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The Friedrich Miescher Institute for Biomedical Research

The FMI is devoted to fundamental biomedical research and the transfer of knowledge gained to pharmaceutical application. We explore basic molecular mechanisms of cells and organisms in health and disease.

As the FMI celebrates its 40th year of existence, our scientists have been more productive than ever before in the institute's history. In 2009-2010, 35 FMI publications appeared in Science, Cell or the Nature family journals and numerous Marie Curie Excellence awards, European Research Council grants and prizes were received.

A key factor in our success has been excellent hiring at the group leader and facility head levels and the exceptional students and postdocs these scientists have attracted to the FMI. My tenure as Director began in 2004, not long after Uli Müller, Willy Krek and George Thomas left the FMI and just prior to the retirements of Barbara Hohn, Thomas Hohn, Fred Meins, Andrew Matus and Denis Monard. The potential for renewal was thus enormous and had already begun with the appointment of four young scientists, Dirk Schübeler, Andreas Lüthi, Thomas Oertner and Antoine Peters. They have all been remarkably productive and have been or will soon be promoted to senior staff. After 2004, junior positions were also accepted by Joy Alcedo, Momo Bentires-Alj, Marc Bühler, Rafal Ciosk, Helge Grosshans, Jan Pielage, Botond Roska and Nico Thomä, while Rainer Friedrich and Filippo Rijli joined as senior staff. We congratulate Botond Roska for his promotion to senior group leader, crowning 5 years of exceptionally creative work on vision restoration. Combined with the success of Witek Filipowicz in defining Dicer and miRNA function and Pico Caroni in neuronal regeneration and ALS, these results have set FMI research standards very high. Further recent papers in Nature, Science or Cell came from Silvia Arber, Rainer Friedrich, Helge Grosshans, Andreas Lüthi and Nico Thomä.

The excellence of FMI technology parallels our prowess in research. Heinz Gut joined us in 2008 as Head of the Protein Structure Facility, Tim Roloff replaced Ed Oakeley as Head of Functional Genomics and one of the world’s best 3D scanning electron microscopists, Christel Genoud, now runs our 3D-EM Facility. Our talented computational biology, imaging, and functional genomics teams have also welcomed new members. Several loyal technical associates retired in 2009-2010, including Herbert Angliker, Marianne Brown-Lüdi, Maria Rita Meins, Matthias Müller, Michel Siegmann and Monique Thomas. We are most grateful for their dedicated work for the FMI.

We congratulate retiring group leader Yoshikuni Nagamine for 27 fruitful years dedicated to the pursuit of the urokinase-type plasminogen activator and more recently, RHAU helicase. Yoshi showed that this AU-repeat recognizing enzyme contributes to message turnover and is needed for the survival of ras-transformed cells, opening a door to pharmaceutical intervention. Loss of RHAU leads to extended telomeres and we could wish Yoshi nothing better than that RHAU inhibition will be his “fountain of youth” as he pursues retirement with a sabbatical in China. Group leader Jan Hofsteenge generously decided to close his lab in 2009 and assume the tasks of Deputy Director and COO. I extend my personal thanks to Jan for freeing me from administrative tasks and for his steady guidance of the 5th floor team.

Erich Schlumpf, one of our highly valued administrative staff, retired as Facility manager in 2009, after training a very competent successor, Peter Thommen. Erich orchestrated the complete renovation and takeover of WRO-1066 in 1995. Rudi Unrau, who led Human Resources for 10 years, has accepted a challenging position as Head of Personnel in a large Basel hospital. Rudi epitomized the FMI spirit with his warm welcome to new colleagues. We wish him great success as we welcome Oliver Fink as his successor. Another important member of our administrative team, Patrick King, will give up his mandate at the end of 2010. A former FMI group leader, Pat returned as a freelancer to manage our biosafety and radiation safety training and facilities. As a talented writer and editor, Pat managed our scientific communication to Novartis and our external website, and produced 30 issues of the “FMI Report”. His final contribution to our
heritage is a comprehensive history of FMI’s first 40 years, a publication that is eagerly awaited. We warmly welcome Sandra Ziegler Handschin, our new communications expert, who has taken over that side of Pat’s duties.

The growth and dynamics of FMI have produced excellent science but have also challenged our core budget. Thankfully, we have seen a significant increase in third-party funding to over CHF 30 million over the next 4 years. In particular, the support of the Swiss National Science Foundation and the Swiss and Basel Cancer Leagues has been crucial, and six FMI scientists have received generous European Research Council grants, namely Silvia Arber, Momo Bentires-Alj, Helge Grosshans, Botond Roska, Dirk Schübeler and Nicolas Thomä. Participation in large EU networks as well as NCCR programs has also helped finance research. Given that competitive funding is as crucial to the maintenance of high scientific standards as is paper peer review, our success at securing funds underscores our excellence in research. Finally, prestigious honors were accorded to Brian Hemmings, who was elected Fellow of the Royal Society of London, and to Nancy Hynes, who received the German Cancer Prize of the German Cancer Society.

FMI interactions with Novartis and the Novartis Institutes of Biomedical Research (NIBR) have increased precipitously in the last 2 years, with over 50 collaborations ongoing. Our scientists participate regularly in FMI-NIBR workshops on new targets in cancer, oncology retreats with NIBR, and epigenetics retreats with the Chinese branch of NIBR. NIBR interest in neuronal degeneration has led to new contacts with FMI neuroscientists, and Novartis Ophthalmology became seriously interested in treating adult-onset blindness caused by retinitis pigmentosa and have licensed crucial patents from Botond Roska and colleagues.

FMI has further marked its presence in Swiss and European science as a participant in the Swiss Institute of Bioinformatics, the Swiss SystemsX program and C-CINA, a new imaging endeavor of the University of Basel. Together with the University, the FMI is spearheading Swiss participation in EuroBioImaging, a program of the European Strategy Forum on Research Infrastructures designed to secure Europe-wide imaging infrastructure. Thus, FMI has broadened its partnerships with both academia and industry, while continuing to occupy its crucial middle ground.

The principles guiding FMI are simple: a commitment to student and postdoc training, fostering of cross-disciplinary research, maintaining sufficient core funding to allow ideas to be pursued independently of peer review, technological innovation, a focus on molecular mechanisms behind rare and common diseases, and the translation of our basic discoveries to disease therapies. Biomedical research today combines genetics and proteomics, structural analyses, and quantitative imaging with functional cellular readout in living organisms. This lends itself to predictive modeling and requires a deeper grasp of mathematics and statistical analyses. Above all, the FMI will continue to promote rigorous scientific insight—both knowledge and its application to medicine—and cross-disciplinary collaboration as the surest way to prosper for the next 40 years.
Neurobiology The formation of neuronal connections during development and their maintenance in adulthood are crucial determinants of nervous system function in health and disease. We use molecular and genetic techniques to explore the cellular mechanisms that determine how circuits are first made and later modified to support animal behaviour.

Silvia Arber  
_Assembly and function of motor circuits_

Pico Caroni  
_Plasticity of neuronal circuits_

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_Neuronal circuits and computations_

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_Transcriptional mechanisms of topographic circuit formation_

Botond Roska  
_Structure and function of local neural circuits_
Assembly and function of motor circuits

INTRODUCTION

Motor behavior is the ultimate output of nearly all nervous system activity but little is known about how neuronal circuits central to this behavior are assembled during development or how they function in the mature nervous system. The gradual organization and function of the nervous system during development relies on the precise sequential assembly of defined neuronal circuits into functional units.

The function of the motor system depends on local circuits within the spinal cord, long distance connectivity with supraspinal centers and sensory feedback from the periphery. We study the acquisition of identity by neuronal subpopulations involved in motor behavior and how this process of neuronal diversification and specification translates into precisely connected neuronal circuits.

Our main aim recently has been to unravel molecular and cellular mechanisms involved in the assembly of the spinal monosynaptic reflex circuit. This circuit constantly updates central spinal circuits with regard to body positioning in space by the formation of precise synaptic connections between proprioceptive sensory neurons and spinal neurons. Motor neurons in the ventral spinal cord are clustered into motor neuron pools, each of which innervates and controls contraction of a defined muscle in the periphery. Proprioceptive sensory neurons relay information back to the central nervous system about the state of contraction of each muscle via connections with neurons in the ventral spinal cord. The precision of these sensory-motor connections is a major factor in the control of motor behavior.

We have shown that precise signaling between neurons and their target tissues and the activity of transcription factors and cell surface molecules play key roles during motor circuit assembly and function in the spinal cord (Pecho-Vrieseling et al. 2009; Friese et al. 2009; Dalla Torre et al. 2008). Our work combines gain- and loss-of-function mouse genetics, high-end microscopy of connectivity and synapses, gene expression profiling of neuronal subpopulations, viral techniques, electrophysiology and behavioral analysis.
MOLecular Codes For SYNAPTic SPECIFICITY

E. Pecho-Vrieseling, M. Sigrist

Spinal reflex circuits assemble with high precision. Synaptic connections between sensory afferents and motor neurons in the spinal cord form with a high degree of selectivity. The initial steps of sensory- and motor axon elaboration are controlled by cell-intrinsic factors and are determined partly at stages before these neurons exit the cell cycle. Different classes of transcription factors control sequential steps in the differentiation hierarchy of motor neurons in the developing spinal cord. In addition to specification of neurons by cell-intrinsic cues, our recent work has shown that signaling interactions with cues encountered by axons en route to the target or from the target region itself also have important roles in the specification of later steps of neuronal connectivity (Dalla Torre et al. 2008). We have identified two molecular pathways linking specific peripherally derived signals to the induction of the ETS transcription factors Pea3 and Er81 in defined subsets of motor neurons (Pea3) and DRG sensory neurons (Er81). Furthermore, we found that these retrograde signals from an intermediate target region act selectively to control the progressive specification and differentiation of distinct neuronal subpopulations through the activation of the ETS transcription factors Pea3 and Er81 (Dalla Torre et al. 2008), both acting at late steps of circuit assembly. Yet, how these dedicated transcriptional programs translate into cell-surface recognition codes expressed by defined neuronal subpopulations as well as the mechanisms by which these in turn regulate the establishment of specific synaptic connections remain unclear.

The organization of spinal reflex circuits exhibits several levels of specificity. We have shown that only certain classes of proprioceptive sensory neurons make direct, monosynaptic, connections with motor neurons (Vrieseling et al. 2006). Those that do are bound by rules of motor pool specificity. They form strong connections with motor neurons supplying the same muscle, but avoid motor pools supplying antagonistic muscles. The pattern of sensory-motor connections is initially accurate and is maintained in the absence of activity, implying that wiring specificity relies on the matching of recognition molecules on the surface of sensory and motor neurons. Nevertheless, determinants of fine synaptic specificity here, as in most regions of the central nervous system, have yet to be defined.

To address the origins of synaptic specificity in mammalian spinal reflex circuits we have used mouse genetic methods to manipulate recognition proteins expressed by subsets of sensory and motor neurons. We found that a recognition system involving expression of Sema3e by selected motor neuron pools and its high-affinity receptor PlexinD1 by proprioceptive sensory neurons is a critical determinant of synaptic specificity in sensory-motor circuits (Pecho-Vrieseling et al. 2009). Specifically, we found within the brachial spinal cord of mice that Sema3e expression is confined to motor neurons innervating the Cutaneous maximum (Cm) muscle and is not expressed by the neighboring Triceps (Tri) or other motor neuron pools at this same segmental level of the spinal cord. In contrast, PlexinD1 expression is restricted to a subpopulation of proprioceptive sensory neurons, where it is expressed by the majority of Cm proprioceptive afferents. To determine whether Sema3e-PlexinD1 signaling plays a role in the establishment of sensory-motor connections, we analyzed mice mutant in these signaling components. We found that mutation of Sema3e in motor neurons or PlexinD1 in sensory neurons leads to specific connectivity defects between Cm proprioceptive afferents and Cm motor neurons in electrophysiological and anatomical assays (Figure 1). Whereas in wild-type mice, no direct connections were present in the Cm-Cm circuit, frequent contacts were established upon mutation of the Sema3e-PlexinD1 signaling pathway. In addition, we found that ectopic expression of Sema3e in Tri motor neurons leads to interference with the establishment of the normally direct sensory-motor connections in the Tri-Tri reflex circuit. In summary, changing the profile of Sema3e-PlexinD1 signaling in sensory and motor neurons leads to a functional and anatomical rewiring of monosynaptic connections, unraveling an important signaling mechanism involved in the establishment of specific synaptic connectivity in the spinal monosynaptic reflex circuit.

Our results indicate that patterns of monosynaptic connectivity in the prototypic CNS circuit of the spinal monosynaptic reflex circuit are con-
structured through a recognition program based on repellent signaling. The presence of Sema3e-PlexinD1 signaling between potential synaptic pairs interferes with the establishment of direct connections through a mechanism of molecular repulsion. In this study, we have uncovered a molecular mechanism directly linking synaptic receptiveness to neuronal function and provide evidence for layers of synaptic specificity in the establishment of synaptic connections.

Since we found previously that Sema3e expression is regulated through the Pea3 transcription factor pathway in Cm motor neurons (Vrieseling and Arber, 2006; Livet et al. 2002), our results also demonstrate how transcriptional pathways intersect with cell-surface recognition codes implementing specificity of synaptic connections. More generally, our data provide evidence that the acquisition of neuronal identity through transcriptional programs plays an essential role in the establishment of synaptic connectivity by regulation of cell-type specific cell-surface recognition codes.

**IDENTIFYING FUNCTIONALLY DISTINCT MOTOR NEURON SUBCLASSES BY TRANSCRIPTION FACTORS**

A. Friese, M. Mielich, M. Sigrist

Spinal motor neurons are specified to innervate different muscle targets through combinatorial programs of transcription factor expression (Dalla Torre et al. 2008). Many of these transcriptional programs, including those described above, obey the functional unit of the motor neuron pool according to the axonal trajectory to innervate a specific muscle in the periphery. However, despite the fact that all motor neurons project peripherally, distinct subpopulations of motor neurons exist even within a given motor neuron pool. In particular, whereas alpha motor neurons innervate extrafusal muscle fibers involved in force generation, gamma motor neurons innervate intrafusal muscle fibers, an integral part of muscle spindles. Gamma motor neurons function in adjusting the properties of the spinal reflex circuit, a function fundamentally different from that of alpha motor neurons involved in muscle contraction.

Whether transcriptional programs also establish these finer aspects of motor neuron subtype identity, notably the prominent functional distinction between alpha and gamma motor neurons reaching beyond the boundary of motor neuron pools, was not known. Recently, we set out to determine whether gamma and alpha motor neurons in the mouse spinal cord are distinguishable on the basis of their transcription factor expression profiles and other molecular markers. In a screen for molecular markers exhibiting subpopulation-restricted expression patterns in the ventral spinal cord, we uncovered two genes with complementary expression profiles in gamma and alpha motor neurons (Friese et al. 2009). The transcription factor Estrogen-related Receptor 3 (Err3) is expressed at high levels in gamma but not alpha motor neurons, whereas the neuronal DNA binding protein NeuN marks alpha but not gamma motor neurons (Figure 2). We found further that signals from peripheral muscle spindles are required to support the differentiation of Err3<sup>on</sup>/NeuN<sup>off</sup> gamma motor neurons in the spinal cord, since gamma motor neurons as identified by this novel molecular code are selectively absent from mice in which muscle spindles are ablated by specific genetic tools, whereas alpha motor neurons are not affected. In contrast, direct proprioceptive sensory input to a motor neuron pool is apparently dispensable for differentiation of gamma motor neurons and the acquisition of the Err3<sup>on</sup>/NeuN<sup>off</sup> transcriptional code in gamma motor neurons.

Together, these findings establish that gamma and alpha motor neurons are molecularly distinct and extend the principle that spinal motor neuron subtype identity has its origins in hierarchical programs of transcription factor expression, even within a single anatomically coherent motor neuron pool.

**Selected publications**


INTRODUCTION

We investigate regulatory mechanisms that control the formation, maintenance and turnover of synaptic connections (structural plasticity) and how this plasticity relates to learning and behavior. We are particularly interested in mechanisms determining the plasticity of defined neuronal circuits, as they may inform us about principles of learning, adaptation, and resilience to disease in the nervous system.

Applying this approach to the hippocampus, a brain structure with a critical role in learning and memory, we investigate how learning and experience specifically influence circuit structure and how that structure in turn impacts on behavior. We are taking a comprehensive approach to hippocampal and cerebellar circuits, with studies ranging from the specification and assembly of defined microcircuits during development to the roles of these microcircuits in adult plasticity. In a second line of research, we investigate mechanisms of disease in neurodegeneration, focusing on mouse models of motoneuron disease.

As well as mouse genetics, mouse behavior, neuroanatomy, single-cell genomics and live imaging, we use transgenic mice expressing fluorescent chimeric proteins in single neurons to visualize neurons and subcellular components in situ.
REGULATION AND ROLES OF STRUCTURAL PLASTICITY IN THE ADULT


Structural plasticity of axons beyond developmental circuit assembly processes and in the absence of physical lesions is a recent discovery and an exciting addition to the plasticity repertoire of mammalian brains. Although the surface has just been scratched so far, it is clear that these novel aspects of brain plasticity may complement the functional impact of long-term plasticity mechanisms at pre-existing synapses. This is mainly due to the different time scales of the phenomena (seconds to hours, versus days to weeks) and to the spatial scale of the modifications to circuits (axons can sample synaptic territories ranging in tens and even hundreds of microns).

Due to its unique anatomical features, the mossy fiber projection by dentate gyrus granule cells onto hippocampal CA3 has provided an attractive model system to investigate axonal structural plasticity in the adult. In CA3, the axons of glutamatergic granule cells (a.k.a. mossy fibers) extend in tight lamellar bundles to establish 8-10 Large Mossy fiber Terminals (LMTs) onto excitatory pyramidal neurons (feed-forward excitation, FFE) and numerous synapses onto inhibitory neurons through en-passant boutons and filopodial extensions of the LMTs (feed-forward inhibition, FFI).

Anatomically, LMTs are Terminal Arborization (TA) structures, which consist of a “parent LMT” (FFE), filopodial extensions (FFI), and “satellite LMTs” (FFE). The connectivity between mossy fiber LMTs and CA3 pyramidal neurons exhibits pronounced structural plasticity in the adult. Most dramatically, adult neurogenesis of granule cells leads to the experience-related incorporation of new projections throughout life. In addition, hippocampal learning can produce spatial expansions of the mossy fiber projection, whereas chronic stress produces global reductions in postsynaptic spine densities. We have provided evidence for balanced turnover of mossy fiber boutons regulated by AMPA receptor activation in slice cultures. In addition, we revealed macroscopically detectable long-term alterations in the adult in vivo. Housing mice under enriched environment (EE) conditions produced a marked increase in dendritic spine lengths and “satellite LMTs”, whereas a gradual life-long shift in LMT size distributions produced increasingly asymmetric distributions of LMT sizes as a function of increasing age.

We recently investigated the synaptic and molecular mechanisms underlying global remodeling of LMTs in response to experience and age in vivo. We found that EE specifically and reversibly produces a robust increase in stratum lucidum synapse numbers and in the densities of synapses per LMT. In parallel, the EE conditions produced a marked increase in CA3 pyramidal neuron Wnt7a/b protein levels. Local inhibition of Wnt signaling in CA3 suppressed the effects of EE on synapse numbers and further reduced synapse numbers in mice housed under control conditions; conversely, the local application of recombinant Wnt7a or of a Wnt agonist was sufficient to mimic the effects of EE in vivo and in slice cultures (Figure 1).

Wnt signaling thus mediates global regulation of synapse numbers in response to experience and age in adult hippocampal stratum lucidum. These sustained global alterations in synapse numbers and connectivities might represent a novel class of structural plasticity mechanisms modifying network properties in response to experience.

SPECIFICATION OF PLASTIC AND STABLE SYNAPSES IN JUVENILE CIRCUITS

E. Bednarek, J. Buetler, I. Galimberti, Y. Deguchi, F. Donato, K. Leptien, S. Ruediger, D. Spirig, A. Udhayachandran

Structural plasticity in the intact adult nervous system includes the formation of new synapses and the loss of existing synapses. This synapse turnover could have major roles for learning and memory, but the mechanisms underlying its regulation and specificity in the adult are poorly understood. Time-lapse imaging studies of synapses in adult mouse and monkey brains have provided evidence for the existence of distinct subpopulations of stable and dynamic synapses. Stable presynaptic boutons or postsynaptic spines appear to persist indefinitely in the adult and represent the largest fractions of total synapses (85-92%). In contrast, dynamic synapses turn over within days or weeks in vivo. In spite of their obvious relevance to the understanding of plasticity in the adult, the mechanisms underlying the distinctions between stable...
and dynamic synapses are currently unknown.

The characteristic arrangement consisting of a core LMT and of satellite LMTs connected to each other through processes identifies some hippocampal LMT complexes as Terminal Arborizations (Figure 2). Such TAs are found in many types of axons, where they consist of a local axonal side-branch that produces multiple local synapses through its secondary side-processes. TAs are particularly structurally plastic and can explore significant volumes of synaptic territory, but the mechanisms that set up and regulate these major sites of structural plasticity in the adult have remained unclear.

