang J., Paragas N., Wallace V.A., Dufort D., Pavlidis P., Jagla B., Kitajewski J., Barasch J. (2007) β-Catenin/TCF/Lef Controls A Differentiation-Associated Transcriptional Program In Renal Epithelial Progenitors. Development. 134(17):3177-90.

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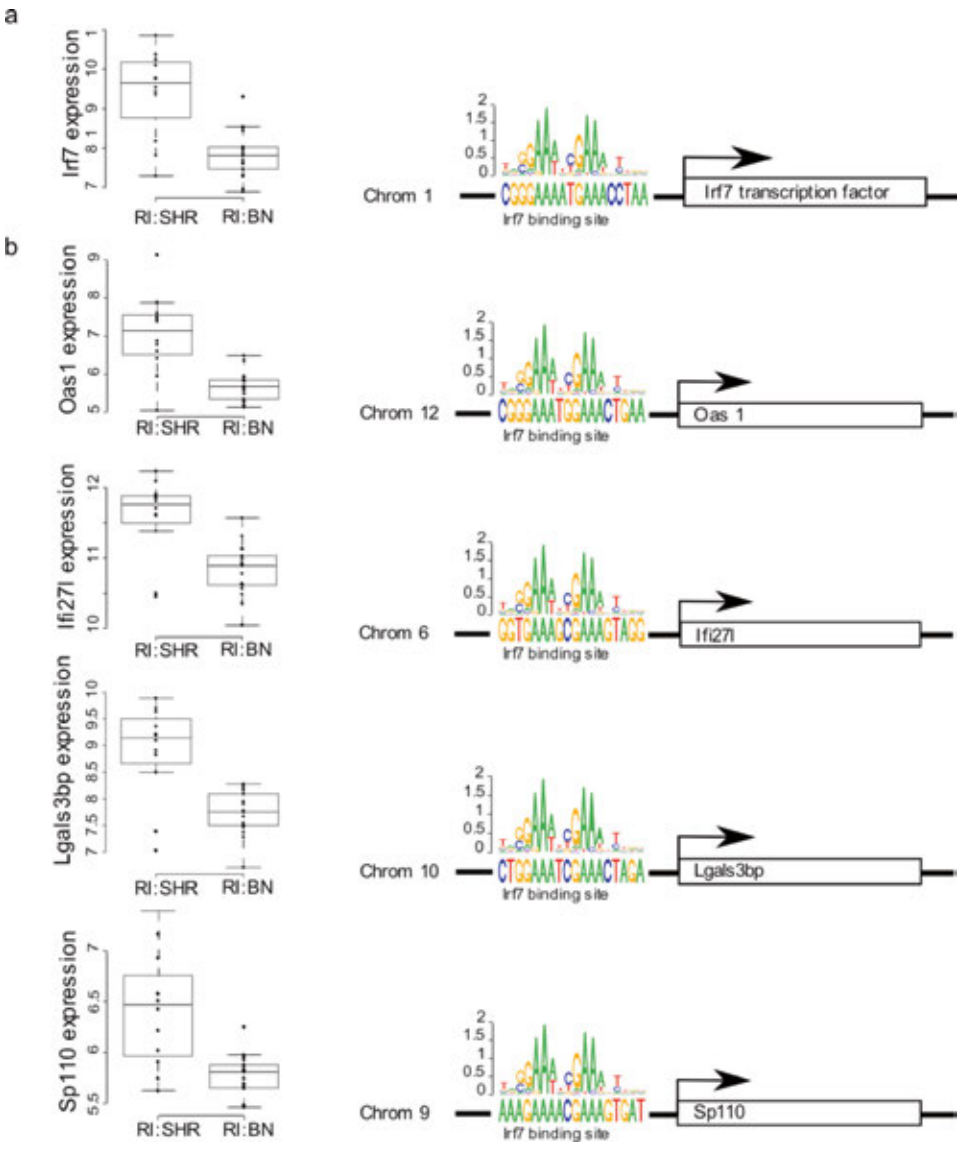
Medical Genomics and Genetics of Cardiovascular and Metabolic Diseases

Many genetic studies in model organisms and humans, including human genome-wide association studies, have pinpointed genomic regions that contribute to susceptibility to common disease. However, to date, these data have provided limited insights into the genes, molecular pathways and mechanisms underlying disease pathophysiology. Our laboratory uses state-of-the-art and emerging large-scale technologies and advanced computation in an expanded multi-disciplinary approach to identify gene networks and genomic mechanisms underlying common cardiovascular and metabolic diseases. We have a particular interest in inflammatory and metabolic genetic risk factors that lead to cardio- and cerebrovascular target organ damage and cardiomyopathies. We propose to use primarily the rat as model organism to identify the major gene pathways for human cardiovascular and metabolic disorders.

We carried out systems approaches that are based on the genetic regulation of gene expression in rat models of the cardiometabolic syndrome and generated the data required at the appropriate scale. In the past, we successfully mapped genetic determinants of gene expression (eQTLs) in left ventricle, skeletal muscle, fat, kidney, and arterial vessels in a panel of rat recombinant inbred (RI) strains. The integrated use of genetic mapping, gene expression, and computational analysis enabled us to identify Ephx2 as a candidate gene for heart failure, Ogn for cardiac hypertrophy, and CD36 for hypertension. It is our goal to reconstruct biological networks underlying complex human cardiovascular and metabolic disorders. In order to model the complexity of comprehensive strain- and tissue specific data, we construct co-expression networks and determine the genomic loci underlying their regulation.

Decoding the genome of the spontaneously hypertensive rat

The spontaneously hypertensive rat (SHR) is the most widely studied animal model of hypertension. Scores of SHR quantitative loci (QTLs) have been mapped for hypertension and metabolic phenotypes. Our collaborators and we have sequenced the SHR genome at more than 20-fold coverage by paired-end sequencing on the Illumina platform. We identified more than 3.6 million high-quality single nucleotide polymorphisms (SNPs) between the SHR and Brown Norway (BN) reference genome, with a high rate of validation. We also identified more than 300,000 short indels between the SHR and reference genomes. These SNPs and indels resulted in several loss of stop codons and frameshifts compared



Analysis of the role of transcription factors (TF) as mediators of genetic perturbations underlying trans-eQTLs identified EBI2 on chromosome 15 that controlled expression of the transcription factor IRF7. Predicted IRF7 targets were enriched in the set of genes that were differentially expressed with respect to the EBI2 geneotype on chromosome 15.

with the BN reference sequence. We also identified more than 10,000 larger deletions that result in complete or partial absence of some 100 genes in the SHR genome compared with the BN reference and more than 500 copy number variants (CNVs) that overlap with the gene regions of 688 genes. Genomic regions containing genes whose expression had been previously mapped as cis-regulated expression quantitative trait loci (eQTLs) were significantly enriched with SNPs, short indels, and larger deletions, suggesting that some of these variants have functional effects on gene expression. This near complete catalog of genomic differences between two extensively studied rat strains provides the starting point for complete elucidation, at the molecular level, of the physiological and pathophysiological phenotypic differences between individuals from these strains.

Gene networks and DNA sequence variation can provide insights into the aetiology of common diseases

We used integrated genome-wide approaches across seven tissues in a panel of rat recombinant inbred (RI) strains that are derived from SHR and BN rats to identify gene networks and the loci underlying their regulation. We constructed a co-expression network based on gene expression in left ventricle, skeletal muscle, fat, kidney, and arterial vessels previously generated by us and our collaborators, which will allow us to discriminate processes that are likely distinct with respect to the myocardium.

For the identification of gene expression networks we used a two-step procedure to integrate eQTL data of transcription factors (TFs) and TF-target genes to identify TF-driven gene networks. TFs act trough transcription factor binding sites (TFBSs) in promoters and enhancers of TF-target genes. We incorporate published data on transcription factors and their experimentally-proven binding sites provided by TRANSFAC. In the first step, we identified TFs with known TFBS models (defined by position weight matrices in TRANSFAC) whose expression mapped to eQTLs across tissues. TFs were mostly (> 90%), under trans-regulatory genetic control. In the second step, we tested for enrichement of predicted TFBSs of transcription factors in the putative promoter sequences of genes that mapped as trans-eQTLs. By the use of co-expression analysis combined with functional enrichment we were able to define an interferon regulatory factor 7 (IRF7)-driven inflammatory network (IDIN) enriched for viral response genes, which represents a molecular biomarker for macrophages and which was regulated in multiple tissues by a locus on rat chromosome 15q25. We subsequently showed that Epstein-Barr virus induced gene 2 (Ebi2, also known as Gpr183), which lies at this locus and controls B lymphocyte migration, is expressed in macrophages and regulates the IDIN. The human orthologous locus on chromosome 13q32 controlled the human equivalent of the IDIN, which was conserved in monocytes. IDIN genes were more likely to associate with susceptibility to type 1 diabetes (T1D) -a macrophage-associated autoimmune disease- than randomly selected immune response genes. The human locus controlling the IDIN was associated with the risk of T1D and was associated with EBI2 (GPR183) expression. These data implicate IRF7 network genes and their regulatory locus in the pathogenesis of T1D. Our data demonstrate that combined analyses of gene networks and DNA sequence variation can provide new insights into the aetiology of common diseases that may not be apparent from genome-wide association studies alone.

Establishment of a mouse model for cerebral ischemic small vessel disease

Cerebral ischemic small vessel disease (SVD) is the leading cause of vascular dementia and a major contributor to stroke in humans. Hypertension is a prominent risk factor for cerebral ischemic small vessel disease but also dominant mutations in NOTCH3 cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a genetic archetype of cerebral ischemic small vessel disease. Progress toward understanding the pathogenesis of this disease and developing effective therapies has been hampered by the lack of a good animal model. We have developed a mouse model for CADASIL via the introduction of a CADASIL-causing Notch3 point mutation into a large P1-derived artificial chromosome (PAC). In a collaboration with Anne Joutel (Paris) we show that in vivo expression of the mutated PAC transgene in the mouse reproduced the endogenous Notch3 expression pattern and main pathological features of CADASIL, including Notch3 extracellular domain aggregates and granular osmiophilic material (GOM) deposits in brain vessels, progressive white matter damage, and reduced cerebral blood flow. Mutant mice displayed attenuated myogenic responses and reduced caliber of brain arteries as well as impaired cerebrovascular autoregulation and functional hyperemia. Further, we identified a substantial reduction of white matter capillary density. These neuropathological changes occurred in the absence of either histologically detectable alterations in cerebral artery structure or blood-brain barrier breakdown. These studies provide in vivo evidence for cerebrovascular dysfunction and microcirculatory failure as key contributors to hypoperfusion and white matter damage in this genetic model of cerebral ischemic small vessel disease.

Selected Publications

McDermott-Roe C et al. Endonuclease G is a novel determinant of cardiac hypertrophy and mitochondrial function. Nature 2011 Oct 5;478(7367): 114-8. doi: 10.1038/nature10490.

Heinig M et al. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 23:460-4, 2010.

Joutel A et al. Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease. Journal of Clinical Investigation 120:433-45, 2010.

Monti J et al. Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. Nature Genetics 40:529-37, 2008.

Saar K et al. SNP and haplotype mapping for genetic analysis in the rat. Nature Genetics 40:560-6, 2008.

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Friedrich C. Luft

Hypertension, Genetics, Eicosanoids, and Cardiovascular Disease

Our goal is to reduce cardiovascular mortality; hypertension is the most important risk factor. We have several projects. First, we are pursuing the molecular genetics of hypertension and brachydactyly. This 15-year project has directed our attention to epigenetics. The brachydactyly phenotype has led us to elucidate cis- and trans-regulatory epigenetic phenomena. Simultaneously, our group actively contributes to the elucidation of the cytochrome P450 (CYP) pathway of eicosanoid formation that has been only recently discovered to play a pivotal role in the pathogenesis of hypertension and target organ damage. Our results offer novel therapeutic strategies based on the mechanisms of CYP-eicosanoid action for the prevention of acute kidney injury and cardiac arrhythmias. Finally, we are collaborating on measuring body sodium stores with a novel magnetic resonance technique.

Genetics

Affected individuals with autosomal-dominant hypertension with brachydactyly type E syndrome develop severe progressive hypertension and, if left untreated, develop stroke by age <50 years. In 1996 we described hypertension and brachydactyly and presented data on adults. We have since identified six families worldwide with this syndrome. We recently revisited our original family and performed further studies. We performed a genome-wide single-nucleotide polymorphism genotyping linkage analysis and confirmed our earlier linkage results. We accrued interesting ancillary data, particularly in the children that we have followed since birth. Substantial hypertension was already present in toddlers and blood pressure increased with age. Thus, blood pressure measurement, rather than brachydactyly, was the most reliable phenotype for the very early diagnosis in children. We have now completed deep sequencing of several families with this disease and are elucidating the rearrangements. The findings will give us insights into basic mechanisms.

A challenge is to understand the brachydactyly phenotype. We have focused on other families featuring brachydactyly type E (BDE) as an isolated phenotype. We studied two distinct families with a t(8;12)(q13;p11.2) and a t(4;12)(q13.2-13.3;p11.2) translocation, respectively. The breakpoints were upstream of the gene encoding parathyroid hormone-like peptide (*PTHLH*) on chromosome 12p11.2. We differentiated fibroblasts from brachydactyly patients into chondrogenic cells and found that *PTHLH* and its targets, *ADAMTS-7* and *ADAMTS-12* were downregulated. These results were the first showing a *cis*-directed breakpoint-dependent *PTHLH* downregulation as primary cause of human chondrodysplasia in the first translocation family. We are now focusing on the second BDE family with the t(4;12) translocation (Fig.1 A-C). To identify the native *PTHLH cis*-regulatory elements, that were dislocated due to the translocation-mediated disruption of

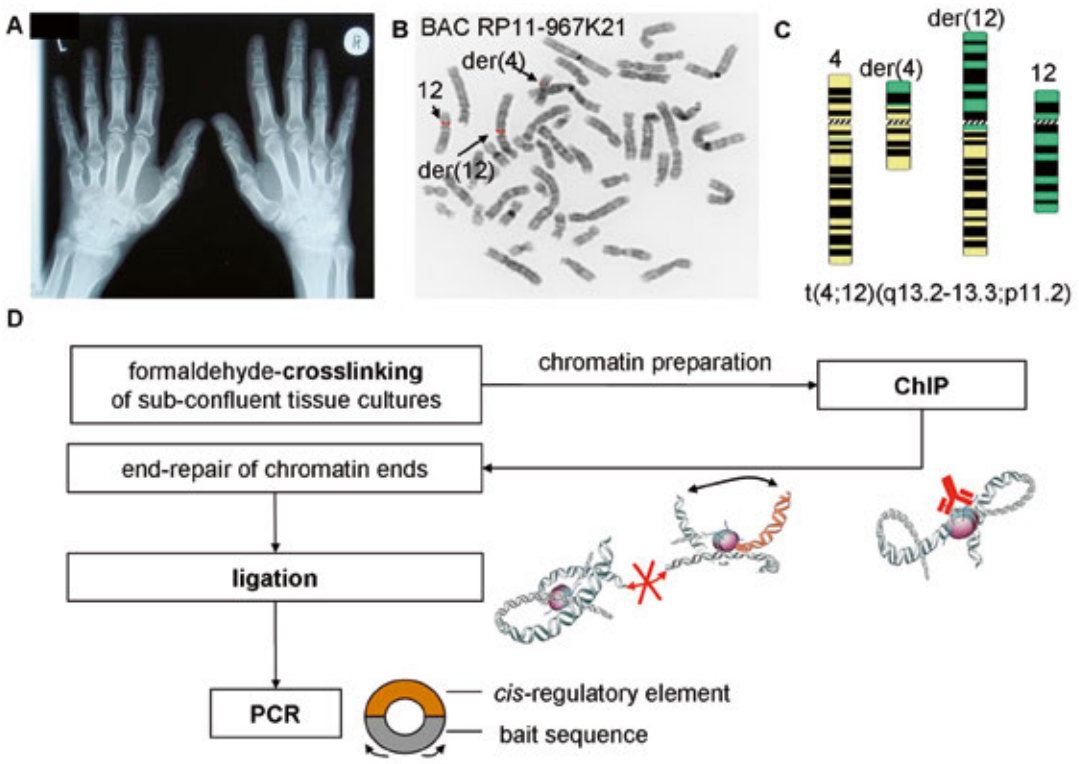

Figure 1. (A) Hands X-ray of one BDE affected subject carrying the translocation t(4;12)(q13.2-13.3;p11.2). The shortened metacarpals IV and V are obvious and define the brachydactyly type E phenotype. (B) FISH with BAC RP11-967K21 on metaphase chromosomes of t(4;12)(q13.2-13.3;p11.2) lymphocytes. Red spots represent hybridization signals of BAC RP11-967K21 of the wildtype chromosome 12 and the two derivative chromosomes der(4) and der(12), what provides the evidence that the BAC covers the breakpoint on chromosome 12. (C) t(4;12)(q13.2-13.3;p11.2) chromosome ideogram. (D) Flow chart of the C6 chromosome conformation capture technique

the chromosome 12 architecture, we designed a novel chromosome conformation capture technique, named 6C (Fig.1 D). *Cis*-regulation of gene expression is mainly done through chromatin loops during interphase. We use human chondrocytes fragmented chromatin and perform chromatin immuno-precipitation (ChIP) with different antibodies against histone modifications. After the circularization of the chromatin ends we use the *PTHLH* promoter sequence as bait. Inverse-orientated oligos PCR-amplify any *cis*-regulatory sequence within the circular chromatin. The results will be not only relevant for the autosomal-dominant brachydactyly and hypertension rearrangement syndrome, but also will have more general regulatory implications in chondrogenesis.

Eicosanoids

Eicosanoids are signaling molecules made by oxygenation of arachidonic acid (AA) and other essential omega-6 and omega-3 polyunsaturated fatty acids (n-6 and n-3 PUFAs). They exert complex control over many bodily systems, mainly in inflammation or immunity, and as second messengers of diverse growth factors and hormones throughout the cardiovascular system. Classical eicosanoids such as prostaglandins, thromboxanes and leukotrienes are produced via cyclooxygenase- or lipoxygenase-initiated pathways. As exemplified by drugs like aspirin, iloprost, and montelukast, these pathways are already clinically targeted to treat fever, pain, asthma, and cardiovascular disease. Our research interest has been focused on the so-called third and most recently discovered branch of eicosanoid formation that is catalyzed by cytochrome P450 (CYP) enzymes. CYP enzymes function as hydroxylases or epoxygenases and convert n-6 and n-3 PUFAs to unique sets of biologically active hydroxy- and epoxy-metabolites, collectively termed CYP-eicosanoids. Prominent examples include 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs). Others and we demonstrated that hypertension and target organ damage are associated with an imbalance of 20-HETE/EET formation. 20-HETE mediates vasoconstriction, inflammation and apoptosis, whereas EETs exert opposite beneficial effects. A major challenge for current research is to identify the initiating steps in CYP-eicosanoid signaling that may serve as novel targets for the treatment of cardiovascular disease. Promising application fields may include ischemic organ damage and cardiac arrhythmias as described in detail below. We collaborate with John R. Falck

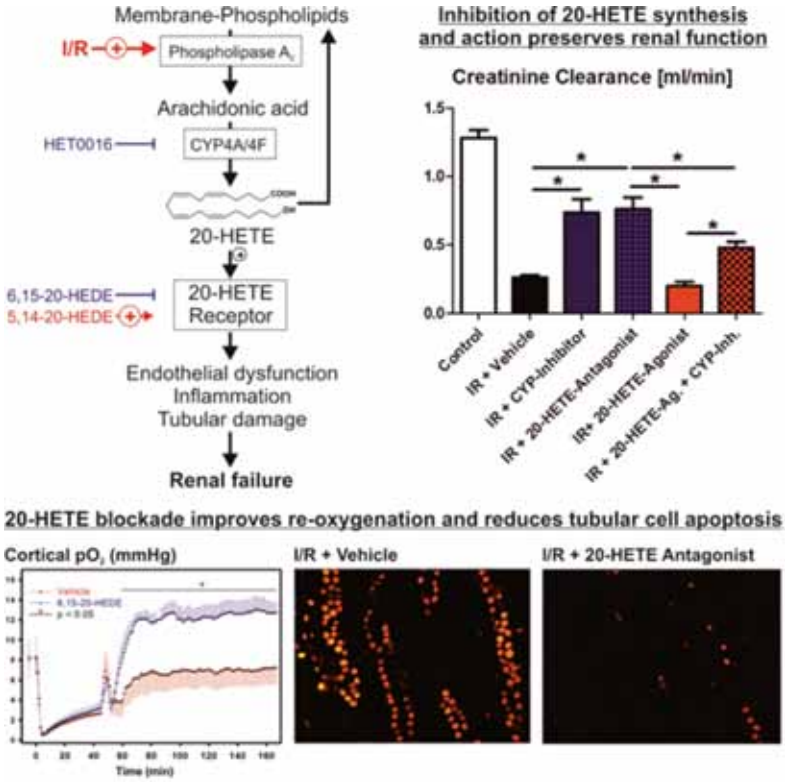

Figure 2. (Upper left panel): Ischemia activates phospholipases A_2 (PLA_2) that release arachidonic acid and preformed 20-HETE from membrane stores leading to accumulation of free 20-HETE in the ischemic tissue. 20-HETE activates pro-inflammatory and pro-apoptotic pathways in vascular endothelial and tubular epithelial cells, likely via a thus far unidentified 20-HETE receptor. (Upper right panel): Ischemia/reperfusion (I/R) caused loss of renal function as reflected by decreased creatinine clearance in a rat model of acute kidney injury. Pretreatment with a selective inhibitor of CYP4A/4F enzymes (HET0016) or a 20-HETE antagonist (6,15-20-HEDE) protected against I/R-induced functional decline. A 20-HETE agonist (5,14-20-HEDE) reversed the beneficial effect of the CYP4A/4F inhibitor. (Lower panel): Ischemia (from 0-40 min) resulted in a severe reduction of cortical (and medullary) oxygenation as measured with microsensors. Reoxygenation was largely improved in the early reperfusion phase after pretreating the animals with the 20-HETE antagonist compared to vehicle control. The 20-HETE antagonist also protected against apoptosis of tubular epithelial cells as revealed by a Tunel-assay detecting DNA fragmentation in the nuclei of apoptotic cells.

(UT Southwestern, Dallas) in developing CYP-eicosanoid derived pharmacological approaches for preventing these disease conditions.

Overproduction of 20-HETE is a common feature of ischemia-reperfusion injury of the heart, brain and kidney, clinically reflected as myocardial infarction, stroke and acute renal failure. In the kidney, 20-HETE is produced by CYP4A/4F isoforms both in preglomerular arterioles and proximal tubules. We used a rat model of acute kidney injury to test the hypothesis that 20-HETE may play a primary role in disease development. LC-MS/MS analysis revealed that ischemia indeed causes a rapid increase of renal 20-HETE levels. Next, we pretreated the animals before inducing renal ischemia with, either an inhibitor of 20-HETE synthesis, a 20-HETE antagonist, a 20-HETE agonist, or vehicle via the renal artery. Pretreatment with either the inhibitor or the antagonist protected from renal failure. The inhibitor and antagonist also markedly reduced tubular lesion scores, inflammatory cell infiltration, and tubular epithelial cell apoptosis. Administering the antagonist accelerated the recovery of renal perfusion, as well as renal medullary and cortical re-oxygenation, during the early reperfusion phase. In contrast, the 20-HETE agonist did not improve renal injury and reversed the beneficial effect of the inhibitor. Thus, 20-HETE generation and its action mediated kidney injury due to ischemia-reperfusion. This finding may offer novel therapeutic strategies for the prevention of acute kidney injury in clinical settings such as cardiovascular surgery and kidney transplantation. This part of the reported studies was performed in close collaboration with Duska Dragun (Charité, Berlin) and other members of the recently established DFG Forschergruppe 1368 (Hemodynamic mechanisms of acute kidney injury). A ProInno-project with Michael Rothe (Lipidomix GmbH, Berlin-Buch) helped us to establish an LC-MS/MS procedure for quantifying 20-HETE and the whole set of other CYP-eicosanoids in biological samples.

Dietary supplementation with the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) protects against cardiac arrhythmia and other cardiovascular diseases by unknown mechanisms. We tested the hypothesis that EPA and DHA may compete with

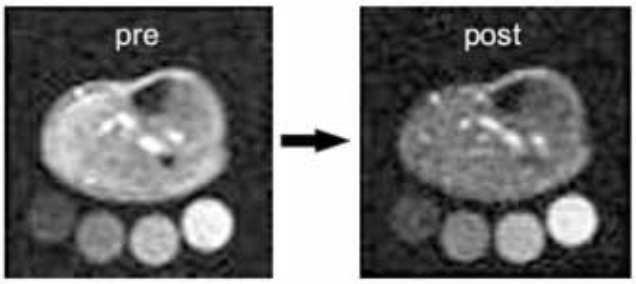

Figure 3. Shown is a 3.0T 23Na magnetic resonance image of a lower calf before and after aldosterone-producing adenoma removal. Intensity reflects tissue sodium content.

AA for the conversion by CYP enzymes, resulting in the formation of alternative, physiologically active, metabolites. CYP2C/2J isoforms converting AA to EETs preferentially epoxidized the ω-3 double bond and thereby produced 17,18-epoxyeicosatetraenoic (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP) from EPA and DHA. We found that these ω-3 epoxides are highly active as antiarrhythmic agents, suppressing the calcium-induced increased spontaneous beating rate of neonatal rat cardiomyocytes at low nanomolar concentrations. Rats given dietary EPA/DHA supplementation exhibited substantial replacement of AA by EPA and DHA in membrane phospholipids in plasma, heart, kidney, liver, lung, and pancreas, with less pronounced changes in the brain. The changes in fatty acids were accompanied by concomitant changes in endogenous CYP metabolite profiles. 17,18-EEQ and 19,20-EDP became the predominant CYP-epoxyeicosanoids in the heart and other tissues after EPA/DHA supplementation. Our results demonstrate that CYP enzymes efficiently convert EPA and DHA to novel epoxy and hydroxy metabolites that could mediate some of the beneficial cardiovascular effects of dietary ω-3 fatty acids. We are currently developing several of these metabolites for first-in-humans studies (see Wolf Schunck and PreGoBio report). Moreover, we are closely collaborating with Dominik Müller (MDC/ECRC), Robert Fischer (Charité, Berlin) and other members of the DFG Forschergruppe 1054 (Sex-specific mechanisms of myocardial hypertrophy) to further investigate the role of CYP-eicosanoids in cardiac hypertrophy and arrhythmia.

Collaboration

Friedrich Luft has a close collaboration between Jens Titze (University of Erlangen) and Thoralf Niendorf (MDC-ECRC). The project involves sodium (Na^+) storage and disposition in hypertensive patients. We have developed a method to measure sodium with magnetic resonance (MR). We used 3Tesla (T), 7T, and 9.4T technology to quantify Na^+ content in skin and skeletal muscle with $^{23}Na^+$ magnetic resonance ($^{23}Na^+$ MRI). We compared $^{23}Na^+$ MRI data with actual tissue Na^+ content measured by chemical analysis in animal and human tissue. We then non-invasively quantified tissue Na+ content in patients with aldosteronism and in patients with refractory hypertension, compared to controls. Skin and muscle Na^+ content with $^{23}Na^+$ MRI showed a high intra-method precision and was closely related to Na^+ measurements by chemical analysis. We found a 29% increase in muscle Na+ content in patients with aldosteronism. Muscle Na^+ excess was mobilized after successful treatment without accompanying weight loss. We conclude that $^{23}Na^+$ MRI allows quantification of hidden Na^+ stores in humans, which otherwise escapes clinical notice. We suggest that this tool will facilitate understanding the relationships between Na^+ accumulation, Na^+ distribution, hypertension, and edema.

Selected Publications

Toka, O., Maass, P.G., Aydin, A., Toka, H., Hübner, N., Rüschendorf, F., Gong, M., Luft, F.C., Bähring, S. 2010. Childhood hypertension in autosomal-dominant hypertension with brachydactyly. Hypertension. 56, 988-94.

Maass, P.G., Wirth, J., Aydin, A., Rump, A., Stricker, S., Tinschert, S., Otero, M., Tsuchimochi, K., Goldring, M.B., Luft, F.C., Bähring, S. 2010. A cis-regulatory site downregulates PTHLH in translocation t(8;12)(q13;p11.2) and leads to Brachydactyly Type E. Hum Mol Genet. 19, 848-60.

Hoff, U., Lukitsch, I., Chaykovska, L., Ladwig, M., Arnold, C., Manthati, V.L., Fuller, T.F., Schneider, W., Gollasch, M., Muller, D.N., Flemming, B., Seeliger, E., Luft, F.C., Falck, J.R., Dragun, D., Schunck, W.H. 2011. Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury. Kidney Int. 79, 57-65.

Arnold, C., Markovic, M., Blossey, K., Wallukat, G., Fischer, R., Dechend, R., Konkel, A., von Schacky, C., Luft, F.C., Muller, D.N., Rothe, M., Schunck, W.H. 2010. Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of {omega}-3 fatty acids. J Biol Chem. 285, 32720-33.

Kopp, C., Linz, P., Wachsmuth, L., Dahlmann, A., Horbach, T., Schöfl, C., Niendorf, T., Müller, D.N., Neininger, M., Eckardt, K-U., Schmieder, R.E., Luft, F.C., Uder, M., Titze, J. 2012. ^{23}Na+ magnetic resonance imaging to approach disorders of internal Na+ balance. Hypertension (in press)

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Cardiovascular Molecular Genetics

The foundation of a great number of adult heart diseases often lies in genetic alterations of distinct structural or functional pathways. The elucidation of specific lesions of the various myocardial defects such as inherited cardiomyopathies, is one focus of our laboratory. Correct fetal programming is known as a prerequisite for normal function in adulthood but little is known on how this is achieved at the molecular level. A second focus of our research is, therefore, the molecular dissection of adequate programming steps in the fetal heart. Interfering with cardiac mitochondrial function during development is our tool to shed light on this issue. Whether or not liver X receptor agonists are potential targets for the treatment of metabolic, inflammatory and cardiovascular diseases and negatively interfere with cytokine-induced nuclear receptor corepressor dissociation from the C-reactive protein promoter, thus maintaining this gene in a repressed state, is another subject of our interest.

Genetics of cardiomyopathies

Sabine Klaassen*, Arnd Heuser

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease predominantly affecting the right ventricle and a prevalent cause of ventricular arrhythmias and sudden death, especially in young adults. Genetically, heterozygous loss-of-function mutations in desmosomal proteins (desmocollin2, desmoglein2, plakoglobin, desmoplakin, and plakophilin2) have been mainly associated with ARVC. We have described mutations in plakophilin2 (PKP2) and desmocollin2 (DSC2) as a cause of autosomal dominant ARVC. PKP2 mutations account for a significant proportion of ARVC cases (10-45%). PKP2 and DSC2 are components of the desmosomal intercellular junction complex (see figure 1) known to be essential for maintaining tissue integrity and increasingly implicated in cell signalling. While the involvement of multiple desmosomal protein genes has led to speculation regarding the sensitivity of myocardium to mechanical disruption, the pathogenic mechanisms leading to ARVC in humans are largely unknown. We currently try to elucidate the genetic and molecular mechanisms of various human PKP2 mutations and their consequences in the pathology of the intercellular junction complex using cell culture experiments and transgenic mouse models. Furthermore, we investigate different knockout models of desmosomal components to define the adhesive and signaling contributions of thesel proteins in the maintenance of cardiac tissue.

Left ventricular noncompaction of the myocardium (LVNC) has recently been recognized as a distinct primary cardiomyopathy with a genetic etiology. LVNC is characterized by a unique congenital cardiac morphology, consisting of numerous, excessively prominent ventricular trabeculations and deep intertrabecular recesses. The heterogeneity of the clinical features includes

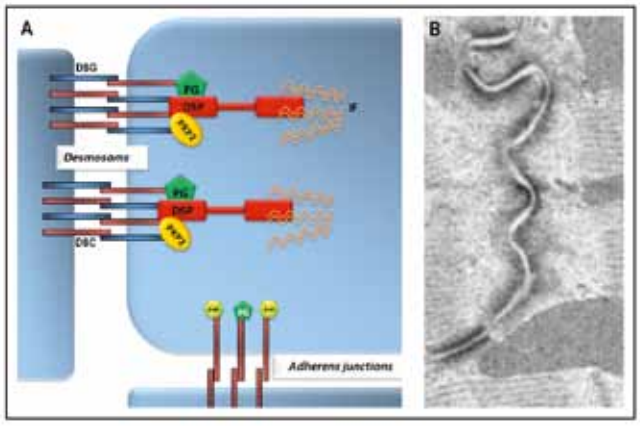

Figure 1. The structural organization of desmosomes. (A) The schematic drawing shows desmosomal cadherins (DSG, desmoglein; DSC, desmocollin); the armadillo-family members plakoglobin (PG) and the plakophilins (PKP); and the intermediate filament (IF)-binding protein desmoplakin (DSP). (B) Electron micrograph of desmosomes from murine heart.

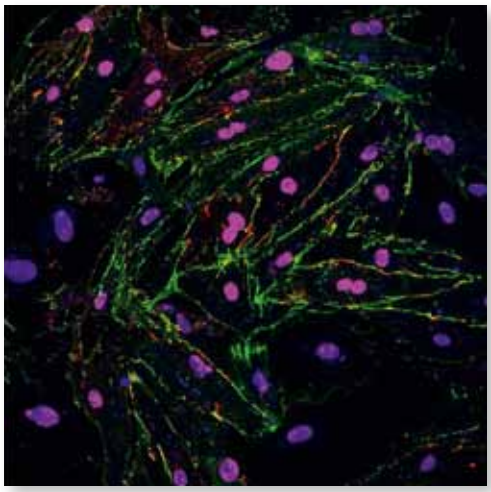

Figure 2. Immunolocalisation of desmosomal proteins Plakophilin2 (green) and Plakoglobin (red) in neonatal rat cardiomyocytes; Nkx2.5 (magenta).

progressive deterioration in cardiac function resulting in congestive heart failure, arrhythmias, thrombembolic events, and sudden cardiac death. The disorder is assumed to result from an intrauterine arrest in the process of compaction of the developing myocardium. We found mutations in genes encoding sarcomere proteins in a significant proportion of LVNC patients. Heterozygous mutations in genes encoding β-myosin heavy chain (*MYH7*), α-cardiac actin (*ACTC1*), cardiac troponin T (*TNNT2*),cardiac myosin-binding protein C (*MYBPC3*), and alpha-tropomyosin (*TPM1*) account for 29% of cases of isolated LVNC in adult patients. MYH7 mutations associated with Ebstein's anomaly and LVNC link structural proteins, cardiomyopathy, and congenital heart malformations.

Mechanisms and consequences of embryonic heart regeneration

Jörg Drenckhahn

The prenatal heart possesses an impressive growth plasticity in response to both endogenous as well as environmental or maternal conditions. In this regard, our recent findings have shown that the embryonic murine heart has a remarkable regenerative capacity. We have inactivated the X-linked gene encoding Holocytochrome c synthase (Hccs), an enzyme essential for normal function of the mitochondrial electron transport chain, specifically in the developing mouse heart. Loss of Hccs activity results in cellular energy starvation causing disturbed cardiomyocyte differentiation and ultimately cellular degeneration. In contrast to the observed mid-gestational lethality of hemizygous Hccs knockout (KO) males, heterozygous females appeared normal during postnatal life with surprisingly few clusters of defective myocardium, considering an expected mosaic of affected and normal cardiomyocytes as a result of random X chromosomal inactivation. However, analyses of heterozygous female (Hccs +/-) embryos revealed the expected 50:50 ratio of Hccs deficient to normal cardiac cells at mid-gestation with a progressive reduction in disease tissue to 10% prior to birth. We could show that this significant change is accounted for by increased proliferation of remaining healthy cardiac cells. These data reveal a previously unrecognised but impressive regenerative capacity of the mid-gestational heart that can compensate for an effective loss of at least 50% of cardiac tissue to enable formation of a functional heart at birth.

Yet despite this regeneration, hearts of neonatal Hccs +/- females are hypoplastic at birth evident as a reduction in heart weight, thinning of left ventricular (LV) walls and a significantly reduced number of cardiomyocytes compared to littermate controls. The reduction in LV mass, however, normalizes until adulthood and this could be attributed to an increase in cardiomyocyte size (hypertrophy) in Hccs +/- females (while prolifera-

tion rates are unaltered suggesting that cardiomyocyte number is not normalized). These data suggest the activation of compensatory cardiac growth mechanisms in the postnatal heart after disturbed heart development. Investigation of the underlying molecular mechanisms revealed alterations in the activation status of several metabolic regulators, such as mTOR and Ampk, as well as activation of ER (endoplasmic reticulum) stress and amino acid metabolism. Current projects intend to thoroughly characterize the metabolic profile of hypoplastic Hccs +/- hearts and clarify the role of certain key metabolic switches.

Unfavourable intrauterine growth conditions have been shown to predispose the heart for cardiac disease later in life, a process referred to as fetal programming. To study the consequences of embryonic heart regeneration for the adult heart we applied different cardiac stress models to Hccs +/- females. Although both Angiotensin II infusion as well as pressure overload did not affect cardiac function when compared to treated controls, they dramatically altered cardiomyocyte growth kinetics as well as molecular stress response (primarily affecting Jak/Stat3 as well as p38 MAP-Kinase signalling) in Hccs +/- hearts. This clearly confirmed that disturbing prenatal cardiac development renders the response of the postnatal heart to pathological conditions. Ongoing research is trying to address the functional relevance of various molecular pathways identified from the above experiments for sustaining normal cardiac function in the hypoplastic heart upon stress.

Finally, we aim to identify the mechanisms that drive regeneration of the embryonic heart in Hccs +/- females, focusing on both induction of proliferation in healthy cells as well as the fate of diseased (Hccs deficient) cells (see Figure 3). One of the most striking observations from these studies was the activation of a multitude of cell protective mechanisms specifically in embryonic Hccs deficient cardiomyocytes, including antioxidative defence, antiapoptotic and cell survival pathways and maintenance of protein homeostasis. To test whether this cardioprotective signalling in response to mitochondrial dysfunction is unique to the prenatal heart, we established an inducible, cardiomyocyte specific Hccs KO model in the adult heart. Strikingly, several cell protective mechanisms identified in embryonic cardiomyocytes cannot be activated in the adult heart upon Hccs deficiency. These data rise the interesting hypothesis that loss of embryonic cardioprotection contributes to disease susceptibility of the postnatal heart. Ongoing projects aim for the functional validation of the identified pathways for survival of embryonic versus adult cardiomyocytes.

Nuclear Receptors as Potential Target for the Treatment and Prevention of Metabolic, Inflammatory and Cardiovascular Disease

Florian Blaschke

Members of the nuclear receptor superfamily of ligand-dependent transcription factors play essential roles in

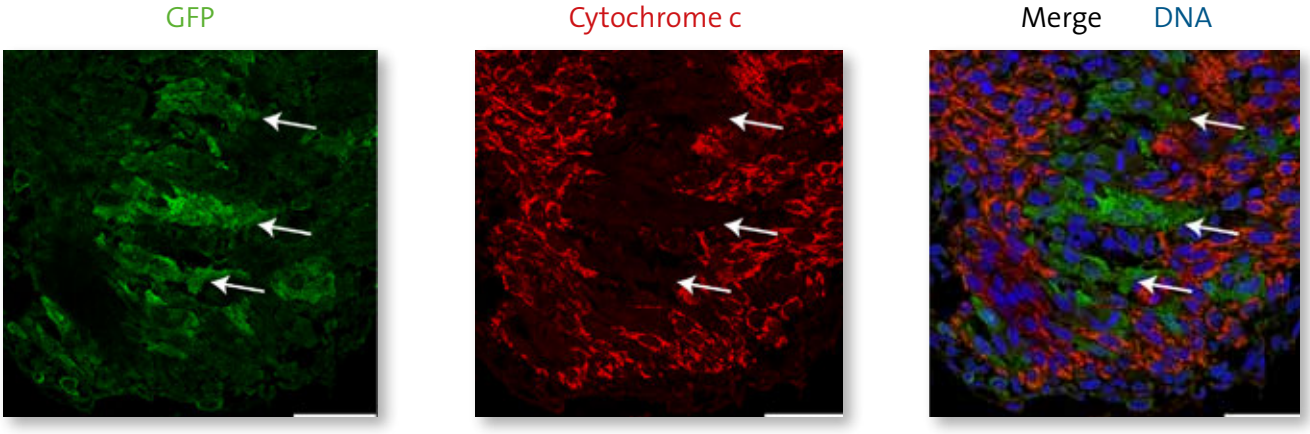

Figure 3. Immunofluorescence staining of an embryonic heart derived from a heterozygous Hccs knockout female (developmental stage 13.5 days post coitum). In these hearts Hccs deficient cardiomyocytes can be identified by the expression of the reporter gene GFP (green fluorescence protein, see arrows in left panel). As Hccs is essential for the assembly of the mitochondrial electron transporter cytochrome c, the latter is completely absent in Hccs deficient (GFP positive) cells (see arrows in middle and right panel) but highly abundant in the surrounding healthy cells (cytochrome c stained in red). (confocal laser scanning microscopy, images taken from the ventricular myocardium, blue nuclei in the right panel are stained with TO-PRO®-3, scalebar = 50 µm)

development, homeostasis, reproduction and immune function. Several members of this family, including the estrogen receptor (ER) and peroxisome proliferator-activated receptors (PPARs) are target of drugs that are used in a variety of clinical settings. The ability of nuclear receptors to switch from a transcriptional repressor to a transcriptional activator by binding of synthetic or natural ligands provided important insight into the mechanism(s) of gene regulation. Given their pleiotropic effects and their activation by specific ligands, new drugs targeting nuclear receptors are emerging as promising therapeutics for the treatment of cardiovascular, metabolic and inflammatory disease.

Activation and proliferation of vascular smooth muscle cells (VSMCs) are recognized to play a decisive role in vascular proliferative diseases such as primary atherosclerosis, postangioplasty restenosis, vein graft graft disease, and transplant vasculopathy. Due to the crucial role of VSMCs in the pathobiology of vascular occlusive disease, a pharmacologic blockade of VSMC activation and the cell cycle machinery is a promising therapeutic approach to prevent and treat vascular proliferative disease. Using both *in vitro* and *in vivo* approaches, we are investigating the role of various nuclear receptors and their activation by synthetic ligands in VSMC activation and cell cycle progression and are characterizing the molecular pathways utilized to regulate gene expression.

Cardiac hypertrophy leading to heart failure is a major cause of morbidity and mortality worldwide. The reasons why cardiac dilatation and failure eventually occur are unknown although alterations in energy status and metabolism are proposed to play an important role. We are elucidating the role of nuclear receptors in cardiac metabolism and hypertrophy both *in vitro* and *in vivo* and characterize the molecular mechanism(s) utilized to regulate gene expression involved in cardiomyocyte growth and energy metabolism.

Selected Publications

Blaschke F, Takata Y, Caglayan E, Collins A, Tontonoz P, Hsueh WA, Tangirala RK. (2006). A nuclear receptor corepressor-dependent pathway mediates suppression of cytokine induced C-reactive protein gene expression by liver X receptor. Circ Res. 99(12):e88-99

Klaassen S, Probst S, Oechslin E, Gerull B, Krings G, Schuler P, Greutmann M, Hürlimann D, Yegitbasi M, Pons L, Gramlich M, Drenckhahn JD, Heuser A, Berger F, Jenni R, Thierfelder L. (2008). Mutations in sarcomere protein genes in left ventricular noncompaction. Circulation.117(22):2893-901

Drenckhahn JD, Schwarz QP, Gray S, Laskowski A, Kiriazis H, Ming Z, Harvey RP, Du XJ, Thorburn DR, Cox TC. (2008). Compensatory growth of healthy cardiac cells in the presence of diseased cells restores tissue homeostasis during heart development. Dev Cell.15(4):521-33.

Postma AV, van Engelen K, van de Meerakker J, Rahman T, Probst S, Baars MJH, Bauer U, Pickardt T, Sperling SR, Berger F, Moorman AFM, Mulder BJM, Thierfelder L, Keavney B, Goodship J, Klaassen S (2011) Mutations in the sarcomere gene *MYH7* in Ebsteins anomaly. Circ Cardiovasc Genet 4: 43-50

Probst S, Oechslin E, Schuler P, Greutmann M, Boyé P, Knirsch W, Berger F, Thierfelder L, Jenni R, Klaassen S (2011) Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. Circ Cardiovasc Genet 4:367-374

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Thoralf Niendorf

Magnetic Resonance (MR)

The group's research concentrates on the development of MR-methodology and MR technology with a focus on new ways of mapping and probing morphology, function, physiology and metabolism together with explorations of the benefits and challenges of ultrahigh field (UHF) imaging to advance cardiovascular, neurovascular, molecular and other MRI applications. These efforts are designed to spatially resolve and characterize (patho)physiological processes and biophysical mechanisms to promote a transfer from basic research to (pre)clinical studies and vice versa. However, signal-to-noise ratio (SNR) and imaging speed have become an increasingly stringent limit in new MRI applications. Promising in this regard is the increase in magnetic field strengths available for both animal (9.4 T) and whole-body MR (7.0 T) scanners, though ultrahigh-field MRI has earned the moniker of being among the most challenging MR applications.

MR-Physics, MR Methodology, RF Coil Technology and Ancillary Hardware for Ultrahigh Field MR

The sensitivity advantage afforded by UHF-MR imaging is the driving force behind several technological developments. One ongoing development pioneered and driven by our group is a move towards multi-channel radiofrequency (RF) coil arrays. This includes the development and clinical evaluation of many element RF coil arrays designed for RF transmission (RX) and signal reception (TX). To this end, the group designed, implemented, evaluated and put to clinical use 4-channel, 8-channel and 16-channel TX/RX configurations (please see Figure 1a) tailored for cardiovascular MRI at 7.0 T. To stay at the forefront of research the group has recently proposed and implemented a 32 channel TX/RX coil array for cardiac MR at 7.0 T. The group's research activities aim to go beyond conventional proton imaging to foster explorations into imaging of sodium, flourine, and other nuclei to gain a better insight into metabolic and (nano) molecular processes. Consequently, various versions of ^{23}Na, ^{19}F, ^{1}H coil arrays – all designed for clinical applications or preclinical studies – were built and used to support national (for example: German Metrology Institute (PTB), Berlin, Germany; ECRC, Charite', Berlin, Germany, University of Erlangen, Erlangen, Germany) and international (for example: NIH, Bethesda, USA) collaborations. Since UHF-MR is still in its infancy the group also focuses on developing ancillary hardware with the ultimate goal to improve image quality for advanced diagnostics. This includes development and clinical evaluation of novel triggering devices/techniques for which members of our group have filed patent applications.

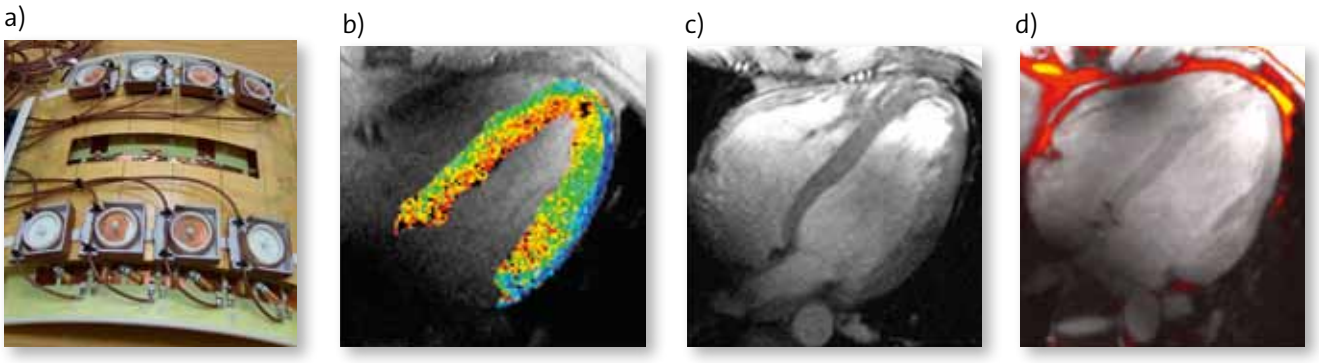

Figure 1. a) Photograph of the anterior section of the transceiver RF coil arry tailored for cardiac MR at 7.0 T. Four chamber views of the beating heart derived from **(b)** high spatial resolution imaging for cardiac chamber quantification, **(c)** parametric T2* mapping used for myocardial tissue characterization and **(d)** fat (red)/water imaging, all enabled by multi-channel transceiver RF-coil technology.

Since MRI accidents are listed as number 9 of the top 10 risks in modern medicine – risks which are even more pronounced at ultrahigh fields – the group is pro-active in reducing if not eliminating these risks by developing mobile warning devices.

Explorations into Cardiovascular Diseases Using UHF-MRI

The field of UHF cardiovascular MRI (CMR) is in the spotlight of the groups MR research. These developments are fueled by the signal-to-noise ratio advantage inherent to higher magnetic field strengths and are driven by explorations into novel MR technology. Such improvements would benefit an ever growing set of indications for CMR. Arguably, the potential of ultrahigh-field CMR is as yet untapped, and the advantages are sometimes offset by a number concomitant physics effects that bear the potential to spoil these benefits. However, emerging applications of UHF CMR – such as (i) high spatial resolution cardiac chamber assessment including morphology and quantification of cardiac function, (ii) myocardial tissue characterization using parametric mapping and (iii) fat/water separated imaging – have been developed by our group as illustrated in Figure 1b-d. This example shows high spatial resolution images of the beating heart derived from healthy subjects. All these efforts are designed to approach the ultimate goal of non-invasive assessment and early diagnosis of cardiac diseases including MR biomarker based myocardial tissue characterization and sub-millimeter spatial resolution imaging of the cardiac and vascular anatomy and function. For this purpose, careful assessment of RF safety of coronary stents at ultrahigh magnetic fields, development of enabling RF technology, implementation of spatially selective excitation techniques to facility zoomed-imaging of subtle cardiac anatomy and tiny vessels and push towards dedicated MR methodology are key objectives of the groups research program. To this end the group builds on strong collaboration with clinical partners (for example: Cardiac MR working group, ECRC, Berlin, Germany) to pursue joint ultrahigh field CMR efforts towards the development of novel imaging guided diagnostic and therapeutic approaches.

Explorations into Neurovascular Diseases Using UHF-MRI

The potential of higher magnetic field strengths for brain imaging in clinical practice and basic research has yet to be fully realized. Here, one important question is the choice of imaging techniques and imaging protocols for microstructural imaging of the brain. To explore this new territory our group has entered into a close collaboration with clinical partners for advanced imaging of neuroinflammatory diseases (Dept. of Neurology and Neurocure, Charite', Berlin) and for diagnostic stroke imaging (Center for Stroke Research (CSB), Berlin). These efforts are tailored to drive clinical applications through MR-physics developments. To this end, rapid spin echo based pulse sequences successfully developed by members of our group offer a distortion-free alternative for susceptibility weighted brain imaging. This technique helps to highlight small cerebral veins with high anatomical detail and can be put to use to gain a better understanding of the pathogenesis of multiple sclerosis by the assessment of perivascular vein density. The challenge to spin echo techniques is avoiding excessive RF

power deposition which will is a focus in the MR-physics research program.

Experimental Imaging and (Nano) Molecular Probing

The ultimate aim of the group is to harmonize research carried out in the area of preclinical animal imaging with that of clinical imaging. For the former the crux of the matter still is adequate characterization and phenotyping of animal models. Realizing this opportunity MRI of small rodents is conceptually appealing since it is non-destructive, provides superb spatial and temporal resolution, offers high reproducibility and is suitable for longitudinal studies. For all these reasons the group became a peer-reviewed member of the *Forschergruppe* FOR 1368 funded by the DFG to study renal hemodynamics and oxygenation by means of a new multimodality approach. At the first stage of the project the group focussed on the assessment of severity of acute kidney injury in an ischemia/reperfusion mouse model using T2 mapping including a comparison with Neutrophil Gelatinase Associated Lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM1). As part of the joints efforts with the ECRC's independent research group on *MR in Immunology* the team has made major progress to study brain inflammation during the pathogenesis of autoimmune encephalomyelitis using the benefits of cryogenically-cooled RF coils for MR microscopy to reveal pre-symptomatic cerebellar lesions and ventricle enlargement in an experimental autoimmune encephalomyelitis (EAE) mouse model. To examine the *in vivo* uptake of fluorine (^{19}F) nanoparticles by inflammatory cells during encephalomyleitis the group investigated the impact of the particle size on 19F-labeling and immunomodulation. The groups experimental cardiac MR research includes assessment of myocardial infarction and myocardial injury, explorations into genetically or pharmacologically induced hypertension, characterization of progression and regression of exercise or pressure overload induced myocardial hypertrophy and identification of sex specific effects of cardiac damage and cardiac (dys)function.

Selected Publications

Dieringer MA, Renz W, Lindel T, Seifert F, Frauenrath T, von Knobelsdorff-Brenkenhoff F, Waiczies H, Hoffmann W, Rieger J, Pfeiffer H, Ittermann B, Schulz-Menger J, Niendorf T. Design and application of a four-channel transmit/receive surface coil for functional cardiac imaging at 7T. J Magn Reson Imaging 2011;33(3):736-741.

Grassl A, Winter L, Thalhammer C, Renz W, Kellman P, Martin C, von Knobelsdorff-Brenkenhoff F, Tkachenko V, Schulz-Menger J, Niendorf T. Design, evaluation and application of an eight channel transmit/receive coil array for cardiac MRI at 7.0T. Eur J Radiol 2011.

Martin C, Frauenrath T, Ozerdem C, Renz W, Niendorf T. Development and evaluation of a small and mobile Magneto Alert Sensor (MALSE) to support safety requirements for magnetic resonance imaging. Eur Radiol 2011;21(10):2187-2192.

Knopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schöfl C, Renz W, Santoro D, Niendorf T, Müller D, Neininger M, Cavallaro A, Eckhardt KU, Schmieder RE, Luft FC, Uder M, Titze J. 23Na magnetic resonance imaging of tissue sodium Hypertension 2011;accepted for publication.

Waiczies H, Lepore S, Janitzek N, Hagen U, Seifert F, Ittermann B, Purfurst B, Pezzutto A, Paul F, Niendorf T, Waiczies S. Perfluorocarbon particle size influences magnetic resonance signal and immunological properties of dendritic cells. PLoS One 2011;6(7):e21981.

Patent applications

T. Niendorf, T. Frauenrath, W. Renz

Magnetic Field Apparatus and Method of Operating a Magnetic Field Apparatus

PCT/EP2011/052801

T. Niendorf, L. Winter

Magnetresonanztomographiesystem und Verfahren zur Untersuchung eines Körpers

EP 11155991.0

T. Niendorf, T. Frauenrath

Magnetic Resonance Tomography (MRT) Apparatus and Method of Operating a Magnetic Resonance (MR) Apparatus

EP 1115600.1

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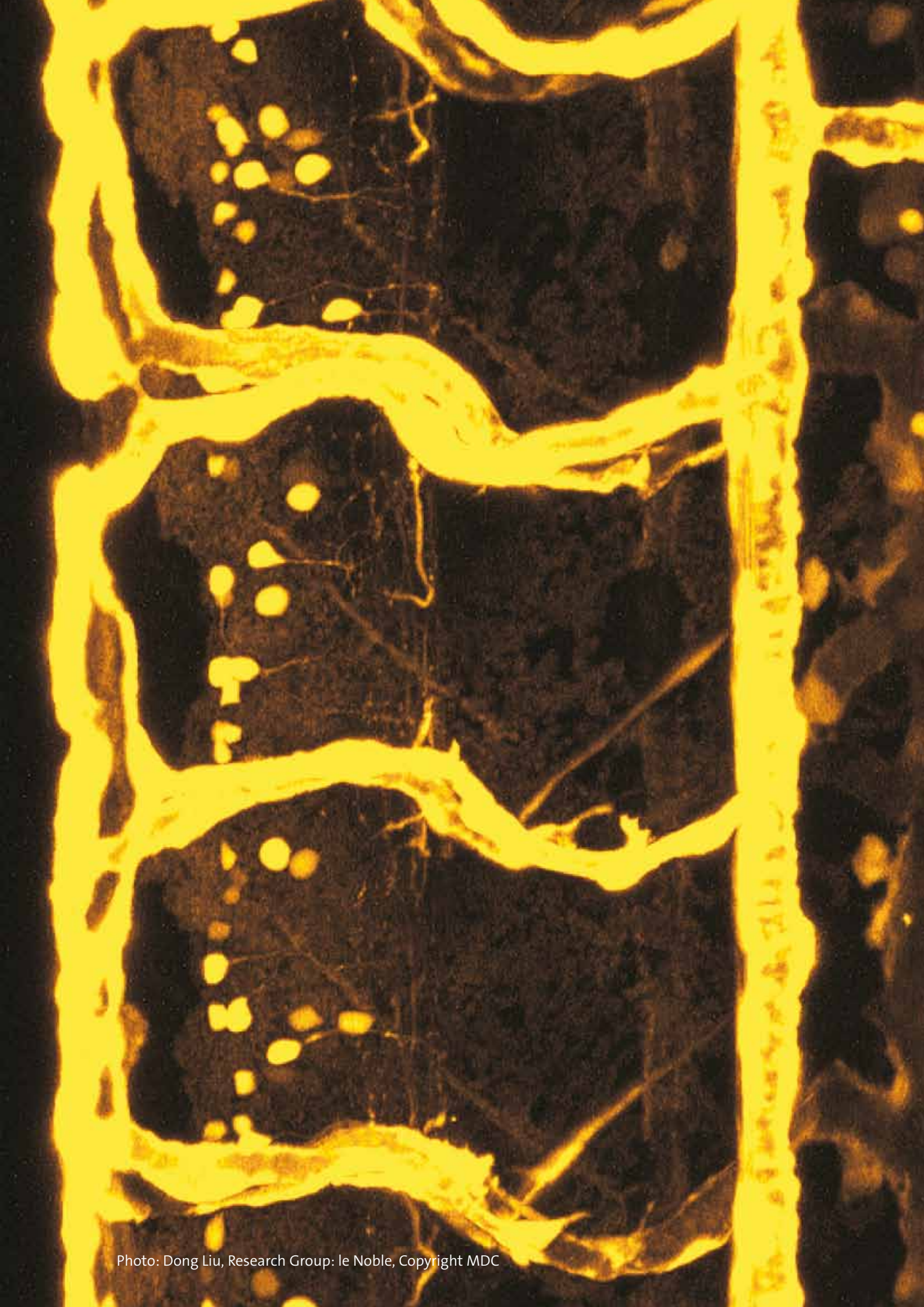

Photo: Dong Liu, Research Group: le Noble, Copyright MDC

Michael Bader

Cardiovascular 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, aiming to apply these fields to the rat.

Renin-angiotensin system

Natalia Alenina, Mihail Todiras, Luiza Rabelo*, Gabin Sihn, Anthony Rousselle, Laura de Souza

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 ACE2, the (pro)renin receptor, angiotensin(1-7) and its receptor Mas, have elucidated the physiological function of these molecules. Together with transgenic rats overexpressing ACE2 or angiotensin(1-7), Mas-knockout mice characterized the ACE2/angiotensin(1-7)/Mas system as a cardioprotective axis that counteracts the classical RAS effects in particular improving endothelial function. Furthermore, these animals showed that angiotensin(1-7) and Mas are important for insulin sensitivity and the pathogenesis of metabolic syndrome.

Kallikrein-kinin system and chemokines

Ines Schadock, Carlos Barros*, Alessander Guimaraes*, Fatimunnisa Qadri, Johan Duchene, Silke Mühlstedt

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 were generated and revealed important functions of this protein in pain perception and inflammation. Moreover, the B1 receptor turned out to be involved in arteriogenesis, sepsis, stroke, multiple sclerosis and high-fat diet induced obesity. Mice lacking both kinin receptors and thereby being devoid of a functional KKS were also generated and

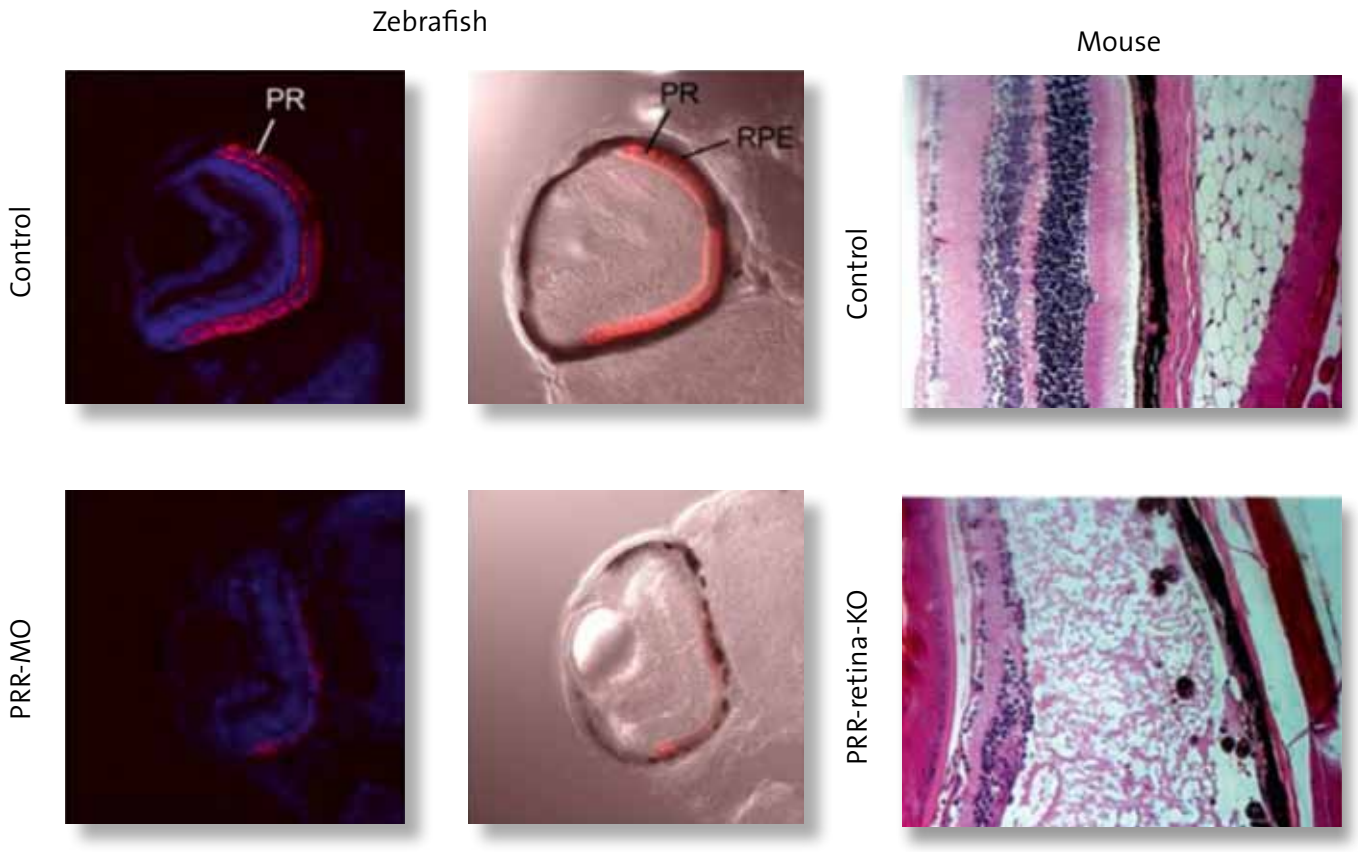

Figure 1. (Pro)renin receptor depletion and eye development in mouse and zebrafish
The expression of (pro)renin receptor (PRR) was depleted by morpholino-antisense oligonucleotides (MO) in zebrafish and Cre-recombinase catalyzed deletion of the PRR gene in the retina of mice, leading to massive alterations in the photoreceptor (PR) and retinal pigment epithelium (RPE) layers.

shown to be completely normal at baseline suggesting that the KKS is irrelevant for development and basic regulation of the cardiovascular system but is involved in the pathogenesis of multiple diseases and, thus, a relevant drug target.

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 in newly developed knockout mouse models. Mice with a local deletion of CXCL12 in cardiomyocytes presented an unexpected deteriorating role of this chemokine for the heart function after cardiac damage.

Natriuretic peptide system

Jens Buttgereit*

There are 3 natriuretic peptides (NP), ANP, BNP, and CNP, which interact with two natriuretic peptide receptors, NPR-A and NPR-B, to induce a multitude of actions in heart, kidney, vessels, brain and other tissues. The receptors are dimeric molecules, which after activation synthesize cyclic GMP. We have shown that dimerization is essential for the activation of the receptors and have designed dominant negative mutants to downregulate the activity of the receptors in cells and transgenic animals. Transgenic rat models expressing a dominant

negative mutant for NPR-B exhibit sympathetic activation and develop cardiac hypertrophy supporting a cardioprotective action of this receptor and its ligand CNP. Moreover, these animals show an impaired bone growth in accordance with the phenotype of knockout mice for NPR-B and CNP and humans with mutations in the NPR-B gene.

Serotonin system

Natalia Alenina, Saleh Bashammakh, Valentina Mosienko, Susann Matthes, Daniel Beis, Ashish Ranjan, Maik Grohmann

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, and cardiovascular parameters. In addition, these mice exhibit increased aggression and maternal neglect. They also show a depression – like phenotype and reduced anxiety in a number of behavioral paradigms. Interestingly, in zebrafish *Tph2* knockdown led to morphogenesis defects during pharyngeal arch formation, supporting an important role of TPH2-derived serotonin as a morphogenetic factor in the development of neural crest derived tissue in this species. Furthermore, we developed protocols for the *in vitro* differentiation of embryonic stem (ES) cells into serotonin-producing neurons and performed gene expression analysis. To evaluate if candidate genes revealed by microarray are crucial for the development of serotonergic neurons *in vivo,* the morpholino-based knockdown of these genes is performed in zebrafish.

Androgen receptor

Gabin Sihn, Silke Mühlstedt

In a collaborative work with the Charité studying gender effects in cardiac hypertrophy and failure, the group generated and characterized animal models with altered androgen receptor expression in distinct cell types of the heart.

Importins

Franziska Rother, Stefanie Hügel, Na Liu, Ariane Schabe, Tatiana Shmidt*, Ilya Chuykin

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 alpha7: Both sexes of these animals are infertile. The molecular basis of the male infertility is currently being analyzed. The female infertility is based on an essential function of importin alpha7 during zygotic genome activation of developing embryos. Furthermore, importin alpha7 is pivotal for influenza virus infection of cells. In addition, we could show that the absence of importin alpha5 during mouse development does not significantly interfere with neuronal differentiation and proper brain development, in contrast to the prediction based on a study in cell culture.

Transgenic and stem cell technology

Alexander Krivokharchenko, Elena Popova, Irina Lapidus, Natalia Alenina, Larissa Vilianovitch, Ilya Chuykin, Jöran Kessler

The group has also a strong emphasis in the field of rat embryology and stem cell research. The rat is the preferred animal 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. These cells are used to explore the signalling cascades underlying mechanisms of pluripotency in the rat, to develop protocols in regenerative therapy, and to establish homologous recombination and thereby allowing gene targeting in the rat. Moreover, to elucidate mechanisms of germ cell self-renewal and differentiation, culture of spermatogonial stem cells was established from Stra8-GFP transgenic rat. Furthermore, transgenic rats

have been produced carrying constructs, which express small interference RNAs suited to downregulate specific genes. The first target gene was the insulin receptor yielding a unique inducible rat model for diabetes mellitus type 2.

Selected Publications

Cui, Y., Niziolek, P.J., MacDonald, B.T., Zylstra, C.R., Alenina, N., Robinson, D.R., Zhong, Z., Matthes, S., Jacobsen, C.M., Conlon, R.A., Brommage, R., Liu, Q., Mseeh, F., Powell, D.R., Yang, Q., Zambrowicz, B., Gerrits, H., Gossen, J.A., He, X., Bader, M., Williams, B.O., Warman, M.L., Robling, A.G. LRP5 functions in bone to regulate bone mass. Nat Med. 2011, 17, 684-691

Alenina, N., Kikic, D., Todiras, M., Mosienko, V., Qadri, F., Plehm, R., Boye, P., Vilianovich, L., Sohr, R., Tenner, K., Hörtnagl, H., Bader, M. (2009) Growth retardation and altered autonomic control in mice lacking brain serotonin. Proc. Natl. Acad. Sci. USA. 106, 10332-10337

Kotnik, K., Popova, E., Todiras, M., Mori, M.A., Alenina, N., Seibler, J., Bader, M. (2009) Inducible transgenic rat model for diabetes mellitus based on shRNA-mediated gene knockdown. PLoS ONE. 4, e5124

Shmidt, T., Hampich, F., Ridders, M., Schultrich, S., Hans, V.H., Tenner, K., Vilianovich, L., Qadri, F., Alenina, N., Hartmann, E., Köhler, M., Bader, M. (2007) Normal brain development in importin alpha 5 deficient mice. Nat. Cell. Biol. 9, 1337-1338.

Langenickel, TH, Buttgereit, J, Pagel-Langenickel, I, Lindner, M, Monti, J, Beuerlein, K, Al-Saadi, N, Plehm, R, Popova, E, Tank, J, Dietz, R, Willenbrock, R, Bader, M. (2006). Cardiac hypertrophy in transgenic rats expressing a dominant-negative mutant of the natriuretic peptide receptor B. Proc. Natl. Acad. Sci. USA 103, 4735-4740.

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Mobile DNA

Transposons ("jumping genes") are discrete segments of DNA that have the distinctive ability to move and replicate within genomes across the tree of life. Transposons offer a new model to study DNA recombination in higher organism, as well as host-parasite interaction. Transposons are also natural gene delivery vehicles that are being developed as genetic tools. Our laboratory is following the strategy of understanding the mechanism of transposition and its regulation and translate this knowledge to derive transposon-based genetic tools for genome manipulation or for gene therapy.

Transposon-host interactions

D. Grzela, A. Deveraj

Transposons occupy a significant portion of our genomes. However, the vast majority of transposons remain silent due to accumulated mutations in their genomes. The transposition of the few, active copies is strongly regulated, but this control is sensitive to environmental stress. Our results show that transposons might exist in a "latent" form in the genome and are able to sense developmental and environmental changes and manipulate stress signaling. Thus, cellular mechanisms that are directly involved in development or stress-response have crucial role in establishing stable host-transposon co-existence.

Genome manipulation – Transposon mutagenesis in rat spermatogonial stem cells

L. Mátés, I. Grabundzija, S. Bashir, A. Osiak

Transposons can be harnessed as vehicles for introducing mutations into genes. Our goal is to establish tools based on SB as well as on the *piggyBac* and *Tol2* transposon systems to manipulate vertebrate genomes (transgenesis, genomic screens) in organisms where this technology was not available before. One particular application relates to loss-of-function insertional mutagenesis in rats with the goal of generating knockout animals. The genes inactivated by transposon insertion are "tagged" by the transposable element, which can be used for subsequent cloning of the mutated allele. With the goal of knocking out genes implicated in disease, we carried out a pilot screen in rat spermatogonial stem cells. The project has enormous potential to develop powerful genomic tools for rat that is the preferred model organism of cardiovascular, as well as toxicology and behavioral studies.

Deciphering the genetic background of hormone-induced breast cancer S. Bashir

The SB transposon is suitable for somatic mutagenesis and emerged as a new tool in cancer research as an alternative to retroviral mutagenesis. Transposon-based insertional mutagenesis screens are able to identifiy both oncogenes and tumor-suppressor genes. We are engaged in two projects aiming at the discovery of novel driver mutations that are associated with neuroblastoma (in mice) and estrogen-induced mammary cancer (in rats). The transposon mutagenesis approach is expected to be a powerful tool to decipher gene regulatory networks cooperating in cancer development, progression and metastasis.

The role of stress-induced transcriptional activation of transposable elements in modifying gene expression in the human genome N. Fuchs

Recent comparative genomics studies have shown that a substantial proportion of constrained noncoding ele-

ments unique to mammals arose from mobile elements, pointing to TEs as a major creative force in the evolution of mammalian gene regulation. We recently set out to investigate the cellular functions of hyper-conserved, TE-derived repeats in the evolution of gene regulation.

Transposon-based, non-viral gene transfer

DNA-based transposons are natural gene delivery vehicles, and molecular reconstruction of SB represents a cornerstone in applying transposition-mediated gene delivery in vertebrate species, including humans. Our recently developed 100-fold hyperactive SB system, SB100X (Molecular of the Year, 2009) opened new avenues for gene delivery approaches.

Transposons as efficient and reproducible vectors for mammalian transgenesis

L. Mátés, A. Deveraj, S. Bashir

Recently, we demonstrated that the SB100X sytem supports efficient transgenesis in various mammaian species. This approach seems to work with similar or improved efficacies to retro/lentiviral strategies, but it is a non-viral technique and is much simpler, safer to use (no elevated security level of laboratory is required), and therefore more accessible to research laboratories. One of the important findings is that transposon-based transgenesis produces trangenics of superior quality in all tested species, in that no major mosaicism is observed in the transgenic animals generated by using transposable elements.

Transposons as non-viral vectors for gene therapy

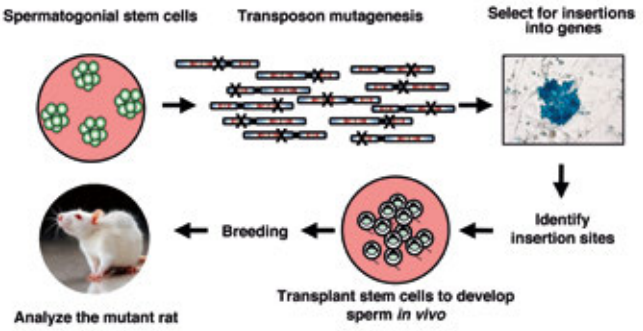
I. Ammar, C. Miskey, K. Voigt, J. Wang, H. Escobar, E. Grueso, M. Swierczek

Using the SB100X system, we are currently performing preclinical studies for gene therapy of Wiskott-Aldrich syndrome, chronic granulomatous disease (CGD), Goucher disease, Fanconi anemia and dysferlinopathy. In collaboration with clinical (U. Aachen, Germany) and industrial partners (NsGene, Denmark), the first clinical trials, using the *Sleeping Beauty* transposon are planned for 2012 to treat age-related macular degeneration (AMD) and Alzheimer disease, respectively .

Transposon-mediated induced pluripotency

I. Grabundzija, J. Wang, N. Fuchs

Due to the superior safety features of the SB-mediated gene delivery, we have established an SB100X-mediated protocol of generating induced pluripotent cells (iPSCs). In order to monitor the genomic stability of the transposon-generated iPSCs, we use low- and high resolution assays to detect genome-wide rearrangements (translocations, inversions, deletions) at a resolution of 500 bp genome.



Generation of knockout rats by insertional mutagenesis with gene-trap transposons in spermatogonial stem cells. Cultured stem cells are transfected with gene-trap transposon and transposase constructs that will lead to thousands of transposon insertions covering all chromosomes. Those cells in which insertions occurred in expressed genes can be selected based on activation of the gene-trap marker, and the insertion sites can be mapped. Cell clones or polyclonal insertion libraries can be transplanted into the testes of sterile males, in which the spermatogonial stem cells will undergo spermatogenesis. These transplanted males are crossed with wild-type females to pass the insertions through the germline and generate transgenic/knockout animals.

Selected Publications

Izsvák, Z., Fröhlich, J., Grabundzija, I., Shirley, J.R., Powell, H.M., Chapman, K.M., Ivics, Z., and Hamra, F.K. (2010) Generating Knockout Rats by Sleeping Beauty Transposon Mutagenesis in Spermatogonial Stem Cells. Nature Methods 7(6):443-5.

Wang, Y., Liska, F., Gosele, C., Šedová, L., Křen, V., Křenová, D., Ivics, Z., Hubner, N. and Izsvák, Z. (2010) A Novel Active Endogenous Retrovirus Family Contributes to Genome Variability in Rat Inbred Strains. Genome Research 20(1):19-27.

Ivics, Z, Li, M.A., Mátés, L, Boeke, JD, Nagy, A, Bradley, A, Izsvák, Z. (2009) Transposon-mediated genome manipulations in vertebrates. Nature Methods 6(6), 415-422.

Mátés, L, Chuah, MK, Belay, E, Jerchow, B, Manoj, N, Acosta-Sanchez, A, Grzela, DP, Schmitt, A, Becker, K, Matrai, J, Ma, L, Samara-Kuko, E, Gysemans, C, Pryputniewicz, D, Miskey,C, Fletcher, B, VandenDriessche, T, Ivics, Z., Izsvák, Z. (2009). Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. Nature Genet. 41(6), 753-761.

Sinzelle, L, Kapitonov, VV, Grzela, DP, Jursch, T, Jurka, J, Izsvák, Z, Ivics, Z. (2008). Transposition of a Reconstructed Harbinger Element in Human Cells and Functional Homology with Two Human Cellular Genes. Proc. Natl. Acad. Sci. USA, 105(12):4715-20

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Young-Ae Lee

Genetics of Allergic Disease

The allergic diseases, eczema, asthma, and hay fever, are among the most common chronic diseases in man. In the industrialized countries, 25-30% of the population are affected. Genetic and environmental factors interact to determine disease susceptibility, and family and twin studies indicate that the genetic contribution is substantial.

Our group is using genetic and genomic approaches to identify genes and genetic variants that predispose to eczema, asthma, and chronic inflammation. Characterization of the genes, molecules, cells, and pathways involved in allergic diseases will not only elucidate disease mechanisms but will also enable the development of disease prediction and prevention algorithms.

Genome-wide association study reveals a common variant on chromosome 11q13 that is associated with eczema and allergic airways disease

To identify genetic variants contributing to eczema, we conducted a genome-wide association study including over 4105 cases and 5472 controls. Highly significant association was found with a common sequence variant (rs7927894) on chromosome 11q13.5 near the gene *C11orf30*. Approximately 13% of European individuals are homozygous for the risk allele, and their risk of developing eczema is 1.47 times that of noncarriers. *C11orf30* encodes the nuclear protein EMSY which has been implicated in breast cancer susceptibility, chromatin modification, DNA repair, and transcriptional regulation. The potential involvement of *C11orf30* in multiple inflammatory and malignant epithelial diseases strongly suggests a role for *C11orf30* in epithelial immunity, growth, and/or differentiation[1].

We analyzed the effect of this risk variant on the general population level in over 9,300 individuals of the prospectively evaluated ALSPAC birth cohort (**A**von **L**ongitudinal **S**tudy of **P**arents **a**nd **C**hildren). Beyond the association with eczema, we demonstrate that rs7927894[T] is also a risk factor for asthma and hay fever. The estimated population attributable risk fractions for eczema, associated atopic asthma, or hay fever were remarkably large at 9.3%, 24.9%, and 23.5%, respectively. Finally in eczema, we found a synergistic interaction of rs7927894[T] with filaggrin gene (*FLG*) mutations which are a major cause of epidermal barrier dysfunction, and replicated the interaction in the German MAS birth cohort (Figure). The synergistic effect of rs7927894[T] and *FLG* mutations on eczema risk, as well as the association of both variants with eczema-associated asthma and hay fever point to an involvement of rs7927894[T] in a functional pathway that is linked to the barrier defect [2].

Systematic association screening in the epidermal differentiation complex (EDC) identifies an *SPRR3* repeat number variant as a risk factor for eczema

Genetically determined skin barrier impairment is a recognized cause of eczema. Our genome-wide association study for eczema revealed association with mark-

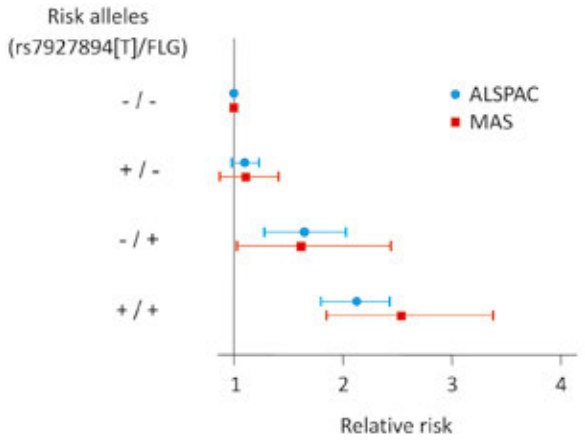

Figure Consistent evidence for synergistic interaction between chromosome 11 and the *filaggrin* mutations in the ALSPAC and MAS birth cohorts. The four risk groups correspond to all combinations of the two risk factors according to the presence (+) or absence (-) of rs7927894[T] and the filaggrin null mutations (*FLG*). Horizontal bars represent the 95% confidence intervals.

ers within the epidermal differentiation complex (EDC) in addition to the known *filaggrin* mutations. Since the EDC contains tens of genes that are important for the maturation and cornification of the epidermis, we screened the NCBI database for putatively functional polymorphisms in the EDC genes and tested them for association with eczema. Of 20 variants with predicted major impact on protein function, 4 were validated: a nonsense mutation in *filaggrin 2* (rs12568784), a stop codon mutation in *LCE1D* (rs41268500), a 24-bp deletion in *SPRR3* (rs28989168), and a frameshift mutation in *S100A3* (rs11390146). Association testing of the validated polymorphisms in 555 eczema patients and 375 controls identified a significant effect of rs28989168 (*SPRR3*) on eczema. The association was replicated in another 1314 cases and 1322 controls, yielding an overall odds ratio of 1.30 (95% confidence interval, 1.12-1.51; *P*=0.00067). Small proline rich proteins (SPRR) are cross-bridging proteins in the cornified cell envelope which provides the main barrier function of stratified squamous epithelia. The extra repeat in the eczema-associated variant of *SPRR3* might therefore disturb the barrier properties of the cornified envelope.

Towards the genetic prediction of childhood asthma

The increasing prevalence of asthma and the lack of curative therapy underscores the need for effective disease prediction and prevention. We have performed the first genetic prediction study for asthma using the *filaggrin* (*FLG*) null mutations and early allergic sensitization to food allergens which are recognized risk factors for asthma.

We found that, in infants with eczema and food sensitization within the first three years of life, the presence of a *FLG* null mutation predicts the future development of asthma with a specificity and a positive predictive value of 100%. The combination of *FLG* mutations and early food sensitization, predicted a sizeable proportion (17.2%) of the infants with eczema who made the transition to asthma later in childhood. Moreover, longitudinal pulmonary function tests demonstrated that this subgroup of asthma children carried a poor prognosis with a steady decline in pulmonary function until puberty. This study demonstrated that the determination of the *FLG* carrier status in infants with eczema and food sensitization within the first three years of life allows the early prediction of asthma before the onset of symptoms and at a critical time of immune development when preventive interventions are likely to be effective.

Selected Publications

Esparza-Gordillo J, Weidinger S, Folster-Holst R, Bauerfeind A, Ruschendorf F, Patone G, Rohde K, Marenholz I, Schulz F, Kerscher T, Hubner N, Wahn U, Schreiber S, Franke A, Vogler R, Heath S, Baurecht H, Novak N, Rodriguez E, Illig T, Lee-Kirsch MA, Ciechanowicz A, Kurek M, Piskackova T, Macek M, Lee YA *, Ruether A. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet 2009;41:596-601. * Corresponding author

Marenholz I, Bauerfeind A, Esparza-Gordillo J, Kerscher T, Granell R, Nickel R, Lau S, Henderson J, Lee YA. The eczema risk variant on chromosome 11q13 (rs7927894) in the population-based ALSPAC cohort: a novel susceptibility factor for asthma and hay fever. Hum Mol Genet 2011;20:2443-2449.

Marenholz I, Rivera VA, Esparza-Gordillo J, Bauerfeind A, Lee-Kirsch MA, Ciechanowicz A, Kurek M, Piskackova T, Macek M, Lee YA. Association screening in the Epidermal Differentiation Complex (EDC) identifies an SPRR3 repeat number variant as a risk factor for eczema. J Invest Dermatol 2011;131:1644-1649.

Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von ME, Farrall M, Lathrop M, Cookson WO, GABRIEL consortium [Charité contributors: Lee YA, Esparza-Gordillo J, Nickel R, Wahn U, Lau S, Marenholz I]. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-1221.

Marenholz I, Kerscher T, Bauerfeind A, Esparza-Gordillo J, Nickel R, Keil T, Lau S, Rohde K, Wahn U, Lee YA. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. J Allergy Clin Immunol 2009;123:911-916.

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Cell Signalling and Mass Spectrometry

Proteins are the chief actors in almost every biological process. While we know a lot about the function of individual proteins there is less information about the system as a whole. Recent developments in mass spectrometry have dramatically improved the analytical power of this technology. We are using quantitative shotgun proteomics to investigate cellular signalling at the protein level on a global scale. Main areas of research are gene expression control, protein-protein interactions and *in vivo* quantitative proteomics.

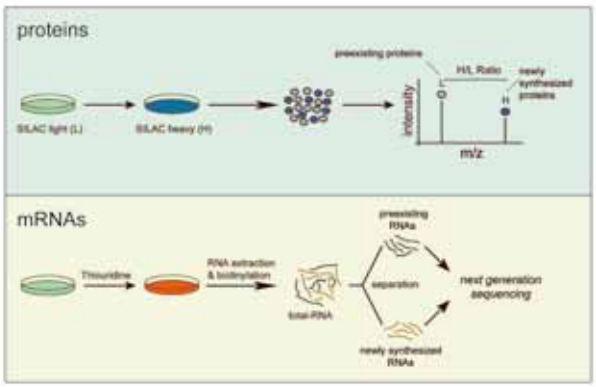
Recently developed quantitative methods make it possible to obtain precise functional information and to monitor temporal changes in the proteome by mass spectrometry. In one approach, named SILAC (for stable-isotope labelling with amino acids in cell culture), cells are differentially labeled by cultivating them in the presence of either normal or a heavy isotope–substituted amino acids, such as ^{13}C-labeled lysine. 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. Quantitative proteomics with SILAC has emerged as a very powerful approach to investigate signaling processes. We are using this technology as our central tool to address fundamental biological questions at the Systems level.

Gene expression control

Olivia Ebner, Björn Schwanhäusser, Erik McShane

The four fundamental cellular processes involved in gene expression are transcription, mRNA degradation, translation and protein degradation. Each of these four steps is controlled by gene-regulatory events. So far, 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 unknown. We are using metabolic pulse labeling approaches to comprehensively quantify gene expression (Fig. 1 A). Protein turnover can be quantified using SILAC. Similarly, newly synthesized RNA can be labelled with nucleoside analogues. Mass spectrometry and next generation sequencing (in collaboration with the group of Wei Chen) then allows us to quantify the absolute abundance of mRNAs and proteins and their half-lives in parallel. These data can then be used to calculate synthesis rates of mRNAs and proteins by mathematical modeling (collaboration with the group of Jana Wolf). Based on this approach we recently obtained the first census of mammalian gene expression (Fig. 1 B). Intriguingly, our data indicates that gene expression is predominantly controlled at the level of translation. Currently, we are investigating how the different levels of gene expression change upon perturbation. In addition, we are using pulsed SILAC (pSILAC) to directly quantify changes in protein synthesis. For example, we are studying the impact of microRNAs and RNA-binding proteins on protein production.

A



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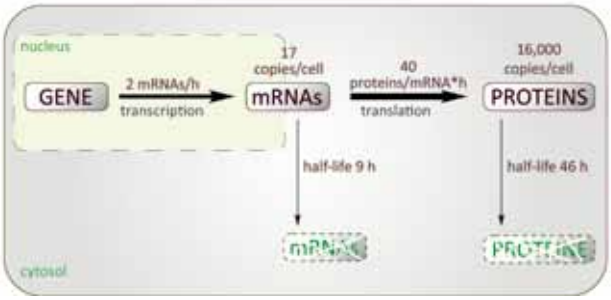

Figure 1. Global quantification of mammalian gene expression by double pulse labelling. (A) Proteins are pulse labelled by transferring cells from culture medium with normal (that is, light) amino acids to medium containing heavy stable isotope-containing amino acids. Newly synthesized proteins and pre-existing proteins can be differentiated in a mass spectrometer to quantify protein half-lives. Similarly, newly synthesized mRNAs are pulse labelled with the nucleoside analogue 4-thiouridine, biotinylated, separated from pre-existing mRNAs and analyzed by next generation sequencing. (**B**) Our data reveals cellular copy numbers, half-lives and synthesis rates for more than 5,000 genes (numbers in the figure are averages). The thickness of the arrows indicates the impact of the respective process for gene expression control. Translation rate constants are the most important factor.

Protein-protein interaction

Fabian Hosp, Florian Paul, Marieluise Kirchner

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 mass spectrometry to analyze protein-protein interactions (PPIs). This approach has two unique advantages. First, accurate quantification allows us to detect interactions with extremely high confidence. Second, quantification reveals how interactions change in response to a stimulus. Currently, we are studying PPIs involved in neurodegeneration, Rho-GTPase signaling and epithelial-mesenchymal transition (collaboration with the lab of Walter Birchmeier).

In vivo quantitative proteomics: The SILAC zoo

Jiaxuan Chen, Matthias Sury

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. While SILAC has enormously improved quantitative proteomics in cultured cells, the method was not yet used in small animal models. We have extended this technology to *Caenorhabditis elegans* (collaboration with the lab of Nikolaus Rajewsky) and *Drosophila melanogaster*. Currently, we are using these models to study gene expression during development and protein-protein interaction *in vivo*.

Selected Publications:

Schwanhausser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2011). Global quantification of mammalian gene expression control. Nature *473*, 337-342.

Paul, F.E., Hosp, F., and Selbach, M. (2011). Analyzing protein-protein interactions by quantitative mass spectrometry. Methods 54, 387-395.

Sury, M.D., Chen, J.X., and Selbach, M. (2010). The SILAC fly allows for accurate protein quantification in vivo. Mol Cell Proteomics *9*, 2173-2183.

Vermeulen, M., and Selbach, M. (2009). Quantitative proteomics: a tool to assess cell differentiation. Curr Opin Cell Biol *21*, 761-766.

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R., and Rajewsky, N. (2008). Widespread changes in protein synthesis induced by microRNAs. Nature *455*, 58-63.

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microRNAs and 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 β-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.

The mechanisms governing gene expression patterns integrate both transcriptional activation, post-transcriptional gene silencing, and post-translational modifications. In the last decade the complex picture of gene regulation has been extended by the discovery of microRNAs. MicroRNAs are short, approximately 22 nucleotide long non-coding RNAs which are thought to be involved in a number of evolutionary conserved regulatory pathways. Many published reports have clearly illustrated a role for individual microRNA sequences in developmental timing, apoptosis, proliferation, differentiation, and organ development. However, in light of the many advances in the fields of microRNAs and RNA interference, many questions remain concerning the functional role of microRNAs in tissues like the pancreatic islet. Our work aims to test the hypothesis that microRNAs expressed in the islet play an important role in the development and function of pancreatic β-cells through their ability to regulate gene expression. Many direct targets of microRNAs expressed in the pancreatic islet have never been studied with respect to β-cell biology and it is important to understand how they contribute to islet function. Using newly developed mouse models, a molecular understanding of the exact nature of microRNAs is necessary to develop therapeutic strategies for the treatment of metabolic diseases like diabetes.

Selected Publications

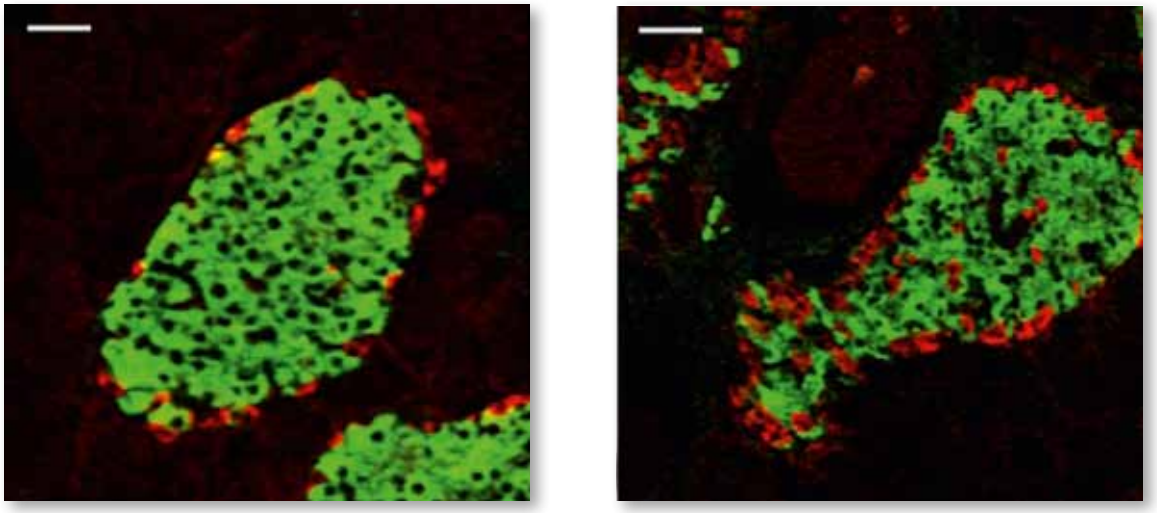
Epithelial microRNAs regulate gut mucosal immunity via epithelium-T cell crosstalk. Biton M, Levin A, Slyper M, Alkalay I, Horwitz E, Mor H, Kredo-Russo S, Avnit-Sagi T, Cojocaru G, Zreik F, Bentwich Z, Poy MN, Artis D, Walker MD, Hornstein E, Pikarsky E, Ben-Neriah Y. (2011) ***Nat Immunol.*** 12(3):239-46.

Poy, MN, Hausser, J, Trajkovski, M, Braun, M, Collins, S, Rorsman, P, Zavolan, M and Stoffel, M. (2009). *miR-375* maintains normal pancreatic α- and β-cell mass. Proc. Natl. Acad. Sci. USA. 106(14):5813-8.

Yi, R, Poy, MN, Stoffel, M, and Fuchs, E. (2008) A skin microRNA promotes differentiation by repressing 'stemness'. Nature. 452(7184):225-29.

Wolfrum, C, Poy, MN, and Stoffel, M. (2005) Apolipoprotein M is required for preβ-HDL formation, cholesterol efflux to HDL and protects against atherosclerosis. Nature Med. 11, 418-422.

Krek, A*, Grun, D*, Poy, MN*, Wolf, R, Rosenberg, L, Epstein, EJ, MacMenamin, P, da Piedade, I, Gunsalus, KC, Stoffel, M, Rajewsky, N. (2005) Combinatorial microRNA target predictions. Nat. Genet. 37(5):495-500. (* equal contribution)



Decreased β-cell mass in miR-375KO pancreatic islets. Representative sections of pancreas from 10-week-old a)wild type and b)miR-375KO mice visualized by immunofluorescence after staining with insulin (green) and glucagon (red). Bar represents 25 μm.

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Mathematical Modelling of Cellular Systems

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

Our group develops and analyses mathematical models of signalling 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 the systems and help to identify most sensitive processes and critical regulations. Another important aspect is the investigation of cell specific differences in signalling and gene-regulatory networks since these are critically involved in the prediction of the efficiency and possible side-effects of drugs.

Modelling mammalian signal transduction pathways: IKK/ NF-κB signalling and Wnt/ β-catenin signalling

Bente Kofahl, Dinto Jose, Janina Mothes, Uwe Benary

In a collaborative approach with the group of Claus Scheidereit at the MDC we aim for a systems level understanding of the IKK/ NF-κB signalling pathway. This pathway consists of a canonical and a non-canonical branch. Both have a distinct timing and distinct biological functions but are interconnected. On the one hand substrates and inducers of the non-canonical branch are produced in the canonical branch, on the other hand a control of the canonical part by the non-canonical branch was reported. We are interested in the regulation of the long-time behaviour of the overall pathway and its malfunction in diseases, e.g. Hodgkin-lymphoma. In particular, we want to dissect the contribution of canonical and non-canonical modules under these conditions. To that end we are investigating the kinetic properties, feedback regulations and interacting modules of both signalling branches.

The Wnt/ β-catenin pathway is another important signal transduction pathway. Its deregulation is associated with various types of cancer. We use mathematical modelling of the canonical Wnt/ β-catenin pathway to analyse the effect of transcriptional feedbacks (e.g. via Axin, β-TrCP/ HOS) and the cross-talk of Wnt/ β-catenin signalling to other pathways, most importantly NF-κB.

Quantification of mammalian gene expression

Dorothea Busse

In a collaborative project with the groups of Matthias Selbach and Wei Chen the multistep process of gene expression including transcription, translations and the turnover of mRNA and protein was for the first time quantified on a genome-wide scale. Our approach, combining mass-spec measurements, next generation

sequencing and mathematical modelling, comprised the simultaneous measurement of absolute mRNA and protein abundance and turnover by parallel metabolic pulse labelling. The synthesis rates of mRNAs and proteins were predicted by mathematical modelling. An important finding is that the cellular abundance of proteins is predominantly controlled at the level of translation. The study also shows that genes with similar combinations of mRNA and protein stability share functional properties.

Metabolic synchronisation by travelling waves in cell layers

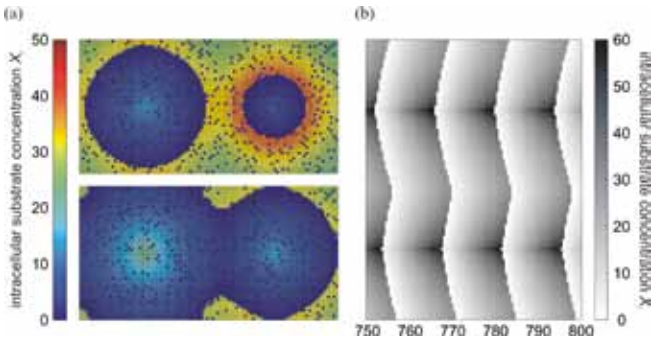
Jana Schütze

The coordination of cellular dynamics is a prerequisite of the functionality of tissues and organs. Generally, this coordination may occur by signal transduction, neuronal control, or exchange of messenger molecules. The extent to which metabolic processes are involved is less understood. Previously, a communicaton between cells via the exchange of metabolites, called dynamic quorum sensing, was shown in stirred cell suspensions. However, it was an open question whether the coupling of cells without stirring would be sufficient for the coordination of cellular behaviour. We studied this question by an experimental-theoretical approach using a simple experimental system, that is, a layer of resting yeast cells. We could for the first time show that a local addition of substrate led to the formation of intercellular glycolytic waves in these cell layers. The theoretical analysis of this phenomenon showed that the radial wave velocity arises from the substrate gradient. Overall, the results demonstrate that metabolic processes introduce an additional level of local intercellular coordination.

Robustness of cellular rhythms

Katharina Baum

Many examples for rhythmic phenomena can be found on the cellular level, e.g. circadian rhythms, the cell cycle, calcium or metabolic oscillations. These rhythms are involved in various functions and react specifically to environmental changes. Our project addresses the question of what determines the balance between sensitivity and robustness of a cellular rhythm. To this end we compare the dynamical properties of various well-established mathematical models for different rhythms. We find models for calcium oscillations to be very sensitive, those for circadian rhythms very robust. These findings correspond well to the biological functions since for calcium oscillations a period-encoded signal transduction was shown, whereas circadian oscillations generate an internal time information. In a subsequent analysis, the impact of local kinetic parameters versus that of the system topology on the robustness is studied.



Simulation of travelling waves in a yeast cell layer.
Local addition of glucose leads to a repetitive formation of metabolic wave fronts with a constant period. The waves annihilate upon head-on collision. (a) Distribution of intracellular substrate shortly before and after the collision of wave fronts, (b) corresponding time-space-plot.

Selected Publications

Schwanhäusser, B, Busse, D, Li, N, Dittmar, G, Wolf*, J, Chen*, W, Selbach*, M. (2011). Genome-wide parallel quantification of mRNA and protein levels and turnover in mammalian cells. Nature 473, 337-342. (*corresponding authors)

Schütze1, J, Mair1, T, Hauser, MJB, Falcke, M, Wolf, J. (2011). Metabolic synchronisation by travelling waves in yeast cell layers. Biophys. J. 100(4), 809-813. (1equal contribution)

Kofahl, B, Wolf, J. (2010). Mathematical modelling of Wnt/ β-catenin signalling. Biochem. Soc. Trans. 38, 1281-1285.

Schütze, J, Wolf, J. (2010). Spatio-temporal dynamics of glycolysis in cell layers. A mathematical model. Biosystems 99, 104-108.

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Mathias Treier

Start of the group: May 2011

Molecular Genetics of Metabolic and Reproductive Disorders

The specification of cell types during organ development has been studied intensively over the last decade. The future challenge will be to unravel how different cell types within one organ function in a concerted action so that an organ can fulfill its physiological task. We study various aspects of mammalian physiology, from the single cell stage to the complex interplay between organs that allow an organism to maintain homeostasis. Ultimately the goal is to understand how mammalian physiology is orchestrated to allow an organism to survive in a changing metabolic environment.

Stem cells, transcription factors and epigenetic regulation

Stem/progenitor cell populations constitute the basic building units from which organs and whole organisms are created. We have identified with the transcriptional regulator SALL4 a key player that is required to maintain the pluripotency state of embryonic stem cells. SALL4 is highly expressed in the inner cell mass (ICM) of the blastocyst which will give rise to the embryo proper and the primitive endoderm. We are currently employing omics technologies to understand the regulation and function of this central player in stem cell biology. Using a biotin/streptavidin system we have unraveled the SALL4 protein complex and its protein interaction network in embryonic stem cells. In addition, we have determined by chromatin immunoprecipitation in combination with high throughput massively parallel sequencing (ChiPseq) the chromosomal localization pattern of these protein complexes. Currently, we try to determine the epigenetic alterations that result from changes of these protein complex activities which are modulated through growth hormone factor signaling.

Ciliopathies

At the organ level we are interested how cells are able to react to changes in their microenvironment. Recently, the cilium has emerged as a crucial sensor of growth factors and physiological parameters in body fluids. With the GLIS family of transcriptional regulators, we have identified molecular players that are involved in transmitting the signal from the cilium to the nucleus allowing i.e. kidney cells to respond to changes in primary urine composition. In particular, we could show that cilia signaling mediated by the transcriptional regulator GLIS2 is essential to maintain kidney architecture. We are now investigating at the molecular level how cilia signal transduction is regulated through post-translational modifications. Understanding cilia signaling in general will have implications for many human ciliopathies that are caused by malfunctioning of this organelle i.e. Bardet-Biedel syndrome which leads to obesity.

Energy homeostasis and eating behaviour

The ultimate goal for any living organism is to maintain energy homeostasis in its quest to survive. We are par-

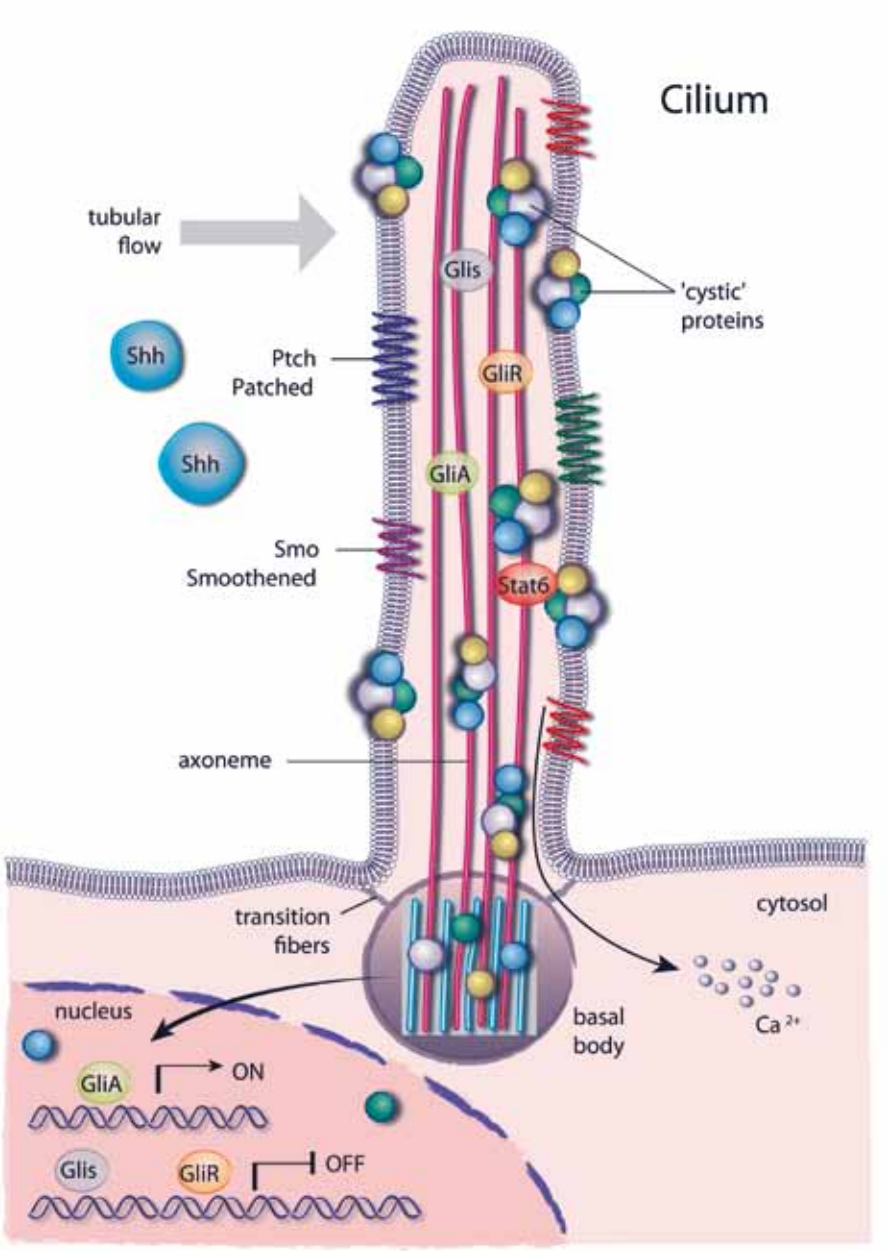

Figure 1. Schematic drawing of a cilium transmitting an extra-cellular signal to the nucleus

A primary cilium consists of a central axoneme made of microtubules enclosed by a distinct cell membrane. Several structural elements such as the periciliary membrane, the transition fibers and basal bodies form a selective barrier at the entrance of the cilium and create a unique environment that allows for compartmentalization. Cilia are sensory organelles that can probe the extracellular environment, but they also act as signaling centers. Bending of the cilium by renal tubular flow causes an intracellular Ca2+ influx that sets off a variety of signaling cascades. Components of the Hedgehog (and other) signal transduction pathway(s) have been shown to depend on ciliary localization. GLI and GLIS transcription factors can move from there to the nucleus to regulate gene expression.

ticular interested in the neuronal circuits of the central nervous system (CNS) that are involved in the regulation of energy homeostasis. We have identified with the brain-specific homeobox protein BSX an essential player marking a neuronal network within the hypothalamus that is central to the regulation of food intake and locomotor activity, the two main components that determine energy balance. We are currently investigating how higher brain centers integrate peripheral signals for satiety and hunger to regulate our drive to eat. Furthermore we try to understand how epigenetic changes through diet regulate metabolic processes in the development of obesity the major risk factor for noncommunicable diseases. Finally we have started to unravel the

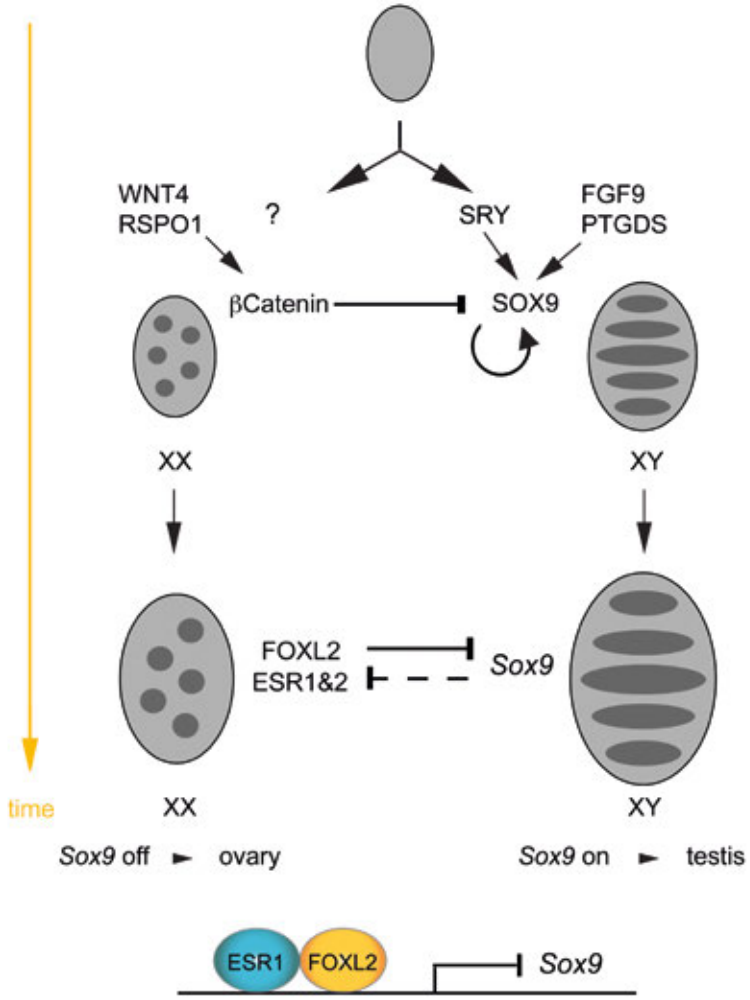

Figure 2. Yin and Yang relationship of FOXL2 and SOX9 in the maintenance of sexual identity in mammals
During initial phases of sex determination, SRY up-regulates *Sox9* expression, and subsequent positive autoregulatory loops involving SOX9 itself, together with FGF9 and prostaglandin D2 signaling, activate and maintain *Sox9* expression in male gonads, whereas β-catenin stabilized by WNT4 and RSPO1 signaling suppresses *Sox9* expression in female gonads. After birth β-catenin activity declines and thus in adult female gonads, FOXL2 and estrogen receptors are required to actively repress *Sox9* expression to ensure female somatic cell fate. The transcriptional repression of *Sox9* by FOXL2 and estrogen receptors is necessary throughout the lifetime of the female to prevent transdifferentiation of the somatic compartment of the ovary into a testis.

reciprocal interactions between hormonal signals and higher cognitive functions in the regulation of eating behaviour.

Sexual reproduction

Reproductive fitness is central for any species to adapt to changing environmental conditions. Sexual reproduction allows the fast reshuffling of genetic information but requires the maintenance of male and female germ lines within a species. We have recently uncovered the underling molecular mechanism of sexual identity in mammals revealing an unexpected plasticity through a YIN and YANG relationship of female and male sexual identity established by the mutually exclusive expression of the two transcriptional regulators FOXL2 and SOX9. We try now to understand the epigenetic code that maintains sexual identity in mammals.

Transcriptional regulators will continue to be at center stage of our investigations. In particular, we have started to look how metabolic changes influence the

epigenetic landscape to modulate transcriptional responses to environmental cues. With a series of mouse models for human diseases that we have created over the last years, we are now in a position to dissect even complicated physiological questions at the organismal level.

Selected Publications

Uhlenhaut N, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier AC, Klugmann C, Klasen C, Holter N, Riethmacher D, Schütz G, Cooney A, Lovell-Badge R & Treier M (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139:1130-1142.

Coldren C, Lai Z, Shragg P, Rossi E, Zuffardi O, Mattina T, Ivy D, Curfs L, Mattson S, Riley E, Treier M & Grossfeld P (2009) Comparative genomic hybrdization mapping suggests a role for BSX and Neurogranin in neurocognitive and behavioral defects in the 11q terminal deletion disorder (Jacobsen syndrome) Neurogenetics 10(2):89-95

Attanasio M, Uhlenhaut NH, Sousa V, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, Seelow D, Nürnberg G, Becker C, Chudley AE, Nürnberg P, Hildebrandt F & Treier M (2007) Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. Nat. Genetics 39:1018-1024.

Sakkou M., Wiedmer, P, Anlag, K, Hamm, A., Seuntjens, E., Ettwiller, L.,Tschop, M & Treier M (2007) A Role for Brain-Specific Homeobox Factor Bsx in the Control of Hyperphagia and Locomotory Behavior Cell Metabolism 5:450-463

Elling, U., Klasen, C., Eisenberger, T., Anlag, K. & Treier, M. (2006) Murine inner cell mass derived lineages depend on Sall4 function. PNAS 103(44), 16319-24.

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Start of the group: July 2011

Electrochemical Signaling in Development and Disease

Physiological cues that include electrical and ionic impulses or metabolic intermediates have been long implicated in the orchestration of organ development during early embryonic patterning. While much is known about genetically encoded developmental pathways, the mechanisms by which the ions and small molecules interact with traditional morphogenetic signals during embryogenesis are poorly understood. We have developed technologies to explore the role of electrochemical and other physiologic stimuli during development in the zebrafish. Utilizing these technologies, we have recently demonstrated that Wnt11 non-canonical signaling, a major developmental pathway regulating tissue morphogenesis and organ formation, patterns intercellular electrical coupling in the myocardial epithelium through effects on transmembrane Ca^{2+} conductance mediated via the L-type calcium channel. This finding offers a new entry point into an aspect of Wnt non-canonical signaling that has been proven difficult to explore.

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

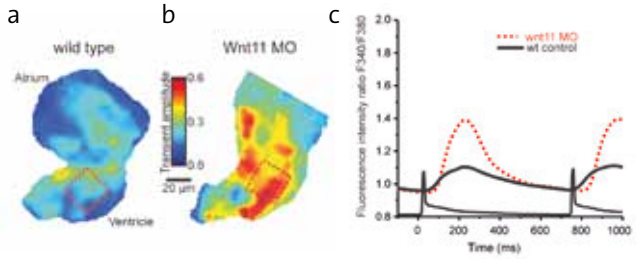
Tareck Rharass

The cardiac L-type Ca^{2+} channel (LTCC) belongs to a family of voltage-gated Ca^{2+} channels that are essentially the major point of entry for Ca^{2+} ions into the cytoplasm. Even though channel opening is primarily regulated by membrane potential, many other molecular mechanisms have been implicated in the modulation of LTCC function. Channel phosphorylation is the most commonly explored way of LTCC activation and Wnt signaling might be a plausible pathway to test for LTCC modulation via specific kinases like PKA, PKC or PKG. Next, Wnt signaling could interfere with LTCC localization to specific membrane microdomains, and thus modulate Ca^{2+} current through LTCC. Alternatively, Wnt signaling might interfere with LTCC channel trafficking. In order to define the molecular mechanism by which Wnt11 regulates LTCC at a subcellular resolution, we have moved from zebrafish embryonic hearts to cell-based systems. This allows us to perform not only immunological experiments, but also more importantly detailed biochemistry.

Characterization of the components of the Wnt non-canonical transduction pathway involved in the L-type Ca^{2+} channel attenuation

Marie Swinarski

The specificity and a wide range of diverse biological effects of Wnt signaling are mediated via binding and affinity of distinct Wnts to their corresponding receptors, Frizzleds. It has been shown that Wnt11 interacts with Fzd7 genetically as well as physically. A crucial step in Wnt signaling is the translocation of Dishevelled (Dsh) from the cytoplasm to the plasma membrane, relaying the Wnt signal to various downstream effectors. Dsh has been implicated so far in all known aspects of Wnt



Wnt11 regulates Ca^{2+} transient amplitudes in cardiomyocytes: a, b, Colour maps of Ca^{2+} transient amplitudes from wildtype hearts (a) and Wnt11 morphants (b). Colour code depicts Ca^{2+} transient amplitudes in flouresence ratio units (F340 / F380). Squares indicate automated ROIs for measurements averaged in c. c, Averaged Ca^{2+} transients from ROIs in a, b.

signaling, canonical and non-canonical. The N-terminal DIX domain regulates Wnt canonical signaling, while the central PDZ and C-terminal DEP domains of Dsh are responsible for the specific activation of the non-canonical, planar cell polarity (PCP) pathway. In addition, the DEP domain has been shown to have the ability to activate Wnt/Ca^{2+} signaling. It has been recently reported that a polybasic stretch within the DEP domain of Dsh is required for Dsh recruitment by Fzd to activate PCP signaling. We aim to define the requirement of the Wnt transduction machinery in the process of attenuation of L-type Ca^{2+} channel by measuring the changes in intracellular Ca^{2+} fluxes in the series of Fzd and Dsh gain- and loss-of-function experiments.

Quantitative analysis of Ca^{2+} compartmentalization by non-canonical Wnt-dependent mechanisms

Alexander Meyer

To further understand the role of Wnt11 signaling in regulating Ca^{2+} fluxes, we decided to take a systematic approach. We are taking advantage of the ratiometric high-resolution Ca^{2+} imaging with Fura-2, that we have developed, to perform quantitative analysis of Ca^{2+} fluxes in both excitable and non-excitable tissues. We will test, what role if any Wnt11 signaling plays on store operated Ca^{2+} in excitable cardiomyocytes. Conversely, it is unknown whether the Wnt11/LTCC pathway exists in non-excitable tissues like epiblast cells of gastrulating zebrafish blastula. We will use the mutants and drug treatments to distinguish between loss and gain of Wnt11 function. We will construct organelle specific Ca^{2+} sensors to determine the role of Wnt11 in Ca^{2+} compartmentalization in *in vivo* experiments. The final aim is to create a comprehensive quantitative atlas of $[Ca^{2+}]_i$ in Wnt/Ca^{2+} signaling in distinct cell types.

Role of Wnt11/Ca^{2+} signaling in cardiac development

We have demonstrated that Wnt11 patterns electrical coupling through regulation of LTCC conductance. However, we do not know what cellular responses precede this patterning. To better understand the patterning of electrical coupling, we first aim to determine whether the observed electrical gradient forms solely as a result of ionic changes across the plasma membrane. Further, due to the fact that gap junctions facilitate action potential propagation between the cells, they are among the most likely candidates that can be targeted by Wnt non-canonical signaling to alter electrical coupling. We have shown that Connexinx43 protein is strongly upregulated at the plasma membrane in the Wnt11 morphant hearts. We will first determine the mechanisms of this upregulation. In addition, we will test whether other cardiac-specific connexins are affected as well. Alternatively, it is feasible to envisage the development of electrical coupling as a process of junction formation. Indeed, Wnt non-canonical signaling has been implicated in regulating cell adhesion and junctional remodeling in many cell biological and developmental contexts. Hence, we have begun addressing the question whether Wnt11/Ca^{2+} signaling is involved in junction formation by testing the localization and abundance of various junctional markers.

Selected Publications

Wang, J, Panáková, D, Kikuchi, K, Holdway, JE, Gemberling, M, Burris, JS, Singh, SP, Dickson, AL, Lin, YF, Sabeh, MK, Werdich, AA, Yelon, D, Macrae, CA, Poss, KD. (2011). The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. Development. 138(16):3421-3430.

Becker, JR, Deo, RC, Werdich, AA, Panáková, D, Coy, S, MacRae, CA. (2011). Human cardiomyopathy mutations induce myocyte hyperplasia and activate hypertrophic pathways during cardiogenesis in zebrafish. Dis Model Mech. 3,400-410.

Boström, P, Mann, N, Wu, J, Quintero, PA, Plovie, ER, Panáková, D, Gupta, RK, Xiao, C, MacRae, CA, Rosenzweig, A, Spiegelman, BM. (2010). C/EBPβ controls exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell. 143(7), 1072-1083.

Panáková, D, Werdich, AA, Macrae, CA. (2010). Wnt11 patterns a myocardial electrical gradient through regulation of the L-type Ca2+ channel. Nature. 466(7308):874-878.

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(November 2011)

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(September 2011)

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Tobias Pischon

Start of the group: December 2010

Molecular Epidemiology

The Molecular Epidemiology Research Group at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch is committed to studying at the molecular level the relationship between lifestyle, genetic, metabolic, and environmental factors with risk and outcome of chronic diseases in human populations. The focus is on molecular biomarkers that have the potential for accurate and precise assessment of exposure, intermediary effects, and early disease development. Our aims are to contribute to the understanding of disease etiology and pathogenesis in humans, to allow a more precise prediction of diseases, and to improve the identification of high risk individuals as well as of the quantification of the effect of interventions, thus envisioning a reduction of chronic disease risk through targeted prevention.

Projects

Role of Metabolic Factors in Chronic Disease Risk (Jürgen Janke, Katharina Nimptsch, Tobias Pischon, Astrid Steinbrecher, in collaboration with Krasimira Aleksandrova and Heiner Boeing, German Institute of Human Nutrition)

Cardiovascular diseases (CVD) and cancer are among the most common chronic diseases in developed countries, and they are the leading causes of death worldwide. Although our understanding of the pathophysiology of CVD and cancer has undergone a remarkable evolution during the past decades, our knowledge about the contribution of metabolic factors to disease risk and of the impact of genetic and non-genetic risk factors on metabolism in humans is rather limited. Such knowledge, however, is important for scientific reasons to gain insight into pathophysiology, and for clinical and public health reasons because it may help to improve the prediction of disease incidence and prognosis, and it may point to targets for prevention. The Molecular Epidemiology Research Group is analysing the role of metabolic factors with risk of chronic diseases in existing and newly established cohort studies. In addition, we are interested in studying their determinants, which may provide insights for measures to reduce disease risk. For example, obesity is among the metabolic factors that are likely to be most relevant on a population level for risk of cardiovascular and other chronic diseases. However, results from large cohort studies suggest that body fat distribution patterns – particularly abdominal adiposity – significantly contributes to risk of disease and premature death beyond obesity per se. Abdominal adiposity is associated with other metabolic abnormalities, such as elevated blood pressure, abnormal glucose metabolism, and dyslipidaemia, which tend to cluster and increase the risk for CVD and type 2 diabetes mellitus, and is described as “Metabolic Syndrome”. Recent evidence suggests that individual components of the Metabolic Syndrome may also be associated with risk of certain types of cancer. In fact, we recently were able to show that the Metabolic Syndrome, as defined by international expert groups, is also associated with a higher risk of colon cancer. Interestingly, this association was largely

accounted for by abdominal obesity and abnormal glucose metabolism, thus again highlighting the role of these factors for risk of chronic diseases. Nevertheless, the underlying mechanisms are not completely understood. Adipose-tissue-derived cytokines and hormones may play a key role in the etiology of metabolic diseases. Thus, fat tissue is not merely a passive triglyceride reservoir of the body, but also produces a vast amount of cytokines and hormones, collectively called adipokines or adipocytokines. For example, we have shown in prospective studies that high levels of adiponectin, an adipocyte derived hormone, are associated with a lower risk of CVD, suggesting a protective role of adiponectin in the development of CVD in humans. In ongoing studies we are further analysing the role of these and other metabolic factors with chronic disease risk.

Genetic Variation in metabolic factors and risk of chronic diseases

(Jürgen Janke, Katharina Nimptsch, Tobias Pischon, Herbert Schulz, in collaboration with Young-Ae Lee and Norbert Hübner, MDC; Krasimira Aleksandrova and Heiner Boeing, German Institute of Human Nutrition; and international partners)

A number of metabolic factors have been identified to be purportedly related to risk of chronic diseases. However, current evidence relies mostly on observational studies, and, therefore, causality remains to be established. One approach to test for causality is to conduct 'Mendelian randomization' studies. This design can assess gene-related risk factors for causal association with clinical outcomes under the assumption that individuals inherit gene variants randomly from their parents. Thus, in principle, if circulating biomarker levels are associated with disease risk then single nucleotide polymorphisms that are related to meaningful variation in biomarker levels should also be associated with disease. Based on this principle, the Molecular Epidemiology Research Group is studying the association of genetic variation in metabolic factors with risk of chronic diseases in observational studies to provide support to the evidence of the causality of exposure-disease relationships.

The National Cohort – A prospective epidemiologic study resource for health and disease research in Germany

(Jürgen Janke, Sabine Mall, Tobias Pischon, Astrid Steinbrecher, in collaboration with partners from other research institutions)

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 Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, plans a large prospective cohort to be used as a common, national resource for studies on risk factors and etiologic mechanisms of major diseases in the German population. The National Cohort will include a total of 200,000 participants, to be recruited through a network of 18 study centers, organized in eight clusters throughout Germany. At each center, a random sample of the general population will be drawn within defined strata of age and sex. All participants will be invited to the study centers to take part in physical and medical examinations, the collection of biomaterials, personal interviews, and to fill in questionnaires. All participants of the National Cohort will be re-invited for a second examination five years after their baseline recruitment. Participants will be re-contacted every 2-3 years and asked to fill in short questionnaires about changes in lifestyle and other characteristics and about the occurrence of selected, major diseases ("active" follow-up). In parallel, a mortality follow-up and systematic record linkage with existing disease registries will be performed periodically ("passive" follow-up). Biomaterials will be stored in a centralized biobank and in decentralized back-up storages at the recruitment clusters. Within this initiative, the MDC coordinates the Cluster Berlin-Brandenburg, which consists of the MDC, the Charité – University Medical Center Berlin, the German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), and the Robert Koch Institute (RKI). The Cluster comprises three study centers that are located at or near the sites of the partner institutions (Berlin-North, run by the MDC; Berlin-Center, run by the Charité; and Berlin-South, run by DIfE). With these study centers the cluster will recruit a total of 30,000 participants (10,000 in each center) into the National Cohort. The study protocol for the National Cohort has been proposed to the Federal Ministry for Education and Research and has been reviewed by an International Referee Panel. Recruitment for the National Cohort is expected to start in early 2013.

Pretests for the National Cohort

(Jürgen Janke, Sabine Mall, Tobias Pischon, Astrid Steinbrecher, in collaboration with partners from other research institutions)

A major foundation for the planning and preparation of the National Cohort is the implementation of pretests to assess the feasibility of methods for recruitment of future study participants and for assessment of study data. In addition, these pretests form the basis to build up recruitment centers and hire qualified personnel for future recruitment of cohort participants. Within these pretests, the Cluster Berlin-Brandenburg recruits 300 participants from the general population of Berlin; 100 recruited by the MDC, 100 by the Charité, and 100 by DIfE. These participants undergo a base set of detailed interviews and physical examination that are standardized across all study centers. In addition, the MDC conducts feasibility studies in collaboration with partner institutions, to evaluate methods to assess physical activity, physical fitness, nutrition, and oral health. Further, the MDC develops methods for shipment and long-term storage of biomaterials in the Cluster Berlin-Brandenburg.

Selected Publications

Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven F, Fedirko V, Rinaldi S, Romieu I, Riboli E, Romaguera D, Overvad KK, Ostergaard JN, Olsen A, Tjonneland AA, Boutron-Ruault MC, Clavel-Chapelon F, Morois S, Masala G, Agnoli C, Panico S, Tumino R, Vineis P, Kaaks R, Lukanova A, Trichopoulou A, Naska A, Bamia C, Peeters PH, Rodriguez L, Buckland G, Sanchez MJ, Dorronsoro M, Huerta JM, Barricarte Gurrea A, Hallmans G, Palmqvist R, Khaw KT, Wareham NJ, Allen NE, Tsilidis KK, Pischon T. (2011). Metabolic Syndrome and Risks of Colon and Rectal Cancer: the European Prospective Investigation into Cancer and Nutrition Study. Cancer Prev Res (Phila). In press.

Montonen J, Boeing H, Schleicher E, Fritsche A, Pischon T. (2011). Association of changes in body mass index during earlier adulthood and later adulthood with circulating obesity biomarker concentrations in middle-aged men and women. Diabetologia. 54:1676-83.

Pischon T, Hu FB, Girman CJ, Rifai N, Manson JE, Rexrode KM, Rimm EB. (2011). Plasma total and high molecular weight adiponectin levels and risk of coronary heart disease in women. Atherosclerosis. In press.

Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjonneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulou A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duijnhoven FJ, Hallmans G, Weinehall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quiros JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. (2008). General and abdominal adiposity and risk of death in Europe. N Engl J Med 359:2105-20.

Weikert C, Westphal S, Berger K, Dierkes J, Mohlig M, Spranger J, Rimm EB, Willich SN, Boeing H, Pischon T. Plasma resistin levels and risk of myocardial infarction and ischemic stroke. (2008) J Clin Endocrinol Metab 93:2647-53.

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Isabell Strobl

Cancer Research

Coordinator: Claus Scheidereit

Signalling Pathways, Cell and Tumor Biology
Structural and Functional Genomics
Tumor Immunology

Cancer Research Program

Claus Scheidereit

Cancer denotes a spectrum of malignant neoplastic diseases with unregulated growth and invasive dissemination. Arising through an accumulation of critical mutations in the genome, cancer cells lose their own self-controls, which normally monitor genomic stability or decide on self-elimination. The malignant cells evade the complex, normal control functions of their tissue environment and proliferate in an uncontrolled manner. They escape surveillance by the immune system and metastasize through the body's blood and lymph system to form secondary tumors. Cancer reveals a high heterogeneity due to different cell type origins as well as in regard to the spectrum of aquired genomic abnormalities. Due to different consequences for disease development and progression, driver and passenger mutations are discriminated in individual cancer genomes. At the level of an individual tumor, cancer stem cells may give rise to further heterogeneity, representing only a fraction of the bulk tumor mass. They may establish and maintain malignant development and, as in the case of tissue stems cells, give rise to a variety of tumor cell types in a given cancer.

Novel options to detect and treat cancer come from discoveries made in basic research after their translation into the clinic. The MDC Cancer Research Program combines a spectrum of interdisciplinary basic research laboratories and clinically oriented groups. The program covers a broad and interdisciplinary research field, with a comprehensive spectrum of experimental expertise, ranging from cell biology, biochemistry, stem cell and developmental biology to immunology, murine tumor models and bioinformatics. Clinical studies are conducted in close collaboration with clinical research groups of the Charité Universitätsmedizin Berlin.

The research activities are subdivided into the three topics (i) Signaling Pathways, Cell Biology and Cancer, (ii) Structural and Functional Genomics and (iii) Tumor Immunology. One aim of the program is the discovery and characterization of genes and molecular mechanisms, that are crucial for the development and treatment of cancer. Another aim is to gain an understanding of the interactions of tumors with the immune system. New insights and discoveries will be important for the development of novel cancer treatments.

In the reporting period, **Oliver Rocks** was recruited as a Helmholtz junior research group leader in 2011 from Mount Sinai Hospital, Toronto. He has made important contributions to the understanding of signaling through Ras and other GTPases. **Klaus Rajewsky** is in the process of moving his laboratory from Harvard University to the MDC. He has laid the foundations for conditional gene recombination techniques in the mouse and made seminal contributions to the understanding of lymphocyte development and lymphomagenesis. **Frank Rosenbauer** has accepted the position of director and full professor at the University of Münster and leaves the MDC at the end of 2011.

Selected scientific highlights

Colorectal carcinoma (CRC) is one of the most commonly diagnosed forms of cancer, with 73,000 people affected in Germany every year. CRC can be caused by mutations in a number of genes that have been identified, including genes of the Wnt/β-catenin pathway. But only a few genes have been associated with CRC metastasis. In the past years, the laboratories of **Walter Birchmeier** and **Peter Schlag** have identified several genes (including S100A4, MACC1, and BAMBI) which play an important role in CRC progression and metastasis; the groups have elucidated their mechanisms of action. S100A4/metastasin is a Wnt/β-catenin target gene. In collaboration with **Robert H. Shoemaker** of the National Cancer Institute (NCI) in Frederick, **Ulrike Stein** and **Peter Schlag** could identify niclosamide, an established antihelminthic drug, as an inhibitor of S100A4 expression. Niclosamide blocks S100A4-induced metastasis formation in a mouse model of colon cancer, has therapeutic potential and is a candidate drug for clinical trials (Sack et al., J Natl Cancer Inst 2011).

The role of the BCL9-2 gene in intestinal tumorigenesis was investigated in the laboratory of **Walter Birchmeier**.

Through expression analysis of normal and transformed epithelia in mice and humans, they showed that BCL9-2 is upregulated in almost all colon tumors. Transgenic overexpression of BCL9-2 increased the formation of adenomas that progressed into invasive carcinoma. BCL9-2 promotes early phases of intestinal tumor progression in humans and in transgenic mice. BCL9-2 increases the expression of a subset of canonical Wnt target genes, but also regulates genes that are required for early stages of tumor progression (Brembeck et al., Gastroenterology 2011)

Epigenetic regulation is the heritable alteration of gene expression through chromatin modifications such as DNA methylation, histone actetylation or methylation. Through sequence-specific DNA binding to genomic sites and recruitment of chromatin-modifying enzymes, C/EBP transcription factors act as key regulators in epigenesis. C/EBPβ is a Ras/MAP kinase signal-sensitive transcription factor and controls genes involved in metabolism, immunity, tumourigenesis and regeneration (Wethmar et al., Genes & Development 2010). The group of **Achim Leutz** showed that the arginine methyltransferase 4 (PRMT4/CARM1) interacts with and methylates C/EBPβ to inhibit its binding to chromatin remodeling complexes and to Mediator, a general transcription coactivator. This inhibition is abrogated upon Ras/MAP kinase signaling and C/EBPβ phosphorylation, resulting in altered gene expression and cellular differentiation. The study revealed the importance of the action of histone-modifying "epigenetic" enzymes on a gene-specific transcription factor, which may be exploited in drug development (Kowenz-Leutz et al., EMBO J 2010).

Cytotoxic cancer therapy by means of chemotherapeutic drugs or irradiation eradicates tumor cells through genotoxic stress with the final generation of DNA double-strand breaks. Therapy resistance may at least partially be caused by the simultaneous activation of the transcription factor 1, a cellular survival factor that counteracts programmed cell death. It was not completely understood how NF-κB is activated by DNA damage. The group of **Claus Scheidereit** has decivered a complex signal transduction pathway that is induced by DNA damage and unleashes the activation of IκB kinases and NF-κB. The pathway is triggered by ATM and by PARP-1 and the laboratory showed how these molecules activate NF-κB in a process that depends on the formation of poly(APD-ribose) and ubiquitin modifications. This pathway contains multiple components, some of which may be target structures for the development of new therapeutic drugs (Hinz et al., Molecular Cell 2010).

During early stages of cancer development, activated oncogenes (e.g. Ras) can cause a permanent growth arrest of tumor cells, called senescence, as a fail-safe program to suppress further malignant development. **Clemens Schmit**t and his colleagues demonstrated that through the initial induction of programmed cell death in a fraction of the tumor cells, certain oncogenes (c-Myc) can drive the remaining tumor cells into senescence, by provoking healthy stroma cells and immune cells to secrete cytokines (TGFβ), which then trigger the senescence program. Tumor cell senescence elicited by the immune system appears to be an important principle of action of cell death that is generated by chemotherapy in cancer treatment (Reimann et al., Cancer Cell 2010). NF-κB signaling can promote oncogenesis and it can counteract cytotoxic therapy through activation of prosurvival genes. However, in contexts where other oncogenes provide pro-survival signals, NF-κB instead enhances sensitivity to cytotoxic chemotherapy, thereby exerting a tumor-suppressor function, as **Clemens Schmitt** and colleagues have found. The observed beneficial action of NF-κB in a mouse model of non-Hodgkin lymphoma was mirrored in patients suffering a subtype of diffuse large cell lymphoma (DLBCL). By cross-species comparison of transcriptome data and clinical data from lymphoma patients, germinal center B-cell-like (GCB) DLBCL with an overexpression of Bcl-2 could be identified as a clinically relevant subgroup, where NF-κB hyperactivity results in a significantly superior therapy outcome (Jing et al., Genes & Development 2011).

The interferon-inducible dynamin-related myxovirus resistance protein A (MxA) is a GTPase that inhibits replication of diverse viruses. On viral infection, intracellular localization of MxA is altered, causing a misdistribution of viral components. The group of **Oliver Daumke** has solved the X-ray structure of MxA, yielding insight into how this cellular factor controls viral proteins. Based on further recent results, a structural model for a mechano-chemical coupling in ring-like MxA oligomers could be established as a fundamental mechanism for this antiviral effector protein. The findings may be improtant for the development of novel antiviral drugs (Gao et al., Nature 2010; Gao et al., Immunity, 2011). Endocytosis is important for the cellular uptake of nutrients, for neuronal signal transmission and in the immune system. Viruses such as HIV also invade cells through endocytosis. A crucial component of the endocytotic machinery is the protein dynamin, which acts as a molecular motor and forms an oligomeric structure surrounding the neck of endocytotic vesicles. Under GTP consumption, dynamin causes detachment of the vesicle from the membrane. **Oliver Daumke** and collaborators also solved the

X-ray structure of GTP-free dynamin, providing a structural model for the mechanochemical coupling that reconciles earlier models of dynamin function (Faelber et al., Nature 2011).

The human genome contains numerous repetitive elements, including long terminal repeats (LTRs); for some time, it has been assumed that they play a role in tumorigenesis. **Stephan Mathas, Bernd Dörken** and their collaboration partners showed that the epigenetic activation of an LTR triggers the aberrant expression of a differentiation gene (Colony-Stimulating Factor 1 Receptor, CSF1R) in Hodgkin's lymphoma cells, which supports the survival of the tumor cells. A similar reactivation of normally silent genes was observed in other related lymphomas as well. Thus, the detection of LTR reactivations could have diagnostic and prognostic implications (Lamprecht et al., Nature Medicine 2010)

Immune-tolerance mechanisms have made it difficult to identify T cell receptors (TCR) of high-affinity T cells which are directed against self-antigens, e.g. of tumor cells. However, TCRs with specificity for foreign human antigens can be identified in the non-tolerant T cell repertoire of the mouse. Thus, mice bearing the human TCR repertoire could be used to screen the entire repertoire against human self-antigens in an unbiased manner. After many years of work, the group of **Thomas Blankenstein** succeeded in establishing transgenic mice with the entire human TCR$\alpha\beta$ locus, which functionally replaces the missing murine TCR repertoire. This transgenic model may be useful in identifying therapeutically applicable TCRs against human tumors, which can then be used in a targeted immune-therapy in human patients (Li et al., Nature Medicine 2010).

Photo: Song Gao and Alexej Dick
Research Group: Daumke, Copyright MDC

Claus Scheidereit

Signal Transduction in Tumor Cells

The major interest of our laboratory is the understanding of gene expression and signal transduction processes in tumor cells and in development. We have characterized various aspects of the regulation of NF-κB transcription factors, whose activities are controlled by IκB proteins and IκB kinases and which bear a wide physiological and medical significance. The NF-κB system controls important steps in immunity and inflammation, as well as cell death, proliferation or metabolism. Our major efforts are to decipher the mechanisms that underlie gene regulation by IKK and NF-κB in development and in the pathogenesis of human diseases.

The molecular constituents of IKK and NF-κB signaling cascades

The NF-κB system controls hundreds of genes, is present in many, if not all cell types of the body and can be activated by a vast array of physiological agents and stress situations. The mammalian NF-κB family comprises five related members, p50, p52, p65, c-Rel and RelB, which form distinct hetero- and homodimers and bind to inhibitory cytoplasmic IκB molecules, IκBα, β or ε, or to nuclear IκB homologues, such as Bcl-3 and MAIL. As a characteristic feature of NF-κB, two of its subunits, p50 and p52, are generated by proteasomal processing of their precursor proteins, p105 and p100. The unprocessed precursors act as IκBs by sequestering other NF-κB subunits in the cytoplasm. Both precursors are subject to signal-induced, ubiquitin- and IκB kinase (IKK) dependent proteolysis. Two distinct IKK pathways are activated by extracellular stimuli and either trigger degradation of IκBs to release prototypic p50-p65 (canonical pathway) or processing of p100 to release p52-containing heterodimers (non-canonical pathway). The two branches depend on IKKβ and IKKγ/NEMO or on IKKα, respectively. A critical role for non-degradative K63-linked and also linear polyubiquitination has been recognized. These ubiquitin-modifications are generated by ubiquitin ligases, such as TRAF2, TRAF6 and LUBAC. These polymers act as recruitment scaffolds for the IKK complex and other components, which have ubiquitin binding motifs. This recruitment is required for T-loop phosphorylation and activation of IKKs. 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.

DNA damage induced NF-κB activation requires a nuclear PARP-1 signalosome and a cytoplasmic ATM-TRAF6-triggered polyubiquitin signaling cascade

Seda Çöl Arslan, Patrick Beaudette, Daniel Heinze, Michael Hinz, Nadine Mikuda, Giulietta Roel, Michael Stilmann

In our recent work we have investigated the mechanisms of IKK and NF-κB activation by DNA double strand breaks, induced by γ-irradiation or chemothera-

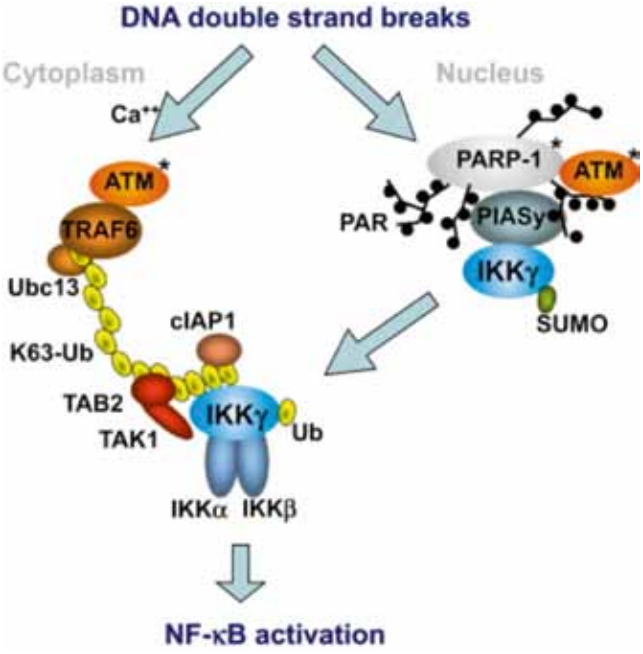

Figure 1. IKK and NF-κB activation by genotoxic stress. PARP-1 senses DNA breaks and synthesizes poly(ADP-ribose) (PAR). PAR-auto-modified PARP-1 recruits PIASy, IKKγ and activated ATM, involving direct PAR binding by PIASy and ATM. Subsequently, IKKγ is SUMOylated by PIASy and exported to the cytoplasm. Another fraction of activated ATM is exported to the cytoplasm and binds directly to TRAF6, resulting in TRAF6 activation and Ubc13-mediated K63-linked polyubiquitination. TAB2-TAK1 and cIAP1 are recruited via polyubiquitin-binding domains, resulting in TAK1 activation. These processes are independent of PIASy and PARP-1. However, exported SUMOylated IKKγ, as well as the cytoplasmic ATM-TRAF6-dependent axis, are both required for monoubiquitination of IKKγ at Lys285. The Lys285 monoubiquitin modification is needed for the catalytic activation of the IKK holocomplex and of NF-κB.

peutic drugs. The activation of NF-κB by genotoxic stress is an integral part of the cellular survival strategy and has been implicated in tumor cell resistance to chemo- or radiotherapy as well as in senescence. A number of pathway components and postranslational modifications underlying DNA damage induced IKK and NF-κB activation could be revealed in the past few years. It was unclear how DNA damage triggers activation of cytoplasmic IKK, and, in turn, nuclear translocation of NF-κB. We could show that activated poly(ADP-ribose) polymerase-1 (PARP-1) acts as a DNA damage sensor, which, along with activated ATM, forms a nuclear signaling complex, where poly(ADP-ribose) acts as a structural scaffold (Fig 1). The signalosome also contains the regulatory IKK subunit IKKγ, ATM and PIASy, which SUMOylates IKKγ as an essential step in the relay of the nuclear signal to cytoplasmic IKK activation. We have found that the PARP-1 signalosome is needed to protect cells from DNA damage-induced apoptosis elicited by irradiation or chemotherapeutic drugs. PARP-1 is essential for NF-κB activation in a number of different tumor cell types and tissues tested. We have also investigated how the DNA damage signal is relayed out of the nucleus. Activated ATM is exported into the cytoplasm, where it binds to the ubiquitin ligase TRAF6, causing TRAF6 activation and Lys-63-linked polyubiquitin formation. The kinase TAK1, along with its ubiquitin-binding adaptor TAB2 and the IKK kinase complex, is recruited to the ubiquitin polymers, to be enzymatically activated. However, final activation of the IKK complex requires SUMOylation of IKKγ and subsequent monoubiquitination of IKKγ at lysine 285, identified by "selected reaction monitoring" (SRM) mass spectrometry (MS) (collaboration with Gunnar Dittmar). In ongoing studies, we investigate characteristics of the genotoxic pathway *in vivo* by bioimaging, taking advantage of NF-κB-luciferase reporter mice. SRM and "stable isotope labeling with amino acids in cell culture" (SILAC) MS is used to characterize posttranslational modifications and to identify further components of the IKK pathway.

The function of non-canonical versus canonical NF-κB in lymphoid tumor cells

Jan Ebert, Eva Kärgel, Kívia A. Pontes de Oliveira, Buket Yilmaz, in cooperation with Miguel Andrade, Gunnar Dittmar, Norbert Hübner, and Jana Wolf

Hodgkin's lymphoma (HL) and many other tumor types rely on IκB kinases (IKK) and NF-κB transcription factors to promote, amongst others, their proliferation and survival. In HL cells, canonical as well as non-canonical IKK/NF-κB signaling is constitutively activated. We investigate at a genome and transcriptome wide level how canonical (p50, p65), non-canonical (p52, IKKα, NIK) and

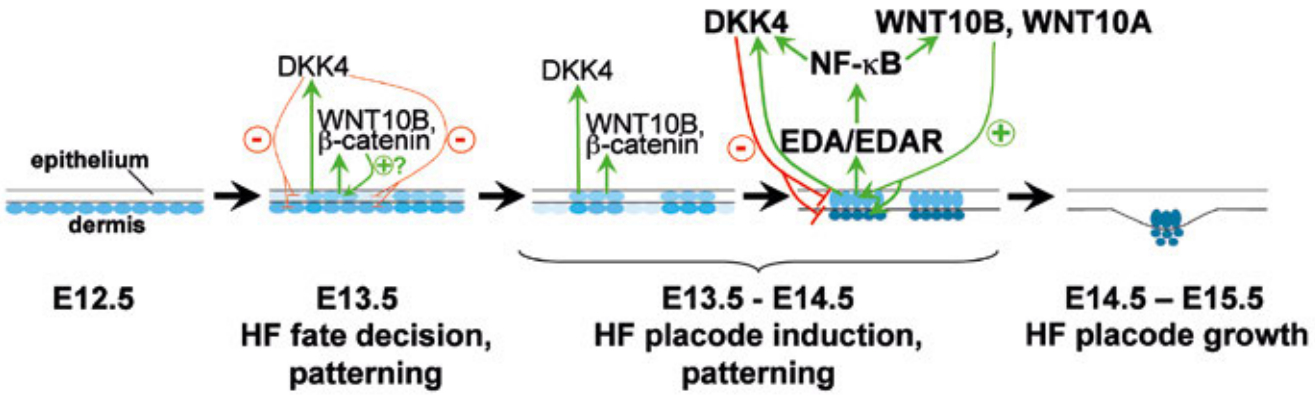

Figure 2. The molecular controls of early hair follicle development in the mouse (see Zhang et al., 2009, for details). At embryonic day E12.5 WNT is uniformly active in the upper dermis. First focal epidermal and dermal WNT activity was observed at E13.5, and is entirely dependent on epidermal WNT/β-catenin activity. The only focally expressed WNT at E13.5 is WNT10b, suggesting that it may be the HF placode inducing WNT. Early *WNT10b* activity initiates expression of the WNT inhibitor Dkk4, which may prevent WNT signaling in the direct surroundings of the developing placode. Between E13.5 and E14.5 Edar expression is focally up-regulated by WNT/β-catenin. This leads to activation of EDA-A1/EDAR/NF-κB signaling, which induces expression of NF-κB target genes, such as Dkk4, Wnt10b and Wnt10a. Higher concentrations of DKK4 protein caused by joint NF-κB and β-catenin transcriptional activity will further refine hair follicle placode patterning. Apart from Dkk4, EDA-A1/EDAR/NF-κB signaling also maintains expression of Wnt10b, and perhaps also of WNT10a and Lef-1, leading to a positive feedback loop between WNT and EDA-A1 signaling.

accessory components (Bcl-3) of the NF-κB system may control distinct tumor-biological functions and how gene specificity is reached. The final aim is a full dissection of the IKK-dependent oncogenic network in HL cells in order to predict novel therapeutic targets. We have determined the complete genomic binding maps for canonical and non-canonical NF-κB species by ChIP-sequencing and characterized their differential transcriptomic impact, using siRNA-mediated knockdown.

In complementary studies we investigate the mechanisms and structures that underlie non-canonical NF-κB signaling, e.g. p100 to p52 conversion, using biochemical analyses, MS-based quantification of pathway components and mathematical modelling.

A functional interplay of TAK1 and RIP1 determines the decision between TNFα-induced apoptosis versus programmed necrosis

Seda Çöl Arslan

One of the best characterized stimuli of canonical NF-κB signaling is TNFα. By binding to TNF receptor I (TNF-RI), TNFα induces the formation of distinct multiprotein complexes, which trigger NF-κB activation, but also lead to apoptosis or receptor-induced programmed necrosis in cells that cannot activate NF-κB mediated gene expression. We have analyzed the molecular network that influences these death decisions. We could demonstrate how TAK1 specifically protects cells from TNFα-induced, RIP1-dependent programmed necrosis. Ongoing studies adress the regulation of RIP1 in this process by postranslational modifications.

Mutual requirements of Eda-A1/Edar/NF-κB and Wnt/β-catenin signaling in early ectodermal organogenesis

Karsten Krieger, Ruth Schmidt-Ullrich, Philip Tomann

To elucidate the *in vivo* function of NF–κB in embryonic development and organogenesis, we use different loss-of-function, gain-of-function and reporter mouse models. Previously, we could demonstrate 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. Hair follicle development requires intense molecular interactions between the early embryonic ectoderm and the underlying mesenchyme. This leads to focal formation of an epithelial thickening (placode), which will grow into the mesenchyme and eventually give rise to a hair follicle. 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*. WNT/β-catenin signaling specifies the initial hair fate decision of epidermal keratinocytes, while NF-κB appears to be required for hair placode growth. We have demonstrated that WNT/β-catenin is initially activated independently of EDA-A1/EDAR/NF-κB, but depends on NF-κB activity for focal hair placode patterning. In contrast, initial EDA-A1/EDAR/NF-κB signaling is not activated in skin of mice expressing the secreted WNT inhibitor DKK1, or lacking epithelial β-catenin. In this context, we have shown that WNT/β-catenin is essential for NF-κB activation and that Edar is a direct WNT/β-catenin target gene. However, at later time points of hair follicle development, localized WNT activity disappears from EDA-A1/EDAR/NF-κB mutant skin, implying that EDA-A1/EDAR/NF-κB is required for the maintenance of WNT signaling (Fig. 2). We found that NF-κB is needed for placodal up-regulation of *Wnt10a* and Lef-1 and that *Wnt10b* is a direct NF-κB target gene. Our data reveal a complex interplay and interdependence of WNT/β-catenin and EDA-A1/EDAR/NF-κB signaling. To unravel further functions of NF-κB in ectodermal organogenesis, we currently investigate NF-κB-dependent target gene signatures in hair follicle development. Our preliminary data suggests that NF-κB has a multifunctional role in hair follicle induction, including the regulation of stem cell markers. The function of NF-κB in hair follicle stem cells is now under investigation.

Selected Publications

Hinz, M, Broemer, M, Çöl Arslan, S, Dettmer, R, Scheidereit, C. (2007). Signal-responsiveness of IκB kinases is determined by Cdc37-assisted transient interaction with Hsp90. J. Biol. Chem. 282, 32311-32319.

Zhang, Y, Tomann, P, Andl, T, Gallant, N, Huelsken, J, Jerchow, B, Birchmeier, W, Paus, R, Piccolo, S, Mikkola, ML, Morrisey, EE, Overbeek, PA, Scheidereit, C, Millar, SE, Schmidt-Ullrich, R. (2009). Reciprocal requirements for Eda/Edar/NF-κB and Wnt/β-catenin signaling pathways in hair follicle induction. Dev. Cell 17, 49-61.

Stilmann M, Hinz M, Arslan SÇ, Zimmer A, Schreiber V, Scheidereit C. (2009). A nuclear poly(ADP-ribose)-dependent signalosome confers DNA damage-induced IkappaB kinase activation. Mol Cell 36, 365-78.

Hinz M, Stilmann M, Arslan SÇ, Khanna KK, Dittmar G, Scheidereit C. (2010). A cytoplasmic ATM-TRAF6-cIAP1 module links nuclear DNA damage signaling to ubiquitin-mediated NF-κB activation. Mol Cell 40, 63-74.

Arslan SÇ and Scheidereit C. (2011). The prevalence of TNFα-induced necrosis over apoptosis is determined by TAK1-RIP1 interplay. PLoS One 6(10):e26069

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* part of the period reported

Walter Birchmeier

Signals Provided by Wnt/β-catenin and Met/Gab1/Shp2 in Development and Cancer

The molecular and functional analysis of cell adhesion and signaling in development and tumor progression has been the major focus of my laboratory. We discovered that β-catenin, which is a central component of canonical Wnt signaling, binds to the transcription factors LEF/TCF and mediates gene expression. We have recently used conditional loss and-gain-of-function mutations of β-catenin in mice to study the role of canonical Wnt signaling in the development of precursor and stem cells in skin, heart, limbs and brain, and in cancer stem cells of the skin. We have also studied Wnt/β-catenin signaling in metastasis of human tumors.

A second interest of the lab is the role of scatter factor/hepatocyte growth factor and Met receptor signaling in development and tumor progression. We showed by genetic means in mice that downstream of Met, signaling of Gab1 through the tyrosine phosphatase Shp2 controls migration of muscle precursor cells to the limbs. Shp2 also acts downstream of many other receptor tyrosine kinases, for instance in Schwann cell development and in kidney branching.

In the present report period, we have performed the following investigations on Wnt/β-catenin and Met/Shp2 signaling:

Combined Met and Wnt Signaling Drive Mammary Gland Cancer Stem Cells

Jane Holland, Klaus Eckert, Balasz Gyorffy (Charité Berlin) and Walter Birchmeier

We generated mice that express gain-of-function mutations of β-catenin and hepatocyte growth factor under the WAP promoter (WAP-ΔNβ-catn; HGF). Compound mutant mice underwent rapid tumor formation as early as one week postpartum. $CD24^{hi}/CD29^{+}$ surface profiling showed that the combined activation of Wnt and Met gave rise to the expansion of mammary cancer stem cells (MaCSCs) at the expense of normal progenitor and stem cell pools. Treatment using pharmacological inhibitors of Wnt and Met on the MaCSC revealed that, remarkably, the Wnt pathway impinges on stemness, while the Met pathway functions to regulate cell fate specification. Gene expression analyses identified several key interacting pathways such as CXCR4, which were regulated by Wnt and Met crosstalk. We also crossed in a loss-of-function CXCR4 mutation into the WAP-ΔNβ-catn; HGF mice, and found that the absence of CXCR4 could significantly delay tumor onset of Wnt/Met driven tumors.

In a cohort of 3,500 patient and 21 public datasets, our mouse signature of 322 genes controlled by Wnt/β catenin and Met was capable of discriminating between molecular subtypes of human breast cancer. The

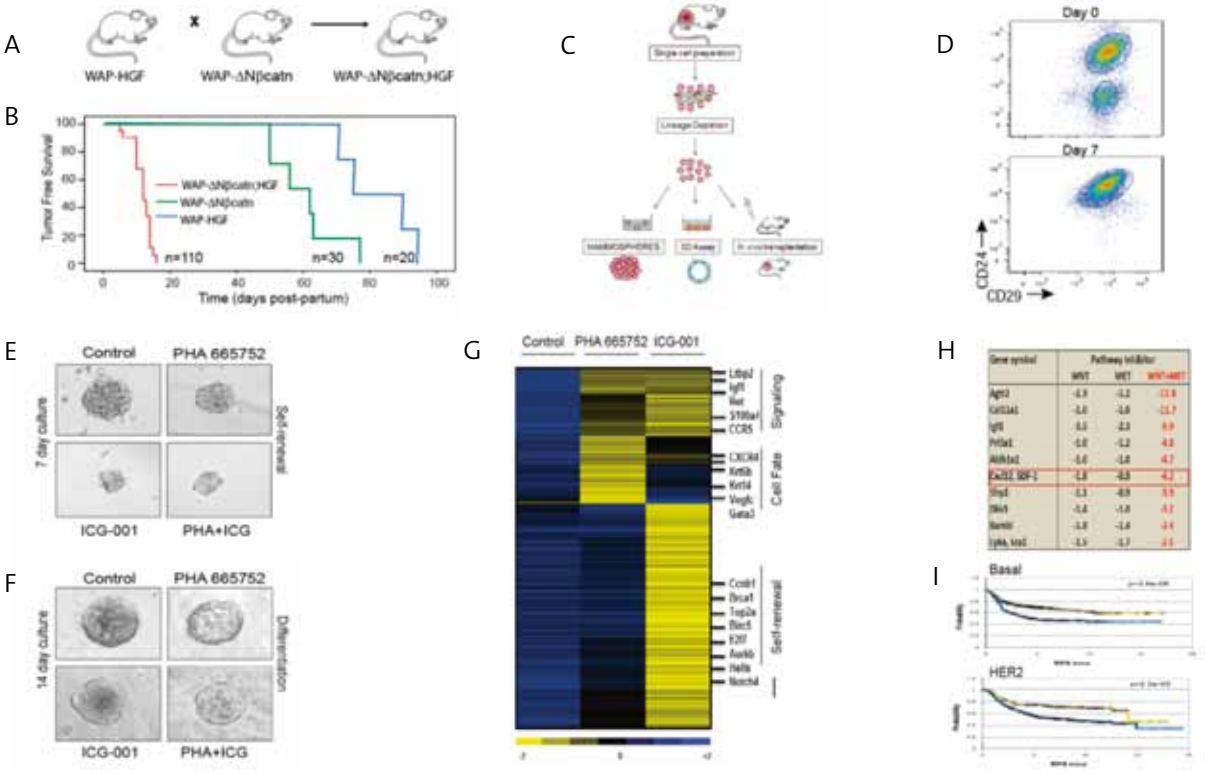

Figure 1. Cooperation of the Wnt and Met Signaling Pathways Drives Mammary Gland Tumorigenesis Through the Activation of Mammary Cancer Stem Cells.

(**A**) Investigation into the cross-talk of the Wnt and Met signaling pathways during mammary gland tumorigenesis was facilitated by the generation of a compound mutant mouse expressing gain-of-function β-catenin and HGF, under the WAP promoter (WAP-ΔNβ-catn;HGF). (**B**) Compound mutant mice underwent rapid tumor formation as early as 1 week postpartum, whilst single mutant mice showed signs of tumor growth after 52 weeks. (**C**) Freshly isolated tumor cells cultured in serum-free, anchorage independent conditions were measured for their ability to form mammospheres (self-renew), grow acini structures in 3D-matrigel conditions (differentiate) or produce outgrowths when transplanted in cleared mammary fat pads of NOD/SCID/Il2$^{-/-}$ mice (tumor recapitulation). (**D**) Isolated mammary cancer stem cells (MaCSCs) grown as mammosphere cultures over 7 days were efficiently enriched for MaCSCs. (**E, F**) Treatment of MaCSCs using inhibitors for the Wnt (ICG-001) or Met (PHA 665752) pathway could show that the Wnt pathway controls proliferation (stemness), while the Met pathway functions to regulate cell fate specification (differentiation), i.e. the inversion from hollow cysts to filled transformed structures. (**G**) Inhibitor treatment was also followed by Affymetrix gene profiling, and a heat map is shown. Stemness genes (Top2a, Birc5 and Hells) and fate-determining genes (CXCR4, Krt6a and Gata3) were indeed downregulated by Wnt and Met inhibitor treatment, respectively. (**H**) Top 10 ranking genes synergistically regulated by the combination of Wnt and Met inhibitors were tabulated. CXCL12/SDF1 importance could be proven genetically (not shown). (**I**) An intrinsic mouse gene set was generated and used to discriminate between molecular subtypes of human breast cancer. This gene signature correlated best to the survival of patients with Basal and HER2 breast cancers.

best correlation to survival was achieved in the Basal and HER2 subgroup of breast cancer patients. Furthermore, the expression of CXCL12 also provided a strong predictor for patient's survival. Taken together, our findings point to an important interplay between Wnt and Met signaling that regulate intrinsic MaCSC programs during mammary gland tumorigenesis.

The tyrosine phosphatase Shp2 acts downstream of GDNF/Ret in branching morphogenesis of the developing mouse kidney

Regina Willecke, Julian Heuberger, Kai Schmidt-Ott, Katharina Walentin, Katja Grossmann (Salk Institute, La Jolla, USA), Odyssé Michos and Frank Costantini (Columbia University, New York, USA) and Walter Birchmeier

We have ablated Shp2 in the developing kidneys of mice using the ureteric bud epithelium-specific Hoxb7/Cre. Mutant mice produced a phenotype that is similar to mutations of the genes of the GDNF/Ret receptor system, that is: strongly reduced ureteric bud branching and downregulation of the Ret target genes Etv4 and Etv5. Shp2 mutant embryonic kidneys also displayed reduced cell proliferation at the branch tips and branching defects, which could not be overcome by GDNF in organ culture. We also examined compound mutants of Shp2 and Sprouty1, which is an inhibitor of receptor tyrosine kinase signaling in the kidney. Sprouty1 single mutants produce supernumerary ureteric buds, which branch excessively. Sprouty1 mutants rescued branching deficits in Ret$^{-/-}$ and GDNF$^{-/-}$ kidneys. We found that Sprouty1; Shp2 double mutants showed no rescue of kidney branching. Our data thus indicate an intricate in-

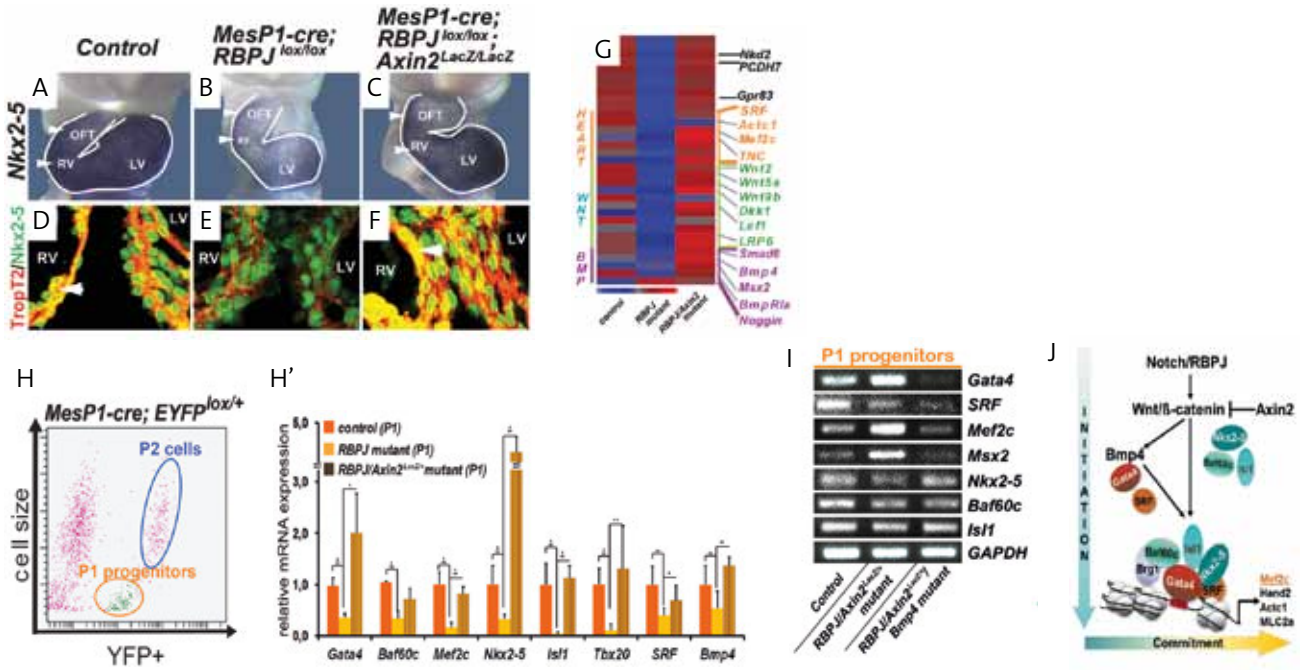

Figure 2. Wnt/β-catenin and Bmp signals control distinct sets of transcription factors in cardiac progenitor cells
In vivo analysis of cardiac morphogenesis and differentiation in control, *RBPJ* single and *RBPJ/Axin2* double mutant embryos at E9.25. (**A-C**) Whole-mount in situ hybridization for *Nkx2-5*. (**D-F**) Immunohistochemistry for TropT2 (red) and Nkx2-5 (green). (**G**) Heatmap of gene expression arrays. The restored expression of particular genes is marked by arrowheads in D,F and on the right in G. Orange in G stands for cardiac-specific genes, green for Wnt genes and magenta for Bmp-controlled genes in *RBPJ/Axin2* double mutant embryos. Note that the *RBPJ* mutation produces severe defects in second heart field (SHF) morphogenesis, but that cardiac morphogenesis and the expression of crucial genes is restored by crossing in the *Axin2* mutation. LV, left ventricle; OFT, outflow tract; RV, right ventricle.

Analysis of isolated cardiac progenitors of controls, *RBPJ* single, *RBPJ/Axin2*$^{LacZ/+}$ double and *RBPJ/Axin2*$^{LacZ/+}$*/Bmp4* triple mutants using (**H**) FACS of YFP+ cells. Two populations of YFP+ cells: P1-progenitors, orange; P2-cells, blue were identified. (**H'**) Relative mRNA expression levels of indicated mRNAs in the P1 progenitor population of controls (orange bars), RBPJ single mutants (yellow bars) and double mutants (yellow-black-striped bars). Note that the expression of crucial cardiac transcription factors and *Bmp4* is attenuated in RBPJ single and is restored in double mutant SHF progenitors (P1). Error bars represent SEM, (*) P<0.05, (**) P<0.01, (***) P<0.005. (**I**) Relative mRNA expression of indicated mRNAs in FACS P1 progenitor cells of controls, *RBPJ/Axin2*$^{LacZ/+}$ double and *RBPJ/Axin2*$^{LacZ/+}$*/Bmp4* triple mutants. Note that the expression of *Gata4, SRF* and *Mef2c* is abolished in SHF progenitors of triple mutants indicating that these are Bmp-controlled genes. The expression of *Nkx2-5, Baf60c* and *Isl1* is unaltered in double and triple mutant SHF progenitors suggesting that these are Wnt-controlled genes. (**J**) Model depicting the sequential regulation of cardiac-specific transcription factors by Notch, canonical Wnt and Bmp signaling in the morphogenesis of the SHF (E9.25).

terplay of Shp2 and Sprouty1 in signaling downstream of receptor tyrosine kinases during kidney development. Apparently, Shp2 mediates not only GDNF/Ret but also signaling by other receptor tyrosine kinases in branching morphogenesis of the embryonic kidney.

Wnt/β-catenin activity is essential in altering the epigenetic state of salivary gland stem cells to create cancer stem cells

Peter Wend, Liang Fang, Qionghua Zhu, Frauke Kosel (MDC), Jörg Schipper and Simone Hindersin (University Clinic Düsseldorf), Christoph Loddenkemper (Charité Berlin), Volker Brinkmann (Max Planck Institute for Infection Biology Berlin), Michael Kahn (University of Southern California, Los Angeles, USA), Ulrike Ziebold* and Walter Birchmeier* (*equal senior authors) (MDC)

Our analysis of aggressive human squamous cell carcinomas (SCCs) of the salivary gland and head and neck in general suggested a link to the Wnt/β-catenin and Bmp signaling systems. Using a genetically modified mouse strain, in which Wnt signaling was upregulated and Bmp suppressed, we found that Wnt/β-catenin promotes the transformation of normal stem cells into cancer stem cells through an epigenetic mechanism. Mouse SCCs of the salivary gland contained high numbers of CD24^{+}CD29^{+} cancer stem cells, of which as few as 500 sufficed to cause tumors when transplanted into NOD/SCID mice. Mice in which only one of the signaling systems was altered, more efficient tissue regeneration but no tumors occurred. We discovered that the difference of normal compared to cancer stem cells in the salivary gland is an upregulation of specific pluripotency genes as well as global changes in trimethylated lysine 4 and 27 of histone 3. This indicates an increase of active

chromatin and a decrease in the repressive form. Cancer stem cells of the salivary gland grew as non-adherent spheres and retained the capacity for differentiation, if β-catenin was suppressed by siRNA or the chemical inhibitor ICG-001. This depended on repressive chromatin, as shown by the fact that 5-azacytidine or HDAC inhibitors restored stemness.

Wnt/β-catenin and Bmp signals control distinct sets of transcription factors in cardiac progenitor cells

Alexandra Klaus, Marion Müller, Herbert Schulz, Yumiko Saga (Natl. Institute of Genetics, Mishima Shizuoka, Japan), James F. Martin (Texas A&M Sytem Heart Science Center, Houston, USA) and Walter Birchmeier

Progenitor cells of the first and second heart fields depend on cardiac-specific transcription factors for their differentiation. Here we define by genetic means the hierarchy of signaling events that controls the expression of cardiac-specific transcription factors in mouse embryos, and show that Wnt and Bmp act downstream of Notch/RBPJ during initiation and commitment of second heart field progenitors. Mutation of *Axin2* enhances canonical Wnt signaling and suffices to rescue the cardiac differentiation arrest caused by loss of RBPJ, the major transcriptional mediator of Notch signals. Analysis of isolated cardiac progenitors, embryo cultures and further genetic experiments defined the crosstalk between Wnt, Bmp and Notch, and classified the expression of heart-specific transcription factors according to their dependence on Wnt (*Nkx2-5*, *Isl1*, *Baf60c*) and Bmp (*Gata4, SRF, Mef2c*) signals.

BCL9-2 Promotes Early Stages of Intestinal Tumor Progression.

Felix Brembeck, Maria Wiese and Nathalie Zatula (University Göttingen) and Tamara Grigoryan, Yiyang Dai, Johannes Fritzmann and Walter Birchmeier

We examined the roles of the two BCL9 and the two Pygopus genes in canonical Wnt signaling of normal and transformed intestinal epithelia in mice and humans. Specific antibodies against the four proteins were generated in rabbits. BCL9 and Pygopus2 proteins were produced in all normal intestinal and colon cancer cells. BCL9-2 was detectable only in the villi, not in the crypts of the normal intestine. However, BCL9-2 was upregulated in adenomas and in almost all colon tumors. Accordingly, transgenic overexpression of BCL9-2 in the intestine of BCL9-2; APC(Min/+) mice increased the formation of adenomas that progressed to invasive tumors, resulting in reduced survival time. Using small interfering RNA analysis, we found that BCL9s and Pygopus are not direct targets of canonical Wnt in colon cancer cells, but Wnt signaling correlated with levels of BCL9-2. BCL9-2 regulated the expression of β-catenin-dependent and independent target genes that have been associated with early stages of intestinal tumorigenesis. We conclude that BCL9-2 promotes early phases of intestinal tumor progression in humans and in transgenic mice. BCL9-2 increases the expression of a subset of canonical Wnt target genes but also regulates genes that are required for early stages of tumor progression.

Selected Publications

Brembeck, FH, Wiese, M, Zatula, N, Grigoryan, T, Dai, Y, Fritzmann, J, Birchmeier, W. (2011). BC 2 Promotes Early Stages of Intestinal Tumor Progression. Gastroenterology, 141, 1359-1370.

Grossmann, K, Wende, H, Paul, FE, Garratt, AN, Fineberg, K, Besser, D, Schulz, H, Selbach, M, Birchmeier, W, Birchmeier, C. (2009). The tyrosine phosphatase Shp2 directs Neuregulin-1/ErbB signaling throughout Schwann cell development. Proc. Natl. Acad. Sci. USA 106, 16704-16709.

Fritzmann, J, Morkel, M, Besser, D, Budczies, J, Kosel, F, Brembeck, FH, Stein, U, Fichtner, I, Schlag, PM, Birchmeier, W. (2009). A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential. Gastroenterology 137, 165-175.

Klaus, A, Saga, Y, Taketo, MM, Tzahor, E., Birchmeier, W. (2007). Distinct roles of Wnt/ß-catenin and Bmp signaling during early cardiogenesis. Proc. Natl. Acad. Sci. USA 104, 18531-18536.

Chmielowiec, J, Borowiak, M, Morkel, M, Stradal, T, Munz, B, Werner, S, Wehland, J, Birchmeier, C, Birchmeier, W. (2007). c-Met is essential for wound healing in the skin. J. Cell Biol. 177, 151-162.

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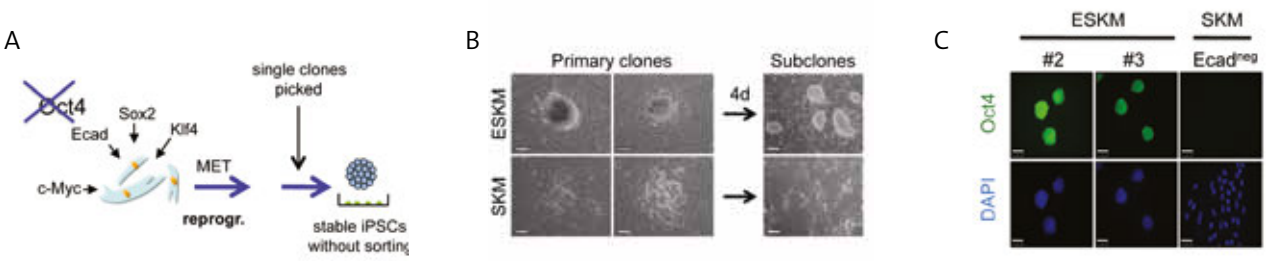
Signaling Mechanisms in Embryonic Stem Cells

Embryonic stem cells (ESC) are pluripotent cells, which can proliferate indefinitely and participate in the formation of most cell types. Studies on human ESCs (hESC) provide an insight into human embryogenesis and the development of tools for pharmacology and regenerative medicine. The focus of our studies is the maintenance of the pluripotent state in murine ESCs (mESCs) and hESCs. We found that the Activin/Nodal pathway is activated in pluripotent hESCs and blocked upon differentiation. Pluripotent cells require these signaling regulating a specific subset of target genes. In addition, the BMP pathway is in the off-state in pluripotent cells and activated upon differentiation. This pathway counteracts the effects of Activin signaling. Moreover, it has been shown that somatic cells for various sources can be reprogrammed by transfection of four defined transcription factors, i.e. Oct4, Sox2, Klf4, and c-Myc, to a pluripotent state. These induced pluripotent stem cells (iPS) are very exciting new cell populations for stem cell research.

Role of TGFβ signaling in pluripotency of embryonic stem cells

(PhD student: Angel Quiroga-Negreira, funded by the DFG, SPP1109 Embryonal and Tissue-specific Stem Cells and HGF-systems biology NW1 SB Cancer DKFZ.I.4)

In this project we are analyzing the effects of TGFβ signaling in ESCs in the regulation of pluripotency and differentiation. Our results suggest that blockage of Activin signaling is able to induce differentiation, at least to a certain extent, in mESCs growing undifferentiated in the presence of LIF. Furthermore, genetic programs modulated after Activin blockage with a chemical inhibitor were shown to be significantly coincident with those regulated upon LIF withdrawal. In addition, global expression comparative analysis of genetic program regulation between human and mouse ESCs revealed an approaching tendency between BMP4 mediated differentiation in hESCs and that caused by LIF removal in mESCs. It was shown that human and mouse ESCs are activating Wnt signaling under these conditions, by up-regulating key transcription factor Lef1 and by modulating the expression of several Wnt ligands and inhibitors. Both cell lines regulated the Cdx/Hox gene cluster in the referred differentiating conditions and this regulation was dependent on Wnt signaling, indicating the importance of this pathway during gastrulation and embryo patterning. Thus, the undifferentiated state may not only be preserved by a high Activin and low Bmp signaling status but also by Wnt inhibition through the expression of Wnt-inhibitors which are rapidly down-regulated upon differentiation. Wnt induction alone is able to reduce pluripotency in hESCs but not in mESCs. A model is put forward in which as an initial step Bmp signaling is required to abandon the undifferentiated state and initiate differentiation into meso-endodermal lineages, and in a second step Wnt signaling is required to induce genes responsible for the embryo pattering organization.



E-cadherin can replace Oct4 in reprogramming. **(A)** Derivation of ESKM-induced pluripotent stem cell clones following viral transduction of MEFs in the presence of Ecad but the absence of Oct4 (ESKM). Transduced MEFs were seeded and embryonic-stem-cell-like colonies were observed. **(B)** Morphology of two independent primary clones photographed 12 days after viral transduction of MEFs with ESKM (upper panel) or SKM (without E-cadherin, lower panel). **(C)** Immunofluorescence analysis of established ESKM (2, 3) and SKM cells for expression of Oct4 (green). Nuclei were stained with DAPI (blue).

Regulation of Oct4: How the regulator is regulated (MDC)

(PhD students: Sebastian Diecke and Angel Quiroga-Negreira)

The maintenance of pluripotency in embryonic stem cells (ESC) is a complex process that is mediated by the interaction of different molecular mechanisms. At the core of these molecular mechanisms lies the autoregulatory complex of the transcription factors Oct4, Sox2 and Nanog. In general differentiation of the ESCs is associated with the downregulation of these core transcription factors. We have shown that spontaneous differentiation of (hESC) leads to morphological and molecular changes without a significant decrease in the expression of the core transcription factor Oct4. Therefore, we hypothesize that posttranslational modifications, such as protein phosphorylation, may decisively influence the function of Oct4. Using two-dimensional gel electrophoresis (2-DG) we established a possible link between the differentiation-associated morphological and molecular changes in the hESCs and the Oct4-phosphorylation state. Furthermore, modulation of the PKA signaling pathway and a point mutation of the potential PKA phosphorylation site caused a change in the Oct4 protein migration pattern in the 2-DG and lead to a change in its DNA binding properties. Finally, we demonstrated that Oct4 phosphorylation not only influences the maintenance of pluripotency in hESCs, but is also important for the induction of pluripotency in somatic cells. In conclusion, this study provides new insights into the regulatory mechanisms of the core transcription factor Oct4 and also provides a direct link between Oct4 phosphorylation and somatic cell reprogramming.

Deciphering mechanisms during reprogramming of mouse embryonic fibroblasts to pluripotent stem cells

(PhD students: Torben Redmer and Sebastian Diecke, funded by START-MSC Consortium, BMBF joint project grant)

We report new functions of the cell-adhesion molecule E-cadherin in murine pluripotent cells. E-cadherin is highly expressed in mouse embryonic stem cells, and interference with E-cadherin causes differentiation. During cellular reprogramming of mouse fibroblasts by Oct4, Sox2, Klf4 and c-Myc (OSKM), fully reprogrammed cells were exclusively observed in the E-cadherin positive cell population and could not be obtained in the absence of E-cadherin. Moreover, reprogrammed cells could be established by viral E-cadherin in the absence of exogenous Oct4 (see figure). Thus, reprogramming requires spatial cues that cross-talk with essential transcription factors. The cell-adhesion molecule E-cadherin has important functions in pluripotency and reprogramming.

Selected Publications

Redmer, T., Diecke, S., Grigoryan, T., Quiroga-Negreira, A., Birchmeier, W., and Besser, D. (2011) E-cadherin is crucial for ESC pluripotency and can replace OCT4 during somatic cell reprogramming. EMBO Rep., 12, 720-726.

Chirasani, S.R., Sternjak, A., Wend, P., Momma, S., Campos, B., Herrmann, I.M., Graf, D., Mitsiadis, T., Herold-Mende, C., Besser, D., Synowitz, M., Kettenmann, H., and Glass, R. (2010) Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 133, 1961-1972.

Haurie, V., Durrieu-Gaillard, S., Dumay-Odelot, H., Da Silva, D., Rey, C., Prochazkova, M., Roeder, R.G., Besser, D., and Teichmann, M. (2010) Two isoforms of human RNA polymerase III with specific functions in cell growth and transformation. Proc. Natl. Acad. Sci. U S A. 107, 4176-4181.

Grossmann, K., Wende, H., Paul, F., Garratt, A., Feinberg, K., Besser, D., Schulz, H., Selbach, M., Birchmeier, W., and Birchmeier, C. (2009) The tyrosine phosphatase Shp2 directs Neuregulin-1/ErbB signaling throughout Schwann cell development, Proc. Natl. Acad. Sci. USA. 106, 16704-16709.

Fritzmann, J.*, Morkel, M.*, Besser, D. *, Budczies, J., Kosel, F., Brembeck, F.H., Stein, U., Fichtner, I., Schlag, P.M., and Birchmeier, W. (2009) A Colorectal Cancer Expression Profile that Includes Transforming Growth Factor Inhibitor BAMBI Predicts Metastatic Potential. Gastroenterology, 137, 165-75 (* equal contribution).

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Regulation of Cell Shape Dynamics by Rho GTPase Proteins

Cells continuously change their shape and their attachment to the environment in order to move or to adopt a specialized structure. These events are fundamental during embryonic development, immune surveillance or wound repair. The reorganization of the cytoskeleton and the adhesion machinery thereby is highly dynamic and requires precise coordination. Any deregulation can contribute to cancer metastasis and various developmental, cardiovascular and neurological disorders. 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 specificity in Rho signaling and thus proper cell responses. Our aim is to identify mechanisms that couple Rho GTPase activity to specific environmental cues and functional contexts in a cell. Insights into this spatio-temporal regulation will provide a deeper understanding of the processes that drive specific cell morphology changes, in normal and disease settings.

Signaling Specificity of Rho GTPases

Rho GTPases are molecular switches that cycle between an 'on' and '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 mediate the sequestration of the membrane anchored Rho GTPases into the cytosol. The Rho family comprises about 20 members of which the most prominent ones are the RhoA, Rac1 and Cdc42 proteins. Numerous Rho-regulated signaling pathways have been outlined over the past years, many of which drive different aspects of cellular morphodynamics. The different members of the Rho family thereby have distinct functions and, in cells, can collaborate or antagonize each other. Cell movement or shape change therefore is the result of a complex interplay of these pathways.

Morphological changes occur in response to different environmental cues and in the framework of very different signaling networks, such as in neuronal cells or lymphocytes. However, Rho proteins do not have any intrinsic features that allow them to select their protein environment for signal transduction. Major challenge in the field therefore is to understand specificity in Rho GTPase signaling. The question is how the cell manages to restrict the activity of these universal switch proteins to a specific subcellular signaling network in response to a specific stimulus, and then to generate the desired biological output. The spatial and temporal information to facilitate such selectivity must be mostly contributed by the RhoGEF/GAP/GDI regulatory proteins. Notably, in contrast to the related Ras family GTPases, deregulated Rho GTPase signaling in pathological settings is mostly caused by alterations at the level of the regulatory proteins and not by mutations of the Rho proteins themselves.

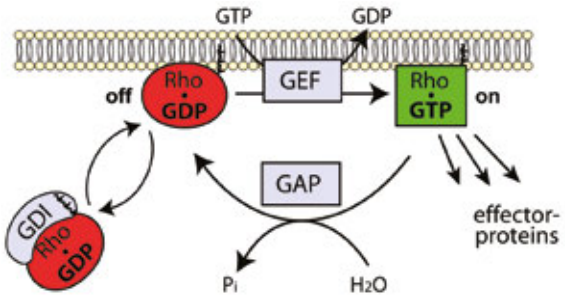

Figure 1. Schematic representation of the molecular switch function of Rho GTPases and the function of their regulatory proteins.

Figure 2. Modular domain architecture of the human RhoGEF and RhoGAP proteins.

RhoGEFs and RhoGAPs

The genome encodes over 145 different RhoGEFs and GAPs which cover a diverse spectrum of subcellular targeting and protein interaction domains. It is conceivable that their multi-domain architecture helps to assemble specific signaling complexes. However, the majority of the RhoGEFs and GAPs is only poorly characterized and systematic studies to identify common functional properties have not been performed so far. Thus, the whole cellular repertoire of context-specific Rho regulation has not been explored and general concepts of signaling specificity are yet to be uncovered. A preceding proteomics project yielded a complete RhoGEF/GAP cDNA library and interactome dataset which we now exploit. Based on this knowledge, we are currently characterizing novel RhoGEFs and GAPs with implications in cell adhesion, growth cone motility and guided cell migration. We use live cell microscopy and biosensors to obtain information on appropriate spatial and temporal microscales and will employ three-dimensional cell culture systems which better reflect biological processes in the organismal context. By expanding our studies to contextually related GEFs and GAPs we aim for a broader understanding of the regulation of these different signaling networks. We therefore explore common functional protein modules in the interactomes and investigate how these modules are individually adapted. In an orthogonal approach, we try to delineate general determinants of signaling specificity that are encoded in the multidomain architecture of the RhoGEFs/GAPs. Individual functional building blocks on these proteins will be used to rewire the signaling properties of unrelated regulatory proteins.

Temporal framework of Rho GTPase signaling

Very little is known also about the time 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 is technically difficult to be studied in cells. We employ state-of-the-art imaging approaches to analyze the dynamics of membrane interaction of Rho GTPases and how it is affected 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 modulation.

Selected Publications

Rocks, O., Gerauer, M., Vartak, N., Koch, S., Huang, ZP., Pechlivanis, M., Kuhlmann, J., Brunsveld, L., Chandra, A., Ellinger, B., Waldmann, H., Bastiaens, PI. (2010) The Palmitoylation Machinery Is a Spatially Organizing System for Peripheral Membrane Proteins. Cell 141(3):458-471

Dekker, FJ.*, Rocks, O.*, Vartak*, N., Menninger*, S., Hedberg, C., Balamurugan, R., Wetzel, S., Renner, S., Gerauer, M., Schölermann, B., Rusch, M., Kramer, JW., Rauh, D., Coates, GW., Brunsveld, L., Bastiaens, PI., Waldmann, H. (2010) Small-molecule inhibition of APT1 affects Ras localization and signaling. Nat Chem Biol 6(6):449-56 *equal contribution

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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Ulrike Ziebold

Genetics of Tumor Progression and Metastasis

The mammal cell cycle has been extensively studied nevertheless its intricacies remain elusive. Recent evidences suggest that in embryonic and cancer stem the cell cycle and the potential for stemness are tightly connected.

To tackle these complex questions, we have modified murine embryonic type stem cells characterizing both transcriptional and signalling modules in stemness, proliferation or differentiation. Similarly, we use mouse models to define the molecular circuitry underlying cancer causing stem cells. Central to our work is the understanding of the molecular consequences of deregulation of the retinoblastoma protein (RB) and E2Fs, since in most human tumors the RB pathway is mutated leading to unfettered activity of the E2F transcription factors. To reveal how E2Fs control growth, we search for interaction partners and analyze chromatin modifiers. Lastly, we analyze the E2F3-specific contribution to tumour progression by identifying novel metastasis specific targets. In summary, our goal is to understand how specific biological processes as cellular stemness, growth control and suppression of tumorigenesis are coupled.

Interaction partners and possible co-activators of E2F3

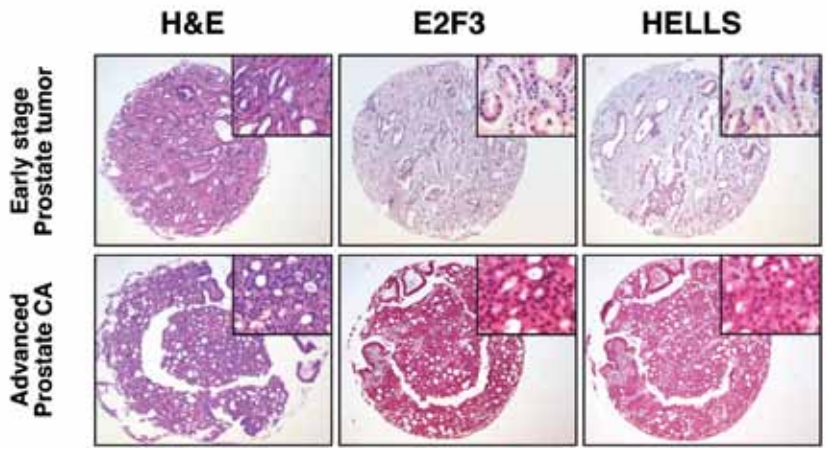
Björn von Eyss and Katharina Möllmann

The RB-tumorsuppressor pathway depends on the activity of the E2F-transcription factors, since E2F3 contributes extensively to the ectopic S-phase and apoptosis in tumor-prone pRB mutant mice. Searching for specific interaction partners of E2F3, we identified HELLS, a SNF2-like helicase with putative chromatin regulatory function. We show that the SNF2-like helicase HELLS interacts with E2F3 *in vivo* and cooperates with its oncogenic functions. Depletion of HELLS severely perturbs the induction of E2F-target genes, hinders cell cycle re-entry and growth. Using chromatin immunoprecipitation coupled to sequencing we identified genome-wide targets of HELLS and E2F3. Our analysis revealed that HELLS binds promoters of active genes, including the trithorax-related MLL1, and co-regulates E2F3-dependent genes. Our analysis is the first to link HELLS with E2F-controlled processes that are critical to establish a proliferative tumour circuitry. Strikingly, just as E2F3, HELLS is overexpressed in human tumours including prostate cancer, indicating that either factor may contribute to the malignant progression of tumours. Our work reveals that HELLS is important for E2F3 in tumour cell proliferation.

E2F target genes in proliferation and metastasis

Kirsten Vormbrock

E2f3 is the only E2F amplified in human tumours, whereby high E2F3 expression marks invasiveness predicting poor patient survival. This led to the hypothesis that E2F3 regulates unique sets of target genes. To search for such targets we use a genetically defined model to study the transcriptional events that are different in benign medullary thyroid carcinomas (MTCs) as compared to metastatic MTCs in pRB/E2F3 deficient mice. We transcriptionally profiled the tumours resulting in a cohort of putative metastatic biomarkers. Importantly, some of these markers are also markers of human metastatic thyroid malignancies. Importantly, these markers all fall into the class of genes responsible for chromosomal instability (CIN). Thus, we link individual E2F-target genes with CIN and the onset of metastasis.



The Snf2-like helicase, HELLS, cooperates with E2F3. It is known that the E2F3 expression increases with the aggressiveness of prostate cancer. Here, we show that the increase also correlates with the expression of HELLS, suggesting that its activity might also be critical for these tumors. Patient biopsies of prostate cancer (PCA) were assembled into a tissue microarray and representative sections were analyzed by Hematoxylin and Eosin (H&E) and also used for immunohistochemistry to detect either E2F3 or HELLS and was counterstained with Hematoxylin.

The miRNA biogenesis and the control of proliferation

Sebastian Memczak in collaboration with Nikolaus Rajewsky

The highly conserved E2F transcription factor family controls cell cycle progression by activation or repression of target genes. Since an aberrant expression of the transcriptional activator E2F3 is associated with unscheduled proliferation leading to cancer, we searched for novel target genes. We identified target genes that are involved in the biogenesis of small non-coding RNAs (miRNAs). The expression of these miRNA-biogenesis-associated proteins was found to be strictly cell cycle controlled on both mRNA as well as on protein level. Since our finding may have consequences for diseases as cancer, we investigated the cell cycle and E2F3 dependent expression and procession of primary miRNA transcripts versus mature miRNAs. To mirror an aberrantly regulated E2F3 activity we are also applying a mouse model that is deficient for pRB, where tumours develop. Our analyses will gain more insight into the interplay between the miRNA and the pRB/E2F-pathway in proliferation and carcinogenesis.

Molecular determinants of cancer stem cells activity

In collaboration with Peter Wend and Walter Birchmeier

Recent evidence suggests that ES as well as cancer stem cells share core transcriptional and signalling modules. This points towards the fact that both cell types share common ancestry. We deployed genetic mouse models and studied Wnt/β-catenin and Bmp signalling in the salivary gland tumours, since these tumours contain significant amounts of cancer stem cells. We characterized these β-catenin/BMP-receptor mutant cancer stem cells and show that they are characterized by embryonic-pluripotency associated transcriptional and epigenetic traits. Significantly, the cancer stem cells strongly depend on wnt/β-catenin-activities to drive stemness, only abrogation of β-catenin allows the cells to epigenetically reprogram and to differentiate. Our results imply that it is β-catenin that is critical to properly control salivary gland stem cells via tethering chromatin remodelling complexes to target promoters. If β-catenin is depleted or rendered inactive via small molecule inhibition the epigenetic state of the cancer stem cells is altered, leading to a loss in tumorigenicity. In the future, we will dissect the exact transcriptional circuitry of these stem cells to decipher the molecular blueprint of cancer stem cells.

Selected Publications

Ziebold, U., Caron, A., Bronson, R., and Lees, J.A. (2003). E2F3-loss has opposing effects on different pRb-deficient tumors, resulting in suppression of pituitary tumors but metastasis of medullary thyroid carcinomas. Mol. Cell. Biol. 18, 6542-6552.

Boden, C., and Ziebold, U. (2004). Cell division – an Overview, in Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine K. Ruckpaul and D. Ganten, Eds. Springer Verlag, Heidelberg.

Tapon, N. and Ziebold, U. (2008). Invasion and metastasis: stem cells, screens and survival. EMBO Rep. 9, 1078-1083.

Grouwels G, Cai Y, Hoebeke I, Leuckx G, Heremans Y, Ziebold U, Stangé G, Chintinne M, Ling Z, Pipeleers D, Heimberg H, Van de Casteele M. (2010). Ectopic expression of E2F1 stimulates beta-cell proliferation and function. Diabetes 59, 1435-1444.

von Eyss B., Maaskola, J., Memczak, S., Möllmann, K., Schuetz, A., Loddenkemper, C., Tanh, M-D., Otto, A., Muegge, K., Udo Heinemann, U., Rajewsky, N., and Ziebold, U. The SNF2-like helicase HELLS mediates E2F3-dependent transcription and cellular transformation. EMBO J. accepted.

Patents

MIT Case No. 8756 "ANTIBODY TO P10".

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Cell Differentiation and Tumorigenesis

Stem cells, differentiation & C/EBP transcription factors

Multi-potency and self-renewal capacity are somatic stem cell features that disappear during lineage commitment/differentiation and recur during tumorigenic conversion through epigenetic mechanisms. An important challenge in the future application of stem cell based bio-medicine is to understand such instructive cues and to manipulate mechanisms of somatic cell differentiation and stem cell maintenance.

CCAAT Enhancer Binding Protein (C/EBP) transcription factors are epigenetic key players and regulate a wide variety of genes involved in stem cell functions, commitment to differentiation, proliferation, regeneration, metabolism, immunity, senescence, and tumorigenesis, in the intestine, liver, pancreas, lung, skin, mammary gland, and in female reproduction. C/EBPs harbor strong trans-differentiation and instructive potential, such as myeloid lineage commitment and monocyte/granulocyte differentiation in the hematopoietic system. Our research is focused on the dynamic regulation of C/EBPs that balances cellular self-renewal capacity versus differentiation.

Research Topics

Structure & Function of C/EBP

C/EBPs comprise a family of 6 genes in vertebrates. Four members, C/EBPα,β,δ and ε, are highly related whereas two others, C/EBPγ and ζ, are more divergent. C/EBPα,β,δ, and ε display high homology in their C-terminal basic DNA binding leucine zipper domain (bZip), which forms a bifurcated coiled-coil structure and determines interaction with distinct DNA sequences. The first third of the C/EBP N-termini represent strong trans-activation domains (TAD) whereas the center sequences harbor regulatory domains (RD). Phylogenetic tree construction suggested early quadruplication of C/EBP genes during vertebrate evolution. Compound gene deletion studies in the mouse revealed that C/EBPα and β, both which are already active in early embryogenesis, are the most important C/EBPs.

How extracellular signals appropriately instruct C/EBPs to orchestrate their multifaceted functions, ranging from regulation of "stemness" on the one hand, to "terminal differentiation" on the other hand, are central questions of our research. C/EBPs interact with many essential co-factor complexes of the cell cycle and of the gene regulatory/epigenetic machinery. Tailor-made C/EBP isoforms are generated by alternative translation initiation and by a multitude of post-translational amino acid side chain modifications. These modifications play key roles in the orchestration of variegated C/EBP functions by instructing differential protein interactions in different settings (Figure 1).

Translational regulation of C/EBP

Valérie Bégay, Jeske J. Smink, Klaus Wethmar

C/EBPα and C/EBPβ are intronless, single-exon genes, yet both genes are expressed as various protein isoforms of different lengths. Both, C/EBPα and C/EBPβ transcripts harbor small upstream open reading frames (uORF) in their mRNAs that sense the activity of the translation initiation machinery and relay translation starts to alternative downstream in-frame initiation

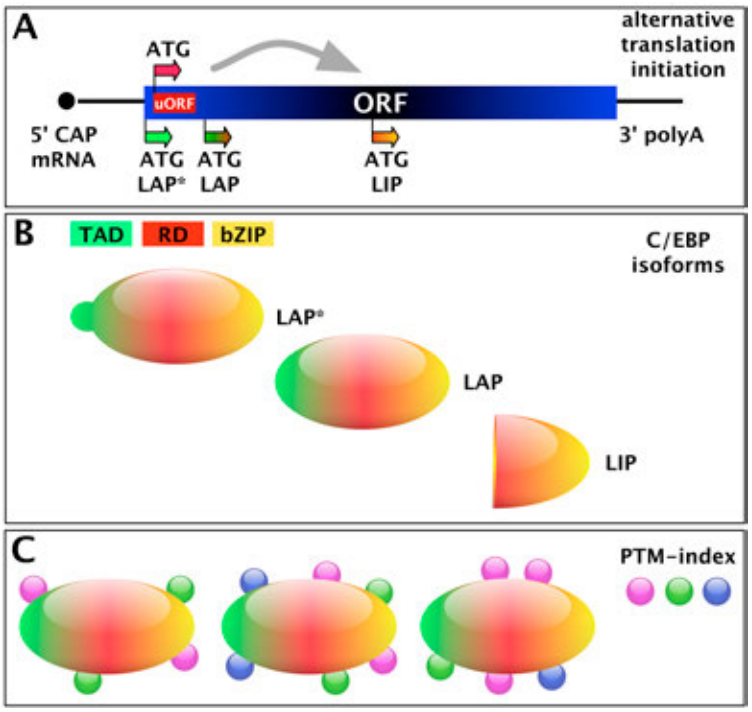

Figure 1: C/EBP modifications
A: C/EBPα,β are intronless genes, yet different protein isoforms are generated. An upstream open reading frame (uORF, red) in the single exon mRNA directs translation initiation to alternative start sites to generate different protein isoforms, termed LAP*, LAP, and LIP in the case of C/EBPβ.
B: LAP*, LAP, and LIP differ in the N-terminal transactivation (TAD, green) and regulatory domains (RD, red) and have common C-terminal DNA-binding bZip domains (bZip, yellow).
C: C/EBPα,β are decorated with multiple post-translational modifications (PTM, shown only for one isoform, as indicated by magenta, green, blue marbles) that may create a PTM-index to determine alternative or sequential functions. PTM entail lysine acetylation and methylation (mono-, di-, trimethylation), arginine methylation (mono-, asymmetric or symmetric dimethylation), and/or phosphorylation (on threonine, serine, or tyrosine) to generate different PTM patterns that may orchestrate co-factor interactions (see also Figure 2).

sites. High activity of the translation machinery results in generation of truncated proteins, which lack part of the N-terminus. Truncated C/EBP isoforms may act as dominant-negative C/EBP products and as genetic repressors. Pharmacological interference with mTOR signaling or removal of the uORF by targeted recombination in the mouse inhibited downstream translation initiation and thus increased the ratio of long C/EBPβ-LAP vs. short LIP isoform. Animals that can no longer switch between C/EBPβ isoforms developed many diseases, including defects in mammary gland development, liver regeneration, bone homeostasis, metabolism, and cancer.

Mice expressing only the truncated LIP isoform developed tumors at various sites, displayed multiple metabolic defects, and enhanced bone turnover. Constitutive expression of truncated LIP isoform in monocytic precursors strongly enhanced the formation of multinucleated osteoclasts, whereas the C/EBPβ LAP isoform inhibited osteoclastogenesis. Profiling of the osteoclast transcriptome revealed MafB as a C/EBP target and as an inhibitor of osteoclastogenesis. The data showed that proper C/EBPβ isoform regulation through mTOR signaling determines the rate of bone turnover and represents a potential target for treatment of osteoporosis and other degenerative bone diseases.

In contrast to the LIP strain, mice deficient for the C/EBPβ uORF initiation codon (C/EBPβΔuORF) fail to initiate translation of the truncated C/EBPβ LIP isoform and only produce long isoforms. C/EBPβΔuORF mice are deficient for branching morphogenesis during mammary gland development and in liver regeneration. C/EBPβΔuORF mice display hyperactive acute phase response and show delayed and blunted entry of hepatocytes into S-phase due to persistent repression of E2F-regulated genes. Thus, switching between C/EBPβ isoforms is important during developmental processes and regeneration. Taken together, deregulated isoform expression may be involved in many types of diseases, including tumor formation.

Epigenetic functions and post-translational C/EBP modifications

Elisabeth Kowenz-Leutz, Günther Kahlert, Maria Knoblich, Qingbin Liu, Marina Scheller, Bilyana Stoilova

Several years ago, we found that C/EBPα and β may instruct even non-hematopoietic cells, such as skin fibroblasts, to express myeloid genes. Accordingly, C/EBPs entail epigenetic competence and gene regulatory functions to orchestrate cell fate. Studies of the last two decades have confirmed that committed cells may retain plasticity to convert to other cell lineages. C/EBPs may also induce lymphoid to myeloid conversion and such lineage switch can actually be observed in several types of lymphoma and leukemia. Employing a mutagenesis approach to elucidate mechanisms of myeloid reprogramming, we identified a region in C/EBPβ that, in conjunction with the bZip domain, is necessary and

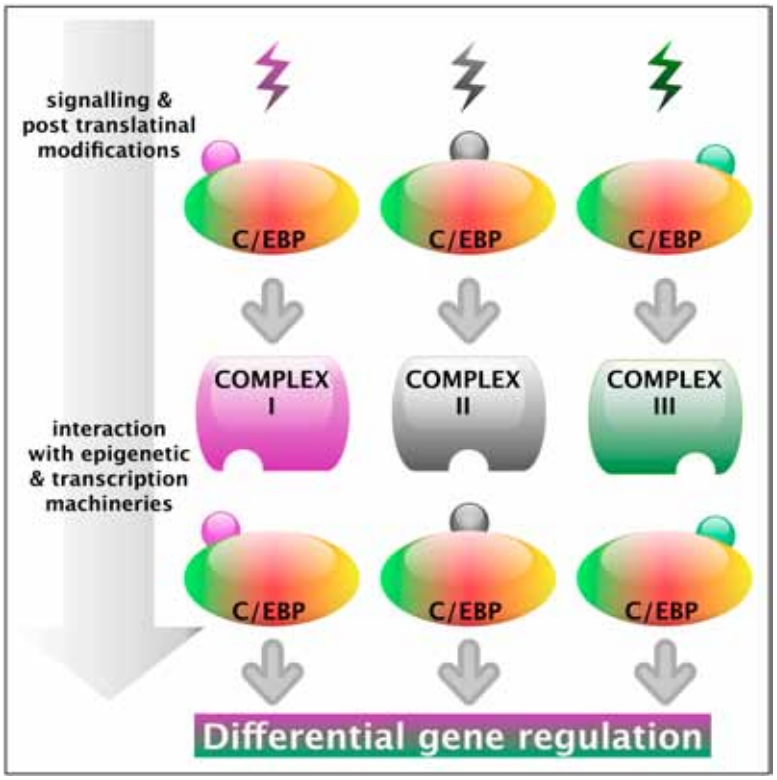

Figure 2: Indexing of C/EBP by post-transcriptional modifications
Various signals cause different post-translational modifications. C/EBP and its post-translational modifications direct recruitment and/or assembly of alternative co-factor complexes (complex I,II,III, etc.) of the epigenetic and transcriptional machinery. Note that for simplicity only one C/EBP isoform and only one alternative modification is shown. Various modifications may develop patterns and crosstalk during sequential and/or alternative co-factor recruitment to orchestrate differential gene expression.

sufficient to induce lymphoid-myeloid conversion. Studies are underway to determine the C/EBPβ interaction partners involved in reprogramming to elucidate the mechanism of trans-differentiation and to address the question whether reprogramming is involved in tumorigenic transformation.

Considering the structural/functional C/EBP features and seeking for an explanation for how C/EBPβ toggles between multiple functions, it came to our attention that several arginine (R) and lysine (K) residues were targets for post-translational modifications (PTM) and that PTM occurrence depended on signaling. Using a systematic mass spectrometric analysis (in collaboration with Gunnar Dittmar, MDC), we identified more than 50 novel modifications on C/EBPα and β from chicken, rat, and human, including K-acetylation and K-, R-methylation. Activation of receptor tyrosine kinase and ras/MAP-kinase signaling pathways induced C/EBPβ phosphorylation, structural conversion, changed K-, R-methylation pattern, and altered interactions with epigenetic protein complexes. Biochemical and molecular genetic analysis revealed that e.g. methylation of N-terminal C/EBPβ R3 abrogated interaction with both, SWI/SNF and Mediator complexes and that activation of ras-MAPK signaling inhibited methylation of R3 by PRMT4/CARM1 or K39 by G9a. Moreover, mutagenesis of R3, K39 or some other R-residues in the transactivation domain enhanced gene activation by more than two orders of magnitude. These data implied the existence of a signal-dependent, intra-molecular crosstalk and modification directed interactions between C/EBPβ and the epigenetic machinery. This notion was confirmed by elimination of methyltransferases or by C/EBPβ mutations that prevented methylation, resulting in enhanced SWI/SNF and Mediator interactions and gene activation. We have now also identified a post-translational modification site in C/EBPα that is recurrently mutated in leukemia. The leukemic mutation enhanced the susceptibility of C/EBPα for inactivation by E2F proteins, which plays a decisive role in tumorigenesis.

Post-translational modifications of histones, transcription factors, and co-factors by methylation of lysine and arginine residues are hallmarks of gene regulation. Such PTMs are thought to promote or obstruct protein interactions that are involved in establishing the epigenetic state of chromatin. We use high-end mass spectrometric methods to explore the existence of a post-translational "Indexing Code" on C/EBPs that may direct protein interactions. The functional consequences of individual PTMs are difficult to discern and require the knowledge of interaction partners for further functional investigations. Accordingly, we have set up a systematic differential screening approach to detect PTM-dependent interactions in the human proteome using chemically unmodified and modified C/EBP peptides. Such peptides were applied in parallel to human pro-

teomic expression libraries. Interactions were detected and quantified by laser scanning of peptide-coupled fluorophores. The interaction screens revealed epigenetically important proteins that exclusively bound to either non-modified or modified peptides. This method complements mass spectrometric methods to detect C/EBP interactions and extends the possibility to fully explore the C/EBP interactome and its dependence on PTMs.

"Indexing Code" hypothesis of signaling coupled epigenetic transcription factor functions

A number of implications and conceptual advances are contained in our results. The multitude of C/EBP modifications suggests an "Indexing Code" of post-translational transcription factor modifications that precedes "Histone Code" modifications on nucleosomes (Figure 2). Initially, signaling events mediate covalent post translational modifications that modulate the structure of C/EBPs (such as through K,R-methylation). Post translationally modified C/EBPs subsequently direct docking of chromatin modulating complexes through "reader" domains in such complexes that recognize C/EBPs and their modification status (such as in SWI/SNF or Mediator interactions with C/EBPβ). The enzymatic activities of such complexes are thus recruited to the sites occupied by C/EBPs on DNA and may alter chromatin structure, histone modifications, and gene expression. Our results imply that writing/reading such a C/EBP "Indexing Code" is downstream of signaling events received from the cell surface, relaying extracellular signals to epigenetic events that finally determine cell fate. This concept is an extension of the "Histone Code" hypothesis to non-histone transcription factor proteins, which are the primary recipients of signals to eventually modulate chromatin and the gene expression status. Accordingly, we surmise that the modifications on C/EBPs orchestrate co-factor interactions in order to direct the gene regulatory and epigenetic machineries in a gene context specific fashion. The multiplicity of C/EBP modifications comprises numerous combinatorial interactions to mediate functional and evolutionary plasticity.

Many mechanistic (gene regulation in development and disease) and medical implications (pharmacology) are contained in this concept. We anticipate that unraveling the PTM-dependent C/EBP interaction network might help to elucidate complex functions in various tissues including cell differentiation, regeneration, and tumorigenesis.

Selected Publications

Smink, J.J, Bégay,V., Schoenmaker, T., Sterneck, E., de Vries, T.J., and Leutz, A. (2009) Transcription factor C/EBPβ isoform ratio regulates osteoclastogenesis through MafB. The EMBO J., 28: 1769-1781

Wethmar, K., Bégay, V., Smink, J., Wiesenthal, V., Zaragoza, K., Dörken, B., Calkhoven, C.F., and Leutz, A. (2010) C/EBPβ$^{\Delta uORF}$ mice – a mammalian model for uORF-mediated translational control. Genes & Development, 24:15-20

Kowenz-Leutz, E., Pless, O., Dittmar, G., Knoblich, M., and Leutz, A. (2010) Crosstalk between C/EBPβ phosphorylation, arginine methylation, and interaction with SWI/SNF and Mediator complexes implies an indexing code during gene regulation. The EMBO J, 29:1105-1115

Leutz, A., Pless, O., Lappe, M., Dittmar, G., Kowenz-Leutz, E. (2011) Crosstalk between phosphorylation and multi-site arginine/lysine methylation in C/EBPs. Transcription, 2:1-6

Pless, O., Kowenz-Leutz, E., Dittmar, G., and Leutz, A. (2011) A differential proteome screening system for post-translational modification-dependent C/EBP interactions. Nature Protocols, 6:359-364

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Cancer, Stem Cells, and Transcription Factors

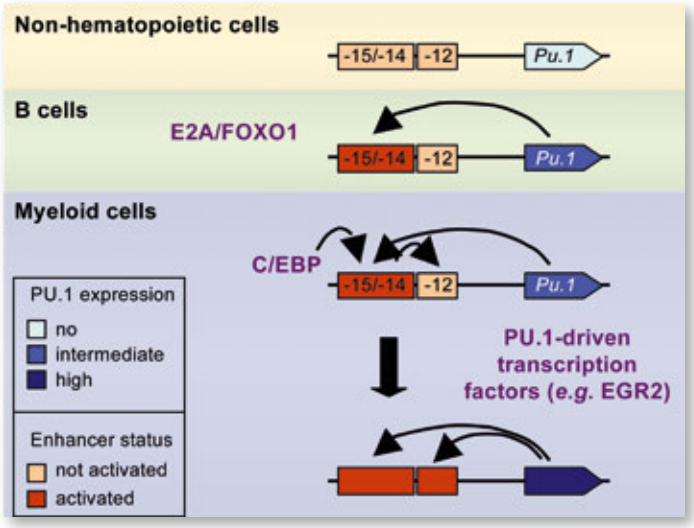
Dynamic PU.1 Expression in Hematopoiesis and Leukemia

One of the main interests of our laboratory is to understand how transcription factors direct normal stem cell functions, such as self-renewal and differentiation, how they program precursors to adopt a certain lineage choice and how disruption of transcription factor activity leads to cancer (stem) cell transformation. Using both transgenic and knockout mouse models, we are particularly interested in discovering crucial molecular up- and downstream mechanisms that regulate the expression and function of transcription factors. A current research focus in our laboratory is on PU.1. The Ets-family member PU.1 is essential for both myeloid and lymphoid lineages. PU.1 knockout mice exhibit early lethality and lack of B-lymphocytes and mature myeloid cells in fetal livers. In addition, PU.1 is important for HSC self-renewal and differentiation into the earliest myeloid and lymphoid progenitors. Furthermore, PU.1 must be properly downregulated in early thymocytes to allow normal T cell development. It was shown that graded changes in PU.1 concentrations have drastic effects on lineage fate decisions. Therefore, a greater understanding of PU.1 gene regulation is the key to deciphering its role in normal hematopoiesis and malignant transformation.

Although the well-controlled tissue-specific expression of PU.1 is essential for hematopoiesis and leukemia, little is known of how this pattern is established. We identified an upstream regulatory *cis*-element (URE) whose targeted deletion in mice decreases PU.1 expression and causes leukemia. However, we showed further that the URE alone is insufficient to confer physiological PU.1 expression, but requires the cooperation with other, previously unidentified elements. Using a combination of transgenic studies, global chromatin assays and detailed molecular analyses we presented evidence that PU.1 is regulated by a novel mechanism involving cross-talk between different *cis*-elements together with lineage-restricted autoregulation. In this model, PU.1 regulates its expression in B cells and macrophages by differentially associating with cell-type specific transcription factors at one of its *cis*-regulatory elements to establish differential activity patterns at other elements (Figure).

Functional cooperation of PU.1 and microRNAs

Whereas PU.1-dependent induction of myeloid-specific target genes has been intensively studied, negative regulation of stem cell or alternate lineage programs remains incompletely characterized. To test for such negative regulatory events, we searched for PU.1-controlled microRNAs (miRs) by expression profiling using a PU.1-inducible myeloid progenitor cell line model. We provide evidence that PU.1 directly controls expression of at least 4 of these miRs (miR-146a, miR-342, miR-338, and miR-155) through temporally dynamic occupation of binding sites within regulatory chromatin regions adjacent to their genomic coding loci. Ectopic expression of the most robustly induced PU.1 target miR, miR-146a, directed the selective differentiation of HSCs into functional peritoneal macrophages in mouse transplantation assays. In agreement with this observation, disruption of Dicer expression or specific antagonization of miR-146a function inhibited the formation of macrophages during early zebrafish (Danio rerio) development. Taken together, we described a PU.1-orches-



Regulated interaction between *cis*-regulatory elements orchestrates the PU.1 expression pattern. Lack of PU.1 expression in non-hematopoietic cells is due to the lack in activation of critical enhancer elements located upstream of the *PU.1* gene. The intermediate PU.1 levels expressed in B cells are driven by the assembly of a B-cell-specific transcription factor complex at the -14 kb URE, which includes E2A and FOXO1, and the formation of a PU.1 autoregulatory loop. However, this complex is obviously not able to activate additional cis-elements in the *PU.1* locus. In contrast, myeloid progenitors express C/EBPα which binds the URE, induces the activation of the -12 kb enhancer to allow formation of a second PU.1 autoregulatory loop and binding of additional PU.1-driven transcription factors, such as EGR2, to increase PU.1 expression levels. Black arrows: Transcription factor interactions/ncRNA. Red arrows: enhancer – promoter interactions.

trated miR program that mediates key functions of PU.1 during myeloid differentiation.

Epigenetic control of hematopoietic stem cell function

DNA methylation is a major epigenetic modification, which in mammals is controlled by at least 3 different DNA-methyltranferases (DNMTs): DNMT3a and -b for de novo methylation and DNMT1 for methylation maintenance. The impact of methylation on stem cell features has been studied in embryonic stem (ES) cells, but little was known about its function in somatic stem cells. We could show that alternative functional programs of HSCs are governed by gradual differences in the methylation level. Constitutive methylation is essential for HSC self-renewal, but dispensable for homing, cell cycle control and suppression of apoptosis. Remarkably, HSCs from mice with reduced DNMT 1 activity fail to suppress key myeloerythroid regulators and as a consequence can differentiate into myeloerythroid but not into lymphoid progeny. We revealed that a similar methylation dosage effect controls stem cell function in leukemia. Thus, our data identified DNA methylation as an essential epigenetic mechanism to protect stem cells from premature activation of predominant differentiation programs and suggest that methylation dynamics determines stem cell functions in tissue homeostasis and cancer. Consequently, these results provide the hope that demethylating drugs may be instrumental to impair the function of cancer stem cells in cancer therapy.

Selected Publications

Ghani S, Riemke P, Schönheit J, Lenze D, Stumm J, Hoogenkamp M, Lagendijk A, Heinz S, Bonifer C, Bakkers J, Abdelilah-Seyfried S, Hummel M, Rosenbauer F. (2011) Macrophage development from hematopoietic stem cells requires PU.1 coordinated microRNA expression. Blood 118, 2275-2284.

Leddin M, Perrod C, **Hoogenkamp M**, Ghani S, Assi S, Heinz S, Wilson NK, Follows G, Schönheit J, Vockentanz L, Mosamam A, Chen W, Tenen DG, Westhead DR, Göttgens B, Bonifer C, Rosenbauer F. (2011) Two distinct auto-regulatory loops operate at the PU.1 locus in B cells and myeloid cells. Blood 117, 2827-2838.

Bröske AM, Vockaentanz L, Kharazi S, Huska M, Mancini E, Scheller M, Enns A, Prinz M, Jaenisch R, Nerlov C, Leutz A, Andrade-Navarro MA, Jacobsen SEW, Rosenbauer F. (2009) DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nature Genet 41, 1207-1215.

Steidl U, Rosenbauer F, Verhaak RGW, Gu X, Out HH, Bruns I, Steidl C, Costa DB, Klippel S, Wagner K, Aivado M, Kobbe G, Valk PJ, Passegué E, Libermann TA, Delwel R, and Tenen DG. (2006) Essential role of Jun family transcription factors in PU.1-induced leukemic stem cells. Nature Genet 38, 1269-1277.