We recently investigated how structural plasticity is set up in individual mossy fibers in CA3 and found that 30-35% of hippocampal mossy fibers exhibit one distinct TA, whereas 10-15% of mossy fibers exhibit >2 distinct TAs. The remaining mossy fibers exhibit no detectable TAs. Mossy fibers with different numbers of TAs reflect distinct subpopulations of granule cells. The TAs account for most structural plasticity by LMTs in CA3 (Figure 2). TAs were specified in juvenile mice through a process leading to the retention of high densities of synaptic sites specifically at core LMTs of TAs. Unexpectedly, mossy fibers established their single or most vigorous TAs at a position along CA3 that is topographically related to the position of the GC along the DG. Specific interference with the activation of the ephrin receptor EphA4 led to the formation of >2 TAs in single-TA mossy fibers and disrupted the topographic specification of TAs. TAs with high plasticity properties are thus specified during a sensitive period in juvenile circuits and they maintain that distinction in the adult, leading to a focusing of structural plasticity at defined positions along CA3. Given that experience and age have pronounced effects on the complexities of TAs, our results suggest that the selection of particular positions along CA3 by TAs might have functional significance for information flow in the hippocampus.

DEVELOPMENTAL MATURATION OF MEMORY STORAGE MECHANISMS

N. Gogolla and P. Caroni, in collaboration with C. Henry and A. Lüthi

Pairing an initially neutral stimulus (CS) with an aversive stimulus (US) leads to the formation of a robust and long-lasting fear memory. Inhibition of conditioned fear responses can be achieved by repeated exposure to the CS in the absence of the US, a process called extinction. Unlike fear conditioning, fear extinction in adult animals is neither robust nor permanent and fear responses can be renewed. In contrast to adult animals, rats younger than 3 weeks do not exhibit renewal of conditioned fear responses. In both adults and young animals, fear extinction depends on the amygdala. The neuronal mechanisms underlying the developmental regulation of fear extinction are not known.

Developmental regulation of brain plasticity is much better understood in sensory systems such as the visual cortex. During the first few weeks of postnatal development (the so-called critical period), monocular sensory deprivation leads to long-lasting functional and structural changes. The assembly of perineuronal nets (PNNs), an organized form of chondroitin sulfate proteoglycans (CSPG)-containing extracellular matrix around parvalbumin-expressing inhibitory interneurons, is thought to contribute to critical-period closure. Consistent with this notion, the degradation of PNNs in adults re-enables the induction of ocular dominance plasticity.

We hypothesized that related developmental plasticity mechanisms may influence emotional learning processes in juveniles. We found that in the amygdala the organization of CSPGs into PNNs coincided with the developmental switch in fear memory resilience. In adults, degradation of PNNs by chondroitinase ABC specifically rendered subsequently acquired fear memories susceptible to erasure. Therefore, distinct neuronal mechanisms mediate the acquisition and extinction of conditioned fear memories during early postnatal development and in adults. Because degradation of PNNs in adult animals re-enabled erasure of fear memories by extinction, the mechanisms underlying extinction-induced fear memory erasure in juveniles are not lost in the adult and fear memories are actively protected from erasure.

Fig. 2. Structural plasticity of an individual mossy fiber projection and all its LMTs in CA3. Camera lucida of the same mossy fiber projection at day in vitro (DIV) 20, 40 and 60 in an organotypic slice culture. The dentate gyrus position is indicated for orientation. Individual LMTs and their processes are shown in different colors; the mossy fiber is in green. Note dramatic structural plasticity predominantly at one individual LMT (yellow)
Type-selective load, subtype-selective ER stress

Fig. 3. Schematic representation of selective vulnerability processes in ALS model mice. Left-to-right: advancing age and disease (shown by darkening background). Disease-causing mutant protein is expressed ubiquitously but α-MNs are affected selectively (type-selective load). Among α-MNs, VUL MNs exhibit progressing ER stress from birth, whereas ER stress in RES MNs only becomes detectable when VUL MNs undergo a UPR and microglia are activated. Growing ER stress leads to axon degeneration and paralysis; SAL Salubrinal by PNNs. Context-dependent renewal of conditioned fear responses is believed to be an important factor contributing to the relapse of pathological fear in patients undergoing therapy for anxiety disorders. Thus, our findings may point to novel strategies for preventing the development of extinction-resistant pathological fear and anxiety.

CELLULAR MECHANISMS UNDERLYING THE DISEASE PROCESS IN MOTONEURON DISEASE

F. Roselli, S. Saxena, P. Wu, L. Xu

Just as experience-dependent rearrangements in neuronal circuitry may mediate learning and the acquisition of new skills, losses in circuitry may play a central role in neurodegenerative and psychiatric diseases. Our studies are based on observations that selective losses of axons and synapses represent key early processes in diseases affecting the nervous system and that disease progression involves increasing failure by neural systems to cope with disease through plasticity and repair.

In neurodegenerative diseases, years of slowly progressing and clinically undetectable alterations and losses set the stage for devastating clinical phases, when treatments have produced disappointing results. Synapses are lost early in disease, but the mechanisms underlying this vulnerability are poorly understood. Because of the experimental accessibility of synapses to individual muscles, motoneuron (MN) disease models provide uniquely advantageous systems to investigate pathways of vulnerability and early disease progression in neurodegeneration. Such information is essential for early detection and the development of more effective treatments against these diseases.

Transgenic mice expressing human SOD1 point mutant proteins associated with Familial Amyotrophic Lateral Sclerosis (FALS) develop paralytic MN disease closely resembling human ALS. The mice provide particularly valuable models of FALS due to their remarkably predictable patterns of disease progression. We established that many peripheral synapses between MNs and muscles are lost in high-expressing SOD1(G93A) mice from P50 on, long before any clinical sign of disease; a detailed study revealed reproducible differences in the timing of denervation of individual muscles. We then identified axons and synapses of first fast fatiguable (FF) and then fast fatigue-resistant (FR) MNs as being selectively vulnerable at well-defined times early in disease and showed that, where present, slow (S) MN axons resist and compensate through sprouting and reinervation.

We recently analyzed motoneurons selectively vulnerable (VUL) and resistant (RES) to motoneuron disease in vivo. This study provided the first longitudinal account of how identified neurons are affected by disease-related mutant proteins and age in a genetic model of a major human neurodegenerative disease. We found that VUL motoneurons were selectively prone to ER stress and ER stress markers were gradually upregulated from birth onwards. Ubiquitin signals increased in VUL and RES motoneurons 25-30 days before the earliest denervations, but an Unfolded Protein Response (UPR) coupled to microglia activation initiated selectively in VUL motoneurons. This transition was followed by selective axonal degeneration and spreading stress. An ER stress-coping agent attenuated all disease manifestations and delayed progression, whereas chronically enhancing ER stress anticipated disease. Therefore, motoneurons are preferentially affected in ALS, but ER stress responses in motoneuron subtypes influence the progressive manifestations of weakening and paralysis (Figure 3). Stress-coping agents also provided protection when applied after the onset of a UPR in VUL MNs and the activation of microglia and up to an advanced disease state, suggesting that they might also be effective during the clinical phase of MN disease in patients.

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EphA4 signaling in juveniles establishes topographic specificity of structural plasticity in the hippocampus. Neuron 65:627-642

Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivity in the adult hippocampus. Neuron 62:510-525

A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. Nat Neurosci 12:627-636

Perineuronal nets protect fear memories from erasure. Science 325:1258-1261

INTRODUCTION

Explaining how higher brain functions emerge from interactions between large numbers of neurons is one of the central challenges in neuroscience. Neuronal circuits in the vertebrate brain consist of thousands or millions of neurons, each a complex signal processor continuously exchanging information with other neurons. Rigorous quantitative insights into the structure and function of such circuits are essential to understand the fundamentals of brain function and its dysfunction in disease. Neuronal circuit research encompasses multiple experimental approaches and scientific disciplines from molecular biology to mathematics and is greatly facilitated by small model systems that allow for the genetic identification and manipulation of defined cell types. In our laboratory, we use a combination of electrophysiological, optical, genetic and other methods to analyze neuronal circuit function in the forebrain of a small vertebrate, the zebrafish.

Our research focuses on the processing of olfactory information in the olfactory bulb and a target area, the posterior zone of the dorsal telencephalon (Dp), that is homologous to olfactory cortex. Our approaches include exhaustive measurements of activity patterns across large numbers of neurons using multiphoton optical methods (Figure 1). Our results have provided insights into the representation and processing of odor-encoding activity patterns and have revealed generic relationships between the architecture of neuronal circuits and their computational properties. Recent results showed that neuronal circuits in Dp integrate information across output channels of the olfactory bulb and create synthetic representations of odor objects. Since Dp is homologous to paleocortical brain areas in mammals, it is a promising model to study basic cortical computations and their neuronal implementations in a small animal model. Current studies exploit optogenetic methods to establish causal relationships between circuit function and behavioral outputs. Moreover, we use optogenetics and 3-D electron microscopy for exhaustive analyses of neuronal connectivity patterns.
PROCESSING OF ACTIVITY PATTERNS IN THE OLFACTORY BULB

J. Niessing, S. Bundschuh, P. Zhu

Odor information is first represented in the brain by stimulus-specific patterns of activation across the input channels of the olfactory bulb, the glomeruli, each of which receives convergent input from sensory neurons expressing the same odorant receptor. Within the olfactory bulb, these input patterns are transformed by a recurrent and predominantly inhibitory network into spatio-temporal patterns of output activity that are transmitted to multiple higher brain areas by the output neurons, the mitral cells. Previous work from our laboratory revealed that this transformation of activity patterns results in multiple computations, including a reduction in the overlap of initially similar activity patterns (“pattern decorrelation”) and the regulation of the population firing rate (“volume control”). Using pharmacological and electrophysiological approaches, we characterized divergent multisynaptic feedback loops involved in volume control and pattern decorrelation.

To further dissect the function of olfactory bulb circuits, we developed a genetic approach based on the Tet system that directs strong gene expression to sparse and defined neuronal subpopulations. Transgenic zebrafish lines generated by this method are currently used to dissect the role of neuronal subtypes for computations using optogenetic and other approaches.

Sensory representations should be sensitive to small changes in a stimulus but at the same time robust against noisy fluctuations, implying that the brain should discriminate between some stimuli but generalize over others. One way to resolve this conflict would be to classify sensory inputs into discrete distinct output patterns with a defined stability range. Such a discretization of neuronal coding space could also subserve higher brain functions such as perceptual switching or decision making and may be achieved by abrupt switching between discrete activity states of neuronal circuits. To test this hypothesis, we gradually varied the concentration or molecular identity of odors (Figure 2A) and found that mitral cell response patterns were relatively insensitive to changes in odor concentration. In contrast, morphing of one odor into another resulted in abrupt transitions between activity patterns (Figure 2B).

These transitions were mediated by coordinated response changes among small mitral cell ensembles, rather than by shifts in the global network state. The olfactory bulb, therefore, classifies odor-evoked input patterns into many discrete outputs, as proposed by attractor models.

BEYOND THE OLFACTORY BULB


Cortical target areas of the olfactory bulb, such as piriform cortex in mammals, are thought to perform basic cortical computations including memory storage and feature integration, but their function is still poorly understood in mechanistic terms. We exhaustively mapped odor responses in the posterior zone of the Dp, the zebrafish homolog of olfactory cortex, and showed that the coarse chemotopic organization of activity patterns in the olfactory bulb is not maintained in Dp. Using opto- and electrophysiological approaches, we found that Dp neurons exhibit broad sub-threshold tuning while supra-threshold responses are quite sparse. Unlike odor responses in the olfactory bulb, responses of Dp neurons to binary odor mixtures could not be predicted from the component responses. Furthermore, we found that action potential firing of Dp neurons is tightly controlled by the balance of excitatory and inhibitory synaptic inputs during an odor response. Together, these results show that neuronal circuits in Dp associate inputs from segregated processing channels in the olfactory bulb and, thereby, create synthetic representations of higher-order olfactory objects. Using optical, physiological and anatomical methods, we are currently analyzing functional connectivity within the Dp and between Dp and other brain areas. The goal of these approaches is to characterize basic computations performed by paleocortical circuits and to analyze the underlying mechanisms at the cellular and circuit level.
One of the main challenges in neuronal circuit research is the identification of cell types and the exhaustive analysis of connectivity patterns between large numbers of neurons. In order to identify and manipulate neurons within a circuit, we explored the Tet system to express fluorescent markers, channelrhodopsin-2 (Chr2) and other proteins in neurons of the olfactory bulb and other brain areas. The Tet system is comprised of a transcriptional activator that is usually expressed under the control of a tissue-specific promoter and of a responder element containing the transgene of interest. Using this two-component system in zebrafish, we found that the expression of the transgene is often restricted to subsets of the cells targeted by the tissue-specific promoter that vary between transgenic lines but are stable over generations. The Tet system is, therefore, an excellent tool to express different transgenes in sharply defined subsets of neurons. Moreover, we found that expression levels were very high, which allows us to reliably evoke action potential firing with high spatial and temporal precision by multiphoton activation of Chr2 (Figure 3A). The combination of Tet system transgenics, multiphoton optogenetics and electrophysiology, therefore, enables the genetic identification of neuron types and the high-resolution mapping of functional synaptic connectivity in the intact circuit (Figure 3B).

Using this approach, we are currently characterizing connectivity between neurons in the olfactory bulb and the Dp. Furthermore, we have used a combination of intracellular electrophysiological stimulation of genetically identified neurons and 2-photon calcium imaging ("forward optical probing") to characterize connectivity patterns between mitral cells and interneurons in the olfactory bulb. We found connectivity to be widespread but not uniform. Together with modeling studies, our results indicate that neuronal computations in the olfactory bulb are unlikely to be governed by short-range lateral inhibitory interactions similar to edge-enhancing circuits in the retina.

Currently, the only method for the complete reconstruction of connectivity matrices is 3-D electron microscopy (3D-EM) as it can provide nanometer resolution throughout large volumes. We, therefore, established serial block face scanning electron microscopy and started to acquire 3D-EM data sets from the olfactory bulb and other brain structures. In collaboration with computer scientists, we are beginning to reconstruct neuronal morphologies and synaptic connections using manual and semi-automated methods. Owing to the enormous size and complexity of 3D-EM data sets, this task can be overwhelming when circuits are large. The small size of neuronal circuits in zebrafish larvae, however, reduces this problem substantially and should allow for the semi-automated circuit reconstruction of complete circuits. We expect that this approach will yield novel and fundamental insights into the synaptic architecture of olfactory circuits that will be important for understanding their function.

MATHEMATICAL ANALYSIS OF STRUCTURE-FUNCTION RELATIONSHIPS

M. T. Wiechert

In order to explore systematic relationships between the structure and functional properties of neuronal circuits, we constructed simplified network models of the olfactory bulb that reproduce computations performed by the biological circuits. Crucial for this approach was a diverse set of neurophysiological data at the single-neuron and population level. We then developed a theoretical framework and mathematically analyzed how computations depend on network variables. This work showed that pattern decorrelation is a generic property of neuronal circuits and is enhanced by recurrent connectivity. The architecture of the olfactory bulb strongly favors pattern decorrelation.

Fig. 2. Responses of mitral cells to gradually varying odors.
A 2-photon imaging of calcium signals in mitral cells evoked by different concentrations of an odor (Lys). Left: Genetic mitral cell marker.
B Dynamics of mitral cell activity patterns evoke by a morphing series of two similar odors (Phe and Trp; blue to green), projected into 3D principal component space. Activity patterns evolve towards steady-states (large circles) that switch abruptly when the odor is morphed.

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One of the most direct strategies to determine causal relationships between neuronal properties, circuit computations, and higher brain functions is to manipulate neuronal activity and quantify the effects on population activity patterns and behavioral outputs. High cell-type specificity and precision of neuronal manipulations can be achieved by (opto)genetic approaches. To exploit the full potential of optogenetics, we used multiphoton activation of Chr2 (Figure 3A, B) and constructed a device based on digital micromirror array technology to project arbitrary spatio-temporal patterns of light into the brain. This device is currently used to manipulate activity patterns in time and space in order to explore the effects on the processing of neuronal activity patterns. Moreover, we screened responses of transgenic zebrafish larvae expressing Chr2 in different sets of neurons to blue-light stimulation and identified lines that respond with distinct motor behaviors (Figure 3C).

Because this approach yields information on defined neurons involved in defined behaviors, it provides an excellent starting point for a mechanistic analysis of the underlying circuits. In order to dissect neuronal circuits involved in more complex behaviors, we are currently establishing odor-guided behavioral tasks in order to examine how the manipulation of neuronal activity patterns interferes with these behaviors. This combination of behavioral assays, circuit physiology and optogenetic manipulations is expected to reveal coding strategies and neuronal computations involved in higher brain functions.

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**Selected publications**


INTRODUCTION

Learning is mediated by long-term functional modifications in brain circuits. We are taking a multidisciplinary approach to investigate the neuronal substrates underlying Pavlovian fear conditioning, a simple form of associative learning that can be studied easily in rodents. Using a combination of cellular and systems electrophysiological and imaging techniques, together with molecular and behavioral manipulations, we aim to understand how function and plasticity of neuronal circuits relates to learning at the behavioral level.

The inability to control or inhibit inappropriate fear responses is a hallmark of human anxiety disorders. We are investigating the cellular mechanisms underlying fear extinction, an associative learning process mediating inhibitory control of inappropriate fear behavior. This aspect of our research not only addresses fundamental mechanisms underlying memory processes in the brain but is also clinically highly relevant, because relapse of pathological fear and anxiety is a major problem in patients suffering from post-traumatic stress disorder and other anxiety disorders.
**PATHWAY-SPECIFIC MECHANISMS OF SYNAPTIC PLASTICITY IN THE LATERAL AMYGDALA**

_E. Fourcaudot, I. Ehrlich, G. Casassus, P. Gastrein_

Auditory fear conditioning requires the induction of NMDA receptor-dependent long-term potentiation (LTP) in the lateral nucleus of the amygdala (LA). Sensory information reaches the LA by two converging glutamatergic pathways originating in the sensory thalamus and cortex. We have shown previously that thalamic and cortical afferent synapses exhibit pathway-specific LTP mediated by distinct mechanisms (Figure 1). The induction of LTP at thalamo-LA synapses is mediated by postsynaptic mechanisms requiring Ca\(^{2+}\) influx through postsynaptic NMDA receptors and R-type voltage-dependent Ca\(^{2+}\) channels. LTP expression involves the recruitment of new AMPA receptors to the postsynaptic membrane. In contrast, cortico-LA LTP is mediated by presynaptic mechanisms. Co-activation of thalamo- and cortico-LA pathways results in NMDA receptor-dependent LTP that is induced and expressed entirely via presynaptic mechanisms. Whereas postsynaptic thalamo-LA LTP is necessary for the acquisition of conditioned fear, studies of mice deficient for selective AMPA and GABA\(_B\) receptor subunits indicate that presynaptic cortico-LA LTP regulates fear generalization, a hallmark of human anxiety disorders.

We have further addressed the molecular mechanisms underlying presynaptic LTP at cortico-LA synapses and have found that signaling via the cAMP/PKA pathway is necessary and sufficient for LTP induction. Downstream of the cAMP/PKA pathway, LTP depends on the active zone protein RIM1\(\alpha\) (Fourcaudot et al. 2008) and on a persistent increase in L-type voltage-dependent Ca\(^{2+}\) channel-mediated glutamate release (Fourcaudot et al. 2009). Our findings implicate functional interactions between RIM1 and presynaptic L-VDCCs in activity-dependent plasticity of release probability and reveal a novel mechanism underlying the expression of presynaptic LTP.

**SWITCHING ON AND OFF CONDITIONED FEAR BY DISTINCT NEURONAL CIRCUITS IN THE BASAL AMYGDALA**

_C. Herry, C. Ciocchi, L. Demmou, V. Senn, C. Müller_

Once encoded, fear memories are resistant to decay. Nonetheless, they can be inhibited in a context-dependent manner after fear extinction. Extinction of conditioned fear can be obtained if the conditioned stimulus is repeatedly presented alone in the absence of any aversive stimulus. While tremendous progress has been made in identifying the mechanisms underlying fear learning, much less is known about the neuronal substrates mediating fear extinction. The amygdala is a potential site of extinction-associated plasticity since we and others have recently shown that intramygdala blockade of NMDA receptors or the MAPK signaling pathway prevents extinction. We used a combination of behavioral, pharmacological and in vivo electrophysiological approaches to study the role of distinct amygdala sub-nuclei in the acquisition and extinction of conditioned fear (Herry et al. 2008).

Single unit recordings in behaving mice revealed that the basal nucleus of the amygdala (BA) contains distinct types of neurons that are specifically activated upon fear conditioning or extinction, respectively. During acquisition of extinction, the activity of “fear neurons” gradually declines whilst “extinction neurons” increase their activity (Figure 2). Conversely, when extinguished fear responses are recovered by placing the animal in an unsafe environment, “extinction neurons” switch off whilst “fear neurons” switch on. Using local micro-iontophoretic injection of the GABA\(_A\) receptor agonist muscimol, we found that inactivation of the BA completely prevents the acquisition of extinction or context-dependent fear recovery, depending on the injection time point. Finally, we could show that “fear neurons” and “extinction neurons” are differentially connected with the medial prefrontal cortex (mPFC) and the ventral hippocampus (vHC), two brain areas involved in context-dependent extinction. In contrast to previous models suggesting that amygdala neurons are active during states of high fear and inactive during states of low fear, our findings indicate that activity in specific neuronal circuits within the amygdala cause opposite behavioral

![Image](image.png)

_Fig. 1. Projection neurons in the LA (grey) receive converging thalamic and cortical sensory afferents. LTP at thalamic and cortical afferents is tightly controlled by GABA released from feedforward interneurons (green). Interneurons are targets of neuromodulators that modify their output activity, which gates the induction of glutamatergic LTP by transiently altering the level of pre- and postsynaptic inhibitory drive._

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outcomes, thus providing a new framework for understanding context-dependent expression and extinction of fear behavior.