Rosenbauer F, Owens BM, Yu L, Tumang JR, Steidl U, Kutok JL, Clayton LK, Wagner K, Scheller M, Iwasaki H, Liu C, Hackanson B, Akashi K, Leutz A, Rothstein TL, Plass C, and Tenen DG. (2006) Lymphoid cell growth and transformation are suppressed by a key regulatory element of the gene encoding PU.1. Nature Genet 38, 27-37.

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Peter M. Schlag

Surgical Oncology

The current challenging concept in cancer research is the identification of molecules and pathways driving tumor progression and metastasis. In this context, the strategy of our group is to find and characterize new molecules, gain insights into their function in cancer cell signaling networks, and verify their importance as biomarkers for clinical diagnosis and for therapy. Most promising molecules are among others MACC1 and S100A4. We already demonstrated their clinical relevance in view to improved subclassification of tumors regarding progression and metastasis. Furthermore, our knowledge on these novel molecular targets is used to develop innovative therapeutic tools. This has been accomplished by employing high throughput screens to find selective small molecule inhibitors or by using gene therapy for molecular intervention. The ultimate goal of all our efforts is the translation and validation of these novel therapeutic strategies in clinical trials.

Subclassification of solid cancer by gene expression profiling and functional analyses

W. Kemmner, S. Förster, Q. Wang, P.M. Schlag, U. Stein.

In cooperation with W. Birchmeier (MDC), M. Dietel, R. Schäfer (Charité), H. Lehrach (Max-Planck-Institute for Molecular Genetics, Berlin), J. Sleeman (Institute for Toxicology and Genetics, Karlsruhe),

P. Malfertheiner (Otto-von-Guericke University Magdeburg), M. Vieth (Klinikum Bayreuth), M. Yashiro (Osaka University, Japan), R.H. Shoemaker, A. Monks (National Cancer Institute-Frederick, MD)

Biomarkers are expected to improve subclassification of solid tumors, guide molecularly targeted therapy and monitor therapeutic response. Transcriptome expression profiling of different tumor entities (e.g. colon, esophageal, gastric, breast cancer) was performed by exploiting our tumorbiobank with more than 10.000 primary tumor samples, normal tissues together with clinical outcome data. The majority of these samples originates from patients treated in clinical phase II/III trials. Most promising differentially expressed and functionally relevant genes are MACC1, S100A4, Bambi, FECH, Claudins 3 and 4. Following the discovery phase, clinical validation studies in independent cohorts are currently in progress, ranging from the final clinical deployment of diagnostic platforms and therapeutic interventions.

MACC1–targeting strategies for a newly identified metastasis biomarker

U. Stein, M. Juneja, F. Schmid, A. Pichorner, C. Lemos, W. Walther, P.M. Schlag.

In cooperation with I. Fichtner, H. Schmidt, F. Rathjen, B. Jerchow, G. Dittmar (MDC), J.P. von Kries (FMP)

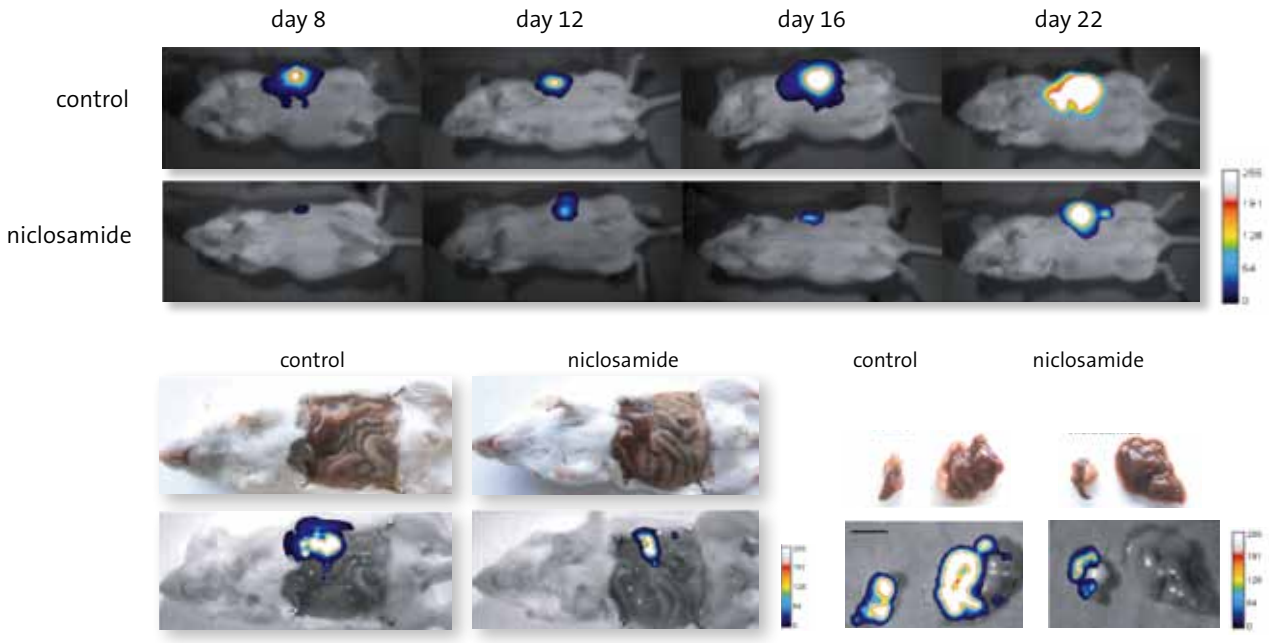

Figure 1. In vivo luminescence monitoring of niclosamide effect on metastasis inhibition in xenografted mice. Colon cancer cells stably expressing firefly luciferase were intrasplenically transplanted into NOD/SCID mice followed by daily intraperitoneal treatment of mice with niclosamide. In situ imaging and imaging of isolated organs was performed, signal intensity of grayscale images (256 scale) were color coded, and overlayed with bright field picture. Tumors at the spleens are observed in the control as well as in the treatment group. Liver metastasis, however, was completely inhibited by niclosamide.

We identified the previously undescribed gene MACC1 (Metastasis-Associated in Colon Cancer 1) in subjects with colon cancer. MACC1 is a stage-independent predictor for metastasis and survival, and allows early identification of high-risk patients. MACC1 induces migration and invasion in vitro, and metastasis formation in several mice models. In order to develop MACC1-specific metastasis inhibitors, we test different intervention strategies: (i) We identified the 5'-regulatory region of MACC1 which demonstrates promoter activity. This region is used for high throughput screenings for identification of small molecules that repress MACC1 expression leading to metastasis inhibition. (ii) We found MACC1 protein binding partners that are essential for MACC1 function to induce cell motility and finally cancer metastasis. Peptides targeting this protein-protein binding are currently tested for their metastasis inhibitory potential. (iii) We identified downstream targets of the transcription factor MACC1, which accomplish MACC1-induced effects such as motility and metastasis induction, thereby serving as targets for restricting metastasis. For monitoring metastasis inhibitory abilities of potential compounds we generated a MACC1-specific xenograft model suitable for non-invasive bioluminescence in vivo imaging. We generated genetically engineered transgenic MACC1 mice. The MACC1 knockout mouse model is underway. Our meta-analyses in human colorectal cancer summarized expression and mutational data (e.g. newly identified MACC1 SNPs) of metastasis-associated genes thereby unveiling crucial combinatorial gene sets for improvement of patients' prognosis but also underlining the outstanding role of MACC1. We also clinically validated the essential impact of MACC1 for tumor progression, invasiveness and metastasis in further human cancer entities. For clinical practice, the potential of the identified MACC1 inhibitors to restrict metastasis formation can be monitored by using our innovative approach of MACC1 transcript determination in patients' blood as biomarker for metastasis, prognosis, and monitoring of therapy response. In cooperation with the pharmaceutical industry we currently prepare the patent-based commercial implementation of MACC1 as biomarker for diagnosis and therapy response for colorectal cancer patients.

Inhibition of colon cancer metastasis by Wnt/β-catenin-pathway-oriented small molecules targeting the metastasis progressor S100A4

U. Stein, U. Sack, M. Dahlmann, W. Walther, P.M. Schlag.

In cooperation with I. Fichtner (MDC), R.H. Shoemaker, N. Scudiero (National Cancer Institute-Frederick, MD)

We previously identified the metastasis-inducing gene S100A4 as target gene of the Wnt/β-catenin signalling pathway. Now we established in vivo intervention strategies targeting S100A4 for metastasis reduction or even

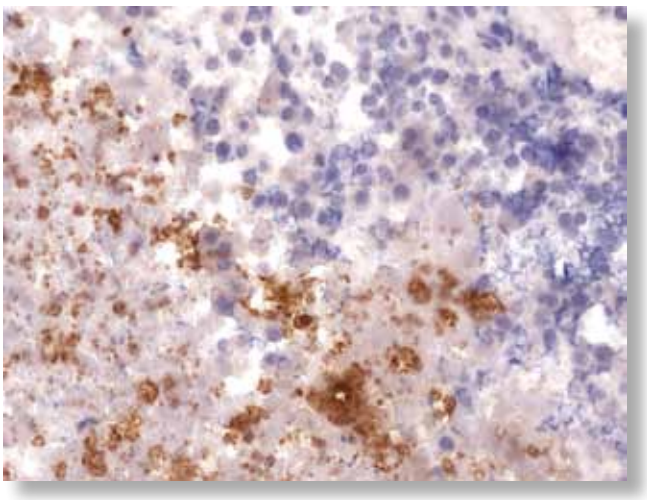

Figure 2. **CPE expression in MCF-7 xenotransplant breast cancer tissue two days after nonviral *in vivo* gene transfer.** CPE expressing areas appear as scattered brown staining within the tumor. CPE expression causes necrosis in the claudin-3 and -4 overexpressing tumor.

prevention. (i) We identified small molecule inhibitors from a high throughput screening of pharmacologically active compounds, employing the S100A4 gene promoter reporter system (e.g. niclosamide). These compounds inhibit S100A4 expression by intervening in the Wnt/β-catenin cascade. (ii) We also used compounds known to interfere with the Wnt/β-catenin signalling pathway (e.g. sulindac). Treatment with these identified S100A4 inhibitors resulted in reduced cell migratory and invasive abilities, and led to reduced metastasis formation in xenografted mice. (iii) We also systemically applied shRNA acting on S100A4 and demonstrated prevention of metastasis formation in mice. Metastasis reduction was monitored over weeks by bioluminescence in vivo imaging. Now we translate our findings on S100A4-specific metastasis prevention/reduction into clinical practice. Our newly established assay for S100A4 transcript determination in patients' blood will be used to monitor treatment success. Together with the National Cancer Institute, MD, USA, we test these already FDA-approved compounds in liver metastasis-resected colorectal cancer patients for inhibition of S100A4-induced recurrence in clinical phase II trials.

Imaging of cancer using a novel RNAi-based detection mechanism

W. Kemmner, K. Wan, P.M. Schlag.

In cooperation with B. Ebert (Department of Biomedical Optics, PTB Berlin) and R. Haag (Free University Berlin)

Tumor growth can be monitored by bioluminescence in vivo imaging as discussed above. Here we introduce a siRNA-based probe that is capable of amplifying an endogenous fluorescence which is selectively accumulated within cancerous tissue. Small interference RNA (siRNA) represents a promising therapeutic option. However, intravenously administered siRNA is rapidly degraded and subjected to renal filtration, resulting in poor cellular uptake and pharmacokinetics. To confer drug-like properties such as stability, cellular delivery, and tissue bioavailability to siRNAs, novel strategies have to be developed. In previous studies, we have demonstrated a significant down-regulation of Ferrochelatase (FECH) mRNA-expression in gastric, colonic, and rectal carcinomas leading to the accumulation of protoporphyrin IX (PpIX), a fluorescent metabolite of the heme synthesis. We were able to show that FECH-siRNA encapsulated within folate-PEG cationic lipoplexes enabled a sensitive *in-vivo* tumor imaging of xenografted tumors in nude mice. This approach opens a new way for monitoring the efficient transportation of siRNA-based probes into cells of the living tissue and may offer new possibilities for simultaneous photodynamic diagnosis and treatment.

Novel targeted suicide cancer gene therapy using the Clostridium perfringens enterotoxin (CPE)

W. Walther, S. Petkov, O. N. Bölling, D. Kobelt, J. Aumann, U. Stein, Peter M. Schlag.

In cooperation with I. Fichtner (MDC), J. Piontek and I. E. Blasig (FMP, Berlin)

Expression arrays revealed, that claudin-3 and -4 are highly overexpressed in numerous human epithelial tumors such as colon, breast, pancreatic, ovarian and prostate cancer. The *Clostridium perfringens* enterotoxin (CPE) is a pore-forming bacterial toxin, which specifically binds to the transmembrane proteins claudin-3 and -4, located in tight junctions of epithelial cells. CPE binding to claudin-3 and -4 triggers formation of membrane pore complexes leading to rapid cell death. This can be exploited for selective tumor cell killing by CPE gene therapy. We constructed CPE expressing vectors to transfect various human claudin-3 and -4 overexpressing tumor cells and tumors. The CPE gene transfer exerted rapid and selective cytotoxicity *in vitro* and *in vivo*. This novel approach clearly demonstrates that CPE can be used for targeted *in vitro* and *in vivo* suicide gene therapy of claudin-3 and -4 over expressing epithelial tumors, mediating rapid and efficient tumor eradica-

tion. Therefore, targeting defined molecules may become an attractive complementary approach to current cancer therapies.

Establishment of nonviral gene therapy to treat solid tumors in clinical application

W. Walther, D. Kobelt, S. Burock, U. Stein, P. M. Schlag.

In cooperation with I. Fichtner (MDC), B. Wittig (Free University, Berlin), M. Schroff and M. Schmidt (MOLOGEN AG, Berlin), U. Trefzer (Charité), LPS GmbH (Hamburg)

In addition to the aforementioned approach we address the development of safe, efficient and applicable gene transfer technologies and made great efforts for the bench-to-bedside translation of nonviral gene therapy of solid tumors. In result of our completed phase I clinical gene transfer trial (DeReGe 62), which showed the limitations of plasmid vectors, we adapted the minimalistic MIDGE vector (Mologen, Berlin) for improved transgene expression and its potential clinical use. This vector system generated pronounced increase in transfer efficiency and in high-level transgene expression, as shown for TNF-α. This is successfully exploited in our chemosensitization strategy for solid tumor treatment, combining nonviral gene therapy with chemotherapy in pre-clinical studies. Currently this gene therapeutic concept will be continued with a phase I gene therapy trial. This trial will evaluate safety and efficiency of local nonviral jet-injection transfer of the TNF-α expressing MIDGE-vector in melanoma patients. At given success, the chemosensitizing potential of TNF-α gene transfer will be further evaluated in a phase II clinical trial for improved therapeutic efficacy of chemotherapy in the local treatment of melanoma patients. In general, establishment of a feasible nonviral gene transfer technology also opens up new horizons for molecular intervention of other specific targets using siRNA/shRNA or new selective transgenes.

Selected Publications

Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, Birchmeier W, Schlag PM. MACC1, a newly identified key regulator of HGF/Met signaling, predicts colon cancer metastasis. Nature Med 15:59-67, 2009

Schuhmacher C, Gretschel S, Lordick F, Reichardt P, Hohenberger W, Eisenberger CF, Haag C, Mauer ME, Hasan B, Welch J, Ott K, Hoelscher A, Schneider PM, Bechstein W, Wilke H, Lutz MP, Nordlinger B, Cutsem EV, Siewert JR, Schlag PM. Neoadjuvant chemotherapy compared with surgery alone for locally advanced cancer of the stomach and cardia: European Organisation for Research and Treatment of Cancer randomized trial 40954. J Clin Oncol 28:5210-5218, 2010

Förster S, Gretschel S, Jöns T, Yashiro M, Schlag PM, Kemmner W, THBS4 a novel heavily accumulated tumor stroma constituent of diffuse-type gastric adenocarcinomas identified by transcriptome-wide expression profiling, Mod Pathol, in press 2011

Sack U, Walther W, Scudiero D, Selby M, Kobelt D, Lemm M, Fichtner I, Schlag PM, Shoemaker RH, Stein U. Novel effect of anti-helmintic niclosamide on S100A4-induced metastasis in colon cancer. J Natl Cancer Inst 103:1018-1836, 2011

Walther W, Petkov S, Kuvardina ON, Aumann J, Kobelt D, Fichtner I, Lemm M, Piontek J, Blasig IE, Stein U, Schlag PM. Novel Clostridium perfringens enterotoxin suicide gene therapy for selective treatment of claudin-3 and -4 overexpressing tumors. Gene Ther, in press 2011

Patents

Stein U, Schwabe H, Walther W, Schlag PM.
Verwendung des neu-identifizierten Gens 7a5/Prognostin für Tumordiagnostik und Tumortherapie.

7a5/Prognostin and use thereof for the diagnostic and therapy of tumors .

Patent, granted	JP 51-9895	2011
Patent, granted	AU 2004259281	2011
Patent, granted	US 7,851,168 B2	2010

Stein U, Walther W, Sack U, Scudiero D, Schlag PM, Shoemaker RH.
Niclosamide for treatment of cancer metastasis

Patent application	EP 11162875.6	2011

Walther W, Kobelt D, Schlag PM, Schmidt M.
Chemosensitivierung von Melanomen mittels Gentransfer eines TNF-alpha exprimierenden minimalistischen MIDGE-hTNF Vektors“

Patent application	EP 10 195 929.4	2010

Stein U, Herrmann P, Burock S, Schlag PM.
Use of metastasis progressor S100A4 transcripts in body fluids of colorectal and gastric cancer patients.

Patent application	PCT/EP2010/001891	2010
Patent application	US 61/261,017	2009

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Cancer Genetics and Cellular Stress Responses in Pathogenesis and Treatment of Lymphatic Malignancies

Our research program is driven by our interest in cellular stress responses (so called 'failsafe mechanisms') that may serve as anti-tumor barriers when challenged by transforming oncogenes, and, in turn, must be bypassed or inactivated before a full-blown malignancy can actually form. Importantly, ultimate stress responses such as apoptosis or cellular senescence – both terminal 'cell-cycle exit' programs – do not only counter tumorigenesis, but are utilized as chemotherapy-induced stress responses as well.

Hence, principles of oncogenesis and mechanisms of treatment sensitivity seem to critically overlap and impinge on each other during tumor formation, cancer therapy and relapsed or progressive disease conditions. We are particularly interested in the connection of failsafe programs and cell metabolism as well as non-cell-autonomous implications of these programs, and, thus, primarily utilize mouse models harboring lymphomas (and other tumor entities) with defined genetic lesions as experimental platforms.

Apoptotic lymphoma cells trigger TGF-β secretion of host macrophages that results in tumor-suppressive lymphoma cell senescence

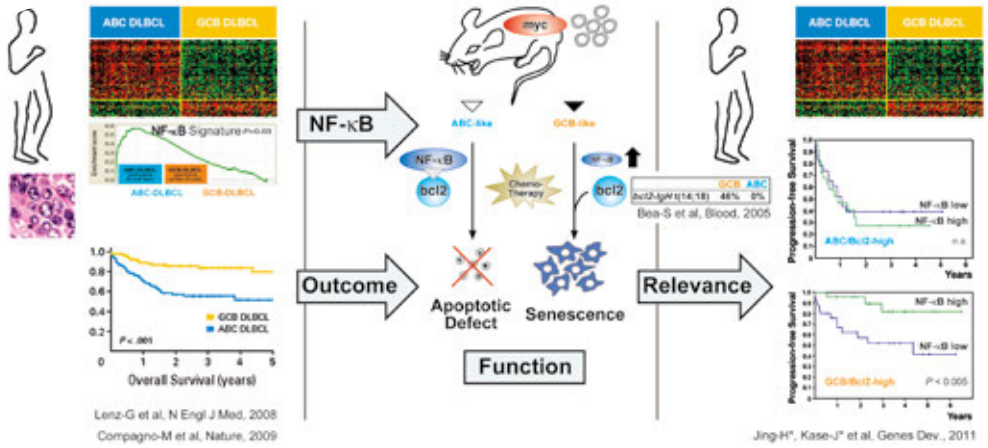
Maurice Reimann, Soyoung Lee, Jan Dörr, Bernd Dörken and collaboration partners

Activated RAS/BRAF oncogenes induce cellular senescence as a tumor suppressive barrier in early cancer development – as previously demonstrated by our group (Braig et al., Nature, 2005) – at least in part, *via* an oncogene-evoked DNA damage response (DDR). In contrast, Myc activation – although producing a DDR as well – is known to primarily elicit an apoptotic countermeasure. Using the Eμ-*myc* transgenic mouse lymphoma model, we identified TGF-β as the pivotal senescence trigger *in vivo* that is delivered by macrophages upon their activation by apoptotic lymphoma cells. These findings, detectable in human aggressive B-cell lymphomas as well, establish a novel network of heterotypic cell-cell interactions in which apoptotic tumor cells launch a paracrine response in non-malignant bystanders of the tumor microenvironment that limits tumorigenesis by cellular senescence.

Opposing roles of NF-κB transcription factors in drug resistance and therapy-induced senescence

Soyoung Lee, Maurice Reimann, Hua Jing, Julia Kase and collaboration partners

Cellular senescence is associated with the production of a broad spectrum of secretable factors, termed the "senescence-associated secretory phenotype (SASP)". SASP include NF-κB- and C/EBPβ-dependent pro-inflammatory cytokines, growth factors and matrix metalloproteases, whose intracellular, auto-/paracrine and heterologous stromal and immune effects are poorly understood. Utilizing a mouse lymphoma model and analyzing transcriptome and clinical data from lymphoma patients, we were able to show that therapy-induced senescence presents with and depends on active NF-κB signaling, whereas NF-κB simultaneously promotes resistance to apoptosis. Further characterization and genetic engineering of primary mouse lymphomas according to distinct NF-κB-related oncogenic networks



Opposing roles of NF-κB in anti-cancer treatment outcome unveiled by cross-species investigations.
Human DLBCL consist of two subgroups, GCB- and ABC (activated B-cell)-type DLBCL, which can be separated by gene expression profiling **(left)**. The ABC subtype is characterized by constitutive NF-κB activity and poor clinical outcome. Based on their genuine NF-κB activity and treatment responsiveness, we proposed an "ABC-like" cohort in primary Eμ-*myc* transgenic mouse lymphomas, and linked their chemo-insensitivity to high NF-κB and anti-apoptotic Bcl2 activity, because *bcl2* is a direct NF-κB target gene **(middle)**. In contrast, "GCB-like" mouse lymphomas engineered to overexpress Bcl2 in an NF-κB-independent manner – as frequently found in human GCB DLBCL due to a Bcl2-activating t(14;18) translocation – responded to DNA-damaging anticancer agents with tumor-controlling cellular senescence in an NF-κB-dependent manner. "Re-application" of these parameters to the human DLBCL transcriptome and clinical outcome data set unveiled the hitherto unknown superior prognosis of NF-κB-hyperactive GCB DLBCL patients with high Bcl2 expression **(right)**.

reminiscent of diffuse large B-cell lymphoma (DLBCL) subtypes guided us to identify Bcl2-overexpressing germinal center B-cell-like (GCB) DLBCL as a clinically relevant subgroup with significantly superior outcome when NF-κB is hyperactive. Our data illustrate the power of cross-species investigations to functionally test genetic mechanisms in transgenic mouse tumors that recapitulate distinct features of the corresponding human entity, and to ultimately utilize the mouse model-derived genetic information to redefine novel, clinically relevant patient subcohorts.

Exploiting senescence-related metabolic alterations as novel therapeutic targets

Jan Dörr, Yong Yu, Soyoung Lee and collaboration partners

Functional non-invasive positron emission tomography (PET)-based imaging using either glucose or thymidine as tracers prompted the discovery of an unexpected feature of cellular senescence: a glucose-avid but thymidine-negative hypermetabolic phenotype. Extensive biochemical and genetic analyzes confirmed boosted glycolysis and oxidative phosphorylation in senescence, and led to the dissection of the underlying mechanism. Senescent cells present with a massive secretory phenotype (SASP, see above) that overwhelms the capacity of proper protein biosynthesis, posttranslational modification and further processing as exosome cargo; as a consequence, senescent cells display signs of endoplasmic reticulum stress, the unfolded protein response, enhanced global ubiquitination – and autophagy to "buffer" proteotoxicity. In turn, energy depletion or inhibition of autophagy results in selective cell death out of senescence, allowing us to utilize senescence and these subsequent, conceptually novel treatment approaches in a "synthetically lethal-like" fashion to eliminate potentially harmful senescent cancer but no normal cells.

Selected Publications

Dörr, J.R., Y. Yu, M. Milanovic, G. Beuster, J.H.M. Däbritz, K. Schleicher, S. Kratzat, B. Purfürst, S. Walenta, W. Mueller-Klieser, U. Keller, A.K. Buck, B. Dörken, S. Lee, and C.A. Schmitt. 2011. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Submitted.

Jing, H., J. Kase, J.R. Dörr, M. Milanovic, D. Lenze, M. Grau, G. Beuster, S. Ji, M. Reimann, P. Lenz, M. Hummel, B. Dörken, G. Lenz, C. Scheidereit, C.A. Schmitt, and S. Lee. 2011. Opposing roles of NF-κB in anti-cancer treatment outcome unveiled by cross-species investigations. Genes Dev. 25: 2137-2146.

Lee, S., C.A. Schmitt, and M. Reimann. 2011. The Myc/macrophage tango: Oncogene-induced senescence, Myc style. Semin Cancer Biol. Epub ahead of print.

Haugstetter, A.M., C. Loddenkemper, D. Lenze, J. Gröne, C. Standfuß, I. Petersen, B. Dörken, and C.A. Schmitt. 2010. Cellular senescence predicts treatment outcome in metastasized colorectal cancer. Br. J. Cancer 103: 505-509.

Reimann, M., S. Lee, C. Loddenkemper, J.R. Dörr, V. Tabor, P. Aichele, H. Stein, B. Dörken, T. Jenuwein, and C.A. Schmitt. 2010. Tumor stroma-derived TGF-β limits Myc-driven lymphomagenesis via Suv39h1-dependent senescence. Cancer Cell 17: 262-272.

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Mechanisms of Protein Quality Control

The endoplasmic reticulum (ER) is a cellular organelle through which a significant proportion of proteins pass on their way to their functional sites in membranes, exocytic and endocytic compartments, or the cell exterior. Far from being a passive traffic way, the ER is home to an array of molecular chaperones, which help proteins to fold and guide their maturation. Despite this support, 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. Malfunction or aggregation of defective proteins challenges the homeostasis of the ER and the cell as a whole. Disturbed protein homeostasis leads to a number of important diseases, among them cancer. As a defense mechanism, evolution has produced a protein quality control (PQC) network that operates on several levels to maintain the integrity of the ER.

The work of this group focuses on how ER homeostasis is maintained (Fig. 1). In other words, how quality control pathways selectively dispose aberrant proteins without jeopardizing correctly folded polypeptides. Signals contained in misfolded proteins of the ER-lumen and membranes are decoded by ubiquitin ligases anchored in the ER membrane. Proteins committed for degradation are exported from the ER lumen or membrane in a process termed protein dislocation. Subsequently, substrate molecules are ubiquitylated and degraded by cytoplasmic 26S proteasomes. This process is referred to as ER associated degradation or ERAD. However, misfolded proteins are not the only substrates of this system. It also plays a regulatory role. For example it eliminates rate-limiting enzymes of sterol synthesis in response of the flux through this pathway.

Since the ERAD pathway appears to be conserved from yeast to mammals, we have used the model organism *Saccharomyces cerevisiae* to investigate the fundamental mechanisms and to identify the key components of this important pathway. These are the HRD ubiquitin ligase and the Doa10 ubiquitin ligase. The HRD-ligase is crucial for turnover of membrane-bound and ER-luminal substrates. Doa10 targets membrane proteins for degradation that carry lesions in their cytoplasmic domains. Both yeast ubiquitin ligases and their identified co-factors are summarized in Fig. 2. The mammalian counterparts of the yeast components are mentioned as well.

In the last years, the group has identified and characterized components of these ligase complexes using genetics, molecular biology, and protein purification strategies. However, to unravel the basic mechanisms

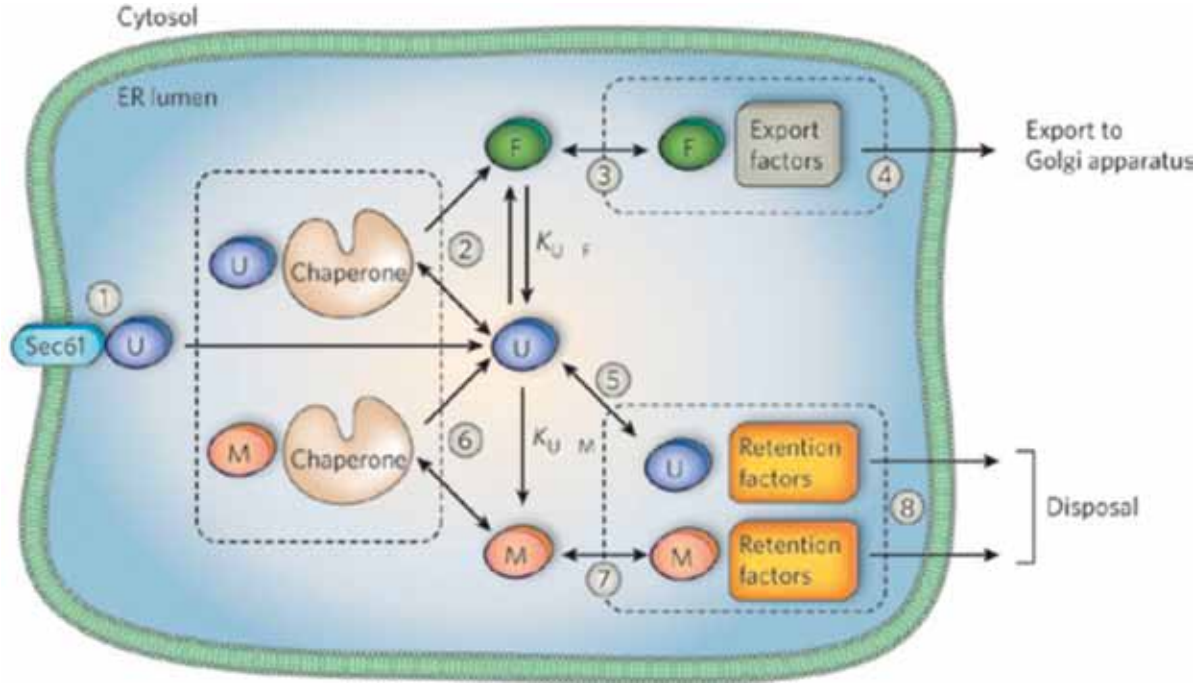

Figure 1. Protein homeostasis in the endoplasmic reticulum. Proteins translocate into the ER via Sec61 (step 1). Unfolded proteins (U) can engage chaperones to fold into their native conformation (step 2). Export factors select correctly folded proteins (F) (step 3) and transport them to the GOLGI (step 4). Retention factors prevent exit of unfolded proteins (step 5). Misfolded proteins (M) are the result of unproductive folding efforts. Chaperones try to remodel these polypeptides into a folding competent state (step 6). Retention factors keep aberrant proteins in the ER (step 7). Eventually, defective proteins (M), along with a fraction of folding intermediates (U), are relegated into the cytosol and disposed (step 8).

how these protein machines work, additional in vitro approaches and quantitative methods have to be established.

Hrd3 acts as a 'Holdase' and substrate receptor of the HRD-ligase

Franziska Zimmermann and Sathish Kumar Lakshimpathy

In order to understand how substrates bind to the ER-luminal sub-module of the HRD ubiquitin ligase (Hrd3, Yos9) we started to work with purified components. Since the function of Hrd3 does neither rely on its membrane anchor nor on its cytosolic carboxyl-terminus this protein can be expressed in insect cells in a soluble form that comprises the entire ER luminal domain. We also expressed Yos9 in insect cells and purified it. Expressed and purified Hrd3 binds Yos9 from yeast extracts, indicating that the protein has a native fold. Using denatured firefly luciferase we demonstrated a direct interaction of Hrd3 with a misfolded protein. Next, we tested whether Hrd3-binding deploys holdase activity by preventing the aggregation of a denatured protein. To this end we diluted denatured luciferase in buffer containing Hrd3 and monitored its aggregation by static light scattering. This experiment clearly shows that Hrd3 prevents aggregation. Moreover, its 'holdase' activity is similar to that of the Hsp70-type ER-chaperone Kar2/BiP. In this assay, Yos9 and a Yos9 mutant defective in the glycan-binding MRH domain also display some 'holdase' activity, indicating that Yos9 averts protein aggregation independently of its lectin function.

Our in vitro data imply that Hrd3 directly binds misfolded proteins. In vivo, Hrd3 was shown to interact with the ER-luminal Hsp70-type chaperone Kar2. We favor the idea that Kar2 segregates bound clients from Hrd3 and mobilizes them after a scanning procedure. The structural rearrangement upon ATP hydrolysis of Kar2 could be required for this process because Hrd3 does not hold an ATPase function.

Future experiments will address the function of Hrd3 and Yos9 in protein aggregation and protein folding and its interaction with the chaperone machinery.

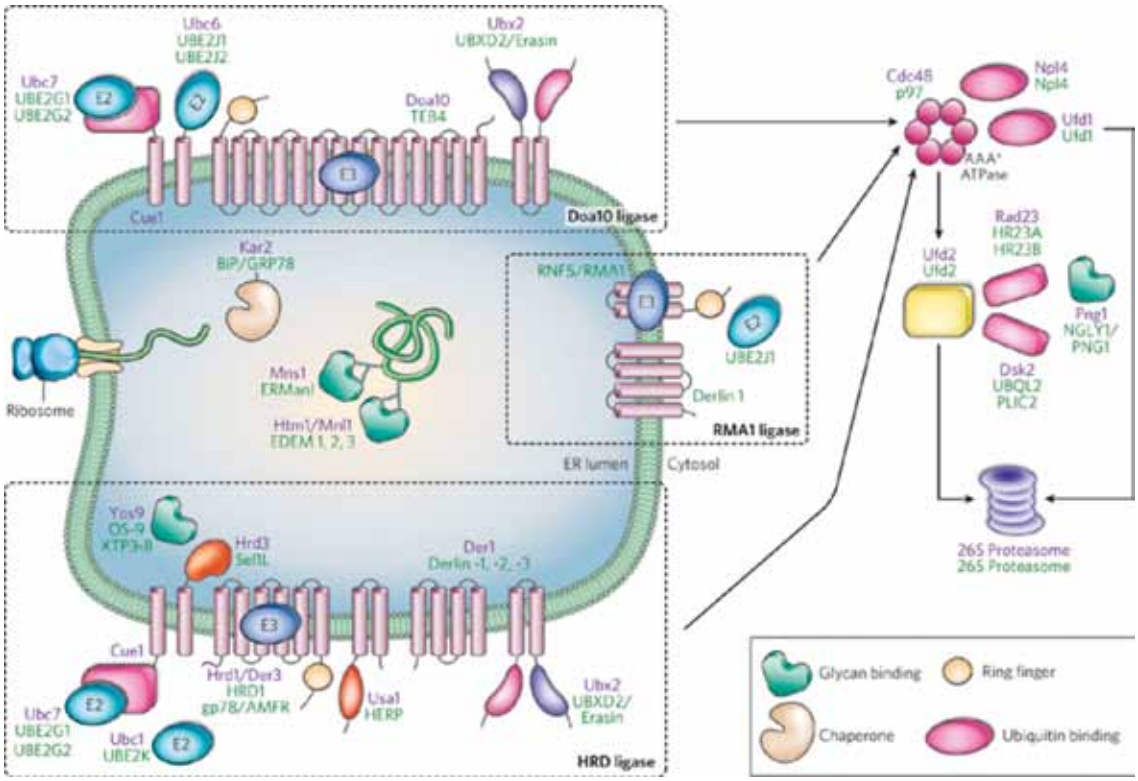

Figure 2. ER-associated protein degradation in yeast and mammalian cells. Molecular chaperones and proteins of the glycosylhydrolase-47 family (Mns1 and Htm1) detect misfolded polypeptides and direct them to membrane bound ligases (Doa10, RMA1, HRD). After dislocation to the cytosolic face of the ER membrane, substrates are ubiquitylated by an ubiquitin ligase. All ligase complexes comprise a central, catalytic active RING finger protein (E3), ubiquitin-conjugating enzymes (E2), and additional factors. The AAA ATPase Cdc48 releases ubiquitylated molecules from the ER membrane. The adapter proteins Rad23 and Dsk2 escort the ubiquitylated substrates to the 26S proteasome for degradation. Concurrently Png1 deglycosylates glycoproteins through its association with Rad23. Proteins containing a glycan-interaction motif or ubiquitin-binding domains are depicted in red and blue, respectively. Proteins are labeled with their yeast names in blue and green letters indicate the mammalian counterpart.

The ubiquitination capacity of the HRD-ligase

Katrin Bagola and Maximillian von Delbrück

One of the major, yet unsolved questions in the field is how proteins are extracted from the ER. We previously could demonstrate that ubiquitination of dislocated substrates is essential for their dislocation, suggesting that ubiquitin conjugation contributes to the directionality of the membrane passage. Furthermore we have shown that over expression of ubiquitinK48R delays ERAD significantly. However, this simple picture of ERAD being executed by attachment of a simple K48-linked polyubiquitin chain to substrates is challenged by recent mass. Spectrometric analyses.

To this end, we have established an in vitro ubiquitin-conjugation assay with purified components. The cytosolic domains of all components of the HRD ligase are expressed in *E. coli*. To make sure that the proteins are soluble, we expressed several of them without their transmembrane segments. Using this system, we were able to demonstrate that ubiquitin-conjugation at the ER-surface is a complex reaction and involves a variety of different ubiquitin chains and not only K48-ubiquitin chains. We will continue this powerful in vitro approach and will include quantitative parameters.

The function of Cdc48/p97 at cellular membranes

Martin Mehnert and Ernst Jarosch

The AAA-ATPase Cdc48, in mammals termed p97 or VCP, is a key player in many ubiquitin-dependent processes like homeotypic membrane fusion, cell cycle control,

proteasome-mediated protein degradation and DNA repair. To fulfill these activities, Cdc48/p97 teams up with a large set of diverse ancillary proteins in a temporally and spatially controlled manner. Yeast Ubx2, for example, recruits $Cdc48^{Npl4/Ufd1}$ to the ER membrane and establishes its interaction with ubiquitin ligase complexes involved in ERAD. In the past this group were among the first to unravel the important function of the yeast $Cdc48^{Npl4/Ufd1}$ complex in ERAD. They demonstrated that a loss of $Cdc48^{Npl4/Ufd1}$ significantly delays ERAD. Notably, mutations in $Cdc48^{Npl4/Ufd1}$ primarily impair the extraction of the substrates from the ER without affecting their polyubiquitylation.

Dfm1 is a small integral protein of the ER membrane that displays strong structural similarities to a subunit of the HRD ligase, Der1. Dfm1 is also associated with at least one of the ERAD ligases. Preliminary results strongly imply that Dfm1 does not bind to $Cdc48^{Npl4/Ufd1}$ but rather to another Cdc48-subcomplex, which may fulfill functions aside from the proteolysis.

In the future we will establish a functional network of membrane-bound Cdc48/p97-related activities and unravel novel mechanisms by which protein ubiquitylation affects the fate of secretory proteins.

Htm1p is a mannosidase that generates N-linked Man7GlcNAc2 glycans to accelerate the degradation of misfolded glycoproteins

Anett Köhler and Christian Hirsch

N-linked glycans are essential for the breakdown of glycoproteins. The covalently attached oligosaccharide structure is used as a signal to display the folding status of the protein. Newly synthesized proteins receive a Glc3Man9GlcNAc2 modification. Such a glycan structure protects a newly synthesized protein from degradation. Subsequently it is trimmed by glucosidases and mannosidases until a specific signal is generated, which is recognized by the quality control ubiquitin ligase. Since trimming of glycans is slow, these processing steps provide a time window in which a newly synthesized protein can adopt its cognate conformation.

To determine if Htm1p acts as a mannosidase in this pathway, Anett Köhler and Christian Hirsch developed an in vitro system consisting of Htm1p purified from yeast cells and the commercially available bovine pancreatic ribonuclease B (RNAse B) as model substrate. This protein has a single glycosylation site at Asn34, which is occupied by heterogeneous oligomannose-type glycans containing 5–9 mannose residues. Additionally, four disulfide bonds stabilize the structure of RNAse B, which allow conversion of RNAse B into a misfolded quality control substrate by reductive denaturation. Using this assay they could demonstrate that a complex of Htm1p and the oxidoreductase Pdi1p converts Man8GlcNAc2 oligosaccharides of the glycoprotein RNAse B to the Man7GlcNAc2 form, which enhances the elimination of aberrant glycoproteins.

Selected Publications

Gauss, R., Jarosch, E., Sommer, T., and Hirsch, C. (2006) A complex of Yos9p and the HRD ligase integrates endoplasmic reticulum quality control into the degradation machinery. Nature Cell Biol., 8, 849-854

Clerc, S., Hirsch, C., Oggier, D. M., Deprez, P., Jakob, C., Sommer, T., and Aebi, M. (2009) HTM1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. J. Cell. Biol. 184, 159-172

Hirsch, C., Gauss, R., Horn, S.C., Neuber, O., and Sommer, T. (2009) The ubiquitylation machinery of the endoplasmic reticulum. Nature 458, 453-460

Horn, S.C., Hanna, J., Hirsch, C., Volkwein, C., Schütz, A., Heinemann, U., Sommer, T., and Jarosch, E. (2009) Usa1 functions as a scaffold of the HRD-ubiquitin ligase. Mol. Cell 36, 782-793

Buchberger, A, Bukau, B, and Sommer, T. (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell 40, 238-252

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Folding Sensors of the Endoplasmic Reticulum

Inevitable by-products of protein synthesis are faulty polypeptides that compromise cellular functions. To avert damage from the cell, most cellular compartments harbour protein quality control systems that recognize misfolded polypeptides and arrange their disposal. Proteins that fail to meet the quality standards of the endoplasmic reticulum (ER) are delivered to the cytosol for proteasomal destruction by a pathway that is termed ER-associated degradation (ERAD). This pathway employs folding sensors to monitor the ER for terminally misfolded polypeptides. Their task is complicated by the fact that the ER is largely dominated by nascent polypeptides that also display structural defects. Consequently, the folding sensors of the ER must reliably discriminate terminally misfolded proteins from the large pool of folding intermediates that will mature productively. In our group, we investigate how the ER folding sensors of the model organism *Saccharomyces cerevisiae* selectively single out terminally misfolded polypeptides for destruction by the proteasome.

Identification and characterization of novel ERAD Substrates

Laura A. Jaenicke, Holger Brendebach, Matthias Selbach and Christian Hirsch

Currently, only few model substrates are available to study ERAD. This limitation restricts our understanding of the ERAD system, since the characterized client proteins represent only a fraction of the folding defects that may arise in the ER. The detection of structural defects that are not represented by existing model substrates may involve additional folding sensors that are not yet characterised. To extend the range of known ERAD substrates, we determined the substrate spectrum of the HRD complex by quantitative proteomics. We identified 132 proteins of the secretory pathway that exhibit elevated protein levels in Δ*hrd1* cells. For most substrates we observed a 1.5 to 2 fold increase in protein abundance. This change appears minor, implying that the folding machinery of the ER is more efficient than anticipated. Among the identified proteins was Erg3p, a glycoprotein involved in sterol-synthesis. Using this substrate, we could demonstrate that the elimination of Erg3p requires Htm1p and Yos9p, two proteins that partake in the glycan-dependent turnover of aberrant proteins. We further showed that the HRD ligase also mediates the breakdown of Erg3p and CPY* engineered to lack N-glycans. The degradation of these non-glycosylated substrates is enhanced by a mutant variant of Yos9p that has lost its affinity for oligosaccharides, demonstrating that Yos9p has a previously unrecognized role in the detection of misfolded proteins that lack N-linked glycans.

In-vitro analysis of the folding sensor Htm1p

Anett Koehler in collaboration with Heike Stephanowitz and Eberhard Krause (FMP, Berlin Buch)

The ER quality control utilizes the mannosidase Mns1p as a timer to discriminate between newly synthesized glycoproteins and those that failed to mature within a given time frame: Initially, Man9GlcNAc2 oligosaccharides protect newly synthesized glycoproteins from degradation by the ER quality control system. Polypeptides that display folding defects after their processing by Mns1p are subjected to ERAD. Because Mns1p acts on glycoproteins regardless of their conformation, Mns1p alone does not suffice to determine the fate of a protein. Otherwise, permanent residents of the ER would be destroyed after their processing by Mns1p. To prevent the ER from cannibalizing itself, two subunits of the HRD complex, Hrd3p and Yos9p form a platform to ensure that the ligase ubiquitylates only proteins which are terminally misfolded. Apparently, Hrd3p recruits potential substrates that expose unstructured regions to the luminal domain of the ligase. Subsequently, the lectin Yos9p queries proteins bound by Hrd3p for a specific Man7GlcNAc2 oligosaccharide. Only substrates that display such a bipartite signal are ubiquitylated by the HRD ligase. How is the Man7GlcNAc2 signal generated? Others and we have demonstrated that Htm1p converts oligosaccharides that were processed by Mns1p to the Man7GlcNAc2 form. We used the glycoprotein RNAse B as a substrate for Htm1p and analysed the single oligosaccharide of RNAse B by matrix-assisted laser desorption/ionization time of flight spectrometry. Incubation of RNAse B with purified Htm1p reduced the amount of detectable Man8GlcNAc2, while Man7GlcNAc2 abundance increased significantly. Since these changes were specific to Htm1p and undetectable in the presence of Htm1p-D279N, a mutant variant that does not support the degradation of aberrant glycoproteins, our results demonstrate that Htm1p generates the glycan structure that is recognized by the lectin Yos9p. Next, the substrate specificity of Htm1p will be investigated with established ERAD substrates such as CPY*, a mutant form of the vacuolar carboxpeptidase Y. We have also reported that Htm1p forms a tight complex with the oxidoreductase Pdi1p. Currently, the function of Pdi1p in this complex is not known. Therefore we will analyse the ability of Htm1p to process aberrant glycoproteins alone or in combination with Pdi1p or functional mutans of this protein in to determine the precise role of each factor during the Htm1p-dependent processing of glycoproteins.

Structure of the HRD ligase

Christian Hirsch in collaboration with Friedrich Förster (MPI Martinsried, Germany)

The HRD ligase is a transmembrane complex that comprises five distinct polypeptides. Together, these subunits link important events on both sides of the ER membrane: substrate recruitment at the luminal side, and protein ubiquitylation at its cytosolic face. It follows that the conduit that exports ERAD substrates to the cytosolic face of the HRD complex should be in the proximity of the ligase. Perhaps the HRD complex itself discharges aberrant proteins into the cytosol. To assess how the individual subunits of the HRD ligase cooperate during substrate selection and ubiquitylation we intend to obtain information on the architecture of the HRD ligase. We can isolate the HRD ligase from detergent solubilized microsomes by affinity purification. In a subsequent separation step on a gel filtration column, the complex elutes in a single peak at a volume that corresponds to a molecular weight of approximately 700 kDa. The molecular weights of the five known subunits of the HRD complex add up to 341 kDa. Since the HRD ligase comprises at least two copies of each subunit, expected and observed molecular weight are in agreement. We will analyse the HRD complex by cryo-electron microscopy in combination with single-particle reconstruction in order to determine the structure of the ligase.

Selected Publications

Jänicke L, Brendebach H, Selbach M, Sommer T, Hirsch C. (2011). Yos9p assists in the degradation of certain nonglycosylated proteins from the endoplasmic reticulum. Mol. Biol. Cell, 22(16):2937-45.

Horn SC, Hanna J, Hirsch C, Volkwein C, Schütz A, Heinemann U, Sommer T, Jarosch E. (2009), Usa1 Functions as a Scaffold of the HRD-Ubiquitin Ligase. Mol. Cell 36(5):782-93.

Hirsch C, Gauss R, Horn SC, Neuber O, Sommer T. (2009). The ubiquitylation machinery of the endoplasmic reticulum. *Nature* 26;458(7237):453-60.

Clerc S, Hirsch C, Oggier DM, Deprez P, Jakob C, Sommer T, Aebi M. (2009). Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. *J Cell Biol*. 184(1):159-72.

Gauss R, Jarosch E, Sommer T, Hirsch C. (2006). A complex of Yos9p and the HRD ligase integrates ER-quality control into the degradation machinery. Nat Cell Biol. 8(8):849-54.

Structure of the Group

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Harald Saumweber

Nuclear Signalling and Chromosomal Domains

We are interested in chromatin based mechanisms in the control of developmental gene expression. In this context we focus on the following topics: 1.) chromatin switches, Notch signaling and cell cycle control 2.) Role of histone phosphorylation in chromatin structure and gene expression 3.) Drosophila heart development. For our studies we use Drosophila, a model system providing us with numerous tools that is amenable to genetic, cytogenetic and molecular analysis. From there we may transfer our knowledge to more human related systems.

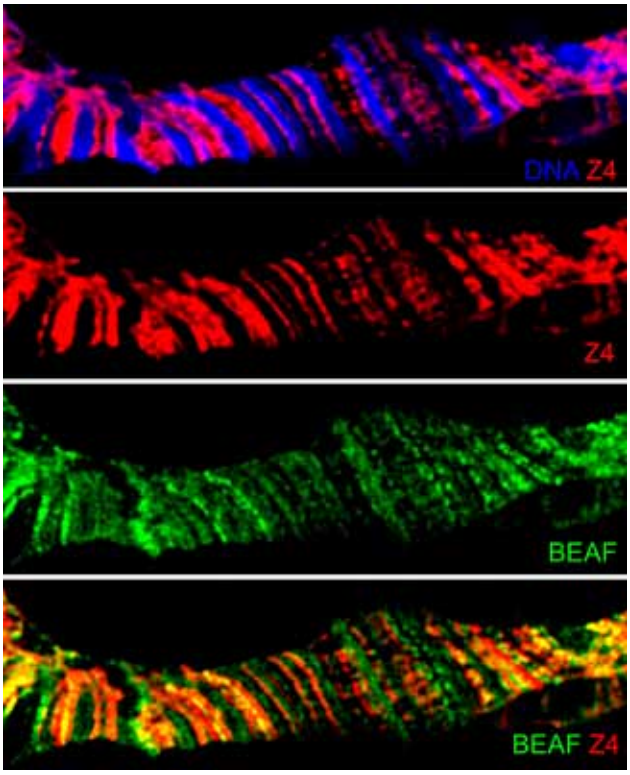
Cell cycle regulation related to Notch signalling

We previously characterized Bx42/SKIP as a conserved chromatin co-activator protein for the Notch pathway (manuscript in revision). Tissue specific knock down of Bx42/SKIP in larval imaginal discs produces *Notch*-like phenotypes and results in the down regulation of several Notch target genes. Bx42/SKIP physically interacts both with the Notch intracellular domain and with the Notch antagonist Hairless. Expression the central SKIP element, the highly conserved SNW region that lacks the Hairless interacting domain, results in dominant negative phenotypes that are suppressed by over-expression of the full length SKIP protein, by Notch gain of function mutations and are enhanced by Notch loss of function alleles and Hairless overexpression. Over-expression of SNW region induces reduced proliferation and interferes with the expression with several cell cycle regulators. In Drosophila S2 cells SKIP down regulation results in an immediate arrest in the cell cycle at the G2/M transition consistent with similar observations on imaginal discs of Drosophila larvae. According to our present knowledge cells are still alive but enter a senescent state. Work is in progress testing the expression of several suitable markers for cell viability, cell cycle control and epigenetic modification.

As a side project we identified the physical interaction of Bx42/SKIP with several isoforms of a Drosphila LIM-domain protein. Over expression data in imaginal discs show a strong phenotype, reminiscent of Notch loss of function indicating a role antagonistic to Bx42/SKIP for these proteins in Notch signalling.

Drosophila heart development

The initial steps in the development of the Drosophila heart tube are remarkably similar in molecular terms to early stages in vertebrate heart development. As a PhD project in the MDC transcard program we initiated a study on Drosophila heart development since this is more easily approachable in a genetically tractable organism. We started a systematic in situ RNA screen for heart expression of a panel of ~150 “heart genes” expressed in vertebrates. In parallel we established strains with heart specific changes in Notch expression. Since such strains show a heart phenotype they will be used to screen for genetic interactors that should enhance or suppress the respective phenotype. Genes identified by this approach should uncover novel genes involved in (upstream/downstream) Notch regulation in the heart.



Section of a polytene chromosome showing the Co-localization of BEAF and Z4 in interbands. Immunofluorescence of Z4 (red), BEAF (green) and DNA staining by Hoechst in blue.

Chromatin domains and boundaries

Formation of boundaries is part of a concept, that eukaryotic genomes are organized into functionally independent chromosomal domains. A well known chromosomal domain is the globin domain in man, mouse and chicken whose boundary function essentially requires the CTCF protein. The *Drosophila* homologue dCTCF is found at a restricted number of interbands on polytene chromosomes and is required for boundary formation in homeotic gene clusters. Due to the determined local change in chromatin condensation at the band/interband interface, chromatin mechanisms and boundary conditions must exist at this interface that are responsible for the difference

Mutation of the interband specific zinc finger protein Z4 results in a dramatic loss of band/interband structure, presumably by affecting the maintenance of chromosomal structure at such boundaries. The Z4 protein is in a complex with known boundary factors (BEAF and CP190), with histone modifying enzymes like the histone kinase Jil-1 and interacts with the novel chromodomain protein Chriz. Chriz is at the core of the complex required for Jil-1 and Z4 binding, H3S10 interphase phosphorylation and the maintenance of chromosome structure [1]. Tethering active but not inactive Jil-1 kinase results in local chromatin decondensation. Normally, Jil-1 binding is dependent on the presence of the Chriz complex. As shown by down regulation in Drosophila salivary glands and S2 cells loss of Chriz results in a significant loss of Jil-1 (and Z4) protein, presumably by altered stability of the unbound proteins. Transcription of Z4 and Jil-1 are not affected under these conditions. Loss of chromosomal Jil-1 then contributes to a loss of chromatin structure in interbands. Z4-mutations may add to the phenotype by a different mechanism. Down regulation of Z4 in imaginal discs has no influence on H3S10 specific histone phosphorylation but affects interband specific H3K4 trimethylation. ChIP data show that the complex is found at many transcriptionally active genes close to the promoter. Currently, using S2 cells we want to elucidate the correlation of the binding of the complex and gene activity. Also work is in progress to purify the whole complex and to sort out the contributions of its constituent proteins.

Selected Publications

Gan M, Moebus S, Eggert H and Saumweber H (2011) The Chriz-Z4 complex recruits JIL-1 to polytene chromosomes, a requirement for interband-specific phosphorylation of H3S10. J. Biosci. 36, 425-438.

Bartkuhn M, Straub T, Herold M, Herrmann M, Rathke C, Saumweber H., Gilfillan GD, Becker PB, Renkawitz R. (2009) Active promoters and insulators are marked by the centrosomal protein 190. EMBO J. 2009 28, 877-888.

[Mohan, M., Bartkuhn, M., Herold, M., Philippen, A., Heinl, N., Leers, J., White, R. A. H., Renkawitz-Pohl, R., Saumweber, H., and Renkawitz, R. (2007) EMBO J 26, 4203-4214.

Mendjan, S, Taipale, M, Kind, J, Holz, H, Gebhard, P, Schelder, M, Vermeulen, M, Buscaino, A, Duncan, K, Mueller, J, Wilm, M, Stunnenberg, Saumweber, H and Akhtar, A (2006) Nucleoporins are involved in the transcriptional regulation of dosage compensation in Drosophila. Mol Cell 21, 1-13.

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Jennifer Jamrath
(since 2009; fellow of the Transcard research program)

Shaza Dehne
(Syrian state PhD support fellow since 2007)

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Alexander Glahs
(HU MSc student since 4/2009)

Miguel Andrade

Computational Biology and Data Mining

Our group focuses on the development and application of computational methods that are used to research the molecular and genetic components of human disease. Recently, our main topics of research have been protein interaction networks, profiling of gene expression during induced cell reprogramming, and the prediction of novel non-coding transcripts.

Study of protein-protein interaction networks

Martin Schaefer and Jean-Fred Fontaine

Knowledge on protein-protein interactions (PPIs) is important to elucidate protein function. Several high-throughput techniques often produce large lists of PPIs, which can be scored for quality with alternative schemes. Often these must be evaluated with just a few known PPIs. To facilitate this evaluation we developed a method that uses a repetitive sampling strategy to assist the selection of the most discriminant scoring and cut-offs. On a related subject, we collaborated with Erich Wanker to generate and study a Yeast-two-Hybrid PPI network focused on 450 signalling-related proteins, which was used to predict modulators of EGF/Erk signaling. We are also studying ways to organize and filter PPI data according to reliability and functional annotations: in collaboration with Tiago Lopez and Hiroaki Kitano we compared six PPI databases for their coverage and topological network properties and explored how the addition of tissue information improves the biological relevance of the data.

Protein sequence analysis

Nancy Mah, David Fournier, Arvind Mer

We study particular aspects of protein function and structure using computational analyses: the C-terminal of cancer-related periostin (with Sebastian Hoersch), repeats in Golgi-component p115 (with Udo Heinemann), the evolution and specificity of the RNA-interacting PAZ domain from murine Piwi in complex with a piRNA (with Bernd Simon), and the function of several zebrafish proteins: the homologs of the human LRP2 receptor (with Thomas Willnow), and the actinodin family, whose loss in the tetrapoda lineage might be related to the emergence of limbs with fingers in tetrapoda (with Marie-Andrée Akimenko and Luis Sánchez-Pulido).

We also develop methods for the analysis of protein sequences and structures such as PDBpaint, which visualizes protein features in structures, and two methods with Carol Perez-Iratxeta: prediction of mycobacterial outer membrane proteins using genomic comparisons; and K2D3 for prediction of protein secondary structure from circular dichroism spectra.

Predictions from large transcript and genomic datasets

Enrique Muro, Paul Krzyzanowski (OHRI group, Ottawa)

The complete genomic sequences from thousands of organisms deposited in public databases can be used to learn about gene function and evolution. We studied the position of bacterial pseudogenes from more than 600 prokaryotic genomes to find that they have a weak but significant tendency to be situated in the last half of operons, whereas essential genes tend to be in the first half of operons (collaboration with Gabriel Moreno-Hagelsieb). This is the first evidence that genes in operons are arranged in decreasing order of importance.

Transcript datasets allow the prediction of novel transcripts. We followed this strategy to find evidence for the origin of natural antisense transcripts (NATs), some of which, trans-NATs, interact in trans with transcripts to which are complementary. The origin of trans-NATs is puzzling since their generation requires the formation

of a large region of complementarity to the target gene. We hypothesized that gene duplication evolving into a pseudogene could result in trans-NATs to the parental gene. Analysis of the Expressed Sequence Tags (EST) data originating from human pseudogenes supported this hypothesis: we observed a number of transcripts antisense to pseudogenes; collectively they displayed a region of higher similarity to the parental gene near their 3' ends.

We developed a method to detect non-coding RNAs (ncRNAs) using evidence of miRNA processing from cDNA-derived EST libraries. To illustrate the application of this approach we built a tiling microarray to test hundreds of predicted ncRNAs in differentiating myoblasts and mES cells (with Michael Rudnicki).

Analysis of gene expression

Nancy Mah, Marie Gebhardt, Mathew Huska

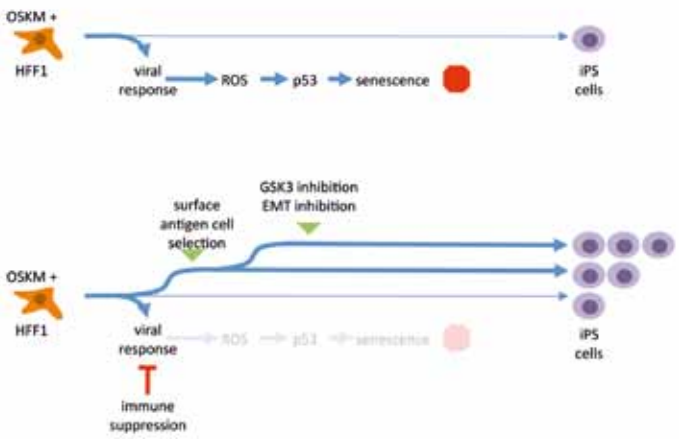
Recently, we have collaborated with several groups on DNA microarray profiling of gene expression: to demonstrate that knock down of DNA methyltransferase 1 pushes haematopoietic stem cells to a myeloerythroid progenitor state (with Frank Rosenbauer), to study the molecular basis of the decrease of muscle regenerative potential associated to ageing in murine skeletal muscle (with Guillaume Grenier), and to demonstrate that the intracellular domain of megalin lacks the capacity to regulate gene expression (with Thomas Willnow).

Research on stem cells pointed to key transcription factors that are now used to reprogram somatic cells into induced pluripotent stem cells (iPSCs). We studied gene expression datasets, including a time series following the first three days of reprogramming of human fibroblasts into iPSCs, to search for factors that could improve the efficiency of the reprogramming procedure (collaboration with James Adjaye and Ralf Mrowka). The main functional effect observed is an immune response, which is triggered by the viral induction protocol and reduces the efficiency of reprogramming. We show the activation of particular pluripotency markers and suppression of genes favoring the epithelial-to-mesenchymal transition and suggest ways to improve the reprogramming protocol (Figure).

Data and text mining

Jean-Fred Fontaine, Adriano Barbosa-Silva

We develop web tools that assist researchers exploring the biomedical literature to understand the function of genes and their relation to diseases. LAITOR (with Jose Miguel Ortega) recognizes interactions between protein and genes from text. Génie prioritizes the complete set of genes from a species according to how their associated literature (or that of their orthologs) matches a topic defined by the user. MLTrends (with Gareth Palidwor) graphs term usage in Medline versus time.



Model for improving the OCT4, SOX2, KLF4 and c-MYC based reprogramming protocol. Top: the standard protocol results in relatively few iPS cells. Bottom: suppression of the initial viral response, combined with FACS-based enrichment of cells expressing ES-specific cell surface receptors and inhibition of GSK3 and EMT, could increase reprogramming efficiency.

Selected Publications

Fontaine, JF, Suter, B, Andrade-Navarro, MA. (2011). QiSampler: evaluation of scoring schemes for high-throughput datasets using a repetitive sampling strategy on gold standards. BMC Research Notes. 4, 57.

Zhang, J, Wagh, P, Guay, D, Sanchez-Pulido, L, Padhi, BK, Korzh, V, Andrade-Navarro, MA, Akimenko, M. (2010). Loss of fish actinotrichia proteins and the fin-to-limb transition. Nature. 466, 234-237.

Mah, N, Wang, Y, Liao, MC, Prigione, A, Jozefczuk, J, Lichtner, B, Wolfrum, K, Haltmeier, M, Flöttmann, M, Schaefer, M, Hahn, A, Mrowka, R, Klipp, E, Andrade-Navarro, MA, Adjaye, J. (2011). Molecular insights into reprogramming-initiation events mediated by the OSKM gene regulatory network. PLoS One. 6:e24351.

Muro, EM, Mah, N, Moreno-Hagelsieb, G, Andrade-Navarro, MA. (2010). The pseudogenes of Mycobacterium leprae reveal the functional relevance of gene order within operons. Nucleic Acids Research. 39, 1732-1738.

Fournier, D, Andrade-Navarro, MA. 2011. PDBpaint, a visualization webservice to tag protein structures with sequence annotations. Bioinformatics. 27:2605-2606.

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Udo Heinemann

Macromolecular Structure and Interaction

In the Heinemann laboratory, crystallographic analysis is combined with biochemical and biophysical experiments to elucidate the molecular basis of central intracellular processes at atomic detail. One strand of research addresses the regulation of gene expression at the levels of transcription initiation or mRNA homeostasis. The transcription factor Klf4 is involved in controlling the differentiation state of cells and has been used in a cocktail with three other transcription factors to re-program terminally differentiated cells to a state of pluripotency. The crystal structure of the DNA-binding domain of Klf4 bound to its cognate DNA sequence reveals the structural basis of DNA sequence recognition by this protein. Proteins regulating the fate of mRNA in cells are often composed of RNA-binding modules. One such module is the cold shock domain which we study in bacterial cold shock proteins and mammalian factors such as YB-1 or Lin28. It has been demonstrated that cold shock domains bind extended DNA or RNA single strands with pyrimidine-preferential contacts at a conserved protein surface with seven distinct sub-sites. A second strand of research uses the same methodology to investigate aspects of intracellular transport. In collaboration with the Sommer and Wanker laboratories, factors involved in ER-associated protein degradation and protein retrotranslocation from the ER into the cytoplasm are studied. We are also interested in the structural basis of vesicular transport, in particular the tethering of ER-derived vesicles to the Golgi membrane. Within the Helmholtz Protein Sample Production Facility we produce proteins, crystals and crystal structure for collaborating laboratories. Our work 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. Small non-coding RNAs play an important role in the latter processes. We are studying the molecular basis of gene-expression control by crystallographic and biochemical analyses of classical transcription factors as well as proteins involved in maintaining mRNA homeostasis.

Klf4 (Krueppel-like factor-4) belongs to the family of SP/Klf zinc-finger transcriptions factors and is indispensable for terminal maturation of epithelial tissues. In a

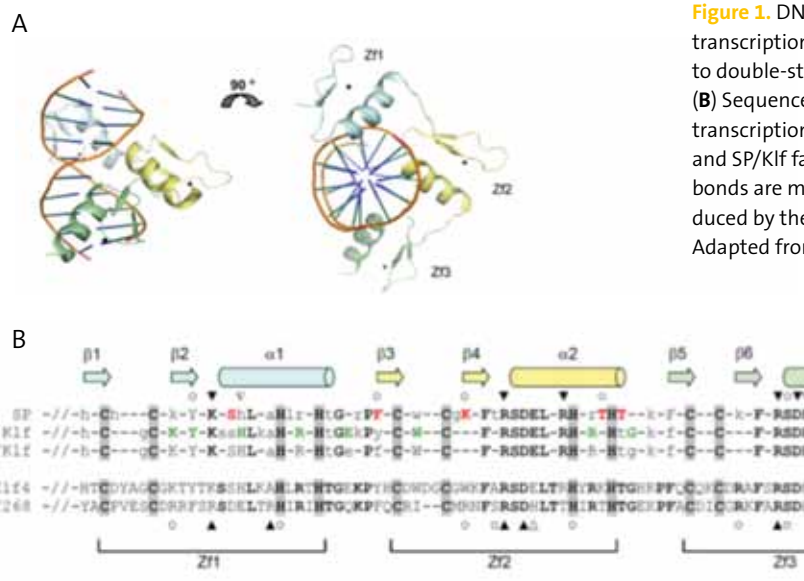

Figure 1. DNA sequence recognition by the zinc-finger domain of transcription factor Klf4. (**A**) Crystal structure of the Klf4 ZFD bound to double-stranded DNA with the consensus binding sequence. (**B**) Sequence alignment of the Klf4 zinc-finger region with the transcription factor Zif268 and consensus sequences for the SP, Klf and SP/Klf families. (**C**) DNA sequence readout by Klf4. Hydrogen bonds are marked by arrows. (**D**) Distortion of the DNA structure induced by the formation of a Klf4 His416·G8·C3' pseudo-base triple. Adapted from Schuetz et al., 2011.

cocktail with transcription factors Oct4, Sox2 and c-Myc, Klf4 can promote an opposite effect, the generation of induced pluripotent cells from differentiated tissues. The domain organization of Klf4 with amino-terminal proline-rich, acidic and PEST domains suggests that only the carboxy-proximal zinc-finger region adopts a compact, globular fold. A crystal structure analysis of the zinc-finger region bound to double-stranded cognate DNA reveals the basis of direct DNA sequence readout by Klf4. Each of the three classical CCHH zinc fingers contacts three base pairs of DNA in the major groove and achieves DNA sequence recognition by side chain-base hydrogen bonding (Figure 1). Six out of the ten base pairs contribute directly to sequence readout by Klf4, and the two carboxy-terminal zinc fingers are required for site specificity. It was shown that the lack of these two zinc fingers blocks the differentiation promoting activity of Klf4.

Cold shock domains are present in bacterial cold shock proteins and several eukaryotic transcriptions factors. They are known to bind DNA and/or RNA single strands and thus integrate the transcription and translation control levels of gene expression regulation. Following earlier work revealing the binding mode of DNA single strands to bacterial cold shock proteins it could be demonstrated that RNA strands share this binding mode by stretching a single strand across a conserved protein surface of the cold shock domain and establishing base contacts at seven distinct sub-sites. For the cold shock protein Bs-CspB we could show that pyrimidine-rich RNA strands bind with tenfold reduced affinity compared to DNA strands of identical sequence. Whereas bacterial cold shock proteins consist of a single cold shock domain, cold shock domains of eukaryotic proteins, such as the transcription/translation factor YB-1

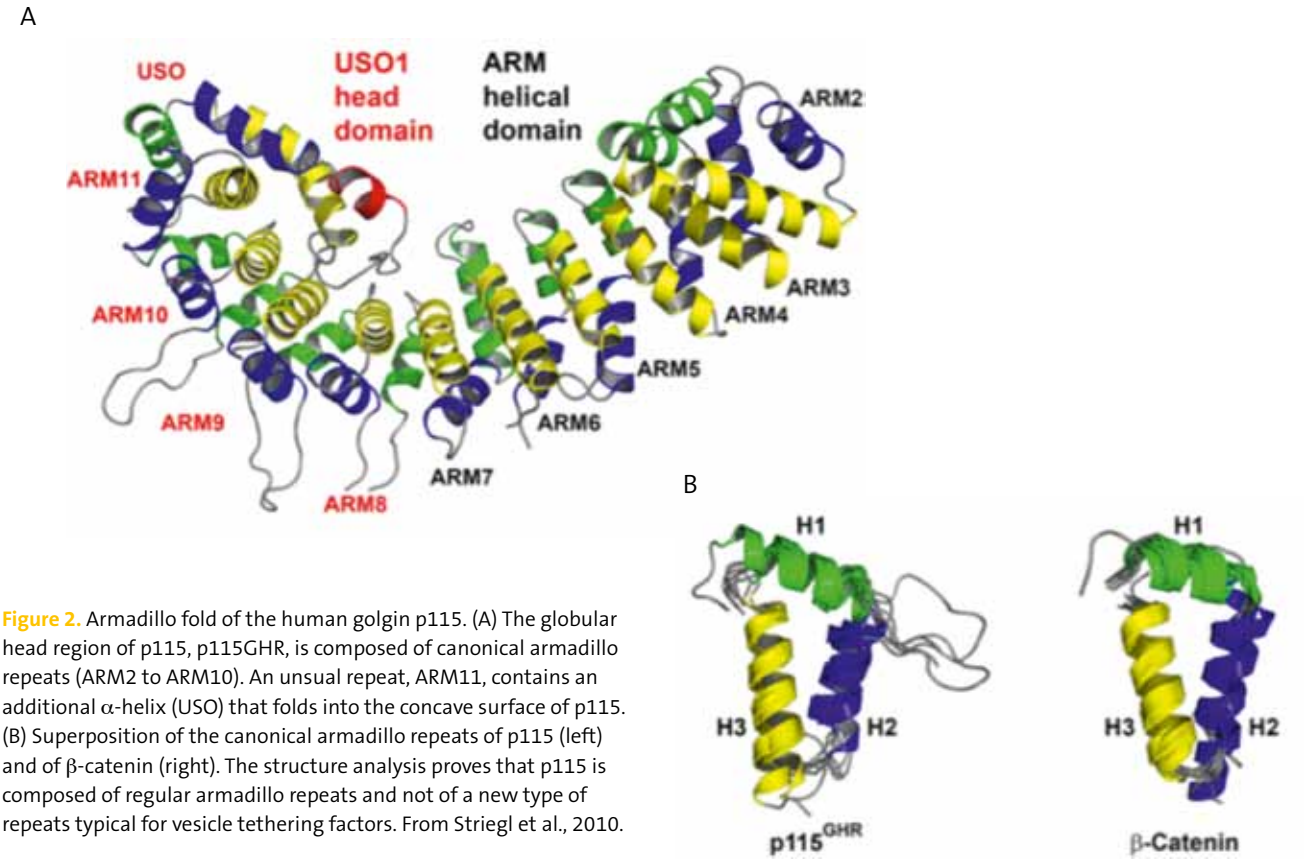

Figure 2. Armadillo fold of the human golgin p115. (A) The globular head region of p115, p115GHR, is composed of canonical armadillo repeats (ARM2 to ARM10). An unsual repeat, ARM11, contains an additional α-helix (USO) that folds into the concave surface of p115. (B) Superposition of the canonical armadillo repeats of p115 (left) and of β-catenin (right). The structure analysis proves that p115 is composed of regular armadillo repeats and not of a new type of repeats typical for vesicle tethering factors. From Striegl et al., 2010.

are often associated with natively unfolded polypeptide regions. An exception to this rule is found in the protein Lin28 where a cold shock domain is present along with a tandem zinc-knuckle domain. Lin28 inhibits the maturation of the let-7 microRNA by two different mechanisms. We could demonstrate that Lin28 binds DNA and RNA in a modus resembling the bacterial cold shock proteins and thereby alters the conformation of let-7 precursor RNA in a chaperonin-like fashion.

Molecular basis of intracellular transport

Eukaryotic cells are compartmentalized, and many proteins and other molecules must travel to their site of activity by crossing membrane bilayers. These transport steps are associated to protein maturation and quality control processes which ensure that defective macromolecules are recognized and degraded along their path. We study two different transport processes in yeast and human cells, the retro-translocation of folding incompetent protein chains from the lumen of the endoplasmic reticulum (ER) into the cytoplasm and the tethering of ER-derived transport vesicles at the membrane of the Golgi apparatus.

In collaboration with the MDC laboratories of Thomas Sommer and Erich Wanker we study the structural basis of the ER-associated degradation of misfolded proteins (ERAD) which is promoted by the HRD ubiquitin-ligase complex. Defective proteins are identified in the ER lumen, ubiquitinated and retro-translocated into the cytoplasm by the HRD-ligase complex and ultimately degraded by the proteasome. The lectin Yos9 is important for the recognition of unfolded proteins in the ER lumen by scanning proteins which still expose hydrophobic patches characteristic for incompletely folded molecules for mature surface carbohydrate structures. Crystal structure analysis showed Yos9 to adopt a dimeric protein structure which likely reflects a dimeric structure of the entire HRD-ligase complex. Evidence for this HRD-ligase dimer was earlier presented when it was shown that the cytosolic subunit Usa1 of the HRD ligase also promoted dimerization. Another protein involved in ERAD is the AAA ATPase p97 (Cdc48 in yeast) whose interaction with regulatory ligands we also study by protein crystallography. Very recently, we could co-crystallize p97 with the human protein ASPL and determine the highly unusual structure of p97-bound ASPL.

Vesicular transport in eukaryotic cells depends on conserved sets of proteins involved in the sequential steps of vesicle budding, uncoating, and tethering to the target membrane, as well as in membrane fusion and cargo release. Vesicle tethering at the target membrane

is regulated by Rab/Ypt GTPases and involves both heteromultimeric tethering complexes and factors characterized by extended coiled-coil regions. We have extended earlier studies of the transport protein particle (TRAPP), a factor required for ER-to-Golgi transport by elucidating the crystal structure of the TRAPP-associated protein Tca17. For the golgin p115, whose crystal structure was determined earlier, we could eliminate a confusion as to the nature of the repeats forming the protein structure by proving unequivocally that p115 is made up of armadillo repeats and not of a new kind of repeats typical for tethering proteins (Figure 2). Furthermore, the structures of the golgin-binding protein GRASP65 and its yeast homolog Grh1 were analyzed by crystallizing their PDZ-like domains predicted to link GRASP65 to p115 via GM130 or Grh1 to Uso1 via Bug1. Collectively, these studies highlight important aspects of vesicle tethering at the cis-Golgi membrane

Protein Sample Production Facility

In close co-operation with the Helmholtz-Zentrum für Infektionsforschung (HZI) in Braunschweig, we operate the Helmholtz Protein Sample Production Facility (Helmholtz PSPF). 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. On the technical side, the PSPF has a focus on the production of transient and other non-covalent protein-protein complexes.

The Helmholtz PSPF offers an ideal opportunity to set up collaborative projects on topics that are initially outside the area of expertise of the Heinemann laboratory. Frequently, these co-operations yield crystal structures and molecular insight on proteins linked to human disease or target proteins for pharmacological intervention with small molecules. Recently, a collaboration with the laboratory of Ludwig Thierfelder has yielded the crystal structure of a mutant form of the desmosomal protein plakophilin-2 that is linked to arrhythmogenic right ventricular cardiomyopathy. In collaboration with the laboratories of Hartmut Oschkinat and Jörg Rademann (both Leibniz-Institut für Molekulare Pharmacology, Berlin) the binding of small-molecule ligands to the PDZ domains of the proteins Shank3 from the postsynaptic density region and Dishevelled-3 involved in Wnt signaling was studied by high-resolution crystallographic analysis. It could be shown that ligand binding to both PDZ domains follows similar principles, and a foundation for the design of ligands binding with higher affinity was provided.

Selected Publications

Striegl H, Andrade-Navarro MA, Heinemann U (2010). Armadillo motifs involved in vesicular transport. PLoS ONE 5, e8991.

Kümmel D, Walter J, Heck M, Heinemann U, Veit M (2010). Characterization of the self-palmitoylation activity of the transport protein particle component Bet3. Cell. Mol. Life Sci. 67, 2653-2664.

Günther S, Schlundt A, Sticht J, Roske Y, Heinemann U, Wiesmüller K-H, Jung G, Falk K, Rötzschke O, Freund C (2010). Bidirectional binding of invariant chain peptides to an MHC class II molecule. Proc. Natl. Acad. Sci. USA 107, 22219-22224.

Schuetz A, Nana D, Rose C, Zocher G, Milanovic M, Koenigsmann J, Blasig R, Heinemann U, Carstanjen D (2011). The structure of the Klf4 DNA-binding domain links to self-renewal and macrophage differentiation. Cell. Mol. Life Sci. 68, 3121-3131.

Sachs R, Max KEA, Heinemann U, Balbach J (2011). RNA single-strands bind to a conserved surface of the major cold shock protein in crystals and solution. RNA, in press.

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Structure and Mechanism of Membrane-Remodeling G Proteins

Membrane-remodeling guanine nucleotide binding proteins (G proteins) include members of the dynamin and septin superfamilies. Whereas dynamin-related G proteins are mechano-chemical enzymes, that use the energy of GTP binding and hydrolysis to deform cellular membranes, septin-related G proteins assemble into linear filaments at their target membranes and orchestrate the recruitment of interaction partners. Our projects aim to elucidate the structural basis of the nucleotide-dependent assembly of these proteins and to understand their detailed mechanism of action. To this end, we use a combination of structural, biochemical and cell-based methods.

Mx GTPases

Mx (myxovirus-resistance) proteins are interferon-induced effector molecules in the innate immune system mediating cellular resistance against a wide range of pathogens including influenza virus. As typical members of the dynamin superfamily, they can tubulate liposomes and oligomerize in ring-like structures around these membrane tubules. Using X-ray crystallography, we determined the structure of the stalk of human MxA which folds into a four-helical bundle. Furthermore, the stalk assembled via three interfaces in a zig-zag fashion in the crystals. We showed by biochemical and antiviral experiments that the arrangement of the stalks in the crystal reflects the assembly of full-length MxA in a physiological context. Based on these results, we suggested a model for ring-like oligomers of MxA and a mechanism for the activation of GTPase activity by dimerization of G domains of neighbouring MxA rings (Fig. 1a).

We also determined the full-length MxA structure. Besides the G domain and the stalk, we identified a third domain, the bundle signalling element (BSE), which is located in the centre of the MxA molecule. Interestingly, the BSE appears to mediate conformational changes between the G domain and the stalk of the neighbouring molecule, thereby suggesting a pathway how structural changes in the G domain are transmitted to the stalk.

Dynamin GTPases

Dynamin is the founding member of the dynamin superfamily. The multi-domain protein oligomerizes around the neck of clathrin-coated vesicles and induces vesicle scission in a GTP hydrolysis-dependent fashion. However, the molecular mechanism of vesicle scission is poorly understood.

Based on our previous work on the MxA stalk, we determined the X-ray structure of dynamin in the absence of nucleotides. Dynamin shows a four-domain architecture composed of the G domain, the bundle signalling element, the stalk and the lipid binding pleckstrin homology domain. Interestingly, an interaction site between the stalk and the PH domain is often mutated in patients suffering from Charcot-Marie-Tooth neuropathy or centronuclear myopathy, two congenital diseases leading to progressive muscle weakness in the limbs. Supported by biochemical and cell-based functional assays, we propose a molecular model for helical dynamin oligomers (Fig. 1b). Furthermore, we suggest how the interplay between the dynamin domains contributes to the mechano-chemical coupling. In the future, we aim to confirm our models by determining structures of reaction intermediates in the mechano-chemical cycle.

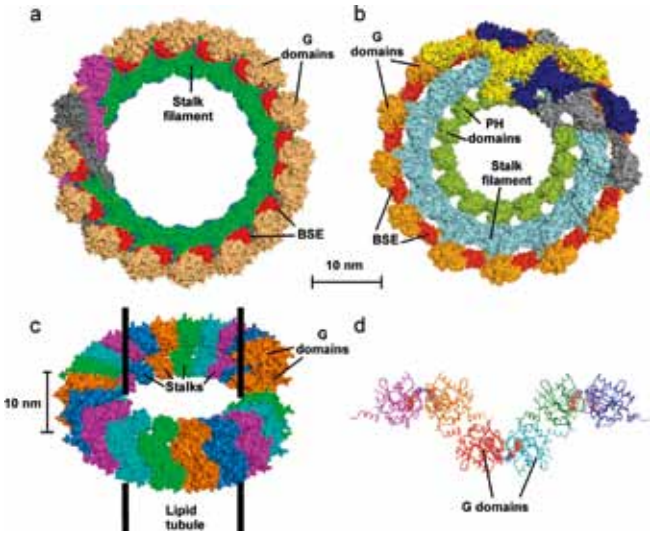

Figure 1: Oligomerization of dynamin and septin-like G proteins.
a) Model of ring-like oligomers of the MxA GTPase. We suggest that MxA assembles via the stalks in ring-like structures around ribonucleoprotein complexes of negative-strand RNA viruses, thereby interfering with viral replication (mod. from ref. 1 and 4).
b) Model of helical dynamin oligomers. Similarly to MxA, assembly takes place via the stalks. The dynamin helix oligomerizes around the neck of clathrin-coated vesicles leading to vesicle scission (mod. from ref. 2).
c) Oligomeric ring model of the EHD2 ATPase, where oligomerization is solely mediated via the G domain, but not by the helical domains / stalks. This assembly mode suggests a different mechanism of membrane remodelling at cellular membranes compared to dynamin (mod. from ref. 5).
d) Linear GTP-dependent oligomer of GIMAP2. Assembly takes place via two interfaces in the G domain (mod. from ref. 3). Instead of the stalk, an amphipathic a-helix might mediate assembly of interaction partners.

Eps15 homology domain containing proteins (EHDs)

EHD members are ubiquitously expressed dynamin-related ATPases which are built of an amino-terminal G domain, followed by a helical domain and a carboxy-terminal regulatory Eps15 homology domain. EHDs can be found at vesicular and tubular structures in the cell, and they are implicated in several trafficking pathways including the exit of cargo proteins from the endocytic recycling compartment.

We showed that also 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 a highly conserved interface in the G domain. The lipid-binding sites in each dimer are located at the tip of the helical domains and create a highly curved lipid interaction site. Based on a biochemical analysis, we suggested a model for EHD2 oligomeric rings (Fig. 1c) whose architecture is remarkably different from the MxA rings and the dynamin helix. Currently, we are characterizing the structural changes associated with lipid binding and oligomerization, using an electron paramagnetic spin resonance approach. Finally, we are interested to study the physiological function of EHD2 using cell-based experiments.

Structure and function of GTPases of Immunity-associated Proteins (GIMAPs)

GIMAPs comprise a septin-related G protein family in humans. The seven members are predominantly expressed in cells of the immune system. Some GIMAPs localize to the mitochondrial membrane and are proposed to regulate apoptosis by controlling the activity of Bcl2 family members.

We determined four X-ray 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 G domains (Fig. 1d). Furthermore, we showed that GIMAP2 in Jurkat cells is located to the surface of lipid droplets which are cellular storage and signalling compartments. Interestingly, GIMAP2 binds GTP with high affinity but does not hydrolyze it. Our future research is directed towards the identification of proteins which regulate the GTPase cycle of GIMAP2. Furthermore, we aim to identify the link between GIMAPs, lipid droplets and apoptosis.

Selected Publications

Gao S, von der Malsburg A, Dick A, Faelber K, Schröder GF, Haller O, Kochs G, Daumke O. (2011) Structure of the interferon-induced MxA GTPase sheds light on the domain interplay during the antiviral function. Immunity, in print.

Faelber K, Posor Y, Gao S, Held M, Roske Y, Schulze D, Haucke V, Noé F, Daumke O. (2011) Crystal structure of nucleotide-free dynamin. Nature, in print.

Schwefel D, Fröhlich C, Eichhorst J, Wiesner B, Behlke J, Aravind L, Daumke O. (2010) Structural basis of oligomerization in septin-like GIMAP2. PNAS 107, 20299-304.

Gao S, von der Malsburg A, Paeschke S, Behlke J, Haller O, Kochs G, Daumke O. (2010) Structural basis of oligomerization in the stalk region of dynamin-like MxA. Nature 465, 502-506.

Daumke O, Lundmark R, Vallis Y, Martens S, Butler PJ, McMahon HM. (2007) Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. Nature 449, 923-927.

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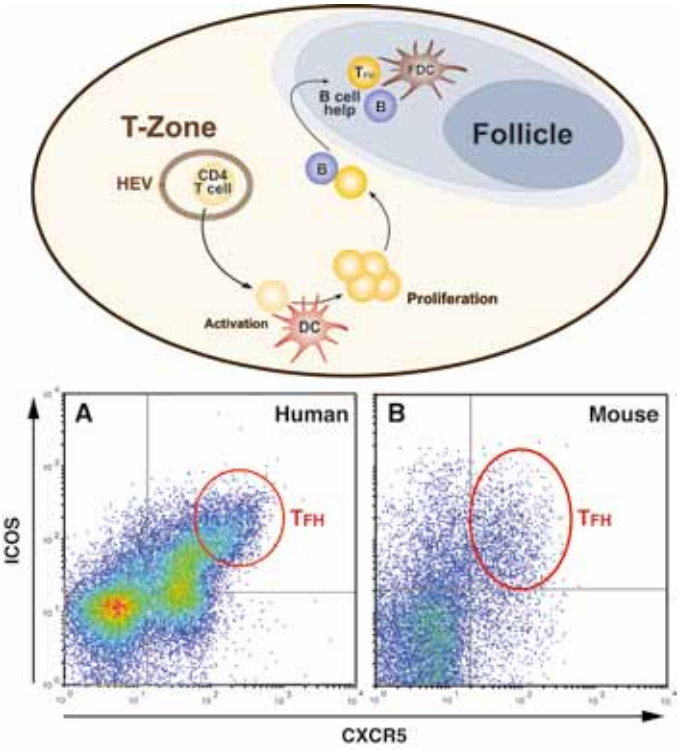
Differentiation and Growth Control in Lymphocyte Development and Immunopathogenesis

Chemokines are essential regulators of lymphocyte migration throughout the body. The chemokine system controls lymphocyte recirculation in immune system homeostasis as well as the activation-dependent and tissue-selective trafficking of lymphocytes and dendritic cells during immune responses. In addition, chemokines are critical factors for the development and organization of secondary lymphoid organs. Our main focus is the role of homeostatic chemokine receptors like CXCR5 and CCR7 in lymphoid organ development, systemic immune responses, and chronic inflammatory diseases. In addition, we are interested in the immune modulatory and growth-inducing functions of chemokine receptors encoded by human herpesviruses, and the function of sphingophospholipid receptors in the immune system.

CCR7 mediates homeostatic lymphocyte recirculation and mucosal immunity

(in collaboration with: Claudia Berek, Deutsches Rheuma-Forschungszentrum, Berlin)

The homeostatic chemokine receptor CCR7 controls not only lymphocyte trafficking to and within secondary lymphoid organs, but also homeostatic migration of T and B lymphocytes through non-lymphoid peripheral tissues. Mice that lack the chemokine receptor CCR7 showed massive gastric accumulation of lymphocytes with the development of tertiary lymphoid organs (TLOs), and histomorphological changes including cystic hyperplasia. Recently, we have been able to identify the formation of gastric TLOs and development of gastric hypertrophy of $CCR7^{-/-}$ mice as a manifestation of an autoimmune gastritis (AIG). Autoreactive $CD4^{+}$ T cells were pivotal for the induction of AIG and lymphoid follicle formation, most likely in cooperation with DCs, macrophages and B cells. The local induction of an inflammatory milieu was followed by a loss of Sonic Hedgehog (Shh)-producing parietal cells and a disturbed differentiation of mucus-producing epithelial cells. Finally, we could show that manifestation of autoimmune gastritis occured in the absence of secondary lymph nodes and preceded the development of tertiary lymphoid organs. Taken together, these findings identify an inflammatory process where gastric autoreactive T cells independent from organized tertiary lymphoid organs and classical lymph nodes can induce and maintain autoimmune gastritis.



Follicular T helper (T_{FH}) cells support B cell differentiation into antibody-secreting plasma cells and memory B cells.
Naive, recirulating CD4 T cells that become activated by antigen-presenting dendritic cells (DC) in the T cell zone of secondary lymphoid organs upregulate the chemokine receptors CXCR5 and CCR7 and migrate to the edges of B cell follicles in search for antigen-experienced B cells. Upon activation, selected cells develop into T follicular helper (T_{FH}) cells. Cognate interaction with antigen-presenting B cells leads to downregulation of CCR7 and enables T_{FH} cells to enter the B cell follicle via CXCR5. T_{FH} cells are typically identified by coexpression of the chemokine receptor CXCR5, which guides these cells into the follicle, and costimulatory molecules such as ICOS, that are required for the differentiation of T_{FH} cells and thus germinal center formation. Coexpression of these molecules is conserved between human (A) and mouse (B) T_{FH} cells.

Differentiation and transcriptional control of CXCR5^{hi}/ICOS^{hi} follicular B helper T cells (T_{FH})

T cell help to B cells is essential to the development of humoral immune responses and required at several stages of B cell differentiation into antibody-secreting plasma cells and the development of immunological memory. B cell help is mediated by a specialized subset of CD4 T cells, called follicular T helper (T_{FH}) cells, that develop independently of classical T helper subsets such as TH1, TH2 or TH17 cells. Cognate interaction of antigen presenting B cells with T_{FH} cells at the edges of follicles in secondary lymphoid organs enables the B cells to enter B cell follicles where they massively expand and start to develop germinal centers. T_{FH} cells are also required during the GC reaction, when class switch recombination and affinity maturation occur, as they control multiple steps of B cell differentiation into high-affinity antigen-presenting plasma cells or long-lived memory B cells. We could show by large-scale gene expression profiling that B helper cell activity is largely confined to CD4 T cells coexperessing the chemokine receptor CXCR5, which guides these cells into B cell follicles, and costimulatory molecules of the CD28 family such as the inducible costimulator ICOS. Currently, we are addressing the molecular mechanisms underlying the different stages of T_{FH} cell differentiation. Based on our observation that T cells express unique patterns of signaling molecules and transcription factors, we are investigating the role of selected candidates genes such as basic helix-loop-helix transcription factors and the Delta Notch signaling pathway on T_{FH} cell differentiation and their impact on humoral immune responses.

Functional role of CXCR5 in the pathogenesis of H. *pylori*-induced chronic inflammatory gastritis

(in collaboration with: Thomas F. Meyer and Anton Aebischer, Max Planck Institute for Infection Biology, Berlin)

Ectopic lymphoid follicles are a key feature of chronic inflammatory autoimmune and infectious diseases, such as rheumatoid arthritis, Sjögren's syndrome, and *Helicobacter pylori*-induced gastritis. Homeostatic chemokines are considered to be involved in the formation of such tertiary lymphoid tissue. In particular, expression of CXCL13, the ligand for CXCR5, has been associated with the formation of ectopic lymphoid follicles and the development of MALT-lymphoma in *H. pylori*-infected patients. We defined the role of CXCR5 in the development of mucosal tertiary lymphoid tissue and gastric

inflammation in a mouse model of chronic *H. pylori* infection. In contrast to wild type mice, CXCR5-deficient mice failed to develop organized gastric lymphoid follicles and exhibited altered Th17 responses to *H. pylori*. Furthermore, CXCR5 deficient mice showed reduced pathogen-specific serum IgG and IgA levels and an overall decrease in chronic gastric immune responses. In conclusion, the development of mucosal tertiary ectopic follicles during chronic *H. pylori* infection is strongly dependent on the CXCL13/CXCR5 signaling axis and lack of de novo lymphoid tissue formation attenuates chronic immune responses.

Development of a novel chronic murine model of rheumatoid arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease with unknown etiology that affects around 1% of the population. In RA, chronic inflammation of the synovium of diarthrodial joints leads to irreversible joint damage, which results in chronic pain and disability. A characteristic feature for RA is the infiltration of the synovial tissue by granulocytes and large numbers of mononuclear cells. Another hallmark is the development of self-reactive T and B cells, leading to autoantibody production. Current animal models of RA have fundamental limitations as they are lacking the complexity of the human disease. Among the restrictions is the failure of most models to induce the formation of RA-specific autoantibodies like ACPA, the rare development of an auto-antigen driven chronic course of the disease, and the restriction to few susceptible strains. Therefore, we developed a novel murine arthritis model by combining classical antigen-induced arthritis (AIA) and the autoantigen collagen type II (CII) used in the collagen-induced arthritis (CIA) model. The rationale behind this set up was to mimic a chronic disease associated with autoantibody production – as observed in human RA – in mouse strains such as BALB/c and C57/BL6 that are commonly used in immunological research. Thus, knock out animals on these backgrounds can be used for arthritis research without the need for backcrossing into more susceptible strains. In BALB/c mice, the novel antigen- and collagen-induced arthritis (ACIA) model leads to a profound synovitis and strong cartilage and bone erosion. Moreover, it is characterized by an efficient and rapid formation of high serum levels of IgG against cyclic citrullinated peptides (CCP) and CII. In comparison between the two mouse strains, arthritis pathology was less pronounced in the C57BL/6 mouse strain and chronic inflammation could not be induced in C57BL/6 mice with the novel ACIA model.

A viral chemokine receptor (vGPCR)-triggered animal model reveals epigenetic mechanisms in immune escape and tumor progression

(in collaboration with: Wei Chen and Gunnar Dittmar, MDC)

The human herpes virus 8-encoded G protein-coupled chemokine receptor (vGPCR) has been implicated in the pathogenesis of Kaposi´s sarcoma (KS) particularly because of its high constitutive signaling activity. We have used retroviral transduction to generate vGPCR-transformed BALB/c-3T3 fibroblasts that are tumorigenic in nude mice, but as expected fail to induce tumors in their immunocompetent counterparts. However, tumor fragments obtained from nude mice grow progressively in BALB/c mice. Unexpectedly, vGPCR-expressing cells established from grafted tumor fragments gave rise to tumors in immunocompetent mice. Short interfering RNA directed at vGPCR abrogated tumorigenesis of tumor-derived cells in nude mice, demonstrating that the tumor development is specifically driven by the vGPCR oncogene, but not by other successive oncogenic mutations. We have now compared gene expression profiles of primary, vGPCR-induced tumors, cell lines established from these tumors as well as the parenteral BALB/c-3T3 and vGPCR-transformed BALB/c-3T3 cells. This approach, in combination with ChIP-on-Chip histone modification analyses, large-scale DNA methylation profiling using Reduced-Representation Bisulfite Sequencing (RRBS), and mass spectrometry-based SILAC proteomics led to the identification of critical gene networks comprising signaling and immunomodulatory genes, which are regulated by epigenetic modifications related to the successive passage of the vGPCR expressing 3T3 cells in nude and immuno-competent mice. Hence, this novel animal model will contribute to our understanding of the role of the tumor microenvironment for the induction of chromatin remodeling and epigenetic changes, which in turn trigger immune escape and progressive tumorigenesis.

Sphingosine-1-phospate receptor 4 (S1P4) deficiency profoundly affects dendritic cell function and TH17-cell differentiation

The group of sphingosine-1-phosphate (S1P) receptors comprises five G protein-coupled receptors mediating a wide variety of biological functions. In order to characterize the as yet unidentified *in vivo* function of the $S1P_4$ receptor that is predominantly expressed on lymphocytic and hematopoietic cells, we have created and analysed two different $S1P_4$-deficient mouse models. The phenotype of $S1P_4$-deficient animals suggest a role of $S1P_4$ in megakaryocyte maturation as well as in T cell biology. $S1P_4$-deficient animals showed normal peripheral lymphocyte numbers and a regular architecture of secondary lymphoid organs. Interestingly, $S1P_4$ only marginally affects T cell function *in vivo*. In contrast, DC migration and cytokine secretion are profoundly affected by $S1P_4$ deficiency. Lack of $S1P_4$ expression on DCs significantly reduces T_H17 differentiation of T_H cells. Furthermore, in various *in vivo* models of T_H1 or T_H2 dominated immune reactions, $S1P_4$ deficiency consistently increased the amplitude of T_H2 dominated immune responses, while those depending on T_H1 dominated mechanisms were diminished. Finally, $S1p_4^{-/-}$ mice showed decreased pathology in a model of DSS induced colitis. In summary, for the first time, we show that $S1P_4$ signaling is involved in the regulation of DC function and T_H17 T cell differentiation. $S1P_4$ mediated S1P signaling also modifies the course of various immune diseases in a murine model. We propose that $S1P_4$ may constitute an interesting target to influence the course of various autoimmune pathologies.

Selected Publications

van de Pavert SA, Olivier BJ, Goverse G, Vondenhoff MF, Greuter M, Beke P, Kusser K, Höpken UE, Lipp M, Niederreither K, Blomhoff R, Sitnik K, Agace WW, Randall TD, de Jonge WJ, Mebius RE (2009). Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. Nat. Immunol., 10:1193-1199 [Epub 2009 Sep 27]

Golfier S, Kondo S, Schulze T, Takeuchi T, Vassileva G, Achtman AH, Gräler MH, Abbondanzo SJ, Wiekowski M, Kremmer E, Endo Y, Lira SA, Bacon KB, Lipp M. (2010) Shaping of terminal megakaryocyte differentiation and proplatelet development by sphingosine-1-phosphate receptor S1P4. FASEB J. 24:4701-4710. [Epub 2010 Aug 4]

Winter S, Rehm A, Wichner K, Scheel T, Batra A, Siegmund B, Berek C, Lipp M, Höpken UE. (2011). Manifestation of spontaneous and early autoimmune gastritis in CCR7-deficient mice. Am. J. Pathol. 179:754-765 [Epub 2010 Aug 27]

Winter S, Aebischer A, Loddenkemper C, Räbel K, Hoffmann K, Meyer TF, Lipp M, Höpken UE. (2010). The homeostatic chemokine receptor CXCR5 is required for mucosa-associated lymphoid neogenesis in chronic Helicobacter pylori-induced inflammation. J. Mol. Med. 88:1169-1180 [Epub 2011 Jun 12]

Schulze T, Golfier S, Tabeling C, Räbel K, Gräler MH, Witzenrath M, Lipp M. (2011) Sphingosine-1-phospate receptor 4 (S1P4) deficiency profoundly affects dendritic cell function and TH17-cell differentiation in a murine model. FASEB J. 11:4024-4036 [Epub 2011 Aug 8]

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Uta E. Höpken
(Delbrück Fellow)

Regulatory Mechanisms of Lymphocyte Trafficking in Homeostasis and Immunopathogenesis

Regulated lymphocytic recirculation is pivotal in immune system homeostasis and immunopathogenesis. We focus on the role of the chemokine receptor system in homeostatic lymphocytic recirculation, mucosal immune responses, and lymphoid neo-organogenesis during chronic inflammatory diseases. Secondly, we aim to dissect cellular requirements and molecular pathways which contribute to the transformation of secondary lymphoid organs toward lymphoma-permissive niches in preclinical mouse models for B cell lymphoma.

Lymphocytic homeostasis and mucosal immunity

Chemokine receptors are central regulators in the maintenance of cellular homeostasis of mucosal tissues

S. Winter, K. Wichner

Homeostatic lymphocytic trafficking through extralymphoid tissues is required for immune surveillance and the establishment of self tolerance. We previously showed that the chemokine receptor CCR7 controls homeostatic recirculation of lymphocytes through peripheral tissues. CCR7 deficiency resulted in massive accumulation of gastrointestinal lymphocytes associated with the development of tertiary lymphoid follicles (TLFs). We now identified the formation of gastric TLFs and development of gastric hypertrophy of CCR7$^{-/-}$ mice as a manifestation of an autoimmune gastritis (AIG). Activated autoreactive CD4^{+} T cells were pivotal for the induction of AIG and the development of TLFs. The local induction of an inflammatory millieu, including the secretion of IFNγ and IL-1β, was followed by a loss of Sonic

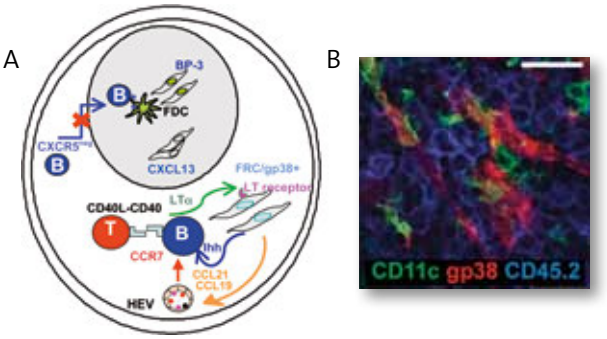

Figure 1. Lymphoma cell homing and cellular interactions in the lymphoma niche. (A) FRCs secrete CCL21 through which CCR7-expressing lymphoma cells are attracted into LNs or spleen and home to FRCs in the T-cell region. Lymphoma B cells secrete LT through which they stimulate LT receptor–expressing FRCs. In the niche, lymphoma cells receive survival signals, i.e. Ihh from FRCs and CD40 stimulation through CD40L-expressing CD4^{+} T cells. B, lymphoma B cell; FRC, fibroblastic reticular cell; Ihh, Indian hedgehog; LT receptor, lymphotoxin receptor; T, T cell. (B) Contact of tumor cells (blue, CD45.2^{+}) with gp38^{+} FRCs (red) and DCs (green) shown by immunofluorescence staining (scale bar, 25 µm).

Hedgehog-producing parietal cells and a disturbed differentiation of mucus-producing epithelial cells. The importance of lymphoid tissues as inductive sites for AIG development was analysed in CCR7/Ltα-double deficient mice which lack LNs and TLFs. Notably, the induction of autoreactive T cell responses could take place independently of organized LNs and TLFs. Taken together, we define the observed pathomorphological phenotype in CCR7$^{-/-}$ mice as an autoimmune disease that results in atrophic gastritis. We show that immuno-pathogenetic steps that lead to AIG can proceed at sites that are separate from classical LNs and organized TLFs.

Chemokine receptor function is required for m ucosa-associated lymphoid neogenesis

S. Winter (collaboration with: T. F. Meyer and A. Aebischer, MPI for Infection Biology, Berlin)

A potential relationship between chronic inflammation, establishment of extranodal TLFs and lymphoma pathogenesis has been inferred from gastric MALT lym-

phomas in humans. Expression of CXCL13, the ligand for CXCR5, has been associated with the formation of TLFs and the development of MALT-lymphoma in *H. pylori*-infected patients. We defined the causative role of CXCR5 in the development of mucosal TLFs and gastric inflammation in a mouse model of *H. pylori*-induced chronic gastritis. CXCR5$^{-/-}$ mice failed to develop organized gastric TLFs. They exhibited impaired Th17 responses, lower *H. pylori*-specific serum Ig levels, and an overall decrease in chronic gastric immune responses. In conclusion, the development of mucosal TLFs during chronic *H. pylori* infection is strongly dependent on the CXCL13/CXCR5 signaling axis and lack of de novo lymphoid tissue formation attenuates chronic immune responses.

Immunosurveillance and interactions between tumor cells and its microenvironment

Targeting the secretory pathway in CTLs to modulate their cytolytic capacity in cancer immunotherapy

(collaboration with A. Rehm and B. Dörken, MDC, Charité; I. Schmitt-Knosalla, Charité-BCRT)

This project studies whether the estrogen-inducible tumor-associated antigen, EBAG9, has a concurrent impact on T cell-mediated tumor immunosurveillance. Cytotoxic T lymphocytes (CTL) are essential for immunosurveillance and score cells for the display of tumor-derived peptides. In EBAG9$^{-/-}$ mice, we characterized the consequences of EBAG9-deletion in CTL-mediated immune responses. Loss of EBAG9 amplifies the release of lytic granules and confers CTLs with an enhanced cytolytic activity. Further proof for the high functional avidity and efficiency of EBAG9-deficient CTLs was obtained from transplantation experiments where minor histocompatibility antigen-mismatched tissues were rapidly rejected. In tumor immunotherapy, modulating the cell biological roadblocks in T cell activation and cytolytic capacity on a single cell level emerges as a strategy to increase avidity and to strengthen anti-tumor T cell efficiency. Our identification of the estrogen tunable repressor of CTL activity, EBAG9, will allow us to modulate CTL efficiency depending on the prevalent estrogen levels. In view of the suggested immunosurveillance function of CTL in cancer, we want to address if estrogen inhibition affects adoptive T cell therapy of tumors in a preclinical model.

Cellular and molecular dissection of the lymphoma-stroma in B cell lymphoma

A. Mensen, K. Schradi (collaboration with A. Rehm, and B. Dörken, MDC, Charité)

The crosstalk between lymphoid tumor cells and their environment provides pivotal signals for the initiation and progression of lymphoma. Histomorphological and immunophenotypical aspects of lymphoma recapitulate features characteristic of benign lymphoid neogenesis. We investigated the cooperation between homeostatic chemokine receptor-controlled dissemination and lymphotoxin (LT)-promoted niche formation in a transgenic mouse model of Myc-driven aggressive B cell lymphoma. We showed that CCR7 regulates Eμ-Myc lymphoma homing to LNs and distinctive microanatomic sites of the spleen. CCR7-controlled access of lymphoma cells to the splenic T cell zone led to a survival advantage compared to CCR7-deficient lymphoma cells which were excluded from this zone. Within the niche, lymphoma cells stimulated a reciprocal crosstalk with gp38^{+} fibroblastic reticular cells. This reciprocal cooperation program was mediated by lymphoma B cell-presented LT which acted on LTβR-bearing stromal cells followed by alteration of stromal cellular composition (Figure 1). Crosstalk inhibition by LTα deletion and by employing a LTβR-immunoglobulin fusion protein impaired lymphoma growth. Currently, we are investigating the role of cellular and molecular elements of secondary lymphoid organs (SLOs) during growth of an indolent lymphoma in mice. Our aim is the dissection of cellular requirements and molecular pathways which contribute to the transformation of SLOs toward lymphoma-permissive niches. 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.

Selected Publications

Rehm A, Mensen A, Schradi K, Gerlach K, Winter S, Büchner G, Dörken B , Lipp M, Höpken UE. (2011). Cooperative function of CCR7 and lymphotoxin in the formation of a lymphoma-permissive niche within murine secondary lymphoid organs. Blood 118, 1020-1033.

Winter S, Rehm A, Wichner K, Scheel T, Batra A, Siegmund B, Berek C, Lipp M, Höpken UE. (2011). Manifestation of spontaneous and early autoimmune gastritis in CCR7$^{-/-}$ mice. Am. J. Pathol. 179, 754-765.

Winter S, Aebischer A, Loddenkemper C, Räbel K, Hoffmann K, Meyer TF, Lipp M, Höpken UE. (2010). The homeostatic chemokine receptor CXCR5 is required for mucosa-associated lymphoid neogenesis in chronic Helicobacter pylori-induced inflammation. J. Mol. Med. 88, 1169-1180.

Höpken UE, Winter S, Achtman AH, Krüger K, Lipp M (2010). CCR7 regulates lymphocyte egress and recirculation through body cavities. J. Leukoc. Biol. 87: 671-682.

Rüder, C, Höpken, UE*, Wolf, J, Mittrücker, H-W, Engels, B, Erdmann, B, Wollenzin, S, Uckert, W, Dörken, B, Rehm, A*. (2009). The tumor-associated antigen EBAG9 negatively regulates the cytolytic capacity of mouse CD8+ T cell. J. Clin. Invest. 119, 2184-2203 *equal contribution

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Angela Mensen
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Katharina Wichner
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Heike Schwede

Klaus Rajewsky

Start of the group: July 2011

Immune Regulation and Cancer

Using conditional targeted mutagenesis in mice as a main tool, we explore mechanisms of normal and malignant development in the immune system. Main topics over the last two years have been the identification of signals mediating B cell differentiation and survival, the modeling of human B cell lymphomas in mice and the analysis of microRNA control in the hematopoietic system. The work was carried out at the Program in Cellular and Molecular Medicine, Children's Hospital, and Immune Disease Institute, Harvard Medical School, Boston, USA. The group will move to the MDC at the end of 2011.

Signaling pathways controlling B cell development and homeostasis

Emmanuel Derudder, Lakshmi Srinivasan, Sandrine Sander, Dinis Calado, Kevin Otipoby, Jane Seagal, Christine Patterson

We have established in published and ongoing work that i) canonical NF-κB signaling is dispensable for early B cell development, but crucial for the maturation of B cells in the peripheral immune system, ii) PI3 kinase delivers a constitutive "tonic" survival signal to mature B cells downstream of the B cell antigen receptor (BCR), and iii) the transcriptional regulator c-Myc is critically involved, in addition to Bcl6, in the differentiation of germinal center B cells (from which most B cell lymphomas originate). In a separate line of work we have shown that contrary to current dogma peripheral B cell subsets do not represent irreversibly committed separate cell lineages. For additional activities of the group in this general area the reader is referred to the literature.

Modeling human B cell lymphomas in mice

Dinis Calado, Baochun Zhang, Tomoharu Yasuda, Sandrine Sander, Shuang Li, Karl Köchert

These experiments go hand-in-hand with our work addressing mechanisms of normal B cell physiology, by studying signaling and transcriptional networks during development and homeostasis of these cells. In this latter context we have concentrated on the canonical and alternative NF-κB and PI3 kinase pathways, which are pivotal in several B cell malignancies. We have developed compound mutant mice modeling the cooperation of the c-Myc oncogene with NF-κB and PI3 kinase signaling by conditionally ablating or overexpressing key members of these pathways. In a general sense, these experiments aim at i) obtaining direct evidence for cooperating oncogenic events in lymphomagenesis in vivo, ii) identifying secondary mutations in the mouse tumors using state of the art technologies (gene expression analysis, SNP array analysis, exome sequencing) and correlating them with the mutational analysis of primary human tumors and iii) studying the role of the immune system in the control of the tumors. Recent work along these lines has established mouse models of Activated B Cell-like Diffuse Large B Cell Lymphoma (ABC-DLBCL) (through concomitant inactivation of the transcriptional repressor Blimp1 and constitutive canonical NF-κB signaling) and of sporadic Burkitt lymphoma (through germinal center B cell-specific deregulation of c-Myc expression and PI3 kinase activity). In addition we successfully modeled plasma cell tumors through c-Myc and canonical NF-κB induction, and of human Epstein-Barr-Virus (EBV) induced Post-Transplant-Lymphoproliferative Disorder (PTLD) through

B cell-specific expression of the EBV protein LMP1. This latter mouse model reproduces not only EBV-driven lymphomagenesis, but also the immune surveillance of these tumors, leading to a novel therapeutic approach based on the recognition of the tumor cells by natural killer cells. Our EBV related experiments are embedded in a broader project dissecting pathogenic mechanisms in Hodgkin lymphoma and are performed in close collaboration with Martin Janz and Stephan Mathas from the Dörken group.

MicroRNA control in the immune system and lymphomagenesis

Changchun Xiao, Lakshmi Srinivasan, Sergei Koralov, Tirtha Chakraborty, Alex Pellerin, Emmanuel Derudder, Siying Peng, Verena Labi, Pavel Volchkov, Robin Graf, Karl Köchert

Focusing on microRNAs specifically expressed and regulated in the hematopoietic system we have used conditional loss- and signal-on mutations of both microRNA genes and putative targets to determine the impact and mechanisms of microRNA control in immune reactions. In combination with cell type-specific ablation of key factors involved in microRNA biogenesis (such as Dicer, Drosha and DGCR8) these experiments showed that microRNAs play a critical regulatory role in a large variety of contexts and revealed basic features of in vivo microRNA control, like dose-response relationships and the targeting of multiple (components of) signaling or transcriptional pathways. Ongoing work focuses on the identification of functional microRNA targets by conditional mutagenesis of seed matches in 3' untranslated regions and the role of specific microRNAs in normal and malignant hematopoietic development.

Selected Publications

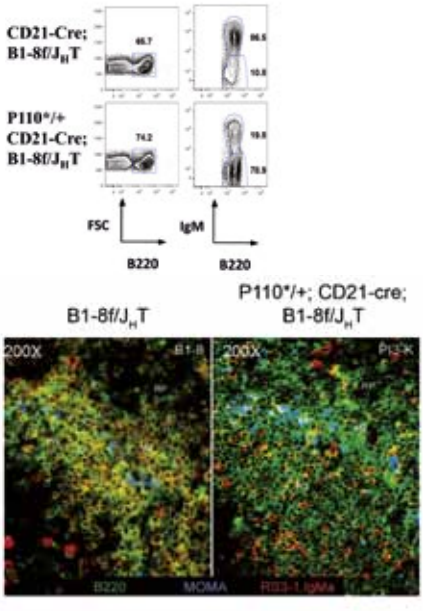
Zhang, B., Kracker. S., Yasuda, T., Casola, S., Vannemann, M., Hömig-Höltzel, C., Wang, Z., Derudder, E., Li, S., Chakraborty, T., Cotter, S.E., Koyama, S., Currie, T., Freeman, G.J., Kutok, J.L., Rodig, S.J., Dranoff, G., Rajewsky, K. (2012). Immune surveillance and therapy of lymphomas driven by Epstein-Barr-Virus protein LMP1 in a mouse model. Cell, in press.

Calado, DP, Zhang, B, Srinivasan, L, Sasaki, Y, Seagal, J, Unitt, C, Rodig, S, Kutok, J, Tarakhovsky, A, Schmidt-Supprian, M, Rajewsky, K. (2010). Constitutive Canonical NF-κB Activation Cooperates with Disruption of BLIMP1 in the Pathogenesis of Activated B Cell-like Diffuse Large Cell Lymphoma. Cancer Cell. 18, 580-589.

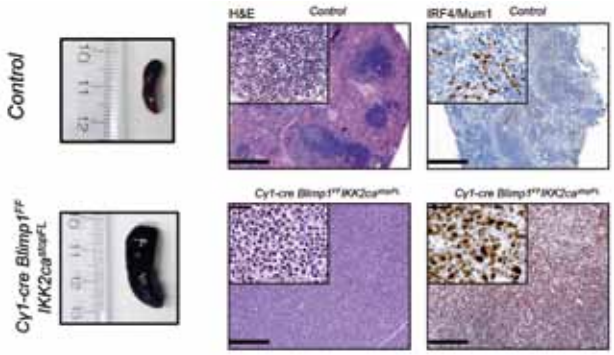
Kuchen, S, Resch, W, Yamane, A, Kuo, N, Li, Z, Chakraborty, T, Wei, L, Laurence, A, Yasuda, T, Peng, S, Hu-Li, J, Lu, K, Dubois, W, Kitamura, Y, Charles, N, Sun, HW, Muljo, S, Schwartzberg, PL, Paul, WE, O'Shea, J, Rajewsky, K, Casellas, R. (2010). Regulation of microRNA expression and abundance during lymphopoiesis. Immunity. 32, 828-839.

Srinivasan, L, Sasaki, Y, Calado, DP, Zhang, B, Paik, JH, DePinho, RA, Kutok, JL, Kearney, JF, Otipoby, KL, Rajewsky, K. PI3 kinase signals BCR-dependent mature B cell survival. (2009). Cell. 139, 573-586.

Xiao, C, Rajewsky, K. (2009). MicroRNA control in the immune system: basic principles. Cell. 136, 26-36. Review.



The PI3 kinase pathway delivers the constitutive "tonic" survival signal in mature B cells. Its constitutive activation (by expression of P110*) rescues BCR (IgM) negative mature B cells (B220 positive) in vivo (FACS analysis) that organize into follicles (FO) and adjacent marginal zone (MZ) within the spleen (immunofluorescent staining) (Srinivasan et al. 2009).



Constitutive canonical NF-κB activation (by expression of Ikk2ca) and block of terminal B cell differentiation (by ablation of Blimp1) results in tumor development. Shown are the spleens of control and tumor bearing mice (left) and the corresponding tissue sections (right, H&E and immunohistochemical staining of IRF4) demonstrating the development of lymphomas which resemble human ABC-DLBCL (Calado et al. 2010).

Structure of the Group*

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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 and exhibit one or more of the following characteristics: the sporadic nature of cancer, knowledge of a tumor antigen to allow analysis of spontaneous anti-tumor responses, non-invasive bioluminescence imaging of tumor or T cells, regulation of oncogene expression *in vivo* or serve as suitable therapy model. 2. The role of the tumor stroma for tumor progression and as target for immunotherapy. 3. Adoptive T cell therapy of cancer. Here, we present projects using several novel transgenic mouse models that allow to address questions related to the three areas.

The immune response to sporadic colorectal cancer in a novel mouse model

Current mouse models do not reflect the sporadic nature of colon cancer and do not allow the analysis of anti-tumor immune response due to the lack of known tumor antigens. Two transgenic mouse models with spontaneous tumor development were generated, directing the expression of SV40 T antigen (Tag) either constitutively (Vil-Cre x LoxP-Tag transgenic mice) or stochastically (Vil-Cre-ER^{T2} x LoxP-Tag transgenic mice) into the putative stem cell region of the crypts. Vil-Cre x LoxP-Tag mice developed multiple adenocarcinomas of the small intestine and colon at an average age of 6 months but also neonatal cytotoxic T lymphocyte (CTL) tolerance. Therefore, Vil-Cre-ER^{T2} x LoxP-Tag mice were established, in which expression of the dormant Tag was induced by stochastic, gastrointestinal-specific activation of Cre recombinase; notably in a tamoxifen-independent fashion. These mice developed highly invasive, metastasizing sporadic colon carcinomas at an average age of 20 months. Colon carcinomas expressed epithelial and/or neuroendocrine markers depending on the grade of differentiation. Young Vil-Cre-ER^{T2} x LoxP-Tag mice had retained CTL responses against epitope IV of Tag. The tumors induced strong anti-Tag IgG responses. We report, for the first time, a mouse model based on stochastic, tissue-specific activation of a dormant oncogene in the colon allowing the analysis of anti-tumor immune response against primary colorectal cancer.

In vivo imaging of an inducible oncogenic tumor antigen visualizes tumor progression and predicts cytotoxic T lymphocyte tolerance

Visualizing oncogene/tumor antigen expression by non-invasive imaging is of great interest for understanding processes of tumor development and therapy. We established transgenic mice conditionally expressing a fusion protein of the SV40 large T antigen and firefly luciferase (TagLuc) that allows monitoring of oncogene/tumor antigen expression by bioluminescent imaging (BLI) upon Cre recombinase-mediated activation. Independent of Cre-mediated recombination the

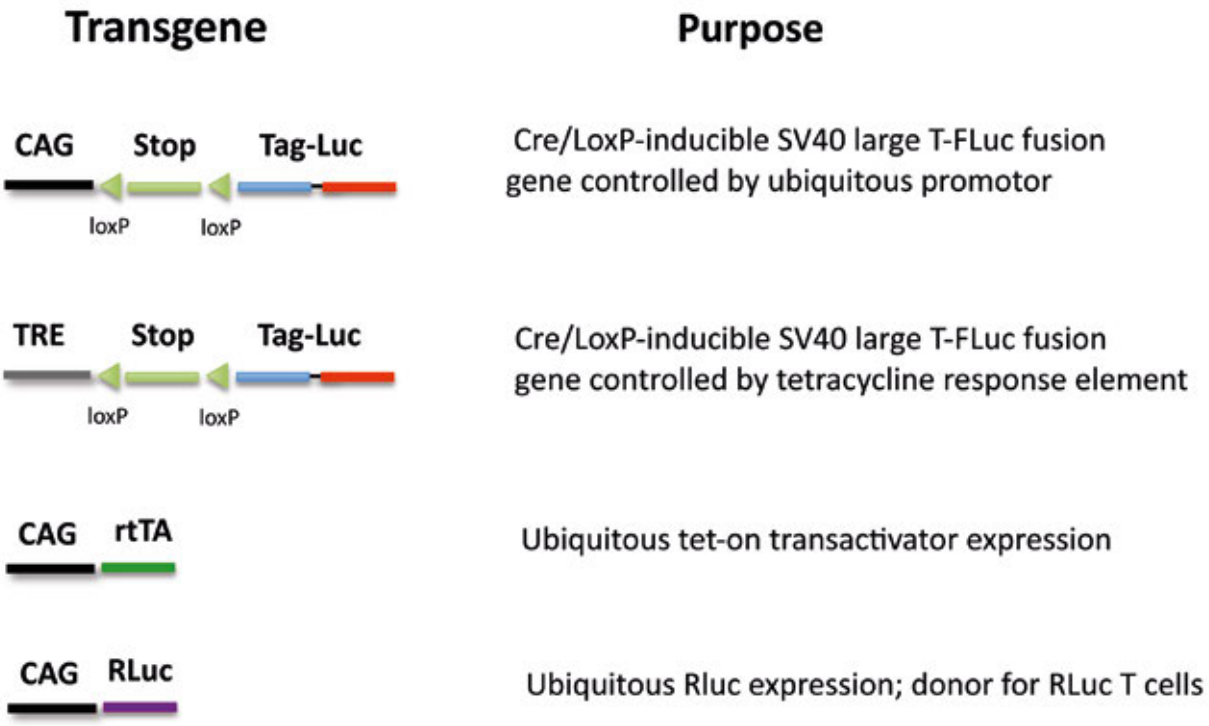

Figure 1. Bioluminescence imaging in vivo of tumor and T cells. Transgenic mice with the indicated constructs were generated. CAG, chimeric promoter composed of CMV enhancer and chicken β-actin rabbit β-globin promoter; Tag, SV40 large T; FLuc, firefly luciferase; RLuc, renilla luciferase; LoxP, signal sequence for Cre recombinase; TRE, tetracycline response element; rtTA, tet-on transactivator.

TagLuc gene was expressed at low level in different tissues, probably due to the leakiness of the stop cassette. The level of spontaneous TagLuc expression, detected by BLI, varied between the different transgenic lines, depended on the nature of the transgenic expression cassette and correlated with Tag-specific CTL tolerance. Following liver-specific Cre-loxP site-mediated excision of the stop cassette that separated the promoter from the TagLuc fusion gene hepatocellular carcinoma development was visualized. The ubiquitous low level TagLuc expression caused the failure of transferred effector T cells to reject Tag-expressing tumors rather than causing graft-versus-host disease. This model may be useful to study different levels of tolerance, to monitor tumor development at an early stage and to rapidly visualize the efficacy of therapeutic intervention versus potential side effects of low-level antigen expression in normal tissues.

T cells targeting the cancer-driving oncogene reject large genetically unstable tumors, while oncogene inactivation selects escape variants

The genetic instability of cancer cells frequently causes drug resistance. We established a cancer model, which allowed targeting of a bioluminescent oncogene by drug-mediated inactivation or mono-specific $CD8^+$ effector T (T_E) cells. Drug therapy of large tumors with high genetic instability was effective but long-term selected resistant clones. In contrast, T_E cells completely rejected large tumors (≥500 mm^3), if the target antigen was expressed in sufficient amounts and a cancer-driving fashion. While drug-mediated treatment selectively killed the cancer cells and left the tumor vasculature intact, which likely facilitated survival and growth of resistant clones, TE cell treatment led to blood vessel destruction and probably "bystander" elimination of escape vari-

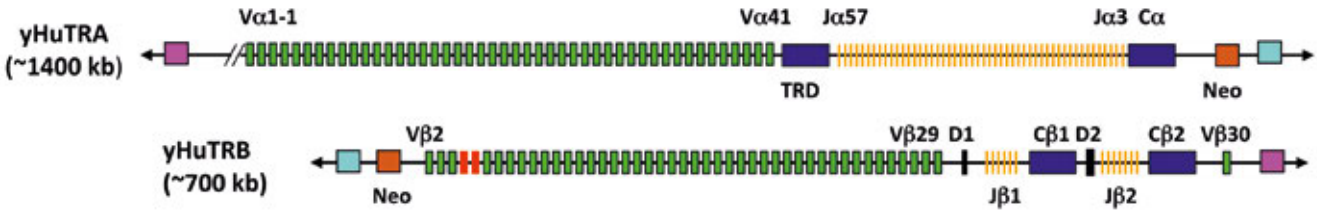

Figure 2. Transgenic mice with a human T cell receptor repertoire. The indicated genomic regions of the TCR-α and TCR-β locus on yeast artificial chromosomes were introduced into the mouse germline by use of embryonic stem cells. V, variable gene; D, diversity gene; J, joining gene; C, constant gene; TRD, TCR-d locus; Neo, neomycin gene; pink and light blue boxes, yeast selectable markers. Size of chromosomal region is indicated in parenthesis.

ants, which did not require antigen cross-presentation by tumor stroma cells.

Visualizing the dynamics of adoptively transferred T cells during tumor rejection

We generated transgenic mice ubiquitously expressing the Renilla luciferase (RLuc) gene, used these mice as donor for bioluminescent T cells and followed fate and migration of transferred T cells during rejection of large established tumors by non-invasive *in vivo* imaging. Transferred T cells preferentially accumulated within antigen positive tumors and expanded efficiently. They did not always expand in a simple linear fashion, but showed an oscillating pattern of expansion and contraction that was often followed by a rebound, until complete tumor rejection was achieved. Furthermore, visualizing the recall response showed that the transferred T cells responded expeditiously, indicating long-term memory for as long as 1 year after rejecting the tumor.

Transgenic mice with a diverse human T-cell antigen receptor repertoire

Due to tolerance mechanisms the T-cell receptor (TCR) of high avidity T cells against self- (e.g. tumor) antigens remains elusive. In mice, TCRs specific for foreign human antigens from the non-tolerant repertoire can be identified. Moreover, if they are constructed to express the human TCR repertoire, one can analyze the unskewed repertoire against human self-antigens. Here, we generated transgenic mice with the entire human TCR-αβ gene loci (1.1 and 0.7 megabases), whose T cells express a diverse human TCR repertoire that compensates mouse TCR-deficiency. A human major histocompatibility class I transgene (HLA-A*0201) increased the generation of $CD8^+$ T cells with human compared to mouse TCRs. Functional $CD8^+$ T cells against several human tumor antigens were induced and those against the Melan-A/MART-1 antigen used similar TCRs as detected in T-cell clones of autoimmune vitiligo or melanoma patients. Together, these mice allow the identification of pathogenic and therapeutic human TCRs. Our goal is to employ TCR gene therapy of cancer with TCRs selected from the transgenic mice.

The only proposed T cell epitope derived from the TEL-AML1 translocation is not naturally processed

Adoptive therapy with TCR-engineered T cells is a promising approach in cancer treatment. While usage of T cells specific for tumor-associated antigens (TAAs) can lead to serious side effects due to autoimmunity, targeting true tumor-specific mutations, such as the products of translocations in leukemias, should reduce such a risk. A potentially ideal target might be the chimeric protein TEL-AML1, which results from the chromosomal translocation 12;21 and represents the most common fusion gene in childhood B cell precursor acute lymphoblastic leukemia (BCP-ALL). Within the fusion region of TEL-AML1, a single epitope has been described by reverse immunology as immunogenic in HLA-A*0201 restriction settings. As a potential source of TCRs specific for this TEL-AML1 epitope, we have used mice expressing a human TCR-αβ repertoire and HLA-A*0201. Surprisingly, we have found that, although a specific functional $CD8^+$ T cell response against this peptide could be evoked, the described epitope was in fact not endogenously processed. Analyses done with a potent antigen presenting cell line, as well as with purified human proteasomes, support the conclusion that this peptide cannot be proposed as a potential target in immunotherapy of ALL in HLA-A*0201-restricted fashion.

Selected Publications

Buschow, C.*, Charo, J.*, Anders, K., Loddenkemper, C., Jukica, A., Alsamah, W., Perez, C., Willimsky, G. and Blankenstein, Th. (2010). In vivo imaging of an inducible oncogenic tumor antigen visualizes tumor progression and predicts CTL tolerance. J. Immunol. 184: 2930-2938. *equal contribution

Li, L.-P.*, Lampert, C.*, Chen, X., Leitao, C., Popovic, J., Müller, W. and Blankenstein, Th. (2010). Trangenic mice with a diverse human T-cell antigen receptor repertoire. Nat. Med. 16: 1029-1034. *equal contribution

Czeh, M., Loddenkemper, C., Shalapour, S., Schön, C., Robine, S., Goldscheid, E., Stein, H., Schüler, T., Willimsky, G. and Blankenstein, Th. (2010). The immune response to sporadic colorectal cancer in a novel mouse model. Oncogene 29: 6591-6602.

Popović, J., Li, L-P., Kloetzel, P.-M., Leisegang, M., Uckert, W. and Blankenstein, Th. (2011). The only proposed T cell epitope derived from the TEL-AML1 translocation is not naturally processed. Blood 118: 946-954.

Anders, K., Buschow, C., Herrmann, A., Milojkovic, A., Loddenkemper, C., Kammertoens, T., Daniel, P., Yu, H., Charo, J. and Blankenstein, Th. (2011). T cells targeting the cancer-driving oncogene reject large genetically unstable tumors, while oncogene inactivation selects escape variants. Cancer Cell, in press

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Wolfgang Uckert

Molecular Cell Biology and Gene Therapy

Over the past decade, the genetic introduction of T cell receptor (TCR) genes into T cells has been developed as a strategy to provide defined antigen-specific T cell immunity. TCR gene transfer aims to target tumor cells or virus-infected cells by genetic modification of T cells to become tumor- or virus-reactive. The potential value of this strategy – designated as TCR gene therapy – was established in mouse models and the feasibility of infusion of autologous TCR gene-modified T cells was shown in phase I clinical trials. However, most studies were accompanied by side effects and revealed first, the importance to select the right candidate as target antigen and second, the need to improve the methodology for TCR gene therapy.

We address questions related to: generation of TCR gene-modified T cells with new antigen specificity and high functional avidity, safety aspects of TCR gene-modified T cells with respect to on- and off-target effects, adoptive transfer of TCR gene-modified T cells in mice as a preclinical model for the application of TCR gene therapy in humans.

Designer TCR for immunotherapy

Daniel Sommermeyer

First clinical trials using TCR gene-modified T cells have shown that a high TCR expression level is crucial for a successful therapy. One promising TCR modification to increase expression on human T cells is the replacement of human TCRα- and TCRβ-chain constant (C) regions by their murine counterparts ("murinization"). Because of the likely immunogenicity of these hybrid constructs, we identified the decisive differences between the human and the murine sequence to reduce the amount of foreign sequences.

For the TCRα-chain C-region, a domain of four amino acids was sufficient for improved TCR expression. Five essential amino acid exchanges were identified in the TCRβ-chain C-region, with exchange of a glutamic acid (human) for a basic lysine (mouse) at position 18 of the C-region, being most important. The minimally murinized TCR variants enhanced expression of human TCR by supporting preferential pairing of transgenic TCR chains. Additionally, the usage of minimally murinized TCR chains improved the function of transduced primary human T cells when compared to cells transduced with wild type TCR. For TCR gene therapy, the utilization of minimal instead of completely murinized C-regions reduces the number of foreign residues by approximately 90 percent and thereby the risk of immunogenicity of transgenic TCR.

Redirection of T cell antigen specificity by TCR gene transfer

Peter Meyerhuber, Lilian Stärck, Matthias Leisegang in collaboration with Helga Bernhard

Human epidermal growth factor receptor 2 (HER2) has been successfully targeted as a breast cancer-associated antigen by various strategies. HER2 is also overexpressed in other solid tumors (e.g. stomach cancer) as well as in hematological malignancies (e.g. acute

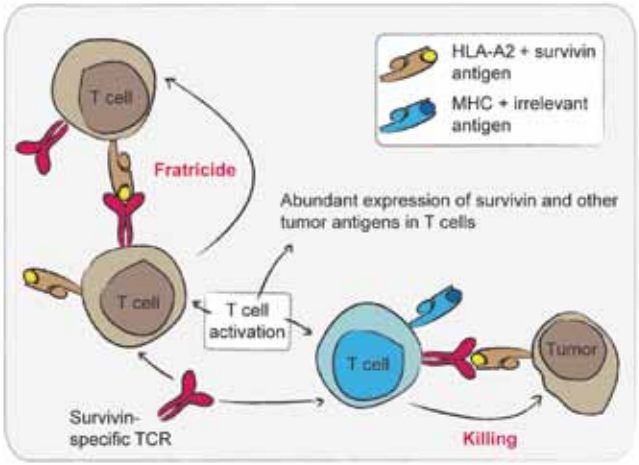

Figure 1. MHC-restricted fratricide limits survivin-based TCR gene therapy.

lymphoblastic leukemia). HER2-targeted therapies are currently under clinical investigation for a panel of malignancies. We isolated the TCR genes from an HLA-A2-allorestricted cytotoxic T cell clone and introduced the TCRα- and β-chain genes into a retroviral vector. Murinization and codon optimization of the HER2-reactive TCR was necessary to achieve efficient TCR expression in primary human T cells. The tumor recognition efficiency of HER2-TCR gene-modified T cells was similar to the parental CTL clone from which the TCR genes were isolated. These results could contribute to the development of TCR gene therapy for the treatment of HER2$^+$ tumors.

Matthias Leisegang in collaboration with Dolores Schendel

The apoptosis inhibitor protein survivin is overexpressed in many tumors, making it a candidate target molecule for various forms of immunotherapy. To explore survivin as a target antigen for TCR gene therapy using survivin-specific transgenic TCR T cells, we isolated HLA-A2-allorestricted survivin-specific T cells with high functional avidity. Transgenic TCR were derived from these T cells and specifically recognized HLA-A2$^+$ survivin$^+$ tumor cells. Surprisingly, HLA-A2$^+$ but not HLA-A2$^-$ T cells expressing transgenic TCR underwent extensive apoptosis over time.

This demise was caused by HLA-A2-restricted fratricide that occurred due to survivin expression in T cells, which created ligands for transgenic TCR recognition (Fig. 1). Therefore, survivin-specific TCR gene therapy would be limited to application in HLA-A2-mismatched stem cell transplantation. We also noted that TCR gene-modified T cells killed T cell clones of various specificities derived from HLA-A2$^+$ but not HLA-A2$^-$ donors. These results raise a general question regarding the development of cancer vaccines that target proteins that are also expressed in activated lymphocytes, since induction of high-avidity T cells that expand in lymph nodes following vaccination or later accumulate at tumor sites might limit themselves by self-MHC-restricted fratricide while at the same time inadvertently eliminating neighboring T cells of other specificities.

Side effects in a mouse model of TCR gene therapy

Ton Schumacher (NCI, Amsterdam, NL) in collaboration with Elisa Kieback

TCR gene therapy is being developed to target tumors and pathogens, and its clinical testing has commenced in patients with cancer. In a mouse model of TCR gene therapy under conditions that closely mimic the clinical setting, we showed that lethal cytokine driven autoimmune pathology can occur. The pairing of transgenic and endogenous TCR chains in TCR gene-modified T cells led to the formation of self-reactive TCR that were responsible for the observed autoimmunity. Furthermore, we demonstrated that adjustments in the design of gene therapy vectors and target T cell populations can be used to reduce the risk of TCR gene therapy-induced autoimmune pathology.

Selected Publications

Sommermeyer, D and Uckert, W. (2010). Minimal amino acid exchange in human T cell receptor (TCR) constant regions fosters improved function of TCR gene-modified T cells. J. Immunol. 184: 6223-6231.

Leisegang, M, Turqueti-Neves, A, Engels, B, Blankenstein, T, Schendel, DJ, Uckert, W*, Noessner, E*. (2010). T cell receptor gene-modified T cells with shared renal cell carcinoma specificity for adoptive T cell therapy. Clin. Cancer Res. 16: 2333-2343. (*Equal contribution).

Meyerhuber, P, Conrad, H, Stärck, L, Leisegang, M, Busch, DH, Uckert, W*, Bernhard, H*. (2010). Targeting the epidermal growth factor receptor (HER) family by T cell receptor gene-modified T lymphocytes. J. Mol. Med. 88: 1113-1121. (*Equal contribution).

Leisegang, M, Wilde, S, Milosevic, S, Spranger, S, Frankenberger, B, Uckert, W*, Schendel, DJ*. (2010). MHC-restricted fratricide of recipient lymphocytes expressing transgenic T cell receptors specific for the apoptosis-inhibitor protein survivin. J. Clin. Invest. 120: 3869-3877. (*Equal contribution).

Bendle, GM, Linnemann, C, Hooijkaas, AI, de Witte, MA, Jorritsma, A, Bies, L, Kaiser, ADM, Pouw, N, Debets, R, Kieback, E, Uckert, W, Song, J-Y, Haanen JBAG, Schumacher, TNM. (2010). Lethal Graft-versus-host disease in mouse models of T cell receptor gene therapy. Nature Med. 16: 565-570.

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Bernd Dörken

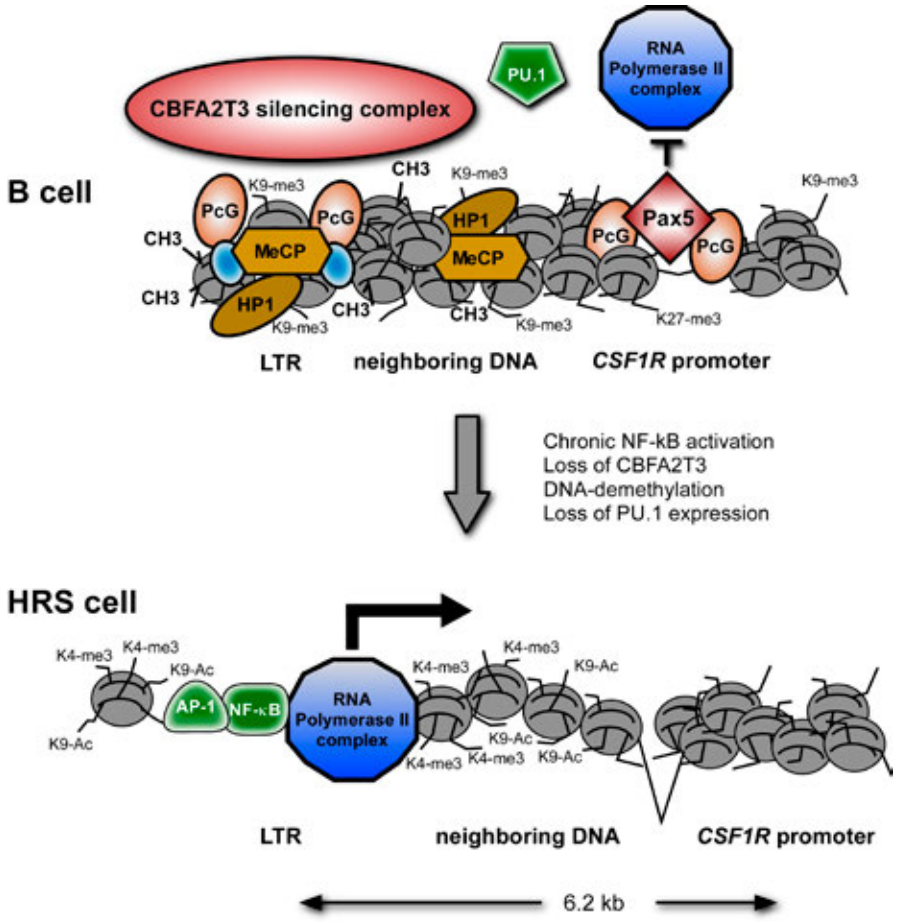
Biology and Targeted Therapy of Lymphoma

A prerequisite to develop new approaches for the treatment of malignant lymphomas is a thorough understanding of the molecular mechanisms that govern oncogenic transformation. Our group is interested in the role of transcription factor networks and signaling pathways with a special emphasis on cellular dedifferentiation and reprogramming of lymphoid malignancies. To this end, we investigate the link between alterations in physiological transcription factor activities, lineage-inappropriate gene expression and oncogenic transformation. In this context, we focus in particular on the transcription factor E2A, which is essential for the development of B and T lymphoid cells. In various lymphoma entities, its function is disrupted by genomic alterations as well as functional antagonism. In addition, it is the aim of the group to translate these findings into new treatment strategies for human B and T cell-derived malignancies.

Deregulated transcription factor networks and their role in dedifferentiation, reprogramming, proliferation and survival of malignant lymphomas

S. Mathas, M. Janz, B. Dörken

Cellular differentiation of hematopoietic stem cells into distinct lineages is controlled by a complex network of transcription factors that establish lineage-specific gene expression and/or suppress alternative developmental fates. During the last two decades, it has become increasingly clear that disruption of the physiological differentiation process is intimately linked to lineage infidelity, cellular reprogramming and eventually tumor development in the hematopoietic system. A number of lymphoid tumors display a phenotype that is in accordance with such a reprogramming process, including classical Hodgkin lymphoma (cHL), primary effusion lymphoma (PEL) and anaplastic large cell lymphoma (ALCL). Among these, cHL constitutes the most prominent example for lineage infidelity and reprogramming: in striking contrast to their origin from B cells, the malignant Hodgkin-/Reed-Sternberg (HRS) cells of cHL have almost completely lost their B cell-specific gene expression program and acquired expression of genes characteristic for other hematopoietic lineages. We have demonstrated that the B cell-associated transcription factor E2A is functionally inhibited in HRS cells by the upregulation of the antagonistic helix-loop-helix proteins ID2 and ABF1, leading to a complete inhibition of the normal B cell-associated E2A DNA binding activity, suppression of B cell-specific genes and activation of non-B cell, i. e. lineage-inappropriate genes,



Model of aberrant endogenous long terminal repeat (LTR) activation in Hodgkin lymphoma cells. In normal mature B cells, DNA upstream of the CSF1R locus including the CSF1R LTR is organized in compact heterochromatin that carries inactive histone marks such as histone H3 K9 trimethylation and methylated DNA. This recruits methyl binding proteins (MeCP), polycomb complexes (PcG) and heterochromatin protein (HP1). The heterochromatic state of the LTR is maintained by the CBFA2T3 silencing complex. The normal CSF1R promoter is repressed by the transcription factor PAX5 which blocks the PU.1-mediated recruitment of RNA polymerase II. In HRS cells, PU.1 and CBFA2T3 expression is lost, silent chromatin is not maintained and DNA is demethylated. In addition, inducible transcription factors such as NF-κB and AP-1 are chronically activated and bind to the LTR. Chromatin is remodelled, leading to the recruitment of RNA polymerase II to the LTR and active transcription.

among them CSF1R, GATA3 and TCF1. Interestingly, we have observed in further studies that disruption of the physiological E2A activity is not limited to cHL, but can also be found in other lymphoma entities such as PEL, ALCL and T cell-derived Sézary syndrome, demonstrating that alterations in E2A activity belong to the most frequent recurrent functional aberrations in lymphoid neoplasms. As a striking example for lineage-inappropriate gene expression, we have identified the overexpression of the myeloid CSF1 receptor in B cell-derived HL, a phenomenon that mediates strong mitogenic and survival signals for HL cells. Remarkably, we observed that expression of the CSF1 receptor is not mediated by the canonical myeloid promoter, but by an upstream long terminal repeat (LTR) that is aberrantly activated due to a loss of epigenetic control.

Identification of survival pathways of lymphoid cells for the development of new therapeutic strategies

M. Janz, S. Mathas, B. Dörken

A second major aim of our work is to translate basic research findings into new approaches for the treatment of lymphoid malignancies, including Hodgkin and non-Hodgkin lymphomas as well as multiple myeloma (MM). In continuation of earlier work, we concentrate on the role of the NF-κB and AP1 transcription factor system in apoptosis resistance and proliferation. For instance, we have shown that overexpression of the NF-κB/IκB family member BCL3 constitutes a novel molecular defect of the NF-κB system in cHL and ALCL. Furthermore, we

have demonstrated that AP1 is involved in the dedifferentiation process of HRS cells by maintaining high expression of the E2A antagonist ID2 and that overexpression of the AP1 family member FRA2 in ALCL cells contributes to their malignant transformation. AP1 activity might be enhanced further by the CREB family member ATF3, which is overexpressed in HRS and ALCL tumor cells. In addition, we have described that p53-dependent apoptosis can be induced in HRS cells by the MDM2-antagonist nutlin-3, indicating that activation of the p53 pathway might represent a novel treatment strategy for cHL. In recent work, we have shown that aberrant expression of the NOTCH coactivator Mastermind-like 2 (MAML2) provides an alternative mechanism to activate NOTCH signaling in human lymphoma cells. Moreover, MAML-derived small-peptide constructs were found to block NOTCH activity and disrupt formation of the NOTCH transcription complex (NTC), suggesting that direct targeting of the NTC can serve as a treatment strategy for NOTCH-dependent malignancies. To identify potential new therapeutic targets in MM, we have used a gene expression profiling approach to characterize the response of myeloma cells to essential growth factors of the bone marrow. These studies demonstrated that SGK1, encoding a serine/threonine kinase highly homologous to AKT, is a prominent transcriptional target of cytokine-induced signaling in myeloma cells promoting their malignant growth.

Targeting the secretory pathway in cytotoxic T lymphocytes to modulate their cytolytic capacity in cancer immunotherapy

Armin Rehm and Bernd Dörken; in collaboration with Uta E. Höpken (MDC), Wolfgang Uckert (MDC), Thomas Willnow (MDC), and Isabela Schmitt-Knosalla, Charite-BCRT

The estrogen-tunable gene EBAG9 tempers lymphocyte killing activity of cytotoxic T lymphocytes (CTLs), implicating that estrogen and its receptor inhibitors could also be intimately linked with T cell-mediated cancer immunosurveillance. We could show that EBAG9 deletion stimulates the cytolytic function of CTLs by negatively regulating cytolytic effector molecule trafficking from the trans-Golgi network to secretory lysosomes, also called lytic granules (Rüder et al., J Clin Invest, 2009).

In an EBAG9-deleted mouse strain we could not only demonstrate improved control of bacterial infections, but also accelerated cytolytic destruction of tumor-peptide pulsed target cells. Further proof for the unusually high functional avidity and efficiency of EBAG9-deleted mice was obtained from transplantation experiments where minor histocompatibility antigen-mismatched tissues were rejected in a rapid manner. Thus, we will exploit an engineered EBAG9-deficient CTL system to perform adoptive transfers in tumor-bearing mice.

Furthermore, in carcinoma cells EBAG9 overexpression delays vesicle transport from the ER and adjacent intermediate compartment to the Golgi complex and causes components of the ER quality control and glycosylation machinery to mislocalize. As a consequence, EBAG9 induces the deposition of tumor-associated glycan antigens on the cell surface, which are thought to contribute to tumor pathogenesis through the mediation of adhesion, invasion, and metastasis (Wolf et al., FASEB J, 2010).

While the function of the sorting receptors SorLA and Sortilin within the neuronal system has been well appreciated, the role of both receptors in the immune system remains enigmatic. In a Sortilin-deficient mouse strain we have elucidated that this sorting receptor plays a pivotal and non-redundant role during the cytotoxic effector response. In contrast to the function of EBAG9 which is restricted to CTLs, all three effector cell populations including CTLs, Th1, and NK cells, were found to be affected in Sortilin KO animals.

From both animal models we infer that the modulation of cell biological roadblocks in T cell activation emerges as a reasonable strategy to increase avidity and strengthen anti-tumor T cell efficiency.

Defining a lymphoma survival niche within secondary lymphoid organs

Armin Rehm and Bernd Dörken; in collaboration with Uta E. Höpken (MDC) and Georg Lenz, Charite-MKFZ

The role of genetic lesions is recognized as being essential for the malignant transformation of lymphocytes. However, there is increasing evidence that a crosstalk between lymphoma cells and their microenvironment, also referred to as tumor stroma, is also critically involved in growth, survival and even chemotherapy-resistance of lymphoma cells.

Malignant lymphocytes phenocopy migratory and lodging behaviour of their benign counterparts; apparently they are also subject to microenvironmental constraints since they only rarely survive ex vivo in cell culture. We have applied transgenic mouse models which deve-

lop oncogene-driven lymphoma to dissect the stroma conditions necessary for lymphoma progression. In the aggressive Eμ-Myc B cell lymphoma model we showed that the chemokine receptor CCR7 regulates access of lymphoma B cells to lymphoma-supporting niches within secondary lymphoid organs. Moreover, lodging within specific microanatomical sites launched a reciprocal cellular crosstalk with fibroblastic reticular stromal cells. This crosstalk was triggered by B cell-derived lymphotoxin, which acted upon the cognate lymphotoxin β-receptor on stromal cells. Subsequently, stromal cells provided the tumor cells with the survival factor Indian Hedgehog, but also with the chemoattractants CCL19 and CCL21 which allowed further recruitment of lymphoma B cells. Immuno-pharmacological interference with this crosstalk employing a lymphotoxin β-receptor immunoglobulin delayed lymphoma growth substantially (Rehm A. et al., Blood, 2011).

In an indolent B cell lymphoma model, we currently extend these observations and aim at a more complete dissection of cellular stromal networks and signaling pathways that are crucial for lymphomagenesis in vivo.

Selected Publications

Fagerli, U-M, Ullrich, K, Stühmer, T, Holien, T, Köchert, K, Holt, RU, Bruland, O, Chatterjee, M, Nogai, H, Lenz, G, Shaughnessy, JD, Mathas, S, Sundan, A, Bargou, RC, Dörken, B, Børset, M, Janz, M. (2011). Serum/glucocorticoid-regulated kinase 1 (SGK1) is a prominent target gene of the transcriptional response to cytokines in multiple myeloma and supports the growth of myeloma cells. Oncogene. 30, 3198-3206.

Köchert, K, Ullrich, K, Kreher, S, Aster, JC, Kitagawa, M, Jöhrens, K, Anagnostopoulos, I, Jundt, F, Lamprecht, B, Zimber-Strobl, U, Stein, H, Janz, M, Dörken, B, Mathas, S. (2010). High-level expression of Mastermind-like 2 contributes to aberrant activation of the NOTCH-signaling pathway in human lymphomas. Oncogene. 30, 1831-1840.

Lamprecht*, B, Walter*, K, Kreher*, S, Kumar, R, Hummel, M, Lenze, D, Köchert, K, Bouhlel, MA, Richter, J, Soler, E, Stadhouders, R, Jöhrens, K, Wurster, KD, Callen, D, Harte, MF, Giefing, M, Barlow, R, Stein, H, Anagnostopoulos, I, Janz, M, Cockerill, P, Siebert, R, Dörken, B, Bonifer, C, Mathas, S. (2010). De-repression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. Nature Med. 16, 571-579. *) equal contribution.

Rehm, A., Mensen, A., Schradi, K., Gerlach, K., Winter, S., Büchner, G., Dörken, B., Lipp, M., Höpken, U.E. (2011). Cooperative function of CCR7 and lymphotoxin in the formation of a lymphoma-permissive niche within murine secondary lymphoid organs. Blood 118, 1020-1033

Wolf, J., Reimer, T.A., Schuck, S., Rüder, C., Gerlach, K., Müller, E.C., Otto, A., Dörken, B., Rehm, A. (2010). Role of EBAG9 protein in coat protein complex I-dependent glycoprotein maturation and secretion processes in tumor cells. FASEB J. 24, 4000-4019

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Molecular Immunotherapy

Goal of our work is the implementation of basic research into clinical trials. We will further develop our gene-modified tumor cell vaccine (developed with Th. Blankenstein and D. Schendel) for treatment of renal cell cancer by combining vaccination with therapies that modulate regulatory T cells (T-regs). A multicenter clinical trial of Dendritic cell vaccination in patients with chronic myeloid leukemia (CML) with persisting minimal residual disease after imatinib treatment will start in early 2012. In preclinical models of DNA vaccination the chemokines CCL19 and CCL21 have been shown to enhance the vaccine activity, we envisage a clinical study using this strategy in Her-2 overexpressing breast cancer patients. Epitopes of the B-cell antigens CD22 and CD79a/b are being evaluated as targets for T-cell receptor (TCR) gene transfer in cooperation with Th. Blankenstein and D. Schendel. In order to avoid the Fc-mediated side-effects of classical immunotoxins and the costs associated with antibody-GMP production we are generating peptides binding to CD22 as vehicle for toxins.

Clinical vaccination studies using dendritic cells or modified tumor cells for the induction of tumor specific immune responses

Jörg Westermann and J. Kopp, in cooperation with Th. Blankenstein, W. Uckert and D. Schendel (GSF, Munich).

A pilot clinical trial using a gene-modified immunogenic tumor cell vaccine that expresses costimulatory molecules and secretes Interleukin-7 has been concluded in renal carcinoma (RCC). Evaluation of the immune response of vaccinated patients and our recent data on regulatory T cells in patients undergoing therapy with the multikinase inhibitors sunitinib and sorafenib have led us to design a follow-up trial including administration of antibodies that deplete regulatory T-cells.

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 are going to start a DC-vaccination trial in CML patients with minimal residual cytogenetic or molecular disease after treatment with tyrosine-kinase inhibitors by early 2012.

DNA Vaccination: preclinical models

Jörg Westermann, Tam Nguyen-Hoai in cooperation with M. Lipp

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 we have explored the possibility of recruiting immune cells by using plasmid DNA coding for the chemokines CCL19 and CCL21 as adjuvants. Coexpression of CCL19 or CCL21 with tumor antigens results in enhancement of a Th1-polarized immune response with improved protective effect. Further improvement of the vaccine potency has been achieved using a gene-gun for intradermal vaccine application (cooperation with O.Hohn/S. Norley, Robert-Koch Institute). A preclinical model in transgenic mice expressing the human breast-cancer associated antigen Her-2/neu as a target antigen is used to evaluate vaccination for breast cancer patients.

Adoptive T-cell therapy for lymphomas

Simone Reuss and Anne-Kathrin Garz in cooperation with Th. Blankenstein, W. Ucker and D. Schendel (GSF Munich).

The B-cell antigens CD22 and CD79a/b are appealing targets for immunotherapy: they are expressed on the large majority of lymphoid leukemias and lymphomas. Recognition of these antigens by transgenic T cells might be a tremendous tool for adoptive immunotherapy. Generation of TCR vectors recognizing presented epitopes of these antigens is being pursued in cooperation with Th. Blankenstein in a murine system with humanized TCR gene sequences coexpressed with a chimeric human HLA-A2 molecule. The TCR repertoire in these mice is not selected for human self-antigens and therefore should give rise to high-affinity T cells after vaccination. For CD22, a further alternative in vitro model is used in cooperation with D. Schendel: dendritic cells from a HLA-A2 negative donor are electroporated with RNA coding for CD22 and HLA-A2 or just CD22 and then used to stimulate autologous T cells. In this way, allo- or auto-restricted antigen-specific T cells are primed and T cell clones can be generated. T cell receptor genes can be isolated and used for TCR gene therapy. Retroviral gene transfer of cloned TCRs should allow generation of therapeutic numbers of CD22- and CD79-reactive T cells able to kill lymphoma/leukemia cells.

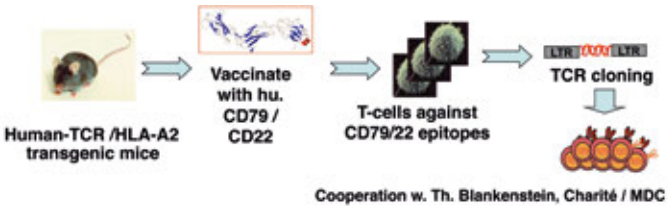
Development of immunotoxins with reduced side-effects against CD22 positive lymphomas

Oliver Schmetzer and Luisa Lindenberg in cooperation with G. Moldenhauer (DKFZ)

The clinical efficacy of immunotoxins targeting B-cell lymphomas has been hampered by considerable toxicity due to unspecific binding to the reticular-endothelial system via Fc-receptor. Moreover, the costs for GMP production of therapeutic antibodies are prohibitive for non-industrial institutions.

B-cell lymphomas are especially sensitive to natural substances such as dithiocarbamate-based compounds or catechins isolated from green tea, which might have reduced systemic side-effects as compared to standard-immunotoxins. We have screened more than 3000 drugs as conjugates with CD22 antibodies to find new non-toxic compounds. In addition, 200 plant extracts used in herbal medicine have been analyzed based on biodiversity and yet undescribed purified compounds have been isolated in cooperation with Prof. Maria José Umbelino Ferreira (Faculty of Pharmacy, University of Lisbon) and the botanical garden of the FU Berlin. The conjugation of these drugs to a CD22-antibody, which is rapidly internalized from lymphoma cells, leads to apoptosis induction in lymphoma cells in vitro and could provide the basis for a new generation of immunotoxins with reduced systemic toxicity.

A further attempt to reduce Fc-mediated side-effects is



based on the use of artificial evolutionary methods to replace antibodies by antigen-binding peptides. mRNA libraries with initially 1016 different sequences have been used to create peptide-mRNA conjugates (phenotype-genotype conjugates) which have then be selected for binding to CD22. Negative selection for binding to other tissue-proteins was done. Repeated transcription-translation cycles were combined with selection-enrichment strategies to increase binding affinity and specificities of the peptides. Several generations are being selected with random mutations increasing the sequence space. Deep-sequencing is applied to determine the mRNA and the corresponding peptide-sequences.

Selected Publications

Subklewe M, Sebelin-Wulf K, Beier C, Lietz A, Mathas S, Dorken B, Pezzutto A. (2007). Dendritic cell maturation stage determines susceptibility to the proteasome inhibitor bortezomib. Hum Immunol. 68: 147-55.

Westermann, J, Kopp, J, van Lessen, A, Hecker, AC, Baskaynak, G, Le Coutre, P, Dohner, K, Dohner, H, Dorken, B, Pezzutto, A. (2007). Vaccination with autologous non-irradiated dendritic cells in patients with bcr/abl+ chronic myeloid leukaemia.Br J Haematol. 137: 297-306.

Westermann J, Hecker AC, Flörcken A, Dörken B, Pezzutto A. (2009).

Granulocyte macrophage-colony stimulating factor plus interleukin-2 plus alpha-interferon plus 5-fluorouracil in the treatment of metastatic renal cell cancer: induction of CD80/86+ T cells indicates adverse outcome.J Immunother. 32: 667-675.

Westermann J, Flörcken A, Willimsky G, van Lessen A, Kopp J, Takvorian A, Jöhrens K, Lukowsky A, Schönemann C, Sawitzki B, Pohla H, Frank R, Dörken B, Schendel DJ, Blankenstein T, Pezzutto A. (2011). Allogeneic gene-modified tumor cells (RCC-26/IL-7/CD80) as a vaccine in patients with metastatic renal cell cancer: a clinical phase-I study. Gene Ther. 18: 354-363.

Nguyen-Hoai T, Baldenhofer G, Sayed Ahmed MS, Pham-Duc M, Vu MD, Lipp M, Dörken B, Pezzutto A, Westermann J. (2011). CCL21 (SLC) improves tumor protection by a DNA vaccine in a Her2/neu mouse tumor model. Cancer Gene Ther. 2011 [Epub ahead of print]

Patent applications:

EpCAM MHC-Klasse-II bindende Peptide und davon abgeleitete Mutanten als Verstärker der zellulären tumorreaktiven immunantwort (MDC 0506 EP)

Peptides regulating the surface expression of the T cell receptor European Patent Application EP1870420

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Clinical and Molecular Oncology

Anticancer drug therapies rely on the induction of cell cycle arrest or cell death through cellular stress programs. Deregulation of such stress pathways in cancer cells by genetic or epigenetic inactivation results in insufficient responses to treatment and poor propgnosis. Genetic pathway analyses therefore provide a rational basis for a molecular understanding of the response to anticancer therapies and the clinical use of cancer therapeutics.

The aim of the group is to identify genetic defects in cancer that result in aggressive disease and resistance to clinical cancer therapy. Recent data indicate that specific defects in cell signaling for apoptosis (type I death) or autophagy (type II death) can be overcome by rational selection of targeted anticancer drugs. Further projects are aimed to gain insights into novel aspects of cell cycle and cell death regulation and their intricate interactions.

Understanding resistance to anticancer therapy

In cooperation with F. Essmann (Interfaculty Institute for Biochemistry, Univ. Tübingen), C. Belka (Clinic and Polyclinic for Radiation Therapy and Radiation Oncology, LMU München), A. Gross (Weizman Institute, Rehovot, IL), R. Preissner, I. Sturm and P. Hemmati (Charité), T. Blankenstein (MDC) and Gunnar Dittmar (MDC)

Many anticancer therapies activate nuclear stress responses to induce cell cycle arrest and DNA repair. When repair fails, the same stress responses trigger cellular senescence or death by apoptosis or autophagy and demise of the affected cell. The molecular basis of these events has been studied extensively during recent years and comprehensive models are now established for large parts of these signaling events. Novel disease-related mechanisms in resistance to cell death, cancer disease prognosis and therapeutic targets are identified by our group in leukemias and solid tumors. In this context, we recently described selective loss of multiple cell death activators, including BH3-only proteins Nbk and Bim, in carcinoma of the kidney. This is a unifying feature of renal carcinoma and appears to be linked to the impressive clinical resistance of this tumor entity to anticancer therapy. Similar events were identified in acute leukemias. Links to epigenetic regulation and mTOR/PI3 kinase signaling are currently addressed.

Regulation of cell death by pro-apoptotic Bcl-2 family members

Apoptosis is mediated through at least three major pathways that are regulated by (1) the death receptors, (2) the mitochondria, and (3) the endoplasmic reticulum (ER). In most cells, these pathways are controlled by the Bcl-2 family of proteins that can be divided into antiapoptotic and proapoptotic members. Although the overall amino acid sequence homology between the family members is relatively low, they contain highly conserved domains, referred to as Bcl-2 homology domains (BH1 to BH4) that are essential for homo- and heterocomplex formation as well as for their cell death inducing capacity. Structural and functional analyses revealed that the proapoptotic homologs can be subdivided into the Bax subfamily and the growing BH3-only subfamily. BH3-only proteins link upstream signals from different cellular or functional compartments to the mitochondrial apoptosis pathway (see figure). Puma, Noxa, Hrk, and Nbk (Bik) are induced by p53 and mediate cell death originating from the nucleus, e.g. upon DNA damage. Nbk localizes to the ER and acti-

vates the pro-apoptotic multidomain protein Bax (but not the homologous Bak) indirectly, through a ER-initiated death pathway that has been recently elucidated by our group.

Aims of our work are (1) to gain structural and functional insights into how Bcl-2 subfamilies promote or inhibit cell death signals and how these properties may be utilized for development of cell death-promoting cancer therapies, (2) to address interactions with oncogene sigalling and p53 dependent and independent sigalling by the $p14^{ARF}$ tumor suppressor and (3) to understand pathway interactions with cell death signalling as resistance mechanisms to targeted cancer therapy, e.g. in mTOR/PI3K survival signalling.

We recently showed that the anti-apoptotic Bcl-2 homolog Mcl-1 is stabilized upon interaction with BH3-only proteins. Mcl-1 thereby intereferes with activation of the Bak pathway during apoptosis induced by BH3-only proteins. Inhibition of the Bak pathway is of general relevance as a resistance mechanism against targeted cancer therapy and was shown to mediate resistance to the death ligand TRAIL in Bax deficient carcinoma cells. Bak re-activation could be achieved by several kinase inhibitors including Sorafenib. We found that such central defects in cell death resistance can be overcome even more efficiently by a new class of apoptosis targeting drugs that mimic the function of the endogenous cell death activator SMAC that interferes with protection from cell death by the Inhibitor of Apoptosis Proteins (IAP) with XIAP playing a dominant role in TRAIL resistance. Interference with the E3-ubiquitin ligase function of XIAP appears to be an important aspect of proteasome targeting drugs such as Bortezomib.

Selected Publications

Zaltsman Y, Shachnai L, Yivgi-Ohana N, Schwarz M, Maryanovich M, Houtkooper RH, Vaz FM, De Leonardis F, Fiermonte G, Palmieri F, Gillissen B, Daniel PT, Jimenez E, Walsh S, Koehler CM, Roy SS, Walter L, Hajnóczky G, Gross A. MTCH2/MIMP is a major facilitator of tBID recruitment to mitochondria. Nat Cell Biol. 2010, 12:553-62.

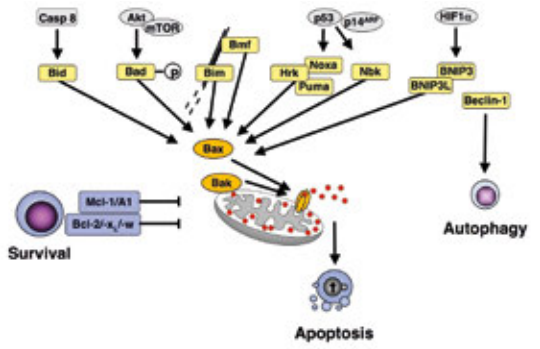
Anders K, Buschow C, Herrmann A, Milojkovic A, Loddenkemper C, Kammertoens T, Daniel P, Yu H, Charo J, Blankenstein T. Monospecific CD8+ effector T cells reject large genetically unstable tumors, while oncogene inactivation selects escape variants. Cancer Cell, 2011, in press

Hemmati PG, Müer A, Gillissen B, Overkamp T, Milojkovic A, Wendt J, Dörken B, Daniel PT. Systematic genetic dissection of p14ARF-mediated mitochondrial cell death signaling reveals a key role for p21CDKN1 and the BH3-only protein Puma/bbc3. J Mol Med. 2010, 88:609-22.

Gillissen B, Wendt J, Richter A, Richter A, Müer A, Overkamp T, Gebhardt N, Preissner R, Belka C, Dörken B, Daniel PT. Endogenous Bak inhibitors Mcl-1 and Bcl-xL: differential impact on TRAIL resistance in Bax-deficient carcinoma. J Cell Biol. 2010, 188:851-62.

Gillissen B, Essmann F, Hemmati P, Richter A, Richter A, Öztop I, Chinnadurai G, Dörken B and Daniel PT. Mcl-1 mediates the Bax dependency of Nbk/Bik-induced apoptosis. J Cell Biol 2007, 179: 701-15.

a



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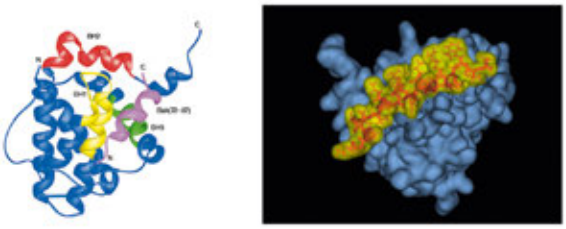

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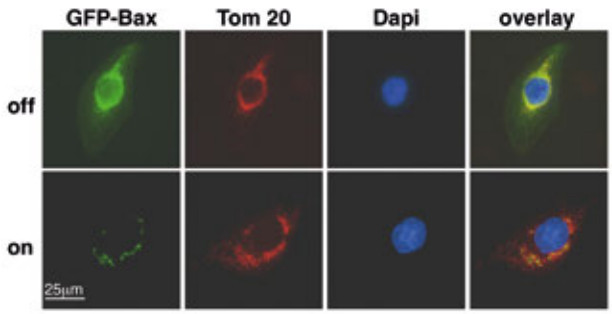

Function of BH3-only proteins as death sensors
A: BH3-only proteins act as functional interface between death signals and the mitochondrial apoptosis pathway or death by autophagy. Anti-apoptotic Bcl-2 proteins put an at least dual layer of protection on activation of Bax/Bak that redistribute upon activation to form pores in the outer mitochondrial membrane for the release of pro-apoptotic factors such as cytochrome c. B (left): Binding of a BH3-domain to Bcl-x_L: Bcl-2 Homology (BH) domains 1 (yellow), BH2 (red) and BH3 (green) of Bcl-x_L form a cleft that binds a-helical BH3 domains (right; violet: Bcl-x_L, yellow/orange: BH3 domain of Bak). BH3-only proteins displace Bax/Bak from binding to e.g. Bcl-x_L or Mcl-1. C: Conditional adenoviral expression of the BH3-only protein Nbk ("on" condition) induces redistribution of GFP-tagged Bax (green) to mitochondria (TOM20, red) and a punctuate formation of Bax clusters due to Bax oligomerization. Blue: DAPI stained nuclei.

Structure of the Group

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*part of the reporting period

Iduna Fichtner

Experimental Pharmacology

Our group further continued with the establishment, characterization and use of patient-derived tumour xenografts. During the recent years, especially colon cancer and lung metastases of different tumour entities were in the focus of interest. These preclinical models remain a high congruence with the original specimen concerning heterogeneity, histology, expression of biomarkers, and response to therapy. With the help of these xenografts dynamic regulations of biomarkers in dependence of therapy were investigated in a standardized way at the genetic and protein level. They were used to evaluate the therapeutic potential of a variety of classical or targeted drugs.

The nanoparticle group developed vesicles with the aim to overcome the blood-brain barrier in order to improve the therapy of brain malignancies.

Within the stem cell project we further established protocols for the in vitro cultivation and teratoma formation with the background to standardize procedures and marker profiles for the estimation of the differentiation status of pluripotent cells.

Potential of patient derived xenografts for the preclinical development of anticancer drugs

In close cooperation with the Evangelische Lungenklinik Berlin-Buch a panel of non-small cell lung cancer xenografts was established and characterized for identity with the original specimens. These xenografts showed a clinically related response profile to classical (Etoposide, Carboplatin, Paclitaxel, Gemcitabine) or targeted (Erlotinib, Cetuximab) therapies.

In a Phase II like approach the therapeutic profile of a novel Epothilone, Sagopilone, was evaluated in 22 different xenografts and showed an overall response in 64% of models (3 stable diseases and 11 partial responses). In addition, the gene expression pattern was determined by Affymetrix chips before and after therapy. Sagopilone induced tubulin isoforms in all tumour samples but genes related to mitotic arrest were found only in responder models. High expression levels of genes for cell adhesion/angiogenesis (EPHA4, integrin alpha 6, carbonic anhydrase 9 and 12) and wild-type TP53 were indicative for resistance of the tumours towards Sagopilone. A combination with drugs targeting vascular endothelial growth factor signaling (Bevacizumab, Sorafenib) restored antitumour efficacy. This example proves the value of the xenograft system to optimize clinical therapies.

In a further study we were interested in the modulation of epigenetic gene regulation and the correlation with response to DNA methylation inhibitors like 5-Azacytidine. We could show in cooperation with the DKFZ Heidelberg that an elaidic acid derivative of azacytidine

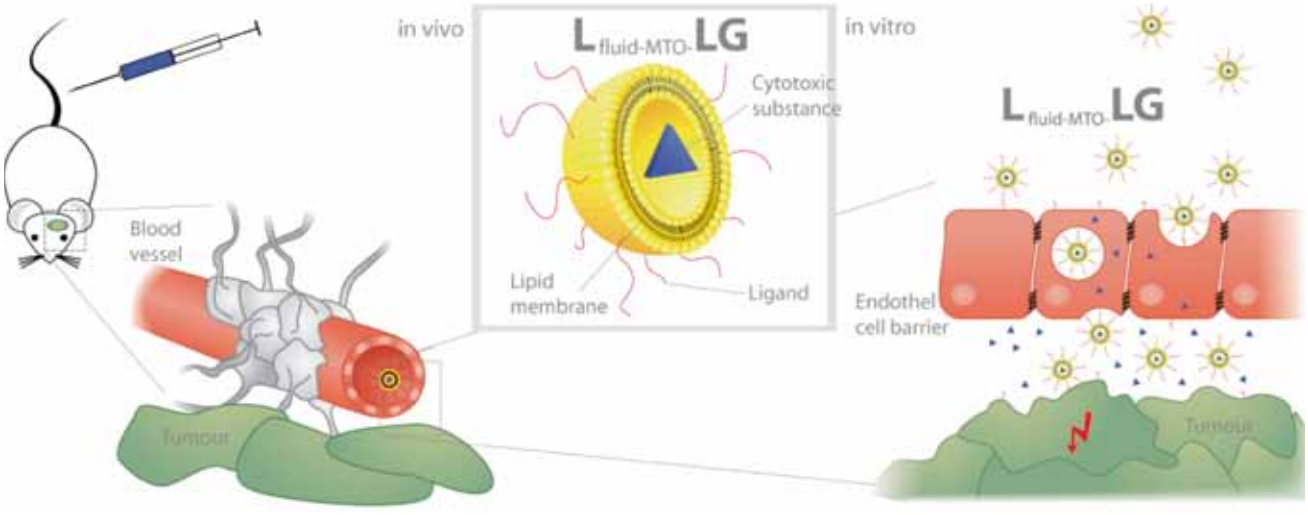

Figure 1. Schematic summary of targeted therapy by fluid membrane liposomes (L= Liposome, LG= Ligand, MTO: Mitoxantrone)

had strong epigenetic modulatory potency in human cancer cell lines and induced a genome-wide DNA demethylation. It had a higher antitumoral activity than the parent drug in an orthotopic mouse tumour model for acute lymphatic leukemia.

Nanoparticles for the treatment of brain malignancies

A systemic treatment of primary and secondary brain tumors remains challenging because of the highly efficient blood-brain barrier preventing the passage of xenobiotics. Liposomes were developed to be used as drug transporters for a therapy of these malignancies.

A broad variety of small liposomes with a rigid or a fluid bilayer were prepared encapsulating Mitoxantrone (MTO). These liposomes were additionally equipped with a 19mer angiopeptide (LG) to target the low density lipoprotein receptor-related protein (LRP), which mediates endocytosis across the blood-brain barrier (Figure 1).

In vitro investigations using epithelial cells revealed that the cellular uptake of liposomes containing calcein was mainly depending on membrane fluidity and vesicle charge. Liposomes with a positive charge and fluid vesicles containing the helper lipid dioleylphosphatidyl-ethanolamine (DOPE) in their membrane were taken up at a 5.5-fold higher rate than rigid vesicles. The fluid, ligand equipped liposomes also led to a 400% higher transcytosis. That effect was positively correlated with membrane fluidity in the outer part of the bilayer as electron paramagnetic resonance measurements revealed.

Human MT-3 breast cancer cells were transplanted into the brain of nude mice in order to test the therapeutic effect *in vivo*. Mice were treated intravenously with 4 mg/kg mitoxantrone or different drug-entrapping liposomes to investigate the pharmacological effect. A treatment with angiopeptide bearing, fluid membrane liposomes resulted in a significant reduction in the tumor volume of 88 % and 73 % of subcutaneously and intracerebrally growing tumors, respectively, in comparison to saline treated control group. In addition, all liposomal formulations reduced the side effects (gastrointestinal toxicity and dehydration) compared with the free drug. Pharmacokinetic investigations demonstrated a clear prolongation of liposome circulation in the blood but no enhanced accumulation of Mitoxantrone in the brain.

These data show that it is possible to significantly improve the therapy of brain metastases with targeted Mitoxantrone containing liposomes.

The targeted liposomes developed in our group represent a platform technology which can be adapted to other receptors or to other drugs in order to optimize a systemic treatment of brain malignancies.

Stem cell research

Further work with stem cells was focusing on the development of standardized procedures for the phenotypic characterization of pluripotency and the evaluation of marker profiles for differentiation.

As for future use of stem cells in regenerative medicine the presence of murine factors is estimated critically, cultivation methods were established avoiding the presence of feeder cells and serum. Marker profile of pluripotency and germ layer differentiation were

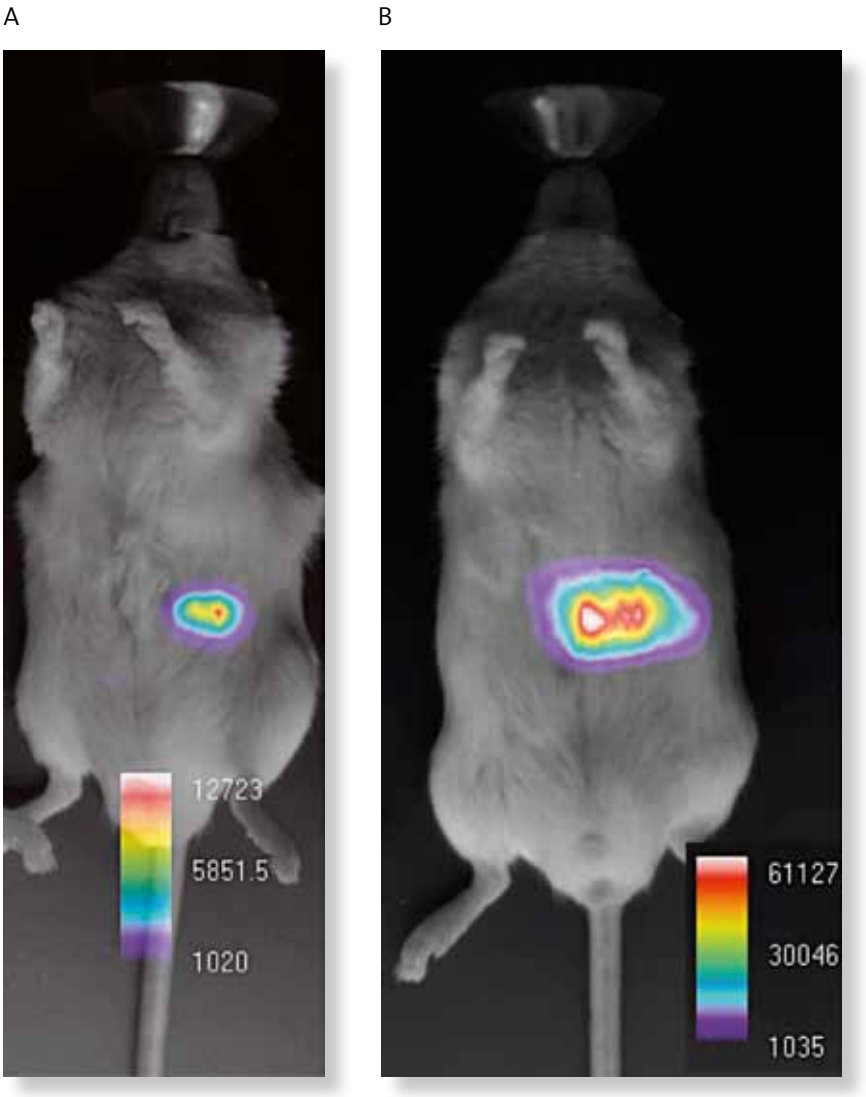

Figure 2. Bioluminescence pictures of NOD/SCID mice four (A) and 35 (B) days after intrahepatic inoculation of murine embryonic stem cells.

measured by RT-PCR and FACS analysis. Undifferentiated and differentiated marker profile expressions were found to be not significantly different using both cultivation methods. Feeder- and serum-free cell cultivation is therefore regarded as a feasible method for cell expansion under standarized conditions for in vitro and in vivo application. A high density of murine embryonic stem cells in reproducible undifferentiated and undirected differentiated state was achieved in a 3D perfusion reactor (Cooperation with Charité Berlin). Histology and immunofluorescence revealed no significant differences between in vitro differentiated cells and teratomas. Directed endodermal/hepatic differentiation of embryonic stem cells was established by a 3-phase-differentiation procedure including dimethylsulfoxide, sodium butyrate and hepatocyte growth factor. Cell differentiation, monitored by RT-PCR and immunohistochemistry revealed efficient cell differentiation into hepatocyte like cells, suitable for in vivo application.

Life imaging bioluminescense technique was applied to follow stem cell engraftment and organ distribution of transplanted cells (Figure 2). Mouse stem cells were transfected with a plasmid containing the luciferase gene. Gene transfer was found to be stable and without significant influence on pluripotent marker expression and cell differentiation capacity in vitro and in vivo. Transfected cells were transplanted subcutaneously, into liver and spleen of NOD/SCID mice. Time dependent cell engraftment was monitored by bioluminescence and cell differentiation into different germ layer cells was measured by RT-PCR. A time dependent expansion of embryonic cells was monitored at the different transplantation sites. Stem cell derived germ layer development into endodermal, ectodermal

and mesodermal cell types were associated with down regulation of pluripotent marker expression in vivo. The differential organ specific milieu had no distinct effect on cell engraftment and differentiation. Therefore we conclude that the transplantation techniques for un-differentiated and differentiated embryonic stem cells and the life imaging methods are applicable for further investigations of the regenerative capacity of stem cells in experimentally injured organs.

Selected Publications

Hammer,S., Sommer,A., Fichtner,I., Becker,M., Rolff,J., Merk,J., Klar,U., and Hoffmann,J. (2010). Comparative profiling of the novel epothilone, sagopilone, in xenografts derived from primary non small cell lung cancer. Clin Cancer Res 16, 1452-1465.

Brückner,B., Rius,M., Markelova,M.R., Fichtner,I., Hals,P.A., Sandvold,M.L., and Lyko,F. (2010). Delivery of 5-Azacytidine to Human Cancer Cells by Elaidic Acid Esterification Increases Therapeutic Drug Efficacy. Mol Cancer Ther 9, 1256-1264.

Orthmann,A., Zeisig,R., Koklic,T., Sentjurc,M., Wiesner,B., Lemm,M., and Fichtner,I. (2010). Impact of membrane properties on uptake and transcytosis of colloidal nanocarriers across an epithelial cell barrier model. J Pharm Sci 99, 2423-2433.

Orthmann,A., Fichtner,I., and Zeisig.R. (2011). Improving the transport of chemotherapeutic drugs across the blood–brain barrier. Expert Rev Clin Pharmacol 4, 477-490.

Gerlach JC, Lübberstedt M, Edsbagge J, Ring A, Hout M, Baun M, Rossberg, Knöspel F, Peters G, Eckert K, Wulf-Goldenberg A, Björquist P, Stachelscheid H, Urbaniak T, Schatten G, Miki, T Schmelzer E, Zeilinger K. (2010). Interwoven four-compartment capillary membrane technology for three-dimensional perfusion with decentralized mass exchange to scale up embryonic stem cell culture. Cells Tissues Organs 192(1), 39-49

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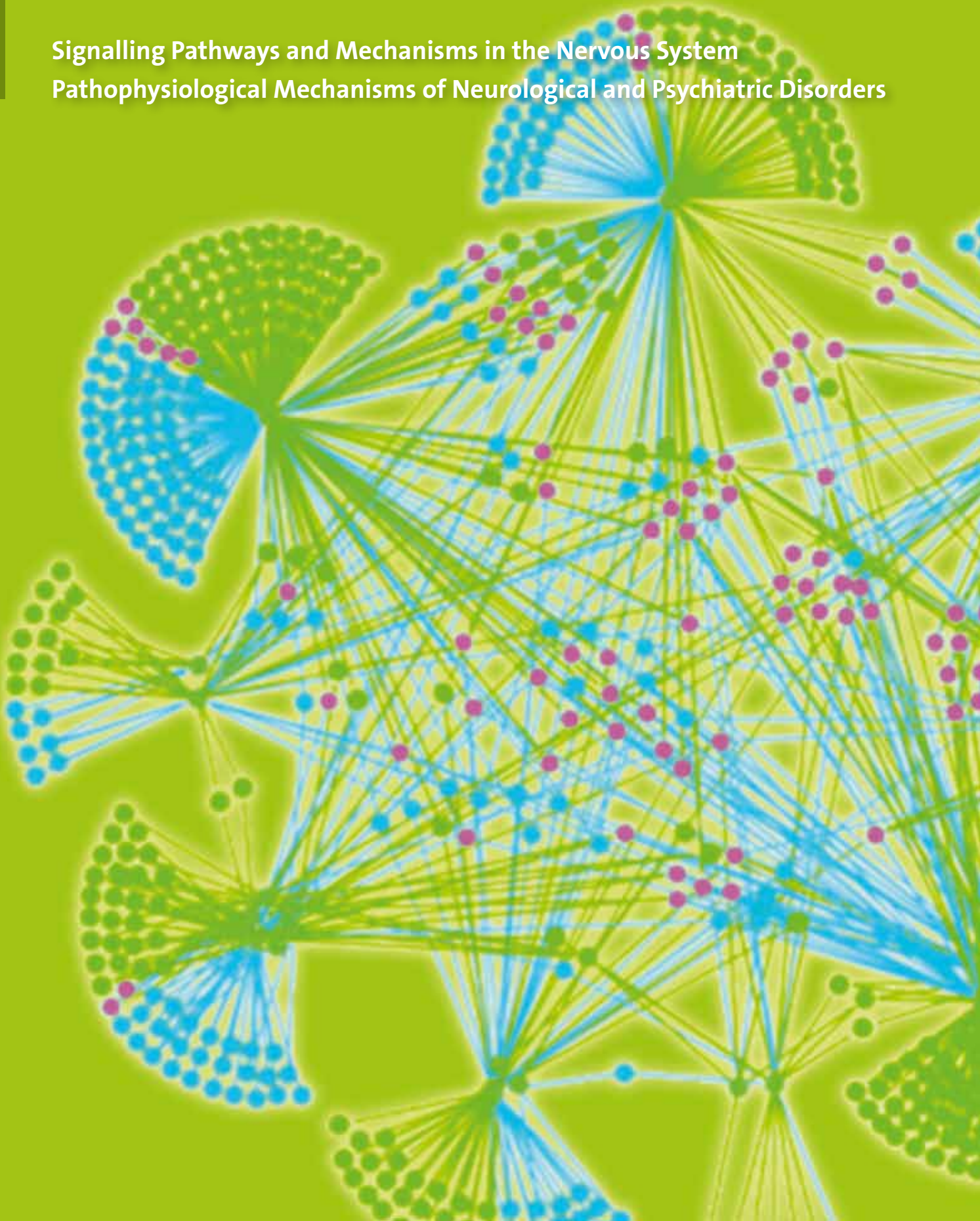
Sylvia Schulz

Diseases of the Nervous System

Coordinator: Carmen Birchmeier

Signalling Pathways and Mechanisms in the Nervous System
Pathophysiological Mechanisms of Neurological and Psychiatric Disorders



Diseases of the Nervous System

Carmen Birchmeier

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 often severely impair afflicted individuals and impose a heavy burden on patients, families, caregivers and society alike. It is crucial to understand the molecular basis of normal nervous system function in order to define the mechanisms and the etiology of neurological and psychiatric disorders. Scientists of the MDC Neuroscience Department aim to understand the molecular mechanisms of normal nervous system function, to elucidate disease mechanisms, and to lay the basis for novel therapeutic approaches to neurological and psychiatric disease.

MolNeuro, the international neuroscience graduate school funded by the Helmholtz Association, was established in 2007 as a joint program of the MDC, Charité and Free University Berlin under the direction of **Gary Lewin**. In 2010, MolNeuro was evaluated for the first time. The expert referees emphasized the international scope of the program, its potential to recruit excellent students, and the high quality of the PhD projects conducted in the program. MolNeuro provides successful candidates with a fully funded position at one of the participating institutions. The curriculum of the program includes a two-year lectures series covering basic and advanced concepts of neurobiology, a student journal club, and practical experimental courses. In addition, the program offers soft skills training provided by the Helmholtz Association and travel grants for students to attend international conferences to present their results. Another graduate school, the International Research Training Group for Myology (Myograd), was established by **Simone Spuler**, a joint member of the Neuroscience Department and of the Experimental and Clinical Research Center. Myograd partner institutions are the Free University Berlin and the Medical Faculty of the Charité in Berlin together with the Université Pierre et Marie Curie in Paris. Students enrolled in the program conduct a bi-nationally supervised doctoral thesis in one of the affiliated research groups and have the opportunity to do a lab rotation between Berlin and Paris. Practical training is complemented by soft-skill training courses, Summer Schools and weekly journal clubs that are broadcast over the internet to all participating laboratories.

Several conferences were organized by scientists of the Neuroscience Department. The Berlin Neuroscience Forum (BNF) is a biennial meeting that brings together neuroscientists from Berlin institutes with key international scientists for two days of lectures and intense discussion. **Gary Lewin** and **Helmut Kettenmann** co-organized the BNF in 2010. Further, in the framework of the Collaborative Research Centre 665 "Function and Dysfunction of the Nervous System," a very successful international symposium was co-organized by the MDC and other members of the Collaborative Research Centre in 2011. The meeting took place in Potsdam and was well attended. The Collaborative Research Centre 665 had been recently approved for a second term of funding by the German Science Foundation (DFG). Participants from the MDC Neuroscience Department include **Carmen Birchmeier**, **Ines Ibanez-Tallon**, **Gary Lewin**, **Fritz Rathjen** and **Dietmar Schmitz**. In addition, a new Collaborative Research Centre, SFB958 "Scaffolding of Membranes," was established in 2011, and several MDC PIs, including two from the Neuroscience Department, contribute projects to this research activity.

Four group leaders of the Neuroscience Department have been awarded prestigious grants by the European Research Council (ERC) during the reporting period. **Gary Lewin** and **Thomas Jentsch** each received a 2.5-Mio Euro ERC Advanced Grant, while **Jan-Erik Siemens** and **James Poulet** were each awarded a 1.5 Mio-Euro ERC Starting Grant. With the grant money, Gary Lewin plans to study pain perception in African naked mole-rats, a eusocial mammal living under extreme conditions that doesn't feel certain types of pain. Jan-Erik Siemens will use his grant to investigate how animals regulate their core body temperature. He is interested in how the brain keeps the body at a constant 37 degree Celsius, and also investigates the molecular mechanisms of "hot"

and "cold" perception. James Poulet plans to investigate brain states and behavior.

Scientific highlights from the Department

Gary Lewin, Stefan Lechner (MDC), Friedrich Luft (ECRC) and Jens Jordan (ECRC, now MHH) were recently able to solve an old mystery: is there an osmoreceptor outside the brain that can regulate blood pressure? Scientists have long been puzzled by the finding that drinking a glass of water can raise blood pressure in patients with neurological damage and in elderly people. The project, undertaken in the mouse, showed that neurons in the liver react to water intake. These osmoreceptors are able to perceive changes in osmolality and then trigger a reflex that stimulates vessels in the liver to raise blood pressure. Discovery of this osmolalic self-regulation mechanism has already led to the recommendation that patients suffering from fainting attacks drink water to maintain a steady blood pressure level (Lechner et al., Neuron 2011).

Electrical activity provides the basis for the communication between neurons, and electrical activity is known to shape the structure of the nervous system during development. The group of **Fritz Rathjen** has analyzed the function of an adhesion molecule of the Ig-CAM family called CAR and its effect on the electrical activity of neurons. This adhesion molecule modulates activity of neurons and regulates calcium levels by releasing it from internal stores. Their study thus revealed an unexpected link between adhesion processes, calcium release and electrical activity (Patzke et al., J. Neuroscience 2010).

According to the World Health Organization, smoking kills more than 5 million people each year. The group of **Ines Ibanez-Tallon** has recently identified one risk factor for nicotine addiction. They investigated an acetylcholine receptor that is expressed in the habenula region of the mid brain and activated by nicotine in smokers. A point mutation in the gene of the receptor is present in many heavy smokers and makes them more prone to becoming addicted to nicotine (Frahm et al., Neuron 2011).

Patients with a form of hereditary hearing impediment display simultaneously an enhanced ability to sense touch. This surprising finding was made in a collaborative analysis performed in the laboratories of **Gary Lewin** and **Thomas Jentsch.** Their study focused on patients carrying a mutation in the potassium channel KCNQ4. KCNQ4 is required for hair cells of the inner ear to restore the resting potential after excitation. When the channel cannot perform this function due to a mutation, the hair cells are continuously stimulated and will eventually perish, resulting in loss of hearing in patients. Interestingly, KCNQ4 is also present in touch receptors of the skin, and here, dysfunction of the receptor does not lead to cell death. Instead, the cells become more sensitive to vibrational stimuli. Apparently, KCNQ4 acts as an attenuator of the excitability of these mechanosensitive neurons, and loss of its function increases their sensitivity. Consequently, patients are more sensitive to vibrational stimuli at low frequencies than their unaffected siblings (Heidenreich et al., Nature Neuroscience 2011).

Jan Bieschke and **Erich Wanker** identified a new natural compound that modulates the formation of amyloid aggregates in cells. Misfolding of proteins and formation of toxic protein aggregates are the reasons for the death of neurons in Alzheimer's disease, a neurodegenerative disorder. It is thought that two types of aggregates exist, small toxic and large, non-toxic aggregates. Screening a large library of natural compounds, Bieschke and Wanker found orcein, a pigment that occurs naturally in certain types of lichen. Orcein and a closely related pigment, O4, bind to misfolded amyloid peptides and facilitate the formation of large, non-toxic aggregates that do not harm neurons. Clinical studies will have to show if these compounds can be utilized as therapeutics in neurodegenerative disease (Bieschke et al., Nature Chemical Biology 2011).

Gliomas represent the most common malignant brain tumor. Patients with gliomas have a very poor prognosis because these tumors are rarely treatable. **Rainer Glass** and **Helmut Kettenmann** found that endogenous neural precursor cells possess anti-tumour activity. Precursor cells produce bone morphogenetic protein-7 (BMP7), a member of the TGF-beta family of growth and differentiation factors. Gliomas contain stem-cell like cells that respond to BMP7 by differentiation, reduced expression of stem-like markers and reduced self-renewal. In addition, these stem-cell like cells show a reduced tumor initiation capacity when treated with BMP7. This anti-tumor response is strongly reduced in older mice, indicating that endogenous neural precursor cells protect the young brain from glioblastoma (Chirasani et al., Brain, 2010).

Glycine is a major inhibitory second messenger in the nervous system whose effects are mediated by glycine receptors (GlyR). GlyR production is regulated by mRNA editing; in this editing process, a nucleotide in the mRNA is modified, leading to the production of a protein with a single amino acid substitution (leucine to proline). The edited version of the receptor has a higher affinity for glycine. The group of **Jochen Meier** was now able to link editing to human disease, and found that patients with temporal lobe epilepsy display an increase in RNA editing (Förstera et al., Brain 2010).

Carmen Birchmeier

Developmental Biology/ Signal Transduction

We investigate signaling systems as key regulators in development and maintenance of the nervous system and muscle. We found previously that muscle progenitor/stem cells rely on Notch signals for maintenance and self-renewal during development and in the adult. In the absence of the Notch signals, progenitor/stem cells differentiate in an uncontrollable manner and are therefore depleted. We are currently defining the target genes that mediate the ability of Notch to maintain progenitor/stem cells in an undifferentiated state. Further, we are analyzing the role of the Neuregulin signaling system in development of the nervous system. Neuregulins represent a family of growth and differentiation factors that bind and activate the ErbB tyrosine kinase receptors. Signals provided by this system control important steps in formation of the nervous system.

Neuregulin signaling

Hagen Wende, Alistair Garratt, Maria Kolanczyk, Cyril Cheret, Katja Grossmann, in collaboration with Matthias Selbach, Florian Paul and Walter Birchmeier

The ErbB tyrosine kinase receptors were originally identified by virtue of their oncogenic potential. Due to their important role in cancer, the structure and activity of ErbB receptors was extensively studied. ErbB receptors mediate signals provided by Neuregulins that act as high affinity ligands. Ligand binding to the extracellular domain of ErbB receptors promotes receptor dimerization and activation of the intracellular tyrosine kinase domain. Activated receptors phosphorylate each other on a number of tyrosine residues, which serve as docking sites for the downstream enzymes or adaptor proteins that mediate further intracellular signal transduction. Our genetic analyses have assigned important developmental functions to ErbB receptors and their ligand, Nrg1.

BACE1 (beta-site amyloid precursor protein-cleaving enzyme 1) is a protease famous for its role in Alzheimer's disease where it is essential for the generation of amyloid-beta peptide. Despite its important role in disease, its normal physiological function was unclear. We found that mutation of BACE1 resulted in the accumulation of unprocessed Nrg1, a neuronally expressed factor required for myelination and induction of the muscle spindle. BACE1-/- mice displayed hypomyelination of peripheral nerves, very similar to that seen in mice with mutations in type III Nrg1. Further, these animals have fewer muscle spindles, similar to mice carrying mutations in type I Nrg1. Thus, BACE1 is required for myelination and spindle induction, two processes that depend on Nrg1 and its correct processing.

Binding of Nrg1 to ErbB2/3 receptors results in the activation of intracellular signal transduction pathways that initiate changes in Schwann cell behavior. We have identified a key Nrg1/ErbB signaling component, Shp2, and defined its role in Schwann cell development and myelination.

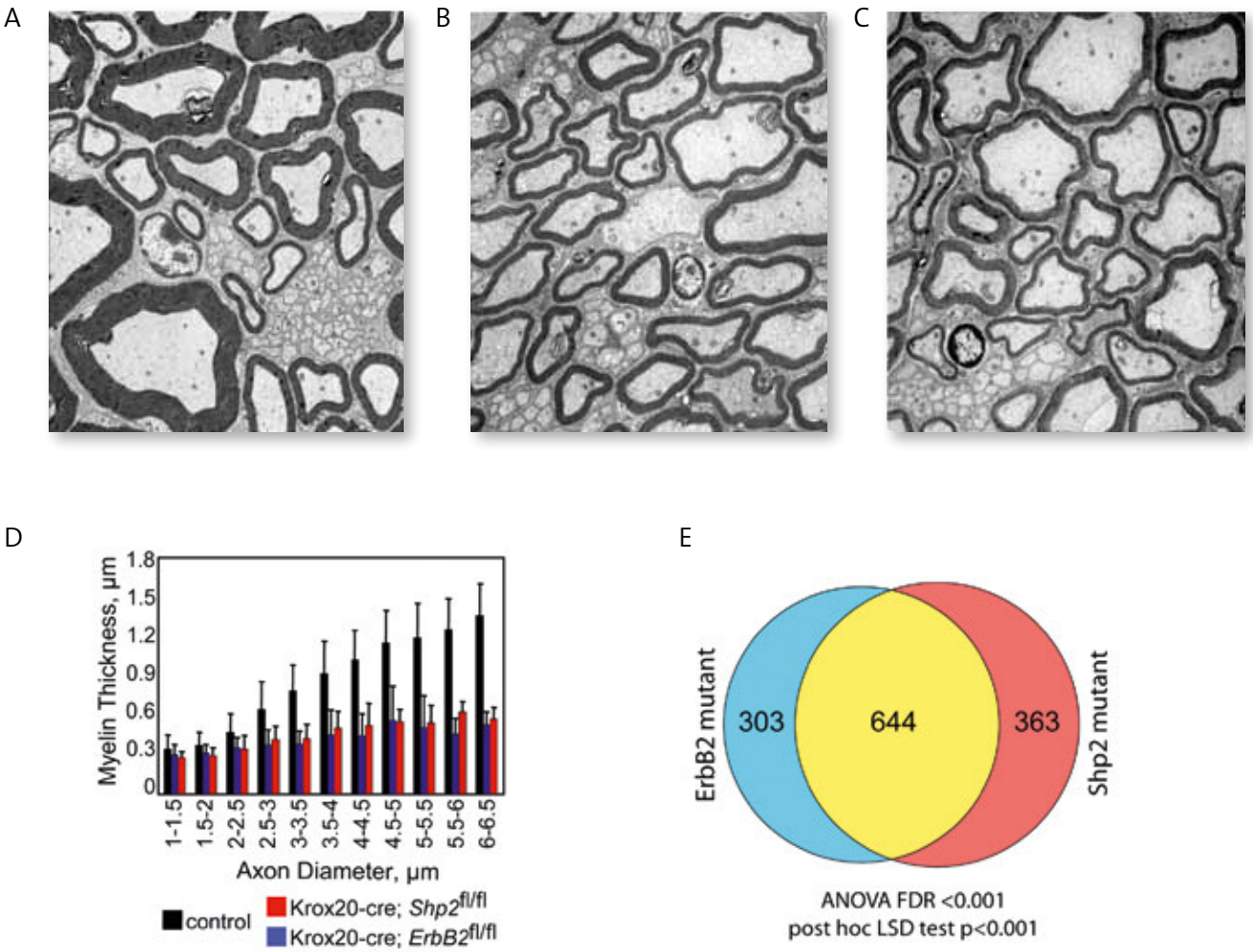


Figure 1. Myelination deficits in conditional ErbB2 and Shp2 mutan

(**A-C**) Electron microscopic analysis of peripheral nerves in control (**A**), conditional Shp2 (**B**) and ErbB2 (**C**) mice. The conditional mutation was introduced into myelinating Schwann cells of peripheral nerves using Krox20cre. (**D**) Comparison of myelin thickness in peripheral nerves of control, conditional Shp2 and ErbB2 mice. (**E**) Comparison of genes whose expression is deregulated in peripheral nerves of control, conditional Shp2 and ErbB2 mice. The percentages of co-regulated genes were determined using a false discovery rate of 10^{-3} as cut-off.

The nonreceptor tyrosine phosphatase Shp2 (PTPN11) participates in the signaling of various tyrosine kinase receptors. We found that conditional mutation of Shp2 in neural crest cells and in myelinating Schwann cells resulted in deficits in glial development that are remarkably similar to those observed in mice mutant for Nrg1 or the Nrg1 receptors, ErbB2 and ErbB3 (Fig. 1). In cultured Shp2 mutant Schwann cells, Nrg1-evoked cellular responses like proliferation and migration were virtually abolished, and Nrg1-dependent intracellular signaling was altered. Pharmacological inhibition of Src family kinases mimicked all cellular and biochemical effects of the Shp2 mutation, implicating Src as a primary Shp2 target during Nrg1 signaling. The major Nrg1-dependent intracellular signaling pathway that depends on Shp2 in this system is the Erk/MAPkinase pathway. Shp2 is particularly important for the sustained activation of Erk1/2. Together, our genetic and biochemical analyses demonstrate that Shp2 is an essential component in the transduction of Nrg1/ErbB signals, and implicate Erk1/2 as a major Nrg1-dependent signaling pathway required for Schwann cell development and myelination. This is also substantiated by recent rescue experiments that demonstrate that expression of activated MAPkinase suffices to rescue the phenotypes of Shp2 mutant Schwann cells.

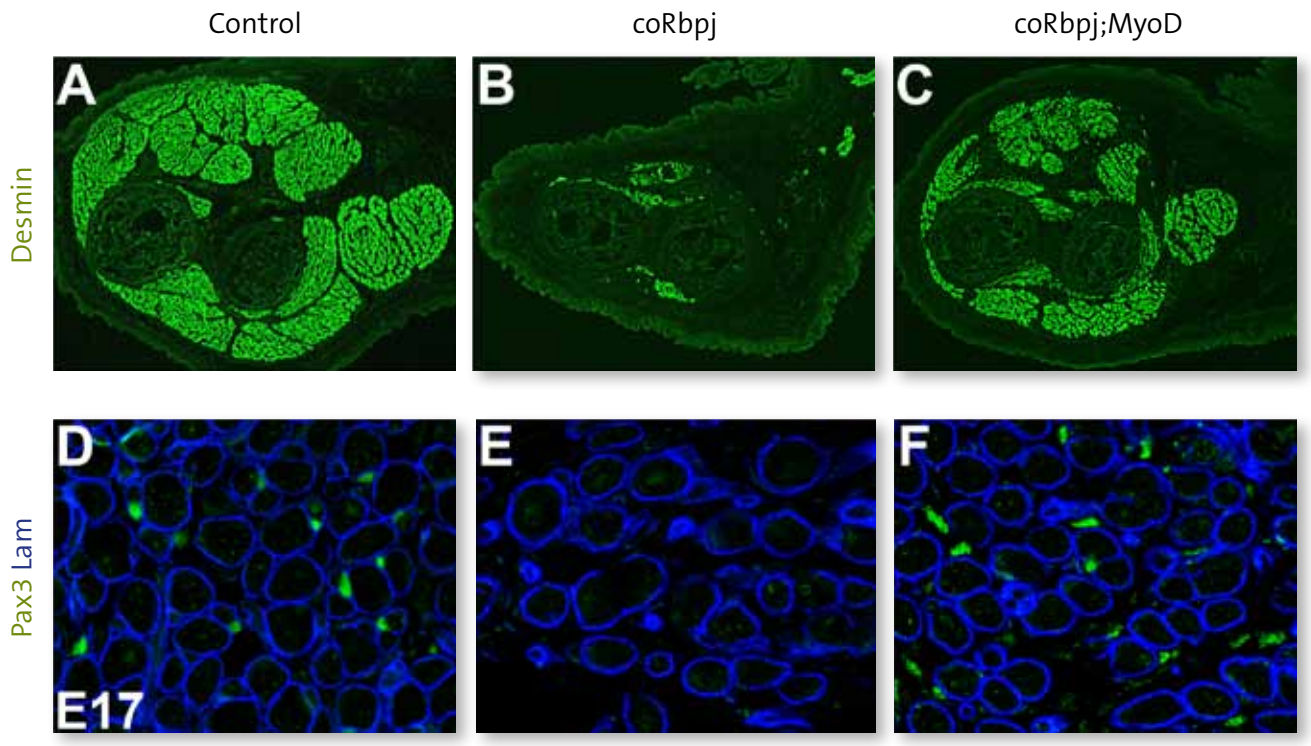


Figure 2. Reduced muscle mass and loss of progenitor cells in the absence of canonical Notch signals

(**A-C**) Muscle in the forelimb of control (**A**), conditional RBPJ (**B**) and MyoD;conditional RBPJ (**C**) mutant mice. The muscle is visualized by staining with anti-desmin antibodies. Note the tiny muscle size in RBPJ mutant mice; the muscle mass is substantially rescued in the RBPJ;MyoD double mutant. (**D-F**) Pax3+ progenitor cells and the laminin-containing extracellular matrix of muscle fibers in the back muscle of control (**D**), conditional RBPJ (**E**) and MyoD;conditional RBPJ (**F**) mutant mice. Note the absence of the Pax3+ cells in the RBPJ mutant muscle; Pax3+ cells are however present in control and are rescued in the RBPJ;MyoD double mutant.

Notch signaling and the maintenance of progenitor/stem cells in the muscle

Dominique Bröhl, Maciej Czajkowski, Claudia Raßek, Joscha Griger, in collaboration with Andranik Ivanov, Wei Chen and Nikolaus Rajewsky

Myogenesis, the formation of skeletal muscle, is a tightly regulated process that occurs during development and regeneration. During mammalian embryonic and postnatal development, myogenic differentiation allows the formation and growth of skeletal muscles. In the postnatal and adult organism, skeletal muscle grows and regenerates by the myogenic differentiation of stem cells, the satellite cells.

Notch genes encode cell surface proteins that are evolutionarily conserved and found in invertebrates like Drosophila melanogaster as well as in all vertebrate species. The transcription factor RBP-J (Rbpsuh) is a primary nuclear mediator of Notch signals. Signals provided by Notch receptors control cell fate decisions, patterning, and they also affect proliferation and maintenance of progenitor cells.

A pool of myogenic progenitor cells is formed in the embryo, and these progenitor cells are maintained during further development. The progenitors reside in the muscle and provide a source of cells for muscle growth. In addition, they generate satellite cells, the stem cells of the postnatal muscle. By the use of conditional mutagenesis in mice, we demonstrated that the major mediator of Notch signaling, the transcription factor RBP-J, is essential to maintain this pool of progenitor cells in an undifferentiated state. In the absence of RBP-J, the cells undergo uncontrolled myogenic differentiation, leading to a depletion of the progenitor pool. This results in a lack of muscle growth in development and to the formation of a tiny muscle (Fig. 2). In addition, due to the absence of progenitors, satellite cells are not formed in conditional RBP-J mutant mice. Thus, canonical Notch signals mediated by RBP-J are required in the developing muscle to set aside proliferating progenitors and

satellite cells. Recent results moreover show that this Notch/RBP-J function is also operative in adult muscle stem cells.

The early depletion of the myogenic progenitor pool that is observed in the absence of canonical Notch signals is accompanied by an upregulated expression of MyoD. We recently found that the drastic effect on progenitor maintenance is rescued by the mutation of the muscle-specific transcription factor MyoD (Fig. 2) Such rescued mutants reveal important new Notch functions in myogenesis.

Selected Publications

Grossmann, K. S., Wende, H., Paul, F. E., Cheret, C., Garratt, A. N., Zurborg, S., Feinberg, K., Besser, D., Schulz, H., Peles, E. et al. (2009). The tyrosine phosphatase Shp2 (PTPN11) directs Neuregulin-1/ErbB signaling throughout Schwann cell development. Proc Natl Acad Sci U S A 106, 16704-9.

Willem, M., Garratt, A. N., Novak, B., Citron, M., Kaufmann, S., Rittger, A., DeStrooper, B., Saftig, P., Birchmeier, C. and Haass, C. (2006). Control of peripheral nerve myelination by the beta-secretase BACE1. Science 314, 664-6.

Newbern, J. and Birchmeier, C. (2010). Nrg1/ErbB signaling networks in Schwann cell development and myelination. Semin Cell Dev Biol 21, 922-8.

Vasyutina, E., Lenhard, D. C., Wende, H., Erdmann, B., Epstein, J. A. and Birchmeier, C. (2007). RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells. Proc Natl Acad Sci U S A 104, 4443-8.

Vasyutina, E., Martarelli, B., Brakebusch, C., Wende, H. and Birchmeier, C. (2009). The small G-proteins Rac1 and Cdc42 are essential for myoblast fusion in the mouse. Proc Natl Acad Sci U S A 106, 8935-40.

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Thomas J. Jentsch

Physiology and Pathology of Ion Transport

Ion transport across cellular membranes is crucial for cellular homeostasis and has integrative functions such as trans-epithelial transport or signal transduction. We study ion transport at various levels: biophysical and structure-function analysis of transport proteins, their role in cellular functions such as cell volume regulation or endocytosis, to the role in the organism. The physiological importance of ion transport proteins is often evident from pathologies resulting from their disruption in mice or men. We have discovered several human 'channelopathies' and have generated and analyzed many mouse models.

We focus on CLC chloride channels and transporters, Anoctamin Ca^{2+}-activated Cl^- channels, KCNQ potassium channels, and KCC potassium-chloride co-transporters. Their mutational inactivation 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 in the role of chloride and pH in endosomes and lysosomes.

(1) CLC chloride channels and transporters

Eun-Yeong Bergsdorf, Sabrina Jabs, Lila Leisle, Carmen Ludwig, Vanessa Plans, Tobias Stauber, Lena Wartosch, Stefanie Weinert

The CLC gene family, discovered in our laboratory in 1990, encodes plasma membrane chloride channels and chloride transporters of intracellular membranes. By means of KO mouse models for most CLCs, in the past couple of years we identified associated β-subunits (barttin and Ostm1), discovered that certain vesicular CLCs are electrogenic Cl^-/H^+-exchangers, performed structure-function analysis, and uncovered several new pathologies resulting from their dysfunction. Vesicular CLCs were believed to be Cl^- channels that facilitate vesicular acidification by shunting proton pump currents, but surprisingly, vesicular CLCs are Cl^-/H^+-exchangers. To determine whether Cl^-/H^+-exchange can be replaced by a Cl^- conductance as in the classical model of vesicular acidification, we have now generated ClC-5 and ClC-7 KI mice in which we converted these exchangers with single point mutations into pure Cl^- conductors. Surprisingly, the phenotypes of these mice largely recapitulated those seen in the respective KOs, pointing to a previously unrecognized role of vesicular Cl^- accumulation. In other projects, we demonstrated that ClC-7 is important for lysosomal protein degradation, but that the enlargement of lamp-1-positive compartments is not a consequence of protein accumulation; that lysosomal acidification is prominently supported by a lysosomal cation conductance; that ubiquitylation is not important for the role of ClC-5 in renal endocytosis, nor is there any compensation by ClC-3 or ClC-4. We have

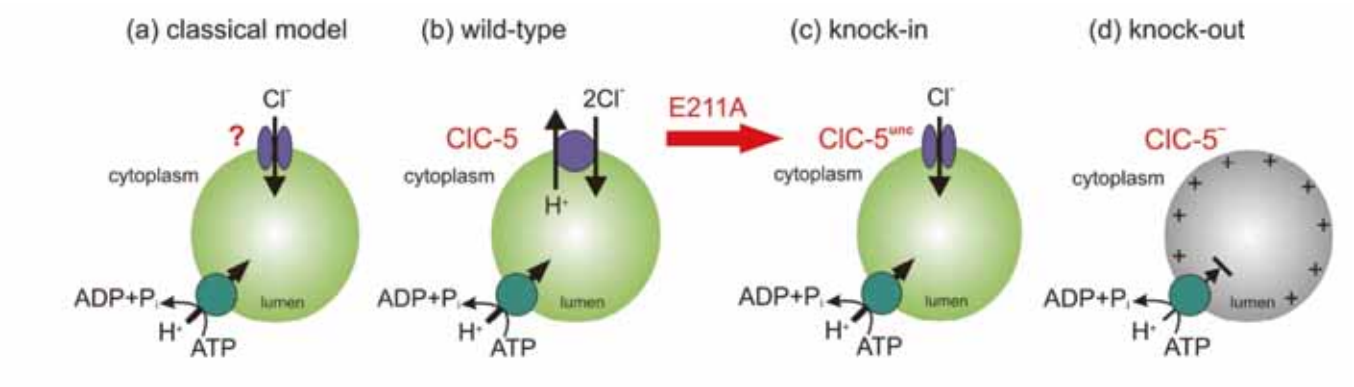

Figure 1. Model for vesicular acidification. (a) In the classical model for vesicular acidification, the current generated by the H^+-ATPase is neutralized by an influx of negatively charged Cl^- ions through a Cl^- channel, the molecular identity of which was unknown. We had hypothesized that ClC-5 to ClC-7 represent these Cl^- channels in various endosomal and lysosomal compartments. **(b)** Our studies, however, revealed that these vesicular CLC proteins are Cl^-/H^+ exchangers, as shown here for ClC-5. **(c)** To elucidate whether the coupling of Cl^- fluxes to a counter-transport of protons is biologically important, we converted ClC-5 (and in other experiments ClC-7) into a pure Cl^- conductance by inserting a single point mutation. We therefore artificially create the 'classical' model shown in (a). Any phenotype observed in knock-in mice carrying these uncoupling (*unc*) mutations cannot be attributed to a defect in vesicular acidification, but specifically to a loss of Cl^-/H^+-coupling. **(d)** When ClC-5 is eliminated in knock-out mice, however, vesicular acidification is impaired.

also investigated sorting signals for all vesicular CLCs and have directed ClC-7 from lysosomes to the plasma membrane where it is amenable to detailed biophysical analysis. We have compared in detail the pathology caused by disruption of ClC-7/Ostm1 and ClC-6, have shown for the first time that ClC-6 is also a Cl^-/H^+-exchanger, and have investigated the selectivity of vesicular CLCs in a structure-function study.

(2) Cation-Cl- cotransporters

Carsten Pffeffer, Guillermo Spitzmaul, Patricia Seja

We have previously knocked-out all KCl-cotransporter isoforms (KCC1-4) in mice and have obtained specific and highly interesting phenotypes. We are continuing our studies on KCCs with conditional KOs. Our major focus is on KCCs expressed in neurons, where KCC2 in particular lowers the cytoplasmic chloride concentration. Such a low concentration is necessary for the inhibitory action of GABA and glycine, which act on ligand-gated chloride channels. We are currently investigating various mouse lines in which KCC2 has been inactivated in specific sets of neurons.

We have also studied the transporter that is the major player in elevating cytoplasmic chloride in neurons before the expression of KCC2 kicks in, namely the NaK2Cl cotransporter NKCC1. NKCC1 raises intraneuronal Cl^- above its electrochemical equilibrium, potentially leading to an excitatory response to these neurotransmitters. We found that NKCC1 is an important, though not the only, transporter elevating intraneuronal chloride. However, in contrast to speculations by others, no morphological changes were observed. Using *Nkcc1*$^{-/-}$ mice, we have now shown that spontaneous neuronal activity is reduced in brain slice preparations of these mice and that the neuronal excitability that is dependent on Nkcc1 drives synaptic network maturation early in development.

(3) KCNQ potassium channels

Matthias Heidenreich, Guillermo Spitzmaul, Pawel Fidzinski, Patricia Preston, Lena Wartosch

There are five different isoforms of KCNQ (Kv7) potassium channels, KCNQ1-KCNQ5. KCNQ2-KCNQ5 mediate 'M-currents' that regulate neuronal excitability. We had previously shown that KCNQ2 and KCNQ3 underlie a form of human epilepsy and that dominant KCNQ4 mutations are a cause of human deafness and have published a few years ago a mouse model for KCNQ4 deafness. We are now investigating possible vestibular phenotypes in these mice as well as the role of KCNQ4 in dorsal root ganglia and in mechanosensation.