EXTINCTION INDUCES CELL TYPE-SPECIFIC NEURONAL PLASTICITY IN AMYGDALA-PREFRONTAL CIRCUITS

V. Senn, C. Herrn, I. Ehrlich, D. Trojer, C. Müller, J. Letzkus

To gain experimental access to the anatomical, physiological and molecular properties of functionally distinct subtypes of amygdala neurons, we are using a combination of in vivo retrograde tracing techniques and expression analysis of the immediate early gene product Fos in animals subjected to fear conditioning or extinction. We performed single and double injections of fluorescent latex beads into mPFC and hippocampus using beads of different colors. Fos staining of bead-labeled neurons revealed that whereas fear conditioning induced Fos in both mPFC and hippocampus-projecting BLA neurons, extinction selectively induced Fos in mPFC-projecting neurons. Given that the prelimbic (PL) and infralimbic (IL) subdivisions of the mPFC are thought to differentially contribute to fear conditioning and extinction, targeted tracer injections into the PL or into the IL were made (Figure 3). We found that BA neurons projecting to the IL exhibited an over-proportional and statistically significant increase in Fos expression after extinction, whereas PL-projecting neurons showed increased levels of Fos expression after fear conditioning, but not after extinction. This indicates that BA neurons projecting to the IL may be specifically involved in extinction learning, whereas PL-projecting cells may rather contribute to fear learning.

We are currently examining fear conditioning- and extinction-induced physiological changes in anatomically defined neuronal subpopulations. Our present data indicate that extinction differentially affects intrinsic properties of PL- and IL-projecting BA neurons. These findings indicate that a change in the balance of activity between two anatomically defined subpopulations of amygdala projection neurons may be involved in the extinction of conditioned fear.

ENCODING FEAR CONDITIONING IN CENTRAL AMYGDALA INHIBITORY CIRCUITS

C. Herrn, S. Ciocchi, F. Grenier, S.B.E. Wolff, I. Ehrlich, C. Müller, J. Letzkus

The central amygdala (CEA), a nucleus predominantly composed of GABAergic inhibitory neurons, is essential for fear conditioning. How the acquisition and expression of conditioned fear is encoded within CEA inhibitory circuits is not understood (Ehrlich et al. 2009). Using in vivo electrophysiological, optogenetic and pharmacological approaches, we found that neuronal activity in the lateral subdivision (CEl) is required for fear acquisition and that expression of conditioned fear responses is gated by stimulus-evoked disinhibition of output neurons in the medial subdivision (CEm) by a discrete subpopulation of spontaneously active CEl neurons. Moreover, plasticity of tonic inhibition in the CEl to CEm pathway regulates generalization of conditioned fear responses. These results identify CEA inhibitory circuits as a major site of plasticity in fear conditioning and indicate that concerted plasticity of phasic and tonic activity in spontaneously active GABAergic circuits may be an important mechanism for regulating generalization of behavioral output in associative learning.

Fig. 2. A Fear-neurons exhibit selective CS+ responses after fear conditioning that are reversed upon extinction. CS+ evoked firing of extinction neurons selectively increases after extinction. B Mean time courses of freezing behavior (grey bars) and neuronal activity (z-scores) of BA fear-neurons (red circles) and extinction neurons (blue circles) during extinction training.

FAITHFUL EXPRESSION OF MULTIPLE PROTEINS VIA 2A-PEPTIDE SELF-PROCESSING:
A METHOD FOR MANIPULATING BRAIN CIRCUITS

I. Ehrlich, S.B.E. Wolff

Establishing causal relationships between the activity of defined amygdala circuits and fear conditioning/extinction in behaving animals requires manipulating their activity in vivo. In collaboration with Rolf Sprengel (MPI, Heidelberg), we have developed a novel strategy to achieve reliable and efficient co-expression of light-responsive ion channels allowing for rapid activation (Channelrhodopsin-2) and silencing (Halorhodopsin/NpHR) in single neurons (Tang et al. 2009). To achieve this, we used viral 2A peptide bridges with auto-proteolytic self-cleaving activity allowing for quantitative co-expression of ChR2 and NpHR from a single neuron-specific promoter. For in vivo expression, we used recombinant adeno-associated virus (rAAV). This method will allow reliable control of the activity of defined neurons in a bi-directional manner in vivo.
**IMPAIRED FEAR EXTINCTION IN MICE LACKING PROTEASE NEXIN-1**

*M. Meins, C. Herry, S. Ciocchi and C. Müller, in collaboration with D. Monard*

The serine protease inhibitor protease-nexin-1 (PN-1) has been shown to modulate N-methyl-D-aspartate receptor (NMDAR)-mediated synaptic currents and NMDAR-dependent long-term potentiation of synaptic transmission. In collaboration with the group of Denis Monard, we analyzed the role of PN-1 in the acquisition and extinction of classical auditory fear conditioning, two distinct forms of learning that both depend on NMDAR activity in the amygdala (Meins et al. 2010).

Immunostaining revealed that PN-1 is expressed throughout the amygdala, primarily in gamma-aminobutyric acid-containing neurons of the central amygdala and intercalated cell masses and in glia. Fear extinction was severely impaired in mice lacking PN-1 (PN-1 KO). Consistent with a role for the basal nucleus of the amygdala in fear extinction, we found that PN-1-deficient mice exhibited decreased numbers of Fos-positive neurons in the basal nucleus after extinction compared with wild-type littermate controls. Moreover, immunoblot analysis of laser-microdissected amygdala subnuclei revealed specific extinction-induced increases in the level of phosphorylated alpha-calcium/calmodulin protein kinase II in the medial intercalated cell masses and in the lateral subdivision of the central amygdala in wild-type mice. These responses were altered in PN-1 KO mice.

Together, these data indicate that lack of extinction in PN-1 KO mice is associated with distinct changes in neuronal activity across the circuitry of the basal and central nuclei and the intercalated cell masses, supporting a differential impact on fear extinction of these amygdala substructures. They also suggest a new role for serine protease inhibitors such as PN-1 in modulating fear conditioning and extinction.

**PERINEURONAL NETS PROTECT FEAR MEMORIES FROM ERASURE**

C. Herry, in collaboration with N. Gogolla and P. Caroni

In adult animals, fear conditioning induces a permanent memory that is resilient to erasure by extinction. In contrast, during early postnatal development, extinction of conditioned fear leads to memory erasure, suggesting that fear memories are actively protected from being erased in adults (Herry et al. 2010). In collaboration with the group of Pico Caroni, we found that this protection is conferred by extracellular matrix chondroitin sulphate proteoglycans (CSPGs) in the amygdala (Gogolla et al. 2009). The organization of CSPGs into perineuronal nets (PNNs) coincided with the developmental switch in fear memory resilience. In adults, degradation of PNNs by chondroitinase-ABC specifically rendered subsequently acquired fear memories susceptible to erasure. This indicates that intact PNNs mediate the formation of erasure-resistant fear memories and identifies a molecular mechanism closing a postnatal critical period during which traumatic memories can be erased by extinction. These findings may point to novel strategies in preventing the development of extinction-resistant pathological fear and anxiety.

This work was carried out in collaboration with D. Anderson (Caltech), P. Caroni (FMI), F. Ferraguti (Medical University of Innsbruck), Y. Humeau (CNRS, Strasbourg), D. Monard (FMI), N. Singewald (Medical University of Innsbruck) and R. Sprengel (Max Planck Institute for Medical Research, Heidelberg).

**Selected publications**


INTRODUCTION

Neurons in the brain communicate through chemical synapses. These synapses are not passive elements like soldering joints on a circuit board, but actively change their strength depending on the activity of pre- and postsynaptic cells. Therefore, synapses not only transmit but can also filter and store information. The adjustment of synaptic strength and the induction of long-term plasticity depend on the activation of complex biochemical signaling networks in the pre- and the postsynaptic terminals. As we have shown recently, the machinery necessary for certain forms of plasticity is not present in all synapses but restricted to specific subsets. As a consequence, identical patterns of activity are interpreted in different ways by individual synapses.

To study local biochemical processes at individual synapses is a technological challenge. We use two-photon laser scanning microscopy to record postsynaptic responses of individual synapses in intact brain tissue. With this optical approach, the activity of single synapses can be monitored over several hundred stimulations, providing information about the probability of transmitter release and about the number of glutamate receptors that become activated on individual dendritic spines. Combining functional imaging with optogenetic stimulation, we can induce plasticity at individual synapses and investigate the underlying electrical and biochemical processes.

Finally, we perform computer simulations of synaptic transmission and electrical signaling. This approach allows us to extract information about important parameters we cannot measure directly, e.g. the depolarization of the spine head during synaptic transmission. The models make certain predictions about the influence of spine morphology on synaptic function that we can subsequently test by pharmacology and correlative electron microscopy (SBFS-EM). This multidisciplinary approach allows us to investigate how information is processed and stored in the brain.
THE SPINE APPARATUS ENABLES SYNAPSES TO UNDERGO mGluR-MEDIATED DEPRESSION

N. Holbro

Organelles in spines could affect the function of local signaling cascades and, thus, differentially regulate the potential for plasticity at individual synapses. We investigated how the presence of endoplasmic reticulum (ER) in dendritic spines, the so-called spine apparatus, affects postsynaptic signaling. The spine apparatus is targeted selectively to large spines containing strong synapses. In ER-containing spines, we frequently observed synaptically triggered calcium release events of very large amplitude. Low-frequency stimulation of these spines induced a permanent depression of synaptic potency that was independent of NMDA receptor activation and specific to the stimulated synapses (Figure 1). In contrast, no functional changes were induced in the majority of spines lacking ER. Both calcium release events and long-term depression depended on the activation of metabotropic glutamate receptors and IP3 receptors. We conclude that the spine apparatus is a reliable indicator of the presence of specific signaling cascades that govern plasticity on a micrometer scale (Holbro et al. 2009). Hyperactivity of this specific signaling cascade has been implicated in Fragile X mental retardation, an example of the devastating consequences of dysregulated synaptic plasticity in humans.

Fig. 1. Endoplasmic reticulum in spines (yellow) enables mGluR-dependent long-term depression of uncaging-evoked excitatory postsynaptic potentials (uEPSC). In spines without ER (red), initial currents were smaller and low-frequency stimulation did not induce plasticity at these synapses.

HOW DO SYNAPSES MEASURE MILLISECONDS?

N. Holbro, A. Grunditz

The connectivity of neuronal circuits in the brain is constantly changing. These changes do not occur randomly, but depend on specific patterns of electrical activity. In 1949, the Canadian psychologist Donald Hebb suggested that for associative learning, connections between nerve cells that fire in rapid succession should be strengthened. This prediction turned out to be entirely correct and, remarkably, the temporal window for Hebbian plasticity is only about 20 ms long. How is coincidence between pre- and postsynaptic activity detected with such high precision? Glutamate receptors of the NMDA-type are highly permeable for calcium, an important trigger signal for synaptic plasticity. Their slow kinetics, however, are at odds with the very brief window for spike-timing dependent plasticity. Combining electrophysiology and two-photon calcium imaging, we could show that the brief but strong depolarization provided by AMPA receptors acts as gate for NMDA receptor-mediated calcium influx (Holbro et al. 2010). Thus, the rapid response of AMPA receptors is the secret behind the millisecond precision of Hebbian plasticity. Furthermore, we could show that this mechanism works best in dendritic spines with high resistance necks. This suggests that the evolution of spines improved the sensitivity of synapses to timing, a prerequisite for rapid associative learning.

SPINE SYNAPSES AS BIOCHEMICAL AND ELECTRICAL COMPARTMENTS

A. Grunditz, C. Vivien, C. Genoud and C. Blumer, in collaboration with T. Vetter (University of Basel) and M. Frotscher (University of Freiburg)

Dendritic spines, which have a volume of only about 0.1 µm³, are able to trap active enzymes and other messenger molecules close to the synapse, making them tiny biochemical reactors (Zhang et al. 2008). The necks of some spines, in addition to their function as diffusional bottlenecks, also have a high electrical resistance and enhance the depolarization of the spine head during synaptic transmission. Spine neck resistances are highly variable, even on a single dendritic branch of a neuron. We could show that diffusion of proteins in and out of spines after dendritic depolarization is slowed down by a factor of 10 or more (Grunditz et al. 2008). This process is triggered by strong calcium influx during depolarization. To assess the impact of spine morphology on synaptic function, we have started to reconstruct entire dendrites after functional characterization of the synapses using serial block face scanning electron microscopy (SBFSEM). This technique allows us to visualize spine structure in 3D at nanometer resolution. Subsequently, we can correlate spine ultrastructure with previously measured functional parameters from the same sample of synapses. Ultimately, every functional change in the nervous system has to result from a modification in structure. How precisely can we predict functional properties of a synapse from its morphology, however, is an open question. We are specifically interested in structures that could be responsible for the difference between plastic and stable synapses, e.g., the shape of the spine neck and the presence or absence of intracellular organelles.
THE OPTOGENETIC INTERFACE: PHYSIOLOGICAL EXPERIMENTS IN UNPERTURBED CELLS

P. Schoenenberger, D. Udvari and S. Wiegert, in collaboration with P. Hegemann (Humboldt University, Berlin)

Modern calcium-sensitive probes make it relatively straightforward to measure functional responses from individual synapses. Stimulating identified synapses in densely packed brain tissue with defined activity patterns, however, remains a big challenge. Recently discovered light-gated ion channels (Channelrhodopsins) can be used to precisely stimulate individual neurons with light. Single cell resolution can be achieved with a focused blue laser that is moved across neuronal tissue (Figure 2). In collaboration with the laboratories of P. Hegemann und G. Nagel, we have characterized and applied designer-channelrhodopsins, in which speed, current amplitude or spectral sensitivity have been altered by rational mutagenesis. Super-sensitive mutants, for example, induced expression of immediate early genes after brief pulses of blue light (Schoenenberger et al. 2009). In addition, we discovered that the photocycle of slow Channelrhodopsins is branched, i.e., a fraction of the activated channels accumulates in a long-lived inactive state. Using these improved Channelrhodopsins, we were able to induce synaptic plasticity at identified contacts and characterize the long-term consequences of these activity patterns days later. The unique combination of high specificity, reliability and non-invasiveness opens the door to complex neuronal stimulation experiments in vitro and in vivo. In combination with two-photon calcium imaging, it is now possible to identify single active synapses in live tissue and to investigate the function of connected pre- and postsynaptic terminals simultaneously (Zhang et al. 2007).

DYNAMIC CONTROL OF VESICLE RELEASE AT HIPPOCAMPAL SYNAPSES

T. Rose

An important aspect of synaptic transmission is the release of neurotransmitter from the presynaptic terminal. Most information about the regulation of this process has been gathered from dissociated neurons growing on glass coverslips. We have developed a new optical strategy and a genetically encoded sensor to examine vesicle release in hippocampal tissue and found a number of interesting differences. Synaptic terminals in dissociated culture use only about half of their vesicles, even when stimulated by more than 1000 action potentials. This observation raised serious questions about the ability of synapses to sustain transmission at high frequencies. In contrast, we could show that synapses in intact tissue use and recycle all of their vesicles. Interestingly, we could induce inactivation of vesicles by prolonged depolarization of hippocampal tissue. Apparently, vesicles were shifted from the actively recycling pool to a dormant “reserve pool” in response to strong and synchronous activity. We suggest that this novel presynaptic mechanism is homeostatic: it keeps neuronal activity within the physiological range by changing the fraction of vesicles available for release.

Selected publications


Fig. 2. Spatial resolution of laser-induced ChR2 currents in a hippocampal neuron. Colors encode photocurrent amplitudes for different positions of the laser spot (red 30 pA, green 17 pA, blue 5 pA)
INTRODUCTION

Neuronal circuits are formed through synaptic connections between defined populations of neurons. While many synapses remain stable over prolonged periods of time, the regulated disassembly of functional synaptic connections is required to ensure precise connectivity during development and to enable plasticity of the mature circuit. In contrast, the inappropriate loss of synaptic connections in response to genetic mutations will lead to a disruption of neuronal circuits and to progressive neurodegenerative disorders. Therefore, identification of the molecular mechanisms controlling synapse stability versus disassembly may help our understanding of neuronal circuit formation and plasticity and may advance our understanding of progressive neurodegenerative disease.

We are using the Drosophila neuromuscular junction (NMJ) as a model system to unravel the molecular mechanisms underlying synapse formation, function and stability. To identify novel genes required for these processes, we perform large-scale genetic screens using a high-resolution imaging assay. The combination of Drosophila genetics, cell biology, electrophysiology and super-resolution imaging techniques enables us to gain detailed insights into the functional and developmental requirements of newly identified genes.

Using these approaches, we recently identified a presynaptic network that regulates synapse formation and stability at the Drosophila NMJ by linking synaptic cell adhesion molecules to the actin and microtubule cytoskeleton. Loss of any molecules of the network results in synapse retraction and finally the degeneration of the NMJ. Importantly, this network is conserved throughout evolution and mutations in mouse or humans result in both learning and memory deficits and neurodegenerative disease. Our emphasis now is on the identification of the extended molecular network and the regulatory mechanisms controlling synapse formation and stability.
PRESYNAPTIC SPECTRIN AND ANKYRIN2
CONTROL SYNAPSE STABILITY
E. Enneking, R. Stephan, E. Moreno

Information processing depends on precise connectivity between appropriate sets of neurons or peripheral targets such as muscles. Many neuronal circuits are refined through the regulated addition or elimination of synaptic connections, both during neural development and during learning and memory. In contrast, the inappropriate loss of synaptic connections will lead to a disruption of neuronal circuits and to progressive neurodegenerative disorders. To understand the mechanisms controlling synaptic plasticity and stability, it is essential that we first identify the molecular framework required for the stabilization of synaptic connections. Proteins within this network might then represent targets of regulatory signaling pathways that alter the dynamics of synapse assembly and disassembly in response to intrinsic and extrinsic stimulation. To identify this molecular network, we developed a high-resolution assay that allows the identification of new genes controlling the integrity and stability of synapses.

We use the Drosophila neuromuscular junction (NMJ) as a model system because it allows analysis of synapse stability at the resolution of individual synapses. We use specific pre- and postsynaptic antibodies to visualize simultaneously the presynaptic nerve terminal, the presynaptic active zone and the postsynaptic glutamate receptors of the muscle. At stable wild-type NMJs there is a precise apposition of pre- and postsynaptic markers but in mutant backgrounds causing synaptic retractions well-organized postsynaptic structures are seen that lack opposing presynaptic antigens, so-called "synaptic footprints" (Figure 1). As induction of postsynaptic structures requires the presence of a presynaptic nerve terminal, the synaptic footprints mark sites from which the presynaptic nerve terminal has retracted, leaving behind an unopposed postsynaptic apparatus that no longer receives motoneuron input. Combination of this light-level analysis with forward and reverse genetics screens allows us to identify novel genes required for the formation and maintenance of synaptic connections. In a second step, we can combine these high-resolution imaging techniques with Drosophila genetics, cell biology, electrophysiological and biochemical approaches to gain detailed insights into the requirements and functions of newly identified genes.

Using these approaches, we recently identified a presynaptic molecular scaffold that is essential for synapse stability and that connects synaptic cell adhesion molecules to the presynaptic microtubule cytoskeleton. The key molecules in this network are the scaffolding proteins α- and β-spectrin and the adaptor molecule ankyrin2 (Ank2L). Absence of either of these molecules results in dramatic alterations in normal synapse development and the elimination of synaptic connections.
Using structured illumination microscopy, a novel super-resolution microscopy technique that allows resolution of details below the diffraction limit of standard confocal microscopy, we were able to demonstrate that Ank2 forms a highly organized lattice in the presynaptic nerve terminal that controls both synapse development and stability (Figure 2). Using biochemical assays, we were able to demonstrate that Ank2L can directly bind to and organize microtubules to stabilize the NMJ. Together with α-/β-spectrin, Ank2L has the potential to provide a direct link from synaptic cell adhesion molecules to the presynaptic microtubule cytoskeleton (Pielage et al. 2005, 2008). Interestingly, a similar spectrin-ankyrin network is present on the postsynaptic side of the Drosophila NMJ. Here, this network is not essential for synapse maintenance or stability but is required for the organization of synapse size, spacing and efficacy (Pielage et al. 2006).

**MOLECULAR MECHANISMS COUNTERACTING LOSS OF SYNAPSE STABILITY**

In a next step, we asked whether mutations in α-spectrin or ank2 represent a cellular stress that triggers an active process of synapse disassembly and retraction at the NMJ, or whether the mutations disrupt cellular mechanisms directly stabilizing the NMJ and, thus, cause a passive structural collapse of the presynaptic terminal. Therefore, we examined means of suppressing synapse retraction in these mutant animals. We were able to define genetic and transgenic conditions capable of conferring enhanced NMJ stability after the loss of presynaptic α-spectrin. First, we demonstrated that NMJ disassembly after loss of α-spectrin can be suppressed by expression of the WldS transgene product, which encodes a protein known to protect severed axons that would otherwise undergo so-called Wallerian degeneration. Thus, mutant NMJ destabilization and degeneration appears not to be a catastrophic collapse of the NMJ but a process that might be mediated through additional signaling processes. Looking for potentially relevant signaling activity, we next demonstrated that an acute cytoskeletal perturbation in Drosophila causes an induction of the immediate early gene fos in motoneurons. Indeed, transgenic overexpression of Fos was sufficient to suppress synapse retraction in α-spectrin mutants. However, the potential stabilizing activity provided by endogenous Fos is only a transient response. Increase in Fos protein induces a negative feedback signaling system via the JNK phosphatase Puckered that ultimately allows synapse retraction and elimination to proceed in the presence of persistent cellular stress. Such a system would favor synapse stability when it is possible to repair cellular damage but favor the elimination of a neuron when damage persists.