Recently we have generated a KCNQ5 KI mouse carrying a dominant negative mutation. Using these mice, we have shown in a collaboration with Roger Nicoll (UCSF) that KCNQ5 mediates a component of the afterhyperpolarization current in the hippocampus. We

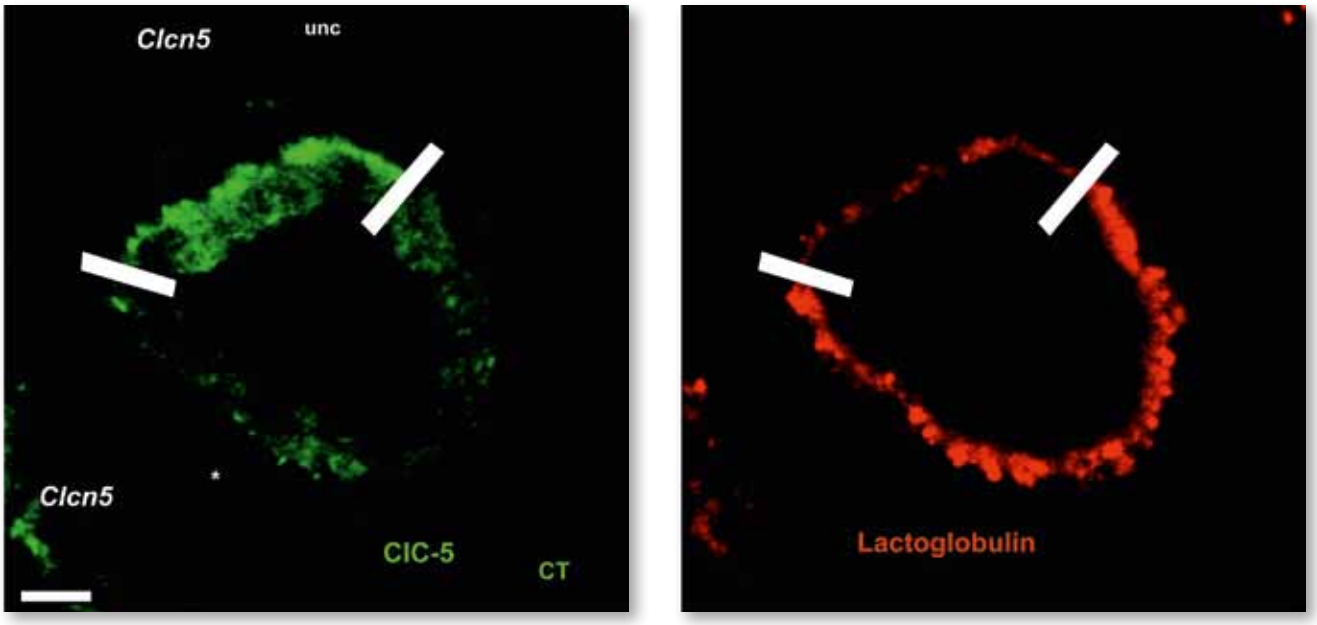

Figure 2. Uncoupling the Cl^- transport of ClC-5 from H^+ countertransport impairs endocytosis. Confocal microscopy pictures of a cross-section of a renal proximal tubule from a female mouse heterozygous for the uncoupling ClC-5^{unc} mutation and for a mutant that changes the C-terminus in two amino-acids (ClC-5^+). This mutated C-terminus is no longer recognized by the ClC-5 antibody against the wild-type ClC-5 C-terminus. Owed to X-linked inactivation of either allele, the proximal tubule is chimeric, expressing in the upper cells the uncoupled ClC-5 (recognized by the antibody) and in the lower part of the picture the ClC-5 with WT $2Cl^-/H^+$-exchange activity, which is not recognized by the antibody (left picture). Uptake of fluorescently labeled lactoglobulin that was injected into the bloodstream is shown in the right panel. Cells in which Cl^-/H^+-exchange has been converted to a Cl^- conductance display a severe cell-autonomous impairment of endocytosis. Taken from Novarino et al., *Science* 2010.

have found that KCNQ4 is not only expressed in sensory cochlear hair cells, but also in sensory neurons that are involved in mechanosensation.

About ten years ago, we had identified KCNE3 as a β-subunit of KCNQ1 which renders this channel constitutively open. We have now generated and analyzed a KCNE3 knock-out mouse and have shown that KCNQ1/KCNE3 channels are important as a recycling pathway for intestinal and tracheal Cl^- secretion.

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

Gwendolyn Billig, Pawel Fidzinski, Balázs Pál, Kristin Schnuppe

We have started new projects to define functions of members of the newly identified Anoctamin family of Ca^{2+}-activated Cl^- channels. As a first result, we have shown that Ano2 is the long-sought Ca^{2+}-activated Cl^- channel of olfactory sensory neurons. This channel has been postulated to provide a roughly 10-fold amplification of olfactory sensitivity. In our Ano2 knock-out mouse model, Ca^{2+}-activated Cl^- currents of olfactory sensory neurons are completely abolished, but only moderate effects are detected at the level of the olfactory epithelium and mice behave normally in behavioral tests for olfaction. Our results require a revision of the text-book model for olfactory signal transduction.

Selected Publications

Billig, GM, Pál, B, Fidzinski, P, Jentsch. TJ. (2011). Ca^{2+}-activated Cl^- channels are dispensable for olfaction. Nature Neurosci 14, 763-749.

Leisle, L, Ludwig, CF, Wagner, FA, Jentsch, TJ, Stauber, T. (2011). ClC-7 is a slowly voltage-gated $2Cl^-/H^+$ exchanger and requires Ostm1 for transport activity. EMBO J. 30, 2140-2152.

Weinert, S, Jabs, S, Supanchart, C, Schweizer, M, Gimber, N, Richter, M, Rademann, J, Stauber, T, Kornak, U, Jentsch, TJ. (2010). Lysosomal pathology and osteopetrosis upon loss of H^+-driven lysosomal Cl^- accumulation. Science 328, 1401-1403.

Novarino, G, Weinert, S, Rickheit, G, Jentsch, TJ. (2010). Endosomal chloride-proton exchange rather than chloride conductance is crucial for renal endocytosis. Science 328, 1398-1401.

Lange P.F., Wartosch L., Jentsch T.J., Fuhrmann J.C. (2006). ClC-7 requires Ostm1 as a β-subunit to support bone resorption and lysosomal function. Nature 440, 220-223.

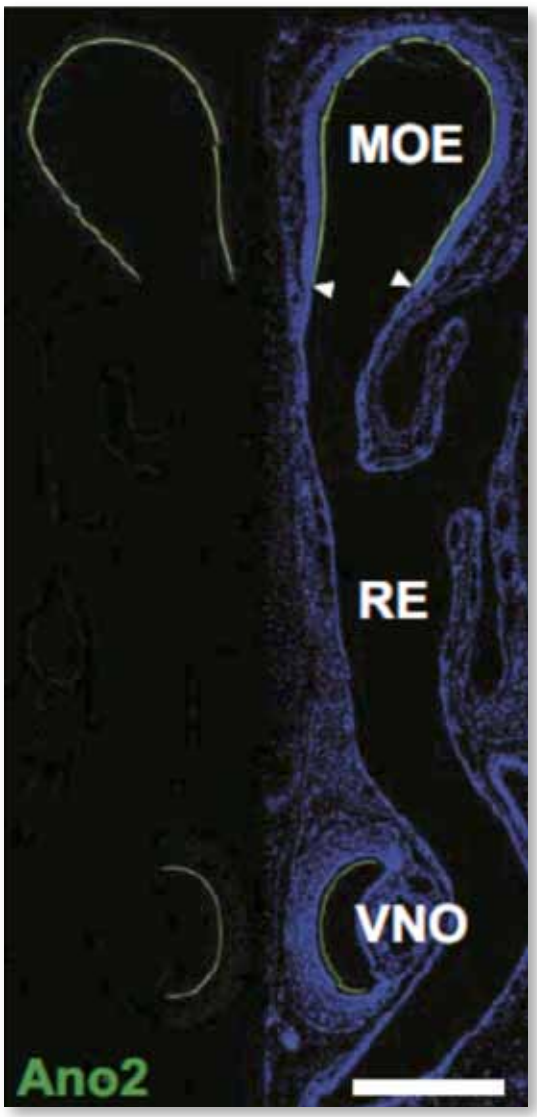

Figure 3. Expression of the Ca^{2+}-activated Cl^- channel Ano2 in olfactory epithelia. Confocal microscopy of a cross-section of murine nose, showing Ano2 immunoreactivity in green. Only in the right part of the figure counterstaining for nuclei (blue) is shown. Ano2 is exclusively expressed on the apical surface of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). It is not found in the respiratory epithelium (RE). Taken from Billig et al., *Nature Neuroscience* 2011.

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Fritz G. Rathjen

Neuronal Connectivity

The complex functions of our nervous system rely on the correct wiring of neurons which is established during embryonic and early postnatal development. The so-called growth cone at the tip of an extending axon is a highly dynamic fan-like structure that explores its environment by protruding and retracting filopodia and lamellipodia. It steers the axon over distances that may stretch over several centimeters to its target region where synaptic contacts are formed. Proper wiring is orchestrated at different levels by multiple cellular and molecular mechanisms. Our research group focuses on two subtopics of the "wiring" problem: the branching of axons and modulation of the neuronal circuitry by electric activity.

Axonal branching is essential to build a complex neuronal circuitry

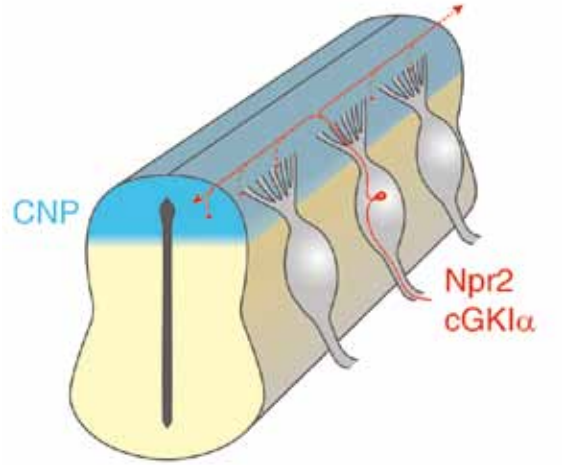
For an integrated processing of information perceived from different places of the body (convergence) or for the divergent distribution of information it is essential that individual neurons establish contacts to multiple neurons that might be located in different parts of the nervous system. To generate this complex circuitry axons are able to branch at specific places during extension and the resulting daughter axons grow to different target regions. Branching that is common to almost every neuron therefore contributes to the enormous complexity of circuits within the nervous system.

A relatively simple and accessible model system for the molecular analysis of axonal branching is the projection of sensory axons into the spinal cord. When entering the cord the growth cone of a sensory axon splits into two arms after which one of the resulting daughter axons grows in rostral whereas the other extends in caudal direction. After a waiting period collaterals are generated from these stem axons that grow to their termination zones where further arborization occurs and synapses are finally established. Collaterals of proprioceptive neurons terminate in the ventral cord, whereas nociceptive and mechanoreceptive collaterals are confined to the dorsal horn.

The natriuretic peptide C (CNP) is required for the bifurcation of sensory axons

Our detailed analysis of cGMP signaling in mutant mice using axon tracing methods with fluorescent dyes or genetic markers revealed that a cGMP signaling cascade is essential for the bifurcation of sensory axons at the entry zone of the spinal cord (Figure 1). Three components of this signal pathway are currently known: the ligand natriuretic peptide C (CNP), the receptor guanylyl cyclase Npr2 (natriuretic peptide receptor 2) and the cGKIα (cGMP-dependent kinase Iα). In the absence of one of these components, sensory axons are unable to bifurcate; instead, all axons simply turn in either rostral or caudal direction. The ligand is released by precursor cells in the dorsal horn and its binding to Npr2 on the

A



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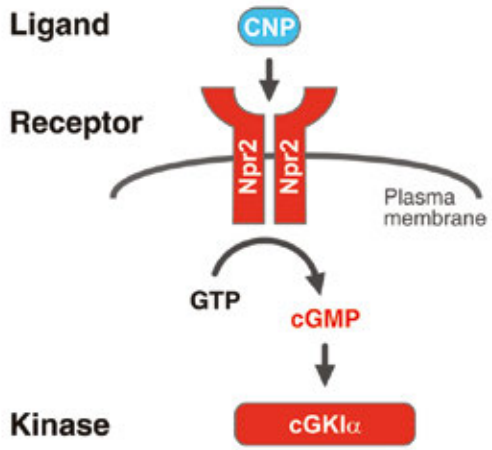

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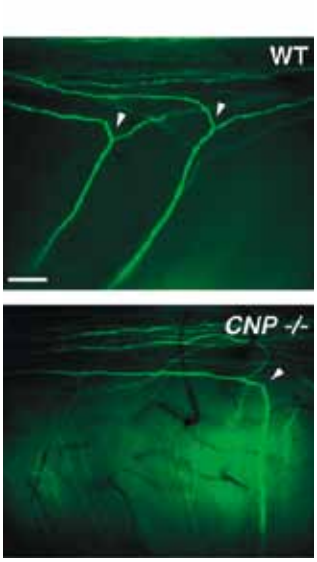

Figure 1. The second messenger cGMP is implicated in the branching of sensory axons when these enter the spinal cord. A) Schematic view of the path taken by sensory axons within the spinal cord. A sensory neuron within the dorsal root ganglion, highlighted in red, enters the spinal cord at the dorsal root entry zone, bifurcates and the two resulting daughter axons grow into rostral or caudal direction. After a waiting period collaterals grow out from the stem axons which terminate either in the dorsal or ventral part of the spinal cord. At the developmental stage when sensory axons reach the spinal cord the ligand CNP is released from precursor cells of the dorsal quarter of the cord – indicated in blue. B) The cGMP-dependent signaling cascade essential for bifurcation consists of the ligand CNP, the receptor guanylyl cyclase Npr2 and the kinase cGKIα. The ligand CNP binds to Npr2 which generates cGMP from GTP on the intracellular side. cGMP in turn activates the kinase cGKIα which phosphorylates so far unknown components. C) In the absence of one of these components sensory axons do not bifurcate. WT – wildtype, upper panel; mutant, lower panel. (Taken from Schmidt and Rathjen, 2010).

surface of sensory growth cones leads to the activation of the intracellular guanylyl cyclase domain of Npr2 that synthesizes cGMP from GTP. cGMP then activates the kinase cGKIα which in turn phosphorylates so far unknown intracellular proteins. Candidates for phosphorylation might be cytoskeletal elements (actin or tubulin associated proteins) that provide the machinery for bifurcation or other intracellular signaling components. Our current efforts focus on the identification of these phosphorylation targets as well as on the question whether axonal branching of other types of neurons also depends on this cGMP signaling pathway.

The cell adhesion molecule CAR establishes a direct link between electric activity and neural cell adhesion at developmental stages.

In the mature nervous system electric activity is the language and therefore provides the basis for the communication between neurons. However, neuroscientists have established evidence that electric activity of neurons is also implicated in shaping the structure of the nervous system during embryonic and postnatal development. Even at very early stages of development neurons reveal on a cell by cell basis spontaneous electrical

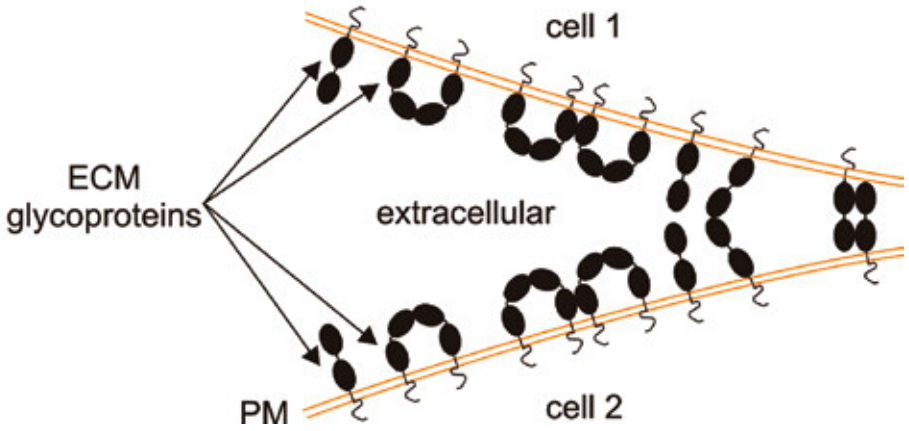

Figure 2. Scheme of the molecular interactions of CAR.
CAR is a transmembrane protein with two Ig domains in its extracellular part (D1 and D2). This extracellular region might exist in different conformations. Possible homophilic interactions might occur through D1-D1 self-association forming a U-shaped structure which might occur between two CAR polypeptides within the same plasma membrane. Other data suggest that homophilic interactions of CAR result from an antiparallel D1-D2 interaction. Heterophilic interactions to ECM glycoproteins are indicated by arrows and are mediated by the membrane proximal domain of CAR (D2). (Taken from Patzke et al., 2010).

activity. As soon as functional circuits form, spontaneous activity becomes correlated between neurons contributing to the refinements of circuits. Although this function of electric activity is known since many years the links between neuronal activity and molecules contributing to the structural remodeling of circuits are less understood.

Cell adhesion molecules (CAM) are considered as candidates to link electric activity to structural changes. CAMs establish initial cell-cell contacts and provide a platform for contact mediated intercellular signaling. Four structural groups of CAMs are expressed in the developing nervous system: cadherins/protocadherin; IgCAMs (immunoglobulin cell adhesion molecules), integrins and neurexins/neuroligins. Of these several IgCAMs are expressed at early stages and reveal a localization on axonal surfaces. We have therefore asked whether IgCAMs influence electric activity itself during development and tested whether the absence of an IgCAM affects the frequency of action potentials. Neurons of different IgCAM-deficient mice were cultivated and their action potentials were analyzed by current clamp recordings. We observed that the IgCAM CAR specifically modulates electric activity while several other IgCAMs do not. Calcium imaging then revealed that CAR regulates calcium levels by releasing it from internal stores. Our studies revealed an unexpected link between adhesion processes, calcium release and electric activity. The IgCAM CAR modulates calcium levels and thereby influences the frequency of action potentials. We hypothesize that CAR coordinates cellular actions with repect to the propagation of electric activity within groups of cells.

Further characterization indicated that CAR is a typical cell adhesion molecule that is primarily expressed in the developing and almost absent in the adult nervous system. It reveals homophilic as well as heterophilic interactions. Our structural, binding as well as adhesion studies predict a flexible ectodomain of CAR allowing conformational shifts for cis or trans homo-

philic interactions on neurons. Within the same plasma membrane CAR might occur as dimer through D1-D1 interactions forming a U-like structure. Conformational changes might enable trans interactions between CAR polypeptides presented from juxtaposed neurons. This trans homophilic interactions can occur between two N-terminal located domains or via D1 and D2 interfaces of CAR in a linear arrangement from opposing neurons (Figure 2).

Selected Publications

Schäfer, M.K., Nam, Y.C., Moumen, A., Keglowich, L., Bouché, E., Küffner, M., Bock, H.H., Rathjen, F.G., Raoul, C., Frotscher, M. (2010) L1 syndrome mutations impair neuronal L1 function at different levels by divergent mechanisms. Neurobiol Dis. 40: 222-237

Patzke,C., Max,K.E., Behlke,J., Schreiber,J., Schmidt,H., Dorner,A.A., Kroger,S., Henning,M., Otto,A., Heinemann,U., and Rathjen,F.G. (2010). The coxsackievirus-adenovirus receptor reveals complex homophilic and heterophilic interactions on neural cells. J. Neurosci. *30*, 2897-2910.

Schmidt,H. and Rathjen,F.G. (2010). Signalling mechanisms regulating axonal branching in vivo. Bioessays *32*, 977-985.

Schmidt,H., Stonkute,A., Juttner,R., Koesling,D., Friebe,A., and Rathjen,F.G. (2009). C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. Proc. Natl. Acad. Sci. U. S. A *106*, 16847-16852.

Schmidt, H., Stonkute, A., Jüttner, R., Schäffer, S., Budgereit, J., Feil, R., Hofmann, F., and Rathjen, F.G. (2007). The receptor guanylyl cyclase Npr2 is essential for sensory axon bifurcation within the spinal cord. J. Cell Biol., 179, 331-340.

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Gary R. Lewin

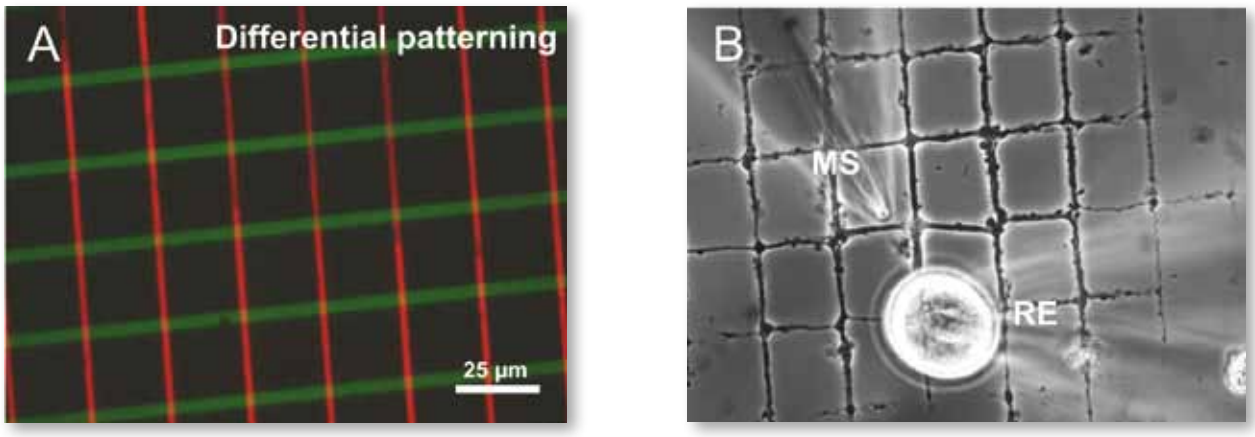
Molecular Physiology of Somatic Sensation

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 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 together with the developmental events that lead to specification of the appropriate sensory neuron sub-types are actively investigated the lab.

Molecular Basis of Mechanotransduction

Yinth Andrea Bernal-Sierra, Liudmilla Lapatsina, Stefan G. Lechner, Kate Poole, Christiane Wetzel

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 a role mammalian orthologs of these genes 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 mechanotransducing channel. We have cloned and characterized vertebrates 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 SLP3 (stomatin like protein-3) was cloned by our group, and we subsequently generated a mouse model with a null mutation of the SLP3 locus. In SLP3 mutant mice many mechanoreceptors (or touch receptors) in the skin do not work in the absence of the SLP3 protein. In order to analyze touch sensation in mice we also developed a novel behavioral assay for touch driven behavior in rodents. This assay is based on the ability of mice to detect and react to gratings, which are fine enough to have a textured quality. We were very pleased to find that SLP3 mutant mice have severe deficits in their ability to detect such textured surfaces. Current work in the lab focuses on the role of related members of the stomatin-domain family in mechanotransduc-



A single sensory neuron growing a check patterned substrate. Top right **(A)** shows the stripes of laminin printed onto a glass surface. Note that different proteins can be printed in the vertical (red) or horizontal axis (green). **(B)** Phase contrast picture of a sensory neuron growing on the patterned substrate. Note that the neuron only grows neurites along the stripes to form a window like pattern of growth. In this picture the neuron was recorded with a patch pipette (RE) and the neurites can be mechanically stimulated with a nanomotor (MS). **(C)** The neurons was filled with a yellow dye via the recording pipette to show that neurites belong to the indicated cell. **(D)** Colour combine showing the pattern together with the yellow neurons and its neurites.

tion, structure function studies and the identification of further essential interaction partners for SLP3. We are also performing molecular and cell biology studies to determine what the structural basis of SLP3 function is in enabling mechanotransduction.

Neuronal nanodetection and micro-patterning, engineering sensory neurons for function

Kate Poole and Ludmilla Lapatsina

The mechanosensitive ion channels that are 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. We have also gathered considerable evidence that the mechanosensitive channels are actually opened via a protein tether that attaches to laminin-containing extracellular matrices. In order to study the influence of different extracellular matrices on mechanotransduction and to quantify the tiny forces that are required to open mechanosensitive channels we have started to a use variety of new micro-fabrication techniques. For example, we have used micro-patterning of matrix molecules in order force neurons in culture to adopt morphologies that better match the in vivo situation. We can also use such patterning to test the local influence of specific matrix molecules on transduction ability or axon branching behavior. We have shown that sensory neurons can be made to grow to produce highly structured patterns in vivo (see Figure 1). Another application of micro-engineering is to make neurons grow on three dimensional surfaces that allow us to gauge the forces needed to open mechanosensitive ion channels in single cells.

Touch, hearing and the development of mechanosensation

Henning Frenzel, Regina Hartl, Jan Walcher, Julia Haseleu, Stefan Lechner, and Simone Pifferi

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 are measuring pyschometric functions in normals and hearing impaired people in order to describe quantitatively differences in the perception of touch. We are also carrying out a large twin study, to examine the heritability of touch acuity in humans. Initial results suggest that genetic factors are very important in determining how good our sense of touch is. We are also pursuing the hypothesis that some of the genetic factors influencing touch may also directly affect the second mechanosensory sense, hearing.

We have examined one gene encoding the potassium channel KCNQ4 which is involved in late onset hearing loss in close collaboration with the group of Thomas Jentsch. This ion channel is only found in very specific types of mechanoreceptors and it was found that pathological mutations lead to a change in the tuning of these receptors so that they are more sensitive than normal to low frequency vibration. Interestingly, patients carrying KCNQ4 mutations displayed altered touch performance in quantitative tests of vibration sensitivity that could be accounted for by the observed changes in touch receptor function.

We have been interested in the development of mechanosensation for many years and it was remarkable how little was known in this area. We have recently shown, in a very detailed study, that sensory neurons acquire their competence to detect mechanical stimuli very early in embryonic development. Interestingly, very distinct developmental mechanisms are used to induce such competence in neurons that underlie touch sensation as opposed to nociception (Painful stimuli). For example, we have shown that NGF plays a critical role in the acquisition of transduction competence by nociceptors.

Tuning pain sensitivity

Stefan Lechner, Ewan St John Smith , Tobias Albert and Rui Wang

Nociception is describes our ability to respond to potentially or actually damaging stimuli. An important aspect of the biology of nociception is that after injury people and animals become much more sensitive to sensory stimulation than before injury. This phenomenon is sometimes called sensitization and it is often desirable to block this process after inflammation to prevent pain becoming pathologically severe. We are interested in the cellular and molecular basis of sensitization. We recently discovered that some endogenous chemicals, such as ATP and UTP that are released from damaged cells during inflammation can potently increase the magnitude of the mechanosensitive current in sensory neurons. This would have the effect of making nociceptors innervating inflamed tissue more sensitive to mechanical stimuli, and such a phenomenon may underlie the tenderness that follows inflammation. Identification of the mechanotransducer as a target of inflammation indicates that, this as yet unknown ion channel, may be an excellent molecular target to block in order to treat pain after inflammation.

The Naked Mole Rat a pain free mammal?

Damir Omerbasic, Ewan St.John Smith, Tania Kovalchuk, Jane Reznick

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 25 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 characterized 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. We were very interested in understanding how naked mole-rats are completely insensitive to acid. In a very recent study 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.

Selected Publications

Chiang L-Y, Poole K, Oliveira BE, Duarte N, Sierra YAB, Bruckner-Tuderman L, Koch M, Hu J, Lewin GR (2011) Laminin-332 coordinates mechanotransduction and growth cone bifurcation in sensory neurons. Nat Neurosci 14:993–1000.

Heidenreich M, Lechner SG, Vardanyan V, Wetzel C, Cremers CW, De Leenheer EMR, Aránguez G, Moreno-Pelayo MA, Jentsch TJ, Lewin GR (2011) KCNQ4 K+ channels tune mechanoreceptors for normal touch sensation in mouse and man. Nature Neuroscience in press.

Hu J, Chiang L-Y, Koch M, Lewin GR (2010) Evidence for a protein tether involved in somatic touch. EMBO J 29:855–867.

Lechner SG, Markworth S, Poole K, Smith ESJ, Lapatsina L, Frahm S, May M, Pischke S, Suzuki M, Ibañez-Tallon I, Luft FC, Jordan J, Lewin GR (2011) The molecular and cellular identity of peripheral osmoreceptors. Neuron 69:332–344.

Smith ESJ, Omerbašić D, Lechner SG, Anirudhan G, Lapatsina L, Lewin GR (2011) The molecular basis of acid insensitivity in the African naked mole-rat. Science in press.

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Ines Ibañez-Tallon

Molecular Neurobiology of Cell-surface Channels and Receptors

The dissection of neuronal circuits underlying a specific function requires both the identification of the cell populations and the profiling and characterization of the ion channels that control the excitability of those cell-types as possible molecular targets for therapeutic interventions. To approach this question, our group focuses on the circuits controlling nicotine addiction and reward, and central and peripheral responses to pain. We have optimized membrane-tethered toxins to silence genetically defined CNS cell types to understand the contributions of specific cell types to circuits controlling behavior. In addition, we have discovered that increased expression of the Chrnb4 subunit dramatically enhances the response of targeted neurons to nicotine and this results in altered nicotine consumption. We are combining these tools with mouse transgenesis and Cre-dependent viral vectors to understand both the biology of nicotine dependence, and to dissect circuits controlling central pain perception.

Silencing neurotransmission with calcium channel specific t-toxins.

Based on the homologies of lynx1 and snake toxins we developed membrane-tethered versions of peptide neurotoxins to inactivate ion channels. Because of their mode of action at the cell-surface, membrane-anchored peptide molecules act only on ion channels and receptors present in the membrane of the cell that is expressing the t-toxin or t-peptide, and not on identical receptors present on neighboring cells that do not express the tethered construct. We have cloned approximately 40 different recombinant t-toxins derived from the venom of several predatory animals and characterized their activity on voltage and ligand-gated ion channels.

By targeting t-toxins to specific neurons we can dissect neuronal circuits in a variety of circuits and organisms. We have developed a system based on constitutive, inducible and Cre-dependent vectors encoding fluorescent membrane-tethered toxins that allows cell autonomous blockade of high-voltage-activated $Ca_v2.1$ and $Ca_v2.2$ calcium channels (Figure 1A). These channels are located at synaptic terminals where they play an essential and joint role in coupling the presynaptic action potential to the neurotransmitter release process. Using these optimized t-toxins we can block each channel individually or simultaneously in neurons. Validation of this strategy in vivo is demonstrated in central and peripheral neurons. Mice injected with lentiviral vectors show selective interference with the dopaminergic nigrostriatal pathway leading to stereotypic circling (Figure 1B). These optimized tethered toxins provide new tools for cell-specific and temporal manipulation of calcium channel mediated activities

Application of t-toxins to the dissection of the nociceptive circuits

Nociceptive sensory neurons innervating the skin are equipped with specific sets of voltage-gated ion channels, which are required for their ability to encode touch,

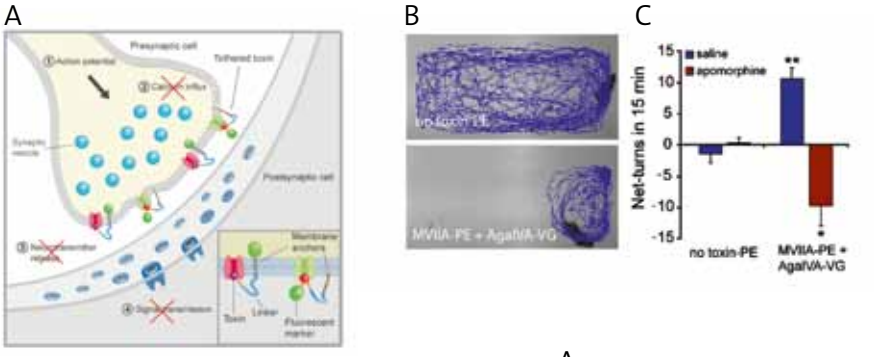

Figure 1. (**A**) Schematic representation of tethered toxins (t-toxins) blocking calcium voltage-gated channels at the presynaptic terminal in neurons; (**B**) Mice microinjected with t-toxin lentivirus show contralateral rotation with saline and ipsilateral rotation with apomorphine treatment, indicating inhibition of dopamine neurotransmitter release. See also Auer et al, 2010 *Nature Methods*

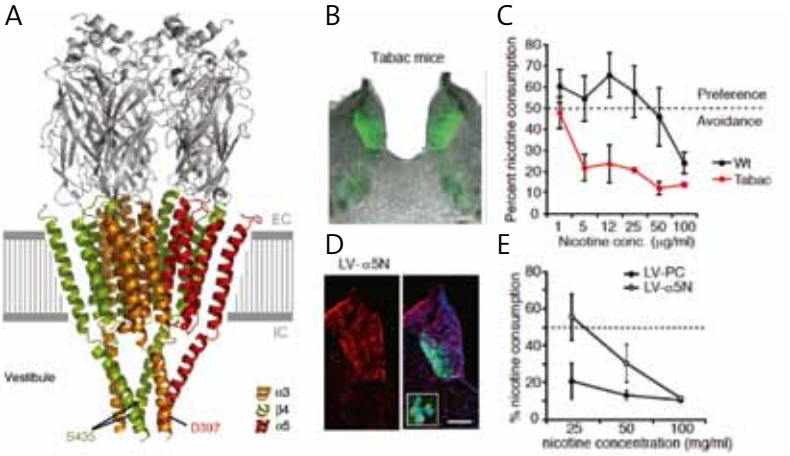

Figure 2. (**A**) Computational model of a3b4a5 nAChR pentamer showing that S435 responsible for potentiation of nicotinic currents by b4 locates within the intracellular vestibule in close proximity to the a5 SNP variant D398N. **B**) Tabac mice overexpress b4 in the MHb **C**) and show strong aversion to nicotine (**D**) Tabac mice injected with a lentivirus encoding the a5-D397N variant (red fluorescence) in MHb (**E**) prefer nicotine containing solution (25 µg/ml).

temperature and pain. We have shown that expression of membrane-tethered MrVIA in nociceptive neurons of transgenic mice. reduces Nav1.8-voltage-gated sodium channel currents, and decreases inflammatory hyperalgesia and sensitivity to noxious cold. Furthermore, we have generated transgenic mice encoding tethered conotoxin MVIIA against $Ca_v2.2$ channels, which are essential for the release of pro-nociceptive neurotransmitters such as substance P to the spinal cord upon inflammatory insult or in response to nerve injury. Consistently t-MVIIA transgenic mice have reduced inflammatory hyperalgesia and protection from neuropathic pain.

Nicotine dependence and consumption.

Recently, we have identified a new molecular mechanism dependent on the beta4 nAChR subunit that controls nicotine aversion in mice. This is interesting given the identification of multiple single nucleotide polymorphisms (SNPs) in the CHRNA5-CHRNA3-CHRNB4 gene cluster that strongly influence nicotine dependence in human populations. We identified a single residue (ser 435) in the β4 subunit that is essential for its ability to potentiate nAChR currents, (Figure 2). This residue lies in the intracellular vestibule in close proximity to the most common SNP linked to heavy smoking: α5 D398N. Functional analysis of this α5 SNP variant indicated reduced nicotine-evoked currents that could be competed by increased levels of b4. Consistently, the nicotine aversion observed in Tabac mice (transgenic mice over-expressing b4) was reversed upon viral-mediated expression of this α5 variant in the medial habenular (MHb) midbrain area of Tabac mice. These studies demonstrated that α3β4α5 receptors contribute to nicotine consumption and identified the MHb as a critical element in the circuitry controlling nicotine-dependent phenotypes.

Selected Publications

Santos-Torres J, Slimak MA, Auer S, Ibañez-Tallon I. (2011) Cross-reactivity of acid-sensing ion channel and Na+/H+ exchanger antagonists with nicotinic acetylcholine receptors . J.Physiol. In press

Frahm S, Slimak MA, Santos-Torres J, Ferrarese L , Santos-Torres J, Auer S, Filkin S, Pons S , Fontaine J-F, , Tsetlin V, Maskos U, Ibañez-Tallon I. (2011) Aversion to nicotine is regulated by the balanced activity of β4 and α5 nicotinic receptor subunits in the medial habenula. Neuron. 70 :522-35

Krasteva G, Canning BJ, Hartmann P, Veres TZ, Papadakis T, Mühlfeld C, Schliecker K, Tallini YN, Braun A, Hackstein H, Baal N, Weihe E, Schütz B, Kotlikoff M, Ibañez-Tallon I, Kummer W. (2011). Cholinergic chemosensory cells in the trachea regulate breathing. Proc Natl Acad Sci U S A. 2011 108 :9478-83.

Stürzebecher AS, Hu J, Smith ES, Frahm S, Santos-Torres J, Kampfrath B, Auer S, Lewin GR, Ibañez-Tallon I. (2010) An in vivo tethered toxin approach for the cell-autonomous inactivation of voltage-gated sodium channel currents in nociceptors. J.Physiol. 588: 1695-1707.

Auer S, Stürzebecher AS, Jüttner R, Santos-Torres J, Hanack C, Frahm S, Liehl B, Ibañez-Tallon I. (2010). Silencing neurotransmission with membrane-tethered toxins. Nat. Methods 7:229-36.

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RNA Editing and Hyperexcitability Disorders

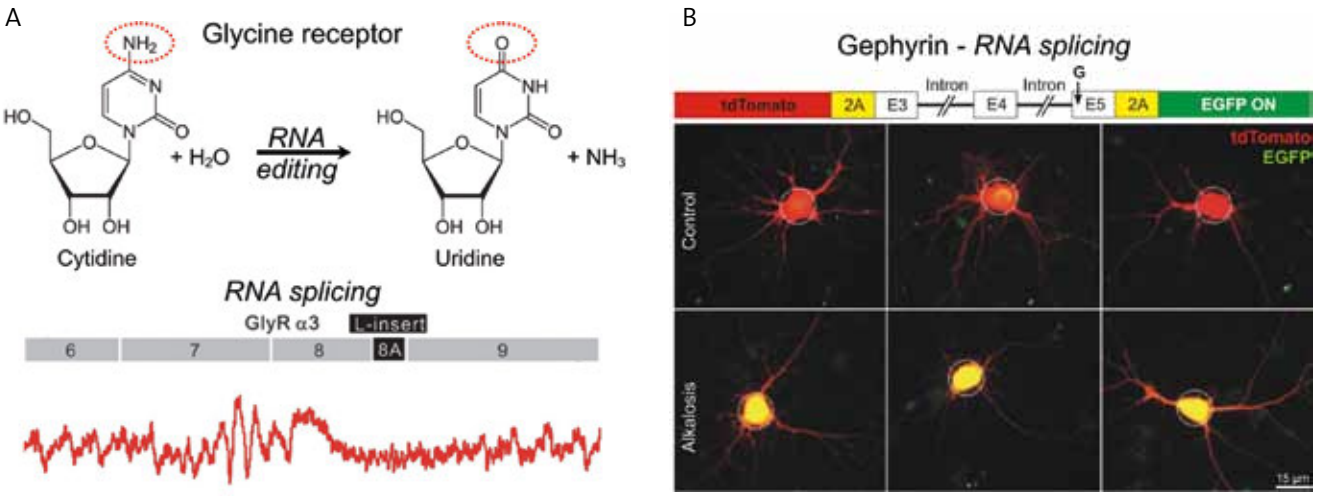
In a healthy organism, a balance is maintained between excitation and inhibition of electrical impulses generated by neurons in the brain. Deregulation of this balance results in nervous system disorders. A core aspect of our work concerns the study of the brain at the molecular level, by investigating RNA editing and RNA splicing. We search for disease-associated alterations in mRNA in the nervous system and elucidate their pathogenic potential at molecular and cellular levels of investigation. Within this context, we are more closely scrutinizing glycine and GABA(A) receptors as well as gephyrin, *id est* the key components of the molecular machine responsible for inhibition of electrical impulses in the brain.

Hippocampal glycine receptors (GlyR) and temporal lobe epilepsy (TLE)

GlyR are well known to mediate synaptic inhibition in the spinal cord and brain stem. Their role in supra-medullar brain areas has remained enigmatic because in the hippocampus, for example, presynaptic glycinergic terminals are rare. Our recent discovery of RNA editing of GlyR gene transcripts (Figure 1A) is disclosing new functional aspects of the resulting gain-of-function GlyR channels. C-to-U RNA editing provokes amino acid substitution leucine for proline within the ligand binding domain, and due to their high agonist affinity, RNA-edited GlyR silence neuronal activity through shunt-type inhibition. We also found that inclusion of exon 8A during RNA splicing leads to preferential association of GlyRα3L with glutamatergic synapses, where glycine is one of the co-agonists at NMDA receptors. Molecular analysis of RNA specimen from patients with intractable TLE furthermore unveiled increased RNA editing of GlyRα3, and the majority of patients express the long splice variant α3L. To address the functional role of RNA-edited GlyRα3L, a knock-in mouse model was generated and allows for cell-type specific expression of these receptors. We are investigating their functional impact on glutamatergic synaptic transmission in the hippocampus and determine whether the hippocampal neuronal network of the knock-in mice is able to generate cognitively relevant network oscillatory activity or whether it is prone to seizure-like events. Moreover, we are screening for specific ligands able to antagonize these receptors as these drugs can be a novel therapeutic option.

Hippocampal gephyrin and TLE

Gephyrin is a multi-domain protein that evolved from fusion of two bacterial proteins involved in molybdenum cofactor (Moco) synthesis, MogA and MoeA. Gephyrin is able to synthesize Moco as it preserved enzymatic activity of the individual *E. Coli* homologous proteins. In mammals, the most important Molybdenum enzyme is sulfite oxidase, which detoxifies cellular metabolites by catalyzing the last step in degradation of sulfur-containing amino acids and sulfatides. Human Moco deficiency is a hereditary metabolic disorder



(**A**) Deamination commutes cytidine to uridine, and C-to-U RNA editing produces gain-of-function GlyR. RNA splicing further increases diversity of GlyR gene transcripts, and in case of the α3 subunit inclusion of exon 8A confers preference of GlyRα3L for glutamatergic synapses. A characteristic of epilepsy patients is increased RNA editing associated with preponderant expression of GlyRα3L. Using a corresponding knock-in mouse line we are investigating whether the hippocampal neuronal network of these mice is prone to seizure-like activity (red trace). (**B**) Skipping of exon 4 during RNA splicing of gephyrin mRNA occurs in epilepsy patients and leads to synthesis of truncated gephyrins which infere with GABAergic synaptic transmission. Seizure-characteristic cellular stress (e.g. alkalosis) induces exon skipping, as visualized with a novel molecular tool consisting of two fluorescent proteins separated by a gephyrin genomic fragment spanning exons 3 to 5 (E3-5). The additional guanine nucleotide ('G') is required to automatically switch on gene expression if E4 is skipped. This tool will now be modified for neuronal self-defence against cellular stress.

characterized by severe neurodegeneration, epilepsy and early childhood death. We have identified a number of gephyrin RNA splice variants deficient in Moco synthesis. In addition, several irregularly spliced gephyrin gene transcripts were isolated out of TLE patients. Epilepsy gephyrins interfere with postsynaptic stabilization of GABA(A) receptors and, accordingly, inhibit synaptic GABAergic transmission. Furthermore, they do not exert enzymatic activity, but whether or not this also contributes to the pathogenesis of TLE remains to be determined. Nonetheless, we could identify some of the reasons underlying irregular gephyrin RNA splicing in the *cornu ammonis* of patients with epilepsy. Cellular stress such as it results from seizure activity elicits exon skipping in gephyrin mRNA. A molecular tool (Figure 1B) was developed, which enables affected neurons to conduct their own defence against cellular stress through compensatory gene expression, and allows us to screen for compounds to prevent exon skipping in gephyrin mRNA. As any gene of interest can be expressed conditionally upon cellular stress, neurons can protect themselves against a variety of harmful conditions, including glutamate excitotoxicity. Thus, we are heading for novel genetic and pharmaceutical therapeutic strategies for treatment of TLE and other neurodegenerative diseases.

Selected Publications

Förstera, B, Belaidi, AA, Jüttner, R, Bernert, C, Tsokos, M, Lehmann, TN, Horn, P, Dehnicke, C, Schwarz, G, Meier, JC. (2010). Irregular RNA splicing curtails postsynaptic gephyrin in the cornu ammonis of patients with epilepsy. Brain 133,3778-3794.

Legendre, P, Förstera, B, Jüttner, R, Meier, JC. (2009). Glycine receptors caught between genome and proteome – Functional implications of RNA editing and splicing. Front. Mol. Neurosci. 2,23.

Eichler, SA, Förstera, B, Smolinsky, B, Jüttner, R, Lehmann, TN, Fähling, M, Schwarz, G, Legendre, P, Meier, JC. (2009). Splice-specific roles of glycine receptor a3 in the hippocampus. Eur. J. Neurosci. 30, 1077-1091.

Eichler, SA, Kirischuk, S, Jüttner, R, Schäfermeier, PK, Legendre, P, Lehmann, T-N, Gloveli, T, Grantyn, R, Meier, JC. (2008). Glycinergic tonic inhibition of hippocampal neurons with depolarizing GABAergic transmission elicits histopathological signs of temporal lobe epilepsy. J. Cell. Mol. Med. 12,2848-2866.

Smolinsky, B, Eichler, SA, Buchmeier, S, Meier, JC*, Schwarz, G*. (2008). Splice-specific functions of gephyrin in molybdenum cofactor biosynthesis. J. Biol. Chem. 283,17370-17379. * Equal contribution.

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Signaling and Transport Processes

Our work focuses on signal and transport processes involving members of the recently identified TMEM16 family of membrane proteins. Mutations in several TMEM16 genes cause human inherited diseases including muscular dystrophy, the rare bleeding disorder, Scott syndrome, and a variant of cerebellar ataxia. Two of the TMEM16 proteins are calcium activated chloride channels (CaCCs), known to be important for various functions including photo transduction, pain perception, and smooth muscle contraction. It is still unknown if other members of this family are ion transport proteins. Working this out is part of our investigation. Using cultured cells and transgenic animals we try to better understand the function of TMEM16 proteins in healthy individuals and diseases associated with these genes.

Introduction

CaCCs are chloride ion channels, which open in response to elevated concentrations of intracellular free calcium. Though CaCCs have first been described in 1982, the molecular correlates of CaCCs have only been identified to be members of the TMEM16 family three years ago. TMEM16 genes are found in most eukaryotes and proteins are expressed in many tissues including nervous system, muscle and epithelia. As CaCCs they depolarize some smooth muscle cells, modify action potentials of neurons, drive fluid secretion in glands, and cause crenation after osmotic challenge. However, their function in other cells, e.g. hepatocytes, is currently not known. Interestingly some members of the TMEM16 family are highly expressed in specific forms of cancer, e.g. gastrointestinal stromal tumors and head and neck squamous cell carcinomas.

Structure / function and cell physiology of TMEM16 proteins

Only TMEM16A and TMEM16B have been shown to be CaCCs yet. We have cloned several other members of the TMEM16 family and determined their subcellular localization. TMEM16F is located in the plasma membrane and we were able to record currents from HEK-293 cells expressing this protein using patch clamp technique (figure 1a). Surprisingly the current only appears several minutes after break in and a robust signal required high calcium concentrations > 10 µM. These striking differences to TMEM16A or TMEM16B mediated currents might be explained by a slow relocation of an interacting factor. We have also been able to construct a mutant with faster appearing currents and faster gating kinetics, showing the current being mediated by TMEM16F directly. Our results established a third member of the TMEM16 family as ion channel. Experiments to identify the potential interacting factor and domains involved in the differences in calcium dependence using chimeras are ongoing.

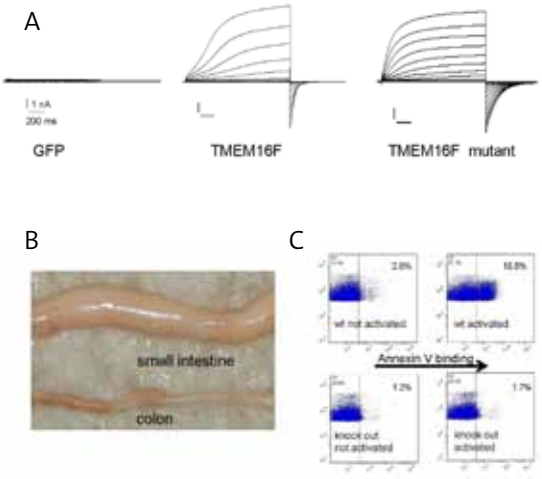

Figure 1 (**A**): Whole cell patch clamp recordings from HEK293 cells transfected with green fluorescent protein (left), TMEM16F (middle) and TMEM16F mutant (right). Starting from a holding potential of -70 mV cells were clamped from -100 mV to +100 mV in 10 mV steps. TMEM16F transfected cells show a slow, depolarization activated current appearing typically 5 minutes after break in under conditions of high calcium in the patch pipette solution. The current in the mutant appears after 2-3 minutes and shows a faster kinetic.

(**B**): Mega-intestine of TMEM16A knock out mouse. The small intestine (top) is much larger than the colon (bottom). This might be a consequence of missing intestinal peristaltic movement due to leak of TMEM16A expression in interstitial cells of Cajal.

(**C**): Phosphatidylserine (PS) expression on the plasma membrane of wild type and TMEM16F knock out platelets measured by flow cytometry. The PS binding protein Annexin V does not attach to resting platelets (left). 10 minutes after activation with thrombin a substantial fraction of wild type (top-right), but not TMEM16F knock out (bottom-right) platelets show Annexin V binding. The negative charged PS in the plasma membrane of activated thrombocytes serves as binding site for proteins involved in blood coagulation.

TMEM16 in vivo studies

Mice deficient for the TMEM16A gene die few days after birth, possibly due to a lung phenotype similar to cystic fibrosis in humans. This it makes it difficult to investigate the physiological role of this channel during later stages of live. To overcome this problem we have, in collaboration with the group of Christian Hübner, University of Jena, generated conditional TMEM16A and other TMEM16 knock out mice.

One interesting phenotype we found in these TMEM16A knock out mice is an enormously enlarged small intestine (figure 1b). This is reminiscent to the megacolon in Hirschsprung's disease, where parts of the bowl are not properly innervated by the enteric nervous system, resulting in intestinal obstruction due to paralysis of the peristaltic movement. Our interpretation is that TMEM16A also plays an important role in peristalsis. Indeed we and others found a robust expression of TMEM16A in interstitial cells of Cajal, which serve as pacemaker cells of the gastrointestinal tract. This finding makes it likely that TMEM16A is required either for the spontaneous activity or coordination of this cell type. Interestingly we observed less severe phenotype in older animals, indicating a change of the way intestinal smooth muscle cells are innervated. Interstitial cells of Cajal like cells do also exist in other organs like urethra and fallopian tube, and it is possible that the chloride channel has a similar role in other hollow organs too.

Inner and outer leaflet of the cellular plasma membrane have different lipid compositions. Especially the negatively charged phospholipid phosphatidylserine is normally located in the inner but not the outer leaflet. During activation of platelets phosphatidylserine is redistributed to the outer leaflet where it provides a binding side for interacting coagulation factors. In Scott syndrome this translocation is disturbed, resulting in impaired blood clotting. Recently a mutation in a splice acceptor site of the TMEM16F gene has been described in a single Scott-patient, suggesting that TMEM16F is involved in phospholipid transport. To analyze the role of TMEM16F in platelets we performed annexin V binding assays on thrombocytes isolated from wild type and TMEM16F knock out mice. Wild type platelets show a robust raise in signal value after activation – indicating an increase in the amount of surface phosphatidylserine, which was almost absent in TMEM16 deficient platelets (figure 1c). With this and other findings we showed that the TMEM16F mouse mimics the situation in Scott patients and confirms the role of TMEM16F for this disease. This mouse is the first animal model for this disorder and analysis of the phenotype, especially regarding other phosphatidylserine expressing cells, e.g. during apoptosis, is still ongoing. In the future we plan to perform electrophysiological measurements and chloride transport assays on platelets to investigate the role of a chloride conductance for this disease.

We hope that our research will not only help to understand the function of various TMEM16 genes, but also help to prevent diseases and conditions like inflammatory bowel disease or ischemia.

Selected Publication

Schroeder, BC, Cheng, T, Jan, YN, Jan, LY. (2008). Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell. 134, 1019-1029.

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Temperature Detection and Thermoregulation

Temperature affects all aspects of life, down to every enzymatic reaction. Thus, internal temperature homeostasis is of critical importance to our health as deviation from a normal, tightly controlled level (37 °Celsius) can cause fatal organ failures. Moreover, clinically controlled reduction of CBT under pathological conditions such as stroke, trauma, and certain surgical procedures has the potential to reduce tissue damage and to improve recovery. We are using multi-disciplinary approaches to analyze peripheral and central temperature sensors on the cellular and molecular level. In this context we are studying members of the TRP family of receptor ion channels that are not only exquisitely sensitive to temperature changes but also respond to inflammatory and painful stimuli. We also seek to identify and characterize neuroendocrine signals involved thermoregulation with the ultimate goal to pharmacologically influence CBT in medically relevant settings.

Introduction

Every organism employs a multitude of regulatory mechanisms to achieve and maintain homeostasis of bodily conditions such as energy expenditure and body temperature. Both, energy expenditure and internal temperature are intricately connected and regulated by neuroendocrine feedback loops that are centered in the hypothalamus. These neuronal networks rely on metabolic as well as temperature information that are provided by sensors residing both centrally and peripherally.

Temperature-sensitive cells in the hypothalamus and in peripheral organs detect temperature changes, which is directly relevant to CBT regulation (Fig. 1). However molecules and mechanisms underlying central temperature detection by hypothalamic neurons are unknown.

Transient Receptor Potential (TRP) ion channel receptors have been found to constitute important components of peripheral temperature sensory neurons. How these receptors mediate their temperature sensitivity on the molecular level is largely unknown.

The Thermosensory TRP Receptor Proteom

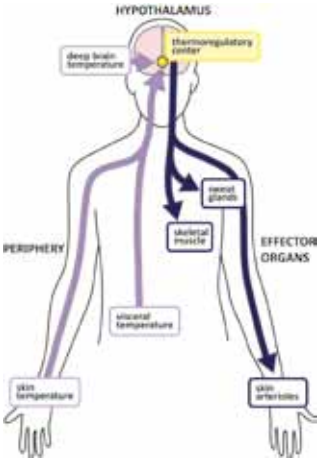
Christina Hanack, Henning Kuich, Sonja Winkler, Jana Rossius (in collaboration with Gunnar Dittmar and Rick Scavetta from the mass spectrometry core facility)

An emerging scheme for any membrane-bound receptor is that they don't function as single autonomous entities but rather in a complex with accessory proteins that shape and regulate receptor function. However, for TRP receptors such accessory subunits have largely remained elusive. We are using genetic-biochemical means to isolate and identify components of thermosensitive TRP channel protein complexes from *in vivo* peripheral somatosensory neurons. We will proceed by characterizing verified components for their role as TRP receptor modulators.

Central Temperature Detection

Dr. Mirko Moroni, Fabian Paul and Kun Song

Within the hypothalamus, the preoptic area (PO) and the anterior hypothalamus (AH) play an important role in detecting and regulating core body temperature. Local PO/AH warming and cooling experiments as well as studies introducing hypothalamic lesions and genetic



Cartoon depicting thermoregulatory pathways. Peripheral thermoreceptors detect environmental and visceral temperatures and report these to the hypothalamus. Hypothalamic temperature receptors detect internal temperature. The thermoregulatory center integrates temperature information and initiates heat-loss or heat gain responses in peripheral organs.

manipulations have shown that neurons in these regions detect changes in local brain temperature necessary for core temperature control. The mechanisms governing central temperature detection and regulation have remained a mystery. Two of the main reasons for this discrepancy are: 1.) Thermo-sensitive detector cells reside deep inside hypothalamic structures of the brain and are thus difficult to access; 2.) Detector cells are also scarce and intermingled with large numbers of neurons involved in other functions, which complicates their analysis. We are employing genetic labeling techniques and functional (calcium-) imaging methods to identify these neurons and electrophysiology & molecular biology to understand their mechanisms detecting temperature change.

Torpor, a model system to study Thermoregulation at the Intersection with Metabolism

Henning Kuich, Fabian Paul

Thermoregulation and metabolism are intricately intertwined, but how and at what level pathways intersect is all but clear. For example, obesity can lead to altered CBT and diabetic patients often have reduced core body temperature. In view of clinical demand for understanding and treating metabolic disorders, it is desirable to pursue novel routes and investigate whether intersection points with thermoregulatory pathways may provide new targets for intervention.

Homeothermic ("warm blooded") animals -including humans- display a decreased core body temperature under caloric restriction. Strikingly, under these conditions, mice can reduce CBT to a few degrees Celsius above ambient temperature (16°C-25°C), a dramatic deviation from their usual CBT, which under normal conditions is centered -similar to humans- around 37°C. This low temperature state is referred to as torpor (or "daily torpor") and exemplifies the intricate connection between metabolism and thermoregulation. The hypothalamus is the integration site for both homeostatic processes and thus plays an important role in orchestrating the torpor response. We are analyzing neuroendocrine mechanisms governing the torpor response with the ultimate goal to utilize this hypometabolic and hypothermic state in medically relevant settings.

Identification of molecules involved in developmental and functional aspects of somatosensory neurons with temperature- and mechanosensitive characteristics.

Dr. Katrin Schrenk-Siemens, Jana Rossius

The developmental program that allows somatosensory neurons to differentiate into a diversity of cell types with very specific traits is largely unknown.

We are employing human embryonic stem cell (hESC) systems to recapitulate developmental programs *in vitro* in order to differentiate hESCs into somatosensory-like neurons. First results show that we are able to obtain neurons with mechanosensitive-like properties. We are currently generating transgenic hESC lines with the goal a) to enhance differentiation efficiency into specific sensory subsets, and b) to visualize subpopulations of sensory neurons by the expression of fluorescent markers.

This line of experiments will allow the production and isolation of enriched/pure populations of sensory neurons, an invaluable tool for the functional characterization of sensory transduction processes that is currently impossible due to the heterogeneity of these cells in sensory ganglia *in vivo*.

Selected Publications

Siemens, J, Zhou, S, Piskorowski, R, Nikai, T, Lumpkin, EA, Basbaum, AI, King, D, Julius, D. (2006). Spider Toxins activate the Capsaicin Receptor to produce Inflammatory Pain. Nature. 444, 208-212.

Bautista, DM, Siemens J, Glazer JM, Tsuruda, PR, Basbaum, AI, Stucky, CL, Jordt, SE, Julius, D. (2007). The Menthol Receptor TRPM8 is the Principal Detector of Environmental Cold. Nature. 448, 204-208.

Trevisani, M, Siemens, J, Materazzi, S, Bautista, DM, Nassini, R, Campi, B, Imamachi, N, Andrè, E, Patacchini, R, Cottrell, GS, Gatti, R, Basbaum, AI, Bunnett, NW, Julius, D, Geppetti, P. (2007). 4-Hydroxynonenal, an Endogenous Aldehyde, causes Pain and Neurogenic Inflammation through Activation of the Irritant Receptor, TRPA1. Proc Natl Acad Sci. 104, 13519-24.

Bohlen, CJ, Priel, A, Zhou, S, King, D, Siemens, J, Julius D. (2010). A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. Cell. 141, 834-45.

Lechner, SG, Siemens, J. (2011). Sensory transduction, the gateway to perception: mechanisms and pathology. EMBO Rep. 12, 292-5.

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Administrative Assistant
Manuela Brandenburg

James Poulet

Neural Circuits and Behaviour

The goal of our lab is to understand principles by which cortical circuits process sensory information and guide adaptive motor behaviour. We focus on cortical regions associated with tactile sensing and movement of the mouse forelimb. Our lab combines electrophysiological and optical neuronal recording techniques with genetically targeted manipulations of neural activity in mice during trained behaviour to investigate the link between neural activity, sensory perception and motor behaviour. We are particularly interested in what role synchronisation of neuronal activity plays in cortical processing and behaviour.

Sensori-motor integration during mouse forelimb behaviour

Group members: Dr Birgit Voigt, Diana Hoffmann

We have developed behavioural methods to train mice to make targeted reaching movements with their forelimb. We record and manipulate cortical neural activity to investigate how cortex controls forelimb movements and processes sensory information during reaching movements.

Somatosensory perception

Group members: Dr Nevena Milenkovic, Leiron Ferrarese

We investigate sensory processing in forepaw primary somatosensory cortex (S1) using whole-cell recordings and stainings in awake mice. We deliver sensory stimuli to the paw and measure neuronal responses in S1 in mice trained to inform us when and what stimuli have been presented. We will go on to use optogenetic techniques to stimulate and inhibit identified populations of cortical neurons to help us unravel how S1 activity results in sensory perception.

The contribution of subtypes of cortical neurons to sensory processing

Group members: Dr. Jean-Sebastien Jouhanneau, Anja Dorrn, Wen-Jie Zhao

We use two-photon microscopy to target whole-cell recordings from cortical neuronal subtypes genetically labelled with GFP to investigate synaptic and network mechanisms of sensory processing. We can visualise neurons in vivo in neocortex and record their activity with micropipettes. For example, in collaboration with Prof. Alison Barth at Carnegie Mellon University USA, we are investigating neurons GFP labelled under the control of the immediate early gene c-fos promoter. The fos-GFP+ve neurons are a highly reactive sub-population of excitatory cortical neurons in primary somatosensory cortex that may drive sensory processing and network activity.

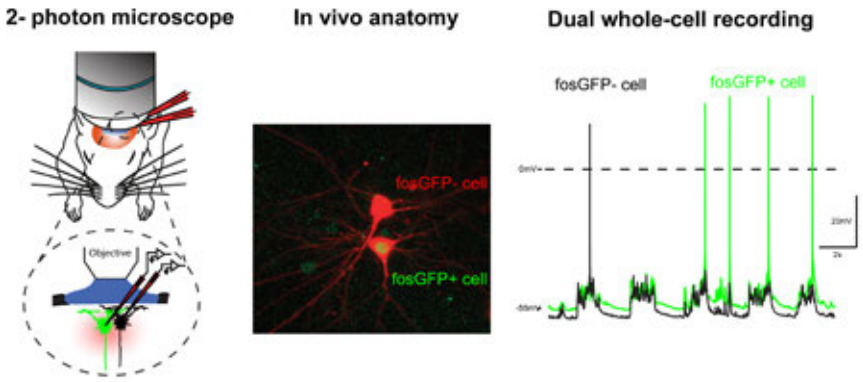

Figure 1. **Left:** In vivo two-photon microscopy setup. Dual whole cell recordings were targeted to nearby cortical neurons. **Middle:** Cells were filled with Alexa594. **Right:** Dual recordings reveal higher spiking rates in fosGFP+ve than fosGFP-ve neurons.

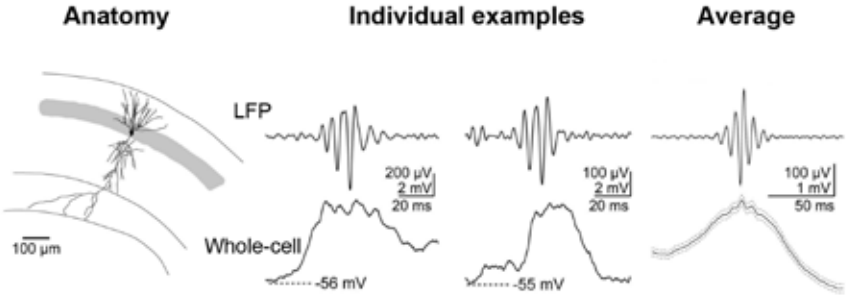

Figure 2. **Left:** Biocytin reconstruction of CA1 pyramidal neuron. **Middle:** (above) Example local field potential recording of hippocampal ripple oscillation and (below) whole-cell membrane potential recordings of stained neuron shows synaptic input during the ripple. **Right:** averaged LFP and membrane potential oscillations during the ripple.

Synaptic mechanisms underlying synchronous hippocampal sharp wave ripple oscillations

Group member: Anja Dorrn

We have made whole cell recordings from hippocampal pyramidal neurons in the awake resting mouse to investigate the network and synaptic mechanisms underlying sharp wave ripple oscillations in a collaborative project with Prof. Dietmar Schmitz at NeuroCure. Sharp wave ripples are fast oscillations (~150 Hz) in hippocampus that occur during sleep and quite wakefulness and have recently been shown to be involved in memory formation. In vitro and in vivo recordings have shown that sharp wave ripples receive phasic excitatory and inhibitory inputs during the ripple.

Selected Publications

Maier N*, Tejero-Cantero A*, Dorrn A, Winterer J., Beed P, Morris G, Kempter R, Poulet JFA*, Leibold C* and Schmitz D* (2011) Coherent phasic excitation during hippocampal ripples. Neuron. in Press *equal contribution

Crochet S, Poulet JFA, Kremer Y, Petersen CC (2011) Synaptic mechanisms underlying sparse coding of active touch. Neuron. 69: 1160-75.

Yassin L, Benedetti BL, Jouhanneau J-S, Wen JA, Poulet JFA, Barth AL, An embedded subnetwork of highly active neurons in the neocortex. (2010) Neuron 68: 1043-50.

Poulet JFA, Petersen CC (2008) Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. Nature. 454: 881-5.

Borgdorff AJ, Poulet JFA, Petersen CC. (2007) Facilitating sensory responses in developing mouse somatosensory barrel cortex. J Neurophysiol. 97: 2992-3003.

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Cellular Neurosciences

Our goal is to understand the role of glial cells in physiology and pathology. We focus on questions as to how neuronal activity is sensed by astrocytes, how astrocytes communicate among each other, and how they feedback on neurons. A second focus addresses the role of connexins, the gap junction proteins, for the formation of myelin and oligodendrocyte function. Thirdly, we study the expression of transmitter receptors in microglial cells and how activation of these receptors influences microglial functions. This is of particular interest within the context of pathology and we are currently studying this question in stroke and gliomas. A fourth line of research addresses the question as to how glioma cells interact with the intrinsic brain cells, specifically microglia and stem cells. We are aiming to understand this interaction on a molecular level, in particular with the hope of identifying therapeutical targets.

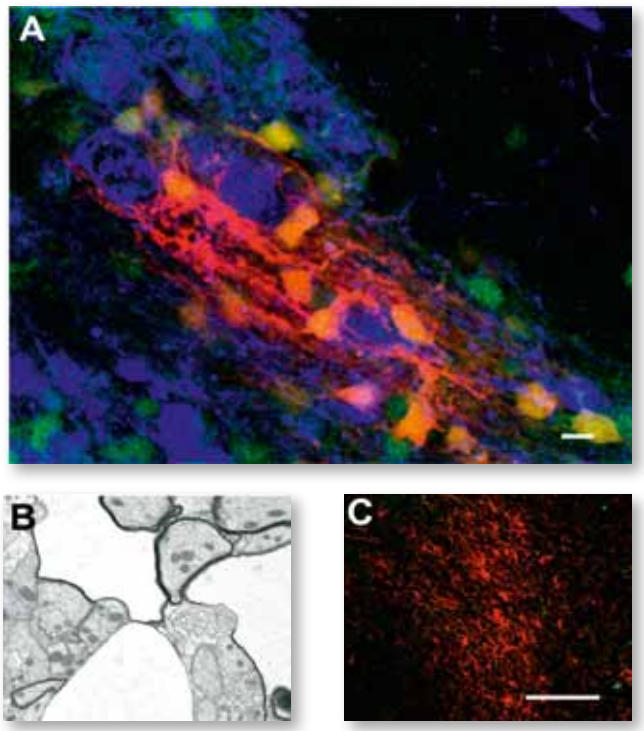
Introduction

The central nervous system contains two major cell populations, neurons and glial cells. The neurons are regarded as the elements mediating the electrical activity in the brain. As a consequence, neuroscience research of the past has focused on this cell type. The functional role of glial cells is not as obvious: while they were first described as cells providing only structural support to neurons, a series of more recent studies on glial cell function has attracted the attention of the neuroscience community. 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 a 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 immuno-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 role of astrocytes in information processing (2) the impact of connexin expression for oligodendrocytes function (3) the response of microglial cells to brain injury and (4) the interaction of gliomas with microglia and stem cells.

Mechanisms of neuron-astrocyte interactions

Vitali Matyash, Bruno Benedetti, Adriana Rocha

This project aims to understand signaling mechanisms between astrocytes and neurons. We recently have focused on the barrel cortex as a model to study neuron-glia interactions. The sensory input of the whiskers in



(A) Oligodendrocyte coupling in the cerebellar white matter of connexin(Cx)47 and Cx30 double deficient mice. Confocal image shows biocytin/streptavidin-Cy3 (red) labelled cells coupled within a network. The gap junction permeable tracer biocytin was injected into a single oligodendrocyte by whole-cell patch clamp. In these mice all coupled cells were positive for eGFP (green), which is selectively expressed by oligodendrocytes under the activity of the Cx47 promoter. No GFAP-positive astrocytes (blue) were detected. Scale bar: 10 µm. **(B)** White matter pathology in cerebellum of P80-P90 $Cx47^{-/-}$ $Cx30^{-/-}$ mice. Ultrathin section depicting severe vacuolation accompanied by myelin unfolding. In these mice degenerating axons were also observed. Magnification: 2500X. **(C)** PDGFRα expression (red) in cerebellar white matter of a $Cx47^{-/-}$ $Cx30^{-/-}$ mouse displaying ataxia symptoms (P47). In this mouse loss of eGFP-positive oligodendrocytes (green) in cerebellar white matter is accompanied by an increase in cells expressing PDGFRα, a specific marker for early oligodendrocyte precursors (in collaboration with Klaus Willecke´s group in Bonn). Scale bar: 100 µm.

rodents is represented in the somatosensory cortex. Each whisker projects into a defined cortical area, the barrel field. These areas are morphologically delineated and can be recognized in acute brain slices without additional staining. The barrel cortex is a well established model for plasticity since removal of whiskers results in changes of the barrel fields. After stimulation in the cortical layer 4, the input to the barrel field, we can record responses in astrocytes and in neurons by using Ca^{2+} imaging and patch-clamp recording. While the neuronal activity spreads beyond barrel borders, the astrocyte activity is restricted to the barrel field. We interfered with intracellular calcium signalling in astrocytes by dialysis with the calcium chelator BAPTA. Such treatment increased excitability of the nearby neurons. The effect of astrocytic calcium chelation was mimicked by pharmacological inhibition of GABA receptors, suggesting that such type of control is GABA-mediated through a combined involvement of $GABA_A$ and $GABA_B$ receptors. This finding demonstrates a role of astrocytes in the regulation of neuronal inhibition in somatosensory (barrel) cortex and adds a new variant to the growing number of pathways with which astrocytes can modulate neuronal networks.

How does connexin expression affect oligodendrocyte function?

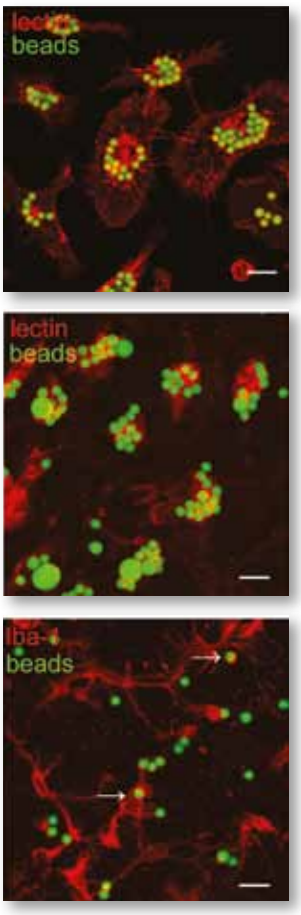
Marta Maglione, Nadine Richter (in collaboration with Klaus Willecke, University of Bonn)

In a collaboration with Prof. Klaus Willecke's group in Bonn we are studying the expression of connexins, the molecular substrate for gap junctions in oligodendrocytes. Gap junctions are communication channels which allow the exchange of molecules between cells. They can also connect different compartments within a given cell. By using different combinations of mouse lines with connexin deletions, we determine which of the connexins are essential to form gap junctions among oligodendrocytes and among astrocytes and oligodendrocytes. This also has an important clinical impact. Mutations in connexins expressed by oligodendrocytes can lead to defects in myelin formation, an important function of oligodendrocytes. We also have studied a mouse mutant which mimics a connexin mutation which in humans leads to leukodystrophy. We are, therefore, interested in the question how coupling determines myelin formation and maintenance (Funded by the Deutsche Forschungsgemeinschaft).

What are the physiological features of microglial cells in brain tissue?

Christiane Nolte, Stefanie Seifert, Grietje Krabbe, Maria Pannell, Julia Parnis, Larisa Bulavina, Susanne Wolf

Microglial cells are the pathologic sensors and represent the immune cells of the central nervous system. 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 and growth factors. They also express a variety of receptors for chemokines and cytokines as expected from a macrophage-like cell. We



Microglial phagocytic activity in vitro and in situ can be analyzed by uptake of microspheres.
Representative confocal pictures of the uptake of microspheres (green) by microglial cells (labeled red) in neonatal cultured microglia (upper), amoeboid microglia *in situ* (middle) and ramified adult microglia in mouse coronal brain slices (lower). Microglial cells in **(A)** and **(B)** were stained with tomatolectin whereas microglia in **(C)** were visualized by Iba-1 immunohistochemistry. Note the high uptake activity of younger microglia including neonatal and amoeboid microglia compared to adult microglia. Scale bar: 10 µm.

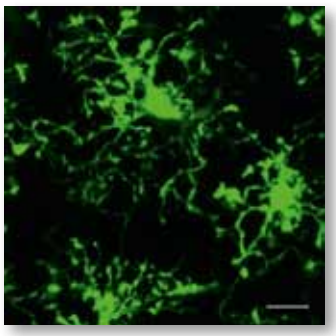
have addressed the question whether microglia would also express receptors to sense neuronal activity. We have recently identified receptors for the neurotransmitters/neurohormones GABA, adrenaline, dopamine, bradykinin, serotonin, endothelin-1, substance P and histamine. We found that activation of these receptors can modulate microglial functions such as migration or cytokine release. To extent our studies from cell cultures to more intact systems, we developed an approach to obtain Ca^{2+} recordings from microglia *in situ*. We injected a retrovirus encoding a calcium sensor into the cortex of mice two days after stimulation of microglial proliferation by a stab wound injury. We recorded transient Ca^{2+} responses to application of ATP, endothelin-1, substance P, histamine and serotonin. The fluorescence amplitude of ATP was increased only at day 6 compared to other time points, while responses to all other ligands did not vary. Only half of the microglial cells that responded to ATP also responded to endothelin-1, serotonin and histamine. Substance P, in contrast, showed a complete overlap with the ATP responding microglial population at day 6, at day 42 this population was reduced to 55%. Cultured cells were less responsive to these ligands. This study shows that *in situ* microglia consists of heterogeneous populations with respect to their sensitivity to neuropeptides and – transmitters.

Recently we studied the impact of serotonin receptor activation on distinct microglial properties. First, we tested the impact of serotonin on the microglial response to an insult caused by a laser lesion in acute slices. In the presence of serotonin the microglial processes moved more rapidly towards the laser lesion which is considered to be a chemotactic response to ATP. Similarly the chemotactic response of cultured microglia to ATP was also enhanced by serotonin in a Boyden chamber assay. Quantification of phagocytic activity by determining the uptake of microspheres showed that the amoeboid microglia in slices from early postnatal animals or microglia in culture respond to serotonin application with a decreased phagocytic activity whereas we could not detect any significant change in ramified microglia *in situ*. The presence of microglial serotonin receptors was confirmed by patch-clamp experiments in culture and amoeboid microglia and by qPCR analysis of RNA isolated from primary cultured and acutely isolated adult microglia. These data suggest that microglia express functional serotonin receptors linked to distinct microglial properties. (funded by Deutsche Forschungsgemeinschaft).

Do microglial cells influence glioma cells?

Rainer Glass, Jitender Kumar, Katyayni Vinnakota, MinChi Ku, Feng Hu, Susanne Wolf, Petya Georgieva

Gliomas comprise the majority of cerebral tumors and patients have a poor prognosis since there is essentially no concept for successful treatment. Gliomas include astrocytomas, oligodendrogliomas, and the most malignant (and untreatable) brain tumor, the glioblastoma multiforme. 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. A factor released by the glioma cells activates toll-like receptors in the surrounding microglial cells and triggers the expression of the MT1-MMP. Thus, glioma cells exploit microglial cells to promote their invasion. Glioma cells obviously need microglia since they can not produce MT1-MMP themselves: A forced expression MT1-MMP in glioma cells leads to their death. Thus interfering with TLR receptors or their intracellular pathways might reduce the rapid expansion of glioma cells and microglia have become a new target for glioma research.



Microglial cells in the acute brain slices from Cx3cr1$^{+/GFP}$ mouse. Image shows the maximum intensity projection of the 60µm-thick volume imaged with 2-photon microscope, scale bar 20 µm.

We found that the clinically approved antibiotic minocycline blocked the increase in MT1-MMP expression and activity in cultivated microglia stimulated with glioma conditioned medium. Glioma growth within an organotypic brain slice preparation was reduced by minocycline and this reduction depended on the presence of microglia. Glioma growth in an experimental mouse model was strongly reduced by the addition of minocycline to drinking water, compared to untreated controls. Coherently, we observed in our orthotopic glioma implantation model, that MT1-MMP was abundantly expressed in glioma associated microglia in controls, but was strongly attenuated in tumors of minocycline treated animals. Overall, our study indicates that the clinically approved antibiotic minocycline is a promising new candidate for adjuvant therapy against malignant gliomas.

Do stem cells influence glioma cells?

Rainer Glass, Kristin Stock, Anika Langenfurth

We have previously observed that gliomas attract neural precursor cells from the subventricular zone. These cells migrate over large distances and enwrap the tumor yet they do not originate from the tumor proper as was previously suspected. This intrinsic anti-tumorigenic response is strongly related to age in an animal model and occurs only during youth when neural precursor cells are more active. Consequently, in older animals this interaction does not occur. The precursor cells inhibit tumor growth and addition of exogenous precursors prolongs the survival rate in older animals.

In the search for a mechanism by which endogenous neural precursor cells exert their anti-tumorigenic effect, we studied bone morphogenic proteins (BMP). We found that endogenous neural precursor cells perform an anti-tumor response by specifically targeting glioma cells with stem-like properties. These recently identified subpopulation of glioma cells control tumor growth and recurrence. In vitro, neural precursor cells predominantly express BMP7; BMP7 is constitutively released from neurospheres and induces canonical BMP signaling in glioma stem cells. Exposure of human and murine glioma stem cells to neurosphere-derived BMP7 induces glioma stem cell differentiation and reduced the ability for self-renewal and the ability for tumor initiation. Neural precursor cell-derived BMP or recombinant BMP7 reduces glioma expansion from glioma stem cells by down-regulating the transcription factor Olig2. In vivo, large numbers of BMP7-expressing neural precursor cells encircle glioma in young mice and induce canonical BMP signaling in glioma stem cells. This anti-tumor response is strongly reduced in older mice. Our results indicate that neural precursor cells protect the young brain from glioma by releasing BMP7, which acts as a paracrine tumor suppressor that represses proliferation, self-renewal and tumor-initiation of glioma stem cells.

Selected Publications

Markovic, DS, Vinnakota, K, Chirasani, SR, Synowitz, M, Raguet, H, Stock, K, Sliwa, M, Lehmann, S, Kälin, R, van Rooijen, N, Holmbeck, K, Heppner, FL, Kiwit, J, Matyash, V, Lehnardt, S, Kaminska, B, Glass, R, and Kettenmann, H. (2009) Glioma induce and exploit microglial MT1-MMP expression for tumor expansion, PNAS 106,12530-12535.

Chirasani, SR, Sternjak, A, Wend, P, Momma, S, Campos, B, Herrmann, IM, Graf, D, Mitsiadis, T, Herold-Mende, C, Besser, D, Synowitz, M, Kettenmann H, Glass R. (2010) Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 133,1961-1972.

Maglione, M, Tress, O, Haas, B, Karram, K, Trotter, J, Willecke, K, Kettenmann H. (2010) Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58, 1104-1017.

Benedetti, B, Matyash, V, Kettenmann, H. (2011) Astrocytes control GABAergic inhibition of neurons in the mouse barrel cortex. J. Physiol. 58, 1159-1172.

Seifert, S, Pannell, M, Uckert, W, Färber, K, Kettenmann H. (2011) Transmitter- and hormone-activated Ca^{2+} responses in adult microglia in situ recorded after viral transduction of a recominant Ca^{2+} sensor. Cell Calcium 49, 365-375.

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Proteomics and Molecular Mechanisms of Neurodegenerative Disorders

The main objective of our work is to understand the functional organization of biological systems and the assignment of single proteins to functional complexes in the context of signaling cascades and disease processes. In particular, we aim to elucidate the molecular mechanisms of protein misfolding and diseases related to it.

More than 35 systemic and neurological diseases are caused by the formation of abnormally folded protein species, among them the late-onset neurodegenerative disorders (NDs) Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). These NDs have several common pathological mechanisms on the symptomatic as well as the molecular level. Therapies available for these illnesses do not address disease-causing molecular mechanisms and have limited effectiveness.

Deposits of misfolded protein species in tissues and disease phenotypes in the non-familial, common forms of AD and PD are observed later in life. The cellular environment in young, healthy cells can efficiently prevent the conversion of mutant, aggregation-prone proteins into toxic forms. Mounting evidence indicates that protein homeostasis, regulated by a highly complex network of molecular interactions, balances biosynthesis, folding, translocation and clearance of cellular proteins. It seems likely that this system also influences misfolding, aggregation and toxicity of neurodegenerative disease proteins (NDPs), in a manner dependent on proteins involved in chaperone pathways, the ubiquitin proteasome system (UPS) and autophagy. However, the precise processes disrupted during neurodegeneration and their connection to disease-associated phenotypes remains unclear. It is also unclear why certain types of cells accumulate misfolded proteins while others do not.

Chemical compounds and proteins that are able to modulate protein misfolding pathways are valuable starting points for therapy development and highly important tools for analysing the complex protein assembly process. For this reason we are interested in the identification of molecules that directly influence the amyloid formation cascade. In a recent investigation we demonstrated that the orcein-related small molecule O4 decreases the concentration of small, toxic Aβ oligomers in aggregation reactions. In another study, we have identified the neuron-specific protein CRMP1 that dramatically influences polyglutamine (polyQ)-mediated huntingtin aggregation in cell-free and *in vivo* disease model systems. We also recently found that the protein MED15 enhances spontaneous polyQ-mediated ataxin-1 aggregation in cell-free assays, and have evidence suggesting that this may be an effect mediated by its coiled-coil domain. We use high throughput interaction screening technologies such as an automated yeast-two-hybrid (Y2H) system to screen for interaction partners of NDPs, generating a first comprehensive PPI network for proteins involved in different ND processes. Finally, we investigated the intracellular signal transduction creating a signaling-related protein-protein interaction (PPI) map, for a better understanding of causal connections between signaling proteins and for the identification of proteins that modulate the flow of information in such networks.

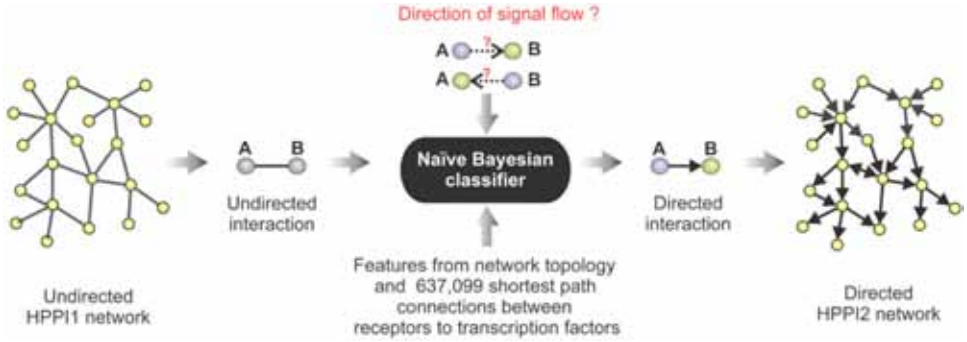

Figure 1. Predicting the potential directions of signal flow in PPI networks.
Inferring edge directions from PPI data. For each interaction in the undirected PPI network (HPPI1), a naïve Bayesian classifier was used to predict the edge direction from topological network properties as well as shortest PPI paths connecting membrane receptors and transcription factors. An activated signaling network (HPPI2) was assembled from all interactions that had a direction assigned.

Identification and characterization of small molecules that modulate protein misfolding pathways

In our studies we aim to find and characterize new mechanisms for modulating protein misfolding pathways that might lead to the discovery of new classes of drugs against protein misfolding diseases such as AD and PD.

In previous studies, we have identified the natural compound (-)-epigallocatechin gallate (EGCG) as a modulator of polyQ-mediated huntingtin aggregation (Ehrnhoefer & Bischke et al., 2008) as well as α-synuclein and amyloid-β amyloid formation (Ehrnhoefer & Bieschke 2008). We found that the compound EGCG is able to redirect the amyloid fibril formation pathway by binding to α-synuclein and amyloid-β monomers and by stimulating the assembly of off-pathway, highly stable oligomers which are non-toxic for mammalian cells. Also, the compound has the ability to convert preformed, mature α-synuclein and amyloid-β fibrils into smaller, amorphous protein aggregates that are non-toxic for mammalian cells (Bieschke et al., 2010). These findings suggest that EGCG is a potent remodelling agent of mature amyloid fibrils and support our hypothesis that the compound has chemical chaperone function.

Several lines of experimental evidence indicate that soluble, pre-fibrillar assemblies of the amyloid-β polypeptide rather than mature, end-stage amyloid fibrils cause neuronal dysfunction and memory impairment in AD. This suggests a new mechanism for detoxifying amyloid-β aggregates: An acceleration of fibrillogenesis might reduce the levels of toxic aggregation intermediates. To address this question, we searched for chemical compounds that promote spontaneous amyloid-β formation. Using a filter retardation assay, the natural dye orcein and related substances were identified. They directly bind to small transient amyloid oligomers and stimulate their assembly into mature amyloid fibrils.

Very recently, we could demonstrate that the acceleration of amyloid-β fibrillogenesis effected by the orcein-related small molecule O4 decreased the concentration of small, toxic Aβ oligomers in aggregation reactions (Bieschke & Herbst et al., 2011). In addition, O4 treatment suppressed inhibition of long-term potentiation by Aβ oligomers in hippocampal brain slices. The molecule directly binds to hydrophobic amino acid residues in Aβ peptides and stabilizes the self-assembly of seeding-competent, β-sheet-rich protofibrils and fibrils. These results support the assumption that small, diffusible pre-fibrillar amyloid species rather than mature fibrillar aggregates are toxic for mammalian cells. They also suggest that conversion of small aggregation intermediates into large amyloid structures might be a viable therapeutic approach to treat protein misfolding diseases.

Identification of proteins that modulate polyQ-mediated huntingtin aggregation

HD is an inherited neurodegenerative disorder that is caused by an expansion of a polyQ tract in the protein huntingtin, which leads to a characteristic accumulation of insoluble Htt aggregates in affected neurons and eventually to cellular dysfunction and toxicity. However, the molecular pathways underlying brain-specific, polyQ-induced neurodegeneration in HD are still unknown. Recently, a large number of interaction partners were identified that associate with the N-terminal domain of huntingtin, which harbours the aggregation-prone polyQ tract. We hypothesized that perturbation of functional huntingtin protein complexes in neurons

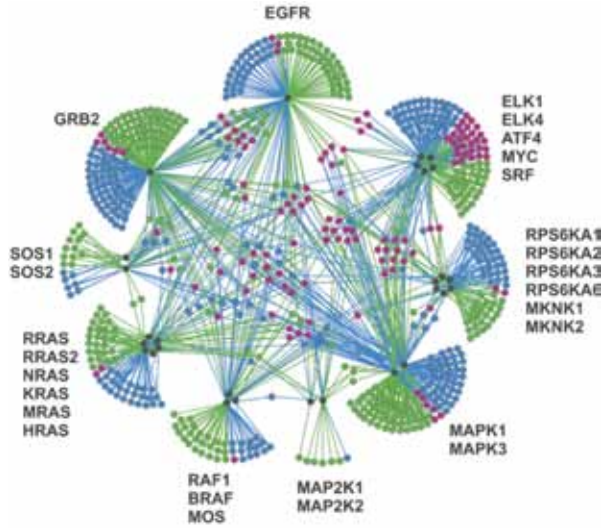

Figure 2. Integration of directed PPIs with dynamic protein phosphorylation data.
A network view of core pathway EGF/ERK proteins with 733 direct-interacting partners. Dark gray nodes represent the 28 known EGF/ERK core pathway proteins (labeled with their Entrez Official Gene Symbols) from EGFR downstream in a counterclockwise arrangement. Blue and green nodes correspond to potential input and output nodes, respectively, that are linked to the core pathway. Proteins that are both input and output nodes are shown in purple.

induces protein misfolding and neurotoxicity. To identify tissue-specific, dysregulated huntingtin protein interactions, a bioinformatic approach was developed. By filtering publically available protein-protein interaction (PPI) data with information from gene expression studies of brain and non-brain tissues, a brain-specific huntingtin PPI network was created, linking 14 potentially dysregulated proteins directly or indirectly to the disease protein. Analysis of published data confirmed the predictive value of this network modelling strategy. Systematic investigations with *in vitro* and *Drosophila* model systems of HD demonstrated that the potentially dysregulated huntingtin interaction partners influence polyQ-mediated protein misfolding and neurodegeneration. The neuron-specific protein CRMP1 e.g. is recruited to inclusion bodies with aggregated huntingtin protein in brains of HD transgenic mice and efficiently inhibits polyQ-mediated huntingtin exon 1 aggregation in cell free assays. Our results offer a new strategy for identifying perturbed, tissue-specific human PPIs and modulators of protein misfolding and aggregation.

Systematic interaction mapping links proteins to neurodegenerative diseases

Neurodegenerative diseases (NDs) have many pathological mechanisms in common, e.g. mitochondrial dysfunction, oxidative stress, neuronal apoptosis, dysfunction of protein homeostasis and accumulation of misfolded protein aggregates.

Previous investigations of PPIs that focused on NDs have provided only single-disease networks with no interconnection. In this study we present the first comprehensive PPI network which links a large number of novel proteins to ND processes. This network was generated by systematic interaction screening using an automated yeast-2-hybrid (Y2H) technology. Utilizing 414 bait proteins involved in neurodegenerative diseases such as AD, PD, HD or ALS we identified ~20,000 PPIs, ~5,000 of which were defined as high quality interactions, applying a bioinformatic confidence scoring system. In addition, ~1,000 Y2H PPIs were validated in an automated cell-based LUMIER co-immunoprecipitation assay. We plan to validate more interactions using the LUMIER and MAPPIT assays, as well as in co-immunoprecipitation from mouse brains.

Finally, in an RNAi screen of 1,143 ND-associated proteins we found that 210 could alter mutant huntingtin aggregation. This information was integrated with the Y2H protein interaction data, and 34 protein clusters containing or closely connected to huntingtin were identified in the resultant network. We selected 12 of these clusters for further study and plan to use overexpression assays to find and validate additional modifiers.

Identification of human proteins that modulate misfolding and proteotoxicity of pathogenic ataxin-1

Proteins with long, pathogenic polyglutamine (polyQ) sequences have an enhanced propensity to misfold and assemble into insoluble protein aggregates. Although modulators of polyQ-mediated protein toxicity and aggregation have been previously identified in lower model organisms, it remains unclear whether their human homologues are relevant for pathogenesis of polyQ diseases. To identify the mechanism of action of polyQ modulators, we computationally predicted 200 human genes that were expected to modulate misfolding and proteotoxicity of polyQ ataxin-1, a protein responsible for the neurodegenerative disease spinocerebellar ataxia type-1 (SCA1). We systematically screened these modulators in cell-based overexpression and RNAi assays and identified 21 human proteins that influence polyQ-induced ataxin-1 misfolding and proteotoxicity. Using Y2H and LUMIER interaction assays, we found that 10 modifier proteins interact with ataxin-1. Analyzing the protein sequences of human modulators, we discovered a recurrent presence of coiled-coil domains in ataxin-1 toxicity enhancers, while such domains were not present in suppressors. Since coiled-coil domains

can mediate protein interactions and multimerization, we studied their effects on polyQ ataxin-1 aggregation in vitro, focusing on the glutamine-rich protein MED15. We found that MED15, for which coiled-coil domains were computationally predicted, enhances spontaneous polyQ-mediated ataxin-1 aggregation in cell-free assays, while no such effect was observed with the glutamine-rich protein Pum1, which lacks coiled-coil regions. These results support recent investigations indicating that coiled-coil domains promote spontaneous polyQ-mediated protein aggregation and suggesting that such domains in interacting proteins might stimulate abnormal misfolding and proteotoxicity of polyQ disease proteins.

A directed protein interaction network for investigating intracellular signal transduction

Cellular signal transduction is a complex process involving protein-protein interactions (PPIs) that transmit information. For instance, signals from the plasma membrane are transduced to transcription factors through series of PPIs to regulate gene expression.

To obtain a global view of cellular signaling and to predict potential signal modulators, we searched for protein interaction partners of more than 450 signaling-related proteins by means of automated yeast two-hybrid interaction mating. The resulting PPI network connected 1126 proteins through 2626 PPIs. After expansion of this interaction map with publicly available PPI data, we generated a directed network resembling the signal transduction flow between proteins with a naïve Bayesian classifier (Fig. 1). We exploited information on the shortest PPI paths from membrane receptors to transcription factors to predict input and output relationships between interacting proteins. Integration of directed PPIs with time-resolved protein phosphorylation data revealed network structures that dynamically conveyed information from the activated epidermal growth factor and extracellular signal-regulated kinase (EGF/ERK) signaling cascade to directly associated proteins and more distant proteins in the network (Fig. 2). From the model network, we predicted 18 previously unknown modulators of EGF/ERK signaling, which we validated in mammalian cell-based assays. This generic experimental and computational approach provides a framework for elucidating causal connections between signaling proteins and facilitates the identification of proteins that modulate the flow of information in signaling networks (Vinayagam et al., 2011).

Selected Publications

Bieschke, J, Herbst, M, Wiglenda, T, Friedrich, RP, Boeddrich, A, Schiele, F, Kleckers, D, Lopez del Amo, JM, Grüning, B, Wang, Q, Schmidt, MR, Lurz, R, Anwyl, R, Schnoegl, S, Fändrich, M, Frank, RF, Reif, B, Günther, S, Walsh, DM and Wanker, EE. (2011) Small molecule conversion of toxic oligomers to non-toxic β-sheet-rich amyloid fibrils. Nat Chem Biol. 2011 Nov 20. Epub ahead of print.

Bieschke, J, Russ, J, Friedrich, RP, Ehrnhoefer, DE, Wobst, H, Neugebauer, K and Wanker, EE. (2010) EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. PNAS USA. 107(17):7710-5.

Ehrnhoefer, DE, Bieschke, J, Boeddrich, A, Herbst, M, Masino, L, Lurz, R, Engemann, S, Pastore, A and Wanker, EE. (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol. 15(6):558-66.

Goehler, H, Dröge, A, Lurz, R, Schnoegl, S, Chernoff, YO and Wanker, EE. (2010) Pathogenic polyglutamine tracts are potent inducers of spontaneous Sup35 and Rnq1 amyloidogenesis. PLoS One. 5(3):e9642.

Vinayagam, A, Stelzl, U, Foulle, R, Plassmann, S, Zenkner, M, Timm, J, Assmus, HE, Andrade-Navarro, MA and Wanker, EE. (2011) A Directed Protein Interaction Network for Investigating Intracellular Signal Transduction. Science Signaling 4 (189: rs8).

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Age-related Protein Misfolding and Detoxification Mechanisms

The misfolding of endogenous protein or peptide fragments to form cytotoxic deposits made from fibrillar protein aggregates characterizes amyloid diseases. The misfolded polypeptides, are specific for each disease, such as amyloid β (Aβ) in Alzheimer's disease (AD), α-synuclein (αS) in Parkinson's disease (PD) or fragments of the huntingtin protein in Huntigton's disease (HD). The specific nature and mechanism of the cytotoxicity are yet unknown. However, a wealth of evidence points to smaller oligomeric aggregates rather than large fibrils being the crucial species for cellular toxicity.

Promoting the formation of large aggregates may therefore be a detoxifying mechanism (Cohen & Bieschke 2006) as an acceleration of fibrillogenesis could reduce the levels of toxic aggregation intermediates. The mechanistic details of protein misfolding, the autocatalytic replication of misfolded protein aggregates, and possible detoxifying mechanisms are the major points of our research.

Endogenous Modifiers of Amyloid Formation

We aim to identify key components of the cellular detoxification machinery and study the influence of endogenous proteins on aggregate assembly and disassembly using several screening based methods:

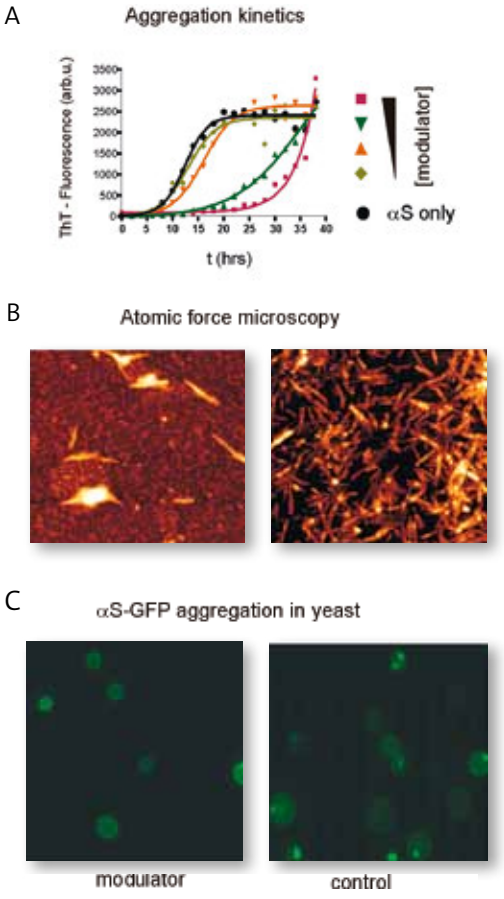

Figure. Effect of modulator protein on αS aggregation **(A)** Thioflavin T fluorescence kinetic assay, **(B)** atomic force microscopy, **(C)**, αS-GFP aggregate formation in yeast.

Cellular aggregation assays and siRNA interference

Katja Welsch

Aggregation-prone fragments of Huntingtin that posess an extended polyglutamine sequence are expressed in mammalian cells (PC12-Htt-ex1-Q103). Target genes

from a library of proteins that are related to protein folding or to the aging pathways are simultaneously downregulated by siRNA during aggregation or after the completion of aggregation to assess their influence on amyloid formation and removal by the cells. We have identified several new modulators of Huntingtin toxicity and validated their mechanistic role by selective cellular downregulation and overexpression.

Genome-wide functional screening for amyloid modulators

Ralf Friedrich, Maliha Shah

A genome-wide library of proteins is recombinantly expressed from E. coli (ca. 14.000 constructs) and added to monomeric or fibrillar forms of the amyloidogenic proteins *in vitro*. Kinetic assays are used to identify proteins that influence Aβ peptide or α-synuclein protein aggregation. We have identified several modulator proteins that efficiently reduce αS aggregate formation in vitro as well as in yeast models (Fig.). Their mechanism of action is currently being studied by biophysical methods and NMR spectroscopy (collaboration with Philipp Selenko, FMP Berlin).

Amyloid Aggregate-Toxicity Relationship in Aβ and tau-Protein

Heike Wobst, Sha Jin

Using model substances, such as EGCG as well as specific oligomer formation protocols, we create fibrillar and oligomeric species of Aβ peptides and tau-protein fragments that are either on-pathway or off-pathway to amyloid formation. We aim to understand the relationship between aggregation state and cytotoxicity of these species and test their capacity to inducue homologous and heterologous amyloid formation.

To that end we are, on one hand, focussing on detailed mechanistic studies using biophysical and biochemical assays, single molecule fluorescence techniques and atomic force microscopy. On the other hand we are correlating these results with aggregate uptake and toxicity assays in mammalian cell culture, to which aggregate sub-populations are added.

Mechanisms of Anti-Amyloid Drug Action

J. Bieschke, G. Grelle, S. Kostka; collaboration with E. Wanker

The collaborative project of the Bieschke / Wanker group aims to find and mechanistically understand new approaches to interfere with the formation of toxic protein aggregates in diseases like Alzheimer's and Parkinson's disease. We have scrutinized several natural compounds for their therapeutic potential.

The flavonoid (-)-epigallocatechin-gallate (EGCG) from green tea and related substances were found to be potent inhibitors of amyloid formation for a variety of polypeptides such as Aβ, α-synuclein and huntingtin. We had previously found that EGCG prevents amyloid formation by a unique mechanism. It stimulates the production of off-pathway oligomers early in the aggregation process and thus diverts the misfolding process away form the toxic species (Ehrnhoefer & Bieschke 2008). We now could demonstrate that EGCG also dissolves preformed amyloid fibrils by binding to the fibrillar form of the protein and remodelling the protein conformation *in situ* (Bieschke 2010).

Soluble oligomers of Aβ rather than mature, end-stage amyloid fibrils are believed to be central to neuronal dysfunction and memory impairment in Alzheimer's disease. Promoting the formation of large aggregates may therefore be a detoxifying mechanism (Cohen & Bieschke 2006) as an acceleration of fibrillogenesis could reduce the levels of toxic aggregation intermediates. By using small molecules derived from Orcein, a natural compound from lichen, we have now provided proof of concept for such a therapeutic strategy (Bieschke & Herbst 2011).

Selected Publications

Bieschke J & Herbst M, et al. Small molecule conversion of toxic oligomers to non-toxic beta-sheet-rich amyloid fibrils (2011) Nature Chem Biol (in press)

Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K, Wanker EE, EGCG remodels preformed ß-sheet rich amyloid fibrils and reduces cellular toxicity, PNAS 107(17):7710-5

Ehrnhoefer DE & Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE. (2008). EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nature Struct Mol Biol. 15, 558-66.

Cohen E & Bieschke J, Perciavalle RM, Kelly JW, Dillin A. (2006). Opposing activities protect against age-onset proteotoxicity. Science 313, 1604-10.

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*part of the period reported

Martin Falcke

Mathematical Cell Physiology

The group „Mathematical Cell Physiology“ develops mathematical models of cellular processes. Current projects comprise second messenger signaling systems (cAMP, Ca^{2+}), membrane potential dynamics, ion transport in the salivary gland, cardiac myocyte cell models, actin dynamics and cell motility. All studies are close collaborations with experimental groups.

In many cell types, the inositol trisphosphate receptor IP_3R is one of the most important components controlling intracellular calcium dynamics, and an understanding of this receptor is necessary for an understanding of the control of gene expression, secretion, muscle contraction and many other processes controlled by calcium. IP_3R form channel clusters on the membrane of the endoplasmic reticulum. In a collaboration with the laboratories of Ian Parker's in Irvine, California, and C.W. Taylor's in Cambridge, UK, we used total internal reflection fluorescence (TIRF) microscopy of two mammalian cell lines to define the temporal relationships between Ca^{2+} puffs on cluster level (inter-puff intervals, IPI) and cellular Ca^{2+} spikes (inter-spike intervals, ISI) evoked by flash photolysis of caged IP_3. We found that IPI are much shorter than ISI, that puff activity is stochastic with a recovery time that is much shorter than the refractory period of the cell, and that IPI are not periodic. We conclude that cellular Ca^{2+} spikes do not arise from oscillatory dynamics of IP_3R clusters, but that repetitive Ca^{2+} spiking with its longer time scales is an emergent property of the dynamics of the whole cluster array.

Intracellular Ca^{2+} dynamics are a stochastic system, but a complete stochastic theory had not been developed yet. We formulated the theory in terms of interpuff interval and puff duration distributions because, unlike the properties of individual channels, we can measure them in vivo. Our theory reproduces the typical spectrum of Ca^{2+} signals like puffs, spiking, and bursting. We find conditions for spiking and calculate ISI distributions. Signal form, average ISI and ISI distributions depend sensitively on the details of cluster properties and their spatial arrangement. In contrast to that, the relation between the average and the standard deviation of ISIs does not depend on cluster properties and cluster arrangement and is robust with respect to cell variability. It is controlled by the global feedback processes in the Ca^{2+} signaling pathway (e.g., via IP3-3-kinase or endoplasmic reticulum depletion). That relation is essential for pathway function because it ensures frequency encoding despite the randomness of ISIs and determines the maximal spike train information content. Hence, we find a division of tasks between global feedbacks and local cluster properties that guarantees robustness of function while maintaining sensitivity of control of the average ISI. We also developed a multiscale cell simulation tool, which confirmed the results on ISI distributions, the role of global feedbacks and robustness properties by an independent method.

We studied also the early stage of Ca^{2+} -induced Ca^{2+} release (CICR) in the diadic cleft of cardiac ventricular myocytes. A crucial question for the understanding of excitation contraction coupling is whether the activation of the ryanodine receptors (RyRs) on the sarcoplasmic reticulum is triggered by one or by multiple open

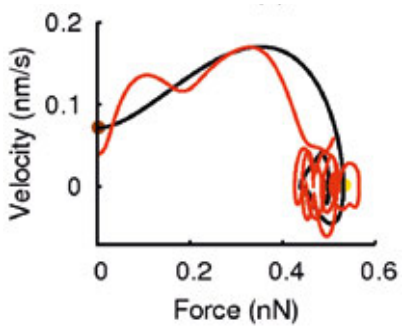

Figure 1. Force velocity relation of a fish keratocyte pushing against a scanning force microscope cantilever. The red line shows experimental results, the black line simulation results.

L-type Ca^{2+} channels (LCCs). We addressed the problem through a modelling approach that allowed for investigation of both possibilities. The model is based on a spatially resolved description of a Ca^{2+} release unit, consisting of the junctional sarcoplasmic reticulum and the diadic cleft. Our study shows that the mechanisms of the early stage of CICR shape measurable properties of CICR in a characteristic way. From here we conclude that the activation of RyRs requires multiple open LCCs.

Our modeling of actin based cell motility aims at suggesting mechanisms which explain the velocity dynamics observed with bacterial propulsion, in reconstituted systems like ActA coated beads and oil droplets, the morphodynamic phenotypes of protrusions observed at the leading edge of a variety of motile cells and the force velocity relation of motile cells. The similarity of the molecular components involved in all of these processes suggests it to be possible to find such a unifying mechanism. Our approach is based on the description of the length and attachment dynamics of actin filaments in the force generating network. We completed the modeling of reconstituted systems with the steady and oscillatory motion of ActA coated beads and published the results. The model reproduces the marked state switches in protrusion morphodynamics found experimentally between epithelial cells in control conditions and cells expressing constitutively active Rac. The model also suggests a mechanistic explanation of experimental distortions in protrusion morphodynamics induced by deregulation of Arp2/3 and cofilin activity.

Cells migrate through a crowded environment during processes such as metastasis or wound healing, and must generate and withstand substantial forces. The cellular motility responses to environmental forces are represented by their force-velocity relation (Figure 1), which has been measured for fish keratocytes but remains unexplained. Even pN opposing forces slow down lamellipodium motion by three orders of magnitude to the velocity values shown in Figure 1. At larger opposing forces, the retrograde flow of actin accelerates until it compensates for polymerization, and cell motion stalls. Subsequently, the lamellipodium adapts to the stalled state. We present a mechanism quantitatively explaining the cell's force-velocity relation; and its changes upon application of drugs that hinder actin polymerization or actomyosin based contractility. Elastic properties of filaments close to the lamellipodium leading edge and retrograde flow shape the force-velocity relation. Our results shed new light on how these migratory responses are regulated, and on the mechanics and structure of the lamellipodium. The results have been submitted for publication.

Selected Publications

Thurley, K., and M. Falcke. 2011. Derivation of Ca^{2+} signals from puff properties reveals that pathway function is robust against cell variability but sensitive for control. Proc Nat Acad Sci USA 108:427-432.

Thurley, K., I. Smith, S. C. Tovey, C. W. Taylor, I. Parker, and M. Falcke. 2011. Time scales of IP_3-evoked Ca^{2+} spikes emerge from Ca^{2+} puffs only at the cellular level. Biophys J in press.

Enculescu, M., M. Sabouri-Ghomi, G. Danuser, and M. Falcke. 2010. Modeling of Protrusion Phenotypes Driven by the Actin-Membrane Interaction. Biophys J 98:1571-1581.

Skupin, A., H. Kettenmann, and M. Falcke. 2010. Calcium Signals Driven by Single Channel Noise. PLoS Comput Biol 6:e1000870.

Schendel, T., R. Thul, J. Sneyd, and M. Falcke. 2011. How does the ryanodine receptor in the ventricular myocyte wake up – by a single or by multiple open L-type Ca^{2+} channels? Eur Biophys J in press.

Structure of the Group

Group Leader
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Scientist
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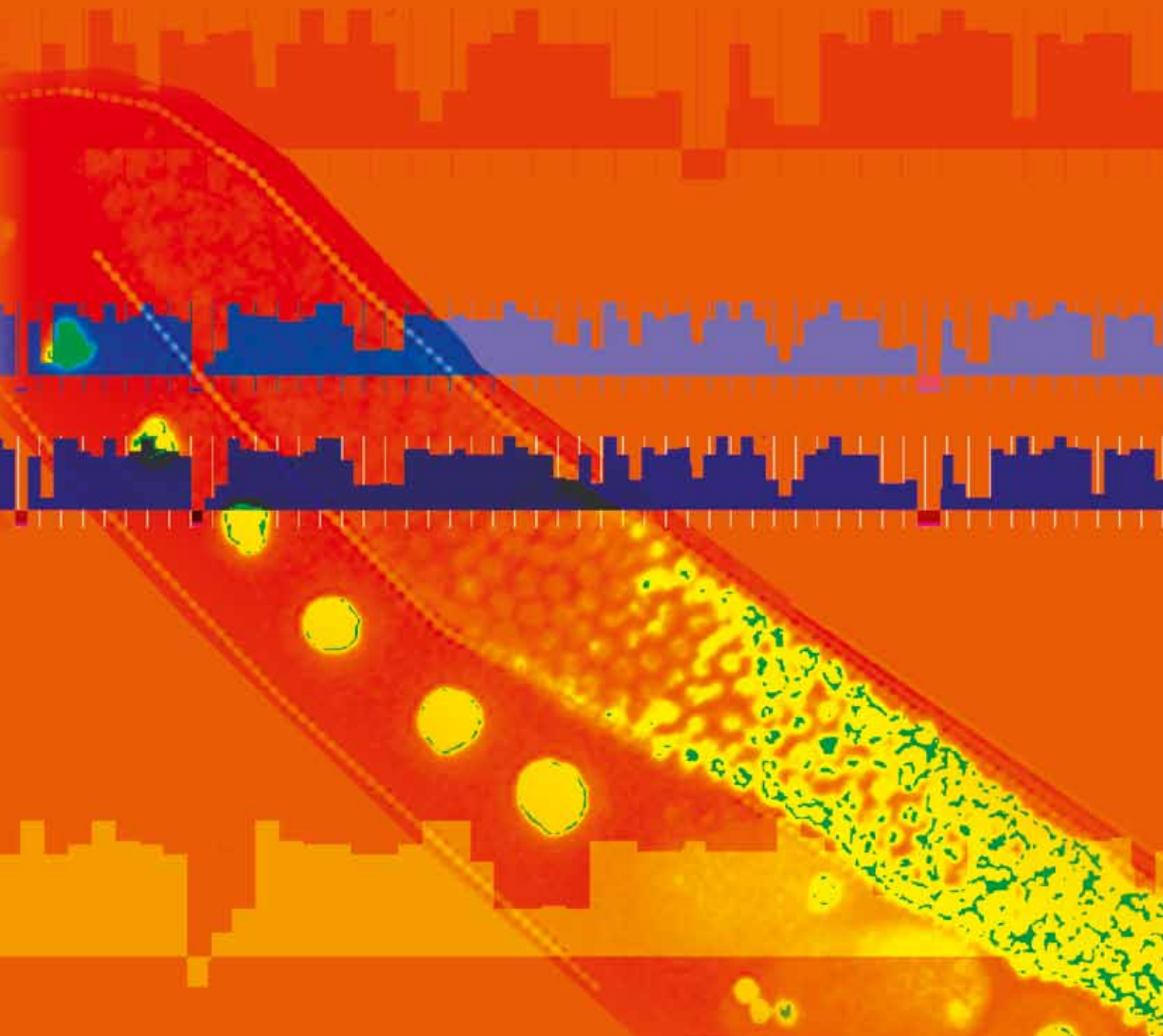
Graduate Students
J. Zimmermann

G. Mönke
T. Schendel
(till June 2011)
B. Gamanut
(till December 2010)

Berlin Institute for Medical Systems Biology

Coordinator: Nikolaus Rajewsky



Berlin Institute for Medical Systems Biology

Nikolaus Rajewsky

The Berlin Institute for Medical Systems Biology was launched in 2008 as a major expansion of the MDC's expertise in molecular medicine in the three major disease areas.

The aim of this initiative is to combine and synergize major systems biology approaches and high-end technologies with relevant disciplines. The availability of huge amounts of data from new types of technologies and high-throughput genomic, proteomic and metabolomic platforms as well as imaging data demand integrated, systematic and quantitative approaches for the understanding and modeling of all scales of life. BIMSB is creating a research environment for excellent scientific groups with interdisciplinary and systems biology approaches at the MDC.

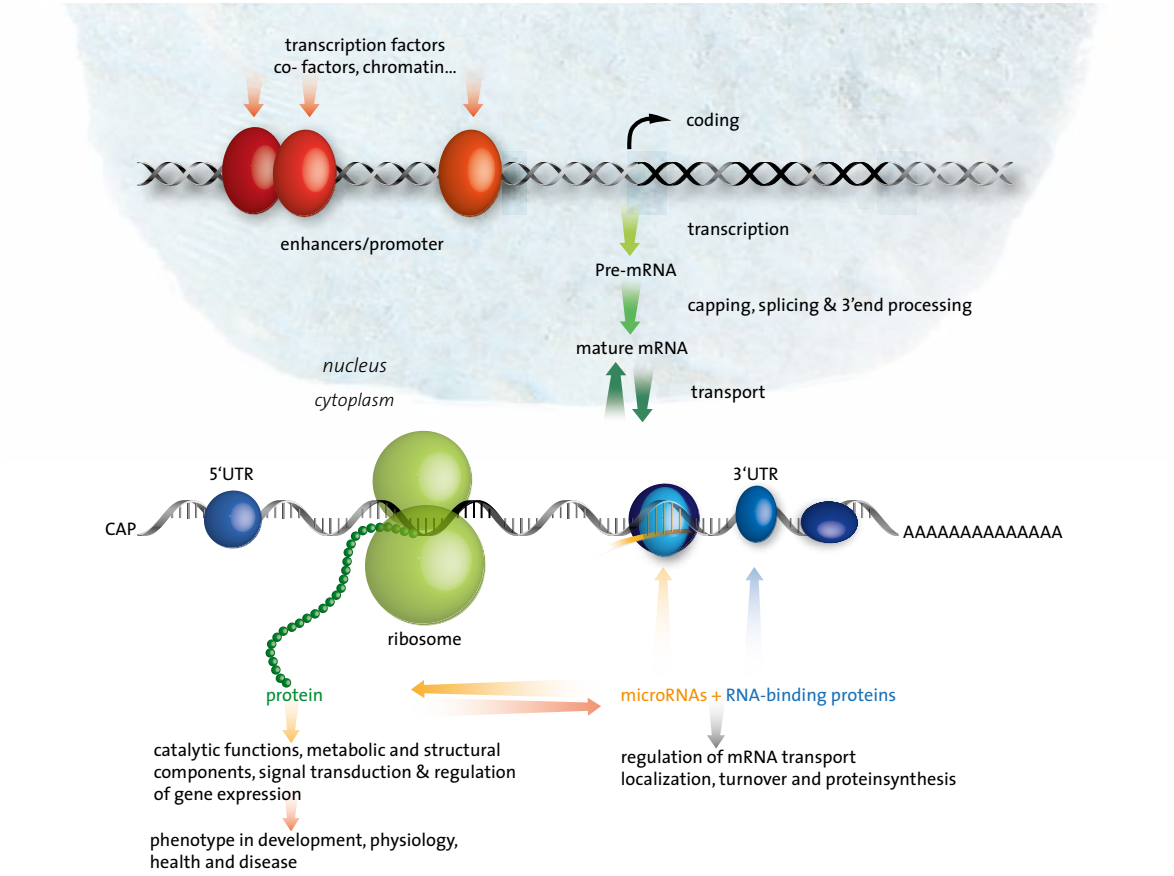
The Berlin Institute for Medical Systems Biology closely collaborates with research institutions and universities in Berlin and is involved in the structuring of an Integrative Research Institute (IRI) with the Humboldt University and the Charité. A new research building will be constructed in Berlin-Mitte on the 'Campus Nord' of the Humboldt University. The signing of a joint Memorandum of Understanding by all three institutions confirms this endeavor. The Senate of Berlin and the BMBF have allocated resources for the BIMSB building and research activities in Berlin-Mitte. A design study for the construction of the building was completed as basis for the architecture architectural competition and to prepare the construction of the new research building.

The central location close to the HU and Charité will develop into an innovative research campus, along with the new Sports Medicine Facility completed in 2010 and a research building for the Institute of Biology to be constructed in the near future.

(Photo: David Ausserhofer/Copyright: MDC)

Chancellor Angela Merkel pushed the start button for a new state-of-the-art DNA sequencer during her visit at the BIMSB of the MDC Berlin-Buch. Looking on are Dr. Jonas Korlach, co-inventor of the technology of Pacific Biosciences and a native Berliner, Dr. Wei Chen (in front), head of the Scientific Genomics Platform at BIMSB. In the background Federal Minister of Education and Research, Annette Schavan, and Professor Nikolaus Rajewsky (l.), Coordinator of the BIMSB.

Post-transcriptional regulatory networks (Copyright MDC)



Initial BIMSB funding was provided by the initiative "Spitzenforschung und Innovation in den Neuen Ländern" (Advanced Research and Innovation in the New States) in 2008-2010. The BIMSB was granted 7.5 million Euros by the BMBF and 4.4 million Euros from the Senate of Berlin for the initial project set-up, covering junior research groups and unique scientific infrastructures. Follow-up funding of the BMBF provided 5.6 million Euros in 2011-2013 and 1.5 million Euros in 2011 from the Senate of Berlin. Increasing central and additional funds from the MDC have been added to sustain and expand the BIMSB.

During the initial phase, the first junior groups have been recruited and an international exchange program has been launched with the Center for Functional Genomics of New York University (NYU), USA. Major investments in high-end technologies enabled the BIMSB to set up state-of-the-art scientific technology platforms for genomics, proteomics, metabolomics and bioinformatics. The most recent investment is the acquisition of a single molecule real-time sequencer, which was inaugurated by German Chancellor Angela Merkel.

Scientific mission

Basic and biomedical research has provided a wealth of information about the role of individual genes in various diseases. And even though new technologies add massive genome wide data of various states of cells and diseases, it becomes an increasing challenge to identify causes and improve therapies of diseases. The most prevalent and devastating diseases such as cancer, cardiovascular disease, diabetes, metabolic diseases and neurodegenerative disorders are multifactorial and can only be understood by quantitative models that predict the interactions and functions of numerous components. Thus systems biology is by nature highly interdisciplinary and combines molecular biology, biochemistry, mathematics, physics and engineering.

The importance of medical systems biology is particularly apparent in the field of post-transcriptional gene regulation and RNA biology (Special issue, Cell 2009). Many new classes of non-coding and small RNAs (such as microRNAs) have recently been discovered and shown to act in the regulatory networks in multicellular organisms. Furthermore, the human genome encodes hundreds of proteins with RNA binding domains. These proteins regulate mRNA localization, mRNA turnover, protein synthesis and epigenetic regulation and are crucial components for the dynamic regulation of gene expression. Small RNAs and RNA binding proteins have been shown to play many roles in the regulation of phenotypes in health and disease and therefore have

a huge potential for medical applications. In short, the grand challenge and overall scientific mission of the BIMSB is to decipher the 'post-transcriptional regulatory code' and to directly integrate it with other major cellular regulatory mechanisms, in particular transcriptional regulatory circuits, epigenetics, signal transduction pathways, protein-protein interaction networks, and post-translational modifications.

Advanced technologies such as next-generation sequencing and mass spectrometry, combined with imaging approaches and biochemical methods, will allow genome-wide quantitative analysis of regulatory mechanisms and resolution of RNA function on many different levels.

BIMSB research groups, scientific platforms and the international PhD exchange program

Young scientists leading independent research groups and scientific platforms in genomics, proteomics and quantitative biology have been appointed at the BIMSB. A few examples from ongoing work reveal how their research complements and expands the MDC's overall mission toward medical systems biology, and demonstrate that the concept of synergies of high-throughput data and multi-expert teams results in top publications.

The following group leaders have been appointed to the BIMSB: Markus Landthaler, an excellent scientist in the field of post-transcriptional regulation and RNA binding proteins, arrived in 2009 from the lab of Thomas Tuschl at Rockefeller University. Alexander Löwer, an expert in signaling dynamics in single cells, joined in 2011 from Harvard Medical School. Baris Tursun, from Columbia University, will join us in 2012 with a group working on gene regulation, cell fate decision and transdifferentiation.

The Genomics Platform is led by Wei Chen, an expert in next-generation sequencing technologies and their application to systems-wide approaches in genomic research. Christoph Dieterich heads the Bioinformatics Platform, where he supports the computational side of genome-wide analysis and bioinformatics. The third platform, headed by Stefan Kempa, enables MDC and BIMSB research groups to analyze the proteome and metabolome through mass spectromentry in a high-throughput format. The integration of the technologies of all of these scientific platforms supports a systems-wide understanding of complex regulatory mechanisms, as shown in highlights from the groups' research.

Researchers at the BIMSB not only use but also develop innovative methods to enhance existing high-end technologies. Published examples are eFACS (fluorescence-activated cell sorting of embryos, Nature Methods 2009), pulsed SILAC (stable isotope labeling by amino acids in cell culture, Nature 2008), detection of microRNAs ('miRDeep', Nature Biotechnology 2008), in vivo PAR-CLIP (Molecular Cell, in press), and automated time lapse-microscopy of living cells and their combination with integrative database approaches and mathematical modeling.

The labs of Jana Wolf, Wei Chen and Matthias Selbach have published a 'first complete census of the cell' in Nature (2011), by combining high-throughput measurements of RNAs and proteins. Their work models the specific steps of gene expression and quantifies transcription, translation and turnover of messenger RNAs and proteins.

The Rajewsky group collaborated with the Selbach and Landthaler labs to perform a transcriptome-wide analysis of regulatory interactions of the disease-relevant RNA-binding protein HuR (Molecular Cell 2011).

The Kempa, Dieterich, Rajewsky and Chen labs established a technology pipeline including massive parallel sequencing and shotgun proteomics for the first comprehensive de novo assembly and validation of an animal transcriptome (planaria)(Genome Research 2011).

In a joint effort by BIMSB groups, data integration is being achieved via the doRINA database combining RNA data, RNA-binding and RNA-protein interactions, i.e. relevant post-transcriptional elements (publication in press).

Another focus of the BIMSB is the international and interdisciplinary training of young scientists, especially PhD students. This led to the development of an exchange program jointly operated by the MDC and New York University (NYU) in the United States, where students are integrated in labs at BIMSB as well as NYU. Other international partnerships, with Kyoto Medical School, Japan, and Weizman University, Israel are currently being developed.

Within the MDC-NYU PhD exchange program, students carry out collaborative projects between the Center for Functional Genomics in New York and the BIMSB in Berlin and may spend up to 50% of their time in either location. They are co-mentored by NYU and MDC faculty and can participate in projects and classes at both institutions. In 2009 and 2010 this collaboration has already led to important publications, and several more are in the pipeline. NYU professors Kris Gunsalus and Stephen Small visited for summer sabbaticals in 2009 and 2010 respectively. Additionally, the BIMSB is active in coordi-

Photographer Alexander Baltz, Copyright MDC

MDC-NYU PhD Exchange students (2011): Back row (from left to right): Fabian Bindel, Marlon Stoeckius, Mathias Munschauer, Jiaxuan Chen
Front row (from left to right): Rina Ahmed, Alexander Baltz, Anna-Carina Jungkamp, Martina Weigt

nating scientific workshops and international conferences (Berlin Summer Meeting), collaborative efforts and third-party funding projects with local, national and international partners.

Partners

By definition, systems biology must be open to the integration of a wide range of expertise. The BIMSB's strategic concept has been developed in collaboration with virtually all local universities and scientific institutions in Berlin as well as other partners in Germany. Several projects funded by the BMBF, Helmholtz Association, the DFG and other sources are being carried out as collaborative projects between BIMSB investigators and partners throughout Berlin.

The major strategic collaboration is the BIMSB's contribution to the HU's Integrative Research Institute (IRI), where joint efforts and additional resources are foreseen and new senior recruitments are planned in cross-cutting topics between the areas of infection and immunology, neuroscience, systems and theoretical biology. BIMSB research and technology also contributes to Graduate School and Excellence Cluster concepts of the HU and Charité (under evaluation in 2011 & 2012).

BIMSB investigators have additionally established industry collaborations for joint projects and technology development (i.e. with life Technologies, Roche, Illumina and Pacific Biosciences).

In addition to Berlin-wide collaborations, BIMSB Investigators interact with research teams throughout Europe and world-wide. The potential of its technologies are also integrated in European research and infrastructure networks.

Future perspectives

The BIMSB will be an institutional branch of the MDC on the HU Campus in Berlin-Mitte, a location that will more closely interconnect the MDC with experimental and theoretical institutions in Berlin including universities, the Charité University hospital, research institutions, the DFG Research Center Matheon and other non-university research institutes. The structure and size of the new building in Berlin-Mitte will offer research space for up to 25 research groups including technology platforms, ample space for visiting scientists and extensive workshop and conference facilities. The start of BIMSBs activities in the new building are scheduled for 2015. Meanwhile, there will be further recruitments of junior and senior faculty. The BIMSB activities as well as the overall scientific concept were positively evaluated in 2009.

Nikolaus Rajewsky

Systems Biology of Gene Regulatory Elements

My lab uses experimental (molecular biology, biochemistry) together with computational methods (bioinformatics, computational biology, etc) to dissect, systems-wide, function and evolution of gene regulation in metazoans. One major focus is to understand more about post transcriptional gene regulation exerted by small RNAs, in particular microRNAs. We are developing predictive models for targets of microRNAs. We also investigate general mechanisms 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*. Furthermore, we have established planaria as a model system in our lab. These freshwater flatworms 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.

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 dieseases 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.

It is clear that a better understanding of gene regulation and in particular of the just emerging universe of

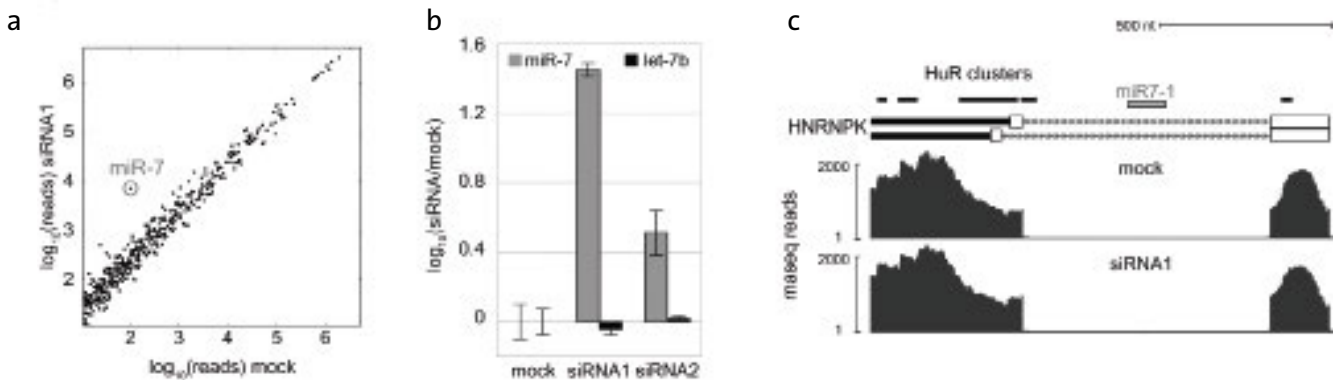


Figure 1. **(a)** expression of human microRNAs before and after HuR knockdown as determined by small RNA cloning and next generation sequencing. Only one human microRNA (miR-7) significantly changes expression levels. **(b)** validation by qPCR. **(c)** The HNRNPK gene harbors miR-7 in one of its introns. HNRNPK expression is unchanged upon HuR knockdown, demonstrating that HuR regulates the post-transcriptional expression of miR-7 (Lebedeva *et al.,* Molecular Cell 2011)

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 as metabolism in mammals, regeneration in planarians, 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 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. More recently, we have shown that microRNAs can 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; see pSILAC website). 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. Part of this work involved collaborations with the Stoffel lab (Rockefeller University, Piano & Gunsalus labs (NYU), Chen and Selbach labs (MDC).

Early embryogenesis 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). However, high-throughput technologies are needed to solve several fundamental problems in embryogenesis, for example how post-transcriptional and later transcriptional regulatory networks drive development. One key problem is that the state of the art method to obtain precisely staged early embryos consists of sorting embryos via mouth pipetting, thus making it impractical to obtain large samples. To overcome this problem,

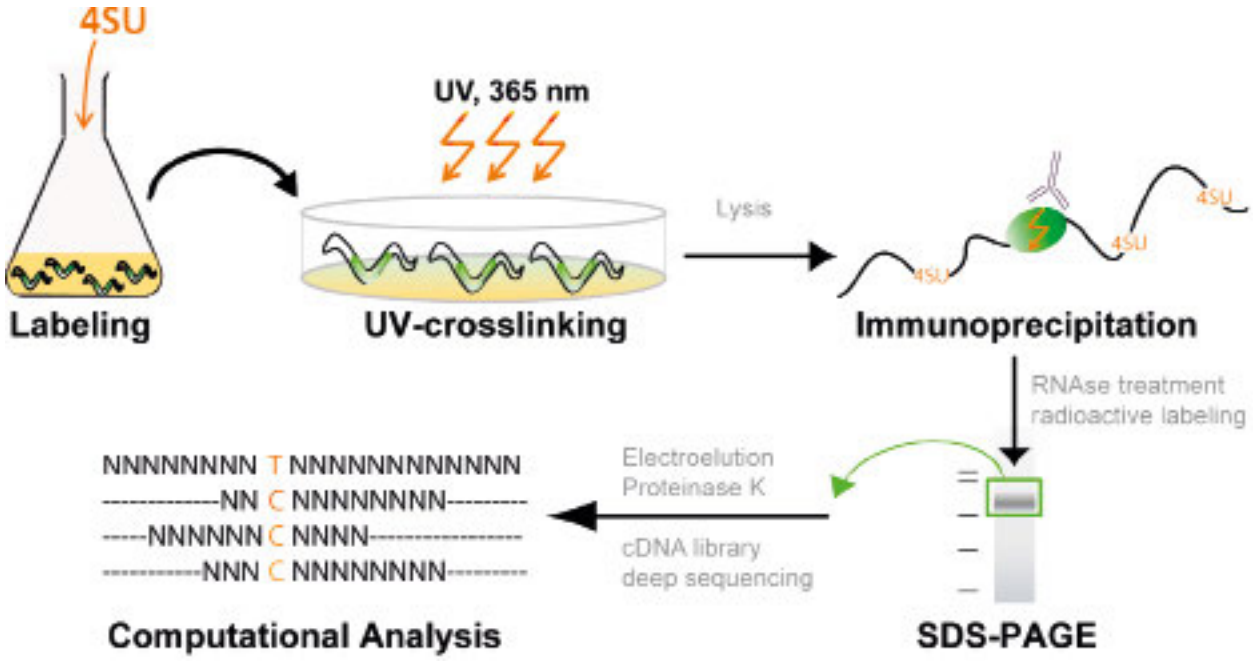


Figure 2. "*in vivo* PAR-CLIP". Biochemical identification of targets of RNA binding proteins at nucleotide resolution. RNAs in *C. elegans* are metabolically labeled with photoreactive nucleosides (4SU). After crosslinking of RNA:Protein interactions, a RNA binding protein of interest is immunoprecipitated and the attached RNA is sequenced via next generation sequencing techniques. Nucleotides in physical contact with the RNA binding protein show conversions of "T" to "C" and thus allow the mapping of the binding sites at nucleotide resolution (Jungkamp *et al.,* Molecular Cell in press).

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 (Stoeckius *et al.*, in preparation). Part of this work involved collaborations with the Piano lab (NYU) and Chen lab (MDC).

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). In an ongoing project, 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.*, in preparation). Part of this work involved collaborations with the Sanchez lab (Utah), Chen, Dieterich, and Kempa labs (MDC).

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 (Lebedeva *et al.,* Molecular Cell 2011), previously unknown functions of HuR. Very inter-

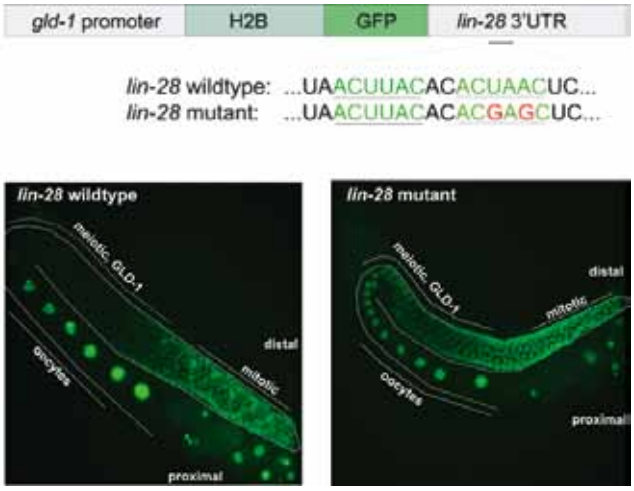


Figure 3. Targets of GLD-1 identified by in vivo PAR-CLIP can be validated in vivo. Transgenic worms were generated which express either the WT 3' UTR of lin-28 (a novel GLD-1 target) or the WT 3' UTR with two point mutations in the binding site identified via PAR-CLIP. The reporter carrying the mutated 3' UTR is de-repressed in the expression domain of GLD-1 (Jungkamp et al., *Molecular Cell* in press).

estingly, we also found that HuR directly and strongly controls the expression of one conserved human microRNA (miR-7, Figure 1). In fact, although we found that HuR directly regulates the expression of >4000 human coding mRNAs, the regulation of miR-7 by HuR is by far the strongest regulatory interaction that we could detect. This finding illustrates the need for studying RNA binding proteins and microRNAs together. We are currently trying to elucidate the function of this interaction in *in vivo* models (zebrafish). In general, 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 in press). This new method ("iPAR-CLIP") allows us to systematically identify the targets and to study the function of any RBP in *C. elegans* (Figure 2, 3). One immediate goal is to identify the targets of DICER and other central components of small RNA pathways *in vivo* (Rybak *et al.*, in preparation). Part of this work involved collaborations with the Landthaler, Selbach, and Kempa labs (MDC).

Selected Publications

Krek, A, Gruen, D, Poy, MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, and Rajewsky N (2005). Combinatorial microRNA target predictions. Nature Genetics 37, 495-500 .

Selbach M; Schwanhaeusser B; Thierfelder N; Fang Z; Khanin R; and Rajewsky N (2008). Widespread changes in protein synthesis induced by microRNAs. Nature 455, 58-63

Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak SD, Gogol-Doering A, Oenal P, Rybak A, Ross E, Sánchez Alvarado A, Kempa S, Dieterich C, Rajewsky N, Chen W (2011). De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics. Genome Research 21, 1193-1200.

Lebedeva S, Jens, M, Theil K, Schwanhaeusser B, Selbach M, Landthaler M, Rajewsky N (2011). Transcriptome-wide analysis of regulatory interactions of the RNA binding protein HuR. Molecular Cell 43, 340-352.

Jungkamp AC, Stoeckius M, Mecenas D, Mastrubuoni G, Kempa S, Rajewsky N (2011). In vivo and transcriptome wide identification of RNA binding protein target sites. Molecular Cell, in press.

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Dr Dominic Gruen
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Toshiaki Kogame
Svetlana Lebedeva
Jonas Maaskola
Sebastian Mackowiak
Pinar Oenal
Marlon Stoeckius
Nadine Thierfelder*
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Technical Assistants
Salah Ayoub
Margareta Herzog*
Signe Knespel*
Jonas Maaskola*
Lena von Oertzen

Secretariat
Alex Tschernycheff
* part of the time reported

Markus Landthaler

RNA Biology and Post-transcriptional Regulation

Our main interest is the understanding of post-transcriptional regulatory networks controlling gene expression in humans. Nascent RNA stably associates with RNA-binding proteins, RNA helicases and nucleases to form ribonucleoprotein complexes. These complexes play a key role in the regulation of spatial and temporal changes in protein synthesis by controlling transport, storage 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 the RNA-protein contacts that allows us to study how these interactions control the fate of cytoplasmic RNA. To achieve this goal we use a novel crosslinking-immunoprecipitation approach (PAR-CLIP) in combination with next-generation sequencing to identify functional RNA-protein interactions at near single-nucleotide resolution.

Post-transcriptional regulation by RNA-binding proteins and microRNAs

Kerstin Baethge, Yasuhiro Murakawa, Alexandra Vasile

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 a large number of RNA-binding proteins the target mRNAs and their function in RNA metabolism are unknown, limiting our understanding of post-transcriptional regulatory processes.

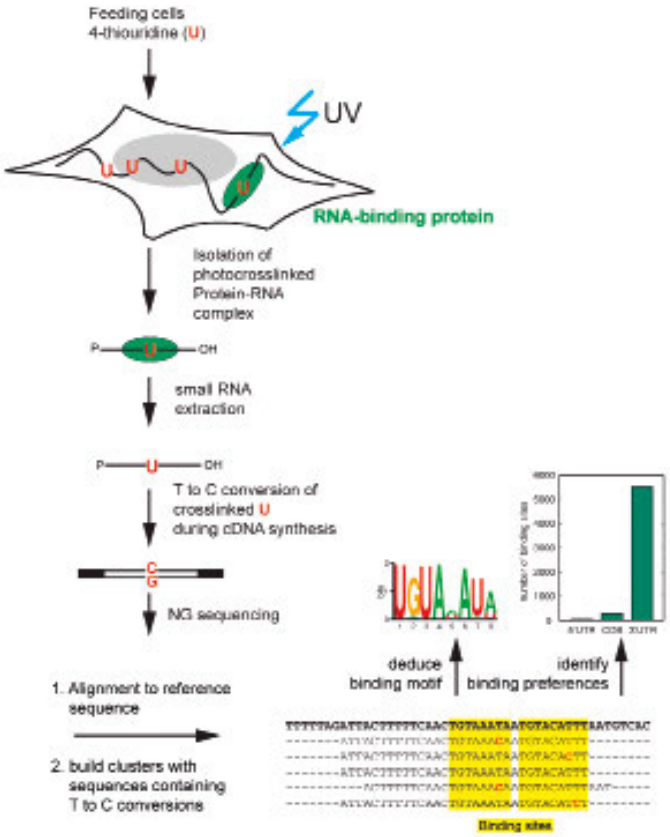
In particular, we are interested in RNA-binding proteins that positively and negatively modulate the activity of microRNAs. By combining maps of functional RNA-protein interactions with cell-based and biochemical assays, we determine the dynamic assembly of RNA-binding proteins and microRNAs on their target mRNAs as well as the elements and mechanisms guiding mRNA maturation, localization, turnover and protein synthesis.

Specificity and function of RNA helicases

Lea Gregersen, Mathias Munschauer

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 tumorgenesis and the replication of viruses. We are using our PAR-CLIP approach to define functional interactions of helicases and RNA that are typically transient in nature.

The identification of RNA target sites provides the foundation for biochemical and reverse genetic approaches



PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation)
Photoreactive 4-thiouridine (^{4S}U), when provided to cultured cells, is incorporated into nascent RNAs and crosslinked to RNA-interacting proteins by long-wave UV irradiation. The protein of interest is immunoprecipitated and the covalently bound RNA reduced to a short protected segment by ribonuclease treatment. In contrast to other CLIP approaches, the sites of crosslinking are identified by mapping T to C transitions residing in the cDNA of libraries prepared from RNA crosslinked to a specific RNA-interacting protein. The identified sequence clusters are used to derive consensus binding motifs and the location of binding sites of RNA-binding proteins.

to investigate the remodeling mechanism of ribonucleoprotein complexes by helicases. These studies will provide insights into the determinants of target RNA selection, functional interactions with other RNA-interacting proteins and the physiological role of RNA helicases.

Global Changes in the mRNA-protein interactome upon cellular stress

Alexander Baltz, Miha Milek, Emanuel Wyler

Systematic understanding of biological networks requires data from all levels of gene regulation; from gene transcription, RNA processing, turnover, translation, and localization to protein modification and abundance. We are developing methods to monitor the dynamic changes of the mRNA-protein interactome during the cellular stress response using state-of-the-art quantitative proteomics and next-generation sequencing approaches. These data will provide insight into the mechanisms that lead to changes in mRNA turnover and in protein synthesis as cellular consequences of stress.

Selected Publications

Zhang, X, Yalcin, S, Lee, DF, Yeh, TY, Lee, SM, Su, J, Mungamuri, SK, Rimmelé, P, Kennedy, M, Sellers, R, Landthaler, M, Tuschl, T, Chi, NW, Lemischka, I, Keller, G, Ghaffari S. (2011) FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. Nat Cell Biol. 13, 1092-1099.

Lebedeva, S, Jens, M, Theil, K, Schwanhäusser, B, Selbach, M, Landthaler, M, Rajewsky, N. (2011) Transcriptome-wide Analysis of Regulatory Interactions of the RNA-Binding Protein HuR. Mol Cell. 43, 340-352.

Ohrt, T, Staroske, W, Mütze, J, Crell, K, Landthaler, M, Schwille, P. (2011) Fluorescence cross-correlation spectroscopy reveals mechanistic insights into the effect of 2'-O-methyl modified siRNAs in living cells. Biophys J. 100, 2981-2990.

Hafner, M*, Landthaler, M*, Burger, L, Khorshid, M, Hausser, J, Berninger, J, Rothballer, A, Ascano, M, Jr., Jungkamp, AC, Munschauer, M, Ulrich, A, Dewell, S, Zavolan, M, Tuschl, T. (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141, 129-141 (* equal contribution)

Hausser, J*, Landthaler, M*, Jaskiewicz, J, Gaidatzis, D, Zavolan, M. (2009) Relative contribution of sequence and structure features to the mRNA-binding of Argonaute/miRNA complexes and the degradation of miRNA targets. Genome Res 19, 2009-2020 (*equal contribution)

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Start of the group: May 2011

Signaling Dynamics in Single Cells

The regulation of proliferation and differentiation is a fundamental challenge for multicellular organisms. The decision between alternative cell fates is therefore tightly controlled by external and internal signals. By now, many molecular players that process these signals have been identified, providing topological maps of the signaling networks. The next challenge is to understand how these networks act dynamically in living cells and how they interact with each other to control the physiological response of a cell. As individual cells within a population often show distinct responses to the same signals depending on their internal state, we focus on the analysis of single cells and investigate the properties that unite them and the sources of variation that make them different. We use time-lapse microscopy of living cells to measure signaling dynamics with high temporal resolution and combine the resulting quantitative data with mathematical modeling to gain a predictive understanding of cellular decision processes.

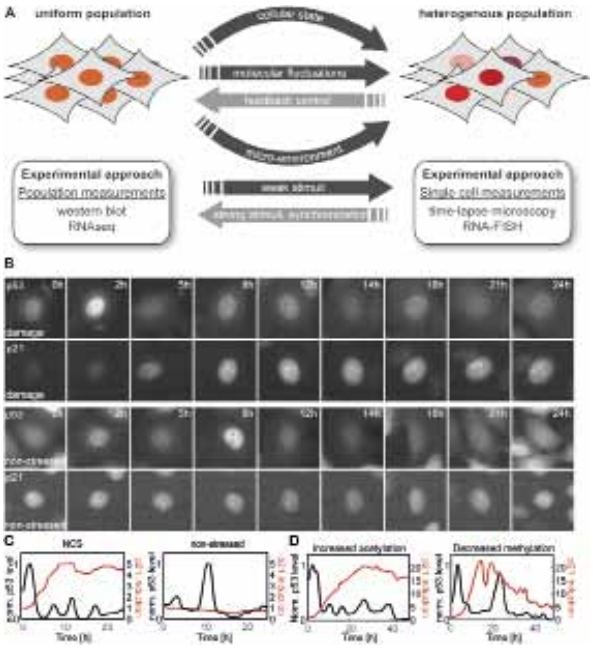
The tumor suppressor p53 is one of the central regulators of cellular proliferation. It is activated by cellular stress, for example damage to the genome, and controls target genes involved in various cellular programs including cell cycle regulation, repair and apoptosis. Among the target genes are regulators of p53 itself, generating positive and negative feedback. These feedback loops shape p53's dynamic response and lead to uniform pulses of protein accumulation, where the extent of damage is solely encoded in the number of pulses ("digital signaling").

Due to its central position in the regulation of proliferation, p53 interacts with various other cellular signaling pathways, for example the TGFβ and NFκB pathways. Focusing on p53 and its interacting signaling networks, the group addresses four fundamental questions:

1) How does the architecture of signaling networks and the function of their feedback loops shape the dynamic response?
2) How is the dynamic activation of a signaling pathway translated into a physiological response of a cell?
3) How do distinct signaling pathways interact to determine cellular decisions?
4) How can we control cell decisions using genetic or chemical perturbations?

Dynamics and function of p53 in cellular homeostasis

Surprisingly, we detected pulses of p53 accumulation in normally proliferating cells. These pulses are triggered by transient intrinsic DNA damage during cell cycle progression. However, pulses in proliferating cells do not lead to the expression of canonical p53 target genes. Instead, they are filtered by further signal processing based on posttranslational modifications. This observation led us to the question whether p53 pulses during proliferation have a specific function in cellular homeostasis. We hypothesize that the filtering mechanism is



A) Multiple internal and external factors increase heterogeneity between individual genetically identical cells of the same type. Experimental techniques that average the behavior of many cells obscure these variations and can result in misleading assumptions about cellular responses (from Loewer et al. (2011) *Curr Opin Genet Dev*). B-D) p53 pulses in cycling cells do not activate p21 expression. B) Time-lapse images of p53 and p21 reporter after damage or in non-stressed conditions C) Trajectories from the cells depicted above showing p53 (black) and p21 (red) dynamics. D) A persistence detector based on post-translational modification filters p53 pulses in cycling cells. When activating acetylation is increased or inhibitory methylation decreased, p53 pulses in non-stressed conditions lead to activation of the p21 promoter as well (from Loewer et al. (2010) *Cell*).

specific for certain p53 target genes, while others respond by transcriptional activation. There is evidence that a class of target genes involved in the regulation of metabolism and the redox state of a cell is induced by basal p53 levels. We will establish fluorescent reporters for these target genes and determine whether their expression correlates with p53 pulses in cycling cells. Using single cell reporters of the cellular redox and energy state, we will determine if p53 pulses play a role in cellular homeostasis. In collaboration with Stefan Kempas group, we will investigate the influence of p53 on metabolism.

Dynamics of the TGFβ pathway

The TGFβ pathway interacts closely with the p53 network to induce a cytostatic response. We will first establish reporter cell lines for central components of the TGFβ network and characterize pathway activation in individual cells in response to different input stimuli. We will determine the variability of the response and investigate the underlying signaling logic. We will focus on identifying the dominant feedback mechanisms that determine the dynamic behavior of the network and validate results by siRNA or small molecule inhibitor mediated perturbations. To induce a cytostatic response, the dynamic activation of the TGFβ pathway must have an impact on the regulatory network controlling cell cycle progression. To date, several interactions between these networks have been identified, but their individual contributions and their interplay on a systems level are less well understood. We will use mathematical modeling in combination with quantitative single cell data to address this question, focusing on the hypothesis that feed-forward loops in the network implement persistence detectors.

Interactions of signaling pathways

In a cell, signaling pathways do not act as isolated units, but are part of larger regulatory networks. To understand how p53 interacts with other pathways, we will generate combined reporter cell lines for the p53 network and the TGFβ and NFκB pathways. This will allow us to correlate how the state of one network influences the activity of another. By stimulating different pathways simultaneously or in varying temporal order, we can determine the interaction logic, for example whether the pathways act synergistically or antagonistically. We will combine quantitative data, modeling and network perturbations to understand the underlying principles of pathway interaction on a molecular level. In the future, we will expand this analysis to additional pathways, including the Wnt and MAPK pathway, to understand how the integrated activity of the cellular signaling network determines cellular decisions.

Selected Publications

Batchelor, E., Loewer, A., Mock, C., Lahav, G. (2011). Stimulus-dependent dynamics of p53 in single cells. Mol. Syst. Biol. 7: 488.

Loewer, A., Batchelor, E., Gaglia, G., Lahav, G. (2010). Basal dynamics of p53 reveals transcriptionally attenuated pulses in cycling cells. Cell 142(1): 89-100

Stempfle, D.*, Kanwar, R.*, Loewer, A.*, Fortini, M.E., Merdes, G., (2010). In vivo Reconstitution of γ-Secretase in Drosophila Results in Substrate Specificity. MCB 30(13): 3165-75

Batchelor, E., Loewer, A., Lahav, G. (2009). The ups and downs of p53: Understanding protein dynamics in single cells. Nat Rev Cancer 9: 371-377

Toettcher, JE.*, Loewer, A.*, Ostheimer, GJ., Yaffe, MB., Tidor, B., Lahav, G. (2009). Distinct mechanisms act in concert to mediate cell cycle arrest. PNAS 106(3): 788-90 * equal contribution

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Novel Sequencing Technology, Medical and Functional Genomics

The recent introduction of massive parallel sequencing technology has revolutionized genomic research. These so-called next generation sequencing platforms, such as Roche/454, Illumina/solexa and ABI/Solid system can sequence DNA orders of magnitude faster and at much lower cost than conventional Sanger method. With their incredible sequencing capacity, my lab has been focused on developing and implementing various genomic assays based on this new generation of sequencers. Apart from offering services to the institute as a core facility provider, we are now applying the assays in transcriptome profiling, characterizing estrogen receptor mediated gene regulation in breast cancer cells, as well as identifying genetic factors underlying human diseases.

Scientific Genomics Platform

The Scientific Genomics Platform has set up all three commercially available next-generation sequencing systems including one Roche/454 FLX titanium, two ABI/SOLiD 4, two Illumina/Solexa GAIIX, four Hiseq2000, and recently implemented the state-of-art single molecule real time sequencing technology (Pacific Bioscience RS). Based on these technologies, we have focused on the development and implementation of various genomic assays. The applications range from *de novo* genome sequencing, genome resequencing, transcriptome sequencing, small RNA sequencing and ChIP-seq. With a maximum capacity of 1.5 x 10^{12} bases sequenced per week, we have been involved in more than 30 internal and more than 10 external projects.

De novo transcriptome assembly

(Yongbo, Xintian You, Andreas Gogol Doering, Mirjam Feldkamp)

To facilitate the genomic research in non-model organisms without reference genome sequences, we have devised an efficient and powerful method that can be successfully used to obtain a high-quality, complex transcriptome without the need to sequence and assemble genomic DNA. The two key ingredients of our methods are (1) the simultaneous usage of complementary sequencing technologies and (2) careful normalization of the cDNA library. Together with research groups led by Nikolaus Rajewsky, Stefan Kempa and Christoph Dieterich, we have de novo assembled a major fraction of the S. mediterranea transcriptome, which dramatically expands and refines planarian gene annotation, demonstrated by validation of several previously unknown transcripts with stem cell-dependent expression patterns. The application of our robust transcriptome characterization pipeline in other organisms without genome assembly, such as grizzly bear is ongoing.

Quantitative profiling of transcriptome dynamics

(Na Li)

Pulse labeling of newly synthesized RNA using nucleoside analogue 4-thiouridine (4sU) allowed the newly synthesized transcripts to be separated from pre-existing fraction. By deep sequencing of the mRNA transcripts in the two fractions, we cannot only globally estimate the absolute level of each transcript at steady state, but also their turnover rates. Together with research groups led by Matthias Selbach and Jana Wolf, based on parallel measurement of protein and mRNA level as well as their half lifes, we have constructed a first genome wide quantitative model on mammalian gene expression. Quantitative information about all stages of gene expression provides a rich resource and helps to provide a greater understanding of the underlying design principles.

Systematic identification of genetic factors underlying monogenic diseases

(Yuhui Hu, Sebastian Froehler, Yongbo Wang, Wei Sunny Sun, Ana Babic, Claudia Langnick, Mirjam Feldkamp, Madlen Sohn)

To expedite the molecular elucidation of genetic factors underlying monogenic diseases, we have applied two strategies, (1) characterization of breakpoints in disease-associated balanced chromosome rearrangements and (2) high-throughput mutation screening by combining target region enrichment and next generation sequencing. So far, together with the group led by Hilger Ropers at Max-Planck-Institute for Molecular Genetics, we have succeeded in the identification of causative genes implicated in intellectual disability or related neurological disorders. The functional characterization of these genes is ongoing.

Global characterization of Estrogen receptor mediated regulatory network in breast cancer cells

(Yuhui Hu, Andreas Gogol Doering, Wei Sun, Martina Weigt, Anna-Maria Stoehl)

About 70% of breast cancers are positive for the nuclear receptor estrogen receptor-α (ER-α) and require estrogen for proliferation. During the genomic action, ER-α binds to the regulatory region of the target genes, modifies the chromatin modification and consequently increases or decreases their expression level. To better understand the transcription regulation mediated by ER-α, we undertook a sequencing-based systematic approach by integrating the genome-wide analysis of ER-α and Pol II binding sites as well as histone modifications, with the mRNA expression profiling in breast cancer cells (MCF-7). By combining the large resources produced by ChIP-seq and mRNA-seq in this study, we are able to dissect, from a multi-dimensional angle, the regulatory network mediated by ER-α. Further understanding of the dynamics and feedbacks of the network may play an important role in optimizing the therapy strategy for patients afflicted with ER-α positive breast cancer.

Selected Publications

Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Püttmann L, Vahid LN, Jensen C, Moheb LA, Bienek M, Larti F, Mueller I, Weissmann R, Darvish H, Wrogemann K, Hadavi V, Lipkowitz B, Esmaeeli-Nieh S, Wieczorek D, Kariminejad R, Firouzabadi SG, Cohen M, Fattahi Z, Rost I, Mojahedi F, Hertzberg C, Dehghan A, Rajab A, Banavandi MJ, Hoffer J, Falah M, Musante L, Kalscheuer V, Ullmann R, Kuss AW, Tzschach A, Kahrizi K, Ropers HH. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature. 2011 Sep 21;478(7367):57-63.

Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J*, Chen W*, Selbach M* Global quantification of mammalian gene expression control Nature. 2011 May 19;473(7347):337-42
*shared corresponding author

Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak SD, Gogol-Doering A, Oenal P, Rybak A, Ross E, Alvarado AS, Kempa S*, Dieterich C*, Rajewsky N*, Chen W*. De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics. Genome Res. 2011 May 2. [Epub ahead of print]
*shared corresponding author

Hu, H.,Wrogemann, K., Kalscheuer, V., Tzschach, A., Richard, H., Haas, S. A., Menzel, C., Bienek, M., Froyen, G., Raynaud, M.,Van Bokhoven, H.,Chelly, J.,Ropers, H., Chen, W. Mutation screening in 86 known X-linked mental retardation genes by droplet-based multiplex PCR and massive parallel sequencing. The HUGO Journal: Volume 3, Issue 1 (2010), Page 41.

Chen W, Kalscheu V, Tzschach A, Menzel C, Ullmann R, Schulz M, Erdogan F, Li N, Kijas Z, Arkesteijn G, Pajares IL, Goetz-Sothmann M, Heinrich U, Rost I, Dufke A, Grasshoff U, Glaeser BG, Vingron M, Ropers HH.(2008) Mapping translocation breakpoints by next-generation sequencing. Genome Res. 18: 1143-1149

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Integrative Metabolomics and Proteomics

Within the past decades biochemical data of single processes, metabolic and signaling pathways were collected and advances in technology led to improvements of sensitivity and resolution of bioanalytical techniques. These achievements build the bases for the so called 'genome wide biochemistry'.

High throughput techniques are the tool for large scale '-omics' studies allowing the obtainment of a nearly complete picture of a determinate cell state, concerning its metabolites, transcripts and proteins. However, single level study of a living organism cannot give a complete understanding of the mechanism regulating biological functions. The integration of transcriptomics, proteomics and metabolomics data with existing knowledge allows connecting biological processes which were treated as independent so far. In this context the aim of our group is to apply metabolomics and proteomics techniques for absolute quantification and to analyze turnover rates of proteins and metabolites using stable isotopes. In addition, the development of data analysis workflows and integrative strategies are in the focus of our interest.

Scientific platform for proteomics and metabolomics

For scientific collaborations and projects, we provide quantitative proteome and metabolome analysis to address complex biological questions by applying standard and customized methodologies for:

- 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)

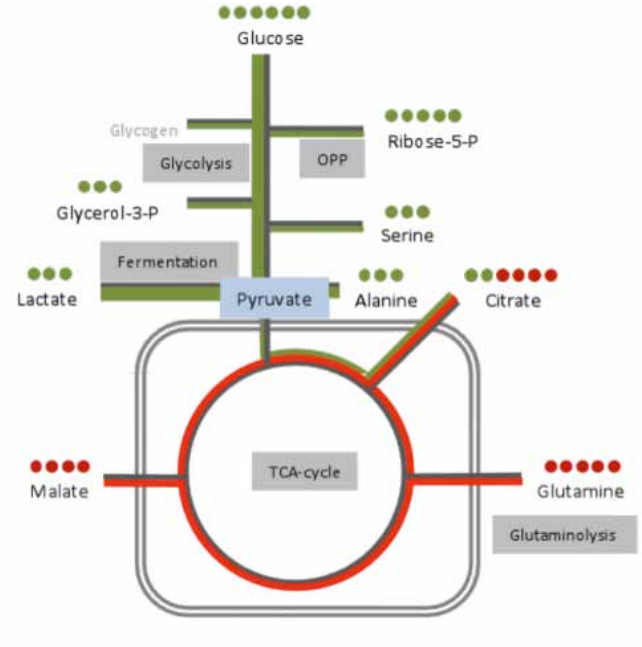
These analyses are performed with three different types of mass spectrometers:

- two dimensional gas chromatography coupled mass spectrometer (GCxGC-TOF MS)
- nano LC / UPLC liquid chromatography coupled triple quadrupol mass spectrometer (LC-QQQ]
- nano LC liquid chromatography coupled LTQ-Orbitrap mass spectrometer (nLC-LTQ-Orbitrap MS)

The combination of technologies allows us to quantify proteins and metabolites from cells, organs and organisms and to monitor their turnover rates in a genome wide scale.

Functional analysis and evaluation of cancer metabolism as therapeutic target

Despite the enormous diversity of cancer, all tumor cells seem to display a de-regulation of the energy metabolism. Already in 1924 the famous Nobel Prize biochem-



Distribution of carbon atoms derived from glucose or glutamine within the central carbohydrate metabolism of cancer cells
Cancer cells were incubated with stable isotope labeled glucose and glutamine and the resulting distribution of carbon atoms was analyzed using stable isotope resolved metabolomics.

ist Otto Warburg described an anaerobic type of metabolism performed by cancer tissues. This phenomenon, called the "Warburg effect", is modulated by the action of well-known oncogenes but the underlying mechanisms are not fully resolved yet. It was known that cancer cells up-regulate the glycolytic pathway but it has now been shown that anaplerotic pathways are also induced at the same time. In this context, a high rate of glutamine consumption by cancer cells was described (Figure). We aim to analyze these events of metabolic reprogramming on a molecular level using proteome and metabolome analyses *in vitro* and *in vivo* and we will evaluate "cancer metabolism" as target for an anti-proliferative therapy.

Stem cell metabolism

Higher organisms consist of multiple cell types forming tissues, organs and the whole organism. Ongoing from the fertilized oocyte cells start to divide and to differentiate. During this process stem cells undergo a reprogramming at all levels e.g. epigenome, transcriptome, proteome and also metabolome. We are interested in how differentiation processes are connected to changes of the metabolism. To answer this question we work with mammalian cell lines and model organisms.

The planarian Schmidtea mediterranea is a well-established model organism at BIMSB. These freshwater flatworms can regenerate all lost body tissue after amputation due to a population of pluripotent somatic stem cells called neoblasts that constitute up to 30 percent of the whole organism. In collaboration with the group of Nikolaus Rajewsky we analyze the biochemistry of neoblasts on a molecular level.

Data integration

Modern "-omics" technologies like transcriptomics, proteomics and metabolomics are established at BIMSB. Those techniques generate large amounts of data and cover a wide range of biological processes. To analyze and store such large amounts of data we, together with other BIMSB groups, are establishing the BIMSB systems database. It will help to structure and synchronize large datasets from multiple analytical platforms and will build up a resource for integrative analyses.

Selected Publications

Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak S, Gogol-Doering A, Oenal P, Rybak A, Ross E, Sánchez Alvarado A, Kempa S, Dieterich C, Rajewsky N, Chen W, (2011) De novo assembly and validation of Planaria transcriptome by massive parallel sequencing and shotgun proteomics. Genome Research 21(7):1193-200

Wienkoop S, Weiß J, May P, Kempa S, Irgang S, Recuenco-Munoz L, Pietzke M, Schwemmer T, Rupprecht J, Weckwerth W, (2010) Targeted proteomics for Chlamydomonas reinhardtii combined with rapid subcellular protein fractionation, metabolomics and metabolic flux analyses. Mol Biosyst 6(6):1018-31

Christian N, May P, Kempa S, Handorf T, Ebenhoh O, (2009) An integrative approach towards completing genome-scale metabolic networks. Mol Biosyst 5: 1889-1903

Kempa S, May P, Wienkoop S, Usadel B, Christian N, Rupprecht J, Weiss J, Recuenco Munoz L, Ebenhöh O, Weckwerth W, and Walther D. (2008) Metabolomics and Proteomics Assisted Genome Annotation and Analysis of the Draft Metabolic Network of Chlamydomonas reinhardtii. Genetics 179: 1–10

Kempa S, Rozhon W, Šamaj J, Erban A, Baluška F, Becker T, Haselmayer J, Schleiff E, Kopka J, Hirt H and Jonak C (2007) A plastid localized glycogen synthase kinase 3 modulates stress tolerance and carbohydrate metabolism. Plant J. 49(6):1076-90

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Bioinformatics in Quantitative Biology

Bioinformatics is a highly dynamic discipline, which operates at the interface of life sciences, computer science and formal sciences. Currently, emerging technologies in nucleic acids sequencing, mass spectrometry and imaging revolutionize biology. For the first time, a holistic quantification of biological systems at the level of genomes, transcripts, proteins and metabolites is in reach. Bioinformatics supports this technology-driven transition of biology into a truly quantitative science.

We use existing and develop novel methods for high-throughput data acquisition, processing, model building and inference in quantitative biology. Computational studies are backed up by experimental work within our group and within active collaborations.

Scientific Bioinformatics Platform

The platform component encompasses scientific computing and hardware-oriented tasks such as overseeing the BIMSB high-performance computing infrastructure. At the time of writing, the computing cluster consists of >800 CPU cores and >700 TB hard disk storage. We provide dedicated hardware for memory-intensive applications (up to 1TB main memory) as well as environments for parallel software development. With this infrastructure, we collaborate with the MDC and external scientists and provide computing expertise for management systems, data exchange and web services. We also assist software development for scientific analysis as well as bioinformatics for workflows and pipelines for experimentalists.

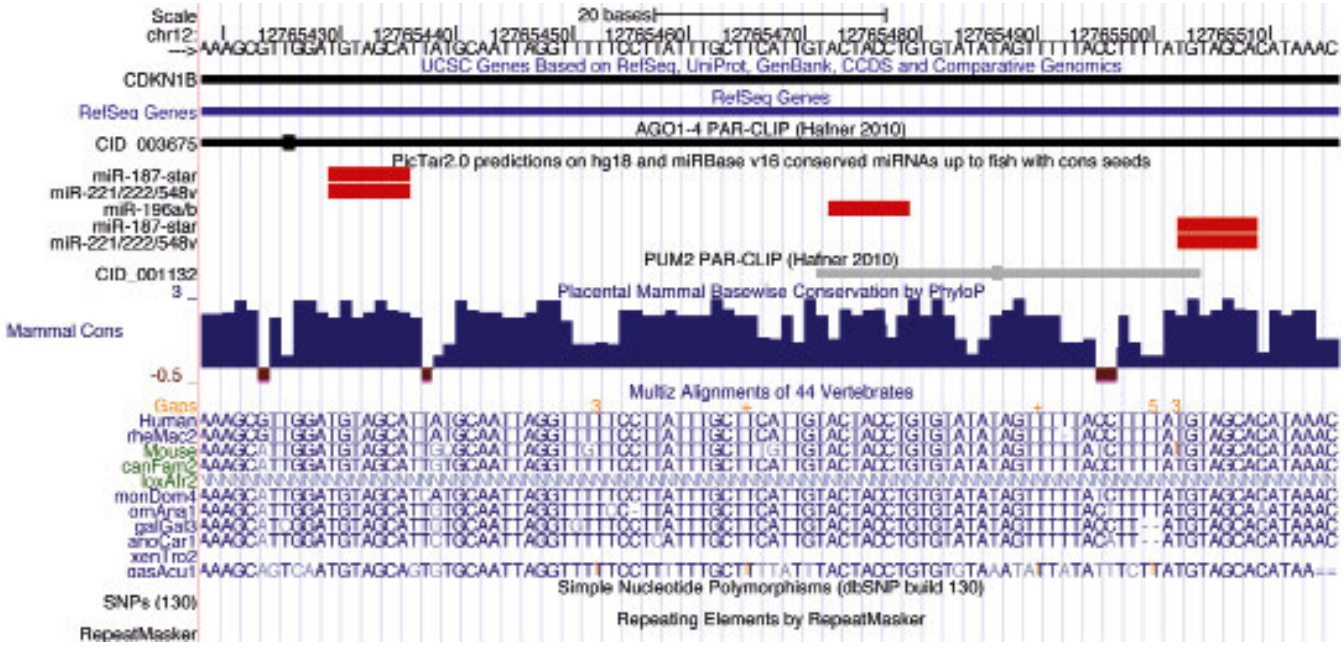
Computational approaches to study eukaryotic gene regulation

The expression of a gene is directly regulated by interactions of proteins and nucleic acids or by RNA-RNA interactions. We work on statistical methods for regulatory motif (e.g. binding site) and module (e.g. promoter elements) prediction in posttranscriptional gene regulation. Additionally, we study the evolution of regulators and binding sites.

We integrate potential effects of these evolutionary changes with expressive graphical models. Moreover, our group implemented the BIMSB database of posttranscriptional regulatory elements (http://dorina.mdc-berlin.de) in a collaborative effort to systematically curate, store and integrate transcriptome-wide binding site data. Another topic are long-ranging interactions between different genomic loci, which are still enigmatic yet of tremendous importance to gene regulation. We support this important endeavor by an experimental design algorithm for the chromatin conformation capture (3C) technique.

Pristionchus – a model system in ecology

Pristionchus pacificus has been established as a satellite system to *Caenorhabditis elegans* over the last years. *P. pacificus* is an omnivorous nematode and lives in a



The doRiNA database – screenshot of the Pumillio 2 target site region in the 3' UTR of CDKN1B.

Kedde2010 *et al.* have shown that p27 (CDKN1B) is post-transcriptionally regulated by mir-221 and mir-222 conditional on an Pumillio-induced RNA structure switch. We independently corroborate this scenario by querying the doRiNA database.

necromenic association with scarab beetles, which is thought to constitute an evolutionary intermediate between free living nematodes and true parasites. To complement our biological knowledge, we sequenced the *P. pacificus* genome and found it to be enriched for gene families involved in stress response and metabolism of xenobiotics. Additionally, there are several instances of lateral gene transfer. For example, cellulase gene predictions were confirmed by experiment and shown to be integrated into the host biology by activity assays. Another set of predicted protein-coding genes did not show any similarity to the known protein universe yet is transcribed and translated. We are functionally investigating gene candidates of lateral gene transfer and *de novo* formation.

Genome evolution – content, order and variation

Phylogenetic profiling of gene content may guide us in studying species adaptation to certain environments. Similarly, gene order is constrained by several partly unknown factors. We invented algorithms for identifying gene order conservation without assigning orthology *a priori*. We are able to look deep into the history of genome structure evolution since these approaches do not rely on a given whole-genome alignment. We are likewise interested in reconstructing the order of these events.

Selected Publications

Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak SD, Gogol-Doering A, Oenal P, Rybak A, Ross E, Sánchez Alvarado A, Kempa S, Dieterich C, Rajewsky N, Chen W.(2011), 'De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics.', Genome Res 21(7):1193-1200.

Fröhler S, Dieterich C. 'ACCUSA – accurate SNP calling on draft genomes.', Bioinformatics 26(10):1364-1365.

Dieterich, C. & Sommer, R. J. (2009), 'How to become a parasite – lessons from the genomes of nematodes.', Trends Genet 25(5), 203-209.

Dieterich, C.; Clifton, S. W.; Schuster, L. N.; Chinwalla, A.; Delehaunty, K.; Dinkelacker, I.; Fulton, L.; Fulton, R.; Godfrey, J.; Minx, P.; Mitreva, M.; Roeseler, W.; Tian, H.; Witte, H.; Yang, S.-P.; Wilson, R. K. & Sommer, R. J. (2008), 'The Pristionchus pacificus genome provides a unique perspective on nematode lifestyle and parasitism.', Nat Genet 40(10), 1193-1198.

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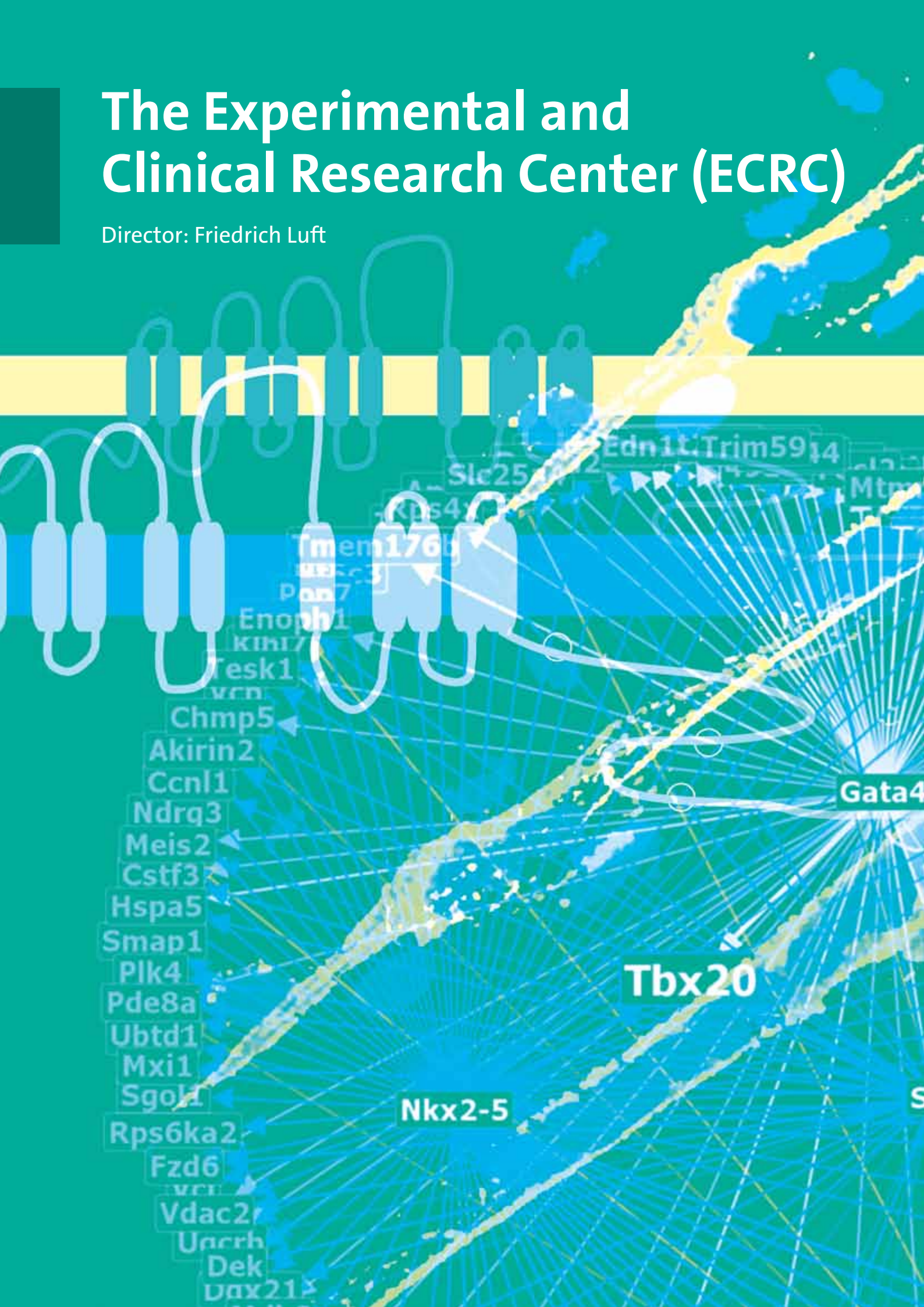


The Experimental and Clinical Research Center (ECRC)

Friedrich Luft

The ECRC is a joint project of the MDC and the Charité. The MDC has as its mission the pursuit of science relevant to medicine. The Charité is obliged to educate medical students and train physicians in all disciplines, including clinical science. The ECRC was conceived to realize those goals in a combined way. Its mission is to accelerate the translation of discoveries from fundamental research into new procedures for diagnoses, prevention, and therapies for common diseases (primarily cardiovascular disease, cancer, and neurological disorders) that threaten our society. The approach to the mission is to train clinician-scientists and PhD scientists who are specifically interested in translational research.

Several instruments have been developed to facilitate this mission. Clinicians or basic researchers can apply for independent project groups to conduct a translational project within the ECRC. Funding is provided for three-five years. Each funding decision is based on a competitive evaluation involving external experts. Extensions are possible predicated on extramural funding and scientific performance. The current ECRC groups presented here and newer groups now getting established follow this track.

Clinicians and researchers of the MDC can jointly submit proposals for collaborative projects, which can be funded through the ECRC after a competitive evaluation. These clinical research cooperative projects (*Klinsches Kooperationsprogramm*) are carried out at the ECRC and/or the MDC.

Since clinical and translational scientists are "made rather than born," we have provided a jointly sponsored MDC and Charité training program for clinicians. This clinical scientist program (*Kliniker Ausbildungsprogramm*) is basically a research fellowship for clinician trainees. The clinicians take a break from their clinical duties and join scientists at one of the MDC laboratories. The program is project oriented and positions are awarded according to peer review. The program frequently results in long-term collaborations between the clinician and host laboratory. The projects are not solely bench science; clinicians can perform patient-oriented research via the CRC and outpatient specialty clinics, (*Hochschulambulanzen*), ECRC-associated "biobanks", and at the high-field magnetic resonance imaging facility.

Scientific projects

Several successful projects have already been carried out within the ECRC framework. Some began as studies of molecular, cellular, or animal model systems related to disease, often at a very basic level. Others began as work in which physicians were serving as caregivers for patients, who served as the starting point to probe mechanisms underlying particular health problems. An example of the latter is the discovery by Ludwig Thierfelder's (MD) group of a connection between mutations in the gene encoding plakophilin and a condition called arrhythmogenic right ventricular cardiomyopathy. Walter Birchmeier (PhD) and his laboratory, while studying WNT signaling, observed that when the gene encoding this protein was disrupted a cardiomyopathy developed in mice. Working together, the groups identified a disease mechanism causing cardiac arrest in affected people. They can be helped by implantation of an automated defibrillator. An analogous example involves the observation that quickly drinking plain water can have surprising effects on blood pressure regulation and metabolism. Work involving Jens Jordan and the CRC physicians (all MDs) and Gary Lewin's laboratory (all PhDs) led to the cloning of a novel osmoreceptor in the portal circulation. Yet another example is the long-term (>15 year) combined pursuit by Bernd Dörken (MD) and his group with Claus Scheidereit (PhD) and his laboratory on the role of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) in the pathogenesis of Hodgkin's lymphoma. Yet other projects are specifically oriented toward treatments. For instance, Thomas Blankenstein (PhD) and Anthony Pezutto (MD) are develop-

Photographer David Ausserhofer, Copyright MDC

German Chancellor Angela Merkel (center) visits a lab in the "Experimental and Clinical Research Center (ECRC)". From left: Philipp DuBois (Charité – Universitätsmedizin Berlin), Dr. Jens Fielitz (Charité – Universitätsmedizin Berlin), and Prof. Friedrich Luft (Director of the ECRC).

Bundeskanzlerin Angela Merkel (Mitte) in einem Labor des „Experimental and Clinical Research Center (ECRC)". Von links: Philipp DuBois (Charité – Universitätsmedizin Berlin), Dr. Jens Fielitz (Charité – Universitätsmedizin Berlin) und Prof. Friedrich Luft (Direktor des ECRC).

ing allogeneically modified tumor cells as a vaccine in patients with metastatic renal cell cancer. Wolfgang Walter and Ulrike Stein (PhDs) are working with Peter Schlag (MD) on novel *Clostridium perfringens* enterotoxin suicide gene therapy in a selective treatment of claudin-3- and -4-overexpressing tumors.

The other success stories are too numerous to list here. Importantly, these collaborations have also spurred a great deal of extramural funding including joint federal grants (*Deutsche Forschungsgemeinschaft*), clinical research groups (*Klinische Forschergruppen*), large program projects (*Sonderforschungsbereiche*), and topic-oriented training grants (*Graduierten Kollegs*).

Main components of the ECRC

The main physical components of the ECRC are a clinical research center (CRC) for studies involving patients and healthy humans and a high-field magnetic resonance facility for the examination of humans and model animals. A third building to house the experimental research center (ERC), whose focus will be disease-oriented experimental research, is nearing completion. Most of the ECRC laboratories and the patient-oriented clinical research center (CRC) are located on the Campus Buch in the former *Robert-Rössle* Clinic of the Charité. Several outpatient clinics have already been established there; namely one for neuroimmunological diseases (multiple sclerosis), another for skeletal muscle disease, another for cardiomyopathies, another for genetic and diabetic renal diseases, another for metabolic diseases, and yet another for pediatric pulmonary and allergic diseases. The skeletal muscle disease clinic was established in the context of a DFG-financed clinical research group and deals with ca. 1200 patients with rare muscle diseases per year.

The CRC has examining rooms, specialized procedure facilities such as microneurography, microdialyis, a metabolic chamber, normobaric hypoxia facilities, and tools for invasive hemodynamic monitoring. Available imaging technologies include ultrasound, echocardiography, Doppler tools, and access to high-field MRI. Personnel include study nurses, dietitians, biostatisticians, and ancillaries. The unit also has an erstwhile bone marrow transplant unit with appropriate safeguards. A "good manufacturing practice" (GMP) laboratory has been

established to provide cellular therapies. At present, preparations are underway to launch a Phase I study for tumor immune therapy using dendritic cells, financed by the Helmholtz Association.

CRC physicians are trained in internal medicine, neurology, clinical pharmacology, and oftentimes a combination of these specialties. Credit towards certification in internal medicine, neurology, and clinical pharmacology is dependent upon German Medical Society (Ärztekammer) approval.

The CRC encompasses not only clinical research group projects but will cooperate closely with the Helmholtz Epidemiological Cohort study, for which 40,000 human subjects will be recruited from the Berlin region, from a total of 200,000 who are being recruited throughout Germany. Tobias Pischon, MD, who heads the new Department of Epidemiology at the MDC leads this endeavor. His activities are located in the ECRC building.

The ultrahigh-field MR facility was opened in January 2009. The facility was recently enlarged to include a second floor housing a new 3 Tesla clinical scanner, in addition to a 9.4 Tesla animal scanner and a 7 Tesla whole body scanner, resulting in a total of 913 square m of research space for MR technologies. Thoralf Niendorf was appointed director of the facility in 2009. A physicist by training, he is a leading expert in MR imaging with research experience in both academia and industry.

To house the ERC, the MDC began construction of a new building in close proximity to the MR facility in spring 2010. The building will be completed in early 2012. The ERC building will comprise ca. 2600 m^2 of highly flexible laboratory and office space for translational research projects.

Educational mission

An important motivation for the project is the need for a new breed of researcher: a "physician-scientist" equally at home in the clinic and the basic research laboratory. Translational research involves all levels of biological organization, from the structures and functions of single molecules to the overall health of an organism over the long term, Physicians and basic researchers have complementary perspectives and expertise that need to be combined, ideally in a single person, to design meaningful basic experiments that will shed light on disease processes in patients.

The ECRC will provide vital infrastructure for some other projects that are currently being planned, such as the establishment of a German Center for Cardiovascular Research (*Deutsches Zentrum für Kardiovaskuläre Forschung*) and a German Institute for Cardiovascular Research (*Deutsches Institut für Kardiovaskuläre Forschung*), in which both the MDC and Charité will participate.

Structure of the ECRC

Director
Friedrich C. Luft

Chief Administrator
Regina Jünger

Program Manager
Cornelia Maurer

Steering committee
Walter Rosenthal (MDC Director) Annette Grüters-Kieslich (Charité Dean) Cornelia Lanz (MDC Administrative Director) Gerrit Fleige (Administrative Head of the Charité Faculty)

ECRC Council (chairman)
Thomas Sommer

Selected publications of ECRC research groups

Sack, U, Walther, W, Scudiero, D, Selby, M, Kobelt, D, Lemm, M, Fichtner, I, Schlag, PM, Shoemaker, RH, Stein, U (2011) Novel effect of antihelminthic Niclosamide on S100A4-mediated metastatic progression in colon cancer. J Natl Cancer Inst. 103, 1018-36.

Steininger, A, Möbs, M, Ullmann, R, Köchert, K, Kreher, S, Lamprecht, B, Anagnostopoulos, I, Hummel, M, Richter, J, Beyer, M, Janz, M, Klemke, CD, Stein, H, Dörken, B, Sterry, W, Schrock, E, Mathas, S, Assaf, C (2011) Genomic loss of the putative tumor suppressor gene E2A in human lymphoma. J Exp Med. 208, 1585-93.

Zelarayán, LC, Noack, C, Sekkali, B, Kmecova, J, Gehrke, C, Renger, A, Zafiriou, MP, van der Nagel, R, Dietz, R, de Windt, LJ, Balligand, JL, Bergmann, MW (2008) Beta-Catenin downregulation attenuates ischemic cardiac remodeling through enhanced resident precursor cell differentiation. Proc Natl Acad Sci U S A. 105, 19762-7.

Knoblauch, H, Geier, C, Adams, S, Budde, B, Rudolph, A, Zacharias, U, Schulz-Menger, J, Spuler, A, Yaou, RB, Nürnberg P, Voit, T, Bonne, G, Spuler, S (2010) Contractures and hypertrophic cardiomyopathy in a novel FHL1 mutation. Ann Neurol. 67, 136-40.

Lechner, SG, Markworth, S, Poole, K, Smith, ES, Lapatsina, L, Frahm, S, May, M, Pischke, S, Suzuki, M, Ibañez-Tallon, I, Luft, FC, Jordan, J, Lewin, GR (2011) The molecular and cellular identity of peripheral osmoreceptors. Neuron. 69, 332-44.

The Clinical Research Center (CRC) of the ECRC

Structure of the CRC

Physician-in-chief
Friedrich C. Luft (Internist, Nephrologist, Intensivist, Clinical Pharmacologist)

Michael Boschmann (Clinical Pharmacologist)
Verena Haas (Nutritionist)
Heidrun Mehling (Clinical Pharmacologist)
Jochen Steiniger (Human Physiologist)

Nadine Krüger (Study Nurse)
Gabriele Rahn (Study Engineer)
Manuela Stendal (Study Nurse)
Anke Strauss (Study Nurse)

Katja Giersch (Doctoral Student)
Anja Mähler (Doctoral Student)
Anna Pakula (Doctoral Student)

Research on human subjects

Patient/proband-oriented research is the final step in bringing medical innovations to humans or in revealing physiological or pathological mechanisms in man, namely "bench-to-beside or bedside-to-bench". A special unit (Clinical Research Center or CRC) that is separate from normal hospital wards is necessary for this work. The CRC is staffed with physicians, nurses, nutritionists, and human physiologists. The facility is available to any Charité group that can benefit from the facility and available expertise. Any approved form of research on human subjects is eligible for CRC support.

The CRC at work

Patient-oriented research is performed in hospitals or outpatient clinics. However, economic pressures, third-party payers, and concerns about cross-financing and interest conflicts have sorely limited human research in these settings. Phase III studies are generally feasible in hospital settings; however, Phase I and Phase II studies require special units outside of the health-care system. Furthermore, studies involving human physiology and disease mechanisms also require a separate setting. Since its founding in 2000, the CRC has produced over 100 peer-reviewed scientific investigations. The CRC features physicians and nurses with special expertise, nutritionists, human physiologists, biostatisticians, and if indicated, other disciplines for success. Our CRC has core personnel encompassing these skills. Furthermore, they are capable of providing certification to satisfy internal review boards (IRB; ethical committees) and regulatory agencies.

Our CRC is situated in an area formerly used as a hospital ward and bone marrow transplant unit. This provides superb facilities suitable for the most demanding studies. We have focused on outpatient and "day" studies, although inpatient investigations could be performed with sufficient prior notice to mobilize the necessary personnel. The primary focus has been on cardiovascular regulation and metabolism. However, any discipline of patient-oriented research is welcome. We are also able to transfer CRC expertise to other Charité sites. Thus, the CRC recently participated in intensive care unit research on critical illness myopathy performed on the Charité Virchow Campus. Access to the CRC facility requires an IRB-approved protocol and funding source, and is subject to peer review by the ECRC steering committee.

The CRC has unique facilities that are not available anywhere else in Germany. One example is a chamber to adjust the inspired oxygen fraction (FIO2) under normobaric conditions. We use this facility to address hypoxia-inducible factor-activated signaling mechanisms. We perform microdialysis to study metabolism at a tissue level in skin, adipose tissue, and skeletal muscle. We have groups able to biopsy these tissues for cell and molecular studies. We recently brought into service a metabolic chamber complete with live-in and exercise

Photographer David Ausserhofer, Copyright MDC

Prof. Dr. Friedrich Luft (ECRC Director) demonstrates the 7-Tesla MR whole body scanner at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.

Prof. Dr. Friedrich Luft (ECRC-Direktor) bei einer Demonstration des 7-Tesla-Magnetresonanztomographen des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.

facilities. This chamber is coupled with mass spectrometry-determined gas measurements so that we can measure metabolism precisely. We perform microneurography, tilt-table lower-body negative pressure, beat-by-beat blood pressure and heart rate measurements, and a complete array of autonomic testing. CRC investigators are particularly interested in cardiac function and therefore we are closely allied to ECRC clinical investigators interested in magnetic resonance imaging and spectroscopy.

The CRC has been the site of numerous investigations into the cardiovascular and metabolic effects of water, leading to the anatomic definition of a novel autonomic spinal reflex sensed by an hepatic osmoreceptor cloned at the MDC (Gary Lewin). Other recent study highlights include insights into metabolic disturbances in patients with Dunnigan's lipodystrophy (LMNA mutations), a biopsy and microdialysis study in the framework of our skeletal muscle research group (KFO192). Additionally, CRC investigators have enrolled a total of 170 overweight and obese, otherwise healthy subjects, who were randomized to either reduced carbohydrate or reduced fat, total energy restricted diet (-30% of energy intake before diet) for 6 months. Body composition was estimated by bioimpedance analyses and abdominal fat distribution by magnetic resonance tomography. Sensitivity to insulin was carefully documented. Subjects were also submitted to fat spectroscopy of liver and oral glucose tolerance testing. In all, 102 subjects completed the diet intervention with measurements of intrahepatic lipid content. Both hypocaloric diets decreased body weight, total body fat, visceral fat, and intrahepatic lipid content. The results showed that a prolonged hypocaloric diet low in carbohydrates and high in fat has the same beneficial effects on intrahepatic lipid accumulation as the traditional low-fat hypocaloric diet. The decrease in intrahepatic lipids appears to be independent of visceral fat loss and is not tightly coupled to changes in whole body insulin sensitivity during 6 months of an energy-restricted diet. The subjects are being followed to watch these trends over the long term. A further focus of CRC investigators has been cardiovascular regulation. The CRC was pivotal in a novel European intervention trial investigating baroreflex activation using a pacemaker-like device. CRC investigators also established the mechanisms by which this therapy might have clinical utility.

Personalia

We congratulate CRC alumni Jens Jordan, Jens Tank, Stefan Engeli, Sven Haufe, and Christoph Schröder (CRC graduates), who have founded a Department of Clinical Pharmacology at the Hannover Medical School (MHH). We welcome Joachim Spranger and Knut Mai from the Department of Endocrinology at the Charité, who now join the ECRC and who have major interests in CRC endeavors.

Recent publications

Boschmann M, Engeli S, Moro C, Luedtke A, Adams F, Gorzelniak K, Rahn G, Mähler A, Dobberstein K, Krüger A, Schmidt S, Spuler S, Luft FC, Smith SR, Schmidt HH, Jordan J (2010). LMNA mutations, skeletal muscle lipid metabolism, and insulin resistance. J Clin Endocrinol Metab. 95, 1634-43.

Haufe S, Engeli S, Budziarek P, Utz W, Schulz-Menger J, Hermsdorf M, Wiesner S, Otto C, Haas V, de Greiff A, Luft FC, Boschmann M, Jordan J (2010) Cardiorespiratory fitness and insulin sensitivity in overweight or obese subjects may be linked through intrahepatic lipid content. Diabetes. 59, 1640-7.

Tank J, Heusser K, Diedrich A, Luft FC, Jordan J (2010) A novel pharmacological approach to determining parasympathetic heart rate reserve in human subjects. Clin Pharmacol Ther. 88, 630-3.

Scheffers IJ, Kroon AA, Schmidli J, Jordan J, Tordoir JJ, Mohaupt MG, Luft FC, Haller H, Menne J, Engeli S, Ceral J, Eckert S, Erglis A, Narkiewicz K, Philipp T, de Leeuw PW (2010) Novel baroreflex activation therapy in resistant hypertension: results of a European multi-center feasibility study. J Am Coll Cardiol. 56, 1254-8.

Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, Hermsdorf M, Mähler A, Wiesner S, Birkenfeld AL, Sell H, Otto C, Mehling H, Luft FC, Eckel J, Schulz-Menger J, Boschmann M, Jordan J (2011) Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. Hepatology. 53, 1504-14.

Simone Spuler

Muscle Research Unit, Clinical Research Group, and MyoGrad

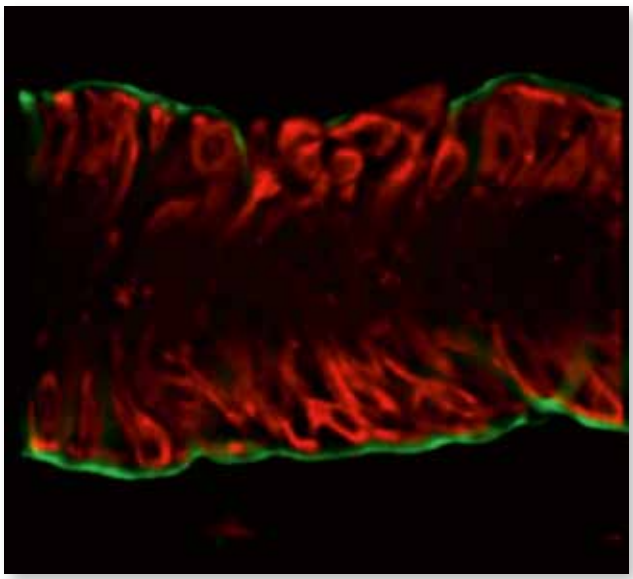
Simone Spuler directs the skeletal muscle disease program project (KFO192, Speaker, Friedrich C. Luft) for the entire Charité. Much of the research activities are located at the ECRC. The outpatient clinic at the ECRC cares for >1000 patients with genetic and acquired skeletal muscle diseases. In 2010, KFO192 was complemented by an international research-training grant for myology, "MyoGrad" (GK1631). This unique training grant crosses national boundaries and unites the Institute de Myologie in Paris, University Paris VI (French Speaker, Thomas Voit), with the Charité and the MDC (German Speaker, Simone Spuler). The dysferlinopathies are the main scientific focus of Simone Spuler's group. Mutations in the gene encoding dysferlin lead to adult-onset progressive proximal and distal muscle weakness and loss of walking ability within 10-15 years. Dysferlin mutations are responsible for most limb-girdle muscular dystrophies. There is no treatment.

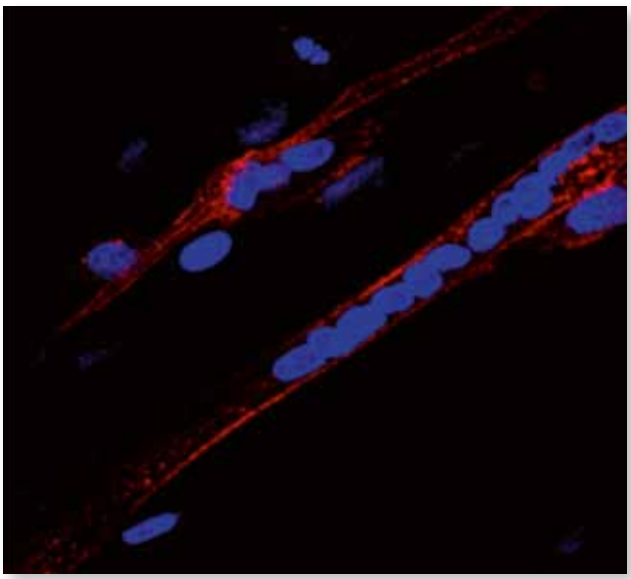
Dysferlinopathies

Dysferlin, a large protein located at the sarcolemma, is involved in membrane repair after microinjury, a physiological consequence of normal muscle activity. Inflammatory changes accompany the dysferlinopathies. We observed that the complement inhibitory factor CD55 is selectively downregulated on dysferlin-deficient skeletal muscle, leading to increased vulnerability to complement attacks. As a result, complement-modulating therapies are being developed. We will head the first international complement-directed treatment trial for dysferlinopathies in 2012. We found that dysferlin mutations lead to misfolding and aggregation of dysferlin and muscle amyloidosis. Dysferlinopathies thereby resemble other protein-misfolding diseases, such as Alzheimer's disease and Parkinson's disease. Verena Schöwel is rescuing missense-mutated dysferlin from degradation and instead relocating the molecule to the plasma membrane. Ute Zacharias is investigating the effect of the potent modulator of skeletal muscle growth, myostatin, on dysferlin-deficient human myotubes. These efforts could lead to novel treatments.

Dystrophies and metabolism

Muscular dystrophies are closely related to generalized disturbances in metabolism. The role of muscle in metabolism (largest bodily organ) is so obvious that muscle diseases were previously considered to be endocrine disorders. Perhaps this idea was prescient. Today, with numerous genes identified, we have good evidence that myopathies are very closely related to metabolic disorders. For instance, patients with Dunnigan's disease feature partial lipodystrophy, early-onset type-2 diabetes mellitus, and non-alcoholic steatosis hepatis. Our group, in conjunction with CRC investigators, has focused on these connections in detail.





Figures: **Left:** Human muscle fiber with proliferating satellite cells. **Right:** Human myotube stained for dysferlin (red) and Hoechst stain (blue)

Satellite cells

Muscle satellite cells have the full potential to proliferate, differentiate, and conduct repair. After muscle injury, these characteristics are highly important. However in tissue culture, these cells rapidly lose these properties. Stephanie Adam isolates and characterizes these unique human satellite cells. She and Carmen Birchmeier at the MDC are focusing in particular on Notch and Wnt signaling pathways. The potential of this work is particularly relevant for the ECRC, since we have available a GMP stem cell laboratory. The laboratory is a core facility for all disciplines at the ECRC, MDC, and Charité. When the time comes, we will "translate", no doubt about it!

We are also interested in acquired myopathies. We have a program within KFG190 to investigate 3-hydroxy-3-methylglutaryl-coenzym-A-reductase inhibitors (statins) and the production of myopathy. Indeed, this drug may be the most common cause of acquired myopathies. We have made inroads on this problem and are now conducting large-scale genetic screens of this disorder with "lipidologists" at the Charité.

Primary funding sources

Deutsche Forschungsgemeinschaft (KFO192 and GK1631).

Selected Publications

Spuler S, Carl M, Zabojszcza J, Straub V, Bushby K, Moore SA, Bähring S, Wenzel K, Vinkemeier U, Rocken C. Dysferlin deficient muscular dystrophy features amyloidosis. *Ann Neurol*, 2008, 63: 323-328

Utz W, Schmidt S, Schulz-Menger J, Luft FC, Spuler S. Cardiac involvement in sporadic inclusion body myositis. *Circulation*, 2010; 121: 706-8

Knoblauch H, Geier C, Adams S, Budde B, Rudolph A, Schulz-Menger J, Spuler A, Ben Yaou R, Nürnberg, P, Voit T, Bonne G, Spuler S. Contractures and hypertrophic cardiomyopathy in a novel FHL1 mutation. *Ann Neurol*, 2010; 67:136–140

Rajab A, Straub V, Seelow D, Varon R, Barresi R, Schulze A, Lucke B, Lützkendorf S, Mohsen K, McCann LJ, Spuler S, Schuelke M. *PTRF-CAVIN* mutations cause generalized caveolopathy with lipodystrophy, rippling muscle disease, and complex cardiac arrhythmias. *PLoS Genetics*, 2010, 6:e1000874

Knoblauch H*, Schöwel V*, Kress W, Kassner U, Spuler S. Another side to statin related side effects. *Ann Int Med,* 2010; 152:478-479

Structure of the Group

Group Leader

Prof. Dr. med. Simone Spuler

Scientists

Dr. med. vet. Stephanie Adams
Lena Brandlhuber (PhD student)
Dr. med. Ulrike Grieben
Dr. rer. nat. Stefanie Grunwald
Christian Herrmann
(M.D., graduate student)
Sarah Keller
(M.D., graduate student)
Séverine Kunz (Ph.D. student)
Dr. rer. nat. Andreas Marg
Susanne Philippi (PhD student)
Dr. med. Verena Schöwel
Dr. Ing. Tobias Timmel
Joanna Schneider
(M.D., graduate student)
Dr. rer.nat. Katrin Wenzel
PD Dr. rer. nat. Ute Zacharias

Technicians

Kornelia Gräning
Stephanie Meyer

Manager of sponsored programs

Susanne Wissler

Ralph Kettritz

Neutrophil Biology in Vascular Diseases

Neutrophil biology in vascular diseases

The group is interested in molecular mechanisms that control the functioning of neutrophils. Neutrophils not only protect the host from infection, but also participate in autoimmunity, cardiovascular diseases, and cancer. We care for patients with a systemic necrotizing vasculitis. Affected patients develop life-threatening pulmonary hemorrhage, upper-airway destruction, and renal failure, all from small-vessel vasculitis. The small-vessel vasculitis is caused by autoantibodies called anti-neutrophil cytoplasmic autoantibodies (ANCA). The neutrophil harbors not only the target ANCA antigens, proteinase 3 (PR3) and myeloperoxidase (MPO), but also is the major effector cell mediating the vascular damage. Standard treatment with steroids and cytotoxic agents has greatly improved survival; however, the treatment is toxic and better options are sorely needed. Improved understanding of disease mechanisms will help to identify novel treatment targets and improve outcomes. Above-and-beyond ANCA vasculitis, we are interested in all aspects of neutrophil biology.

CD177 and PR3 form a larger signaling complex that allows neutrophil activation by PR3-ANCA

We discovered that the ANCA antigen, PR3, is expressed on the membrane surface of a neutrophil subset via the GPI-anchored CD177 (neutrophil antigen B1, NB1). However, transmembrane molecules are needed to initiate signal transduction and cell activation. In collaboration with our partners in the MDC proteomics facility, we identified several candidates including the heterodimeric transmembrane receptor Mac-1 (CD11b/CD18). We showed co-localization, co-immunoprecipitation and direct interaction of NB1 and Mac-1 using surface plasmon resonance analysis (SPR). Interestingly, SPR showed direct protein-protein interactions with both CD11b and CD11a. In contrast, when these integrins were presented as heterodimeric transmembrane proteins on transfected cells, only CD11b/CD18 (Mac-1) transfected cells adhered to immobilized NB1 protein. We subsequently establish a functional role of the NB1-Mac-1 receptor interaction for PR3-ANCA mediated neutrophil activation.

We performed a high-throughput screen together with J. P. von Kries at the FMP to identify compounds that inhibit the NB1-PR3 interaction. We generated NB1-transfected HEK293 cells that expressed the NB1 receptor on the cell surface. We observed that two selected compounds demonstrated a dose-dependent inhibition of PR3 binding to NB1 that was also seen when human neutrophils were used. In contrast, the compounds did not decrease mPR3 expression on resting neutrophils, nor did they prevent a TNF-α-mediated increase in mPR3 on $NB1^{pos}$ neutrophils, suggesting that binding of degranulated PR3 to NB1-expressing neutrophils plays no major role in activation-mediated increased mPR3 expression.

A mouse model allows testing novel mechanisms and treatment targets

We established an animal model for ANCA-induced necrotizing crescentic glomerulonephritis (NCGN). We tested the hypothesis that the PI3Kinase gamma isoform (PI3Kγ) is pivotal to ANCA-activated neutrophil

functions and in disease induction. We found that specific PI3Kγ inhibition by the small molecule AS605240 abrogated ANCA-induced respiratory burst, degranulation, and GM-CSF-mediated neutrophil migration. Moreover, we observed that bone marrow transplantation from PI3Kγ$^{-/-}$ mice protected from NCGN and that oral treatment of WT bone marrow transplanted mice with AS605240 was also protective. These data suggest that specific inhibition of PI3Kγ may provide a novel treatment target.

ANCA are generated by plasma cells. Thus, we hypothesized that the proteasome inhibitor bortezomib (BTZ) reduces MPO-specific plasma cells and thereby protects from NCGN. After disease induction, mice either received a steroid/cyclophosphamide (S/CYC) combination or BTZ, respectively. All untreated control mice developed urine abnormalities and NCGN. S/CYC and BTZ significantly reduced urine abnormalities, NCGN, and leukocyte infiltration. BTZ significantly diminished total and MPO-specific plasma cells in spleen and bone marrow whereas B lymphocytes, total, naive and effector CD4, and CD8 T cells were spared. We show the utility of the mouse model to compare efficiency and adverse events of new drugs to standard protocols.

Does the neutrophil participate in Renin-Angiotensin-Aldosterone mediated effects?

Mineralocorticoid receptor (MR) activation by aldosterone controls salt homeostasis and inflammation in several tissues and cell types. We investigated the hypothesis that aldosterone modulates inflammatory neutrophil responses via the MR. We found that neutrophils possess MR. Preincubation with aldosterone dose-dependently inhibited NF-κB activation in IL-8 and GM-CSF treated neutrophils on fibronectin. Aldosterone had no effect on TNF- and LPS-mediated NF-κB activation or on IL-8 and GM-CSF induced ERK, p38 MAPK, and PI3K/Akt activation. Spironolactone prevented NF-κB inhibition, indicating an MR-specific aldosterone effect. Aldosterone completely prevented NF-κB-dependent TNFα, generation by IL-8. Conditioned medium from IL-8-treated neutrophils increased ICAM-1 expression on endothelial cells and subsequently the adhesion of IL-8-treated neutrophils to endothelial cells. These effects were reduced when conditioned medium from aldosterone-pretreated neutrophils was used and spironolactone blocked the aldosterone effect. Our data indicate that a functional MR exists in neutrophils controlling inflammation.

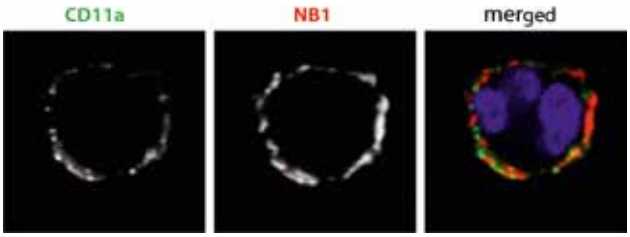


Figure 1. The PR3 receptor NB1 forms a signaling complex with the b2-integrin Mac-1 on the neutrophil membrane.

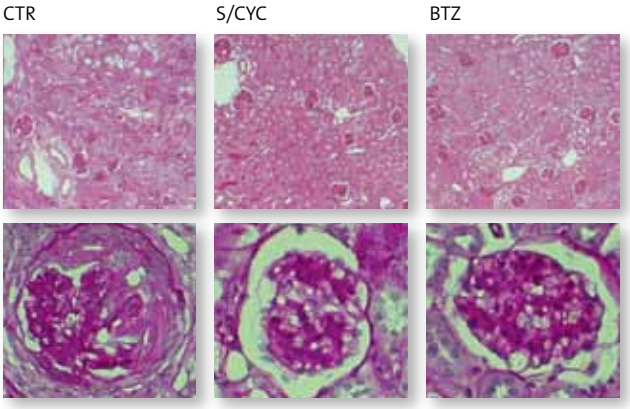


Figure 2. Proteosome inhibition by Bortezomib protects from necrotizing crescentic glomerulonephritis in a ANCA mouse model.

Selected Publications

Jerke, U., Rolle, S., Dittmar, G., Bayat, B., Santoso, S., Sporbert, A., Luft, F., and Kettritz, R. (2011). Complement Receptor Mac-1 Is an Adaptor for NB1 (CD177)-mediated PR3-ANCA Neutrophil Activation. J Biol Chem *286*, 7070-7081.

Bontscho, J., Schreiber, A., Manz, R.A., Schneider, W., Luft, F.C., and Kettritz, R. (2011). Myeloperoxidase-specific plasma cell depletion by bortezomib protects from anti-neutrophil cytoplasmic autoantibodies-induced glomerulonephritis. Journal of the American Society of Nephrology: JASN *22*, 336-348.

Bergmann, A., Eulenberg, C., Wellner, M., Rolle, S., Luft, F., and Kettritz, R. (2010). Aldosterone abrogates nuclear factor kappaB-mediated tumor necrosis factor alpha production in human neutrophils via the mineralocorticoid receptor. Hypertension *55*, 370-379.

Choi, M., Eulenberg, C., Rolle, S., von Kries, J.P., Luft, F.C., and Kettritz, R. (2010). The use of small molecule high-throughput screening to identify inhibitors of the proteinase 3-NB1 interaction. Clin Exp Immunol *161*, 389-396.

Schreiber, A., Rolle, S., Peripelittchenko, L., Rademann, J., Schneider, W., Luft, F.C., and Kettritz, R. (2010). Phosphoinositol 3-kinase-gamma mediates antineutrophil cytoplasmic autoantibody-induced glomerulonephritis. Kidney Int *77*, 118-128.

Structure of the Group

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Technical Assistants
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Cardiovascular Magnetic Resonance

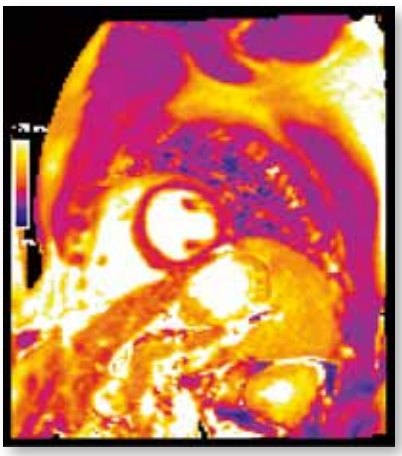
The CMR group at the Experimental Clinical Research Center (ECRC) and Clinics for Cardiology and Nephrology of the HELIOS Clinics Berlin Buch has focused its research on the in vivo assessment of functional and structural myocardial abnormalities related to inflammatory diseases and coronary heart disease. We are focusing on clinical research using a 1.5 clinical MRI-scanner with dedicated cardiac software. We developed new approaches for the differentiation of tissue changes in myocardial diseases. The application as research tools and the translation into a clinical setting are the main interests of the group. The successful work led to the introduction of the University Professorship in Cardiology for Noninvasive Imaging focused on Cardiovascular magnetic resonance (CMR).

Since 2009 the group was able to expand their research activities by developing translational research tools applying advanced imaging modalities in close collaboration with the Berlin Ultra-High-Field Facility at MDC.

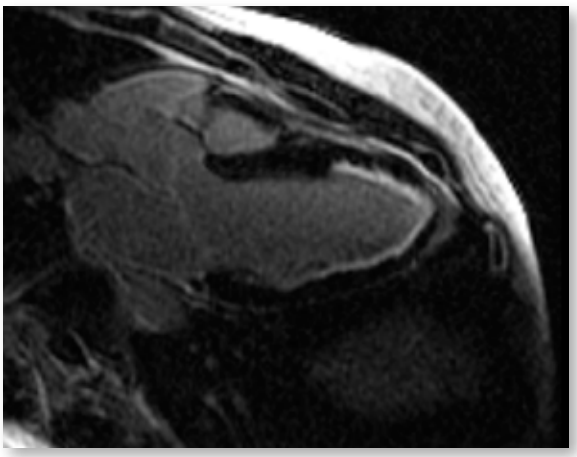
Myocardial Injury

The diagnosis of myocardial inflammation in different diseases and the assessment of myocardial tissue changes during follow-up is a challenging task in cardiovascular research and clinical cardiology. Clinical presentation of patients with myocarditis or myocardial involvement in systemic disorders often mimics other disorders and may vary from flu-like symptoms or subclinical disease to acute heart failure and sudden cardiac death. Cardiovascular magnetic resonance (CMR) has the capability to differentiate between the various forms of myocardial injuries (e.g. edema, hyperemia and fibrosis). In the late 90s we developed an approach for the noninvasive detection of acute myocarditis by CMR. In 2008/09 we published the noninvasive detection of myocardial edema and the capability of a multi-sequential approach for follow-up. Myocardial inflammation also has a high prognostic impact in different systemic diseases. However early assessment is difficult. We applied CMR technology in patients with different systemic disorders including Churg Strauss syndrome and were able to detect myocardial involvement already patients with preserved left-ventricular function. Interestingly, applying that approach it is also possible to detect subtle reversible myocardial injury after bindge drinking. It is well known, that that development of fibrosis is of potential prognostic impact in hypertrophy. Using contrast enhanced CMR we were able do differentiate the pattern in various non-ischemic disease and visualize fibrotic tissue in hypertrophy caused by different diseases.

Furthermore we tried to get insight the gender-related remodeling process in hypertrophic cardiomyopathy.



Cardiac short axis view: Quantitative T2-mapping



long axis view: contrast-enhanced CMR showing subendocardial scar

Coronary Artery Disease and Arteriosclerosis

The detection of coronary artery stenosis is a growing field in CMR. Quantitative analysis of perfusion is a crucial step for evaluation of significant ischemia and development of new therapeutic strategies. In 2009 we could show that the lipid-apheresis has an impact on myocardial perfusion in young patients with elevated LPa. In 2009 we published, that the quantitative dual bolus perfusion is of higher accuracy having the potential to reduce the sample size. The field is our step into Ultra-High-Field research. In collaboration with the University of Oxford we published our first paper on CMR-driven infarct-detection in mices. Recently, we could show that the CMR-driven quantification of scar chronic myocardial infarction can identify high risk patients.

Cardiac MR at Ultra Highfield

The close collaboration with BUFF at MDC allowed to publish the world-wide first paper assessing cardiac function in volunteers at 7.0Tesla.

Based on further technical improvement new noninvasive steps into myocardium will take place in a strong collaboration with the group of the Ultra-Highfield-Facility

Selected Publications

Zagrosek A, Abdel-Aty H, Boye P, Wassmuth R, Messroghli D, Utz W, Rudolph A, Bohl S, Dietz R, Schulz-Menger J. Cardiac magnetic resonance monitors reversible and irreversible myocardial injury in myocarditis. *JACC Cardiovasc Imaging*. 2009;2(2):131-138.

Zagrosek A, Messroghli D, Schulz O, Dietz R, Schulz-Menger J. Effect of binge drinking on the heart as assessed by cardiac magnetic resonance imaging. *JAMA*. 2010;304(12):1328-1330.

Rudolph A, Abdel-Aty H, Bohl S, Boye P, Zagrosek A, Dietz R, Schulz-Menger J. Noninvasive detection of fibrosis applying contrast-enhanced cardiac magnetic resonance in different forms of left ventricular hypertrophy relation to remodeling. *J Am Coll Cardiol*. 2009;53(3): 284-291.

Bohl S, Medway DJ, Schulz-Menger J, Schneider JE, Neubauer S, Lygate CA. Refined approach for quantification of in vivo ischemia-reperfusion injury in the mouse heart. *Am J Physiol Heart Circ Physiol*. 2009;297(6):H2054-2058.

Boyé P, Abdel-Aty H, Zacharzowsky U, Bohl S, Schwenke C, van der Geest RJ, Dietz R, Schirdewan A, Schulz-Menger J. Prediction of life-threatening arrhythmic events in patients with chronic myocardial infarction by contrast-enhanced CMR. JACC Cardiovasc Imaging. 2011 Aug;4(8):871-9.

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Maik Gollasch

Blood Vessel Function and Target-Organ Damage

Current projects

Our group focuses on ion channels, primarily in vascular smooth muscle cells (VSMC), to clarify mechanisms contributing to hypertension and cardiovascular disease. Calcium-activated potassium channels (K_{Ca}) and transient receptor potential (TRP) channels have received special attention. We investigated the role of TRP ion channels in agonist-independent $G_{q/11}$ protein-coupled receptor activation. We showed that $G_{q/11}$-coupled receptors function as membrane stretch receptors in VSMC. We are currently studying angiotensin (Ang) II type 1a receptors ($AT_{1a}R$) and ryanodine receptor isoforms (Figure 1) in VSMC myogenic tone. Michael Bader is helping us here. We collaborate with Wolf-Hagen Schunck, who has peaked our interests in eicosanoids. We found that epoxyeicosatrienoic acids are vasodilatory, largely through their ability to activate endothelial NO synthase and NO release. We also collaborate with Huang Yu, Hong Kong, China. We found that endothelium-derived contracting factors (EDCF) depend on cyclooxygenase-2 and induce inflammation. We also have a project focusing on the vascular adventia as a source for relaxing factors. Finally, in collaboration with the German Institute of Human Nutrition (DifE), we are studying human diabetic nephropathy with a focus on genetics.

TRP channels

TRPC1 and TRPV1 channels are expressed in the vasculature. We used TRPC1 deficient mice and found that TRPC1 downregulates endothelial-derived hyperpolarizing factor (EDHF)-type vasodilatations and thereby contribute to blood pressure regulation. The findings suggest that TRPC1 inhibition may lower blood pressure. We have also examined TRPV1 channels in regulating renal blood flow. TRPV1 channels and their natural activator 20-HETE may contribute to ischemia/reperfusion (I/R) -induced kidney injury.

Eicosanoids

EETs serve as endothelial-derived hyperpolarizing factors (EDHF), but may also affect vascular function by other mechanisms. Our current research in this area is directed towards identifying red blood cells as source of vasodilatory EETs and natural inhibitors of sEH/TRPV1 channels. Hypertension and vascular dysfunction result in the increased release of EDCF. We found that endothelial cyclooxygenase (COX)-2 can generate EDCF and identified the possible EDCF candidate. The results support a role for prostaglandin (PG)F(2alpha) in endothelium-dependent contractions. We also identified protein kinase Cδ and cytokine monocyte chemoattractant protein-1 participation in these processes.

Vasodilator signals from perivascular adipose tissue

We have identified a vasorelaxing factor produced in the vascular advential (ADRF). Our recent work showed that KCNQ (K_v7) 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. Alterations in the paracrine control of arterial tone by periadventitial adipose tissue have been found in animal models of hypertension and metabolic disease. Cystathionine gamma-lyase deficient mice are

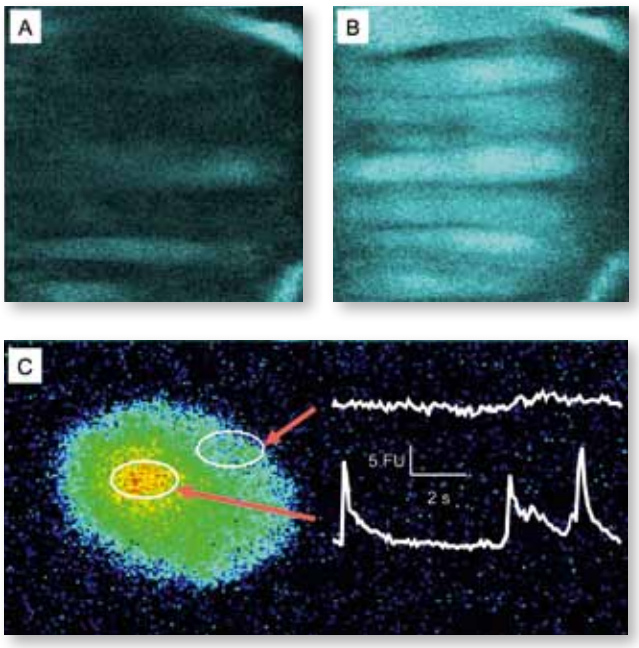

Figure 1. Intracellular calcium release events visualized by calcium sensitive dyes in the vessel wall of intact resistance arteries (panels A and B) and in freshly-isolated arterial smooth muscle cells (panel C) of mice. Global calcium elevations cause vasoconstriction (panels A, B) whereas calcium sparks (panel C) control vasodilation. Both calcium signals are caused by opening of ryanodine receptor calcium release channels in smooth muscle. 2-D calcium sparks are measured by Nipkow spinning disc laser scanning microscopy.