It is interesting to note that Fos expression is also upregulated in response to acute brain injury in mammals and it appears in general that the molecular mechanisms underlying synapse maintenance are conserved throughout evolution. Thus, information about synapse stability in Drosophila could provide insight into the molecular processes underlying neural degeneration in vertebrate model systems and humans. For example, mutations affecting spectrin or ankyrin in vertebrates show clear evidence for progressive neurodegeneration in the central and peripheral nervous systems. Furthermore, human genetic studies have shown that mutations in βIII-spectrin can lead to human spinocerebellar ataxia type 5.
**HOMEOSTATIC CONTROL OF NEUROTRANSMITTER RELEASE VIA THE Eph-RECEPTOR, EPHEXIN AND Cdc42**

In addition to the control of morphological synaptic stability, we are interested in understanding how functional stability of neurotransmitter release at synapses is controlled at the molecular level. Homeostatic signaling systems are believed to interface with the mechanisms of neural plasticity, ensuring that neural function remains stable over time. At the *Drosophila* NMJ, homeostatic mechanisms control excitability of the postsynaptic muscles through modulation of presynaptic neurotransmitter release. For example, impaired postsynaptic neurotransmitter receptor function initiates a rapid compensatory increase in presynaptic release that offsets impaired receptor function, thereby restoring muscle excitation. Using a forward genetic screen, we recently identified core components of a presynaptic signaling system that is necessary for the homeostatic modulation of presynaptic release. Mutations in *Drosophila* Ephexin (a Rho-type guanine nucleotide exchange factor) disrupt the homeostatic enhancement of presynaptic release following impairment of postsynaptic glutamate receptor function but do not alter other aspects of NMJ development. Ephexin is sufficient presynaptically for synaptic homeostasis and localizes to regions close to the active zone. During synaptic homeostasis Ephexin functions primarily with the small GTPase Cdc42 in a signaling system that converges upon the presynaptic Cav2.1 calcium channel. Ephexin binds the *Drosophila* Eph receptor and indeed loss of Eph receptor function also impairs synaptic homeostasis. Together, our data support a model in which the presynaptic Eph-receptor relays a retrograde signal via Ephexin and Cdc42 to presynaptic calcium channels to precisely modulate presynaptic release.

**NOVEL GENES CONTROLLING SYNAPSE FORMATION AND STABILITY**

I. Kieweg, E. Enneking, V. Bulat, R. Stephan

The identification of the presynaptic spectrin-Ank2 network represents a first step towards the molecular identification of the regulatory system controlling synapse formation and stability. We are currently performing large-scale genetic screens aimed at the identification of the extended molecular network controlling synapse formation and stability in response to intrinsic or extrinsic signals.

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**Selected publications**

Frank CA*, Pielage J*, Davis GW (2009)
A presynaptic homeostatic signaling system composed of the Eph receptor, Ephexin, Cdc42 and Cav2.1 calcium channels. Neuron 61:556-569 (*co-first authors)

Massaro CM, Pielage J, Davis GW (2009)

A presynaptic giant Ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion. Neuron 58:195-209

Pielage J, Fetter RD, Davis GW (2005)
Presynaptic spectrin is essential for synapse stabilization. Curr Biol 15:918-928

A postsynaptic spectrin scaffold defines active zone size, spacing and efficacy at the *Drosophila* neuromuscular junction. J Cell Biol 175:491-503
INTRODUCTION

Neuronal circuits are characterized by topographic maps of coordinated axonal connections in which the positional coordinates of spatially ordered input neurons are precisely mapped onto spatially ordered sets of target neurons. To understand the molecular mechanisms of topographic map formation, we focus on the development of neural circuits in the vertebrate hindbrain, which has a vital role in the integration of motor, sensory and vegetative functions. In early development, the hindbrain region is transiently segmented along the anteroposterior axis into rhombomeres but little is known about the impact of this early segmental plan on the assembly of neuronal groups and their connectivity. In addition, subdivision of the hindbrain underlies the segmental specification of the cranial neural crest cells (NCCs) contributing to cranial sensory ganglia and the derivatives of the pharyngeal arches, and giving rise to most of the facial and neck skeleton. A high level of integration is maintained between rhombomeres, their derived NCCs, and pharyngeal arches during development. The resulting precise spatial relationships between skeletal, muscle and neuronal elements of similar axial origin show this to be a key mechanism in the construction of the vertebrate head and prompt the question of its molecular control.

Current work addresses the involvement of homeodomain transcription factors of the Hox family in late aspects of circuit formation, e.g., neuronal migration, axon pathfinding, and topographic axon branching. We use genetic tools, imaging of fluorescently labeled neurons and axonal tracts, in utero electroporation, molecular phenotype analysis and gene expression profiling and focus on the development of the mouse whisker-to-barrel somatosensory system as well as the auditory and precerebellar circuitry. This may increase knowledge of the molecular mechanisms establishing precise connectivity between neurons in the developing central nervous system and the etiology of brainstem and craniofacial disorders and their relationship to developmental abnormalities.
MOLECULAR MECHANISMS OF WHISKER MAP FORMATION IN MOUSE TRIGEMINAL BRAINSTEM

C. Laumonnerie, A. Bechara, L. Parra, N. Vilain

In the mouse trigeminal pathway, sensory inputs from the face are topographically mapped onto the somatosensory cortex via relay stations in the thalamus and hindbrain. Somatotopic representations are generated at each level of the neuraxis in which distinct facial structures, such as whiskers or lower jaw and lip, are mapped on different scales. The molecular mechanisms generating such maps are poorly understood. We focus on the wiring of the facial pattern at the level of the brainstem, the first relay station of the trigeminal pathway. We showed previously that the gross topographic organization of the face map in the brainstem is related to the positional (rhombomeric) origin along the rostrocaudal axis of the second order neurons of the principal (PrV) nucleus and to their graded Hoxa2 expression (Oury et al. 2006; Figure 1).

To further understand the mechanisms underlying the proper formation of trigeminal somatosensory face maps, we are currently investigating the respective roles of face- and hindbrain-derived signals in determining the positional identity of first order sensory neurons of the trigeminal ganglion (TG) and their peripheral and central connections. To investigate the role of face signals in trigeminal circuit patterning, we are analyzing the connectivity and molecular pattern of TG neurons in ednrA-, edn1-, and Dlx5/6-deficient mice. These mutants present a homeotic transformation of the lower jaw in an upper jaw-like structure, including a partial duplication of the whisker pad. To characterize the involvement of target-derived signals in defining central whisker map formation, we are currently studying the effects on trigeminal afferent connectivity of Hox gene loss- and gain-of-function selectively at the brainstem level.

Hox Gene Function in Auditory Circuit Development

Y. Narita, K. Karmakar

Just as in other sensory systems, topographic connectivity can also be observed in the auditory system. In the auditory circuit, sound information from the inner ear is transmitted to the cochlear nucleus (CN) in the brainstem via spiral ganglion (SG) neurons. The connectivity between these components is organized topographically in a frequency-specific manner, such that a topography of frequency-selective sound responses is generated along the apico-basal axis of the cochlea, which is depicted and preserved in the cochlear neurons in the form of a frequency-specific map, the «tonotopic» map. The information from the cochlear nucleus is transmitted to higher relaying centers in the brain through several parallel pathways, representing several independent ways of computation of the sound (e.g., sound intensity, sound localization). However, very little is known about how the development of such pathways to relaying centers is controlled or how the development of tonotopic connectivity is organized.

We found that some Hox genes, including Hoxa2, are expressed in the CN throughout its development until postnatal stages. Furthermore, we found that Hoxa2 is also expressed in SG cells at the stage when connectivity between inner ear and CN is established. Thus, Hox genes might play important roles in auditory circuit development. As an entry point to investigate the molecular and cellular mechanisms of auditory circuit development in terms of tonotopic organization or projection to higher relaying centers, we are in the process of analyzing conditional Hox mutant mouse employing several methods, including neuronal labeling and gene profiling.

Hox-Dependent Control of Pontine Neuron Migration by Slit-Robo Signaling

M. Geisen, T. Di Meglio and S. Ducret, in collaboration with A. Chedotal (Institut de la Vision, Paris)

In the developing central nervous system, neurons migrate sometimes over long distances from their birthplace to their final location, where they condense in specific nuclei. The precise final positioning of migrating neurons is critical to the building of ordered connectivity with their target partners. Little is known about how exposure of migrating neurons to simultaneous attractive and repulsive guidance cues may be integrated at the transcript-
To begin addressing this issue, we recently focused on the molecular mechanisms regulating the directionality of long-distance migration of pontine neurons in the mouse brainstem (Figure 2). Such neurons belong to the so-called precerebellar system, which is essential for coordinated motor activity, and provide the principal input to the cerebellum. By using rhombomere-specific transgenic lines, we first mapped with a high degree of precision the migratory route of pontine neurons through distinct rhombomere territories. We next provided evidence for the implication of Hoxa2 and Hoxb2 in the control of pontine neuron migration along the rostrocaudal axis. We identified the guidance receptor Robo2 as a direct target gene of the Hoxa2 gene. We further showed that repulsive signaling mediated through the Robo2 receptor expressed in migrating neurons and its ligand Slit2 secreted from the facial motor nucleus are key components of the molecular guidance system maintaining rostrocaudal migration and preventing premature attraction towards the brainstem ventral midline. Our data provide a conceptual framework to understand how transcriptional regulation of the response to environmental guidance cues controls stereotyped neuronal migratory behavior in the developing mammalian brain.

**A DEFAULT PATTERNING PROGRAM UNDERLIES ASSEMBLY OF FACIAL AND THROAT STRUCTURES**

M. Minoux, in collaboration with G. Antonarakis and D. Duboule (University of Geneva)

Although morphologically distinct, face and throat structures such as jaw, hyoid, and thyroid cartilages develop from a rostrocaudal metameric series of pharyngeal arches, colonized by the cranial neural crest cells (NCCs). Colinear Hox gene expression patterns underlie arch-specific morphologies, except in the first (mandibular) arch, which is devoid of Hox gene activity. We have shown previously that the mandibular and second (hyoid) arches share a common Hox-free ground (default) patterning program. However, it was not known whether posterior pharyngeal arch neural crest derivatives giving rise to throat structures are also patterned according to a similar ground plan.

We found that the simultaneous inactivation of all Hoxa genes in NCCs resulted in multiple jaw and first arch like structures, partially replacing second, third, and fourth arch derivatives. Such a phenotype was not enhanced by further deleting the Hoxd complex. Thus, a Hox-free ground patterning program is shared by rostral and caudal arches and corresponds to that of the rostral most element of the series, the mandibular arch. Moreover, we found that Hoxa2 and Hoxa3 act synergistically to pattern third and fourth arch derivatives.

These results strengthen the notion that a segmental pattern underlies the development and morphogenesis of the pharyngeal region of the vertebrate head. They also provide novel insights into the molecular mechanisms that control how facial and throat structures are assembled and integrated together during mammalian head development.

**Selected publications**

- **Chedotal A, Rijli FM (2009)**
  Transcriptional control of tangential neuronal migration in the developing forebrain. Curr Opinion Neurobiol 19:139-145

- **Erzurumlu RS, Murakami Y, Rijli FM (2010)**
  Mapping the face in the somatosensory brainstem. Nat Rev Neurosci 11:252-263

  Hox paralogue group 2 genes control the migration of mouse pontine neurons through slit-robo signaling. PLoS Biol 6:e142

- **Minoux M, Antonarakis G, Knita M, Duboule D, Rijli FM (2009)**
  Rostral and caudal pharyngeal arches share a common neural crest ground pattern. Development 136:637-645

  Hoxa2- and rhombomere-dependent development of the mouse facial somatosensory map. Science 313:1408-1413
INTRODUCTION
The function of the brain can be studied at many different hierarchical levels. We are interested in how neurons interact in local neuronal networks to compute behaviorally relevant functions. We use the mammalian retina as a model system because the input, a dynamically changing light pattern, is well defined and can be easily manipulated experimentally. Moreover, the activity of each neuron can be recorded during retinal light stimulation. Our experimental approach is interdisciplinary: we combine physiological, molecular, viral and computational approaches to reveal the structure and function of retinal circuits. We use molecular techniques to genetically identify cell types in the network and label them using transgenic technologies. The connections of labeled cells are revealed using trans-synaptic viruses. Next, we study the function of a genetically isolated circuit with physiological and two-photon laser imaging tools. Finally, we use computational methods to predict the behavior of an isolated circuit in natural conditions. Basic understanding of retinal circuits could lead to rational therapies in certain forms of blindness. Using circuit-specific targeting of a light-activated channel, channelrhodopsin-2, or the light-activated pump halorhodopsin, we recently restored some visual function in vivo in mouse models of retinal degeneration and in vitro in human retinal explant cultures.
RESTORING COMPLEX VISUAL RESPONSES IN RETINITIS PIGMENTOSA BY GENETIC REACTIVATION OF CONE PHOTORECEPTORS WITH HALORHODOPSIN


Retinitis pigmentosa refers to a diverse group of hereditary diseases affecting two million people worldwide that lead to incurable blindness. As a common pathology, rod photoreceptors die early whereas light-insensitive, morphologically altered cone photoreceptors persist longer. It was not known whether these cones are accessible for therapeutic intervention. We showed that expression of archaeabacterial halorhodopsin in light-insensitive cones can substitute for the native phototransduction cascade and restore their light sensitivity in mouse models of Retinitis pigmentosa. Resensitized photoreceptors activate all retinal cone pathways, drive sophisticated retinal circuit functions, including directional selectivity, activate cortical circuits and mediate visually guided behaviors. Using human ex vivo retinas, we showed that halorhodopsin can reanimate light-insensitive human photoreceptors. Finally, we identified blind patients with persisting, light-insensitive cones for potential halorhodopsin-based therapy.

APPROACH SENSITIVITY IN THE RETINA PROCESSED BY A MULTIFUNCTIONAL NEURAL CIRCUIT

T.A. Münch, S. Siegert and T.J. Viney, in collaboration with R.A. da Silveira and G.B. Awatramani

The detection of approaching objects, such as looming predators, is necessary for survival. We combined genetic labeling of cell types, two-photon microscopy, electrophysiology and theoretical modeling to address the question of which neurons and circuits mediate this function. We identified an approach-sensitive ganglion cell type in the mouse retina, resolved elements of its afferent neural circuit and described how these confer approach sensitivity on the ganglion cell. The circuit’s essential building block is a rapid inhibitory pathway that selectively suppresses responses to non-approaching objects. This rapid inhibitory pathway, which includes AII amacrine cells connected to bipolar cells through electrical synapses, was previously described in the context of nighttime vision. In the daytime conditions of our experiments, the same pathway conveys signals in the reverse direction. The dual use of a neural pathway in different physiological conditions illustrates the efficiency with which several functions can be accommodated in a single circuit.

GENETICALLY TIMED, ACTIVITY SENSOR AND RAINBOW TRANSSYNAPTIC VIRAL TOOLS FOR THE ANALYSIS OF NEURAL CIRCUITS


The mammalian brain is composed of billions of neurons organized into functional neural pathways that perform distinct computational tasks. However, as the elements of functionally distinct circuits are spatially intermingled, novel techniques are necessary to monitor activity from only those neurons that belong to the same circuit. We have
developed retrograde, transsynaptic PRVs with genetically encoded activity sensors (Activity Sensor PRVs) that both mark connected elements and optically report the activity of multiple neurons belonging to the same functional circuit. Other PRVs were equipped with a dichromatic genetic system expressing two differentially colored fluorescent proteins in a time-shifted manner (Timer PRVs), which allows definition of a time window early after infection to investigate neural activity. The activity sensor and timer properties were also combined into one PRV that can be used as a versatile tool in neuronal circuit tracing. Finally, we have engineered multiple-colored PRVs (Rainbow PRVs) for differentiating and dissecting the complex architecture of brain regions (Figure 2).

**GENETIC ADDRESS BOOK FOR RETINAL CELL TYPES**

S. Siegert and B.G. Scherf, in collaboration with K. Del Punta, N. Didkovsky and N. Heintz

The mammalian brain is assembled from thousands of neuronal cell types organized in distinct circuits to perform behaviorally relevant computations. Transgenic mouse lines with selectively marked cell types would enable the dissection of functional components of complex circuits. We carried out a screen for cell type-specific green fluorescent protein expression (Figure 3) in the retina using BAC transgenic mice from the GEN-SAT project. Among others, we identified mouse lines in which the inhibitory cell types of the night vision and directional selective circuits were selectively labeled. We quantified the stratification patterns to predict potential synaptic connectivity between marked cells of different lines and found that some lines enabled targeted recordings and imaging of cell types from developing or mature retinal circuits. Our results suggest the potential use of a stratification-based screening approach for characterizing neuronal circuitry in other layered brain structures, such as the neocortex.

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**Selected publications**


Epigenetics  Epigenetic modifications are potentially heritable but reversible alterations in gene expression. They determine cell fate, help maintain genome integrity, and play a role in diseases, including cancer and neuronal disorders. Our interdisciplinary approach exploits various model organisms to examine the molecular mechanisms underlying epigenetic regulation.

Marc Bühler  
*Non-coding RNAs and epigenetic genome regulation*

Rafal Ciosk  
*Cell fate determination and reprogramming in animal development and disease*

Witold Filipowicz  
*Mechanisms and regulation of microRNA function and metabolism in mammalian cells*

Susan Gasser  
*Nuclear organization in genome stability and epigenetic patterning*

Hele Grosshans  
*Function and regulation of animal microRNAs*

Patrick Matthias  
*Transcription factors and epigenetic networks in mammalian cells*

Antoine Peters  
*Epigenetic control of mammalian germ line and early embryonic development*

Dirk Schübeler  
*Epigenetic modulation of genome function*
INTRODUCTION

Data from a variety of organisms have shown that the assembly of silent chromatin coincides with the presence or absence of non-protein-coding RNAs (ncRNAs). These range from long ncRNAs, which have been classically implicated in the regulation of dosage compensation and genomic imprinting, to small ncRNAs involved in heterochromatin assembly via the RNA interference (RNAi) pathway. This raises the question of how common ncRNAs are used to control gene expression at the level of chromatin.

Research in our group is focused on the mechanisms and conservation of epigenetic processes that are under the control of ncRNAs, in the conviction that a better understanding of the role of RNA in cellular mechanisms will yield promising new drug targets and tools for the treatment of human disease. Our approach is to first study fundamental processes in simple model organisms and here we take advantage of the power of fission yeast. The insights gained from our work with yeast are then investigated in higher eukaryotes.

We are working currently on three major projects. In a first project, we study the role of the RNAi pathway in the assembly of heterochromatin. Here we focus on spatial and temporal aspects by employing parallel genomics, biochemical, and live-cell imaging approaches with yeast and mammalian systems. In a second project, we investigate a novel mode of gene silencing that we refer to as “co-transcriptional gene silencing” (CTGS). Finally, we are working on Friedreich’s Ataxia (FRDA), a neurodegenerative disorder caused by an expansion of trinucleotide repeats in the non-protein coding region of a gene. Despite many years of research, the precise mechanism underlying FRDA is not well understood and we hope to contribute towards a better mechanistic understanding of this devastating disease.
PROBING PHYSICAL ASSOCIATIONS OF THE RNAi PATHWAY WITH YEAST AND MAMMALIAN GENOMES

K. Woolcock and R. Stunnenberg, in collaboration with D. Gaidatzis

RNAi is a highly conserved, sequence-specific gene regulatory mechanism in eukaryotes that is triggered by double-stranded RNA (dsRNA). The mechanism of silencing involves the generation of small RNA molecules of ~22 nucleotides from the longer dsRNAs by an enzyme called Dicer. These small interfering RNAs (siRNAs) then load onto effector complexes and are best known for triggering post-transcriptional silencing of target mRNAs.

In addition to its role in post-transcriptional gene silencing, RNAi has also been implicated in transcriptional gene silencing. Recent studies have suggested that RNAi-mediated heterochromatin formation is cell-cycle regulated and that RNAi can induce transient, cell cycle-specific changes in the fission yeast epigenome. We are using a genome profiling technique that allows us to profile weak and transient interactions of the RNAi machinery with the fission yeast genome. Combined with high-throughput Solexa sequencing of small RNA libraries, this revealed novel genomic targets of the fission yeast RNAi machinery. We are now extending our analysis to mammalian cells in the hope of finding direct evidence for a role of RNAi in chromatin-dependent gene regulation in higher eukaryotes.

SPATIAL ORGANIZATION OF THE FISSION YEAST RNAi PATHWAY

S. Emmerth, H. Schober, R. Stunnenberg

Biochemical and genetic analyses in recent years have greatly improved our understanding of how the RNAi pathway contributes to heterochromatin assembly in S. pombe. However, little work has been done at the cell biology level and important questions such as the subcellular localization and temporal regulation of the RNAi pathway have remained largely unanswered. In order to get a more comprehensive understanding of the spatial organization of the fission yeast RNAi pathway, we analyze RNAi factors and their associated proteins in the respective complexes by direct epifluorescence in living cells.