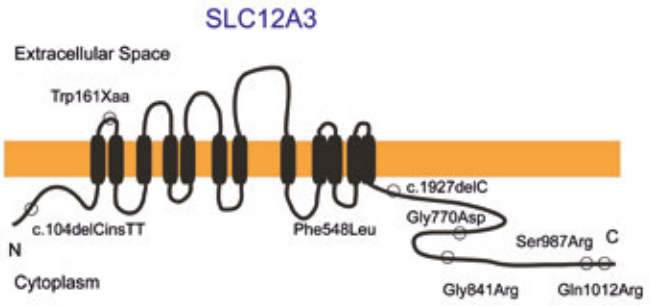

Figure 2. Mutations detected in symptomatic patients with Gitelman syndrome.

available to us to clarify the role of H_2S. ADRF, or perhaps its putative targets, might represent exciting new targets for the development of drugs for treatment of cardiovascular and metabolic disorders.

Genetic renal diseases

An outgrowth of Maik Gollasch's clinical responsiblities has been a focus on clinical genetics related to renal diseases. We discovered novel SLC12A3 mutations in symptomatic patients with Gitelman syndrome (Figure 2) and mutations in CLDN16 in familial hypomagnesemia with hypercalciuria and nephrocalcinosis. We have performed functional analyses of mutations causing familiar kidney diseases with specific emphasis on TRPC6 channels in focal and segmental glomerulosclerosis (FSGS). In collaboration with the German Institute of Human Nutrition, we are studying genetic factors, which contribute to human diabetic nephropathy. For this purpose, we recently established the Registry of Diabetic Nephropathy (http://www.charite-buch.de/rdn/), and are contineously recruiting patients for our study.

Primary funding

The Deutsche Forschungsgemeinschaft (Go 766/15-1, GO 766/13_1, GO 766/12-1) and cooperative projects between the MDC and ECRC have funded this work.

Selected Publications

Wong SL, Lau CW, Wong WT, Xu A, Au CL, Ng CF, Ng SS, Gollasch M, Yao X, Huang Y. Pivotal role of protein kinase Cdelta in angiotensin II-induced endothelicyclooxygenase-2 expression: a link to vascular inflammation. Arterioscler Thromb Vasc Biol. 2011 May;31(5):1169-76.

Wong SL, Leung FP, Lau CW, Au CL, Yung LM, Yao X, Chen ZY, Vanhoutte PM, Gollasch M, Huang Y. Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with increased impact during aging. Circ Res. 2009 Jan 30;104(2):228-35.

Roser M, Eibl N, Eisenhaber B, Seringer J, Nagel M, Nagorka S, Luft FC, Frei U, Gollasch M. Gitelman syndrome. Hypertension. 2009 Jun;53(6):893-7.

Essin K, Gollasch M, Rolle S, Weissgerber P, Sausbier M, Bohn E, Autenrieth IB, Ruth P, Luft FC, Nauseef WM, Kettritz R. BK channels in innate immune functions of neutrophils and macrophages. Blood. 2009 Feb 5;113(6):1326-31.

Mederos y Schnitzler M, Storch U, Meibers S, Nurwakagari P, Breit A, Essin K, Gollasch M, Gudermann T. Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. EMBO J. 2008 Dec 3;27(23):3092-103

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Galyna Dubrovska
Oluwatosin Adaramoye (guest scientist)
Friedrich C. Luft (advisor)

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Yoland-Marie Anistan

Silke Rickert-Sperling

Start of the group: April 2011

Cardiovascular Genetics

Most cardiovascular disease have complex genetic and environmental origins. Our lab studies molecular mechanisms underlying cardiac development and function using molecular biological techniques and bioinformatics expertise. We focus on the transcriptional regulation process, which plays a key role for normal and abnormal cardiogenesis leading in the latter case to congenital heart disease (CHD). A rapidly growing number of factors have been shown to be involved in regulating the pattern and timing of expression of genes responsible for the cardiac lineage determination, heart chamber formation, valvulogenesis and conduction-system development. Spatiotemporal and quantitative regulation of cardiac transcription factors must occur in a precise manner to ensure fine regulation of downstream targets. However, the ability of transcription factor binding to DNA is highly influenced by the chromatin status and epigenetic mechanisms have an important role in establishing and maintaining transcriptional programs. To understand networks directing gene expression, the interplay between different transcription factors, co-regulatory elements and epigenetic factors has to be considered.

Regulation of cardiac gene expression

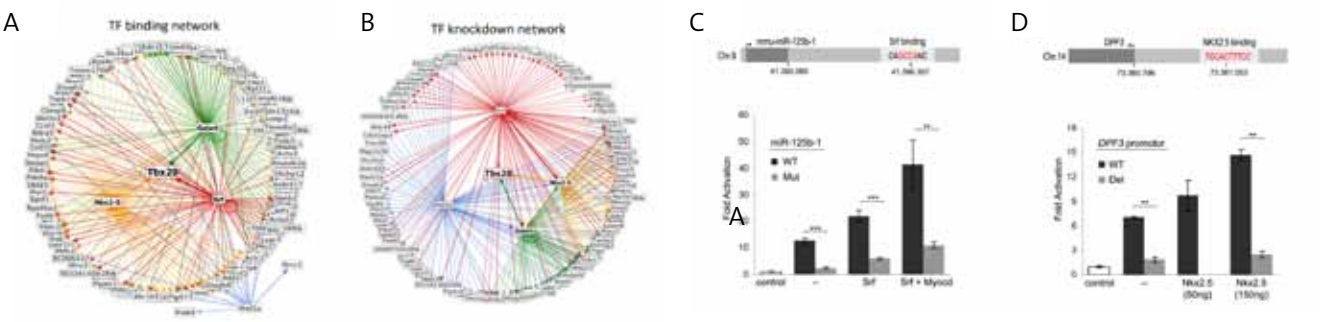
Jenny Schlesinger, Markus Schüler, Marcel Grunert, Vikas Bansal

We performed a systems biology study in murine cardiomyocytes integrating mRNA profiles with DNA–binding events of key cardiac transcription factors (Gata4, Mef2a, Nkx2.5, and Srf), activating histone modifications (H3ac, H4ac, H3K4me2, and H3K4me3), and microRNA profiles obtained in wild-type and RNAi–mediated knockdown. Even these non-paralogous transcription factors partially compensate each other's function. Srf and Gata4 driven gene expression is highly dependent on the co-presence of H3ac, whereas Nkx2.5 and Mef2a activate transcription in a H3ac independent manner. A significant proportion of indirect Srf downstream targets are potentially regulated through Srf-regulated microRNAs. These findings show the impact of interdependencies between different factors and layers of regulation on the cardiac transcriptome. Further functional tests are required to evaluate regulatory circuits on a single gene basis. It is of interest to study how the interplay of the different factors stabilizes the overall function of a given network, and how this contributes to the resistance to external disturbances as well as pathogenic mutations missing phenotypic impact.

Chromatin-remodelling factor DPF3 (BAF45c)

Martin Lange, Katherina Bellmann, Huanhuan Cui, Qin Zhang

We have identified the first chromatin-remodelling factor, namely DPF3 (BAF45c), which is capable of binding



Regulation of the cardiac transcriptome by combinatorial binding of transcription factors (TF) Gata4 (green), Mef2a (blue), Nkx2.5 (yellow) and Srf (red). Shown are in total 1671 genes, which are targets of at least one factor. **(A)** Direct downstream targets identified by chromatin-immunoprecipitation. **(B)** Direct and indirect downstream targets identified by siRNA knockdown of respective factors. Promoter analyses of miR-125b-1 **(C)** and DPF3 **(D)** using luciferase reporter gene assays show regulation by Srf and Nkx2.5 respectively.

acetylated as well as methylated histone modifications through its double plant-homeodomain (PHD-finger). Thus it bridges distinct regulatory signals of the histone code and enables a tissues-specific read-out based on its neural, cardiac and muscle specific expression. DPF3 is associated with the BAF chromatin-remodelling complex and came to our attention due to its differential expression in hypertrophic hearts of Tetralogy of Fallot. DPF3 is evolutionarily conserved and knockdown of dpf3 in zebrafish leads to incomplete cardiac looping and severely reduced ventricular contractility, with disassembled muscular fibers. Changes in chromatin structure and gene transcription are frequently induced by external stimuli. In particular phosphorylation of chromatin-associated proteins is mediated by different kinases, such as p38, CaMK and CKII, and represents a powerful interface for the transmission of extracellular signals to chromatin. So far, the upstream signalling pathway of DPF3 is undiscovered and a particular focus of our research.

Congenital heart disease

Cornelia Dorn, Martje Tönjes, Ilona Dunkel, Susanne Thomsen, Sascha Werner

Cardiac malformations represent a broad panel of in part overlapping phenotypes, reflecting the modular background of cardiogenesis. The biological network modifying the impact of key regulators is still widely a black box. One important lesson learned from the study of animal models, as well as patients with complex CHD is that our primary hope that one gene would simply refer to one phenotype is not fulfilled. Even one particular mutation can be associated with a panel of different cardiac malformations and the majority of CHD is not following Mendelian inheritance. Nevertheless, it is undoubtful that there is a clear genetic impact. We currently enter a novel era of research, which is characterized by fast evolving technological advances enabling the generation, study and interpretation of more and more complex data. This will allow the study of congenital heart disease in a manner, which provides insights into the underlying biological network influenced by genetic, epigenetic and environmental factors, and stochastic events.

Selected Publications

Schlesinger, J, Schueler, M, Grunert, M, Fischer, JJ, Zhang, Q, Krueger, T, Lange, M, Tönjes, M, Dunkel, I, Sperling, SR. (2011). The cardiac transcription network modulated by Gata4, Mef2a, Nkx2.5, Srf, histone modifications, and microRNAs. PLoS Genet. 7, e1001313.

Sperling, SR. (2011). Systems biology approaches to heart development and congenital heart disease. Cardiovasc Res. 91, 269–278.

Lange, M, Kaynak, B, Forster, UB, Tönjes, M, Fischer, JJ, Grimm, C, Schlesinger, J, Just, S, Dunkel, I, Krueger, T, Mebus, S, Lehrach, H, Lurz, R, Gobom, J, Rottbauer, W, Abdelilah-Seyfried, S, Sperling, S. (2008). Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. Genes Dev. 22, 2370–2384.

Toenjes, M, Schueler, M, Hammer, S, Pape, U, Fischer, J, Berger, F, Vingron, M, Sperling, S. (2008) Prediction of cardiac transcription networks based on molecular data and complex clinical phenotypes. Mol Biosyst, 4, 589–598.

Purmann, A, Toedling, J, Schueler, M, Carninci, P, Lehrach, H, Hayashizaki, Y, Huber, W, Sperling, S. (2007). Genomic organization of transcriptomes in mammals: Coregulation and cofunctionality. Genomics. 89, 580–587.

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Joachim Spranger

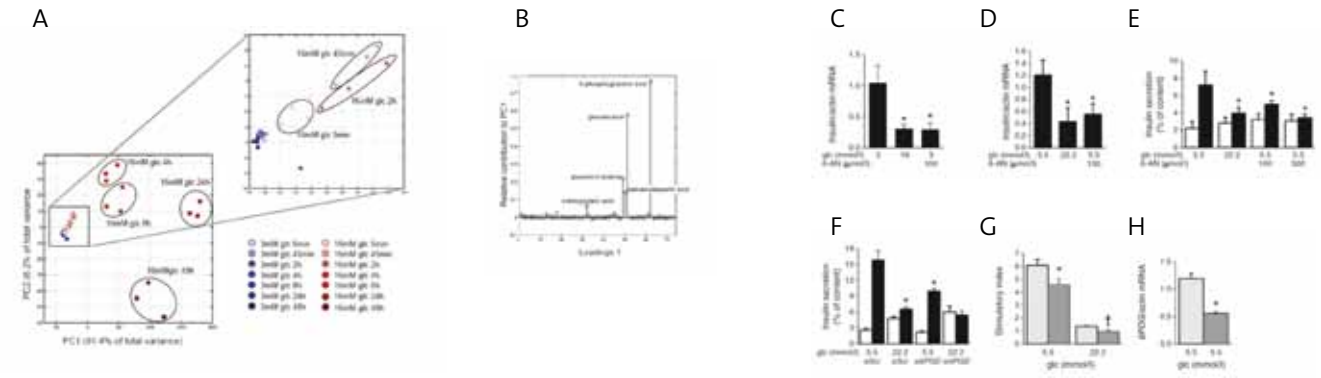
Start of the group: August 2011

Endocrinology, Diabetes and Nutrition

Ageing of the population as well as increases in the prevalence of obesity, type 2 diabetes, and physical inactivity contribute to the projection that cardiovascular disease but also cancer will remain among the major causes of death in Germany. Against the background of an individual genetic make-up, environmental risk factors interact with metabolic/endocrine pathways to finally modify disease risk. Apparently, the endogenous reaction to environmental stimuli depends on numerous non-modifiable factors such as age, gender, but also stage of disease and existing co-morbidities. Thus, an age-related metabolic inflexibility to exogenous stimuli is likely to contribute to metabolic and cardiovascular ageing. We therefore aim to identify novel and further elucidate known endogenous metabolic modifiers of cardio-metabolic health. We analyze the interrelation between environment, specific molecular targets and cardio-metabolic risk in relation to age and co-morbidities to finally translate those findings into preventive practice. The group interacts with several other research groups in Berlin and outside and is partner of several joint research projects from the DFG, BMBF and EU.

Metabolism and brain

Weight reduction is recommended in obese individuals at increased CV risk, but long-term consequences are unclear. Neuroendocrine counter-regulation may induce an unfavorable metabolic phenotype associated with an increased CV risk. We aim to elucidate the relevance of those central-nervous circuits by delineating the effects on metabolism and cardiovascular risk. A DFG-funded clinical research coordinated by members of this research group is devoted to investigate the endocrine regulation of body weight maintenance. Among others we currently perform a large human intervention trial investigating the relation of lifestyle to body weight maintenance. In collaboration with other partners from the MDC and Charite, we study hormonal regulators of energy metabolism by analysing the relevance of novel hormones such as thyronamines, but also the impact of estrogen receptor signaling and the role of miRNAs. Together with partners from the Charite Center for Advance Neuroimaging and the Bernstein Institute of Computational Neuroscience, we study behavioural measures associated to central-nervous reward systems using fMRI technology. Our group established experimentally deep brain stimulation of specific brain regions associated with the development of obesity and analyse short- and longterm effects of such treatment in diet- but also genetically obesity-prone mice. We hypothesize that the deep brain stimulation may be a useful therapeutic approach in morbidly obese individuals, specifically by modification of central-nervous reward systems.



The group made use of metabolite profiling technologies to predict disease status in prospective observation studies and to detect intracellular pathways associated with metabolic inflexibility. Exemplarily we were able to identify the Pentose Phosphate Pathway as one bottle neck affecting glucotoxic β-cell dysfunction (Göring et al, Diabetologia 2011).

Metabolites of the PPP drive differences in the metabolic intracellular patterns of INS-1E cells acutely and chronically treated with low (3 mmol/l, blue circles) and high (16 mmol/l red circles) glucose. PCA score plots (**A**) illustrate separation of samples according to culture conditions and exposure time. Each data point represents the entire metabolite profile of a sample. Loading plot (**B**) describes the contribution of each metabolite to PC1. The PPP metabolites glucono-δ-lactone, 6-phosphogluconic acid and gluconic acid, the pyrimidine pathway intermediate carbamyl-aspartic acid and the TCA cycle intermediate α-ketoglutaric acid were the most dominant factors driving the observed separation. Colour intensity of circles relates to duration of exposure, with lightest dots corresponding to 5 min and darkest to 48 h. (**C, D**) Insulin mRNA levels significantly decreased during high glucose or 6-AN (an inhibitor of 6PGD) treatment. INS-1E cells were exposed to 6-AN for 48 h (**C, D**) and human islets for 72 h (**E**). Glucose-stimulated insulin secretion is impaired in 6PGD-depleted human islets (**E**). Glucose-stimulated insulin secretion was performed in islets 96 h after transfection with 100 nmol/l si6PGD at 5.5 or 22.2 mmol/l glucose. (**F**) Basal and stimulated insulin denote the amount of insulin secreted during 1 h at 2.8 or 16.7 mmol/l glucose (basal [white bars] and stimulated [black bars], respectively). (**G**) Stimulatory index is given as the ratio of stimulated to basal insulin. (H) 6GPD mRNA levels are expressed relative to β-actin. (**G, H**) Light grey bars, scramble siRNA; dark grey bars, si6PGD. Data shown are means±SE; *p<0.05 vs 5.5 mmol/l glucose; †p<0.05 vs 22.2 mmol/l glucose. siScr, scramble siRNA

Metabolism and peripheral tissues

Adipose tissue and muscle are highly active endocrine organs, secreting a vast amount of hormones affect whole body metabolism and function. Exemplarily some so-calles “inflammatory” cytokines are secreted by adipose tissue, but also muscle. A considerable number of individuals do not develop cardio-metabolic disease, despite being overtly obese. Immunological mechanisms within the adipose appear to contribute to this phenomenon, which is among others also supported by the observation that patients with chronic inflammatory disorders such as rheumatoid arthritis often die from cardio-metabolic disease. Subclinical inflammation regularly accompanies metabolic diseases (i.e. obesity and T2DM). We therefore analyse the interaction of inflammatory and metabolic pathways and investigate those relations in adipose tissue, but also muscle, i.e. by investigating effects of specific anti-inflammatory treatment approaches on metabolic traits. Based on those hypotheses, the group will implement lifestyle-based intervention trials within the DZHK to provide further evidence which environmental parameters affect cardio-metabolic risk.

Selected Publications

Goehring I, Sauter NS, Catchpole G, Assmann A, Shu L, Zien KS, Moehlig M, Pfeiffer AF, Oberholzer J, Willmitzer L, Spranger J*, Maedler K* (* contributed equally). Identification of an intracellular metabolic signature impairing beta cell function in the rat beta cell line INS-1E and human islets. Diabetologia. 2011 Oct;54(10):2584-94.

Mai K, Andres J, Bobbert T, Assmann A, Biedasek K, Diederich S, Graham I, Larson TR, Pfeiffer AF, Spranger J. Rosiglitazone increases fatty acid Δ9-desaturation and decreases elongase activity index in human skeletal muscle in vivo. Metabolism. 2011 Jul 6. [Epub ahead of print]

Biedasek K, Andres J, Mai K, Adams S, Spuler S, Fielitz J, Spranger J. Skeletal muscle 11beta-HSD1 controls glucocorticoid-induced proteolysis and expression of E3 ubiquitin ligases atrogin-1 and MuRF-1. PLoS One. 2011 Jan 31;6(1):e16674.

Bobbert T, Raila J, Schwarz F, Mai K, Henze A, Pfeiffer AF, Schweigert FJ, Spranger J. Relation between retinol, retinol-binding protein 4, transthyretin and carotid intima media thickness. Atherosclerosis. 2010 Dec;213(2):549-51.

Mai K, Bobbert T, Groth C, Assmann A, Meinus S, Kraatz J, Andres J, Arafat AM, Pfeiffer AF, Möhlig M, Spranger J. Physiological modulation of circulating FGF21: relevance of free fatty acids and insulin. Am J Physiol Endocrinol Metab. 2010 Jul;299(1):E126-30.

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Ralf Dechend

Start of the group: February 2011

Dominik N. Müller

Mechanisms of Hypertension-Induced Target Organ Damage

Ralf Dechend and Dominik N. Müller lead a group of young investigators pursuing the question of how hypertension induces target-organ damage. In a translational approach, the Dechend/Müller lab focuses primarily on the placenta, vessels, heart and kidneys. The primary mediator that has captured their attention is the renin-angiotensin system. The group also cooperates closely with MDC and Charité scientists. The group has also been a resource for young clinicians and doctoral students beginning their careers in experimental cardiovascular research. In 2011, Dominik N. Müller received a professorship for Experimental Medicine at the University of Erlangen.

The immune system, salt and hypertension-induced target organ damage

Hypertension induces target-organ damage; however, the mechanisms are unclear. We hypothesize that immunological processes are important. Angiotensin (Ang) II induces inflammation, fibrosis, and growth remodeling via the AT1 receptor. Innate immunity is pivotal in Ang II-related target-organ damage. Nevertheless, our understanding concerning adaptive immunity and target-organ damage is rudimentary. T cells, macrophages, and dendritic cells all harbor the AT1 receptor. Ang II stimulates T cell proliferation and dendritic cell migration. We found that regulatory T cells modulate Ang II-induced target-organ damage to a point of therapeutic utility. We also have shown that direct renin inhibition greatly ameliorates autoimmune encephalitis in mice, a non-hypertensive model for multiple sclerosis. Interestingly, non-specific immunosuppression ameliorates all aspects of target-organ damage in our models.

Together with the Titze lab (Erlangen/Vanderbilt) we provided evidence that sodium a known risk factor for cardiovascular disease interacts with the immune system. Macrophages are actively regulating the homeostatic process necessary to allow the body to store sodium in the skin. Upon sodium excess, sodium accumulates in the skin and activates the osmotic stress gene TonEBP and VEGF-C secretion promoting the clearance of hypertonic fluid from the interstitium. This circuit being critically dependent on macrophages wards off hypertension in case of excess sodium supply. In the same collaboration together with the group of Thoralf Niendorf, we have set skin and muscle Na^+ content with $^{23}Na^+$ MRI. This technology will allow us in the future to quantify the tissue sodium in humans with cardiovascular disease. In collaboration with Norbert Hübner, we are investigating the role of sodium on epigenetic regulation of macrophage differentiation. In collaboration with Markus Kleinewietfeld (Yale), we study the role of salt on T cell activation.

Cardiovascular and non-cardiovascular function of (pro)renin receptor (PRR)

Our understanding about the role of the prorenin receptor (PRR) in physiology and pathology changed dramatically. Initially, it was believed that the PRR is a cardiovascular receptor regulating the renin-angiotensin system. However, recent data (by others and us) demonstrate a fundamental non-cardiovascular role of the PRR. In collaboration with Michael Bader, we learned that complete knockout of PRR in mouse embryonic stem cells fails to generate chimeras when injected into blastocysts. We

have set up podocyte-specific PRR knockout mice (cKO), which died ~2-3 weeks after birth. Within 14 days, cKO animals developed nephrotic syndrome, albuminuria, due to podocyte foot process fusion, and cytoskeletal changes. Podocyte-specific PRR deletion also led to disturbed processing of multivesicular bodies and enrichment of autophagosomal and lysosomal markers. Our findings indicated a functional block in autophagosome-lysosome fusion and overload of the proteasome protein degradation machinery suggesting that the PRR is essential for podocyte function and survival. The PRR exists also as a soluble receptor (sPRR). We have set up an ELISA and currently study the role of sPRR as a biomarker in cardiovascular disease and in cancer patients.

Cypchrome P450 derived Eicosanoids and target organ damage

In a DFG-funded Forschergruppen project (FOR 1054) together with Wolf Schunck (MDC) and Robert Fischer (Charité), we investigated the role of CYP-derived epoxyeicosatrienoic acids (EETs) on maladaptive cardiac hypertrophy, electrical remodeling and sudden death. We found that eicosanoids play a pivotal role in electrical remodeling. We have taken this concept also into humans. At the ECRC, together with AG Schunck, AG Fischer and AG Boschmann, we conduct a fish oil study in human volunteers to determine eicosanoid levels. We also performed a detailed structure analysis for the antiarrhythmic potency of CYP-derived eicosanoids leading a patent application "Novel Eicosanoid Derivatives", which is the basis for the foundation of a company for the development of new drugs for atrial fibrillations. We also investigate their role of eicosanoids in the pathogenesis of preeclampsia. The project is run by the senior post-doc of our group Florian Herse and supported by his DFG grant.

Pathogenesis of preeclampsia

We studied AT1 receptor activating antibodies (AT1-AA), their role in preeclampsia and their participation in a form of humoral transplant rejection and in systemic sclerosis. We could show that endothelin-1 is an important mediator in the AT1-AA induced preeclamptic phenotype. We were the first to establish state of the art diagnostic procedures performed in the clinic for preeclamptic women in a rodent model for preeclampsia, establishing a causal link between reduced trophoblast invasion and placental perfusion. Subsequently we provided experimental in-vivo evidence that AT1-AA account for the observed reduced Ang II sensitivity observed in preeclamptic patients. We could demonstrate that circulating and local, uteroplacental Ang II are responsible for different phenotype in pregnancy. In clinical studies, we began to investigate physiological parameters and novel biomarkers during and after preeclampsia, which help to explain the increased cardiovascular risk of former preeclamptic patients. Besides AT1 receptor antibodies, we also investigated the role of alpha adrenergic receptor autoantibodies In a translational approach we performed a clinical pilot study in refractory hypertensive patients with immunoadsorption, isolated their autoantibodies and compared them to similar antibodies generated with immunization in rabbits. In a subsequent study we generated autoantibodies in rats in long-term immunization experiments and characterized the relevance for hypertension induced target organ damage in vivo.

Selected Publications

Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park J-K, Beck F-X, Müller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze. J. (2009) Macrophages regulate salt-dependent volume and blood pressure by a VEGF-C dependent buffering mechanism. Nat Med 15, 545-52.

Kvakan, H, Kleinewietfeld, M, Qadri, F, Park, JK, Fischer, R, Schwarz, I, Rahn, HP, Plehm, R, Wellner, M, Elitok, S, Gratze, P, Dechend, R, Luft, FC, Muller, DN. (2009) Regulatory T cells ameliorate angiotensin II-induced cardiac damage. Circulation. 119, 2904-2912.

Herse F, Fain JN, Janke J, Engeli S, Kuhn C, Frey N, Weich HA, Bergmann A, Kappert K, Karumanchi SA, Luft FC, Muller DN, Staff AC, Dechend R. (2011) Adipose Tissue-Derived Soluble Fms-Like Tyrosine Kinase 1 Is an Obesity-Relevant Endogenous Paracrine Adipokine. Hypertension. 58, 37-42.

Wenzel K, Rajakumar A, Haase H, Geusens N, Hubner N, Schulz H, Brewer J, Roberts L, Hubel CA, Herse F, Hering L, Qadri F, Lindschau C, Wallukat G, Pijnenborg R, Heidecke H, Riemekasten G, Luft FC, Muller DN, Lamarca B, Dechend R. (2011) Angiotensin II type 1 receptor antibodies and increased angiotensin II sensitivity in pregnant rats Hypertension. 58:77-84.

Riediger F, Quack I, Qadri F, Hartleben B, Park JK, Potthoff SA, Sohn D, Sihn G, Rousselle A, Fokuhl V, Maschke U, Purfürst B, Schneider W, Rump LC, Luft FC, Dechend R, Bader M, Huber TB, Nguyen G and Muller DN. (2011) Prorenin receptor is essential for podocyte autophagy and survival. J Am Soc Nephrol. in press

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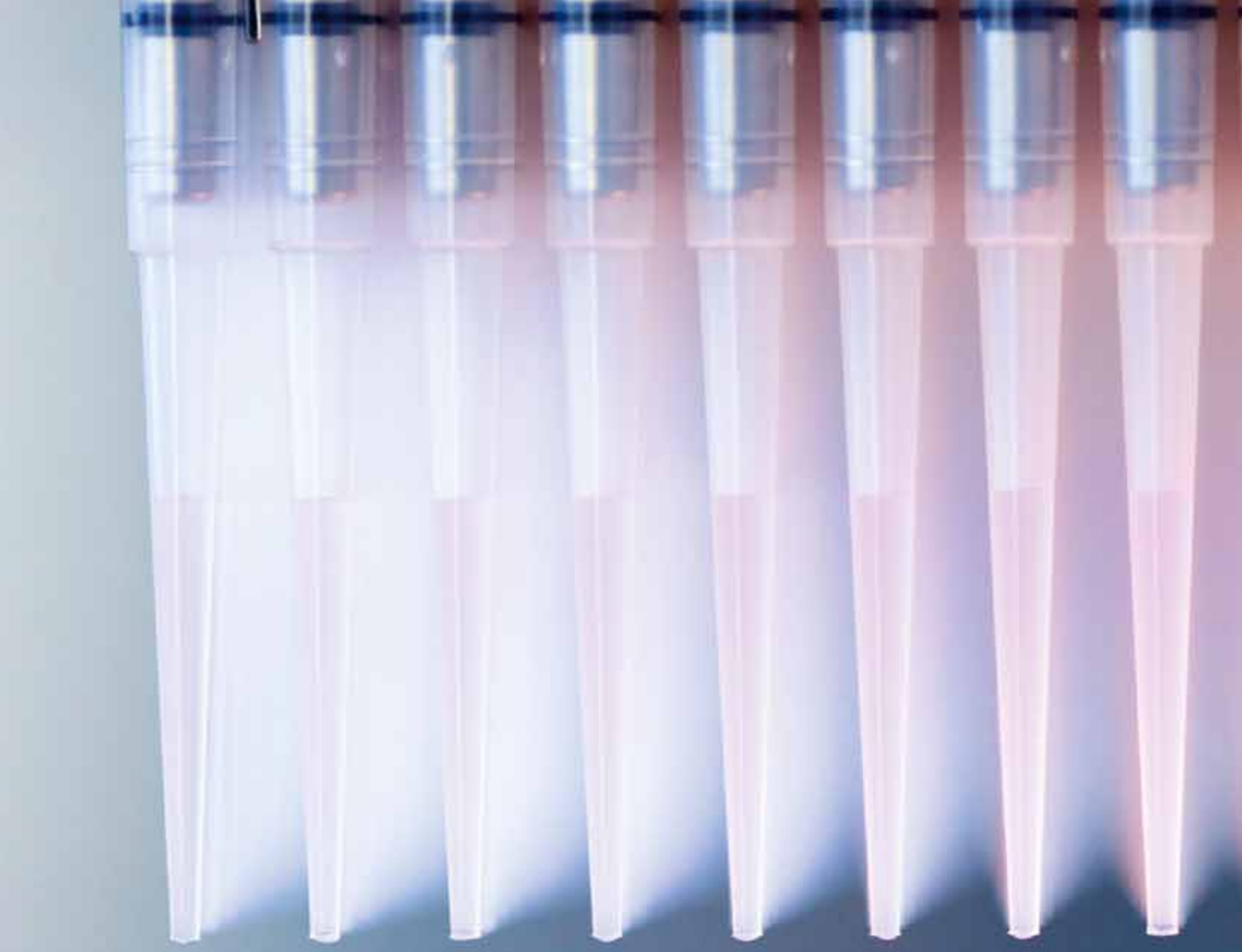


Technology Platforms

Mass Spectrometry
Confocal and 2-Photon Microscopy
Preparative Flowcytometry
Electron Microscopy
Transgenics

Gunnar Dittmar

Mass Spectrometry

Cellular signaling

Cells interact 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. The identification of proteins, their expression levels and the state of post-translational modification provides insight into the regulation of these networks. This requires the identification of several hundreds or thousands proteins and their quantification in a complex mixture. In addition the information has to be rapidly collected. All these requirements are matched by modern mass spectrometry and result in the methods rise to be the default method for large-scale protein identification in life sciences.

The core facility mass spectrometry offers a wide range of mass spectrometry methods for the identification and quantification of proteins and peptides. Besides the identification of proteins in gel slices, the mass spectrometry core facility uses a number of proteomic techniques in different collaborations with groups at the MDC. We are working closely with these groups to optimize the methods for the different projects.

Targeted proteomics

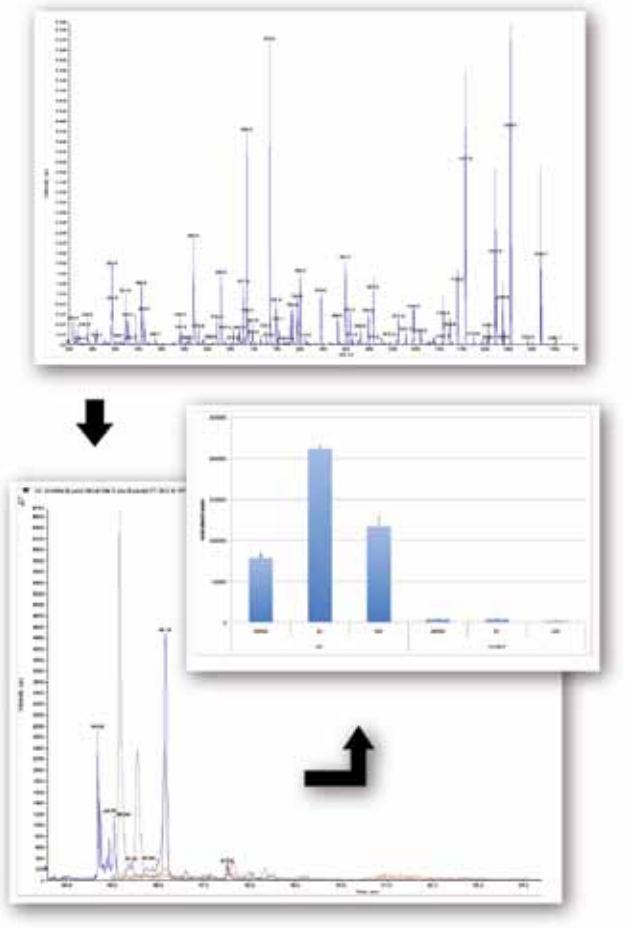
Monitoring the regulation of proteins under different conditions leads to a better understanding underlying signal transduction cascades of regulatory networks. For many enzymatic cascades and interaction networks are the major players already identified. In order to study the responses of an interaction network it is sufficient to monitor just these proteins. This avoids the sequencing of unrelated, not regulated proteins in the cell. A method which allows the selection of a limited number of proteins is selected reaction monitoring (SRM). The technique has the advantage of a high sensitivity combined with short run times on the liquid chromatography systems. This opens 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 SRM- experiments. The core facility has access to two different mass-spectrometers capable for this type of experiments (Q-Trap 5500 and Q-Trap 4000).

Non-targeted proteomic approaches

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 (SILAC – stable isotope labeling in cell culture). The additional mass of the amino acids can later be detected by mass spectrometry. Comparison of two different samples is possible by analyzing the sample on a high accuracy mass spectrometer (LTQ-Orbitrap). The results of these measurements are relative ratios for each protein detected in the two different samples. This technique is currently used by the core facility for the quantification of immunoprecipitations and large scale protein identifications.

Development of an SRM method for quantification of peptides. Starting from the initial MS/MS spectrum of the peptide of interest the SRM-transitions are constructed. Elution profile of different peptides deducted from SRM measurements, which lead to the quantification of the peptide of interest.



Identification of post-translational modifications

Besides the translational regulation of proteins, another layer of regulation exists, which is mediated by post-translational modifications. 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. 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, e.g. phospho-ion scan with polarity switching for improved sensitivity.

Selected Publications

Schwanhäusser, B. et al. Global quantification of mammalian gene expression control. Nature 473, 337–342 (2011).

Jerke, U. et al. Complement receptor Mac-1 is an adaptor for NB1 (CD177)-mediated PR3-ANCA neutrophil activation. J Biol Chem 286, 7070–7081 (2011).

Pless, O., Kowenz-Leutz, E., Dittmar, G. & Leutz, A. A differential proteome screening system for post-translational modification-dependent transcription factor interactions. Nat Protoc 6, 359–364 (2011).

Hinz, M. et al. A cytoplasmic ATM-TRAF6-cIAP1 module links nuclear DNA damage signaling to ubiquitin-mediated NF-κB activation. Mol Cell 40, 63–74 (2010).

Stolz, A. et al. The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. Nat Cell Biol 12, 492–499 (2010).

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Confocal and 2-Photon Microscopy

The aim of the Confocal and 2-Photon Microscopy Core Facility (MCF) at the MDC is to provide researchers at the MDC and ECRC with high-end microscopy systems to support advanced light microscopy research. The MCF offers individual, customized support including project planning, sample preparation, image acquisition and image analysis for a wide range of specimens from fixed cells and tissue sections to live organisms and animals. The MCF provides access and training for different microscope systems: confocal laser scanning microscopy, intravital and 2-Photon microscopy, wide-field fluorescence microscopy, TIRF microscopy, laser-assisted micro-dissection and catapulting, as well as stereo fluorescence microscopy. In addition, several computer workstations with advanced image analysis software and customised image processing tools are available.

Available techniques & Selected applications

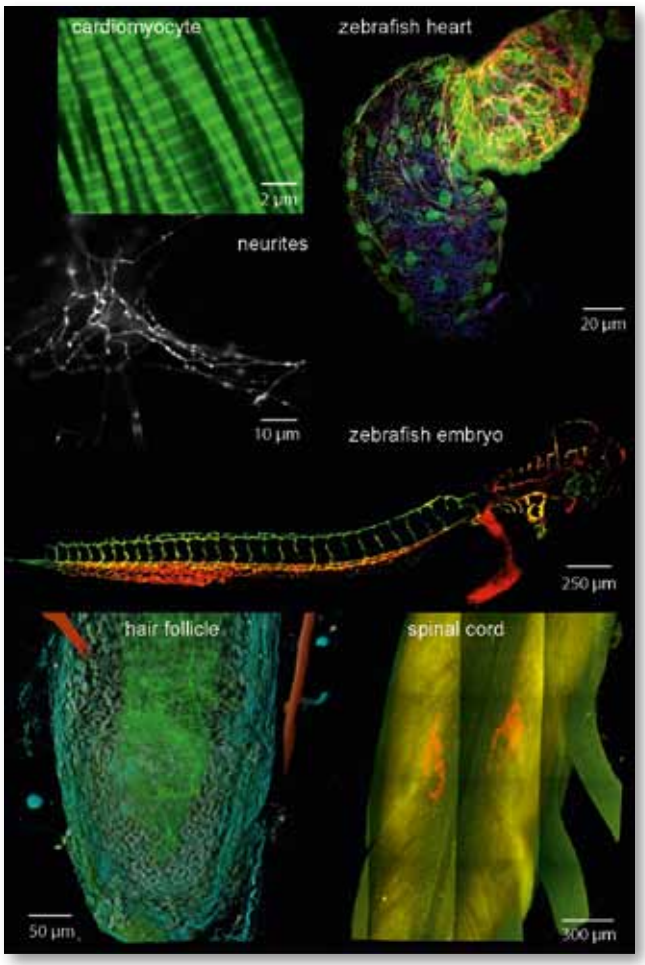
Confocal microscopy with efficient separation of spectrally overlapping fluorophores and optical z- sectioning is beneficial for multi-colour, 4D fluorescence imaging of structures from the subcellular level (Fig.A) to the morphology of small organisms (Fig. C/D) and large tissue areas (mosaic scanning, Fig.F). Microscope stage incubators to control CO_2 concentration, humidity and temperature allow for advanced live specimen experiments.

2-Photon (2-P) microscopy is generally used to image thick, turbid specimen, e.g. mouse and zebrafish embryos and large tissue preparations. The pulsed infrared (IR) lasers used penetrate deeper into biological samples and are less harmful in terms of photo damage than visible lasers. Special setups allow for intravital imaging, e.g. to monitor cell trafficking of the lymphatic system, or migration of cells into tissues. 2-P systems are also able to measure second harmonic generation (SHG) signals, emitted by highly ordered biological structures, e.g. collagen, thus providing a label-free imaging technique for such tissue types (Fig. E).

TIRF-(Total Internal Reflection Fluorescence) Microscopy only excites fluorophores that are located close to the coverslip. This makes TIRF an ideal technique to image dynamic processes close to the cell membrane, e.g. vesicle transport, endocytosis and exocytosis, and receptor-ligand interactions.

Laser microdissection microscopy uses a laser to cut out selected cells or tissue areas and lift them up to be captured by a collection device. Isolated live or fixed cells or tissue parts can be processed further, e.g. by extracting DNA or RNA.

In addition to routine user training and equipment maintenance, we also work in collaboration with several research groups from the MDC/ECRC. We contribute to advanced imaging projects, for instance quantifying



A: M-band and z-disk in rat cardiomyocyte (F-actin staining)

B: live mouse neuron after synaptic activation (pHfluorin, courtesy of M. Rohe/AG Willnow)

C: zebrafish heart (courtesy of C. Otten/AG Seyfried)

D: vasculature of whole zebrafish embryo (flt1YFP, flkCherry, courtesy of S. Kunert/AG LeNoble)

E: autofluorescence and SHG of human hairfollicle (2-Photon imaging)

F: dorsal horn of mouse spinal cord (labeled cholera toxin subunit B, courtesy of J. Haseleu/AG Lewin)

the distribution and colocalisation of surface markers on neutrophils (AG Kettritz), imaging and simulating zebrafish heart development (AG Seyfried, Fig. C), measuring the mobility of sarcomere proteins (AG Gotthardt), monitoring the activation of neuronal synapse proteins (AG Willnow, Fig. B) and of mitochondrial proteins (AG Müller) in living cells, analysing calcium waves upon receptor activation in intact retina (AG Schröder), measuring cell volume (AG Lewin, Fig. F), and imaging human skin and hair follicles (R. Paus/University Lübeck, Fig. E).

Development of Core Facility Management tools

Quality assessment tests:

To ensure the optimal and reproducible performance of the microscopes hosted by the MCF, we developed test routines that we apply at regular intervals to monitor important microscope parameters, including laser power, objective quality, and detector registration. We plan to further extend these test routines and documentation into a tool for microscope quality assessment for core facilities.

Customized core facility management software:

We developed a customized Core Facility management tool box including a user database, online user registration form, online microscope booking calendar, user contact form with email distribution lists, trouble ticket system and MCF wiki. These software tools will make the MCF administrative tasks more time-efficient, the access of users to MCF easy and transparent and ensure a better contact between MCF users and staff to enable e.g. an individual user support and faster troubleshooting of microscope problems.

Selected Publications

Zoltan Cseresnyes, Fabian Kriegel and Anje Sporbert "A customised web-based tool box for microscopy core facility management" Imaging & Microscopy Issue 4 Vol 13 2011

Jerke U, Rolle S, Dittmar G, Bayat B, Santoso S, Sporbert A, Luft F, Kettritz R. Complement receptor Mac-1 is an adaptor for NB1 (CD177)-mediated PR3-ANCA neutrophil activation. J Biol Chem. 2011 Mar 4;286(9):7070-81.

Cseresnyes Z, Schwarz U, Green CM. Analysis of replication factories in human cells by super-resolution light microscopy. BMC Cell Biol. 2009 Dec 16;10:88.

Bischoff M, Cseresnyés Z. Cell rearrangements, cell divisions and cell death in a migrating epithelial sheet in the abdomen of Drosophila. Development. 2009 Jul;136(14):2403-11

Schmidt V, Sporbert A, Rohe M, Reimer T, Rehm A, Andersen OM, Willnow TE. SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem. 2007 Nov 9;282(45):

Structure of the Group

Group Leader
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Scientists
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(since 2010)

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(part time)

Hans-Peter Rahn

Preparative Flowcytometry

Fluorescence-activated cell sorting (FACS) is a specialized type of flowcytometry. It was designed in 1971 by Len Herzenberg to provide a fast physical separation method for fractionating a heterogeneous mixtures of cells into distinct subsets, based upon the light scattering and fluorescent characteristics of different cell types.

The FACS Core Facility assists researchers at the MDC with two state-of-the-art, digital high-speed "FACSAria" sorters and one analog high-speed "FACSVantage SE" sorter from BD. The facility has been used by more than 30 scientific groups at the MDC. Sorted cells are often used for microarray- or sequencing-based gene expression analysis, quantitative real-time PCR, DNA sequencing, live cell imaging or adoptive transfer experiments in animals.

Recent Projects:

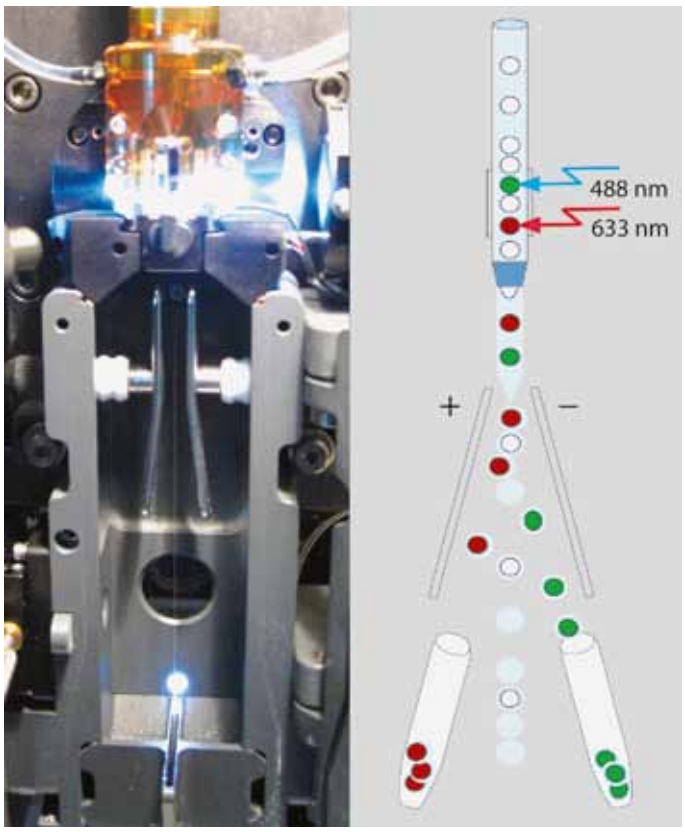
Sophisticated FACS applications are used in the research group of Frank Rosenbauer, with the theme "Cancer, Stem Cells, and Transcription Factors". The interest of this group is to integrate hematopoitic stem cell characteristics with underlying genetic and epigenetic regulatory mechanisms. To identify specific differentiation states of hematopoetic and leukemic cells, the group developed new staining methods using up to nine colors. Routinely, seven color sorts of mouse bone marrow cells are used to analyse hematopoetic cells in vivo and in vitro.

The "Molecular Tumor Genetics and Immunogenetics" group of Martin Lipp investigates the function of T- and B- cells during the development of acute and chronic inflammation. The group focuses on distinct memory and effector T cell populations which are important for the development of antibody-based adaptive immune responses. In this connection central memory, effector/memory und follicular B helper T cells are enriched by FACS using specific patterns of surface markers on these cells.

The "Hematoloy, Oncology and Tumorimmunology" group of Bernd Dörken "Hematoloy, Oncology and Tumorimmunology" uses FACS enrichment of tumor and primary murine hematopoetic cells in order to analyse the function of ectopically expressed genes in comparison to the endogenous expression levels in lymphoma cells.

Together with the research group of Manfred Gossen, FACS was used to create long term stable transgenic cell clones, without the influence of additional anitbiotic resistance genes.

To study early embryogenesis employing high-throughput genomics as well as biochemistry assays, the "Systems Biology" lab of Nikolaus Rajewsky devised a new method to collect large amounts of precisely staged C. elegans embryos by FACS. This method will make a contribution towards a more complete understanding of gene regulatory networks during early C. elegans development.



Left: Inside view of a FACSAria 2 (flow cell with sort block and deflection plates), Right: Schematic view of the separation principle

Selected Publications

Broske, A.M., Vockentanz, L., Kharazi, S., Huska, M.R., Mancini, E., Scheller, M., Kuhl, C., Enns, A., Prinz, M., Jaenisch, R., et al. (2009). DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nat Genet 41, 1207-1215.

Rasheed, A.U., Rahn, H.P., Sallusto, F., Lipp, M., and Muller, G. (2006). Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression. Eur J Immunol 36, 1892-1903.

Mathas, S., Kreher, S., Meaburn, K.J., Johrens, K., Lamprecht, B., Assaf, C., Sterry, W., Kadin, M.E., Daibata, M., Joos, S., et al. (2009). Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. Proc Natl Acad Sci U S A 106, 5831-5836.

Kaufman, W.L., Kocman, I., Agrawal, V., Rahn, H.P., Besser, D., and Gossen, M. (2008). Homogeneity and persistence of transgene expression by omitting antibiotic selection in cell line isolation. Nucleic Acids Res 36, e111.

Stoeckius, M., Maaskola, J., Colombo, T., Rahn, H.P., Friedlander, M.R., Li, N., Chen, W., Piano, F., and Rajewsky, N. (2009). Large-scale sorting of C. elegans embryos reveals the dynamics of small RNA expression. Nat Methods 6, 745-751.

Structure of the Group

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Bettina Purfürst

Electron Microscopy

There is a constant need for high resolution microscopy from a wide range of research teams on the campus. The EM facility provides tools and expertise for various collaborations like advice in preparation techniques, service, common research projects and training of guests. Especially high is the demand for phenotyping on the ultrastructural level to characterize genetically modified specimen. Due to the use of high speed digital camera systems it is now increasingly possible to make large picture series and, hence, quantify morphological details. In the period reported, we performed more than 18 EM collaborations using different specimen like cell cultures, embryos, heart, aorta, skin, kidney, pancreas, nerves, muscle and structures in negative contrast. Three examples are given here:

Phenotyping: Changes in podocyte morphology in the kidney

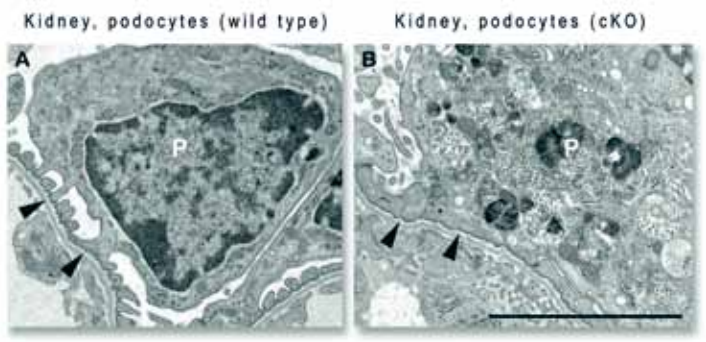
(in collaboration with Fabian Riediger and Dominik N. Müller, ECRC/MDC)

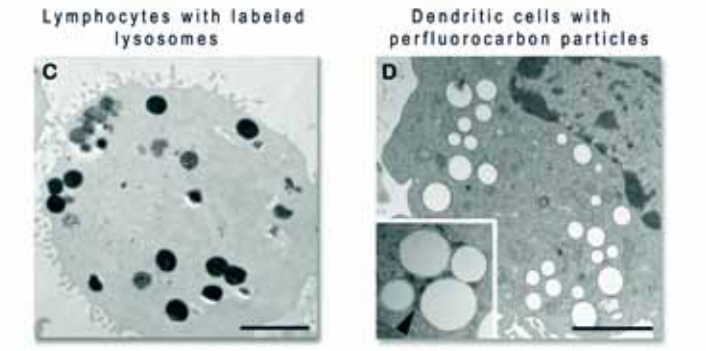
The podocyte-specific inactivation of the prorenin receptor, a new member of the renin-angiotensin system, leads to severe glomerular damage, renal failure and early death in mice. We could show by classical embedding methods that the podocytes in conditional prorenin knock out mice reveal severe foot process effacement (Figure A, B, arrowheads) without major alterations of the glomerular basement membrane diameter. Moreover, in numerous podocytes we could identify a massive accumulation of multivesicular bodies, different procession stages of lysosomes and an expanded endoplasmic reticulum (Figure B). These data support a range of other findings that the prorenin receptor may be essential for protein turnover and autophagy homeostasis.

Quantification of the lysosomal compartment in lymphocytes

(in collaboration with Constantin Rüder and Armin Rehm, MDC)

In this case, we examined the role of EBAG9 (estrogen receptor-binding fragment-associated antigen 9) in the process of granule biogenesis and maturation in $CD8^+$ T-lymphocytes (CTLs). Wild type and $EBAG9^{-/-}$ lymphocytes were labeled over night with the endocytic tracer horseradish peroxidase to load lytic granules. Following DAB staining, this compartment is directly visible in the electron microscope (Figure C) and can be quantified. The relative size distribution revealed a statistically significant shift towards a smaller granule diameter of the lytic granules in EBAG9-deficient cells, indicating that EBAG9 modulates the ultimate generation of secretory granules in this kind of cytotoxic T lymphocytes.





A,B:
Conditional prorenin receptor knock out mice develop podocyte damages in the kidney.
In wild type animals (A), the podocytes (P) show normal morphology and foot processes (arrowheads), whereas in cKO mice (B) the foot processes fuse, and massive protein degradation associated vacuolization occurs within the cytoplasm of the podocytes.

Epon-embedding, contrast uranyl acetate / lead citrate, bar = 2 µm.

C:
Visualization of the lytic granule compartment in mouse $CD8^+$ T-lymphocytes (CTLs). Cells were labeled with the endocytic tracer horseradish peroxidase, and the dark stained, electron dense lysosomes were quantified.

Epon-embedding, contrast uranyl acetate (cell) and DAB staining (lysosomes), bar = 2 µm.

D:
Localization and size determination of perfluorocarbon particles in dendritic cells. Numerous bright spheroids are distributed within the cytoplasm, often arranged in clusters with an amorphous grey compartment (inset, arrowhead).

Epon-embedding, contrast uranyl acetate / lead citrate, bar = 2 µm.

Localization of perfluorocarbon particles in dendritic cells

(in collaboration with Sonia Waiczies, Experimental Ultrahigh-Field MR)

Perfluorocarbon particles are used in magnetic resonance imaging for the non-invasive tracking of cells in vivo. To characterize the efficiency of cellular labeling and putative impacts of these particles on cellular dynamics, we performed a first set of experiments to localize them in dendritic cells (Fig. D). The fluorine-rich particles are easy to detect as bright spheroids in the cytoplasm of the immune cells. They have a membrane-like surface, some of them are clustered and have an amorphous grey compartment (see inset, arrowhead). Electron microscopy allowed an exact determination of the particles size, a feature not only important for the labeling efficiency but also for the immunological status of the cells. Further experiments are planned to optimize the labeling conditions in other cell types (eg. T cells and stem cells) as well as localize the particles within target organs using the relevant preclinical mouse models.

Selected Publications

Rüder, C, Höpken, UE, Wolf, J, Mittrücker, H-W, Engels, B, Erdmann, B, Wollenzin, S, Uckert, W, Dörken, B, Rehm, A. (2009). The tumor-associated antigen EBAG9 negatively regulates the cytolytic capacity of mouse $CD8^+$ T cells. J Clin. Invest. 119, 2184-2203.

Werth, M, Walentin, K, Aue, A, Schönheit, J, Wuebken, A, Pode-Shakked, N, Vilianovitch, L, Erdmann, B, Dekel, B, Bader, M, Barasch, J, Rosenbauer, F, Luft, FC, Schmidt-Ott, KM. (2010). The transcription factor grainyhead-like 2 regulates the molecular composition of the epithelial apical junctional complex. Development 137, 3835-3845.

Vaegter, CB, Jansen, P, Fjorback, AW, Glerup, S, Skeldal, S, Kjolby, M, Richner, M, Erdmann, B, Nyengaard, JR, Tessarollo, L, Lewin, GR, Willnow, TE, Chao, MV, Nykjaer, A. (2011). Sortilin associates with Trk receptors to enhance anterograde transport and signalling by neurotrophins. Nature Neurosc. 14, 54-61.

Waiczies, H, Lepore S, , Janitzek, N, Hagen, U, Seifert, F, Ittermann, B, Purfürst, B, Pezzutto, A, Paul, F, Niendorf, T, Waiczies, S. (2011). Perfluorocarbon particle size influences magnetic resonance signal and immunological properties of dendritic cells. PLoS One 6, e21981.

Riediger, F, Quack, I, Qadri, F, Hartleben, B, Park, J-K, Potthoff, S, Sohn, D, Sihn, G, Rousselle, A, Fokuhl, V, Maschke, U, Purfürst, B, Schneider, W, Rump, LC, Luft, FC, Dechend, R, Bader, M, Huber, TB, Nguyen, G, Müller, DN. Prorenin receptor is essential for podocyte autophagy and survival in mice. JASN (J of the American Society of Nephrology), in press

Structure of the Group

Group Leader
Dr. Bettina Purfürst

Technical Assistants
Marianne Vannauer
Margit Vogel (part-time)

Boris Jerchow

Transgenics

The Transgenic Core Facility (TCF) offers expertise in all kinds of mouse Assisted Reproductive Technologies (ART). Starting at the planning phase, we support scientists from the MDC and their external collaborators with the design and layout of their projects. One focus of our work is the generation of genetically modified mouse lines. This is accomplished either by gene targeting in embryonic stem (ES) cells and subsequent generation of chimeric mice from recombinant ES cell clones or by microinjection of plasmid or BAC type transgenes into the pronuclei of fertilized oocytes.

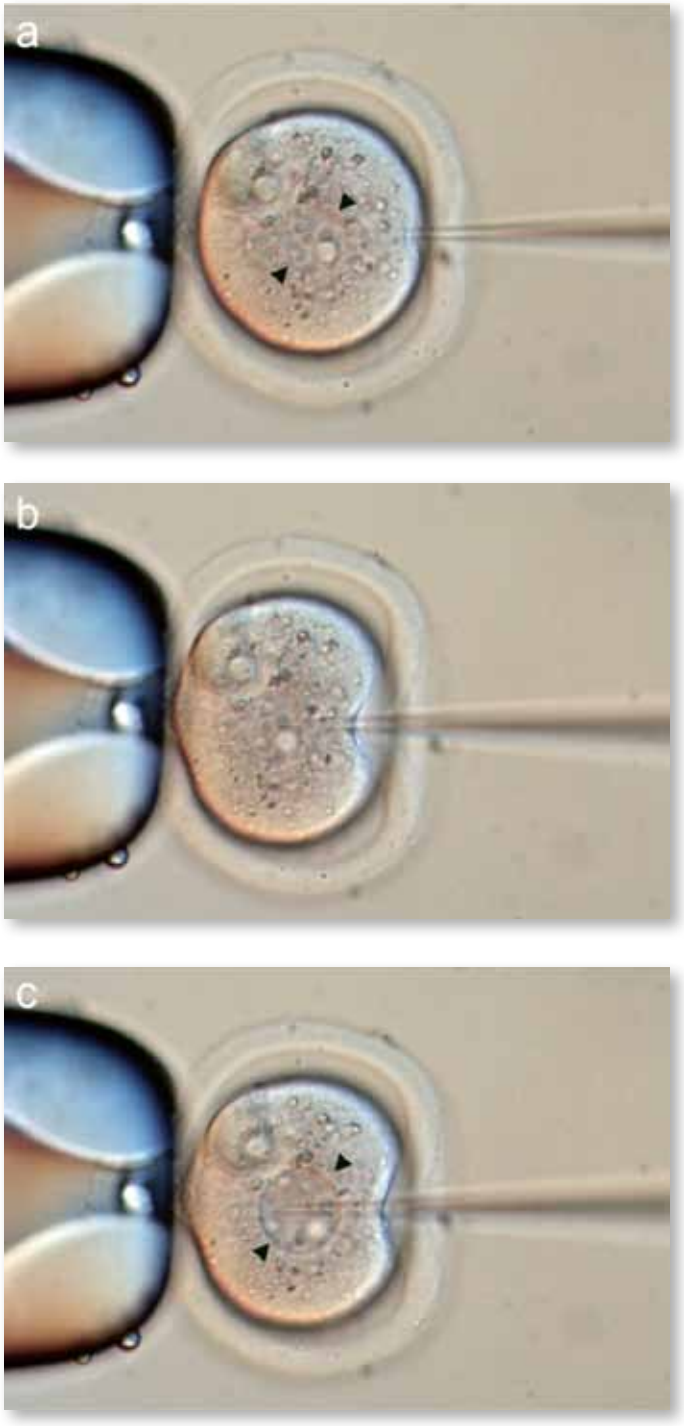
A second focus of our work is the conservation of precious mouse lines and the rederivation of conserved lines. This service has been continuously expanded during the last years both in quantity and in the different protocols that have been optimized in the lab. Since most groups choose to conserve mouse sperm this is the main form of germ plasm we freeze down and store in liquid Nitrogen. However, we also offer the long term cryopreservation of pre-implantation embryos. Due to the growing number of mouse models worldwide, we see an increase in the number of organizations who offer to send frozen material instead of live mice. These lines are revitalized at the MDC following a variety of protocols depending on the method of cryoconservation.

In addition to the above, we have started a project to rederive a number of immune-compromised lines by embryo transfer to reach an elevated standard above the regular MDC status. In addition in 2011 many new lines will be introduced by *in vitro* fertilization directly to this elevated standard.

There are individual projects that do not fit 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, BAC preparation, ES cell culture, ES cell strain background, coat color genetics, ES cell derivation, and help with the selection of targeted ES cell clones from international knock out consortia for the generation of the corresponding gene targeted mouse lines.

Record 2010:

- 8 transgenic lines
- 5 recombinant ES cell lines
- 12 lines from recombinant ES cells
- 44 sperm freezings
- 15 embryo freezing
- 1 hygienic rederivation
- 4 rederivations of cryoconserved lines



Pronuclear microinjection. When a zygote is fixed to the holding capillary, both the injection needle filled with DNA solution as well as one of the pronuclei are brought into focus. Then the *zona pellucida* is penetrated with the injection capillary aiming at one of the pronuclei (a). The injection capillary is moved further to the far side of the pronucleus and then slightly pulled back to place the capillary's tip in its middle (b). When the injection pressure is applied a swelling of the injected pronucleus has to be clearly visible (compare arrowheads in a to those in c).

Structure of the Group

Group Leader
Dr. Boris Jerchow

Katja Becker
Andrea Leschke
Christine Krause

Technology Transfer

Technology Transfer

Technologietransfer

Since 2007, the MDC has been supporting a technology transfer program to stimulate the transfer of results from basic research to commercialization. The MDC technology transfer program funds practically oriented projects with the aim to open up new career perspectives in applied research or in business. MDC scientists may apply for funding for their own position and the position of a technical assistant as well as for consumables amounting to a maximum of 30,000 euros per year. The program provides funding for 18 months with the possibility of extension for up to 36 months.

Until 2010, only projects in the early commercialization phase could be funded, thus enabling the acquisition of extramural funding, for instance from GO-Bio, following the MDC grant. Since January 2011, however, the MDC grant can also follow expired GO-Bio funding and can thus promote projects that are in later commercialization stages. Through the funding of both early and late commercialization stages, the program supports the overall improvement of commercialization opportunities at the MDC.

Early-Stage Commercialization Funding

Currently, seven early-stage projects are being funded. One project from the first round of funding ("Development of Small Molecule Antagonists for Chemokine Receptors" of Gerd Müller, Lipp Research Group) has now been completed. Another project from the first round ("TPH2 activator: A drug for depression" of Saleh Bashammakh, Bader Research Group) has received an 18-month extension in funding. Furthermore, following an interim evaluation, the projects from the second round of funding ("Eicosanoid-like drugs for the prevention and treatment of cardiac arrhythmias" of Wolf-Hagen Schunck, Luft Research Group, and "TAT-ARC protein transduction is a novel therapeutic approach for fulminant liver failure in mice" of Stefan Donath, Dietz Research Group) will continue to be funded for an additional year and a half. In the third call for proposals, the

Seit 2007 unterstützt das MDC den Transfer wissenschaftlicher Ergebnisse aus der Grundlagenforschung hin zur kommerziellen Verwertung. Das MDC-interne Technologietransferprogramm fördert anwendungsnahe Projekte und soll Wissenschaftlern eine Karriereperspektive in der angewandten Forschung oder der Wirtschaft eröffnen. MDC-Wissenschaftler können die eigene Stelle, eine TA-Stelle und bis zu 30 T€ Sachmittel pro Jahr zunächst für 18 Monate einwerben. Eine Verlängerung auf insgesamt 36 Monate ist möglich.

Bis 2010 konnten nur Projekte der frühen Verwertungsphase gefördert werden und so die Möglichkeit eröffnen, im Anschluss an die MDC-Förderung Drittmittelförderung, wie z.B. Go-Bio, einzuwerben. Seit Januar 2011 kann die MDC-Förderung aber auch an eine ausgelaufene Go-Bio-Förderung anschließen und kommt damit auch späteren Verwertungsphasen zugute. Durch die Förderung von sowohl frühen als auch späten Verwertungsphasen dient das Programm nun insgesamt der Verbesserung der Verwertungsmöglichkeiten am MDC.

Frühe Verwertungsförderung

Derzeit werden sieben Projekte in einem frühen Stadium gefördert. Ein Projekt aus der ersten Förderrunde ("Entwicklung von Antagonisten für den Chemokinrezeptor CXCR5P" von Gerd Müller, AG Lipp) ist mittlerweile abgeschlossen. Ein weiteres Projekt aus der ersten Runde ("Tryptophanhydroxylasen als pharmakologische Zielstrukturen" von Saleh Bashammakh, AG Bader) hat für eine 18-monatige Anschlussförderung erhalten. Auch die Projekte aus der zweiten Förderrunde („Development of anti-arrhythmic drugs based on the structure of novel endogenous cardioprotective eicosanoids" von Wolf-Hagen Schunck, AG Luft und "TAT-ARC Proteintransducktion als innovativer Therapieansatz bei Herz-und Hirninfarkt sowie akutem Leberversagen" von Stefan Donath, AG Dietz) werden nach einer Zwischenevaluierung für 1,5 Jahre weitergefördert. In der dritten Ausschreibungsrunde wurde das

project “Specific inhibitors of the protein tyrosine phosphatase Shp2: improvement by medicinal chemistry and test in xenotransplanted tumor mice” (Stephanie Grosskopf, W. Birchmeier Research Group) was recommended for funding. In the fourth round of applications in 2010, the projects “Activation of PPARdelta as Novel Strategy for the Prevention of Restenosis after Angioplasty “ of Florian Blaschke (Thierfelder Research Group) and “TAT-ARC protein transduction is a novel therapeutic approach for fulminant liver failure in mice” of Christiane Wetzel (Lewin Research Group) were funded.

Late-Stage Commercialization Funding

The project of Annett Böddrich (Erich Wanker Research Group) “Protein Misfolding in Alzheimer’s and Huntington’s Disease, Enabling Technologies for Drug Discovery” was funded from 2007-2009 through the GO-Bio initiative. Following this grant by the BMBF, Erich Wanker acquired funds for his project from the Helmholtz Enterprise Fund (HEF). On March 23, 2011, it was decided to fund his project for a further 12 months from an internal MDC program, the Pre-GO-Bio Program.

Spin-Offs

Currently, one spin-off project is being funded at the MDC. Thoralf Niendorf and his team acquired 100,000 euros from the Helmholtz Enterprise Fund to found a company to manufacture novel MRI components.

Projekt „Shp2-Inhibitoren als neuartige Therapeutika zur Behandlung von SHP-2-abhängigen Krankheiten“ (Stephanie Grosskopf, AG W. Birchmeier) zur Förderung empfohlen. In der vierten Antragsrunde 2010 werden das Projekt „Activation of Nuclear Receptors as Novel Strategy for the Prevention of Restenosis after Angioplasty“ von Florian Blaschke (AG Thierfelder) und das Projekt „Exploitation of novel transduction targets for pain relief “ von Christiane Wetzel (AG Lewin) gefördert.

Spätere Verwertungsförderung

Das Projekt von Annett Böddrich (AG Erich Wanker) „Inhibition of amyloidogenesis: Development of a drug therapy for the treatment of Huntington’s and Alzheimer’s disease” wurde von 2007-2009 durch GO-Bio-Mittel gefördert. Im Anschluss an die BMBF-Förderung konnte Erich Wanker für sein Vorhaben Mittel aus dem Helmholtz-Enterprise-Fonds einwerben. Am 23. März 2011 wurde auf eine Weiterfinanzierung für 12 Monate aus dem MDC-internen Pre-GO-Bio-Programm entschieden.

Ausgründungen

Aktuell wird ein Ausgründungvorhaben am MDC gefördert. Thoralf Niendorf und sein Team konnten 100 T€ zur Gründung einer Firma für neuartige Komponenten für die Magnetresonanztomographie (MRT) aus dem Helmholtz-Enterprise-Fonds (HEF) einwerben.

Figures for Technology Transfer in 2010 / Kennzahlen zum Technologietransfer 2010

Patent applications / Patentanmedlungen	12
Patent rights / Schutzrechtsbestand	309
License agreements / Lizenzverträge (Neuabschlüsse)	4
License revenues / Lizenzerträge	405,000€
R&D commissions (number) / FuE-Aufträge (Anzahl)	15
R&D commissions (proceeds) / FuE-Aufträge (Erträge)	388,000€
R&D cooperations (number) / FuE-Kooperationen (Anzahl)	468
R&D cooperations (proceeds) / FuE-Kooperationen (Erträge)	197,000€

Saleh Bashammakh

TPH2 activator: A drug for depression

Saleh Bashammakh[1], Susann Matthes[1], Katja Tenner[1], Anja Schütz[2], Jens von Kries[3], Maik Grohmann[1], Michael Bader[1]

[1]MDC; [2]PSPF; [3]FMP

Serotonin (5-HT) is synthesized by two different tryptophan hydroxylases, TPH1 and TPH2. TPH1 is responsible for the biosynthesis of 5-HT in peripheral tissues, where 5-HT functions as a hormone. TPH2, discovered by our group, initiates the 5-HT biosynthesis in the brain. Central 5-HT works as a neurotransmitter playing a role in behavioral and autonomic control. Low 5-HT levels in the brain have been linked to depressive disorders in humans and, therefore, substances increasing 5-HT at synapses are the most frequently prescribed antidepressant drugs. The aim of our project is to find small chemicals, which exclusively activate TPH2 in order to increase the 5-HT concentration in the brain without interfering with the peripheral functions of 5-HT. Since TPH2 harbours a unique self-inhibitory domain, this should be possible. By high throughput screening of 37,000 compounds with an enzymatic activity assay we found several substance families, which increase TPH2 activity. The most effective substance S9863 increased TPH2 activity about 3 fold *in vitro*. TPH2-expressing PC12 cells treated with S9863 showed a 2.5 fold increase in 5-HT content. This effect was TPH2-specific since 5-HT levels were not affected in TPH1-expressing BON cells. Further experiments will test this substance *in vivo* and aim to improve it by chemical modifications for high efficacy and bioavailability. Due to the specificity for the 5-HT system in the brain TPH2 activators promise a better treatment of depressive disorders with less side effects than the currently available drugs and could represent a novel class of antidepressant drugs.

Florian Blaschke

Activation of PPARdelta as Novel Strategy for the Prevention of Restenosis after Angioplasty

Florian Blaschke and Ludwig Thierfelder (MDC) in collaboration with Dominik N. Müller (ECRC), Wolf-Hagen Schunck (MDC), and Friedrich Jung (BCRT)

Balloon angioplasty and, subsequently, coronary stenting has revolutionized the perspective of stable and unstable coronary artery disease management in the last decades. However, the long term results of stent usage have been blighted by the dual problems of in-stent restenosis and stent thrombosis. Our approach differs from the principle of the currently clinically available drug-eluting stents. Instead of an aggressive pharmacologic cytotoxic and cytostatic effect, our approach is based on a selective inhibition of proliferation and migration of vascular smooth muscle cells, an acceleration of re-endothelialization and an inhibition of thrombocyte activation and aggregation.

The nuclear receptor PPARdelta can both activate and repress gene expression in a cell type-specific manner. This project is based on our findings that PPARdelta ligand coated drug-eluting stents inhibit in-stent restenosis in a rabbit model of experimental atherosclerosis. In collaboration with Dr. Marcus Weber from the Konrad-Zuse-Zentrum in Berlin and John R. Falck from the University of Texas Southwestern, we will develop novel synthetic PPARdelta activators with improved release kinetics and evaluate the ligand in a porcine coronary artery stent model.

Annett Böddrich

Protein Misfolding in Alzheimer's and Huntington's Disease, Enabling Technologies for Drug Discovery

Annett Böddrich, Sigrid Schnögl, Thomas Wiglenda, Babila Tachu, Sandra Neuendorf, Nancy Schugardt, Daniela Kleckers, Erich E. Wanker

Alzheimer's and Huntington's disease are neurodegenerative disorders for which no effective causal treatment exists to date. Both diseases are believed to be caused by misfolding and aggregation of disease-relevant polypeptides. Under this hypothesis, we have started drug discovery efforts to identify first-in-class modulators of neurotoxic misfolded protein species. Small molecule modulators of aggregation of the amyloid-beta polypeptide in Alzheimer and the huntingtin protein in Huntington's disease have been found. The most effective compound emerging from these screenings showed efficacy in two mouse models transgenic for Alzheimer's disease. In the course of these studies we established a variety of *in vitro* and *in vivo* assays that shall be further developed into a technology platform for the identification and characterisation of chemical and biological substances that interact with the protein misfolding and aggregation cascade in Alzheimer or other protein misfolding disorders, like e.g. Parkinson's disease.

Until 2011 the project was supported by the GO-Bio initiative (Gründungsoffensive Biotechnologie) of the Federal Ministry of Education and Research (BMBF) as well as the Helmholtz Enterprise Fund, a funding instrument established by the Helmholtz Association to promote technology transfer. The Pre/Post-Go-Bio program of the MDC will provide funds for the continuation of the activities. Further financing for technology development and transfer into a commercial framework is presently being sought.

Stefan Donath

TAT-ARC protein transduction is a novel therapeutic approach for fulminant liver failure in mice

Junfeng An, Katarzyna Pogodzinski, Rainer Dietz, Stefan Donath

The apoptosis repressor with caspase recruitment domain (ARC) is a recently discovered death repressor that inhibits both death receptor and mitochondrial apoptotic signaling. Acute liver failure (ALF) is associated with massive hepatocyte cell death and high mortality rates. Therapeutic approaches targeting hepatocyte injury in ALF are hampered by the activation of distinct stimulus-dependent pathways, mechanism of cell death and a limited therapeutic window.

Here, we investigated the in vivo effects of ARC fused with the transduction domain of HIV-1 (TAT-ARC) on Fas- and TNF-mediated murine models of fulminant liver failure. Treatment with TAT-ARC protein completely abrogated otherwise lethal liver failure induced by Fas-agonistic antibody (Jo2), concanavalin A (ConA) or D-galactosamine/lipopolysaccharide (GalN/LPS) administration. Importantly, survival of mice was even preserved when TAT-ARC therapy was initiated in a delayed manner after stimulation with Jo2, ConA or GalN/LPS. ARC blocked hepatocyte apoptosis by directly interacting with members of the death-inducing signaling complex. TNF-mediated liver damage was inhibited in a JNK-dependend manner. TAT-ARC protein transduction was well tolerated in the animals and no signs of hepatotoxicity or relevant immunologic side effects were seen. The efficacy of TAT-ARC protein transduction in multiple murine models of ALF demonstrates its therapeutic potential for reversing otherwise lethal liver failure.

Stefanie Grosskopf

Specific inhibitors of the protein tyrosine phosphatase Shp2: improvement by medicinal chemistry and test in xenotransplanted tumor mice

Stefanie Grosskopf, Sandra Miksche, Chris Eckert, Walter Birchmeier (MDC) and Silke Radetzki, Carola Seyffarth, Andreas Oder, Edgar Specker, Jörg Rademann, Jens von Kries (FMP), in cooperation with Oncotest GmbH, Freiburg.

The small molecule PHPS1 was previously identified by our labs as an inhibitor of the tyrosine phosphatase Shp2 (PTPN11), which acts as an oncogene product in several human tumor types. A four-step synthesis procedure was developed to prepare a small library of potentially improved inhibitors of PHPS1, which was evaluated by SAR using *in vitro* and *in vivo* experiments. Specific site modifications of PHPS1 with aromatic and heterocyclic moieties were examined in particular. The most active Shp2 inhibitor now available shows in enzyme assays an IC_{50} in the nanomolar range, which is 20-fold better than PHPS1. In addition, this inhibitor is highly selective against related tyrosine phosphatases, i.e. 29- and 45-fold more active against Shp2 than against SHP-1 and PTP1B, respectively. The most active Shp2 inhibitors also block HGF-stimulated epithelial-mesenchymal transition of MDCK-C cells as well as of human pancreatic tumor cells HPAF II in the low micromolar range. The best compounds also show inhibition of colony formation in soft agar with Shp2-dependent human lung tumor cells. *In vivo* testing of the best inhibitor with human lung tumor cell xenografts in mice blocks tumor growth, with a T/C value of 43 %. This compound is well tolerated in the animals and is thus suitable for further development as an agent against cancers like juvenile myelomonocytic leukemia or carcinomas.

Gerd Müller

Development of Small Molecule Antagonists for Chemokine Receptors

Mathias Koch, Hendrik Falk, Florian Weigend, Jens-Peter von Kries[1], Martin Lipp and Gerd Müller

MDC and [1]FMP

Homeostatic chemokine receptors and their ligands play a vital role in the development and organization of secondary lymphoid tissues, as well as the recruitment of lymphoid cells in either innate or acquired immune responses. Moreover, they have been implicated in a host of clinically important diseases such as cancer metastasis and inflammation and, consequently, are deemed as suitable targets for the development of novel therapies.

The project focuses on the development of small molecule compounds that modulate chemokine receptor function for therapeutic use in chronic inflammatory autoimmune diseases and non-Hodgkin lymphoma. It covers the early phase in drug development from drug discovery by high throughput screens of compound libraries to preclinical development of the most promising candidate substances.

Wolf-Hagen Schunck

Eicosanoid-like drugs for the prevention and treatment of cardiac arrhythmias

Christina Westphal and Wolf-Hagen Schunck (MDC) in collaboration with Dominik N. Müller, Robert Fischer, Gerd Wallukat and Friedrich C. Luft (ECRC)

This project is based on our previous finding that 17,18-epoxyeicosatetraenoic acid (EEQ), a cytochrome P450-dependent eicosapentaenoic acid metabolite, contributes to the antiarrhythmic effects of dietary omega-3 fatty acids. The omega-3 epoxyeicosanoid activates a thus far unidentified Gi protein-coupled receptor in cardiomyocytes and thereby triggers an endogenous signaling pathway that protects the heart against electrical and structural remodeling. In laboratory tests, 17,18-EEQ exerted antiarrhythmic effects with EC50-values of 1-2 nM and was 1000-fold more potent than the parental omega-3 fatty acid. The natural compound is prone to autoxidation, rapid metabolic inactivation, and degradation. In collaboration with John R. Falck from University of Texas Southwestern, we have developed synthetic agonists that display improved chemical and metabolic stability, enhanced biological activity, and increased water-solubility. Our initial *in vivo* studies documented the capacity of this novel class of compounds to reduce the incidence and severity of myocardial infarction-induced ventricular arrhythmia in rodent models. Ongoing studies are aimed at developing suitable drug candidates for the prevention and treatment of atrial fibrillation.

Christiane Wetzel

Exploitation of novel transduction targets for pain relief

Christiane Wetzel and Gary R. Lewin

There is an unmet need for more effective analgesics and novel strategies for analgesic drug development. With Stomatin-like protein-3 (STOML-3) we have identified a potential molecular target that directly participates in the transduction of noxious and innocuous mechanical stimuli in sensory neurons. We found that STOML3 deficient mice have many mechanoreceptors and nociceptors which are essentially insensitive to mechanical stimulation. Interestingly, STOML3 deficient mice show only very minor symptoms of neuropathic pain and this may be due to the impairment of touch reception in these mice. We are developing novel high throughput screens screens for small molecules that disrupt STOML3 function.

For further validation and lead optimization of small molecules we employ a set of experimental paradigms. For example we can test whether candidate compounds block mechanosensitive currents in isolated sensory neurons using the whole-cell patch-clamp technique. We can further test if local application of our compounds to the skin will block mechanosensitivity at the receptive endings of single primary afferents in the skin using the in vitro skin-nerve technique. Eventually we will perform behavioral tests in order to determine if the application of novel, potentially analgesic compounds lead to a lack of hypersensitivity to touch evoked pain in an animal model of neuropathic pain.

Academics

Akademische Aktivitäten

Photographer Katharina Bohm, Copyright MDC

Academic Appointments 2010-2011

Berufungen 2010-2011

2010

Prof. Michael Bader appointed to W3 professorship at the Charité

Professor Michael Bader, research group leader at the MDC and associate professor at the Charité, has been offered W3 positions as Professor of Pharmacology at the University of Heidelberg Medical School and Professor of Pharmacology and Toxicology at the University of Lübeck Medical School. In a counteroffer, the MDC appointed him to a W3 professorship at the Charité. Michael Bader has been a research group leader at the MDC since 1993. His work focuses on the elucidation of active regulatory mechanisms of various hormones such as angiotensin, bradykinin and serotonin. These hormones play an important role in the regulation of the cardiovascular system and pathological changes in blood pressure.

Dr. Zsuzsanna Izsvák receives permanent group leader position at the MDC

Dr. Zsuzsanna Izsvák came to the MDC in 1999, initially as a postdoc in the research group of her husband Dr. Zoltán Ivics. After receiving a EURYI Award in 2005, she established her own group. In Winter 2011, following an excellent review by external experts, she was offered a tenured position as leader of her research group at the MDC.

Prof. Matthias Selbach receives W2 professorship at the Charité

Prof. Matthias Selbach studied biology at the University of Münster and received his PhD degree from Humboldt University Berlin. He then went as postdoc to the laboratory of Matthias Mann, Max Planck Institute for Biochemistry in Martinsried. Since 2007, Prof. Matthias Selbach has led an independent junior research group at the MDC. Within a very short time, he furthered the development of mass spectrometry at the MDC. He is engaged in various collaborations with research groups of

2010

Prof. Michael Bader auf ordentliche W3-Professur an die Charité berufen

Prof. Michael Bader, Forschungsgruppenleiter am MDC und außerordentlicher Professor an der Charité, hat Rufe auf die W3-Professur für Pharmakologie an der Medizinischen Fakultät der Universität Heidelberg und auf die W3-Professur für Pharmakologie und Toxikologie an der Medizinischen Fakultät der Universität Lübeck erhalten. Zur Rufabwehr konnte das MDC ihn auf eine W3-Professur an der Charité berufen. Prof. Michael Bader ist seit 1993 Forschungsgruppenleiter am MDC. Schwerpunkte seiner Arbeit sind die Aufklärung von Wirk- und Regulationsmechanismen verschiedener Hormone, wie z. B. Angiotensin, Bradykinin und Serotonin. Diese Hormone spielen insbesondere in der Regulation des Herz-Kreislaufsystems und bei pathologischen Veränderungen bei Bluthochdruck eine wichtige Rolle.

Dr. Zsuzsanna Izsvák erhält permanente Gruppenleiterposition am MDC

Dr. Zsuzsanna Izsvák kam 1999 zunächst als Postdoc in die Arbeitsgruppe ihres Ehemanns Dr. Zoltán Ivics an das MDC. Mit einem EURYI Award konnte sie 2005 ihre eigene Gruppe aufbauen. Nach vorangegangener exzellenter Begutachtung durch externe Gutachter wurde ihr im Winter 2011 von den Gremien des MDC die Entfristung ihrer Stelle angeboten.

Prof. Matthias Selbach erhält W2-Professur an der Charité

Prof. Matthias Selbach studierte Biologie an der Westfälischen Wilhelms-Universität Münster und promovierte an der Humboldt-Universität zu Berlin. Danach wechselte er als Postdoc in das Labor von Matthias Mann, Max-Planck-Institut für Biochemie, Martinsried. Seit 2007 ist Prof. Selbach Leiter einer unabhängigen Nachwuchsforschungsgruppe am MDC. In kürzester Zeit hat Prof. Selbach die Massenspektrometrie am MDC aufge-

the Charité and the MDC and has published extensively. The methods of quantitative mass spectrometry developed by Matthias Selbach are crucial for proteomics research. There are only a few scientists worldwide who have been so instrumental in driving this technology forward.

2011

Helmholtz Junior Research Group for Dr. Daniela Panáková

Dr. Panáková's main area of research is the regulation of calcium currents via electrochemical signals and their impact on the formation of the heart and the development of cardiovascular disease. Dr. Panáková comes from Slovakia and studied at Comenius University in Bratislava. She did her doctoral work at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, where she continued to work for two years as a postdoc after completing her doctorate. In 2007, she went to Harvard Medical School in Boston. First, she worked in the cardiovascular research center at the Massachusetts General Hospital and then from 2009 at the Brigham and Women's Hospital. In 2008, she was awarded a grant in recognition of her achievements by the Human Frontier Science Program (HFSP).

Helmholtz Junior Research Group for Dr. Oliver Rocks

Dr. Oliver Rocks studies proteins (Rho proteins) which play a crucial role in signal transduction in the organism and, thus, regulate important cellular processes. If these processes are disrupted, cancer cells can metastasize, or cardiovascular diseases and neurological disorders can result. Born in Meppen, Germany, Dr. Rocks studied biochemistry in Bielefeld and obtained his PhD degree from the University of Bochum. In 2005, he received the Otto Hahn Medal of the Max Planck Society for outbaut, ist in vielfältige Kooperationen mit Forschungsgruppen der Charité und des MDC eingebunden und publiziert außerordentlich erfolgreich. Die von Prof. Matthias Selbach entwickelten Methoden der quantitativen Massenspektrometrie sind entscheidend für die Proteomics-Forschung. Es gibt weltweit nur wenige Wissenschaftler, die in der Lage sind, diese Technologie so voranzutreiben.

2011

Helmholtz-Nachwuchsgruppe für Dr. Daniela Panáková

Dr. Daniela Panáková erforscht vor allem die Steuerung von Kalziumströmen durch elektrochemische Signale und ihre Auswirkungen auf die Entwicklung des Herzens sowie auf die Entstehung von Herz-Kreislauf-Erkrankungen. Dr. Panáková stammt aus der Slowakei und studierte an der Comenius Universität in Bratislava. Sie machte ihre Doktorarbeit am Max-Planck-Institut für molekulare Zellbiologie und Genetik in Dresden, wo sie zwei Jahre nach ihrer Promotion als Postdoktorandin arbeitete. 2007 ging sie an die Harvard Medical School nach Boston, USA. Zunächst arbeitete sie dort am Herz-Kreislauf-Forschungszentrum des Massachusetts General Hospital und ab 2009 am Brigham and Women`s Hospital. 2008 erhielt sie aufgrund ihrer Leistungen eine Förderung durch das internationale Human Frontier Science Program (HFSP).

Helmholtz-Nachwuchsgruppe für Dr. Oliver Rocks

Dr. Oliver Rocks erforscht Proteine (Rho-Proteine), die eine entscheidende Rolle bei der Weiterleitung von Signalen im Organismus spielen und damit wichtige zelluläre Prozesse steuern. Sind diese Prozesse gestört, können Krebszellen metastasieren oder Herz-Kreislauf-Krankheiten sowie neurologische Erkrankungen die Folge sein. Dr. Oliver Rocks stammt aus Meppen. Er studierte Biochemie in Bielefeld und promovierte an der Universität Bochum. 2005 erhielt er die Otto-Hahn-Me-

standing scientific achievement. This was followed by research stays at various Max Planck Institutes in Munich and Dortmund as well as at the Weizmann Institute in Rehovot, Israel and at the European Molecular Biology Laboratory (EMBL) in Heidelberg. From 2007 to 2010, he received a HFSP fellowship to conduct research at the Samuel Lunenfeld Research Institute at Mount Sinai Hospital in Toronto, Canada.

BIMBS Junior Research Group for Dr. Alexander Löwer

Alexander Löwer began in May 2011 as a new junior group leader at the Berlin Institute for Medical Systems Biology (BIMSB). During his postdoc in the Department of Systems Biology at Harvard Medical School he studied the regulatory networks of the tumor suppressor p53 using state-of-the-art imaging methods.

Professor Tobias Pischon appointed to a W3 professorship in epidemiology

Prof. Tobias Pischon studied medicine at the Free University of Berlin (FU) and health care systems at the Technical University of Berlin (TU). He earned his doctorate at the Free University and then worked as postdoc at the Charité, where he also completed his *Habilitation*. From there Prof. Tobias Pischon went on a postdoctoral fellowship to the Harvard School for Public Health in Boston. From 2008 to 2010, he led the biomarkers research group at the German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE) and during this period developed his strong internationally competitive research profile. Tobias Pischon studies the role and function of various molecular and other biomarkers in cardiovascular and metabolic diseases and is also responsible at the MDC for the National Cohort within the Helmholtz Association.

Prof. Mathias Treier comes to the MDC from EMBL

Prof. Mathias Treier earned his doctorate at the European Molecular Biology Laboratory (EMBL) and then joined the lab of Dirk Bohmann, also EMBL, as a postdoc. His research contributions on the function and ubiquitination of c-Jun were widely recognized. He then went to the University of California at San Diego, USA, working as a postdoc under the supervision of Michael Rosenfeld. There, with his analyses of transcription factors that are important in embryonic development and endocrinology, he made important contributions to understanding the formation of the pituitary gland. Prof. Matthias Treier has conducted groundbreaking research, for example on the transcription factor FOXL2, which is responsible during embryonic development for the formation of the

daille der Max-Planck-Gesellschaft für außerordentliche wissenschaftliche Leistungen. Es folgten Stationen an verschiedenen Max-Planck-Instituten in München und Dortmund sowie am Weizmann-Institut in Rehovot, Israel, und am Europäischen Labor für Molekularbiologie (EMBL) in Heidelberg. Von 2007 bis 2010 forschte er mit einem HFSP-Stipendium am Samuel Lunenfeld Research Institute am Mount Sinai Hospital in Toronto, Kanada.

BIMSB-Nachwuchsgruppe für Dr. Alexander Löwer

Dr. Alexander Löwer begann im Mai 2011 als neuer Nachwuchsgruppenleiter am Berlin Institute for Medical Systems Biology (BIMSB). Während seiner Postdoc-Zeit in den USA untersuchte er mit neuesten Imaging-Methoden an der Harvard Medical School im Department of Systems Biology die Regulationsnetzwerke des Tumorsupressors p53.

Prof. Tobias Pischon wird auf eine W3-Professur in Epidemiologie berufen

Prof. Tobias Pischon studierte Medizin an der Freien Universität Berlin (FU) und Gesundheitswesen an der Technischen Universität Berlin (TU). Er promovierte an der FU und arbeitete zunächst als Postdoc an der Charité, wo er auch habilitierte. Mit einem Stipendium wechselte Prof. Tobias Pischon als Postdoc an die Harvard School for Public Health, University of Harvard, Boston, USA. Von 2008 bis 2010 leitete er die Arbeitsgruppe Biomarker am DIfE und entwickelte während dieser Zeit sein exzellentes, international kompetitives Forschungsprofil. Prof. Tobias Pischon erforscht die Rolle und Funktion verschiedener molekularer und anderer Biomarker bei kardiovaskulären und metabolischen Erkrankungen und ist am MDC u.a. auch für die Nationale Kohorte innerhalb der Helmholtz-Gemeinschaft zuständig.

Prof. Mathias Treier wechselt vom EMBL ans MDC

Prof. Mathias Treier promovierte am EMBL, wechselte dann als Postdoc ins Labor von Dirk Bohmann, EMBL, und machte dort mit den Beiträgen zur Funktion und Ubiquitinierung von c-jun auf sich aufmerksam. Als Postdoc bei Michael Rosenfeld, University of California, San Diego, USA, leistete er mit seinen Analysen von Transkriptionsfaktoren, die in der Embryonalentwicklung und Endokrinologie von Bedeutung sind, wichtige Beiträge zur Aufklärung der Hypophysenentwicklung. Prof. Mathias Treier gelangen wegweisende Beiträge z.B. zu dem Transkriptionsfaktor FOXL2, der während der Embryonalentwicklung für die Ausbildung des Ovars und im erwachsenen Organismus für den Erhalt der Funktionalität des Ovars verantwortlich ist. Der Transkriptions-

ovary and in the adult organism for the maintenance of ovary functionality. The transcription factor also prevents ovary-to-testes transdifferentiation. In May 2011, Prof. Mathias Treier joined the MDC where he leads the research group "Genetics of Metabolic and Reproductive Disorders".

Prof. Klaus Rajewsky returns to Germany from the U.S.

The immunologist Prof. Klaus Rajewsky returned to Germany after ten years' research activity at Harvard University in Boston in order to establish a research group at the MDC at the end of 2011. The internationally renowned researcher is thus the first German scientist to return to Germany to conduct research after going to the U.S. upon reaching the mandatory retirement age of 65 in Germany. In 2001, Professor Rajewsky accepted an offer from Harvard University because as emeritus professor in Germany he would only have been able to continue working to a limited extent at the University of Cologne. Klaus Rajewsky has conducted fundamental research on the development and function of B cells, the antibody factories of the immune system. In his laboratory in Cologne in the mid-nineties, together with his student Ralf Küppers and the pathologist Martin-Leo Hansmann, he succeeded in identifying B cells as the origin of Hodgkin's lymphoma, the most common cancer of the lymphatic system. Another research focus of Klaus Rajewsky is the significance of microRNAs for the development and function of the immune system. MicroRNAs are small, highly diverse molecules, which consist of ribonucleic acid and are involved in the regulation of almost all life processes. Klaus Rajewsky works closely with researchers at the MDC and the Charité both in the field of microRNAs and Hodgkin's lymphoma.

faktor verhindert außerdem eine Transdifferenzierung in die Testes. Seit Mai 2011 hat Prof. Mathias Treier am MDC eine Forschungsgruppe „Genetik metabolischer und reproduktiver Störungen".

Prof. Klaus Rajewsky kehrt aus den USA nach Deutschland zurück

Der Immunologe Prof. Klaus Rajewsky kehrt nach zehnjähriger Forschungstätigkeit an der Universität Harvard in Boston, USA, nach Deutschland zurück und wird bis Ende 2011 eine Forschungsgruppe am MDC aufbauen. Der international renommierte Forscher ist damit der erste deutsche Wissenschaftler, der nach Überschreitung des Pensionsalters aus den USA nach Deutschland in die Forschung zurückkehrt. 2001 hatte er ein Angebot der Harvard Universität angenommen, da er in Deutschland nach seiner Emeritierung im Alter von 65 Jahren nur eingeschränkt an der Universität Köln hätte weiterarbeiten können. Prof. Klaus Rajewsky hat grundlegende Arbeiten über die Entwicklung und Funktion der B-Zellen, den Antikörperfabriken des Immunsystems, vorgelegt. Mitte der neunziger Jahre war es ihm zusammen mit seinem Studenten Ralf Küppers und dem Pathologen Martin-Leo Hansmann in seinem Labor in Köln gelungen, B-Zellen als Ursprungszellen des Hodgkin-Lymphoms, des häufigsten Lymphdrüsenkrebses, zu identifizieren. Ein weiterer Forschungsschwerpunkt von Prof. Klaus Rajewsky ist die Bedeutung der microRNAs für die Entwicklung und Funktion des Immunsystems. MicroRNAs sind kleine, hochdiverse Moleküle, die aus Ribonukleinsäure bestehen und an der Kontrolle fast aller Lebensprozesse beteiligt sind. Sowohl auf dem Gebiet der microRNAs als auch bei der Erforschung der Hodgkinschen Krankheit arbeitet Prof. Klaus Rajewsky eng mit Forschern am MDC und der Charité zusammen.

Awards
Preise

2010

Oliver Daumke
Bayer Early Excellence in Science Award
Bayer Science & Education Foundation

EMBO Young Investigator Programme (YIP)

Elisa Kieback (Research Group: Wolfgang Uckert)
Nachwuchswissenschaftler/in-Preis
Forschungsverbund Berlin e. V.

Friedrich Luft
Elected honorary member
British Hypertension Society

Thoralf Niendorf
Helmholtz-Förderung zur Firmengründung, Impuls- und Vernetzungsfonds der Helmholtz-Gemeinschaft
Bereich Helmholtz-Enterprise

James Poulet
ERC Starting Grant
European Research Council

Matthew Poy
ERC Starting Grant
European Research Council

Nikolaus Rajewsky
Berliner Wissenschaftspreis
Regierender Bürgermeister von Berlin

Elected member EMBO
European Molecular Biology Organization

Matthias Selbach
Analytica Forschungspreis
Gesellschaft für Biochemie und Molekularbiologie e.V., Roche

EMBO Young Investigator Programme (YIP)

Salim Seyfried
Heisenberg-Stipendium der Deutschen Forschungsgemeinschft (DFG)

Ulrike Stein (Research Group: Peter Schlag)
Monika Kutzner-Preis
Berlin-Brandenburgische Akademie der Wissenschaften

Arbeitsgemeinschaft Internistische Onkologie-Wissenschaftspreis der Deutschen Krebsgesellschaft

2011

Katja Herzog (Research Group: Thomas Willnow)
"For Women in Science" Award
L'Oréal Deutschland, die Deutsche UNESCO-Kommission und die Christiane-Nüsslein-Volhard-Stiftung

Walter Rosenthal
Elected member Leopoldina
Nationale Akademie der Wissenschaften
German National Academy of Sciences Leopoldina

Klaus Rajewsky
ERC Advanced Research Grant
European Research Council

Nikolaus Rajewsky
Gottfried Wilhelm Leibniz-Preis
Deutsche Forschungsgemeinschaft (DFG) 2012

Thomas Willnow
Franz-Volhard-Preis
Deutsche Gesellschaft für Nephrologie (DGfN)

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Prof. Dr. Andreas Busch, Head of Global Drug Discovery at Bayer Pharmaceuticals, presents the Bayer Early Excellence in Science Award to Dr. Oliver Daumke, MDC, on March 24, 2011.

Prof. Dr. Andreas Busch, Leiter der Global Drug Discovery bei Bayer Pharmaceuticals, überreicht den Bayer Early Science Award an Prof. Oliver Daumke, MDC, am 24. März 2011.

Post-Doctoral Programs

Postdoktorandenförderung

Max Delbrück and Cécile Vogt Postdoctoral Programs

At the MDC, the traditional framework for promoting the early independence of young researchers has been and continues to be the Max Delbrück Program with its Delbrück Fellowships. In 2010 and 2011 four postdocs – Dr. Uta Höpken, Dr. Annette Hammes, Dr. Jan Bieschke and Dr. Christian Hirsch – received funding from the Max Delbrück Program. In addition, since the beginning of 2011, female researchers may apply for a fellowship from the Cécile Vogt Program (Cécile Vogt Fellows) to finance their own position. The Cécile Vogt Program is designed specifically to enable the early independence of female researchers and to support the visibility of their achievements. In contrast to the three-year fellowships in the Max Delbrück Program, the fellowships in the Cécile Vogt Program are for four years in order to take potential maternal/parental leave into account. The researcher's own position is financed in both programs. A budget for consumables is also available.

Career Pathways Seminars

In November 2010, the lecture series "Career Pathways" was resumed. In 2010, four speakers gave talks about alternatives to an academic career. In 2011, there were ten speakers, among them three MDC alumni. Workshops and half-day seminars were also offered on the topics of applications and research fellowships. On "Career Development Day", the postdocs obtained information on research fellowship programs from the MDC advisers for the EU and the international programs (Dr. Sabine Baars and Dr. Oksana Seumenicht) and representatives of the Alexander von Humboldt-Foundation, the German Academic Exchange Service (DAAD), European Molecular Biology Organization (EMBO) and the Human Frontier Science Program (HFSP) as well as from the EU Liaison Office of the German Research Organizations (KoWi).

Postdoctoral Association

Since 2011, the postdocs at the MDC have organized themselves to form the "MDC Postdoctoral Association". The spokespersons (currently Dr. Anne-Sophie Carlo and Dr. Rick Scavetta) participate in the Scientific Council of the MDC. along with additional elected postdocs.

Max-Delbrück- und Cécile-Vogt-Postdoktoranden-Programme

Die Förderung einer frühzeitigen Unabhängigkeit von Nachwuchswissenschaftlern (sogenannten Postdoktoranden) erfolgt am MDC bislang im Rahmen des Max-Delbrück-Programms („Delbrück Fellowships"). In den Jahren 2010 und 2011 wurden die vier Nachwuchswissenschaftler Dr. Uta Höpken, Dr. Annette Hammes, Dr. Jan Bieschke und Dr. Christian Hirsch über das Max-Delbrück-Programm gefördert. Seit Anfang 2011 können Wissenschaftlerinnen auch im Rahmen des Cécile-Vogt-Programms („Cécile Vogt Fellows") ihre eigene Stelle beantragen. Das Cécile-Vogt-Programm soll gezielt die frühe Selbstständigkeit von Wissenschaftlerinnen ermöglichen und die Sichtbarkeit ihrer Leistungen unterstützen. Anstatt der dreijährigen Förderdauer des Max-Delbrück-Programms werden im Rahmen des Cécile-Vogt-Programms vier Jahre gefördert, um eventuellen Familienzeiten Rechnung tragen zu können. In beiden Programmen wird die eigene Stelle gefördert. Ein Sachmittelbudget steht zur Verfügung.

„Career Pathways" Seminare

Im November 2010 wurde die Vortragsserie „Career Pathways" (Karrierewege) wieder aufgenommen. Im Jahr 2010 haben vier Referenten des Deutschen Akademischen Austauschdienstes und im Jahr 2011 zehn Referenten, darunter 3 MDC-Alumni, über Alternativen zu einer wissenschaftlichen Karriere berichtet. Des Weiteren wurden Workshops und halbtägige Seminare zu Bewerbungs- und Forschungsförderungsthemen angeboten. Auf dem „Career Development Day" informierten die MDC-Referenten für EU- und Internationale Programme (Dr. Sabine Baars und Dr. Oksana Seumenicht) sowie Vertreter der Alexander von Humboldt-Stiftung (DAAD), der Europäischen Organisation für Molekularbiologie (EMBO), des Human Frontier Science Programm (HFSP) und der Kooperationsstelle EU der Wissenschaftsorganisationen über Forschungsförderprogramme.

Post-Doctoral Association

Die Postdoktoranden des MDC organisieren sich seit 2011 in der „MDC Post Doctoral Association". Ihre Sprecher (derzeit Dr. Anne-Sophie Carlo und Dr. Rick Scavetta) nehmen mit weiteren gewählten Postdoktoranden am Wissenschaftlichen Rat des MDC teil.

PhD Program

PhD-Programm

The MDC considers the training of new generations of researchers in Molecular Medicine as a basic prerequisite for sustainable development and international scientific success. In 2003, the MDC and Humboldt University (HU) established an International PhD Program to offer training and interdisciplinary education to graduate students. The programme combines academic research in molecular medicine, education and training, and provides high-end technology and interdisciplinary project opportunities, including many collaborations between research groups on Campus.

Following the success of the international PhD program, the MDC and its university partners were awarded a substantial 6-year grant from the Helmholtz Association to establish the Helmholtz Graduate School of "Molecular Cell Biology" in 2007. The Helmholtz Graduate School offers structured support and training, and education opportunities to students conducting their thesis project at the MDC together with partners at the Humboldt University, the Freie University, and the Leibniz-Institut für Molekulare Pharmakologie (FMP). Currently, 350 PhD students work towards their PhD degree at the MDC and benefit from the structured program and research school training. One third of these students come from abroad, representing 39 countries.

The graduate school consolidates various activities including:

- Welcome centre to assist international students at their start in Berlin
- University interface
- PhD committee meetings to regularly review project progress
- Lectures and seminar series in molecular cell biology and specialized areas
- Advanced methods courses
- Introduction to new technologies to complement the research skills training

Das MDC betrachtet die Ausbildung neuer Generationen von Forschern auf dem Gebiet der molekularen Medizin als grundlegende Voraussetzung für eine nachhaltige Entwicklung und für internationalen wissenschaftlichen Erfolg. Im Jahr 2003 starteten das MDC und die Humboldt-Universtität zu Berlin (HU) ein internationales PhD-Programm, um graduierten Studenten eine interdisziplinäre Ausbildung zu bieten. Das Programm verbindet wissenschaftliche Forschung auf dem Gebiet der molekularen Medizin mit Aus- und Weiterbildung. Es bietet High-End-Technologie und interdisziplinäre Projektmöglichkeiten einschließlich vieler Formen der Zusammenarbeit zwischen Forschungsgruppen auf dem Campus.

Nach dem Erfolg des internationalen PhD-Programms wurde dem MDC und seinen Universitätspartnern durch die Helmholtz-Gemeinschaft eine bedeutende Förderung für sechs Jahre zugesprochen, um 2007 die Helmholtz-Graduiertenschule Molecular Cell Biology zu gründen. Den Studenten, die ihr Dissertationsprojekt am MDC zusammen mit Partnern an der Humboldt-Universität, der Freien Universität und dem Leibniz-Institut für Molekulare Pharmakologie (FMP) durchführen, bietet die Helmholtz-Graduiertenschule strukturierte Förderung und Ausbildungsmöglichkeiten. Gegenwärtig arbeiten am MDC 350 Doktorandinnen und Doktoranden an ihrer Promotion und profitieren von dem strukturierten Programm und der Forschungsausbildung. Ein Drittel dieser Studierenden kommt aus dem Ausland und zwar aus insgesamt 39 Ländern.

Die Graduiertenschule vereinigt verschiedene Aktivitäten wie

- Welcome Center, um internationalen Studierenden ihren Start in Berlin zu erleichtern
- eine Schnittstelle zu den Universitäten
- Treffen der Doktorandenkomitees zur regelmäßigen Überprüfung des Projektfortschritts
- Vorlesungen und Seminarreihen auf dem Gebiet der molekularen Zellbiologie und Spezialgebieten

Photographer David Ausserhofer, Copyright MDC

- Soft skill training and career development program to complement the scientific training
- Annual Campus Symposium and 3-day PhD Retreat are organized by our students and promote networking and exchange of scientific ideas
- Building of an alumni network

To help students keep track of various activities and courses during their PhD and facilitate the organization of individual training needs and interests, the Graduate School operates a credit point system which summarizes the students' achievements in the MDC PhD-Certificate. Additional and more specialized research schools have been established and are integrated within the Helmholtz Graduate School. These research schools specialize in cardiovascular disease (*TransCard)*, molecular neurobiology (*MolNeuro)* and systems biology (*BIMSB-NYU exchange program)*:

Close collaboration between the MDC and the Freie University (FU) led 2007 to the establishment of the Helmholtz Research School in Molecular Neurobiology *MolNeuro*. The training curriculum focuses on basic and advanced concepts of Molecular Neurobiology. The 'Berlin Brain Days', an annual student's conference, offer students all over Berlin to meet and discuss their

- Kurse in fortgeschrittenen Methoden
- Einführung in neue Technologien zur Ergänzung der Forscherausbildung
- Training in Soft Skills und ein Karriere-Entwicklungsprogramm zur Ergänzung der wissenschaftlichen Ausbildung
- jährliches Campus-Symposium und dreitägiger PhD-Retreat werden durch unsere Studierenden organisiert – eine Förderung der Vernetzung und des Austauschs von wissenschaftlichen Ideen
- Aufbau eines Alumni-Netzwerks

Die Graduiertenschule hat ein Leistungspunktsystem (European Credit Transfer System), in dem die Leistungen der Studierenden während ihres PhD-Studiums im MDC-Promotionszeugnis aufgeführt werden. Das hilft den Studierenden, die Übersicht über verschiedene Aktivitäten und Kurse zu behalten und ihre individuellen Ausbildungserfordernisse und Interessen zu berücksichtigen.

Zusätzliche und weiter spezialisierte Kollegs (Research Schools) wurden gegründet und sind in die Helmholtz-Graduiertenschule integriert. Diese Research Schools sind spezialisiert auf kardiovaskuläre Krankheiten

Photographer David Ausserhofer, Copyright MDC

research projects. In 2009 the Helmholtz Research School in Translational Cardiovascular and Metabolic Medicine *TransCard* was established from Funds of the Helmholtz-Association. The Programme offers lectures, summer schools and e-learning in the Area of Cardiovascular and Metabolic Research. Also in 2009, the Berlin Institute Medical Systems Biology and the Center for Genomics and Systems Biology of the New York University started their joint *PhD-Exchange-Program*. The students are working on collaborative projects with the scientific focus on post-transcriptional gene regulation in systems biology. All students are, in addition to their MDC supervisor, under supervision of a NYU faculty member and conduct their research in the respective laboratories both, in Berlin and New York.

Calls for applications to the International PhD Program are announced twice per year. The International PhD Program receives about 1,500 applications per year, of which approximately 120 candidates are invited for on-site assessment by MDC research group leaders in Berlin and about 40 of these candidates will be offered a PhD position in one of the MDC or University laboratories. Successful candidates will find excellent research facilities and outstanding training opportunities in one of Germany's leading research centers. In addition to the semi-annual International PhD Program recruitment rounds outstanding candidates may present a dedicated research project together with one of the MDC research group leaders anytime to apply for an MDC-scholarship.

(*TransCard*), molekulare Neurobiologie (*MolNeuro*) und das Austauschprogramm Systembiologie (*BIMSB-NYU*).