Inter alia, this work revealed that the S. pombe RNAi protein Dicer is a predominantly nuclear protein that is enriched at the nuclear periphery. Importantly, we found evidence that Dicers in general might be nucleo-cytoplasmic shuttling proteins. Shuttling of S. pombe Dicer is mediated by its double-stranded RNA-binding domain (dsRBD) and is under the control of a novel motif that we call “C33”. In the absence of C33, Dicer accumulates in the cytoplasm, the formation of heterochromatin is impaired and the protein acts promiscuously (Figure 1). In accordance with its nuclear function in fission yeast, nuclear retention of Dicer is thus a prerequisite for proper assembly of heterochromatin at centromeric repeats.

THE ROLE OF NUCLEAR ORGANIZATION FOR DE NOVO ASSEMBLY OF HETEROCHROMATIN VIA siRNAs

H. Schober, R. Stunnenberg, Y. Shimada

One of the key features of RNAi-mediated heterochromatic gene silencing in fission yeast is its cis restriction. We have demonstrated that newly siRNA-programmed RITS complex is very inefficient in silencing the expression of a second allele in trans under physiological conditions. Nuclear organization can create micro-environments facilitating chromatin-mediated repression and we are, therefore, aiming to unveil whether subnuclear localization of the target locus is a crucial determinant for its susceptibility to siRNA-mediated de novo assembly of heterochromatin. We are also conducting genetic screens in order to find conditions that would promote siRNA-mediated heterochromatin formation in trans.

POST-TRANSLATIONAL REGULATION OF DICER

R. Kulasegaran, T. Punga, Y. Shimada and R. Villasenor, in collaboration with H. Gut (FMI) and F. Allain (ETH Zurich)

Our recent results suggest that regulation of nucleo-cytoplasmic localization of fission yeast Dicer...
Epigenetics

is mediated by conformational changes in Dicer itself. In order to gain novel mechanistic insights into Dicer shuttling and its regulation, we are working towards a 3D structure of fission yeast Dicer by crystallography and NMR. We have also identified several post-translational modifications of Dicer that might be important for regulating both its activity and subcellular localization.

CO-TRANSCRIPTIONAL GENE SILENCING

C. Keller, K. Woolcock

The insertion of reporter genes within or adjacent to heterochromatic regions results in clonally inherited gene-silencing phenomena exhibiting the characteristic properties of classical position effect variegation (PEV). PEV has been thought to be mediated by transcriptional repression of the marker genes because physically condensed chromatin (heterochromatin) has been assumed to be transcriptionally repressed. However, this view has been challenged by our recent observations in S. pombe, which suggest that heterochromatin can be a relatively accessible structure and in some situations silencing occurs by a mechanism that does not prevent the association of RNApolII with endogenous or transgene promoters within heterochromatin. Clearly, some promoters can be transcribed within heterochromatic domains, but the resulting RNA is most likely degraded by a mechanism that we call co-transcriptional gene silencing (CTGS). We are currently focusing on the mechanistic and functional dissection of this chromatin-dependent RNA degradation pathway.

DECIPHERING THE UNDERLYING GENE SILENCING MECHANISM IN FRIEDREICH’S ATAXIA

T. Punga, in collaboration with the Genomics Institute of the Novartis Research Foundation

Friedreich’s ataxia (FRDA) is an autosomal recessive disease in which most patients have a pathogenic expansion of a trinucleotide GAA repeat within the first intron of the frataxin (FXN) gene that results in FXN silencing (Figure 2). Resembling PEV observed in yeast and flies, GAA triplet repeat expansions causing FRDA are associated in mammalian cells with the accumulation of heterochromatic markers such as methylation of H3K9 and hypoacetylation of histones H3/H4. It is widely assumed that the H3K9 methylated chromatin causes transcriptional silencing of the FXN gene in FRDA. Our most recent results demonstrate that silencing can occur independently of H3K9 methylation and that the main problem is most probably an aberrant DNA structure formed by long GAA repeat tracts that is difficult for RNA polymerase II to transcribe. Therefore, finding novel strategies to overcome the problem of transcribing long GAA repeat tracts is of prime medical importance.

We are collaborating currently with the Genomics Institute of the Novartis Research Foundation (GNF), where we use a molecular model of FRDA for high-throughput RNAi and compound screens. We hope that this will not only shed new light on the mechanism of heterochromatic gene silencing but will also provide new insight into therapeutics to treat FRDA.

Selected publications

Buhler M (2009)
RNA turnover and chromatin-dependent gene silencing. Chromosoma 118:141-151

Buhler M, Gasser SM (2009)
Silent chromatin at the middle and ends: lessons from yeasts. EMBO J 28:2149-2161

Nuclear retention of fission yeast Dicer is a prerequisite for RNAi-mediated heterochromatin assembly. Dev Cell 18:102-113

Proteomic and functional analysis of the noncanonical poly(A) polymerase Cid14. RNA 16:1124-1129

Punga T, Bühler M (2010)
Long intronic GAA repeats causing Friedreich’s Ataxia do not affect transcription initiation but provoke abortive transcription. EMBO Mol Med 2:120-129

Fig. 2. A Friedreich’s Ataxia (FRDA) is caused by expansion of a trinucleotide repeat in the first intron of the FXN gene.
B Western blot showing reduction in FXN protein in an FRDA patient-derived cell line.

Buhler M(2009)
RNA turnover and chromatin-dependent gene silencing. Chromosoma 118:141-151

Buhler M, Gasser SM(2009)
Silent chromatin at the middle and ends: lessons from yeasts. EMBO J 28:2149-2161

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Proteomic and functional analysis of the noncanonical poly(A) polymerase Cid14. RNA 16:1124-1129

Punga T, Bühler M (2010)
Long intronic GAA repeats causing Friedreich’s Ataxia do not affect transcription initiation but provoke abortive transcription. EMBO Mol Med 2:120-129
INTRODUCTION

During animal development, cells acquire distinct identities allowing the formation of complex structures such as the human body. Occasionally, however, cells can push the developmental clock back, which can lead to diseases such as cancer. The mechanisms controlling cell fate commitment are complex and the cells undergoing fate reprogramming are often difficult to pinpoint. For these reasons, we are using a genetically tractable model – the nematode *Caenorhabditis elegans*.

The germ line is the only cell lineage that generates all types of cells following the fusion of an egg and sperm. This underlying potential, or totipotency, of germ cells is revealed in certain germline tumors called teratomas, where the germ cells aberrantly differentiate into many kinds of somatic tissue. However, to date the mechanisms controlling totipotency are poorly understood.

Our previous findings demonstrated a critical role for RNA regulation in controlling germ cell totipotency, as mutations in the conserved RNA-binding proteins GLD-1 and MEX-3 induced a worm equivalent of the human teratoma (Ciosk et al. 2006). This observation raised many questions; for example, how do these proteins regulate mRNA metabolism, what are their mRNA targets, and how does the deregulation of their targets induce germ cells to adopt somatic fates? Our recent results, outlined below, are beginning to shed light on these questions.

In our future research, we will continue to investigate developmental decisions in the germ line. Our ultimate goal is to understand the regulation of germ cell totipotency and our findings may impact on the treatment of degenerative diseases and cancer.
GLD-1 belongs to the STAR (signal transduction and activation of RNA) family of RNA-binding proteins that includes mammalian Quaking and Sam68 (Biedermann et al. 2010). In the gld-1 mutant, germ cells progressing through meiosis abnormally re-enter mitosis, forming a tumor. Remarkably, some cells in the gld-1 mutant germ line differentiate into various somatic cell types, bypassing the normal program of oocyte formation and fertilization (Figure 1A). This worm teratoma is enhanced by the loss of another conserved RNA-binding protein, MEX-3 (Giosk et al. 2006). Both GLD-1 and MEX-3 can function as translational repressors and, therefore, abnormal expression of their target mRNA(s) in the germ line is predicted to cause re-entry into mitosis and teratomatous differentiation. However, the identity of the mRNA(s) whose repression is critical to prevent mitosis and the relation between the cell cycle and the loss of germ cell identity remained unclear.

We have been focusing mostly on the role of GLD-1 because it is an essential protein and a tumor suppressor. We found that GLD-1 acts as a tumor suppressor, at least in part, by translational repression of cye-1/cyclin E mRNA. In the gld-1 mutant, derepression of CYE-1 results in ectopic activation of CYE-1/CDK-2 activity, which promotes precocious entry into the M phase of the cell cycle and consequently the induction of a germline tumor. Remarkably, reactivation of the cell cycle leads to the loss of germline-specific markers and transcriptional activation of genes that are normally expressed only in the early embryo. This appears to reflect precocious onset of embryonic gene activation (EGA) (Biedermann et al. 2009). Thus, CYE-1/CDK-2-mediated re-entry into mitosis induces a switch in cell identity. Previously, differentiation into muscles in a worm teratoma was shown to depend on the maternal transcription factor PAL-1/Caudal (Giosk et al. 2006), whose translation in the wild-type germ line is repressed by GLD-1. However, PAL-1 is occasionally expressed in the wild-type germ line but is apparently insufficient to induce muscle differentiation. Thus, we propose that the acquisition of embryonic-like identity, manifested by the onset of EGA, is required for somatic fate determinants including but not limited to PAL-1, and the induction of teratomatous differentiation into various somatic cell types (Figure 1B). Whether a GLD-1 related or another RNA-binding protein plays a role similar to GLD-1 in the mammalian germ line is currently unknown. In a mouse model for testicular teratoma (the 129 family of inbred strains), loss of...
the RNA-binding protein DND1 dramatically increased teratoma incidence. Interestingly, DND1 has been demonstrated to stabilize and promote expression of the p27Kip1 mRNA that encodes a CDK2 kinase inhibitor. Thus, DND1 (-) germ cells are expected to have low levels of p27Kip1 and higher CDK2 activity. Therefore, it is possible that derepression of CDK2 activity may also induce proliferation, EGA, and consequently teratomatous differentiation in the mammalian germ line.

This study links for the first time cell cycle regulation with the germ line versus soma fate decision and provides a paradigm for the possible origin of human teratomas. However, a number of important questions remain to be answered; most important, how reactivation of the cell cycle triggers the onset of embryonic transcription. Because cyclin/Cdk-mediated phosphorylation is known to regulate, among other targets, major cell fate determinants such as the myogenic transcription factor MyoD, it is possible that CYE-1/CDK-2 might drive embryonic transcription directly by phosphorylation of a transcriptional regulator involved in EGA.

RNA CODE FOR mRNA TARGET SELECTION BY GLD-1

J. Wright

Several mutations that reduce or alter GLD-1 function are within the RNA-binding STAR domain, demonstrating that RNA binding is critical for GLD-1 function. To date, few GLD-1 targets have been described and RNA sequence selection by GLD-1 is unclear. To address these questions, we undertook a ribonomic approach.

Much of the control of gene expression in the germ line lies with RNA binding proteins (RBPs) and important germ line RBPs such as FBF/Pumilio and CGH-1/Dhh-1 associate with large numbers of mRNAs, implying large-scale mRNA regulation. Impressively, we found that GLD-1 interacts and potentially regulates a large part of the germ-line transcriptome (up to 20% of germline mRNAs). Some RBPs have been suggested to co-regulate mRNAs encoding functionally related proteins. Somewhat consistent with this idea, we see several members of functional groups enriched among GLD-1 targets, such as cytokinesis, cell division, embryonic development, reproductive processes, cell cycle and DNA replication. This global analysis suggests that, apart from cyclins (Biedermann et al. 2009), GLD-1 may also regulate cyclin “effectors” such as DNA replication factors. Thus, we expect that at least some biological roles of GLD-1 may reflect the regulation of networks rather than individual proteins.

Recent efforts from several labs using global approaches have begun to unravel the “RNA-binding code” for various RBPs. The scale of GLD-1/mRNA interactions allowed us to determine rules governing GLD-1 target selection and to derive a predictive model where GLD-1 association with mRNA is based on the number and strength of 7-mer GLD-1 binding motifs (GBMs) within UTRs. We verified this model both in vitro and in live animals, including by “transplantation” experiments where “weak” and “strong” GBMs imposed translational repression of increasing strength on a non-target mRNA. Importantly, we have obtained a unique quantitative picture of how an RBP interacts with its mRNA targets. As combinatorial regulation by multiple RBPs is thought to regulate gene expression, quantification of RBP/mRNA interactions should be a way to predict and potentially modify the biological outcome of complex posttranscriptional regulatory networks; our analysis suggests that such an approach is possible.

MOLECULAR FUNCTION OF GLD-1

C. Scheckel

Intriguingly, GLD-1 and related proteins from other organisms, called Quaking-related (QR) proteins, coordinate the cell cycle and differentiation in undifferentiated progenitors (Biedermann et al. 2010). However, this similarity might reflect an ancient function rather than a common mechanism. Although human QKI-6, if overexpressed in worms, can substitute for GLD-1 in translational regulation, under normal circumstances different QR proteins appear to regulate diverse aspects of mRNA regulation, ranging from splicing to mRNA stability. How proteins utilizing the same RNA recognition module have evolved the ability to elicit different molecular responses is an interesting question. As the conservation between the QR proteins is limited to the STAR domain, it is likely that additional domain(s) within QR proteins determine the molecular outcome of RNA binding. Another possibility is that the subcellular distribution of a QR protein may influence its molecular function.
Over the past decade, RNA/protein cytoplasmic granules have been shown to play an important role in the storage and/or degradation of mRNAs (Anderson and Kedersha 2009). A variety of proteins involved in mRNA surveillance, degradation, translational control and RNA interference have been shown to localize to granules called processing bodies (P bodies). P body assembly occurs in an mRNA-dependent manner and is enhanced by mRNA repression, supporting the idea that mRNA degradation and translational repression takes place in these structures. Other types of RNPs present in the germ line are P granules and storage granules. These granules have been suggested to control various aspects of mRNA metabolism including stability and translation. Curiously, GLD-1 is present in the germline granules and teratoma formation in gld-1 (mex-3) mutants is preceded by P granule disintegration (Ciøsk et al. 2006), suggesting the possibility that GLD-1 may control expression of some mRNAs through association with germline granules.

Irrespective of the possible involvement of RNPs in GLD-1-mediated regulation, we are interested in understanding precisely how GLD-1 represses translation. Reportedly, one GLD-1 target message, pal-1, co-migrates with polyribosomes in sucrose density gradients, suggesting that pal-1 repression occurs at the level of translational elongation or that the message is a component of RNPs that have the density of polyribosomes. In contrast, tra-2, another GLD-1 target that encodes a female sex determinant, has been shown to associate mainly with monosomal fractions, indicating that GLD-1 might inhibit initiation of translation. To resolve this apparent discrepancy, we are conducting similar experiments on additional target mRNAs.

Selected publications

Anderson P, Kedersha N (2009)

Biedermann B, Wright J, Senften M, Kalchhauser I, Sarathy G et al. (2009)
Translational repression of cyclin E prevents precocious mitosis and embryonic gene activation during C. elegans meiosis. Dev Cell 17:355-364

The quaking family of RNA-binding proteins: coordinators of the cell cycle and differentiation. Cell Cycle 9:1929-1933

Translational regulators maintain totipotency in the Caenorhabditis elegans germline. Science 311:851-853
INTRODUCTION

Epigenetic control of gene expression and post-transcriptional silencing by RNA interference (RNAi) and microRNAs (miRNAs) have emerged recently as extraordinarily important and interesting areas of molecular biology. These reactions contribute greatly to the developmental and tissue specificity of gene expression and also indicate a key role for hundreds of novel non-coding RNAs in the regulation of gene expression. Our research is focused on mechanistic and regulatory aspects of miRNA function and on the metabolism of miRNAs in mammalian cells. miRNAs are ~21-nt RNAs involved in the regulation of development, differentiation and many other fundamental processes. Several hundred different miRNAs are encoded in genomes of metazoa. Unlike siRNAs, which guide endonucleolytic degradation of target mRNAs, miRNAs in metazoa generally imperfectly base-pair to mRNA 3'-untranslated regions and inhibit protein synthesis, either by repressing mRNA translation or causing mRNA deadenylation and degradation (Figure 1).

miRNA-mediated regulation is a very complex process involving dozens of different proteins and intersecting with many other cellular pathways. miRNAs function in the form of RNPs (miRNPs, also known as miRISC), with Argonaute (Ago) and GW182 proteins being the most important protein components. The biogenesis of miRNAs is also a complex reaction, involving two enzymes of the RNase III family, Dicer and Drosha. In addition, many accessory factors regulate miRNA biosynthesis at different steps. miRNAs are generally assumed to have a very long half-life but our recent studies indicate in some cells, in particular in neurons, that miRNAs turn over very fast and that miRNA turnover is likewise the subject of sophisticated regulation.
ROLE OF DICER IN RNAi AND miRNA REACTIONS

M. Doyle, L. Jaskiewicz, S. L. Sinkkonen, T. Hugenschmidt and C. Artus-Revel, in collaboration with U. Kutay (ETH, Zurich), M. Zavolan (University of Basel) and P. Svoboda (Institute of Molecular Genetics, Prague)

Dicer is a key enzyme involved in RNAi and miRNA pathways. It is required for the biogenesis of both siRNAs and miRNAs. Human Dicer is a large ~220-kDa multi-domain protein containing RNA helicase/ATPase, DUF283, and PAZ domains, two neighboring RNase III-like domains (RIIIa and RIIIb) and a C-terminal dsRNA-binding domain (dsRBD). The RNase III domains resemble the catalytic domain of the bacterial RNase III, which also has specificity for dsRNA. Our previous work with different mutants of human Dicer indicated that the enzyme functions as an intramolecular dimer of RIIIa and RIIIb domains, assisted by two RNA-binding domains, dsRBD and PAZ. We have also studied the role of Dicer in the differentiation of mouse embryonic stem (ES) cells. As reported by others, the loss of Dicer in mouse ES cells results in miRNA depletion and growth and differentiation defects in vivo and in vitro. We found that differentiation defects in Dicer-/- cells are due to downregulation of de novo DNA methyltransferases (Dnmts) (Figure 2). The downregulation is mediated by retinoblastoma-like 2 (Rbl2), a direct target of miRNAs specifically expressed in ES cells.

REGULATORY FACTORS AND INTRA-CELLULAR LOCALIZATION OF miRNA EVENTS

J. Bethune, I. Lödige, M. de la Mata, T.-M.-P. Nguyen, S.N. Bhattacharyya and A. Haase, in collaboration with E. Bertrand and F. Rage (CNRS, Montpellier), R. Gherzi (University of Genua), M. Rosenfeld (University of California, San Diego) and R. Sack (FMI)

Translationally repressed mRNAs accumulate in cytoplasmic processing bodies, P-bodies, where they are either stored for future reutilization or degraded. However, other cellular structures such as endoplasmic reticulum (ER) or multivesicular bodies (MVBs) are also implicated in miRNA repression. We are using different cell fractionation and biochemical approaches to analyze the association of miRNA processing enzymes (e.g. Dicer) and miRNP components such as Ago and GW182 proteins with different cellular organelles.

Intracellular mRNA transport and local translation play a key role in neuronal physiology. Translationally repressed mRNAs are transported as part of RNP particles to distant dendritic sites, but the properties of different RNP particles and the mechanisms of their repression and transport remain largely unknown. We identified a new class of RNP-particle, the dendritic P-body-like structures (dlP-bodies), which are present in the soma and dendrites of mammalian neurons and have both similarities and differences to P-bodies of non-neuronal cells. These particles, containing Ago2, show motorized movements along dendrites and relocalize to distant sites in response to synaptic activation. The dlP-bodies may regulate local translation by storing repressed mRNPs in unstimulated cells and releasing them upon synaptic activation. We have also tagged many additional components of the miRNA pathway and study their localization and transport as a function of neuronal activity.

Using monoclonal and polyclonal antibodies raised against human Dicer, we previously identified TRBP, a protein with three dsRNA-binding domains, as a protein partner of Dicer and demonstrated that TRBP is required for optimal RNA silencing mediated by siRNAs and miRNAs. Another identified Dicer-interacting protein, KSRP, is required for efficient processing of several primary miRNA transcripts (pri-miRNAs) and precursor miRNAs (pre-miRNAs) by the Drosha and Dicer complexes, respectively. The protein binds to the loop sequence of pri/pre-miRNA hairpins. We are using immunoprecipitation and other approaches to identify additional proteins interacting with Dicer and other miRNP components. One of the proteins characterized so far is Ro-52 (TRIM21) and we are investigating its potential role in miRNA-mediated repression.
MECHANISM OF TRANSLATIONAL REPRESSION BY MAMMALIAN miRNAs

M. Chekulaeva, H. Mathys, J. Zipprich, J. Attig, S. Bhattacharya and M. Colic, in collaboration with N. Sonenberg (McGill University, Montreal) and R. Parker (University of Arizona, Tuscon)

The mechanistic details of miRNA function in repressing protein synthesis are still poorly understood. miRNAs can affect both the translation and stability of mRNAs. Our data indicate that miRNAs in mammalian cells and in extracts derived from them repress translation at the initiation step. Consistent with the initiation process being targeted by miRNAs, we find that repressed mRNAs are less efficiently recruited to polysomes. Experiments performed with an in vitro system from mouse Krebs2 ascites cells, which recapitulates many features of the miRNA-mediated in vivo effects, also indicate that miRNAs repress translation at the early step of initiation, probably involving cap recognition. MiRNAs also lead to deadenylation and partial degradation of target mRNAs. Deadenylation of reporter mRNAs in the in vitro system from Krebs2 cells requires the C-terminal domain of GW182 protein. This domain recruits a poly(A) binding protein, PABP, which is absolutely essential for deadenylation. Kinetic experiments indicate that translational repression precedes the deadenylation.

Proteins of the GW182 family play an important role in executing miRNA repression in metazoa. They interact directly with Ago proteins, components of miRNPs, and also form part of P-bodies. They induce both translation repression and mRNA deadenylation and decay. Dissection of the Drosophila GW182 (dGW182) revealed that it contains three independent repressive domains. Each of these domains also functions independently of poly(A) tails, indicating that mRNA repression is not through deadenylation. Interaction of GW182 with Ago proteins is mediated by GW/WG repeats, which are conserved in many Ago-binding proteins involved in RNAi and miRNA silencing. Analysis of the N-terminal repressive domain of dGW182 demonstrated that its function in repression requires intact GW/WG repeats, but does not involve interaction with the Ago1 and is independent of the mRNA polyadenylation status. These results revealed a novel role for the GW/WG repeats as effector motifs in miRNA-mediated repression.

In contrast to a single GW182 protein in Drosophila, three GW182 paralogs, TNRC6A, TNRC6B, and TNRC6C, are encoded in mammalian genomes. We found that all three proteins are important for efficient miRNA repression. As in the case of dGW182, repression is due to a combination of effects on mRNA translation and mRNA level. Through deletion and mutagenesis, we identified the C-terminal part of TNRC6C encompassing the RRN-binding motif and its flanking sequences as a key effector domain mediating protein synthesis repression by TNRC6C.

ROLE OF HuR IN REVERSIBILITY OF TRANSLATIONAL REPRESSION BY miRNAs

P. Kundu, L. Sokol, C. Artus-Revel, T. Hugenschmidt and J. Zipprich, in collaboration with S. N. Bhattacharya (IIICB, Kolkata) and N-C. Meisner (NIBR, Basel)

We showed previously that miRNA-mediated translational repression is a reversible process in mammalian cells. Target mRNAs with AU-rich regulatory elements (AREs) in the 3’UTR can be relieved of miRNA repression in response to different forms of cellular stress applied to human hepatoma Huh7 or HeLa cells. The ELAV family protein HuR, an ARE-binding protein containing three RRM domains, translocates from the nucleus to the cytoplasm in response to stress and its binding to target mRNA is essential for the relief of miRNA-mediated inhibition. The derepression is associated with the exit of mRNA from P-bodies and its recruitment to polysomes for active translation.

We are investigating the mechanism of the HuR-mediated effect on miRNA repression in human cells. Using mutants of HuR accumulating in the cytoplasm in the absence of stress and tumor cell lines constitutively accumulating endogenous HuR in the cytoplasm, we were able to uncouple the HuR effect on miRNA repression from stress. We also found that Ago2 and HuR do not interact with each other and that their binding to target mRNA appears to be mutually exclusive. We are using recombinant wild-type and mutant HuR proteins and an in vitro miRISC assay to find out whether HuR has an effect on binding and activity of miRISC.
Fig. 3. Regulation of miRNA metabolism in neurons. In both retinal photoreceptors (left panel) and non-retinal neurons (right panel) miRNAs turn over much more rapidly than in other cell types. In photoreceptors, expression of some miRNAs is induced in response to light. In hippocampal and cortical neurons, miRNA turnover is accelerated by neuronal stimulation and inhibited by blocking of action potentials.

miRNA FUNCTION AND METABOLISM IN RETINAL and NON-RETINAL NEURONS

J. Krol, I. Markiewicz and S. Ribi, in collaboration with the B. Roska group, M. Stadler and T. Oertner (FMI), M. Bibel and J. Richter (NIBR, Basel) and G. Schratt (University of Heidelberg)

The mammalian retina consists of three neuronal body and two synaptic layers representing a complex network of many different cell types. It is an excellent system to study formation of neuronal circuits in development and the regulation of gene expression in response to different intrinsic and extrinsic factors, including exposure to light. We are investigating the role of miRNAs in the light/dark adaptation of mouse retina. Deep sequencing and other methodologies identified miRNAs that respond to different light conditions in mouse retinal neurons, independent of the circadian clock. Levels of the sensory neuron-specific miR-182/183/96 cluster and miR-204 and -211 were downregulated during dark adaptation and upregulated during light adaptation as a result of rapid miRNA decay and increased transcription, respectively. We identified the voltage-dependent glutamate transporter Scl1a1 as a target of the light-regulated miR-182/183/96 cluster in photoreceptor cells. Many miRNAs in retinal neurons turn over much faster than miRNAs in other cell types (Figure 3).

ROLE OF miR-122 IN CHRONIC HEPATITIS C

J. Krol and I. Markiewicz, in collaboration with the M. Heim group (University Hospital Basel)

Liver-specific miRNA miR-122 was reported previously to be required for the replication of hepatitis virus C (HCV) RNA in Huh7 hepatoma cells. Several miRNAs, including miR-122, have also been implicated in the response of HCV replication to interferon-α (IFN-α) in human hepatoma cells. Our analysis of liver biopsies from patients with chronic hepatitis C (CHC) undergoing IFN-α therapy showed no correlation of miR-122 expression with viral load but, unexpectedly, a marked decrease in miR-122 in patients not responding to IFN-α therapy. Other miRNAs showed only very limited changes. These observations may be used to predict the outcome of IFN therapy and also have implications for the targeting of miRNAs in CHC therapy.

Selected publications


This result led us to investigate miRNA turnover in non-retinal neurons. Fast decay of many miRNAs was found in cultures of dissociated hippocampal and cortical neurons, in mouse brain cultured hippocampal slices and in neurons differentiated from mouse ES cells in vitro. In all these systems, addition of transcription inhibitors resulted in a rapid decrease in neuron-specific miRNAs such as miR-124, -128, -134, and -138. In contrast, we observed no appreciable decay of miRNAs in NIH3T3 or RPE-1 cultured cells, or in ES cells.

Experiments with neuronal cells indicated that miRNA turnover may be subject to complex activity-dependent regulation. In four neuronal cultures tested, blocking action potentials with tetrodotoxin (TTX) prevented rapid turnover of miRNAs. Blocking glutamate receptors with NBQX/CPP likewise prevented turnover of miR-124, -128, -134, and -138 in hippocampal and ES cell-derived neurons, whilst the addition of glutamate accelerated it (Figure 3). These data indicate that active miRNA metabolism may be essential for the function of neurons, for example, for regulation in response to synaptic activity of the translation of neuronal miRNAs located at dendritic spines.
INTRODUCTION

Genes alone are not the blueprint for an organism’s body. Supplementing the coding capacity of the linear DNA molecule is a series of “epigenetic” factors that regulate the accessibility of DNA for transcription, replication and repair events. These factors include compaction of DNA around histones, covalent modification of histones on cores and tails, methylation of cytosine residues in DNA, and folding of chromatin into higher-order structures. This “epigenetic code” does not replace the genetic code but complements and extends it, establishing cell-type specific patterns of gene expression and mechanisms for the inheritance of expression states. Unlike the coding sequence, however, epigenetic changes can arise from internal and external signals such as hormones, developmental cues, nutrients and environmental insults.

Not surprisingly, the enzymes that set and remove epigenetic marks are directly implicated in many human diseases, including aging and cancer. Thus, a thorough understanding of epigenetic control over gene expression will be important in tissue engineering, to reprogram gene expression in diseased cells and alter cell fate decisions. Despite this obvious biomedical use, most of our insight into epigenetic control stems from work with model organisms such as yeast, worms, plants, flies and mice. Model organisms have allowed us to examine the inheritance of epigenetic information under different environmental conditions and genetic manipulation has shown how epigenetic control mechanisms influence disease.

In our laboratory, we pursue the mechanisms that maintain genomic stability through replication and those that mediate epigenetic inheritance of transcriptional states through cell division and development. Addressing these questions at the molecular and systemic levels, we have had particular success in extending approaches optimized in budding yeast to the nematode C. elegans.

Susan Gasser
Nuclear organization in genome stability and epigenetic patterning
SUBNUCLEAR COMPARTMENTS IN BUDDING YEAST AND THEIR FUNCTION

A. Taddei, H. Schober, H. Ferreira

As in all organisms, the budding yeast genome is divided into open zones of active gene transcription and closed zones of heterochromatin. In many organisms, including yeast, repressive zones are found adjacent to the nuclear envelope. These micro-environments are thought to facilitate repression by accumulating repressive histone-binding factors and in yeast they are created by the clustering of telomeric repeats. In contrast to the repressive zones, the association of active genes with nuclear pores can actually enhance transcription (Taddei et al. 2006). Pore association increases mRNA elongation, its processing or export rather than initiation. Similar mechanisms may fine-tune chromosomal expression in higher eukaryotes, for example the pore-association of up-regulated genes on the X-chromosome in male flies (Ahktar and Gasser 2007).

We have characterized a novel pathway for the anchoring of telomeric repeats and their associated heterochromatin through the highly conserved Sad1-UNC-84-Nesprin (SUN) domain protein Mps3 (Schober et al. 2009). This pathway depends on the catalytic yeast telomerase subunits Est2/Est1 and Tcl1, the RNA template that binds Yku80. Mps3 is the principal membrane anchor for this pathway. If we impair anchoring to the Mps3 N-terminus in a tel1 deletion background, we generate deleterious levels of recombination between subtelomeric repeats. This argues that telomere binding to the nuclear envelope helps protect sequences from recombination. It also illustrates how a specialized structure requires proper spatio-temporal localization to fulfill its biological role and suggests a novel pathway of telomere protection.

Loss of telomeric anchoring has several consequences. In a yeast strain that lacks both the Yku-anchoring pathway and a second one based on Enhancer of silent chromatin 1, the Silent Information Regulatory (SIR) proteins 2, 3 and 4 were dispersed throughout the nucleus rather than sequestered in peripheral foci. Under these conditions, 60 genes were reproducibly misregulated. Of those with higher expression, 22 % were near telomes, confirming that telomere anchoring helps repress subtelomeric genes. In contrast, loci that were down-regulated by dispersed SIR factors were distributed across all chromosomes. Further controls allowed us to conclude that released SIR factors can repress promiscuously. Thus, clustering of repeats in heterochromatic foci serves to favor subtelomeric repression and to prevent promiscuous effects of dispersed repressors. Crucially, these findings show that subnuclear compartments help regulate gene expression patterns.

RECONSTITUTION OF HETEROCROMATIN: SIR FACTOR ASSEMBLY IN VITRO

M. Oppikofer, S. Küng, M. Tsai, F. Martino

Although we know much about the genetics of silencing, the structure of repressed chromatin has remained obscure. To examine this, we set up an in vitro system allowing reconstitution of nucleosomes with the yeast heterochromatin proteins, Sir2, Sir3 and Sir4. We add the yeast Sir2-3-4 complex purified from baculoviral-infected insect cells (Figure 1) to nucleosomal arrays created from recombinant histones. Loading of the SIR complex is cooperative and generates a stable structure bearing one SIR complex (Sir2-3-4 in 1:1:1 ratio) that protects the linker DNA between adjacent nucleosomes. Importantly, the affinity of Sir3 binding is affected by the N-terminal tail of histone H4 and the acetylation of H4K16. Sir3 also interacts with the face of the nucleosome, where it requires unmodified lysine 79 on histone H3. Intriguingly, the by-product of Sir2-mediated NAD hydrolisis, O-acetyl ADP ribose, greatly stimulated assembly of SIR proteins onto nucleosomes and, in particular, enhanced the binding of Sir3 (Martino et al. 2009).

The impact of histone modification and active histone deacetylation on heterochromatin assembly is being examined by mapping the precise contributions of specific domains of Sir3 and Sir4 as they bind histones and DNA. We propose that O-AADPR binds the AAA+ helicase-like domain of Sir3, inducing a conformation change that stabilizes the chromatin-SIR complex. These changes are being analyzed by tomographic electron microscopy, with the aim of modeling the 3D structure of repressed chromatin.
CELL FATE DETERMINATION AND NUCLEAR ORGANIZATION IN C. ELEGANS

P. Meister, B. Pike, B. Towbin, S. Rohner

Although it is known that heterochromatin accumulates as cells differentiate, to define how nuclear compartments regulate gene expression and repression during metazoan development is a formidable challenge. We approach this by exploring how nuclear compartments in *C. elegans* change during development and whether genes expressed tissue-specifically assume unique positions in the nuclei of differentiated cells.

By integrating LacO binding sites that recruit GFP-lacI, we tracked the position of integrated transgenes containing cell-type specific promoters (Figure 2). In differentiated cells but not early embryos, nuclei are functionally compartmentalized, with silent genes bound to the nuclear periphery and active genes sequestered in the nuclear lumen. Importantly, developmentally regulated promoters themselves are sufficient to direct intranuclear positioning of large domains, even those bearing heterochromatic histone marks. We demonstrated this principle in different cell types of mesodermal, endodermal and ectodermal origin (Meister et al. 2010). Even prior to differentiation, binding of the MyoD homologue Hlh-1 overrides the peripheral sequestration of heterochromatin, shifting it inwards. Intriguingly, however, promoters of housekeeping genes do not carry “intranuclear address labels”. Thus, it is not transcription per se but factors recruited by tissue-specific promoters that cause the internal shift.

A genome-wide RNAi screen for factors derepressing and/or relocating large heterochromatic arrays revealed a role for lysine methylation in peripheral anchoring. The anchored heterochromatic arrays carry methylation on both histone H3K9 and H3K27 (targets of the Suv3-9 or Polycomb histone methyl transferases, respectively). We are testing exactly which marks are involved and what recognizes them.

In contrast to developmentally regulated promoters, experiments with the heat-shock promoter *hsp-16.2* showed that stress-activated arrays do not shift inwards upon transcriptional induction but remain at the nuclear periphery. Both high-level transcription and association with the nuclear periphery require heat-shock transcription factor Hsf-1. Taken together, our data on *C. elegans* nuclear organization argue that transcriptionally active domains in the nuclear interior harbor differentiation-induced genes during cell-fate acquisition, whilst the nuclear periphery consists of inactive zones of heterochromatin and active sites for stress-induced genes at nuclear pores.

RecQ HELICASES AND ATR KINASE RESCUE STALLED REPLICATION FORKS

A.M. Friedel, K. Shimada and N. Davoodi, in collaboration with N. Thomä (FMI) and R. Movva (NIBR)

During S phase, all genomic DNA is copied precisely once. During this process, replication forks frequently encounter obstacles, such as tightly bound protein-complexes or DNA damage, and stall. This forms fragile DNA structures with the potential for DNA double-strand break (DSB) formation and aberrant homologous recombination (HR; Figure 3). Fork collapse is prevented by a surveillance mechanism, the intra-S phase checkpoint, that maintains polymerase binding at forks and regulates cell cycle progression, DNA repair and late origin firing. Fork instability increases during oncogenic transformation, making the intra-S phase checkpoint a major gate-keeper preventing fork-associated rearrangements that aggravate the oncogenic potential of cells.
Two important proteins stabilizing arrested replication forks are the checkpoint kinase ATR (called Mec1 in budding yeast) and the Bloom’s Syndrome helicase (BLM), which has a single homologue in yeast, the RecQ helicase Sgs1. Loss of both the ATR- and BLM-mediated pathways leads to a dramatic increase in fork collapse and gross chromosomal rearrangements, even in the absence of fork-stalling lesions. We hypothesized that these two pathways in budding yeast converge on Replication Protein A (RPA), the single-strand binding heterotrimer necessary for replication and repair. Indeed, RPA recruits Mec1-Ddc2 to stalled replication forks and was shown earlier to tightly bind Sgs1. Under conditions of fork arrest induced by low rNTP levels provoked by the cytosolic drug hydroxyurea (HU), we examined how these factors cooperate to stabilize stalled replication forks, ensuring both checkpoint activation and appropriate fork recovery.

We mapped sites of Sgs1 and RPA interaction to an acidic domain in the Sgs1 N-terminus and the first oligonucleotide binding (OB) fold of the largest RPA subunit, Rpa70. We tested the importance of this interaction for fork stability in a mutant, sgs1-1, lacking the binding interface. The mutation leaves Sgs1 active but completely disrupts Rpa70 binding in two hybrid assays, although residual interactions occur between full-length proteins. Consistent with a role for this interaction in DNA polymerase stability, we found that sgs1-1 partially displaces DNA pol α from stalled replication forks. Secondary mutations in Sgs1 showed that, in addition to RPA-binding, the helicase activity of Sgs1 is necessary to ensure association of DNA pol at stalled replication forks.

Intriguingly, the region of Sgs1 that interacts with RPA is also a target of Mec1-mediated phosphorylation. Phosphorylated Sgs1 binds tightly to the downstream checkpoint kinase Rad53 (Chk2 in mammals) and contributes to the activation of Rad53 by Mec1 at stalled replication forks. Thus, a conserved acidic N-terminal domain of Sgs1 binding RPA serves a second function in checkpoint activation. These two functions may be mutually exclusive and regulated by Mec1 phosphorylation, depending on the nature of the lesion at the replication fork.

The major binding site for Sgs1 in the N-terminal OB fold of Rpa70 was also mapped and characterized by in vitro binding studies. A basic cleft on the folded OB structure, reported to mediate p53 binding in human cells, is implicated in binding Sgs1 protein in yeast. A charge reversal mutation in rfa1-t11 that points into the basic cleft disrupts binding to Sgs1 in two-hybrid assays, displays a genome-wide replication defect in response to replication stress, and affects DNA pol α association at HU-stalled replication forks (Figure 3).

Intriguingly, there is an epistatic relationship between rfa1-t11 and mutants such as mre11 and rad51, which mediate homologous recombination. Thus impaired recombination between newly replicated sisters (strand-switching synthesis) may cause fork restart failure in rfa1-t11 cells. This link between RPA and the recombination machinery suggests a new pathway for maintaining replication fork integrity.

One goal of this project is the deliberate, selective provocation of fork collapse in cancer cells, which are hypersensitive to perturbation of replication. This is pursued in collaboration with Novartis using a chemicogenetic approach. Compounds that selectively kill cells on low concentrations of HU and in the presence of sgs1Δ or mec1-100 mutations have helped us identify novel pathways for intervention at the replication fork.

**GENOME-WIDE MAPPING OF CHROMATIN REMODELERS AND THEIR ROLES IN DNA REPAIR**

K. Shimada and T. Yoshida, in collaboration with M. Harata (Tohoku University, Sendai, Japan)

In earlier studies, we used Chromatin Immunoprecipitation (ChIP) at specific sites of DNA damage to study chromatin remodeling at DNA double-strand breaks (DSB) and replication fork lesions (Van Attikum et al., 2004, 2007; Dubrana et al., 2008). Using genome-wide approaches, we found that the INO80 chromatin remodeling complex is present at origins of replication and rRNA
genes throughout the yeast genome, but is particularly enriched at stalled replication forks near early firing origins (Shimada et al. 2008). The INO80 complex is dispensable for origin firing and maintenance of the replication bubble upon HU-induced arrest, but resumption of DNA replication was impaired in ino80 or ino80 complex mutants (Shimada et al. 2008). These mutants accumulated DSBs during resumption of replication after fork arrest. We are examining whether INO80 removes nucleosomes at stalled forks, as it does at breaks (van Attikum et al. 2007).

We have extended genome-wide mapping activities to map the binding sites of a closely related complex, the SWR1 remodeling complex (SWR-C or SRCAP in mammals). Although both SWR-C and INO80 are recruited to DSBs, their binding patterns in unperturbed yeast cells do not overlap. The SWR-C complex is found at promoters, where it deposits the histone H2A.Z variant. Surprisingly, although the Ino80 actin-related subunits Arp5 and Arp8 always co-localize with catalytic subunit Ino80, the Swr1 actin-related component Arp6 has both SWR-C-dependent and -independent functions in gene expression. The latter is achieved by anchorage of chromatin domains to the nuclear pore complex. Intriguingly, among genes anchored to pores by Arp6 (but not by SWR-C) is a subset of highly expressed ribosomal protein genes. Loss of ribosomal protein gene positioning at the nuclear pore helps fine-tune its expression level. This implicates actin-related proteins and nuclear pores in long-range chromatin organization.

**A NOVEL PORE-ASSOCIATED UBIQUITIN LIGASE AIDS DSB REPAIR**

S. Nagai, K. Shimada, H. Schober, H. Ferreira, V. Kalck and M. Tsai, in collaboration with N. Krogan (University of California at San Francisco)

Nuclear pores were further implicated in the processing and repair of DSBs and collapsed replication forks, if homologous recombination pathways fail (Nagai et al. 2008). Unrepaired DSBs and broken replication forks associate with a subcomplex of the nuclear pore containing Nup133, Nup84 and Nup60, as well as a SUMO-recognizing, ubiquitin-conjugating complex of Slx8 and Slx5 (Rnf4 in mammals). The relevance of this pathway for repair was confirmed by an increase in gross chromosomal rearrangements and sensitivity to DNA damage after mutation of these proteins in yeast and human cells alike. We aim to define the molecules involved in the relocalization event and to identify the type of repair facilitated by the action of Slx5/Slx8 ubiquitination.

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**Selected publications**


Reconstitution of yeast silent chromatin: multiple contact sites and O-AADPR binding load SIR complexes onto nucleosomes in vitro. Mol Cell 33:323-334


Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322:597-602

Schober H, Kalck V, Ferreira H, Gehlen LR, Gasser SM (2009)

Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. Genes Dev 23:928-938


Ino80 chromatin remodeling complex promotes recovery of stalled replication forks. Curr Biol 8:566-575
INTRODUCTION

MicroRNAs (miRNAs) are a novel class of regulatory genes. In a typical animal, hundreds of different miRNAs jointly regulate thousands of messenger RNAs (mRNAs). Thus, they constitute an important layer of gene regulation that affects diverse processes such as cell differentiation, apoptosis, and metabolism. Accordingly, dysregulation of miRNAs has been implicated in numerous diseases, including various cancers.