Die enge Zusammenarbeit zwischen dem MDC und der Freien Universität (FU) führte 2007 zur Gründung der Helmholtz Research School in Molecular Neurobiology *MolNeuro*. Im Fokus des Ausbildungscurriculums stehen Grundkonzepte und fortgeschrittene Konzepte der Molekularen Neurobiologie. Die jährlich stattfindende Studierendenkonferenz „Berlin Brain Days", bietet Studierenden in ganz Berlin die Möglichkeit zu einem Treffen und zur Diskussion ihrer Forschungsprojekte. 2009 wurde die Helmholtz International Research School in Translational Cardiovascular and Metabolic Medicine *TransCard* aus Mitteln der Helmholtz-Gemeinschaft gegründet. Das Programm umfasst Vorlesungen, Sommerkurse und E-Learning auf dem Gebiet der kardiovaskulären Forschung und Stoffwechselforschung. Das Berlin Institute for Medical Systems Biology und das Center for Genomics and Systems Biology (BIMSB) der New York University starteten ebenfalls im Jahr 2009 ihr gemeinsames PhD-Austauschprogramm. Die Studierenden arbeiten an gemeinschaftlichen Projekten mit dem wissenschaftlichen Fokus auf post-transkriptionelle Genregulation in der Systembiologie. Zusätzlich zu ihrem Betreuer vom MDC haben alle Studierenden ein Fakultätsmitglied der New York University als Betreuer und betreiben ihre Forschung in den entsprechenden Laboren sowohl in Berlin als auch in New York.

Ausschreibungen für das internationale PhD-Programm werden zweimal jährlich veröffentlicht. Dafür werden jedes Jahr rund 1500 Bewerbungen eingereicht. Etwa 120 Kandidatinnen und Kandidaten werden zu Assessment-Interviews durch MDC-Forschungsgruppenleiter nach Berlin eingeladen. Von diesen wird etwa 40 Personen eine Doktorandenstelle in einem der MDC- oder Universitätslabore angeboten. Erfolgreiche Kandidatinnen und Kandidaten finden dann hervorragende Forschungseinrichtungen und Ausbildungsmöglichkeiten in einem der führenden Forschungszentren Deutschlands. Zusätzlich zu den halbjährlich stattfindenden internationalen Bewerbungsrunden für das PhD-Programm können herausragende Kandidatinnen und Kandidaten jederzeit ein bestimmtes Forschungsprojekt bei einem der MDC-Forschungsgruppenleiter vorstellen, um sich für ein MDC-Stipendium zu bewerben.

Conferences and Scientific Meetings

Kongresse und Wissenschaftliche Tagungen

2010

29 January	**Neujahrsempfang des Campus** Organizer: Campus Öffentlichkeitsarbeit
22-24 March	**9th Transgenic Technology Meeting 2010 (TT2010)** **Konferenz der International Society for Transgenic Technologies (ISTT)** Organizer: Dr. Boris Jerchow
16 April	**1st Annual Scientific Symposium Ultrahigh Field Magnetic Resonance Clinical Needs** Organizer: Prof. Thoralf Niendorf
22-23 April	**6. Laborrunde-Konferenz** Organizer: Ralf Streckwall
26 April-31 May	**Ausstellung „EVA YEH In den Raum – Objekte aus Plexiglas“** Organizer: MDC Vorstand
3-7 May	**Basic Gene Mapping Course** Organizer: Gruppe Bioinformatik
5 June	**Lange Nacht der Wissenschaften (Long Night of the Sciences)** Organizer: Campus Öffentlichkeitsarbeit
11 June	**Preisverleihung „Land der Ideen“** Organizer: BBB Management GmbH Campus Berlin-Buch
24-26 June	**3rd Berlin Summer Meeting: Computational & Experimental Molecular Biology** Organizer: Prof. Nikolaus Rajewsky
29 September	**Cancer Day** Organizer: Prof. Claus Scheidereit
30 September-2 October	**115. Jahreskongress der Deutschen Gesellschaft für Physikalische Medizin und Rehabilitation** Organizer: Prof. Friedrich Luft
22-29 October	**Basic Gene Mapping Course** Organizer: Gruppe Bioinformatik
26-27 October	**Deutsch-Israel Symposium** Organizers: Prof. Thomas Sommer, Dr. Almut Caspary
1-4 November	**Brain Days** Organizer: Prof. Helmut Kettenmann
8 December	**Festveranstaltung Arnold Graffi und Nikolaj Timoffeev Ressovsky** Organizer: MDC Vorstand

2011

20 January	**Neujahrsempfang des Campus** Organizer: Campus Öffentlichkeitsarbeit
22 February	**Wissenschaft und Wirtschaft: Treffpunkt Campus Berlin-Buch** Organizer: Freundeskreis des MDC
23 March	**Bayer Early Excellence in Science Award for Oliver Daumke** Organizer: Bayer Healthcare, MDC, Freundeskreis des MDC
28 March	**Geburtstagsveranstaltung zum 70. Geburtstag von Prof. Dr. Detlev Ganten, Gründungsdirektor des MDC** Organizer: MDC Vorstand
14-15 April	**7. Laborrunde-Konferenz** Organizer: Ralf Streckwall
25 May	**Benefizkonzert: Musik für Japan aus Berlin-Buch** Organizer: MDC Vorstand
28 May	**Lange Nacht der Wissenschaften (Long Night of the Sciences)** Organizer: Campus Öffentlichkeitsarbeit
16-17 June	**Brain Tumor Meeting** Organizer: Prof. Helmut Kettenmann
24 June	**2nd Annual Scientific Symposium** **Ultrahigh Field Magnetic Resonance Clinical Needs** Organizer: Prof. Thoralf Niendorf
23-25 June	**4th Berlin Summer Meeting: Computational & Experimental Molecular Biology** Organizer: Prof. Nikolaus Rajewsky
11-14 September	**Stem Cell Meeting** Organizers: Dr. Daniel Besser, Prof. Carmen Birchmeier, Dr. Ulrike Ziebold
8-13 September	**40. EMC – European Muscle Conference** Organizer: Prof. Ingo Morano
21-25 September	**EMBO Conference: Ubiquitin and Ubiqutin-like Modifiers (Location: Dubrovnik)** Organizer: Prof. Thomas Sommer
27-28 October	**Symposium zum Schwerpunkt „Tea & Health“** Organizer: Franz-Volhard-Centrum für Klinische Forschung am Experimental & Clinical Research Center (ECRC)
7-9 December	**Berlin Brain Days** Organizers: International Graduate Program Medical Neurosciences and NeuroCure Berlin School of Mind and Brain International Doctoral Program Computational Neuroscience Helmholtz International Research School Molecular Neurobiology GRK 1123 Learning and Memory International Graduate School Languages of Emotion

Seminars 2010

Seminare 2010

First name	Last name	Organization	Talk title
Antoine	**Adamantidis**	Department of Psychiatry and Behavioural Sciences, Stanford University	Optogenetic probing of hypothalamic regulation of arousal
Jochen	**Balbach**	Universität Halle/Wittenberg	Structural exploration of protein energy landscapes by NMR
Ovidiu	**Baltatu**	Grigore T. Popa University of Medicine and Pharmacy, Romania	Endothelin Antagonism – A Case of Reverse Translation Research
Michael	**Banner**	Trinity College, Cambridge	Public attitudes towards animals – a UK perspective.
Gavin	**Bendle**	The Netherlands Cancer Institute	Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy
Carsten	**Beta**	University of Potsdam/ Biological Physics	Microfluidic tools for the study of cell motility and chemotaxis
Andreas	**Beyer**	TU Dresden	The power of data integration: molecular interpretation of functional screens
Lutz	**Birnbaumer**	National Institute of Environmental Health Sciences, North Carolina	Gazing at the mammalian methylome with a Solexa sequencing machine: defining thousands of novel unmethylated regions with methylation-sensitive restriction enzymes
Stefan	**Blankenberg**	Universitätsmedizin der Johannes-Gutenberg-Universität Mainz	Translational Concepts in Cardiovascular Medicine – From Genome to Clinical Application
Camille	**Boutin**	The Developmental Biology Institute of Marseilles	Postnatal electroporation of the forebrain
Thomas	**Brand**	Faculty of Medicine, Imperial College London	The Popeye genes, a novel class of heart rate regulators
David	**Brunner**	ETH Zürich, Institute for Biomedical Engineering	What is next in MR Imaging: Traveling and other Mysterious Waves
Juan A.	**Bueren**	Hematopoietic Gene Therapy Division, Madrid	Gene Therapy and Cell Reprogramming in F anconi Anemia
Dmitry	**Bulavin**	Institute of Molecular and Cell Biology, Singapore	Wip1 phosphatase at the crossroads of cancer and aging
Guido	**Cavaletti**	Dipartimento di Neuroscienze e Tecnologie Biomediche, Monza	Physiology and Molecular Biology of Drug induced Neuropathies
Ehud	**Cohen**	The Institute for Medical Research Israel-Canada, Hebrew University Medical School	Reducing Insulin/IGF signaling as a novel neurodegeneration therapy: current knowledge and future prospects
Ruud	**Delwel**	Erasmus University Rotterdam	Uncovering the patho-physiology of human acute myeloid leukemia with defects in the transcription regulator C/EBPalpha

Seminars 2010

Seminare 2010

First name	Last name	Organization	Talk title
Marian	**Difiglia**	MGH Harvard	Early Membrane Trafficking Defects in Huntington's Disease
Oliver	**Ebenhoeh**	University of Aberdeen	Entropic principles in carbohydrate metabolism
Gaetano	**Gargiulo**	The Netherlands Cancer Institute (NKI)	Polycomb complexes repress developmental regulators in murine neural progenitor cells
Ivan	**Gesteira Costa Filho**	Center of Informatics, Federal University of Pernambuco	Inferring (Epi-)genetic Gene Regulation in Cell Differentiation
Christian	**Göritz**	Karolinska Institutet Stockholm	Adult neural stem and niche cells and their response to injury
Núria	**Gresa**	Cerebral Isquemia and Neurodegeneration department, Barcelona	Anti-inflammatory and neuroprotective effects of C/EBP delta inhibition in microglia
Atan	**Gross**	Weizmann Institute of Science	Balancing cell life and death decisions
Christel	**Herold-Mende**	University of Heidelberg	Differentiation resistance: an unforeseen therapeutic challenge
Wiebke	**Herzog**	Westfälische Wilhelms University Münster	Endothelial cell migration in zebrafish
Mark S	**Hipp**	Stanford University	Proteostasis and the ubiquitin proteasome system in neurodegenerative disease
Thomas	**Jahn**	University of Cambridge	Molecular Mechanisms of Protein Aggregation: from the test tube into Drosophila
Ralf	**Jauch**	Genome Institute of Singapore	How proteins understand genomes – the structural biochemistry of transcription factors
Christoph	**Kaether**	Leibniz Institute for Age Research, Fritz Lippmann Institute, Jena	Assembly and quality control of gamma-secretase, a key enzyme in Alzheimer´s disease and Notch signaling
Michael	**Kahn**	University of Southern California	Differential Coactivator Usage in Stem Cells and Cancer Stem Cells
Oktay	**Kirak**	Whitehead Institut for Biomedical Research, Cambridge	„Next Generation" Mouse Models with pre-defined T and B Cell Receptor obtained via Somatic Cell Nuclear Transfer
Thomas	**Langmann**	University of Regensburg, Institute of Human Genetics	Microglial activation in retinal degeneration
Nathan D.	**Lawson**	University of Massachusetts Medical School	How to make a blood vessel sprout

First name	Last name	Organization	Talk title
Serge	**Leyvraz**	Centre Pluridisciplinaire d'Oncologie Lausanne	Adoptive transfer therapy in the clinic: the Lausanne experience
Carsten	**Linnemann**	The Netherlands Cancer Insitute	Identifying new T cell receptors for T cell receptor gene therapy
Dima	**Lukatsky**	Ben-Gurion University of the Negev	Multi-scale sequence correlations are evolutionary selected to increase protein promiscuity
Jörg	**Männer**	Georg-August-Universität Göttingen	Cardiac looping in higher vertebrate embryos: an uneasy deal with morphology and chirality
Manuela	**Marega**	Molecular Oncology Laboratory, University of Milano-Bicocca	Molecular Mechanisms for the Progression of Chronic Myeloid Leukemia
Stephen F.	**Marino**	Max Planck Institute of Biophysics, Frankfurt/M.	High-level production and characterization of a G-protein coupled receptor signalling complex
Jochen	**Meier**	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	RNA plasticity in hyperexcitability disease
Amir	**Orian**	The Ruth and Bruce Rappport Faculty of Medicine, Israel Institute of Technology	Role of SUMO Targeted Ubiquitin Ligases in transcription during Development
Anant	**Parekh**	University of Oxford	Store-operated calcium channels: gating and function in health and disease
Francesca	**Peri**	EMBL Heidelberg	Microglia: the guardians of the developing brain
Valentina	**Perissi**	University of California San Diego	The many faces of the NCoR corepressor complex.
Juri	**Rappsilber**	University of Edinburgh	From stable cores to elusive peripheries – increased vision into protein complexes by cross-linking and mass spectrometry
Oliver	**Rocks**	Samuel Lunenfeld Research Institute, Toronto	Spatio-temporal control of Rho signalling
Jürgen	**Sandkühler**	Center for Brain Research, Vienna	Learning and Memory in Pain Pathways
Annika	**Schäfer**	Klinik für Urologie, Charité – Universitsmedizin Berlin	MiRNA Regulation in Prostate Carcinoma: Diagnostic, Prognostic and Therapeutic Implications
Marc	**Schmidt-Supprian**	Max Planck Institute of Biochemistry, Martinsried	The role of the tumor suppressor A20 in B cell physiology
Gunnar F.	**Schröder**	Forschungszentrum Jülich	Structure refinement at low-resolution

Seminars 2010

Seminare

First name	Last name	Organization	Talk title
Stephan	**Schulz**	Institut für Pathologie der TUM	Combining Morphology, Immunofluorescence and Bioluminescence in vivo to clarify Migration of allogeneic T Cells
Licia	**Selleri**	Weill Medical College of Cornell University	Genetic and Transcriptional Control of Organ Size: Lessons from the Spleen
Arndt	**Siekmann**	Max Planck Institute for Molecular Biomedicine Münster	Role of chemokine signaling during angiogenesis in zebrafish embryos
Robert	**Slany**	Department of Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg	Errors in Hematopoiesis – MLL Fusions in Acute Leukemia
Daniel	**Speiser**	Ludwig Institute for Cancer Research	Avidity and clonality of human tumor-specific CD8 T cells
Julia	**Stindl**	University of Regensburg	Calcium signaling mediated by oxytocin in cultured hypothalamic neurons
Leslie M	**Thompson**	University of California	Therapeutic strategies to treat Huntington's disease
Mathias	**Treier**	Universität Köln	A hypothalamic network connecting appetite, activity and memory
Erik	**van Nimwegen**	Biozentrum, University of Basel	Motif Activity Response Analysis: Inferring genome-wide transcription regulation in mammals
Sachdev	**Vinay**	School of Life sciences, University of Skövde	Alternate signaling pathways underlying the cause of Hypoxic Brain damage
Renate	**Voit**	German Cancer Research Center, Heidelberg	AMP-activated protein kinase and SIRTUINs: Master regulators of transcription
Falk	**Weih**	Leibniz Institute for Age Research, Fritz Lippmann Institute, Jena	Regulation of T cell development in thymus by RelA and RelB
Katrin	**Willig**	Max Planck Institute for Biophysical Chemistry	Live-cell STED microscopy
F. Gregory	**Wulczyn**	Charité – Universitätsmedizin Berlin	Glimpses of a parallel universe: Regulation of miRNA biogenesis in stem cells
Thomas	**Wunderlich**	Institute for Genetics, University of Cologne	Dissecting inflammatory signaling pathways in obesity-associated diseases.
Katharina	**Zimmermann**	Institut für Physiologie und Pathophysiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg	Is inhibition of outward potassium currents sufficient to initiate cold transduction in nociceptor nerve endings?

Seminars 2011

Seminare 2011

First name	Last name	Organization	Talk title
Susanne	**Arnold**	Institute for Neuroanatomy, RWTH Aachen	Power of Life – Mitochondria and their role in neurodegeneration
Brendan James	**Battersby**	Biomedicum, Helsinki	Role of GIMAPs and other factors in tissue-specific regulation of mitochondrial genetics
Rui	**Benedito**	Max Planck Institute for Molecular Biomedicine Münster	Creating top Notch differences among equals during vascular development
Willem	**Bintig**	Institut für Biophysik, Leibniz Universität Hannover	Purinergic receptors, gap junctions, and voltage gated ion channels – overview of different projects
Rico	**Bongaarts**	Union Biometrica, Belgium	Analysis and sorting of model organisms (C. elegans, Drosophila, Zebrafish) using Union Biometrica's Copas™ and Biosorter® large particle flow cytometry instruments
Denis	**Burdakov**	Department of Pharmacology, University of Cambridge	Controlling the switches of brain state: regulation of orexin neurons
Dinis	**Calado**	Harvard Medical School	Genetic Interactions that Determine Mature B-cell Tumor Type
Maren	**Eckey**	Adolf Butenandt Institut, München	The chromatin remodeler ISWI in mammals – two orthologues with opposing functions
Ute	**Felbor**	Institute of Human Genetics, University of Greifswald	Genetic and clinical aspects of cerebral cavernous malformations
Dean W.	**Felsher**	Stanford School of Medicine, Division of Oncology	Oncogene Addiction Inside and Out
Christian	**Gaser**	Structural Brain Mapping Group, Dept. of Psychiatry, University of Jena	MR-Morphometry for assessment of structural brain plasticity
Susana	**Godinho**	Dana-Farber Cancer Institute, Boston	Centrosome amplification: >> cancer's friend or foe?
Achim	**Gossler**	Institute for Molecular Biology Hannover	Function and regulation of the homeobox gene Noto
Dolores	**Hambardzumyan**	Stem Cell Biology and Regenerative Medicine, Lerner Research Institute, Cleveland	Radiation resistance and cancer stem-like cells in brain tumors
Thorsten	**Hoppe**	Institute for Genetics, University of Cologne	Life and Destruction: Ubiquitin-mediated Proteolysis in Longevity and Age-related Diseases
Mia	**Horowitz**	University of Tel Aviv	EHDs and endocytosis
Roland	**Jahns**	Universitätsklinikum Würzburg	A New Therapeutic Approach in Heart Failure: Cardioprotective Cyclic Peptides

Seminars 2011

Seminare 2011

First name	Last name	Organization	Talk title
Sergey	**Kasparov**	Department of Physiology and Pharmacology University of Bristol	Opto-genetic analysis of central chemosensitivity
Kai	**Kessenbrock**	University of California, San Francisco	Proteolytic control of inflammation and cancer
Friedemann	**Kiefer**	Max Planck Institute for Molecular Biomedicine Münster	Morphogenetic events during the birth of lymphatic vessels.
Fabian	**Kiessling**	RExperimental Molecular Imaging, RWTH Aachen	Characterizing tissue vascularisation by multimodal imaging from the molecular to the macroscopic scale
Darius Vasco	**Köster**	Institute Curie, Paris	The role of Caveolae in Membrane Mechanics
James G.	**McNally**	National Cancer Institute, Bethesda	Measuring the live cell kinetics of transcription: at the crossroads of binding, movement and structure
Sebastian	**Meijsing**	Max Planck Institute for Molecular Genetics Berlin	The site code: DNA-binding site sequence directs Glucocorticoid receptor structure and activity
Henning	**Morawietz**	University Hospital Carl Gustav Carus, University of Technology Dresden	Arteriovenous differentiation and mechanosensitive gene expression in endothelial cells
Daniel J.	**Murphy**	Theodor Bovery Institut, Universität Würzburg	Exploiting oncogene induced dependency to target Myc in cancer
Pavel I.	**Nedvetzky**	Mostov Laboratory; School of Medicine, Dept. of Anatomy; University of California, San Francisco	The role of cAMP-dependent protein kinase in epithelial morphogenesis in vitro
Cajetan	**Neubauer**	Laboratory of Molecular Biology Cambridge	ReIE and tmRNA: structural insights into the rescue of stalling ribosomes
Paul	**Nghiem**	Fred Hutchinson Cancer Research Center, Seattle	Merkel Cell Carcinoma
Ulrike	**Nuber**	Lund University, Stem cell gene regulation	Therapeutic translation of molecular mechanisms in two brain diseases: Rett syndrome and rhabdoid tumors
Hanspeter	**Pircher**	Universitätsklinik Freiburg	Mouse models for studying virus- and tumor-specific T cell responses in vivo
Gerrit	**Praefcke**	Center for Molecular Medicine Cologne	Lipid modification in the dynamin-like GBP1
Stephan	**Preibisch**	Max Planck Institute of Molecular CellBiology and Genetics, Dresden	In-toto reconstruction of Drosophila embryogenesis by processing Selective Plane Illumination Microscopy (SPIM) images
Klaus	**Rajewsky**	Harvard Medical School	Conditional signal-on mutants in the analysis of lymphocyte homeostasis and transformation

First name	Last name	Organization	Talk title
Eva	**Rosenbaum**	Institut für Strukturbiologie Grenoble	Intracellular Proteolysis and Extremophilic Adaptation in Archaea
Aurélien	**Roux**	University of Geneva	How membrane curvature regulates dynamin-mediated fission
Hermann	**Schindelin**	Institute of Structural Biology Würzburg	From A to O – Proteins in the Endoplasmic Reticulum
Marion	**Schmidt**	Albert Einstein College of Medicine, New York	Proteasome-mediated regulation of ribosome biogenesis
Diane	**Scott**	Imperial College London	Induction of antigen-specific allograft tolerance by intranasal peptide treatment
Elizabeth	**Simpson**	Imperial College London	Minor H antigens: tools for reprogramming rejection responses
Andrei	**Sommer**	Institute of Micro and Nanomaterials, University of Ulm	The Light-Cell-Pump: Forcing Cells to Uptake Small Molecules, e.g., EGCG
Peter	**Stadler**	Universität Leipzig	Beyond microRNAs: treasures in short-read sequencing data
Kunchitha-padam	**Swaminathan**	National University of Singapore	Structural insights of CRFR1, Pac1R and PKA in the urocortin and adenylate cyclase mediated cell signaling events
Maria Elena	**Torres-Padilla**	Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), France	Epigenetic mechanisms in early mammalian development
Gleb	**Turchinovich**	Cancer Research UK	Molecular control of gamma/delta T cell functional selection
Dirk	**Winnemöller**	Miltenyi Biotech, Bergisch Gladbach	New Methods in Stem Cell Research – Enabling the Reprogramming Workflow
Susanne	**Wolf**	University of Zurich	Impact of in utero events on neurogenesis
Yang	**Ye**	Shanghai Institute	"Identification of bioactive compounds responsible for therapeutic benefits of traditional herbal medicine"
Hua	**Yu**	City of Hope Duarte, California	STAT3 in tumor immunity and cancer inflammation
Dan	**Zhu**	Institute of Micro and Nanomaterials, University of Ulm	The Light-Cell-Pump: Forcing Cells to Uptake Small Molecules, e.g., EGCG

Research Projects 2010-2011

Forschungsprojekte 2010-2011

DFG Research Grants / DFG Sachbeihilfe

Adenoviraler Gentransfer kostimulatorischer Moleküle in Tumorzellen zur Hemmung von T-Zell-Delection und Steigerung der T-Zell-Zytotoxizität gegen Tumore

Aktivitäts-abhängige Regulation von neuralen Zelloberflächenproteinen und ihre Funktion bei der Synaptogenese: Funktionelle Charakterisierung von CALEB

Analyse der Funktion alternativ gespleißter schwerer Myosinketten im glatten Muskel der Maus durch Knock-Out

Analyse von cis und trans Elementen der DNA Replikationsinitiation in Drosophila melanogaster

Angiotensin II-induzierte Entzündungsreaktionen und ihre Kontrolle durch P450-abhängige Metabolite mehrfach ungesättigter Fettsäuren

Bedeutung der a2A-Adrenorezeptoren für die autonomen Funktionsstörungen bei Diabetes mellitus

Bedeutung der Delta-Isoformen der Ca+/Calmodulin-abhängigen Proteinkinase II für Differenzierung und Funktion von Myozyten

Biologische Funktionen des Proteinkinase A-Ankerproteins AKAP18

Biologische Funktionen von neuartigen Proteinkinasen A-Ankerproteinen (Ht31/Rt31), die cAMP- und RHO-Signale integrieren können

Blutdruckregulation und Nierenfunktion/Interaktion von Renin-Angiotensin- und NO-System: Untersuchungen an AT2-Rezeptor-defizienten Mäusen

Cell biological characterization of EBAG9/RCAS1 as a modifier of tumor-associated O-linked glycan expression

Cellular functions of the transposon-derived domesticated SETMAR gene in human cells

Characterization of the Hub1 protein modification system

Charakterisierung eines neuen, löslichen Cytochrom-P450-Systems und eines am Aromatenumsatz beteiligten Cytochrom-P450-Systems in der Hefe Trichosporon spec. SBUG 752

Charakterisierung von transgenen Mäusen zur Untersuchung der Immunmodulationsfähigkeit von HCMV-Genprodukten

Diabetes, abdominal obesity, weight change, metabolic factors and risk of liver cancer in the European Prospective Investigation into Cancer and Nutrition

Die Bedeutung von Cytochrom P450 (CYP) 2J2 und deren Metabolite in der Pathogenese der Präeklampsie

Die Funktion des Dynamin II am trans-Golgi-Netzwerk, die Bedeutung der Prolin-reichen Domäne und die Charakterisierung der mit ihr interagierenden Proteinkomplexe

Die Niere als Gegenregulator/Stabilisator von Blutdruckanstiegen vermittelt durch G-Protein-gekoppelte Rezeptoren. Untersuchungen an RGS2- und Spinophilin-defizienten Mäusen

Die physiologische Signifikanz des hepatischen Fettsäurebindungsproteins

Dysfunktion des Noradrenalin-Transporters (NET) als Ursache von Kardiovaskulären Erkrankungen

Editierung Glycin-Rezeptor alpha-spezifischer mRNAs unter physiologischen und pathophysiologischen Bedingungen

Funktionelle Analyse des neuralen Zelloberflächenproteins Neurotractin durch Gen-Inaktivierung in der Maus und Identifikation heterophiler Interaktionspartner

Funktionelle Analyse des OPA1-Gens in OPA1-defizienten Mausmutanten und im Zellkulturmodell

Genetics of endocardial-myocardial interactions during zebrafish heart development

Genetische Analyse eines Mausmodells für das menschliche Blepharophimosis/Ptosis/Epicanthus Inversus Syndrom (BPES)

Golgi-spezifische Proteinkinase C-Bindungsproteine und -Substrate und ihre Bedeutung für die Bildung konstitutiver Transportvesikel

Identification of phosphorylation targets of zebrafish atypical protein kinase C using chemical genetics

Identifizierung des an Chromosom 11p15 gekoppelten Krankheitsgens für autosomal-dominant vererbte Noncompaction des linksventrikulären Myocards

Identifizierung und funktionelle Charaktersierung neuer Zytostatika-Resistenzgene mittels 2D-Protein-Elektrophorese und Mikrosequenzierung

in vivo Beobachtung von manipulierten dendritischen Zellen in Tumor-Modellen

Investigations on the roles of Claudins during zebrafish cardiovascular development

Klonierung und Charakterisierung des Gens für Hypertonie und Brachydaktylie auf dem kurzen Arm von Chromosom 12

Konformation, Stabilität und Wechselwirkungen von Plasmid pSM19035-kodierten Proteinen

Künstliche neuronale Netzwerke zur Analyse von Gen-Gen-Interaktionen im Lipidstoffwechsel

Lipid sorting and formation of distinct plasma membrane domains during cell polarization in Drosophila

Mechanismen der Protein-Qualitätskontrolle des endoplasmatischen Retikulums

Mechanismus der Cytochrom P450 Katalyse: FT-Infrarotspektro-skopische Untersuchungen am Häm-Thiolat-Protein und Redox-partnern

Modulation der Claudinoligomerisierung zur Beeinflussung der Blut-Hirnschranke

Molekularbiologische Analyse von Gab1, einem spezifischen Substrat der Rezeptor-Tyrosinkinase c-Met

Molekulare Charakterisierung cis-aktiver Genregulation Brachydaktylie-relevanter Gene

Molekulare Interventionsstrategien zur Verminderung der S100A4-induzierten Metastasierung des kolorektalen Karzinoms

Neue Thymidin-Analoga als Proliferationsmarker für die Tumordiagnostik mittels PET und die endogene Radiotherapie

Regulationsmechanismen und Funktion der RING-Finger E3 Ubiquitinligasen MuRF1 und 3 in der kardialen Hypertrophie.

Roles of DNA repair pathways in Sleeping Beauty transposition in vertebrate cells

Rolle von LRP2/Megalin in der Entwicklung des Vorderhirns

Sensibilisierung Therapie-resistenter Tumorzellen durch Hyperthermie und Kontrolle Apoptose-regulierender Gene

Sleeping Beauty transposon-mediated transgenesis in the pig genome

Stable transfection in Schistosoma mansoni using transposons

Struktur der Protein-RNA Wechselwirkungen in Ribosomen. Domänen ribosomaler RNA-Protein-Kontaktstellen sollen durch Bindungsstudien und Kristallisation in ihrer Struktur untersucht werden

Strukturelle und funktionelle Charakterisierung von β-Amyloid Aggregations-Intermediaten

T-Zellrezeptor-Gentransfer zur Konstruktion von Cancer-Testis Antigen-spezifischen T-Zellen für die Behandlung von multiplen Myelomen

Targeting the secretory pathway in cytotoxic T lymphocytes to modulate their cytolytic capacity in cancer immunotherapy

The Activator (Ac) element from maize: transposition mechanism and regulation and advancement as a mutagenesis tool

The molecular causes of autosomal-dominant hypertension with brachydactyly (OMIM 112410)

The role of the cytoskeleton in the vasopressin-induced aquaporin-2 shuttle in renal collecting duct principal cells

Tierexperimentelle Untersuchungen zur Effizienz eines Hyperthermie-induzierbaren Vektors für die nicht-virale Gentherapie

Transgene Tiere zur Untersuchung der in vivo-Funktion der Interaktion zwischen essentiellen leichten Myosinketten und Aktin im Herzen

Untersuchungen der physiologischen und klinischen Funktionen essentieller leichter Myosinketten-Domänen im Herzen mittels transgener Ratten

Untersuchungen zu Kernexportprozessen in der Hefe Saccharomyces cerevisiae

Wechselwirkung zwischen Astrozyten und Subtypen von Neuronen – ein morpho-physiologischer Ansatz mit neuen transgenen Tiermodellen

Zellzyklusregulation, Apoptoseresistenz und klinische Resistenz kolorektaler Adenokarzinome gegenüber multimodaler Therapie

Zur Rolle der löslichen Epoxidhydrolase bei der Hypertonieentstehung

Zwei Target-Strategie zur Hemmung der menschlichen Telomerase

Emmy Noether-Programm Forschungsstipendium:

Charakterisierung der Zielzellen des Proteins 24p3/NGAL im metanephrischen Mesenchym

Emmy Noether-Programm Nachwuchsgruppe:

Die Rolle β-Catenin/TCF-abhängiger transkriptioneller Netzwerke bei Morphogenese und Regeneration renaler Epithelien

Exzellenzcluster:

EXC 257: NeuroCure - Towards a better outcome of neurological disorders

Forschergruppe:

FOR 427: Pathogenese der spinozerebellären Ataxie Typ 3 (SCA3)

Forschungsstipendium:

Adoptiver Transfer von verschiedenen dendritischen Zelluntergruppen (CD8alpha+ und CD8alpha- dendritische Zellen) in Verbindung mit verschiedenen immunogenen und tolerogenen Stimuli

Einfluß von (n-3) Fettsäuren auf das TNF-alpha- und Leptin-System und deren Bedeutung für die Entstehung der Insulinresistenz und des Typ 2-Diabetes beim Menschen: Eine epidemiologische Analyse der Health Professionals Follow-up Studie

Listeria monocytogenes infection and the host cell cycle

Modulation der Signalwege von Sonic hedgehog und Notch bei cerebellären Vorläuferzellen zur Induktion von primitiven neuroektodermalen Tumoren

Recombinant, Truly Tumor-Specific, Very High-Affinity T Cell Receptor for Cancer Therapy

Graduiertenkolleg:

GRK 80: Modellstuduien zu Struktur, Eigenschaften und Erkennung biologisch relevanter Moleküle auf atomarer Ebene

GRK 238: Schadensmechanismen im Nervensystem: Einsatz von bildgebenden Verfahren

GRK 268: Dynamik und Evolution zellulärer und makromolekularer Prozesse

GRK 276: Signalerkennung und -umsetzung

GRK 331: Temperaturabhängige Effekte für Therapie und Diagnostik

GRK 426: Molekularbiologische Grundlagen der Therapie

GRK 754: Geschlechtsspezifische Mechanismen bei Myokardhypertrophie

GRK 865: Vaskuläre Regulationsmechanismen

GRK 1360: Genomische und systembiologische Analyse molekularer Netzwerke

GRK 1631: Internationales Graduiertenkolleg für Myologie

GRK 1772: Computergestützte Systembiologie

Graduiertenschulen:

GSC 86: Berlin School of Mind & Brain

GSC 203: Berlin-Brandenburg School for Regenerative Therapies

Collaborative Research Centers:

SFB 366: Cellular Signal Recognition and Transduction
SFB 449: Structure and function of membrane-integral receptors
SFB 507: The role of non-neuronal cells in neurological disease
SFB 515: Mechanisms of Developmental and Experience-Dependent Neural Plasticity
SFB 577: Molecular Basis of Clinical Variability in Mendelian Disorders
SFB 618: Theoretical Biology: Robustness, modularity and evolutionary design of living systems
SFB 633: Induction and Modulation of T-Cell Mediated Immune Responses in the Gastrointestinal Tract
SFB 665: Developmental Disturbances in the Nervous System
SFB 740: From Molecules to Modules: Organisation and Dynamics of cellular Functional Units
SFB 958: Scaffolding of Membranes: Molecular Mechanisms and Cellular Functions

Transregio:

TRR 3: Mesial Temporal Lobe Epilepsies
TRR 19: Inflammatory Cardiomyopathy - Molecular Pathogenesis and Therapy
TRR 36: Principles and Applications of Adoptive T Cell Therapy
TRR 43: The brain as a target of inflammatory processes
TRR 52: Transcriptional Programming of Individual T Cell Subsets
TRR 54: Growth and Survival, Plasticity and cellular Interactivity of lymphatic Malignancies

DFG Förderung des MDC (SFB; TRR) – Stand November 2011

Sonderforschungsbereich:

SFB 366: Zelluläre Signalerkennung und -umsetzung
SFB 449: Struktur und Funktion membranständiger Rezeptoren
SFB 507: Die Bedeutung nicht-neuronaler Zellen bei neurologischen Erkrankungen
SFB 515: Mechanismen entwicklungs- und erfahrungsabhängiger Plastizität des Nervensystems
SFB 577: Molekulare Grundlagen klinischer Variabilität monogen bedingter Krankheiten
SFB 618: Theoretische Biologie: Robustheit, Modularität und evolutionäres Design lebender Systeme
SFB 633: Induktion und Modulation T-zellvermittelter Immunreaktionen im Gastrointestinaltrakt
SFB 665: Entwicklungsstörungen im Nervensystem
SFB 740: Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten
SFB 958: Einrüstung von Membranen: Molekulare Mechanismen und zelluläre Funktionen

Transregio:

TRR 3: Mesiale Temporallappen-Epilepsien
TRR 19: Inflammatorische Kardiomyopathie - Molekulare Pathogenese und Therapie
TRR 36: Grundlagen und Anwendung adoptiver T-Zelltherapie
TRR 43: Das Gehirn als Zielorgan von entzündlichen Prozessen
TRR 52: Transkriptionelle Programmierung individueller T-Zell-Populationen
TRR 54: Wachstum und Überleben, Plastizität und zelluläre Interaktivität lymphatischer Neoplasien

EU Projects FP7/ EU Projekte FP7

EU Cooperation

SET-DEV FP7-SCIENCE-IN-SOCIETY-2007-1
Science, Ethics and Technological Responsibility in Developing and Emerging Countries
01.03.2008 – 31.07.2010

SFMET FP7-HEALTH-2007-A
HGF/SF and MET in metastasis
01.04.2008 – 31.03.2011

PERSIST FP7-HEALTH-2007-B
Persisting Transgenesis
01.01.2009 – 31.09.2011

EURATRANS FP7-HEALTH-2009-two-stage
European large-scale functional genomics in the rat for translational research
01.04.2010 – 30.09.2014

SynSys FP7-HEALTH-2009-two-stage
Synaptic Systems: dissecting brain function in health and disease
01.07.2010 – 30.06.2014

EUROSPIN FP7-HEALTH-2009-single-stage
European Consortium on Synaptic Protein Networks in Neurological and Psychiatric Diseases
01.01.2010 – 31.12.2013

EU People – Marie Curie

InduStem FP7-PEOPLE-IAPP-2008
Comparative stem cell research in mouse and humans
01.01.2009 – 31.12.2012

ATTRACT FP7-PEOPLE-ITN-2008
Advanced Teaching and TRaining for Adoptive Cell Therapy
01.10.2009 – 30.09.2013

EVONET FP7-PEOPLE-2007-1-1-ITN
Evolution of gene regulatory networks in animal development
01.10.2008 – 30.09.2012

ENDOPANC FP7-PEOPLE-IRG-2008
Novel signals guiding endodermal progenitor cells toward a pancreatic fate
01.07.2009 – 30.06.2013

MuRF and hypertrophy FP7-PEOPLE-IRG-2008
Regulation and function of the E3 ubiquitin ligases Muscle RING finger 1 and 3 in cardiac hypertrophy
01.01.2010 – 31.12.2013

Kinase crosslinking FP7-PEOPLE-2009-IOF
Capturing kinase-substrate pairs in intact mammalian cells by using unnatural amino acid mutagenesis and photocrosslinking
01.02.2011 – 31.01.2014

Touch in situ FP7-PEOPLE-2009-IEF
Mechanotransduction in situ
01.08.2010 – 31.07.2012

Atherochemokine FP7-PEOPLE-2009-IEF
Investigation of the role of CXCL5/CXCR1 pathway in atherosclerosis
1.7.2010 – 30.08.2012

EU Ideas – ERC

HEPATOPANCREATIC ERC-2009-StG
MECHANISMS UNDERLYING CELL FATE DECISION BETWEEN PANCREAS AND LIVER
01.11.2009 – 31.10.2014

IsletVasc ERC-2010-StG
Molecular Mechanisms Regulating Pancreatic Islet Vascularization
01.11.2010 – 31.10.2015

BrainStates ERC-2010-StG
Brain states, synapses and behaviour
01.02.2011 – 31.01.2016

LYMPHOMA ERC-2010-AdG
Modeling lymphoma pathogenesis in mice – from basic mechanisms to pre-clinical models
Start: 01.07.2011 – 30.06.2016

CardioSplice ERC-StG-2011
A systems and targeted approach to alternative splicing in the developing and diseased heart: Translating basic cell biology to improved cardiac function
01.01.2012 – 31.12.2016

ThermoReg ERC-StG-2011
Peripheral and Central Mechanisms of Temperature Detection and Core Body Thermoregulation
01.02.2012 – 31.01.2017

Extremophile Mammal ERC-AdG-2011
Molecular exploitation of an extremophile mammal
01.04.2012 – 31.03.2017

TRANSPOSOstress ERC-AdG-2011
Impact of stress-induced transposon activities on human disease
2012 – 2017

EU Capacities – Infrastructures:

EU-OPENSCREEN; EuroBioImaging

Overview
Überblick

The Helmholtz Association

Die Helmholtz-Gemeinschaft

With a staff of about 31,000 employees working in 17 research centers, the Helmholtz Association is the largest research organization in Germany. It has an annual budget of around 3.3 billion euros, 30 percent of which comes from externally funded grants from the public and private sectors.

The research centers belonging to the Helmholtz Association conduct research in six core fields: (1) Energy (2) Earth and Environment (3) Health (4) Key Technologies (5) Structure of Matter and (6) Aeronautics, Space and Transport.

Eight Helmholtz centers cooperate in the research field of Health in six programs: cancer research, cardiovascular and metabolic diseases, function and dysfunction of the nervous system, infection and immunology, environmental health and systemic analysis of multifactorial diseases. The most important research activities are carried out at five centers: the *German Cancer Research Center* (DKFZ) in Heidelberg, at the *German Center for Neurodegenerative Diseases* (DZNE) in Bonn, which was founded in 2009, at *Helmholtz Zentrum München – German Research Center for Environmental Health* (HMGU), at the *Helmholtz Center for Infection Research* (HZI) in Braunschweig and at the MDC. In addition, the Helmholtz centers *Forschungszentrum Jülich* (FZJ), the *Helmholtz Centre for Heavy Ion Research* (GSI) in Darmstadt, the *Helmholtz-Zentrum Geesthacht Centre for Materials and Coastal Research* (GKSS) and the *Helmholtz Centre for Environmental Research* (UFZ) in Leipzig make important contributions. The focus is on a strong, excellent and dynamic basic research and the transfer of experimental insights to the clinic. The MDC participates in three programs. The program Cardiovascular and Metabolic Diseases is coordinated by the MDC.

Program-Oriented Funding

In the autumn of 2001, all research activities of the Helmholtz Association were restructured and bundled

Die Helmholtz-Gemeinschaft ist mit ihren rund 31.000 Mitarbeiterinnen und Mitarbeitern in 17 Forschungszentren sowie einem Jahresbudget von rund 3,3 Milliarden Euro, davon ca. 30% Drittmittel aus dem öffentlichen und privatwirtschaftlichen Bereich, die größte Wissenschaftsorganisation Deutschlands.

Die Forschungszentren der Helmholtz-Gemeinschaft arbeiten in sechs Forschungsbereichen: Energie, Erde und Umwelt, Gesundheit, Schlüsseltechnologien, Struktur der Materie sowie Verkehr und Weltraum.

Im Forschungsbereich Gesundheit kooperieren acht Helmholtz-Zentren in sechs Forschungsprogrammen: Krebsforschung, Herz-Kreislauf- und Stoffwechselerkrankungen, Funktion und Dysfunktion des Nervensystems, Infektion und Immunität, Umweltbedingte Störungen der Gesundheit sowie Systemische Analyse von multifaktoriellen Erkrankungen. Die wichtigsten Forschungsaktivitäten sind an 5 Zentren angesiedelt: am *Deutschen Krebsforschungszentrum* (DKFZ) in Heidelberg, am *Deutschen Zentrum für Neurodegenerative Erkrankungen* (DZNE) in Bonn, das 2009 neu gegründet wurde, am *Helmholtz Zentrum München für Gesundheit und Umwelt* (HMGU), am *Helmholtz-Zentrum für Infektionsforschung* (HZI) in Braunschweig und am MDC. Darüber hinaus leisten die Helmholtz-Zentren: *Forschungszentrum Jülich* (FZJ), *Helmholtz-Zentrum für Schwerionenforschung* (GSI) in Darmstadt, *Forschungszentrum Geesthacht* (GKSS) und *Helmholtz-Zentrum für Umweltforschung* (UFZ) in Leipzig, wichtige Beiträge. Der Fokus liegt auf einer starken, exzellenten und dynamischen Grundlagenforschung und der Übertragung der gewonnenen Erkenntnisse in die Klinik. Das MDC ist an drei Programmen beteiligt. Das Programm ‚Herz-Kreislauf- und Stoffwechselerkrankungen' wird federführend vom MDC getragen.

Die Programmorientierte Förderung

Im Herbst 2001 wurde die gesamte Forschung der

Photographer Katharina Bohm, Copyright MDC

in the six core fields mentioned above. Thematic programs were defined within these core fields to be carried out by one or more centers.

Since this reorganization, resources are no longer provided to the individual institutions, but rather are awarded on a competitive basis to research programs that may involve several centers. A strategic evaluation is the basis for selecting which research programs are to be funded. This task is carried out by renowned experts from around the world. Their reports form the basis for the decision on the amount of funding and for determining the funding ratio between the federal and state governments for each program. In addition, as a result of the last evaluation process, two strategic cross-program initiatives are receiving funding upon recommendation of the reviewers. One is translational research, which seeks to transfer findings from basic research as quickly as possible into clinical applications. The other is the establishment of the Helmholtz Cohort, a population-based study which seeks to analyze and monitor a large number of individuals over a 20-year period to improve the prevention and early diagnosis of the common diseases.

The first funding period was from 2003 to 2008. The second program period is also for a term of five years,

Helmholtz-Gemeinschaft neu strukturiert. Die Helmholtz-Gemeinschaft hat ihre Forschungsaktivitäten in den sechs oben genannten großen Bereichen gebündelt und innerhalb dieser Forschungsbereiche thematische Programme definiert, die von einem oder mehreren Zentren getragen werden.

Ressourcen werden nicht mehr einzelnen Institutionen, sondern zentrenübergreifenden Forschungsprogrammen, die sich untereinander im Wettbewerb befinden, zur Verfügung gestellt. Eine strategische Begutachtung bildet die Basis für die Finanzierung der Forschungsprogramme. Diese Aufgabe übernehmen renommierte Experten aus aller Welt. Ihre Gutachten bilden die Grundlage für die Entscheidung, in welcher Höhe und in welcher Aufteilung Bund und Länder die einzelnen Programme fördern. Ein weiteres Ergebnis des letzten Begutachtungsprozesses sind zwei programmübergreifende strategische Initiativen, die auf Empfehlung der Gutachter gefördert werden. Dies sind die Translationale Forschung, um Ergebnisse aus der Grundlagenforschung schneller für die klinische Anwendung zu nutzen, sowie der Aufbau einer großen Populationsstudie „Helmholtz-Kohorte“ über die kommenden zwei Jahrzehnte, um Vorbeugung und Frühdiagnostik bei den großen Volkskrankheiten zu verbessern.

Die erste Förderperiode lief von 2003 bis 2008. Die

2009/2010 to 2013/2014, depending on the year of the review. The reviews for the second funding period took place in the spring of 2008 (Aeronautics, Space and Transport, Health, Earth and Environment) and in spring 2009 (Energy, Structure of Matter, Key Technologies). The program reviews for the third funding period are scheduled to take place in spring 2013 and 2014.

Review of the Centers

The MDC performed extremely well and received excellent marks from the reviewers in all three program reviews for the second funding period. The results of these evaluations impressively confirm the results of the center evaluation from October 2006.

The reviews of the individual centers at the research group level take place between the evaluations for the program-oriented funding. The last evaluation of the MDC research groups took place in October 2006. In these reviews the positive development of the MDC was impressively confirmed. During two days, sixteen experts from Germany and other countries evaluated the work of all MDC research groups and were impressed by the scientific achievements of the MDC. Of the total of 40 peer-reviewed research groups at the MDC, 48 percent – almost half – received the mark of "excellent", and 35 percent of the groups an "outstanding". At the same time, the reviewers stressed that the MDC, as one of the great non-university research institutions in the field of the life sciences in Germany, is also one of the most successful. According to the reviewers, the special orientation of the MDC with its focus on cardiovascular and cancer research and the neurosciences facilitates the understanding of the fundamental molecular causes across various diseases.

The next center review is scheduled for March 2012.

zweite Programmperiode beträgt ebenfalls fünf Jahre, 2009/2010 bis 2013/2014, je nach Zeitpunkt der Begutachtung. Die Begutachtungen für die zweite Förderperiode erfolgten im Frühjahr 2008 (Verkehr und Weltraum, Gesundheit, Erde und Umwelt) und im Frühjahr 2009 (Energie, Struktur der Materie, Schlüsseltechnologien). Die Programmbegutachtungen für die dritte Förderperiode stehen im Frühjahr 2013 und 2014 an.

Begutachtung der Zentren

Das MDC hat in allen drei Programmbegutachtungen im Rahmen der zweiten Förderperiode äußerst erfolgreich abgeschnitten und Höchstnoten von den Gutachtern erhalten. Die Ergebnisse dieser Begutachtungen bestätigten eindrucksvoll das Ergebnis der Zentrumsbegutachtung vom Oktober 2006.

Zwischen den Begutachtungen im Rahmen der programmorientierten Förderung finden die Begutachtungen der einzelnen Zentren auf der Ebene der Forschungsgruppen statt. Die Forschungsgruppen des MDC wurden zuletzt im Oktober 2006 begutachtet. Dabei konnte die positive Entwicklung des MDC eindrucksvoll bestätigt werden. 16 Experten aus dem In- und Ausland haben an zwei Tagen die Arbeiten aller Forschungsgruppen des MDC evaluiert und sich von den wissenschaftlichen Leistungen des MDC beeindruckt gezeigt. Von den insgesamt 40 begutachteten Forschungsgruppen des MDC wurden insgesamt 48 %, also knapp die Hälfte, als „excellent", und 35% der Gruppen als „outstanding" („herausragend") eingestuft. Gleichzeitig hoben die Gutachter hervor, dass das MDC als eines der größten außeruniversitären Forschungszentren auf dem Gebiet der Lebenswissenschaften in Deutschland auch zu den erfolgreichsten zählt. Die besondere Ausrichtung des MDC mit seinen Forschungsschwerpunkten Herz-Kreislaufforschung, Krebsforschung und Neurowissenschaften macht es nach Auffassung der Gutachter möglich, die molekularen Ursachen krankheitsübergreifend zu verstehen.

Die nächste Zentrenbegutachtung wird im März 2012 erfolgen.

The Campus-Berlin-Buch

Der Campus-Berlin-Buch

Biotechnology Park with Innovation and Founders' Center

The basic and clinical research activities on the Berlin-Buch Campus and in the neighboring hospitals provide a stimulating environment for the development of the Biotech Park with its Innovation and Founders' Center (IGZ), which together have evolved into one of the largest such centers in Germany. To date, more than 60 million euros have been invested in the expansion and modernization of the campus infrastructure, the shared facilities, and the Biotech Park. This sum includes GA funds from the program "Joint task – improvement of the regional economic structure" and ERDF funds (European Regional Development Funds). Overall, since

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In front of the Max Delbrück Communications Center (MDC.C) on the Campus Berlin-Buch: Dr. Andreas Mätzold (Managing Director, BBB Management GmbH Campus Berlin-Buch), Prof. Walter Rosenthal (MDC Scientific Director), Dr. Ulrich Scheller (Managing Director, BBB Management GmbH Campus Berlin-Buch) and Klaus Wowereit (Mayor of Berlin) (from left).

Vor dem Max Delbrück Communications Center (MDC.C) auf dem Campus Berlin-Buch: Dr. Andreas Mätzold (Geschäftsführung der BBB Management GmbH Campus Berlin-Buch), Prof. Walter Rosenthal (Vorsitzender des MDC-Stiftungsvorstands), Dr. Ulrich Scheller (Geschäftsführung der BBB Management GmbH Campus Berlin-Buch) und Klaus Wowereit (Regierender Bürgermeister von Berlin) (v. l.).

Biotechnologiepark mit Innovations- und Gründerzentrum

Grundlagenforschung und klinische Forschung auf dem Campus sowie benachbarte Kliniken bilden ein stimulierendes Umfeld für die Entwicklung des BiotechParks mit seinem Innovations- und Gründerzentrum (IGZ). Der BiotechPark mit IGZ hat sich zu einem der größten in Deutschland entwickelt. In den Ausbau und die Modernisierung der Campusinfrastruktur, der Gemeinschaftseinrichtungen des Campus und in den Biotechnologiepark sind bislang mehr als 60 Millionen Euro investiert worden. Die Fördermittel kommen aus der Gemeinschaftsaufgabe zur Verbesserung der regionalen Wirtschaftsstruktur (GA) und aus dem Europäischen Fonds für Regionale Entwicklung (EFRE). Die angesiedelten Unternehmen selbst haben seit Mitte der 90er Jahre rund 180 Millionen Euro investiert.

Im BiotechPark forschen und produzieren auf einer Fläche von rund 27.500 Quadratmetern gegenwärtig 51 kleine und mittelständige Unternehmen. 38 davon sind im Bereich der Biomedizin tätig. Die Firmen beschäftigen etwa 770 Mitarbeiter. Fünf Unternehmen siedelten sich im Jahre 2010 neu an. Die aktuelle Auslastung (2011) im BiotechPark liegt mit 92% höher als im Vorjahr (86%).

Von Bedeutung für die nachhaltige Entwicklung des BiotechParks wie auch für den Gesamtstandort Berlin ist, dass sich das Wachstum der Bestandsfirmen kontinuierlich fortsetzt. Von ganz besonderem Stellenwert ist die Entscheidung der Eckert & Ziegler AG, die Produktionskapazitäten am Standort Berlin-Buch in den nächsten Jahren um ca. 10.000 Quadratmeter zu erweitern. Die Campusnutzergemeinschaft hat deshalb unter Zustimmung des Landes Berlin beschlossen, für diese Erweiterung ein Teilgrundstück am Campuseingang Robert-Rössle-Straße 10 an die EZAG zu veräußern. Der Spatenstich für die Errichtung der ersten Baustufe mit 5.000 Quadratmeter fand im Mai 2011 statt. Campusfirmen wie Glycotope, Celares, Invitek und Bavarian

the mid-nineties, the investment volume of the companies located on campus has amounted to approximately 180 million euros.

Fifty-one small and medium-sized companies, 38 of which are in the biomedical sector, are currently engaged in research and production in the Biotech Park. Approximately 27,500 square meters of rental commercial space are provided for the young firms, which altogether have about 770 employees. In 2010 five new companies moved to the park. The current occupancy rate (2011) is 92%, which is higher than in the previous year (86%).

The continual growth of the companies on campus is important for the sustainable development of the Biotech Park and for the location of Berlin as a whole. The decision of Eckert & Ziegler AG to increase production capacity at the Berlin-Buch site and to expand its facilities by about 10,000 square meters in the next few years is of special importance. With the approval of the State of Berlin, the campus user community has therefore decided to sell part of a piece of property at the campus entrance at Robert-Rössle-Str. 10 to Ernst & Ziegler AG. The groundbreaking ceremony for the first phase of construction with 5,000 square meters took place in May 2011.

Other campus companies such as Glycotope, Celares, Invitek and Bavarian Nordic have also expanded their lab and office space within the Founders' Center.

In 2010, in its role as operation and development company for the Berlin-Buch Campus, BBB Management GmbH organized a competition for the best concept for the urban development of the campus. This concept will allow the existing total floor area to be nearly doubled within the next decades through an orderly expansion. In addition, the attractiveness of the campus will continue to increase and internal communication relationships will be improved significantly.

BBB has been working intensively to find viable solutions to securing the supply of electrical energy for the Campus. BBB has continued and expanded its networking activities on all levels to support campus companies and to strengthen the image of the location. As in previous years, the Berlin-Buch Campus was showcased at numerous national and international fairs and events. Event programs were organized and carried out for 32 visitor groups comprising representatives from science, business and industry, the political arena and the media as well as for individual visitors from 12 countries. To promote sustained campus development, BBB continued to work intensively to

Photographer Katharina Bohm, Copyright MDC

Nordic erweiterten ihre Labor- und Büroflächen innerhalb des Gründerzentrums.

Als Betreiber des Campus hat die BBB Management GmbH im Jahr 2010 gemeinsam mit den großen Campusnutzern ein Leitkonzept zur städtebaulichen Weiterentwicklung des Campus erarbeiten lassen. Dazu führte die BBB im Auftrag der Campusnutzergemeinschaft einen städtebaulichen Wettbewerb durch. Das Leitkonzept gestattet, die auf dem Campus vorhandene Bruttogeschossfläche in den nächsten Jahrzehnten durch geordneten und planmäßigen Ausbau nahezu zu verdoppeln, die Attraktivität des Campus weiter zu steigern sowie die inneren Kommunikationsbeziehungen wesentlich zu verbessern.

Intensiv hat die BBB daran gearbeitet, für die sichere Grundversorgung des Campus mit Elektroenergie machbare Lösungen zu finden. Fortgeführt und erweitert hat die BBB auch die Netzwerkarbeit auf allen Ebenen zur Unterstützung von Unternehmen und zur Stärkung des Standorts. Der Standort wurde erneut international und national auf Messen und Veranstaltungen präsentiert. Für 32 Besuchergruppen mit Vertretern aus Wissenschaft, Wirtschaft, Politik und Medien sowie Besuchern aus 12 Ländern wurden Veranstaltungs-

heighten the profile of Berlin-Buch as a Health Region, for example by applying for EUR 500,000 in funding for a period of three years.

BBB Management GmbH Campus Berlin-Buch was founded by the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. The Leibniz-Institut für Molekulare Pharmakologie (FMP) and Bayer HealthCare Pharmaceuticals are co-shareholders.

Life Science Learning Lab

In 2010 the Life Science Learning Lab, Berlin's most well-known extracurricular learning location, offered challenging lab courses and advanced training courses for around 21,000 children, secondary school students, teachers, specialists in the field and participants from the general public. The topics covered included current methods and applications of genetic research, cell biology and molecular medicine. The Life Science Learning Lab, which is run by BBB Management GmbH, offered courses on the Berlin-Buch Campus that were attended by 9,500 students. Experimental courses offered at Berlin day care centers and elementary schools were attended by 11,000 kindergarten and elementary school children.

The MDC has supported these activities significantly, for example by funding a lab for middle and high school students as well as holiday courses and a science camp for staff children to help facilitate the combination of work and family life.

In 2010 the Life Science Learning Lab received EUR 385,000 in funding from the Economic Stimulus Program II to establish a second gene lab, to renovate the common areas and exhibition spaces and to upgrade the presentation equipment in the training area.

On the basis of a long-term agreement with the campus institutions regarding public relations work, in 2010 the Life Science Learning Lab organized and carried out event programs for more than 35 visitor groups with representatives from science, business and industry, the political arena and the media. As in previous years, central activities of the Campus PR/Life Science Learning Lab team included the editing and publication of the newsletter "CampusNews", the publication of booklets and flyers for location marketing as well as the successful realization of events such as the New Year's reception of the campus institutions and the "Long Night of the Sciences," a strong magnet for visitors. One highlight was the participation of the Berlin-Buch Campus in the Year of Science 2010. In addition, BBB is actively engaged in the network of its Berlin partners and in the

programme organisiert und betreut. Zugunsten einer nachhaltigen Campusentwicklung hat sich die BBB erneut intensiv in die weitere Profilierung des Stadtteils Berlin-Buch zu einer Gesundheitsregion eingebracht und dafür Fördermittel in Höhe von 500.000 € für drei Jahre beantragt.

Die BBB Management GmbH Campus Berlin-Buch ist eine Gründung des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch. Mitgesellschafter sind das Leibniz-Institut für Molekulare Pharmakologie (FMP) sowie die BayerAG.

Das Gläserne Labor

Das BBB Management GmbH betriebene Gläserne Labor hat als bekanntester außerschulischer Lernort Berlins im Jahre 2010 ca. 21.000 Kindern, Schülern, Lehrkräften und einem breiten Laien- und Fachpublikum anspruchsvolle Laborkurse und Fortbildungen zu aktuellen Methoden und Anwendungen der Genforschung, Zellbiologie und molekularen Medizin angeboten. Davon besuchten 9.500 Schüler die Laborkursen auf dem Campus; für 11.000 Kita-Kinder und Grundschulkinder bot das Gläserne Labor Experimentierkurse in Berliner Kitas und Grundschulen an.

Das MDC hat diese Aktivitäten maßgeblich unterstützt, z.B. durch die Finanzierung eines Schülerlabors sowie von Angeboten zur naturwissenschaftlichen Ferienbetreuung für Mitarbeiterkinder zur Verbesserung der Vereinbarkeit von Beruf und Familie.

Das Gläserne Labor erhielt 2010 aus dem Konjunkturprogram II Fördermittel in Höhe von 385 T€ für Einrichtung eines zweiten Genlabors, für die Erneuerung der Aufenthalts- und Ausstellungsbereiche sowie der Präsentationstechnik im Schulungsbereich.

Auf der Grundlage einer langfristigen Vereinbarung mit den Campus-Einrichtungen zur Öffentlichkeitsarbeit des Campus betreute das Gläserne Labor im Jahre 2010 über 35 Besuchergruppen mit Vertretern aus Wissenschaft, Wirtschaft, Politik und Medien. Dafür organisierte und begleitete sie entsprechende Veranstaltungsprogramme. Zu den zentralen Aktivitäten der Campus-Öffentlichkeitsarbeit zählte wie in den Vorjahren die Gestaltung und Herausgabe der Mitarbeiter- und Imagezeitung „CampusNews", die Publikation von Broschüren zum Standortmarketing des Campus sowie die Organisation und erfolgreiche Durchführung von Veranstaltungen wie dem Neujahrsempfang der Campus-Einrichtungen und der äußerst erfolgreichen und besucherstarken „Langen Nacht der Wissenschaften."

communication platforms and activities of the Berlin-Brandenburg Technology Cluster.

In "CampusSterne", the campus day care center initiated by BBB Management which opened five years ago, all 30 available places available at the time are filled. In a joint endeavor in 2010, the CampusSterne management and the MDC succeeded in soliciting funds to expand the day care center to provide places for 60 children and to strengthen the profile and quality of its offerings.

Leibniz-Institut für Molekulare Pharmakologie (FMP)

The Leibniz-Institut für Molekulare Pharmakologie (FMP) conducts basic research in the field of molecular pharmacology. Due to the close spatial proximity to the MDC, the already existing collaborations between the two institutes have been considerably intensified. The research concepts of the MDC and the FMP complement each other: while molecular medicinal research at the MDC is particularly dedicated to diseases or clinical symptoms and their molecular explanations, the focus of the FMP research is on the one hand the search for new substances which interact with proteins and change their functions. On the other hand, protein systems are studied for their suitability as targets for drug development. This qualifies them as research tools as well as starting molecules for new drugs.

The close connection between the two research establishments extends into the organizational level. Guest scientist contracts make it possible for scientists of one institute to use the equipment in the other. Both establishments manage the Timoféeff-Ressovsky-Haus which houses the Screening Unit. MDC and FMP send representatives to important committees of the other establishment respectively. The planning of costly and long-term research projects as well as the appointment of leading scientists take place in joint agreement. The MDC and the FMP arrange and finance joint events for their PhD students.

Ein Veranstaltungshöhepunkt war die Beteiligung des Campus am Wissenschaftsjahr 2010. Darüber hinaus bringt sich die BBB aktiv in die Aktivitäten und Kommunikationsplattformen des Berlin-Brandenburger Technologie-Clusters sowie des Netzwerkes der Berlin Partner ein.

Die von der BBB initiierte und vor fünf Jahren eröffnete betriebsnahe Kindertagesstätte „CampusSterne" ist mit 30 verfügbaren Plätzen ausgebucht. Gemeinsam mit dem Kita-Betreiber und dem MDC ist es 2010 gelungen, Fördermittel für die räumliche Erweiterung der Kita auf 60 Plätze einzuwerben sowie Profil und Qualität der Betreuungsangebote zu verbessern.

Das Leibniz-Institut für Molekulare Pharmakologie (FMP)

betreibt Grundlagenforschung auf dem Gebiet der molekularen Pharmakologie. Begünstigt durch die räumliche Nähe auf dem Campus Berlin-Buch bestehen intensive Forschungskooperationen zwischen MDC und FMP. Während sich die molekular-medizinische Forschung des MDC besonders mit Krankheiten oder klinischen Symptomen und ihren molekularen Grundlagen beschäftigt, steht im Mittelpunkt der FMP-Forschung auf der einen Seite die Suche nach neuen Substanzen, die mit Proteinen in Wechselwirkung treten und deren Funktion ändern können. Auf der anderen Seite werden Proteinsysteme auf ihre Eignung als Ziele für Wirkstoffentwicklungen untersucht. Dadurch kommen sie als Werkzeuge für die Forschung sowie als Ausgangsmoleküle für neue Arzneimittel in Frage.

Kooperationsverträge machen es möglich, dass Wissenschaftler des einen Instituts Geräte des anderen verwenden. Beide Einrichtungen bewirtschaften das Timoféeff-Ressovsky-Haus, das die Screening Unit beherbergt. MDC und FMP entsenden Vertreter in die wichtigsten Gremien der jeweils anderen Einrichtung. Kostspielige, langfristige Forschungsprojekte werden gemeinsam geplant, führende Wissenschaftler in gegenseitigem Einvernehmen ernannt. Auch organisieren und finanzieren MDC und FMP gemeinsam Veranstaltungen für ihre Promotionsstudenten.

Organizational Structure

Organisationsstruktur

The Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch is a foundation under public law of the State of Berlin with the purpose of pursuing medical research at the molecular and cellular levels and implementing its clinical and practical application.

Board of Trustees

The Board of Trustees is the supervisory body of the MDC and monitors the conduct of operations with respect to legality, appropriateness, economic efficiency and financial viability. The Board decides on general research objectives as well as important research policy and financial matters of the Foundation.

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Das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch ist eine Stiftung des öffentlichen Rechts des Landes Berlin mit dem Zweck, medizinische Forschung auf molekularer und zellulärer Ebene und ihre klinische Anwendung und praktische Umsetzung zu betreiben.

Das Kuratorium

Das Kuratorium ist das Aufsichtsgremium des MDC. Es überwacht die Rechtmäßigkeit, Zweckmäßigkeit und Wirtschaftlichkeit der Geschäftsführung. Es entscheidet über die allgemeinen Forschungsziele und über wichtige forschungspolitische und finanzielle Angelegenheiten der Stiftung.

Mitglieder des Kuratoriums

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Max Planck Institut für Molekulare Zellbiologie und Genetik, Dresden*

Prof. Dr. Michael W. Linscheid (bis 2010)
Vizepräsident für Forschung der Humboldt-Universität zu Berlin

Prof. Dr. Peter A. Frensch (seit April 2011)
Vizepräsident für Forschung der Humboldt-Universität zu Berlin

Prof. Dr. Thomas Lüscher (seit August 2010)
Universitätsspital Zürich, Klinik für Kardiologie*

Prof. Dr. Renato Paro
Center of Biosystems, ETH Zürich, Basel*

Regierungsdirektorin Marianne Pyrczek
Bundesministerium der Finanzen, Berlin

Prof. Dr. Monika Schäfer-Korting
Freie Universität Berlin

Senatsdirigent Martin Schmahl
Senatsverwaltung für Gesundheit, Soziales, Berlin

Dr. Birgit Schnieders
Bundesministerium für Gesundheit, Bonn

Prof. Dr. Matthias Selbach
MDC Berlin-Buch

* zugleich Mitglieder des Wissenschaftlichen Ausschusses

Scientific Committee

The Scientific Committee of the Board of Trustees prepares the decisions of the Board of Trustees in scientific matters. The Scientific Committee is responsible for the ongoing evaluation of the results of the research work of the MDC through scientific assessment. Together with the scientific members of the Board of Trustees, up to seven external specialists sit on the Scientific Committee.

Members of the Scientific Committee

Prof. Dr. Mathias Hentze
EMBL Heidelberg (Chair)*

Prof. Dr. Renato Paro
Center of Biosystems, Swiss Federal Institute of Technology Zurich, Basel (Vice-Chair)*

Prof. Dr. Ruedi Aebersold
Swiss Federal Institute of Technology Zurich, Institute of Molecular Systems Biology

Prof. Dr. Corinne Antignac
INSERM, Hopital Necker – Enfants Malades, Paris, France

Prof. Dr. Frederico Caligaris-Cappio
(since September 2011)
University Scientific Institute San Raffaele, Department of OncoHematology

Prof. Dr. Anna Dominiczak
University of Glasgow, Cardiovascular Research Centre, Glasgow, United Kingdom

Prof. Dr. Martin Eilers (since September 2011)
University of Würzburg, Theodor Boveri Institute, Biocenter

Prof. Dr. Magdalena Götz
Helmholtz Zentrum München*

Prof. Dr. Roger Goody
Max Planck Institute of Molecular Physiology, Dortmund*

Prof. Dr. Christoph Huber (until September 2011)
University of Mainz

Prof. Dr. Nancy Hynes (since August 2010)
Friedrich Miescher Institute, Basel*

Prof. Dr. Elisa Izaurralde
Max Planck Institute for Developmental Biology, Tübingen*

Prof. Dr. Elisabeth Knust
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden*

Der Wissenschaftliche Ausschuss

Der Wissenschaftliche Ausschuss des Kuratoriums bereitet die Entscheidungen des Kuratoriums in wissenschaftlichen Fragen vor. Er trägt die Verantwortung für die fortlaufende Ergebnisbewertung der Forschungsarbeiten des MDC durch wissenschaftliche Begutachtung. Dem Wissenschaftlichen Ausschuss gehören neben den wissenschaftlichen Mitgliedern des Kuratoriums bis zu sieben externe Fachwissenschaftler an.

Mitglieder des Wissenschaftlichen Ausschusses

Prof. Dr. Mathias Hentze
EMBL Heidelberg (Vorsitzender)*

Prof. Dr. Renato Paro
Center of Biosystems, ETH Zürich, Basel (Stellv. Vorsitzender)*

Prof. Dr. Ruedi Aebersold
ETH Zürich, Institut für Molekulare Systembiologie

Prof. Dr. Corinne Antignac
INSERM, Hopital Necker – Enfants Malades, Paris, Frankreich

Prof. Dr. Frederico Caligaris-Cappio
(seit September 2011)
University Scientific Institute San Raffaele, Department of OncoHematology

Prof. Dr. Anna Dominiczak
University of Glasgow, Cardiovascular Research Centre, Glasgow, United Kingdom

Prof. Dr. Martin Eilers (seit September 2011)
Julius-Maximilians-Universität Würzburg, Theodor-Boveri-Institut, Biocenter

Prof. Dr. Magdalena Götz
Helmholtz Zentrum München*

Prof. Dr. Roger Goody
Max-Planck-Institut für molekulare Physiologie, Dortmund*

Prof. Dr. Christoph Huber (bis September 2011)
Universität Mainz

Prof. Dr. Nancy Hynes (seit August 2010)
Friedrich Miescher Institut, Basel*

Prof. Dr. Elisa Izaurralde
Max Planck Institut für Entwicklungsbiologie, Tübingen*

Prof. Dr. Elisabeth Knust
Max Planck Institut für Molekulare Zellbiologie und Genetik, Dresden*

Prof. Dr. Kerstin Krieglstein
University of Freiburg, Institute of Anatomy and Cell Biology

Prof. Dr. Thomas Lüscher (since August 2010)
University Hospital Zurich, Cardiology Clinic*

* Also members of the Board of Trustees

Executive Board

The Executive Board manages the Max Delbrück Center and consists of a scientific member, Prof. Dr. Walter Rosenthal, and an administrative member, Cornelia Lanz. The Chair of the Executive Board is Prof. Dr. Walter Rosenthal.

Scientific Council

The Scientific Council advises the Executive Board in matters of fundamental scientific importance.

Members of the Scientific Council:

Dr. Salim Abdelilah-Seyfried
Prof. Dr. Michael Bader
Dr. Daniel Besser
Prof. Dr. Carmen Birchmeier-Kohler
Prof. Dr. Walter Birchmeier
Prof. Dr. Thomas Blankenstein
Prof. Dr. Oliver Daumke (Vice-Chair)
Prof. Dr. Bernd Dörken
Dr. Iduna Fichtner
Prof. Dr. Michael Gotthardt
Prof. Dr. Udo Heinemann
Prof. Dr. Norbert Hübner
Dr. Inés Ibanez-Tallon
Dr. Zsuzsanna Izsvák
Prof. Dr. Thomas Jentsch
Prof. Dr. Helmut Kettenmann
Dr. Markus Landthaler
Dr. Stefan Lechner
Prof. Dr. Ferdinand le Noble
Prof. Dr. Achim Leutz
Prof. Dr. Gary R. Lewin (Chair)
PD Dr. Martin Lipp
Prof. Dr. Friedrich C. Luft
PD Dr. Jochen Meier
Prof. Dr. Ingo L. Morano

Prof. Dr. Kerstin Krieglstein
Universität Freiburg, Institut für Anatomie und Zellbiologie

Prof. Dr. Thomas Lüscher (seit August 2010)
Universitätsspital Zürich, Klinik für Kardiologie*

* zugleich Mitglieder des Kuratoriums

Der Stiftungsvorstand

Der Stiftungsvorstand leitet das Institut und besteht aus einem wissenschaftlichen Mitglied, Prof. Dr. Walter Rosenthal, und einem administrativen Mitglied, Cornelia Lanz. Vorsitzender des Stiftungsvorstands ist Prof. Dr. Walter Rosenthal

Wissenschaftlicher Rat

Der Wissenschaftliche Rat berät den Stiftungsvorstand in den Angelegenheiten von grundsätzlicher wissenschaftlicher Bedeutung.

Mitglieder des Wissenschaftlichen Rates:

Dr. Salim Abdelilah-Seyfried
Prof. Dr. Michael Bader
Dr. Daniel Besser
Prof. Dr. Carmen Birchmeier-Kohler
Prof. Dr. Walter Birchmeier
Prof. Dr. Thomas Blankenstein
Prof. Dr. Oliver Daumke (Stellv. Vorsitzender)
Prof. Dr. Bernd Dörken
Dr. Iduna Fichtner
Prof. Dr. Michael Gotthardt
Prof. Dr. Udo Heinemann
Prof. Dr. Norbert Hübner
Dr. Inés Ibanez-Tallon
Dr. Zsuzsanna Izsvák
Prof. Dr. Thomas Jentsch
Prof. Dr. Helmut Kettenmann
Dr. Markus Landthaler
Dr. Stefan Lechner
Prof. Dr. Ferdinand le Noble
Prof. Dr. Achim Leutz
Prof. Dr. Gary R. Lewin (Vorsitzender)
PD Dr. Martin Lipp
Prof. Dr. Friedrich C. Luft
PD Dr. Jochen Meier
Prof. Dr. Ingo L. Morano

Dr. Thomas Müller
Prof. Dr. Thoralf Niendorf
Dr. Cristiane Nolte
Prof. Dr. Tobias Pischon
Dr. James Poulet
Dr. Matthew N. Poy
Prof. Dr. Nikolaus Rajewsky
Prof. Dr. Fritz G. Rathjen
Dr. Armin Rehm
Dr. Oliver Rocks
Dr. Frank Rosenbauer
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
Dr. Ruth Schmidt-Ullrich
Dr. Björn Christian Schroeder
Dr. Anja Schütz
Prof. Dr. Matthias Selbach
Dr. Jan-Erik Siemens
Prof. Dr. Thomas Sommer
Dr. Francesca Spagnoli
Prof. Dr. Ludwig Thierfelder
Prof. Dr. Mathias Treier
Prof. Dr. Erich Wanker
Prof. Dr. Thomas Willnow
Dr. Jana Wolf

Staff Council

The Staff Council is the collective representation of the employees of the MDC, based on the Staff Representation Act of Berlin.

The Staff Council is involved in decisions regarding personnel and social matters and monitors the observance of labor rights, which are regulated in laws, collective agreements, internal agreements and administrative provisions. The Staff Council receives suggestions and complaints of the employees and clarifies these together with the responsible departments or the Executive Board.

The Staff Council currently has 11 members from all employee groups at the MDC. Since the MDC will have more than 1,000 employees at the time of the next election in November 2012, hence 13 members will be elected to the Staff Council.

Dr. Thomas Müller
Prof. Dr. Thoralf Niendorf
Dr. Cristiane Nolte
Prof. Dr. Tobias Pischon
Dr. James Poulet
Dr. Matthew N. Poy
Prof. Dr. Nikolaus Rajewsky
Prof. Dr. Fritz G. Rathjen
Dr. Armin Rehm
Dr. Oliver Rocks
Dr. Frank Rosenbauer
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
Dr. Ruth Schmidt-Ullrich
Dr. Björn Christian Schroeder
Dr. Anja Schütz
Prof. Dr. Matthias Selbach
Dr. Jan-Erik Siemens
Prof. Dr. Thomas Sommer
Dr. Francesca Spagnoli
Prof. Dr. Ludwig Thierfelder
Prof. Dr. Mathias Treier
Prof. Dr. Erich Wanker
Prof. Dr. Thomas Willnow
Dr. Jana Wolf

Personalrat

Der Personalrat ist auf der Grundlage des Personalvertretungsgesetzes des Landes Berlin die kollektive Interessenvertretung der Mitarbeiterinnen und Mitarbeiter des MDC.

Der Personalrat ist an Entscheidungen zu personellen und sozialen Belangen beteiligt und überwacht die Einhaltung von Arbeitnehmerrechten, die in Gesetzen, Tarifverträgen, Dienstvereinbarungen und Verwaltungsvorschriften geregelt sind. Er nimmt Anregungen und Beschwerden der Mitarbeiter entgegen und klärt diese in Zusammenarbeit mit den zuständigen Abteilungen oder dem Vorstand.

Der Personalrat hat bis zur nächsten Wahl elf Mitglieder aus allen Beschäftigtengruppen des MDC. Da das MDC bei der nächsten Wahl im November 2012 über 1.000 Beschäftigte haben wird, werden dann dreizehn Mitglieder in den Personalrat gewählt.

Members of the Staff Council

Ingo Kahl (Chair)
Robby Fechner (Vice-Chair)
Lutz Else (Vice-Chair)
Dagmar Gerhard (Vice-Chair)
Manuela Adloff
Gitta Blendinger
Prof. Dr. Oliver Daumke
Carola Bernert
Daniela Keyner
Siegne Knespel
Hagen Rostalski
Brigitta Wedekind (Secretary)

Women's Representative

The Women's Representative is responsible for matters and measures concerning equal opportunities for women at the MDC. She advises not only in the planning but also in the decisions of the Board and other organizational units, in particular with regard to personnel, welfare, and organizational decisions. In her work, the Women's Representative follows the legal requirements of the Berlin State Equal Opportunities Act (LGG) (http://www.berlin.de/sen/frauen/landesdienst/lgg.html) and the "Research-Oriented Gender Equality Standards" of the German Research Foundation (DFG) (http://www.dfg.de/foerderung/grundlagen_dfg_foerderung/chancengleichheit/index.html) and monitors and ensures their observance. The current Women's Representative at the MDC is Dr. Christiane Nolte.

Mitglieder des Personalrates

Ingo Kahl (Vorsitzender)
Robby Fechner (Stellv. Vorsitzender)
Lutz Else (Stellv. Vorsitzender)
Dagmar Gerhard (Stellv. Vorsitzende)
Manuela Adloff
Gitta Blendinger
Prof. Dr. Oliver Daumke
Carola Bernert
Daniela Keyner
Siegne Knespel
Hagen Rostalski
Brigitta Wedekind (Sekretariat)

Frauenvertreterin

Die Frauenvertreterin ist für Angelegenheiten und Maßnahmen der Gleichstellung der Frauen am MDC zuständig. Sie wird sowohl bei der Planung als auch bei Entscheidungen des Vorstands und anderer Organisationseinheiten, insbesondere bei personellen, sozialen und organisatorischen Entscheidungen beratend beteiligt. In ihrer Arbeit orientiert sich die Frauenvertreterin eng an den rechtlichen Vorgaben des Berliner Landesgleichstellungsgesetzes (LGG) (http://www.berlin.de/sen/frauen/landesdienst/lgg.html), sowie an den „Forschungsorientierten Gleichstellungsstandards der DFG" (http://www.dfg.de/foerderung/grundlagen_dfg_foerderung/chancengleichheit/index.html) und achtet auf deren Einhaltung. Derzeit nimmt Dr. Christiane Nolte die Funktion der Frauenvertreterin am MDC wahr.

Communications
Kommunikation

Communications at the MDC

Communication is a vital part of science, and the ultimate aim of the MDC Communications Department is to support and enable science in the best possible way. This is achieved through traditional communication routes including scientific conferences and press releases, as well as through new means of exchange such as social media. Media relations are a special issue and are thus covered in a section of their own (see page 283). The communications team, headed by Josef Zens, deals with visiting groups, internal communications, online activities, teacher training, and event management with an emphasis on scientific conferences and public affairs, i.e. strategic and political communication.

The Communications Department hosted 17 visitor groups in 2011, as well as 14 events including scientific conferences and the yearly "Long Night of Sciences" with over 3,000 visitors (see p. 255). A particular high-

Kommunikation am MDC

Kommunikation ist ein essentieller Teil der Wissenschaft. Ziel der Kommunikationsabteilung am MDC ist, die Wissenschaft zu unterstützen und dazu beizutragen, möglichst optimale Bedingungen für die Forschung zu schaffen. Dies kann auf traditionellem Weg geschehen, beispielsweise durch Kongressorganisation oder Pressearbeit, aber auch durch neue Wege des Austauschs, etwa über Social Media. Pressearbeit ist ein eigenes Feld und wird daher von einer eigenen Einheit abgedeckt (siehe S. 283). Das Kommunikations-Team von Josef Zens befasst sich mit der internen Kommunikation ebenso wie mit Besuchergruppen, Online-Aktivitäten, Lehrerausbildung, Event-Management mit einem Schwerpunkt auf wissenschaftlichen Konferenzen, und mit „Public Affairs", d.h. mit strategischer und politischer Kommunikation.

Photographer Katharina Bohm, Copyright MDC

Scientists interact with visitors of all ages during the "Long Night of the Sciences", May 28, 2011.

Besucherinnen und Besucher aller Altersgruppen lassen sich bei der "Langen Nacht der Wissenschaften" am 28. Mai 2011 Forschung erklären.

Photographer Katharina Bohm, Copyright MDC

Visitors view the "Best Scientific Image Contest" during the "Long Night of Sciences" on May 28, 2011.

Besucher der Langen Nacht der Wissenschaften (28. Mai 2011) betrachten die Bilder des Wettstreits "Best Scientific Image Contest"

light was the visit of the German Chancellor, Dr. Angela Merkel, who came in September 2011, along with Berlin's Senator of Education and Research, Jürgen Zöllner, and Dr. Annette Schavan, Federal Minister of Education and Research. We had numerous other VIP guests including the Governing Mayor of Berlin, Klaus Wowereit, members of Berlin's Senate and the German Parliament, high-ranking diplomats, and delegations from all over the world.

In 2011 a few new communications projects were launched, including iMDC, our magazine for staff members, guests, alumni and friends of MDC (editor-in-chief Dr. Barbara Urban), "Labor trifft Lehrer" our teacher-training program run by Dr. Luiza Bengtsson in close cooperation with the Buch Life Sciences Learning Lab "Gläsernes Labor", and several community-building efforts that took place both online and in the real world (conceived by Dr. Lucy Patterson) including sports activities, a lemonade stand and a "Glühwein" stand.

In the upcoming two years our communication efforts will concentrate on strategic issues such as the development of a new common institutional platform for translational research at the MDC and Charité, public affairs with an emphasis on political contacts, and our web presence. We will of course continue to provide various other types of support and offer even more services for MDC's scientists.

The members of the communications team are:

Dr. Luiza Bengtsson, Pamela Cohen, Lien-Georgina Dettmann, Russ Hodge, Michaela Langer, Dr. Lucy Patterson, Dr. Timkehet Teffera, Dr. Barbara Urban, Josef Zens (head).

Contact: communications@mdc-berlin.de

Die Kommunikationsabteilung betreute 2011 insgesamt 17 Besuchergruppen sowie 14 Veranstaltungen, darunter wissenschaftliche Konferenzen und die jährlich stattfindende „Lange Nacht der Wissenschaften" mit über 3.000 Besuchern (siehe S. 255). Der Höhepunkt war die Visite der deutschen Bundeskanzlerin Angela Merkel im September 2011. Sie besuchte das MDC zusammen mit Berlins Wissenschaftssenator Jürgen Zöllner und der Bundesministerin für Bildung und Forschung Annette Schavan. Viele weitere bedeutende Persönlichkeiten waren zu Gast am MDC, darunter Berlins Regierender Bürgermeister Klaus Wowereit, Mitglieder des Berliner Senats und des Bundestages sowie hochrangige Diplomaten und wissenschaftliche Delegationen aus der ganzen Welt.

Im Jahr 2011 wurde eine Reihe von neuen Kommunikations-Projekten ins Leben gerufen; dazu zählen das iMDC, unser Magazin für Mitarbeiterinnen und Mitarbeiter, Gäste, Alumni und Freunde des MDC (Chefredakteurin ist Dr. Barbara Urban), „Labor trifft Lehrer", ein Fortbildungsprojekt für Lehrer von Dr. Luiza Bengtsson, das in enger Zusammenarbeit mit dem „Gläsernen Labor" läuft, sowie Vorhaben zur Stärkung der MDC- bzw. Campus-Identität, die sowohl online als auch in der echten Welt realisiert wurden (konzipiert von Dr. Lucy Patterson). Selbst wenn manches informellen Charakter hat, ist der Austausch am Limonaden- oder Glühweinstand nicht zu unterschätzen.

In den kommenden beiden Jahren wird sich die Kommunikationsarbeit auf strategische Themen konzentrieren, in erster Linie auf die gemeinsame institutionelle Plattform für die translationale Medizin zwischen MDC und Charité, sowie auf Public Affairs und auf den Web-Auftritt. Wir werden natürlich weiter wie bisher Unterstützung für die Forschung anbieten und wollen den Service noch ausbauen.

Im Kommunikationsteam sind:

Dr. Luiza Bengtsson, Pamela Cohen, Lien-Georgina Dettmann, Russ Hodge, Michaela Langer, Dr. Lucy Patterson, Dr. Timkehet Teffera, Dr. Barbara Urban, Josef Zens (head).

Kontakt: communications@mdc-berlin.de

Press Office
Pressestelle

Press Office

Primarily supported by taxpayer contributions, the MDC strives to communicate its work and progress to a broad public. In the period cited, the Press Office published 150 press releases, among them 50 in English. The press releases resulted in approximately 120 interview requests. MDC news was covered by the media in Europe as well as abroad. The Press Office published six press reports documenting part of this coverage.

Pressestelle

Hauptsächlich finanziert von Steuergeldern bemüht sich das MDC die Medien über seine Forschungsarbeit zu informieren. In den vergangenen beiden Jahren veröffentlichte die MDC-Pressestelle 150 Pressemitteilungen, davon 50 in Englisch. Sie waren Anlass für rund 120 Interviewanfragen. MDC-Pressemitteilungen fanden Resonanz sowohl in den Medien in Europa als auch in Übersee. Die Pressestelle gab sechs Pressespiegel heraus, in denen ein Teil der Medienberichterstattung dokumentiert ist.

Photographer David Ausserhofer, Copyright MDC

The press and German Chancellor Angela Merkel (third from right) in front of the "Experimental and Clinical Research Center (ECRC)" of the MDC and the Charité. German Minister of Research, Annette Schavan, Prof. Annette Grüters-Kieslich (Deacon, Charité – Universitätsmedizin Berlin), Prof. Jürgen Mlynek (President, Helmholtz Association of German Research Centers), Prof. Detlev Ganten (Chair, Charité Foundation Council), Cornelia Lanz (Administrative Director, MDC), Prof. Walter Rosenthal (Scientific Director, MDC) and Berlin's Science Senator Jürgen Zöllner (from left).

Presse und Bundeskanzlerin Angela Merkel (3. v. r.) vor dem „Experimental and Clinical Research Center (ECRC)" von MDC und Charité. Bundesforschungsministerin Annette Schavan, Prof. Annette Grüters-Kieslich (Dekanin der Charité – Universitätsmedizin Berlin), Prof. Jürgen Mlynek (Präsident der Helmholtz-Gemeinschaft Deutscher Forschungszentren), Prof. Detlev Ganten (Vorsitzender des Charité-Stiftungsrats), Cornelia Lanz (Administrativer MDC-Vorstand), Prof. Walter Rosenthal (Vorsitzender des Stiftungsvorstands des MDC) und Berlins Wissenschaftssenator Jürgen Zöllner (v. l.).

The Press Office promoted five scientific conferences, two of which it organized. For all five conferences, the Office researched and wrote extensive press material. In addition, the Office invited journalists to the visit of German Chancellor Angela Merkel and Annette Schavan, Federal Minister of Education and Research, provided them with background material and published press releases. Together with the office of the Director, the Communications Department, and the BBB Management GmbH, the Press Office organized the visit of Klaus Wowereit, Berlin`s Governing Mayor, and published a press release.

The Press Office contributed to the exhibition "WeltWissen" which celebrated 300 years of science and research in Berlin and developed an exhibit for the research ship "MS Wissenschaft". It organized lab visits for the "Long Night of the Sciences" as well as the programs for ten visits of students from the USA, a high-ranking Chinese delegation, and a group of science journalists from Europe and provided for background information. It also organized more than 30 photo shootings.

The Press Office members are: Barbara Bachtler (head), Ann-Kathrin Schöpflin

Die Pressestelle begleitete fünf Kongresse mit ihrer Pressearbeit, davon bereitete sie zwei auch inhaltlich und organisatorisch vor. Sie lud die Hauptstadtpresse zum Besuch von Bundeskanzlerin Angela Merkel und Bundesforschungsministerin Annette Schavan und betreute die Journalisten während des Besuchs. Mit dem Vorstand, der Abteilung Kommunikation und der BBB Management GmbH bereitete sie den Besuch von Berlins Regierendem Bürgermeister Klaus Wowereit vor und machte ebenfalls die Pressearbeit.

Die Pressestelle beteiligte sich an der Ausstellung „WeltWissen" zu 300 Jahren Wissenschaft und Forschung in Berlin und erarbeitete ein Exponat für die „MS Wissenschaft". Sie organisierte Laborbesichtigungen für die „Lange Nacht der Wissenschaften", machte das Programm für zehn Studentengruppen aus den USA, für eine hochrangige chinesische Delegation sowie für eine Gruppe von Journalisten aus mehreren europäischen Ländern. Weiter organisierte sie über 30 Fototermine.

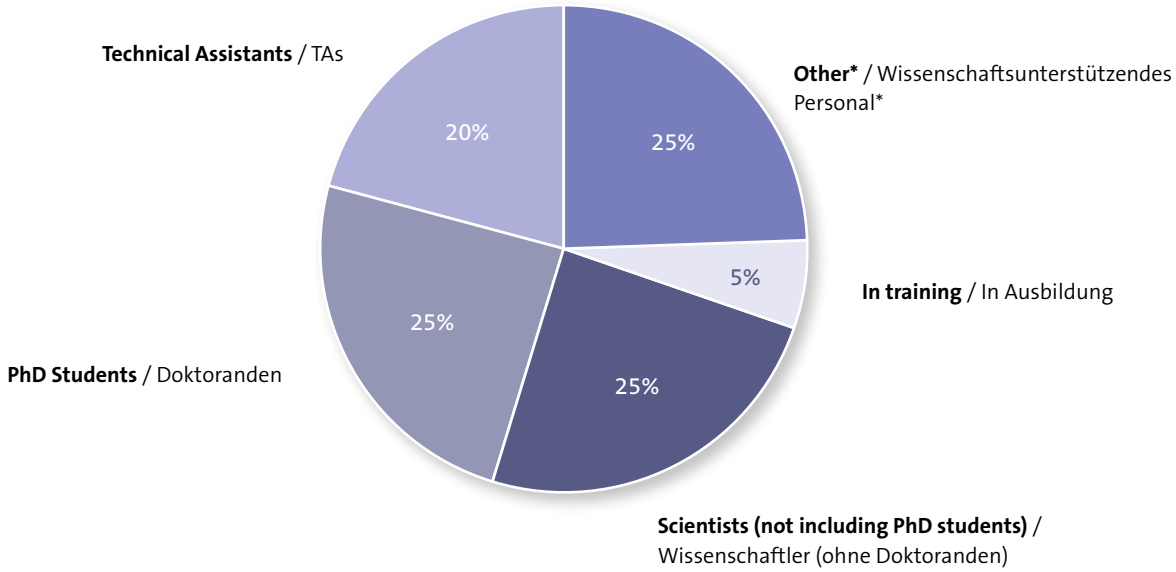
In der Pressestelle arbeiten: Barbara Bachtler (Leitung), Ann-Kathrin Schöpflin

Facts and Figures
Fakten und Kennzahlen

Personnel / Personal

Personnel (as of December 31, 2010) / Personalstand in Köpfen mit Stichtag 31.12.2010

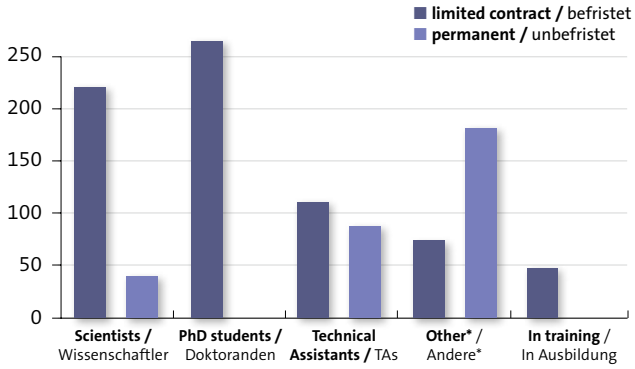
	Total/ Gesamt	**limited contract/** befristet	**permanent/** unbefristet	**in-house financing/** grundfinanziert	**third-party financing/** drittmittelfinanziert
Scientists (not including PhD students) Wissenschaftler (ohne Doktoranden)	254	214	40	144	110
PhD students Doktoranden und studentische Hilfskräfte	262	262	0	121	141
Technical Assistants (TAs) in the scientific sphere Technische Angestellte im wissenschaftlichen Bereich	202	114	88	144	58
Other / Wissenschaftsunterstützendes Personal inklusive Tierhauspersonal, Infrastruktur, Administration und Technologietransfer.	259	74	185	251	8
In training / In Ausbildung	48	48	0	48	0
Total / Summe	**1025**	**712**	**313**	**708**	**317**



***Infrastructure, administration, and technology transfer**/ *Infrastruktur, Verwaltung und Technologietransfer

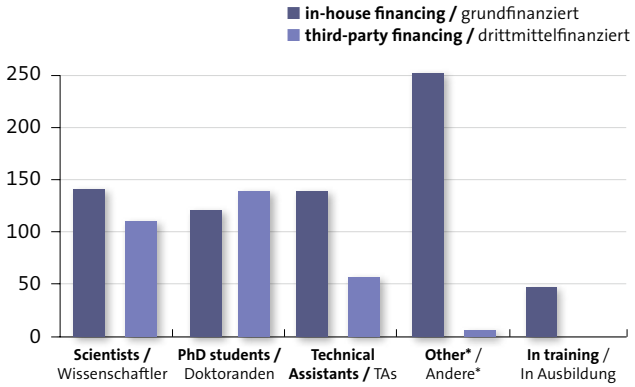
Type of Employment Contract / Art der Verträge

	limited contract / befristet	**permanent /** unbefristet
Scientists / Wissenschaftler	214	40
PhD students / Doktoranden	262	0
Technical Assistants / TAs	114	88
Other* / Andere*	74	185
In training / In Ausbildung	48	0

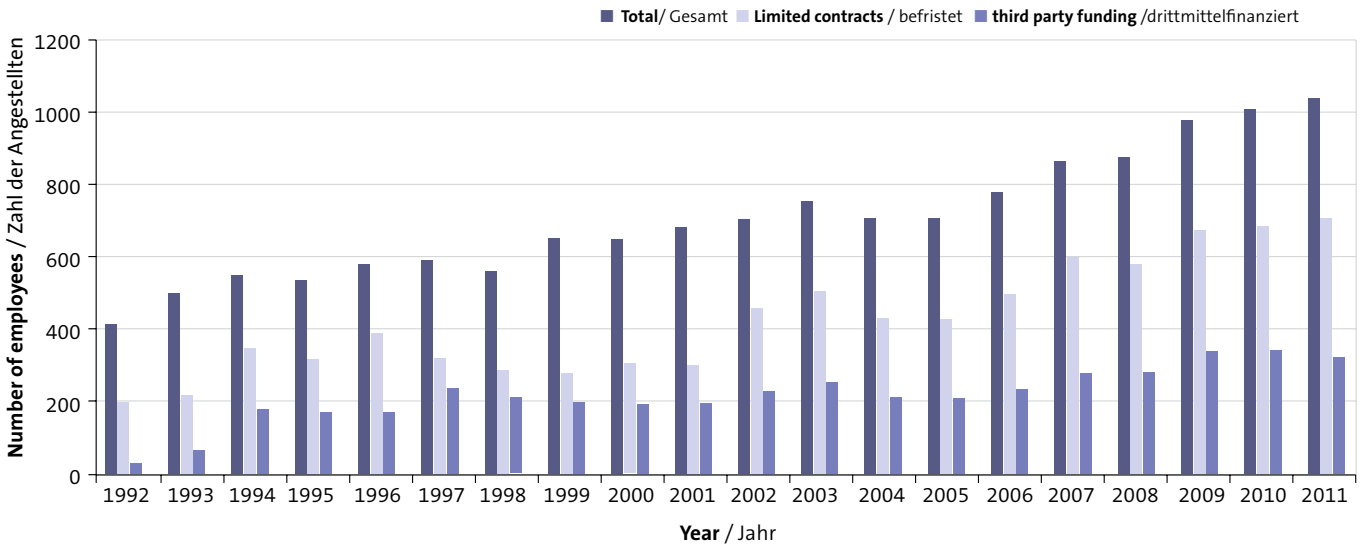


Type of Financing / Art der Finanzierung

	in-house financing/ grundfinanziert	third-party financing/ drittmittelfinanziert
Scientists / Wissenschaftler	144	110
PhD students / Doktoranden	121	141
Technical Assistants / TAs	144	58
Other* / Andere*	251	8
In training / In Ausbildung	48	0



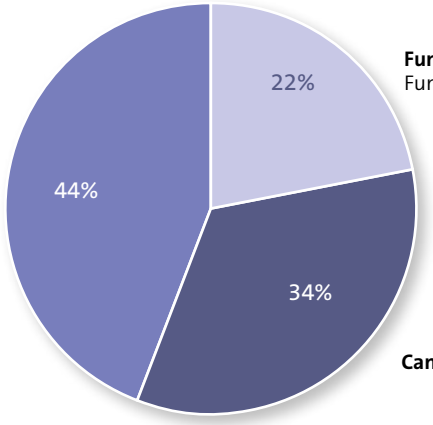
Personnel Development at the MDC / Entwicklung des Personals MDC



Financing / Finanzierung

Costs of research programs in 2010 (in thousands of €) / Kosten der Forschungsprogramme 2010 in T€ (Vollkosten)
Vollkostendarstellung ohne Einbeziehung der programmungebundenen Forschung

Programs & Categories / Programme & Kategorien	**Costs** / Kosten
Cancer Research / Krebsforschung	18,394
Cardiovascular and metabolic disesases / Herz- Kreislauf- und Stoffwechselerkrankungen	23,758
Function and dysfunction of the nervous system / Funktion und Dysfunktion des Nervensystems	12,070
Total Research Area Health / Gesamt	**54,222**



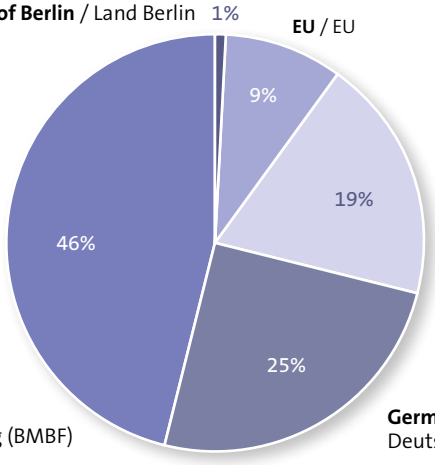
Function and dysfunction of the nervous system / Funktion und Dysfunktion des Nervensystems

Cardiovascular and metabolic disesases / Herz- Kreislauf- und Stoffwechselerkrankungen

Cancer Research / Krebsforschung

Extramural funding in 2010 (in thousands of € / Drittmittelfinanzierung 2010 in T€

Extramural funds / Drittmittelgeber	**Amounts /** Drittmittelausgaben
Federal Ministry of Education and Research / Bundesministerium für Bildung und Forschung (BMBF)	10,627
German Research Foundation / Deutsche Forschungsgemeinschaft	5,747
Industry and other organizations / Industrie und sonstige Organisationen	4,483
EU / EU	2,087
State of Berlin / Land Berlin	127
Total / Gesamt	**24,008**



State of Berlin / Land Berlin

EU / EU

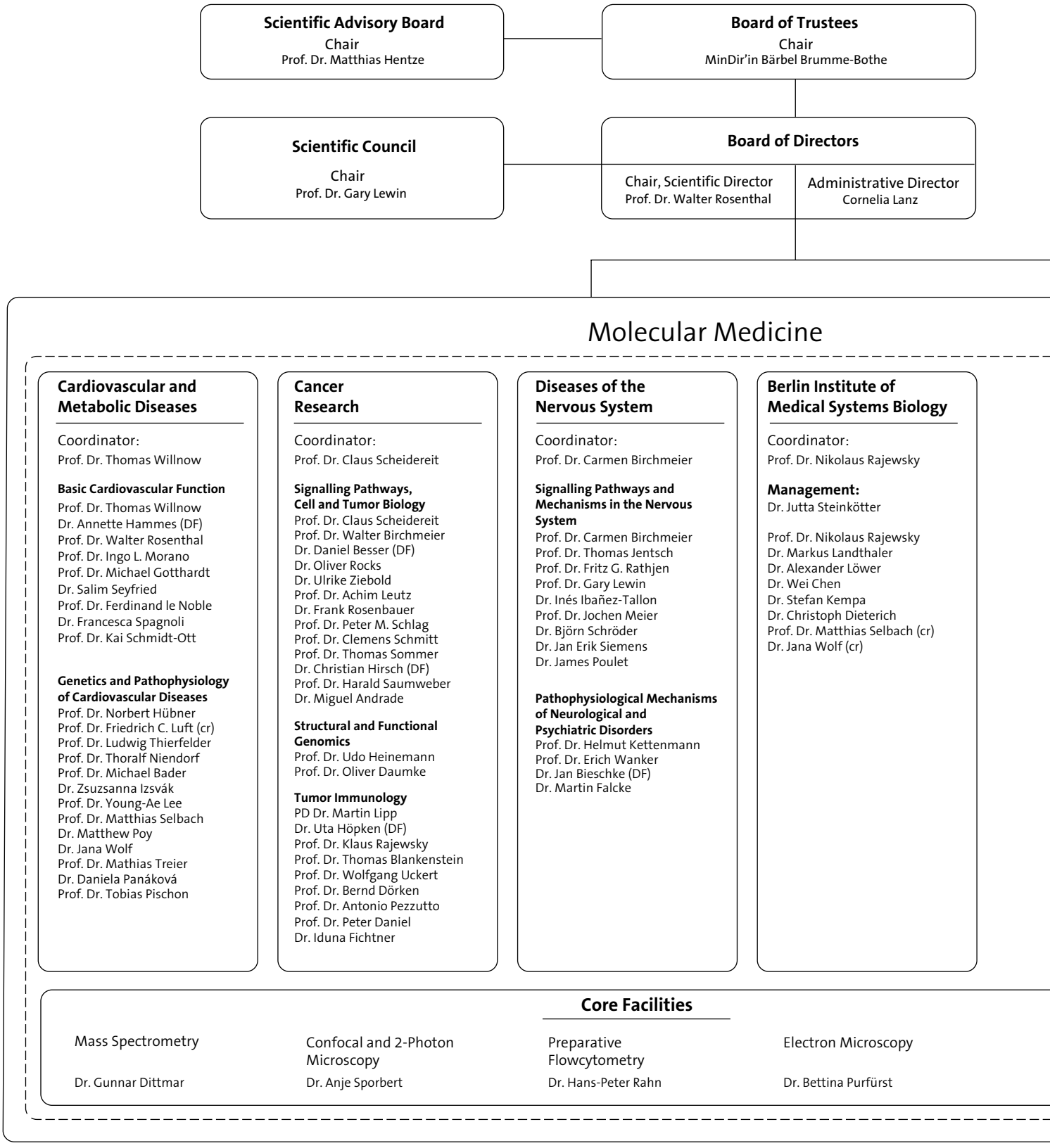
Industry and other organizations / Industrie und sonstige Organisationen

Federal Ministry of Education and Research / Bundesministerium für Bildung und Forschung (BMBF)

German Research Foundation / Deutsche Forschungsgemeinschaft

Organization Chart of the Max Delbrück Center for Moleculare Medicine

Foundation under public law



Legend
DF = Delbrück Fellow
cr = cross-reference

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN BERLIN-BUCH IN DER HELMHOLTZ-GEMEINSCHAFT e.V.

Staff Council

Chair
Ingo Kahl

Representatives

Woman's Representative:	Dr. Christiane Nolte
Data Protection Officer:	Christine Rieffel-Braune
Anti-Corruption Officer:	Ina Herrmann
Ombudsman:	Prof. Dr. Jens Reich
Contact Person for PhDs:	Prof. Dr. Thomas Sommer

ECRC (jointly with Charité)
Clinical Reasearch

Director:
Prof. Dr. Friedrich C. Luft

Head of administration:
Dr. Regina Jünger (Charité)

Program-Management:
Dr. Cornelia Maurer

Prof. Dr. Friedrich C. Luft
Prof. Dr. Simone Spuler
Prof. Dr. Ralph Kettritz
Prof. Dr. Jeanette Schulz-Menger
Prof. Dr. Maik Gollasch
Prof. Dr. Silke Rickert-Sperling
Prof. Dr. Joachim Spranger
Prof. Dr. Dominik N. Müller/
Dr. Ralf Dechend

Transgenics

Dr. Boris Jerchow

Science Administration

Scientific Coordination
Dr. Christina Quensel
Dr. Almut Caspary
Dr. Iwan Meij

Press Office
Barbara Bachtler

Communications
Josef Zens

EU / Third Party Funds
Dr. Sabine Baars

International Office
Dr. Oksana Seumenicht

PhD-Coordination
Dr. Michaela Herzig

Safety

Biological Safety:	Dr. Regina Dietl
Radiation Protection & Laboratory Safety:	Dr. Frank-Peter Kirsch

Administration and Infrastructure

Administrative Coordination
Dana Lafuente
Katrin Rosswog

Internal Audit
Uta Geng

Personnel
Dr. Hans-J. Seehrich

Finances / Controlling
Andrea Winnefeld

Animal Facilities
Dr. Karin Jacobi

Library
Dr. Dorothea Busjahn

IT
Bernd Lemke

Technical Facility Management
Construction: Ralf Streckwall
Operation: Michael Arnold

Purchasing and Central Services
Wolfgang Groll

Legal Affairs and Technology Transfer
Christine Rieffel-Braune

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Campus Map

Campusplan

Campus Berlin-Buch
Der Gesundheit verpflichtet

Robert-Rössle-Str. 10
13126 Berlin
Tel.: +49-30-9489-2920
Fax: +49-30-9489-2927
www.campus-berlin-buch.de

☐ Common Facilities

A 8 Gate House with Café Max and apartments
A 9 Reception gate
A 13 Life Science Learning Lab; CampusInfoCenter
A 14 Cafeteria

Guesthouses of the MDC

B 54 Hans-Gummel-Guest House
B 61 Salvadore-Luria-House with kindergarten

■ Research

Max Delbrück Center for Molecular Medicine (MDC)

C 27 Walter-Friedrich-House
C31.1-3 Max-Delbrück-House
C 83 Max-Delbrück-Communications Center
C 84 Hermann-von-Helmholtz-House
C 87 Timoféeff-Ressovsky-House
C 71, B 63, B 64 } Research services
C 88 Ultrahigh Field Facility
A 10 Library

Leibniz-Institut für Molekulare Pharmakologie

C 81 Leibniz-Institut für Molekulare Pharmakologie (FMP)

Shared Facilities by MDC and FMP

C84.1 Research services
C 87 Timoféeff-Ressovsky-House

■ Clinical Research

B 42-45 Experimental and Clinical Research Center (ECRC)

■ Companies

A 15 car mechanics, EZAG, Charles River, WISAG

B 55 **Oskar und Cécile Vogt House**
BBB-post office, 8sens.biognostic, FILT, MRW, ConGen, E.R.D.E., HUMAN, neptuntec, ART-CHEM, TECAN, Dr. Scherrer, LIPIDOMIX, Roboklon, Fresenius, Patent lawyer Dr. Baumbach, GHP, niontec, MRI, Octropharm, ART-CHEM, NIKON, Institut E & G

B 64 epo

D 16/23 Eckert & Ziegler AG, NEMOD, Eurotope, Glykotope, BEBIG, Eckert Consult, Isotope Products

D 72 Bavarian Nordic

D 79 **Erwin Negelein House**
Akademie der Gesundheit, Isotope Products, BioTeZ, imaGenes, BG Berlin-Genetics,

D 80 **Otto Warburg House**
ALRISE, Silence Therapeutics, Evotec AG, Dreyer & Bosse, Celares

D 82 **Karl-Lohmann-House**
Eckert & Ziegler, BEBIG, AJ Innuscreen

D 85 **Arnold Graffi House**
BBB, I.M.S.M., stratec, aokin, Biosyntan, L.O.S., emp, Klin.Forschung, Prof.Wanker,MerLion, ProteaImmun, GLYCOTOPE, Nutrineu, ascenion, S.Langmacker accountant

How to find your way to the MDC

Wie gelangen Sie zum MDC

HELMHOLTZ ASSOCIATION