Despite such critical roles, deciphering the mechanism of action of miRNAs has been difficult, leading to multiple, partially contradictory, models of miRNA activity. Moreover, adding an additional layer of complexity, it is emerging that miRNAs themselves are regulated by various mechanisms that we are only beginning to understand.

We aim to find out how miRNAs achieve repression of their target mRNAs and how miRNAs are made and regulated. To address these issues, our studies focus on a whole-animal model, the roundworm Caenorhabditis elegans. In addition to exploiting its traditional strengths in genetics and cell biology, we are developing and applying biochemical assays. These complementary approaches allow us to obtain insights into both the molecular mechanisms of new pathways and their physiological relevance. For example, we have been able to identify an miRNA degradation pathway that prevents detrimental increases in mature miRNA levels and to recapitulate this process biochemically.

Given the importance of miRNAs in development and disease, identifying the mode of action and the regulators of these tiny gene regulators will continue to be both of scientific interest and biomedical relevance.
A NOVEL NUCLEAR EXPORT ROUTE FOR miRNAs

I. Büssing, in collaboration with J.-S. Yang and E.C. Lai (Sloan Kettering Institute, New York)

The biogenesis of mature, cytoplasmic microRNAs (miRNAs) is a complex, multi-step process. According to the current model, miRNAs are transcribed by RNA polymerase II as long primary transcripts of several hundred nucleotides (nt), the so-called pri-miRNAs. Still in the nucleus, the pri-miRNAs are processed by the RNase Drosha into ~70 nt-long pre-miRNA. Subsequently, the pre-miRNAs are exported from the nucleus by Exportin-5 and delivered to the cytoplasmic RNase Dicer. Processing of the pre-miRNA by Dicer yields the mature, functional miRNA of ~22 nt.

Drosophila orthologue of XPO-1, Embargoed does not possess an Exportin-5 orthologue, suggesting that our understanding of miRNA biogenesis is incomplete. Using a genetic approach, we have identified CRM1/XPO-1 and the heterodimeric cap binding complex CBC consisting of CBP-80 and CBP-20 as joint mediators of miRNA nuclear export in C. elegans (Büssing et al. 2010). Depletion of any of these factors causes cell differentiation and cell cycle exit defects similar to those seen with loss of activity of core mRNA machinery genes (Figure 1A, B). Indeed, the levels of various miRNAs are reduced in these mutants (Figure 1C). Unexpectedly, however, pre-miRNA levels are also low, whereas pri-miRNAs accumulate, thus identifying pri-miRNAs as the export substrates.

In flies, Exportin-5 mediates nuclear export of pre-miRNAs. Nonetheless, we find that the Drosophila orthologue of XPO-1, Embargoed (Emb), is also required for efficient miRNA biogenesis in Drosophila S2 cells. Rather than establishing a parallel nuclear export pathway, however, Emb appears to function upstream of Exportin-5, possibly in intranuclear pri-miRNA transport. Thus, our results not only identify XPO-1 and CBC as novel factors in miRNA biogenesis, but also add to a growing body of evidence suggesting that highly conserved miRNA pathway genes can be appropriated to distinct miRNA-related functions in diverse organisms.

TRANSLATIONAL REPRESSION OF ENDOGENOUS let-7 TARGET GENES IN VIVO

X. Ding, B. Hurschler, M. Hunkeler

Mature miRNAs, bound to an Argonaute protein, silence their target (“cognate”) mRNAs through an antisense mechanism that involves binding to partially complementary sequences in the mRNA. However, the precise mechanism(s) of action employed to achieve decreased accumulation of the cognate protein has remained controversial. Regulation at the level of mRNA stability, translation, polyadenylation, and/or protein stability has been proposed. It is likely that different miRNAs use different mechanisms and that an individual miRNA may use some of these mechanisms redundantly. However, even experiments employing identical miRNA and target reporter pairs have yielded mutually exclusive results on the apparent mechanism of action. Moreover, few of these mechanisms have been validated in vivo under physiological conditions and virtually nothing is known about the molecules mediating them.

We investigated the possibility that miRNAs regulate their cognate mRNAs by blocking translation. Using a polyribosome-profiling assay to investigate the translational status of endogenous let-7 miRNA target mRNAs, we found that loss of let-7 function significantly enhanced translation of cognate genes whilst not affecting genes not under control of let-7 (Ding and Grosshans 2009).

Reporter gene experiments demonstrated that these effects were direct as they depended not only on endogenous let-7 but also on the presence of let-7 binding sites in the 3’ untranslated region of the target mRNA. Our findings further reveal that endogenous let-7 blocks translation at the initiation step on its targets in vivo. We demonstrated this mechanism to function also for additional miRNAs, including lin-4, previously thought to repress its target at a step downstream of translation initiation.

In addition to causing translational repression, the presence of miRNAs typically resulted also in a decrease in cognate mRNA levels. As the extents of translational repression and transcript degradation did not correlate, it appears that they constitute two independent mechanisms. We are currently seeking to identify conditions in which they can be uncoupled.

Unexpectedly, despite the distinctiveness of the two mechanisms, the GW182-like proteins AIN-1 and AIN-2 are required for both translational silencing and transcript degradation. These proteins are known to bind to Argonaute proteins and our results suggest that they are indeed essential effectors of miRNAs. We are now investigating the mechanisms and molecules by which AIN-1 and AIN-2 effect target mRNA repression.

![Image of nuclear export receptor XPO-1 and the cap-binding protein CBP-80 promote miRNA biogenesis. A] Seem cells, a subset of skin cells highlighted with green fluorescent protein, differentiate and fuse in wild-type adult worms. B As in other miRNA pathway mutant animals, seam cell differentiation fails when xpo-1 is depleted by RNAi (arrows unfused cells). C RNAi against xpo-1 or the cebp-80/cbp-1 subunit of the nuclear cap-binding complex decreases levels of mature miRNAs. Modified from Büssing et al. (2010)
ACTIVE DEGRADATION OF MATURE miRNAs
S. Chatterjee, S. Rüegger, M. Fastler, N. Antih

MicroRNAs have long been held to be exceptionally stable molecules with half-lives of 1 day or more. Nonetheless, miRNA expression profiles during development tend to be quite dynamic, with some miRNAs being upregulated, others downregulated. We postulated, therefore, that active turnover pathways for miRNAs exist that are used to clear away no longer needed mature miRNAs. Using a directed genetic screen of candidate genes, we identified XRN-2 as a ribonuclease (RNase) that can degrade mature miRNAs in vivo and in cell-free lysates (Chatterjee and Grosshans 2009). Inactivation of this “miRNase” causes an increase in the levels of mature miRNAs but not their precursors. The accumulating miRNAs are functional, indicating that the role of XRN-2 is to restrict miRNA activity.

The interaction of miRNAs with Argonaute proteins is known to be very stable. Intriguingly, however, we found that XRN-2, in conjunction with an unknown factor, can mediate release of miRNAs from Argonaute, enabling their degradation by XRN-2. When examined in a C. elegans lysate, miRNA release and, thus, also degradation are blocked in the presence of target miRNAs. This type of regulation might, therefore, establish a means of aligning the levels of Argonaute-bound miRNA with that of its targets, as well as aiding in the recycling of Argonaute (Figure 2).

In our ongoing research, we are investigating the physiological relevance of target miRNA-mediated miRNA protection. We are also seeking to identify the miRNA release factor using biochemical as well as genetic approaches. Moreover, we are interested in identifying the cellular compartment(s) in which miRNA degradation occurs. Finally, we are investigating to what extent miRNA degradation by XRN-2 or other “miRNases” is regulated in a tissue- or developmental-specific manner.

IDENTIFICATION OF NOVEL miRNA PATHWAY GENES
M. Rausch, B. Hurschler, M. Ecsedi, X. Ding

During the past 10 years, we have witnessed an explosion in knowledge about miRNAs, yielding a well-supported model of the core steps of miRNA biogenesis and function. However, we still lack many details of these processes, as well as insight into the numerous events that appear to regulate them. To identify such factors, we are using genetic interaction screens that identify factors modulating miRNA activity in genome-wide, RNAi-based approaches. For example, a mutation in let-7 reduces the function of this miRNA and causes animals to die by bursting. In a pilot experiment, we have screened through > 2000 genes located on C. elegans chromosome I to identify those whose knockdown by RNAi restores the viability of let-7 mutant animals. This screen revealed the existence of an intricate balance between global translational activity and let-7 as a requirement for normal cell differentiation (Ding et al. 2008). Moreover, it identified several factors with possible roles in miRNA biogenesis and function or their regulation. We are currently extending this screen to a genome-wide level, as well as following up on individual suppressors already identified.

Selected publications


INTRODUCTION

Stem cells give rise to differentiated cells through gradual establishment of lineage-specific gene expression programs that are the result of a complex interplay between signal transduction cascades and genetic hierarchies of transcription factors and epigenetic chromatin modifiers. This creates a molecular code unique to each cell type that defines its properties and fate. We are interested in the molecular mechanisms underlying cellular plasticity and cell-specific gene expression in a genetically tractable system and concentrate on the transcriptional and epigenetic control of lymphoid (B) cell development. For this, we study several transcription factors and epigenetic regulators that may be relevant for B cell development. One of the factors we have studied intensively is OBF1, a B cell-restricted coactivator that associates with Oct factors. We also examine the role of other regulatory factors in this biological paradigm.

In addition, we increasingly focus on histone deacetylases (HDACs). Acetylation of histones is a crucial epigenetic annotation of chromatin that contributes significantly to the regulation of gene expression. HDACs remove acetyl groups from histone N-terminal tails and thereby contribute to chromatin condensation and to the modulation of gene expression. Furthermore, acetylation/deacetylation of non-histone proteins has become implicated in an expanding range of cellular processes. Although the biological role of HDACs in mammals is still poorly understood, it is clear already that these proteins have major medical importance. Small molecule inhibitors of HDACs have beneficial effects in cancer and other diseases, such as autoimmunity or neurodegeneration. HDACs are, thus, potential therapeutic targets in diverse pathological settings. In this context, our studies aim at a better understanding of the role of individual HDACs in normal physiology and in disease situations.
TRANSCRIPTIONAL AND EPIGENETIC REGULATORS IN B CELL DEVELOPMENT

C. Du Roure, M. Kaller and A. Manoharand, in collaboration with M. Rebhan (NIBR), M. Stadler (FMI) and A. G. Rolink (University of Basel)

To generate a global view of the regulatory networks potentially playing a role in B cell differentiation, we have undertaken an analysis of the transcription factors and epigenetic regulators differentially expressed during B cell development. For this, the transcriptome of B cells at different developmental stages was analyzed by microarray. Subsequently, an extensive bioinformatics analysis filtered the data for known or potential chromatin regulators and for transcription factors. Distinct expression profiles were generated to which the factors were assigned. This data set is a valuable source of information that forms a general framework for our studies and allows us to formulate hypotheses, for example, regulators were identified with reciprocal expression patterns through B cell development, suggesting that they may have unique functions. Also, members of known multi-protein complexes were found to vary in expression, indicating that the composition of the corresponding complexes may be more dynamic than anticipated.

In this context, we have analyzed the role of the de novo DNA methyltransferases Dnmt3a and Dnmt3B. We have generated mice lacking these two enzymes specifically in B cells and tested the impact on B cell development and function. In addition, we have examined gene expression and genomic methylation in B cells derived from these mice.

ANALYSIS OF THE COACTIVATOR OBF1

A. Bordon, C. Du Roure, A. Versavel, M. Dalvai, C. Cao and G. Matthias, in collaboration with A. G. Rolink (University of Basel)

We showed previously that the absence of the coactivator OBF1 leads to multi-focal defects in the lymphoid system. The most prominent phenotype in OBF-1-/- mice is a severely impaired immune response correlating with a lack of germinal centers (GC) in secondary lymphoid organs and there are also several developmental defects. In addition, OBF-1 has several isoforms, one of which is a nuclear protein (p34) whilst the other (p35) is generated from a precursor and, because of the myristoylation of its N-terminus, is localized at the cytoplasmic membrane. To define the biological role of these different isoforms, we used BAC modification technology to generate mice sele-

![Fig. 1. A Early B cell differentiation stages showing the expression of markers or immunoglobin (IgM) at the surface. Curved arrows Relative cellular proliferation activities. Top Pattern of cre expression of deleter mice. B Flow cytometry of single (HDAC1^F/F + mb1-cre) or HDAC2^F/F + mb1-cre) or double (HDAC2^F/F + mb1-cre) knockout mice. Individual dot plots for spleen cell suspensions stained with the indicated antibodies. Numbers are percentage cells in the respective quadrant.](image)
tively expressing each isoform at normal endogenous levels. Analysis of the resulting mice, as well as gene expression profiling of cells that only express the nuclear isoform p34 has allowed us to define specific functions for different OBF-1 isoforms.

Friend of GATA1 (FOG1) is a co-regulator of the transcription factor GATA1 that interacts with various epigenetic regulators, such as CtBP or the NuRD complex. It has been shown previously to be critical for the development of megakaryocytes and erythroid cells, and it can also modulate cell plasticity. However, its expression or function in B cells has not been addressed so far. We found that FOG1 is expressed in early B cells, where its transcription is indirectly controlled by OBF1. We have manipulated the expression of FOG1 in B cells and revealed an important novel role for this factor in B cell progenitors. Furthermore, since GATA factors are not expressed in B cells, our studies identify a novel mode of action for FOG1.

GENOMIC REGULATION BY HDAC1 AND HDAC2

T. Yamaguchi, F. Cubizolles, N. Reichert, G. Matthias, A. Manoharand and C. Cao, in collaboration with C. Seiser (Biocenter and Medical University of Vienna, Austria)

Given the important role of protein acetylation in mammals, we have begun a genetic analysis of several histone deacetylases. The class I enzymes HDAC1 and HDAC2 are highly homologous nuclear proteins that are usually co-expressed and found as components of chromatin-regulating complexes such as Sin3, NuRD and Co-REST. Recruitment of these HDAC1/2-containing complexes to promoter regions usually results in gene repression. While HDAC1 activity is essential during mouse embryogenesis, HDAC2 is not. Absence of HDAC2 leads to partial perinatal lethality but also to viable mice of reduced size. Thus, these two enzymes can not entirely compensate for each other during embryogenesis and are likely to have specific functions in diverse biological systems.

We have generated mice conditionally targeted at the HDAC1 or HDAC2 loci and used these to generate cell-specific deletion of these enzymes. We have examined the role of these proteins in particular in the hematopoietic system and in B lymphocytes. Our studies have identified several critical functions for HDAC1 and HDAC2 in these systems. In the absence of both enzymes, the development of multiple hematopoietic lineages was found to be dramatically affected, in part owing to impaired proliferation of hematopoietic progenitor cells. In the absence of only HDAC1 or HDAC2, hematopoietic development was largely normal. Within the B cell lineage, we found these enzymes to be required at distinct stages of early B cell differentiation; they also have a strong impact on cell cycle progression. In the absence of both enzymes, peripheral mature B cells were absent and a developmental block was observed at the preB stage in the bone marrow. The rare preB cells that could be isolated exhibited a strong cell cycle block in the G1 phase (Yamaguchi et al. 2010).

Furthermore, HDAC1 and 2 were found to play an important role in the development of a normal immune response and to also contribute to immunoglobulin gene regulation. To perform mechanistic studies, we ablated HDAC1 and/or HDAC2 in primary mouse embryo fibroblasts (MEFs). In these cells, the absence of HDAC1 or HDAC2 had slightly different effects on cellular proliferation. However, in the absence of both HDAC1 and HDAC2, a G1 cell-cycle block was observed, as in B lymphocytes. In this case, we demonstrated that this is due to upregulation of the small CDK inhibitors p21 and p57 and showed that the corresponding genes are normally under direct control of HDAC1 and HDAC2. In the absence of these two enzymes, wide-ranging changes in gene expression and also histone modification patterns were observed, revealing complex feedback mechanisms between different histone modifications such as acetylation and methylation.
NON-GENOMIC REGULATION BY HDAC-6

O. True, G. Matthias, Y. Miyake, SH Kwon and C. Cao, in collaboration with S. Khochbin (Institut Albert Bonniot, Grenoble)

HDAC-6 is a class II histone deacetylase with a unique primary structure comprising two conserved hdac domains. Unlike most other HDACs, it is not found in the cell nucleus but resides mostly in the cytoplasm, where it partly co-localizes with the microtubule network. Importantly, HDAC-6 binds with high affinity to ubiquitin or ubiquitinated proteins and can, thus, have an impact on processes involving ubiquitination, such as proteasome-mediated protein degradation. We demonstrated previously that HDAC-6 deacetylates microtubules both in vitro and in vivo and is, therefore, a tubulin deacetylase (TDAC) regulating microtubule acetylation.

While it has been known for a long time that microtubules can be reversibly acetylated, the significance of this modification for microtubule function is still not fully understood. We generated MEFs and also mice lacking HDAC-6 function and found, surprisingly, that HDAC-6-/- mice are viable and appear largely normal. In various organs of these mice, as well as in HDAC-6-deficient MEFs, microtubules were heavily hyperacetylated, thereby confirming that tubulin is a physiological substrate of HDAC-6 and that this enzyme is the main physiological TDAC. Remarkably, the increase in tubulin acetylation was found to vary greatly between different organs, ranging from almost no change in the brain to a 20-fold increase in the testis. In spite of this, testis function was normal. Our studies also identified novel functions for HDAC-6 in responses to stress. In particular, HDAC-6 was found to sense the accumulation of misfolded proteins and to control the ensuing cellular response. Furthermore, we found that HDAC-6 is a novel component of the stress granules (SGs) that form rapidly in response to most kinds of stress and in which mRNAs are protected from degradation. We showed that SGs also contain ubiquitinated proteins and hypothesize that HDAC-6 may play a role in regulating the assembly of SGs through its capacity to bind to microtubules as well as ubiquitinated proteins.

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Antoine Peters

Epigenetic control of mammalian germ line and early embryonic development

INTRODUCTION

Mammalian development starts with the fusion of two highly differentiated, transcriptionally silent germ cells, leading to the formation of the totipotent embryo. Totipotency enables embryonic cells to proliferate and ultimately differentiate into a multitude of distinct cell types with specialized functions. The identity of the descendant cells is the result of interplay between transcriptional and post-transcriptional regulatory mechanisms translating the genetic information and directing somatic development.

The molecular mechanisms underlying the acquisition of totipotency are largely unknown. Genomes undergo major changes in chromatin states during the early phase of pre-implantation development. It is widely thought that these changes enable acquisition of totipotency. The potential of such “epigenetic reprogramming” was illustrated by nuclear transfer experiments, where the epigenome of a fully differentiated somatic cell is reprogrammed by the cytoplasm of an enucleated oocyte, resulting in living offspring. The success rate of reproductive cloning is, however, very low compared with natural reproduction. In contrast to terminally differentiated cells, the genomes in germ cells may hold and/or acquire during germ cell development certain chromatin traits that provide gametes the competence to support the major changes in cell identity occurring after fertilization.

Using conditional mouse mutants, we study the role of histone methyltransferases and Polycomb group proteins for epigenetic programming in primordial germ cells, maturing oocytes, developing male germ cells and pre-implantation embryos. We further investigate the transgenerational contribution of certain chromatin modifications to the regulation of gene expression in early embryos. These studies will contribute to our understanding of the regulatory role of chromatin in defining cellular identity in conditions of health and disease.
Epigenetics

PcG PROTEINS AND EARLY EMBRYONIC DEVELOPMENT

R. Kunzmann, P. Nestorov, E. Posfai

In early embryos, the two parental genomes differ greatly in chromatin constitution, reflecting the history of chromatin maturation during oogenesis and spermatogenesis. Whereas the maternal genome is transmitted in a nucleosomal configuration, carrying many post-translational modifications on histone proteins, the paternal genome is largely packaged by protamines in a more condensed configuration. Shortly after gamete fusion, protamines are rapidly exchanged for maternally provided histones that become post-translationally modified in a temporally and spatially coordinated but mechanistically unknown manner during the following cell cycles. The first few cell cycles may represent a “window-of-opportunity” for epigenetic reprogramming of certain histone modifications to equalize differences between parental genomes. These complex changes in chromatin states are remarkably similar to chromatin reprogramming events occurring during primordial germ cell development.

During recent years, several maternal effect genes have been identified that regulate chromatin states and gene expression in the early embryo. In such cases, maternal transcripts and/or proteins are essential for embryogenesis. We recently identified transgenerational transmission of methylated histones as a novel form of maternal contribution (Puschendorf et al. 2008). We further revealed that in 1- to 8-cell embryos chromatin at the maternal genome is characterized by repressive histone H3 lysine 9 (H3K9) tri-methylation, whereas the paternal genome is extensively decorated by proteins of the Polycomb Reppressive Complex 1. We aim to dissect the mechanism and functional significance of parental asymmetry in repressive chromatin states for the start of global transcription at the late one-cell and two-cell stages and for subsequent lineage specification. In particular, we focus on Polycomb group (PcG) proteins that serve evolutionary conserved roles in regulating gene expression and maintaining cell identity in stem cells and during cellular differentiation.

PATERNAL TRANSMISSION OF EPIGENETIC INFORMATION

U. Bryczyszynska, M. Hisano and S. Erkek,
in collaboration with M. Stadler, D. Schübeler (FMI) and L. Ramos (Radboud University, Nijmegen)

At the end of spermatogenesis, about 90% and 99% of histones present in developing human and mouse germ cells are substituted by smaller, arginine-rich protamine proteins that mediate chromatin condensation and direct the structural reorganization of the sperm nucleus into the elongated form required for motility. After fertilization, protamines are re-exchanged for maternally provided histones that then acquire post-translational modifications such as methylation at conserved histone lysine residues. The mechanism targeting histone methyltransferases to specific regions of the paternal genome is unknown. Paternally inherited modified histones may influence the acquisition of modifications in cis on newly deposited histones after fertilization, thereby coordinating transcriptional activation of the paternal genome in one- and two-cell embryos.

To study whether histones and their potential modifications function in paternal transmission of epigenetic information, we determined the genomic distribution of nucleosomes in spermatozoa of fertile human individuals using high-throughput sequencing. In all individuals, we found nucleosomes along the entire genome, with moderate enrichment at gene regulatory regions, suggesting a limited potential for transgenerational transmission of histone-encoded information via sperm (Figure 1). We also studied the genomic distribution of H3K4 di-methylation (H3K4me2) and H3K27 tri-methylation (H3K27me3) in human spermatozoa, two modifications important for Trithorax and Polycomb-mediated gene regulation during somatic development. For this, we performed chromatin immuno-precipitation with modification-specific antibodies and detection of precipitated DNA by hybridization to micro-arrays (ChIP-chip methodology). Both modifications showed methylation-specific distribution at regulatory regions in human sperm. H3K4me2-marked promoters control gene functions in spermatogenesis and cellular homeostasis, indicating that this mark reflects germline transcription. In contrast, H3K27me3 marked promoters of key developmental regulators in sperm as in soma. Importantly, comparison with genome-wide expression data sets showed in particular that genes with extensive H3K27me3 coverage around transcriptional starts sites in spermatozoa are not expressed during male or female gametogenesis, or in pre-implantation embryos. Moreover, promoters of orthologous genes were similarly modified by H3K4me2 and H3K27me3 in mouse spermatozoa.
These data are compatible with an evolutionary conserved role for Polycomb proteins in repressing somatic determinants across generations, potentially in a variegating manner (Brykczynska et al. 2010).

We now aim to identify the mechanism underlying the enhanced retention of modified histones at regulatory regions of genes. We will examine whether modified histones in spermatozoa are part of an intrinsic transgenerational developmental program and/or whether they function in response to environmental cues experienced by the father.

**PcG Proteins and Primordial Germ Cell Development**

*S. Yokobayashi*

In mammals, the germ line is newly formed from a pool of differentiating epiblast cells in gastrulating embryos. Germ cell formation is a long developmental process characterized by several waves of genome-wide epigenetic reprogramming processes. It starts with the specification of primordial germ cells (PGCs), which is characterized by major changes in gene expression, with repression of the somatic program and activation of a germ cell program. PGCs subsequently migrate from the epiblast via the hindgut endoderm towards the future gonads. At some point during their migration, PGCs become arrested in the G2 phase of the cell cycle and enter a transcriptional quiescent state. During this phase, PGCs extensively remodel the genome-wide landscape of repressive chromatin modifications. For example, they start to erase genome-wide DNA methylation and H3K9 dimethylation and acquire high levels of H3K27me3. Upon re-entry into the cell cycle and arrival into the gonads, PGCs further erase DNA methylation at repetitive sequences and imprinting control regions and undergo an additional remodeling of chromatin states. The mechanisms and biological significance of the various waves of chromatin reprogramming are currently not well understood. It is, however, widely speculated that the epigenetic reprogramming processes may contribute to the acquisition of totipotency (Figure 2). Our research is focused on elucidating the role of Polycomb group proteins and H3K27me3 in epigenetic reprogramming during PGC development.

**Epigenetic Regulation of Genomic Imprinting**

*R. Terranova, S. Yokobayashi*

In placental mammals, about 80 genes are expressed during development in a parent-of-origin specific manner. Most such “imprinted” genes are involved in placental and embryonic development or in postnatal neurological processes. Mono-allelic imprinted expression implies that each gamete, male or female, provides information that directs the transcriptional state for an allele after fertilization. Correct establishment and maintenance of allele-specific transcriptional states is vital and deregulation of imprinted gene expression is associated with developmental defects, cancer and behavioral syndromes in humans.

Most imprinted genes are clustered in large chromosomal domains and are controlled by cis-acting “imprinting control regions” (ICRs). At all known imprinted loci, the ICR is differentially methylated, harboring allelic DNA methylation inherited from the maternal or paternal gamete. Following acquisition during oogenesis or spermatogenesis, these DNA methylation marks are maintained in the zygote and reliably transmitted throughout development. Ultimately, the epigenetic features at ICRs are translated in various ways to ensure proper parental allele-specific silencing of neighboring genes. At each generation, DNA →

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**Fig. 2.** Expression of the pluripotency marker Oct3/4 in germ cells in female and male gonads at embryonic day 12.5 of development

**Fig. 3.** Genomic contraction of the paternal Kcnq1 imprinted cluster in trophodermal cells of the early mouse embryo. Green and red signals represent DNA regions flanking the Kcnq1 cluster, whilst the yellow signal denotes paternal Kcnq1ot1 expression.
methylations, forming a discrete repressive nuclear compartment devoid of RNA polymerase II. We noted a similar configuration at the Igf2r imprinted cluster. In RNA–DNA FISH experiments, we observed that the paternal Kcnq1 cluster exists in a three-dimensionally contracted state within the repressive compartment (Figure 3). Conditional mutational analyses showed that PcG proteins Ezh2 and Rnf2 are independently required for genomic contraction as well as imprinted silencing in vivo. The data suggest that the formation of a parental-specific higher-order chromatin organization renders imprint clusters competent for mono-allelic silencing. Further, our data assign an unprecedented role to PcG proteins in organizing large gene clusters for repression (Terranova et al. 2008).

**DOMINANT MALE STERILITY**

U. Brykczynska, in collaboration with S. Haueter and P. Pelczar (University of Zurich)

Transgenic mice are vital tools in both basic and applied research. Unfortunately, the transgenesis process and other assisted reproductive techniques involving embryo transfer rely on vasectomized males to induce pseudopregnancy in surrogate mothers. Vasectomy is a surgical procedure associated with moderate pain. To eliminate the need for vasectomy, we developed a transgene-based approach. Overexpression of a protamine1-EGFP fusion protein during spermiogenesis caused dominant sterility in male mice due to impaired spermatid maturation affecting sperm viability and motility. To facilitate genotyping, we linked the sterility trait to whole-body EGFP expression (Figure 4). This double-transgenic approach is a reliable and cost effective “genetic vasectomy” procedure making surgical vasectomy obsolete (Haueter et al. 2010).

**Selected publications**


INTRODUCTION

Individual cell types are defined by the particular set of genes they express. How is the genome organized so that the right gene is activated when needed and kept silent at other times? Part of this organization relies on changing the packaging of DNA by modifying proteins within nucleosomes (histones) or by methylating the DNA without changing its sequence. These epigenetic modifications provide an additional layer of information that, in concert with the genetic sequence, regulate genome readouts.

Such epigenetic information can restrict or enhance sequence-specific recognition of DNA and thus can modulate cellular responses to external and internal cues. Current models suggest that this epigenetic information specifies cellular identity and potency and that it behaves highly dynamically during cellular differentiation and transformation. Indeed misregulation of proteins involved in epigenetic regulation of chromatin and DNA have been linked to many human diseases, including cancer.

We are using Drosophila cells and mammalian stem cell models to ask how chromatin structure, DNA methylation and replication crosstalk to transcription. Towards this goal, we combine molecular biology and functional genomic approaches. This enables us to monitor the epigenome and its dynamics in an unbiased way and to generate regulatory models from genome-wide datasets, which we test in cellular models by genetic perturbation. Our aim is to understand the control of epigenetic gene regulation and its role in cellular reprogramming events during development and disease.
TEMPORAL PLASTICITY AND REGULATION OF GENOME REPLICATION

M. Schwaiger and C. Wirbelauer, in collaboration with M. Stadler and H. Kohler

Duplication of eukaryotic genomes during S phase is coordinated in space and time. In order to identify zones of initiation and both cell-type and gender-specific plasticity of DNA replication, we profiled replication timing, histone acetylation and transcription throughout the *Drosophila* genome. We observed two waves of replication initiation, with many distinct zones firing in early S phase and multiple, less-defined peaks at the end of S phase, suggesting that initiation becomes more promiscuous with time. A comparison of different cell types revealed widespread plasticity of replication timing on autosomes. Most occur in large regions but only half coincide with local differences in transcription. In contrast to confined autosomal differences, a global shift in replication timing occurs throughout the single male X chromosome. Unlike in females, the dosage-compensated X chromosome replicates almost exclusively early. This difference occurs at sites that are not transcriptionally hyperactivated but show increased acetylation of lysine 16 of histone H4. This suggests a transcription-independent yet chromosome-wide process related to chromatin. Importantly, H4K16ac is also enriched at initiation zones as well as in early replicating regions on autosomes during S phase. Together, our data reveal novel organizational principles of DNA replication of the *Drosophila* genome and suggest that H4K16ac is more closely correlated with replication timing than is transcription (Figure 1; Schwaiger et al. 2009).

We further demonstrated that the integrity of genome replication depends on HP1, an important protein constituent of heterochromatin. Our studies revealed that HP1 is required to maintain late replication of repetitive DNA but also important for the correct initiation of very early initiation events during S-phase (Schwaiger et al. Genome Res, in press). This further supports the notion that chromatin structure plays important roles in the temporal and spatial organization of genome duplication.

GENOME ACCESSIBILITY AS A FUNCTION OF CHROMATIN STRUCTURE

O. Bell, M. Schwaiger and F. Lienert, in collaboration with M. Stadler

Histone modifications are thought to regulate gene expression in part by modulating DNA accessibility and higher-order chromatin structure. However, there is limited direct evidence to support structural differences of chromatin fibers in the nucleus. To ask how histone modifications relate to chromatin compaction, we have developed an approach to measure DNA accessibility throughout the genome. We expose nuclear chromatin from *Drosophila* cells to a bacterial DNA methyltransferase (SssI) and measure the resulting DNA methylation by immunoprecipitation (MeDIP-footprint). Using this approach, we could show that DNA accessibility in the *Drosophila* genome reflects differential distribution of active and repressive histone modifications. Active promoters are highly permissive to M.SssI activity but inactive chromosomal domains decorated with H3K27me3 are least accessible, providing in vivo evidence for Polycomb-mediated chromatin compaction. Conversely, DNA accessibility is increased at chromosomal regions marked with H4K16ac and at the dosage-compensated male X-chromosome, suggesting that transcriptional dosage compensation is facilitated by more permissive chromatin structure. Interestingly, early replicating chromosomal regions and sites of replication initiation also show higher accessibility, linking temporal and spatial control of genome duplication to the structural organization of chromatin.

These complex patterns of differential chromatin accessibility challenge the binary distinction into euchromatin and heterochromatin and argue that differential histone modifications and the organization of transcription and replication have distinct and measurable effects on the exposure of the DNA template (Bell et al. Nat Struct Mol Biol, in press).

**Fig. 1.** Interplay between chromatin and DNA replication in a 1.3-Mb stretch of *Drosophila* chromosome 2L. y-axis replication timing (a high positive value indicates replication early in S-phase); x-axis chromosomal position; blue, upper graph presence of histone acetylation at lysine 16 of histone H4 (H4K16ac); green, lower graph presence of RNA; arrow a region replicating early, showing a high level of H4K16ac but no transcriptional activity
EPIGENETIC REPROGRAMMING IN THE MAMMALIAN GENOME

F. Mohn, F. Lienert, L. Hoerner, T. Roloff, V. Tiwari, S. Tippmann, T. Baubec, R. Murr and R. Ivanek, in collaboration with M. Stadler (FMI) and M. Bibel (Novartis)

Stem cells and multipotent progenitor cells face the challenge of balancing the stability and plasticity of their developmental states. Their self-renewal requires the maintenance of a defined gene-expression program that must be stably adjusted towards a new fate upon differentiation. Recent data imply that epigenetic mechanisms can confer robustness to steady-state gene expression but can also direct the terminal fate of lineage-restricted multipotent progenitor cells.

We have tested these models by dissecting epigenome reprogramming during stem cell differentiation. Mammalian stem cells not only allow the study of pluripotency but also provide a unique cellular model to understand how stable developmental gene expression patterns are acquired and maintained. We have used a murine system that progresses from stem cells to lineage-committed progenitors to terminally differentiated neurons in order to identify sites of epigenetic reprogramming in the genome. Our results show that several hundred promoters, including pluripotency and germline-specific genes, become DNA methylated in lineage-committed progenitor cells, suggesting that DNA methylation contributes to repression of pluripotency already in progenitor cells (Figure 2, Mohn et al. 2008).

We also studied the Polycomb system during in vitro neurogenesis of stem cells as a separate repressive pathway that functions via histone modifications. Our studies revealed extensive reorganization entailing loss and acquisition of Polycomb-mediated histone modifications at additional targets in both progenitor and terminal states. Together, these findings suggest a model of how de novo DNA methylation and dynamic switches in Polycomb targets restrict pluripotency and define the developmental potential of progenitor cells (Mohn et al. 2008). In ongoing efforts, we aim to further understand how epigenetic repression contributes to cellular identity and how development-specific targets are specified. In particular, we test individual DNA sequences for their ability to mediate cell-type-specific states of DNA methylation and chromatin in order to define the crosstalk and regulatory logic between genetic and epigenetic pathways.

Selected publications


Signaling and cancer Our growth control program strives at a comprehensive understanding of signaling circuits and mechanisms regulating the growth, division and death of cells. A deeper knowledge of these processes should further the development of innovative, mechanism-based therapeutics to counter many human diseases.

Joy Alcedo
Sensory mechanisms that influence animal physiology and longevity

Mohamed Bentires-Alj
Biology of breast development, cancer and metastasis

Ruth Chiquet-Ehrismann
Cell communication in growth control and cancer

Brian A. Hemmings
Targeting the cancer kinome

Nancy Hynes
The molecular basis of breast cancer

Nicolas Thomä
The molecular basis of genome maintenance
INTRODUCTION

An animal’s sensory system interprets complex environments and accordingly generates appropriate behavioral and physiological responses. Interestingly, recent studies have shown that the sensory systems of *C. elegans* and *Drosophila* not only influence the behavior and physiology of these animals but also their lifespan. This sensory influence involves subsets of gustatory and olfactory neurons that either shorten or lengthen lifespan, which suggests that some of the cues affecting lifespan are food-derived and that these cues can exert different lifespan effects. Since restricting food levels can increase lifespan, it is possible that the sensory system has an influence simply by regulating general food intake. Indeed, the sensory system has been implicated in lifespan effects of food-level restriction in *Drosophila*. However, the sensory influence on lifespan, at least in *C. elegans*, can be uncoupled from the sensory effects on feeding rate, development and reproduction. Since the effect of food-level restriction on lifespan has been linked to decreases in feeding and developmental rates and reproductive output, this suggests that the sensory system also affects lifespan through other mechanism(s).

Sensory neurons influence lifespan by modulating signaling pathway activities that control animal physiology. One such pathway is insulin/IGF-1, which has been proposed to affect aging by coordinately regulating the expression of many lifespan-influencing genes. Still, the mechanisms that would mediate the sensory influence on lifespan remain unclear. Thus, we aim to determine (1) the nature of the sensory cues that affect lifespan, (2) the signaling pathways regulated by these cues and the mechanisms by which they integrate sensory information to influence lifespan, and (3) the exact nature of the physiological changes mediating this influence on lifespan.
A NEUROMEDIN U RECEPTOR ACTS WITH THE SENSORY SYSTEM TO MODULATE FOOD TYPE-DEPENDENT EFFECTS ON *C. ELEGANS* LIFESPAN

W. Maier, B. Adilov, M. Regenass, A. Neagu, J. Alcedo

The sensory system has previously been shown to mediate the effects of food intake on lifespan (Figures 1 and 2). However, the lifespan of an animal is affected not only by the level of its food intake but also by the type of food source. Therefore, we examined the role of the sensory system in food-source influence on *C. elegans* lifespan and the signaling pathway(s) that might be involved in this process.

We found that different subsets of sensory neurons alter the effects of different *E. coli* food sources on *C. elegans* longevity. We also showed that the sensory system acts with a homologue of mammalian neuromedin U receptors, nmur-1, to affect lifespan in a food source-dependent manner. Wild-type nmur-1, which is expressed in the somatic gonad, sensory neurons and interneurons, shortens lifespan only on specific *E. coli* strains, an effect dependent on the type of *E. coli* lipopolysaccharide (LPS) structure. Moreover, unlike the effect of food-level restriction, the food type-dependent effect of nmur-1 on lifespan can be uncoupled from nmur-1 effects on feeding rate, development and reproduction.

Together, our data suggest that (1) sensory neurons recognize food types and by doing so affect lifespan, and (2) the nmur-1 neuropeptide signaling pathway is involved in this process. Furthermore, our study leads us to propose that the two forms of dietary influence on lifespan – food type-dependence and food-level restriction – employ distinct but overlapping mechanisms. Thus, nmur-1 appears to process information from specific food cues, such as *E. coli* LPS structure, and thus influence lifespan and other aspects of physiology.

Currently, we are studying the cellular and molecular bases by which NMUR-1 modulates *C. elegans* physiology and lifespan in a food source-dependent manner. We aim to identify the cellular sites of nmur-1 function not only in lifespan but also in other processes, which should further help determine the type of sensory or non-sensory cues that might regulate the different activities of this gene. We also aim to identify and characterize the NMUR-1 neuropeptide ligand(s), as well as the regulators and downstream effectors of NMUR-1, in order to understand how this pathway integrates environmental information with signaling pathways known to regulate physiological responses affecting lifespan.

Finally, the food source-dependent effects of nmur-1 raise the intriguing likelihood that additional neuropeptide signaling pathways also influence lifespan and/or other physiological processes under different environmental conditions. This may suggest that the large repertoire of neuropeptides and their receptors in *C. elegans* and other animals serve to translate the complexity of the environment into physiological responses that optimize survival.

FUNCTIONAL CHARACTERIZATION OF *C. ELEGANS* INSULIN-LIKE GENES IN DEVELOPMENT AND LIFESPAN

A. Cornils, M. Glöck, P. Fardel, M. Thomas, J. Alcedo

The sensory influence on *C. elegans* longevity is largely mediated by the insulin/IGF-1/DAF-2 pathway. The worm genome is predicted to have forty insulin-like genes divided into four classes and many of these genes are expressed in partly overlapping sensory neurons. However, it is not known whether all or only a subset of these genes mediate the sensory influence on lifespan.

The numerous predicted insulin-like ligands suggest a complex regulation of the DAF-2 pathway, which controls a number of processes such as development, metabolism and lifespan. Hence, some of these ligands may act redundantly or the release of each ligand may be triggered by a different sensory cue. In addition, the ligands perhaps act on DAF-2, the worm insulin/IGF-1 receptor, in different tissues or at different times. Finally, the ligands may bind the DAF-2 receptor with different affinities and promote different levels of DAF-2 activity, which would lead to the expression of different target genes. Surprisingly, whereas some of the insulin-like genes (ins-4, ins-6, ins-7 and daf-28) have been proposed to encode DAF-2 agonists, at least two further insulin-like genes (ins-1 and ins-18) have been proposed to encode DAF-2 antagonists. Thus, understanding the function of the various predicted DAF-2 ligands should elucidate...
Fig. 2. Specific gustatory and olfactory neurons influence C. elegans lifespan. 

A Certain gustatory neurons inhibit longevity, but other neurons promote longevity; both classes appear to modulate insulin/IGF-1 signaling and thus affect worm lifespan.

B Diagram of gonad precursors showing that germ-line cells (green circles) inhibit longevity; somatic gonad (blue circles) promotes longevity in an olfactory neuron-dependent manner.

How DAF-2 signaling is modulated to regulate different biological processes.

We have analyzed the developmental and lifespan phenotypes of null deletion mutations that affect a subset of insulin-like genes. Such an approach allows direct study of the relative contributions of different insulin-like peptides to C. elegans development and lifespan, in contrast to earlier studies involving indirect manipulations of insulin-like gene function. Accordingly, unlike previous results, our data not only suggest that the insulin-like peptides have distinct and non-redundant functions but also indicate the existence of an insulin-like peptide code that regulates different physiological processes in response to specific sets of environmental cues. In the future, we will test further how different sensory stimuli regulate the function of each insulin-like peptide. We will also compare the transcriptional read-outs of the different peptides. Finally, we plan to test whether the variation in peptide signaling activities results from differences in receptor-binding affinities.

The Sensory Influence on Lifespan is Conserved in Drosophila

I. Ostojic and J. Alcedo, in collaboration with W. Boll and M. Noll (University of Zurich)

Many biological processes are conserved between C. elegans and higher organisms. For example, the insulin/IGF-1 pathway regulates the physiology and consequently the longevity of worms, flies and mice. Thus, the sensory systems of other animals might also influence their lifespan.

We have tested this hypothesis by determining whether Drosophila taste mutants also have lifespan phenotypes. We have found that flies lacking a subset of taste inputs live long and exhibit physiology that does not resemble animals with restricted food intake. These lifespan-influencing taste inputs originate from fly mouthparts known as labella and are innervated by taste neurons that extend processes to the subesophageal ganglion (SOG), a region in the brain that relays gustatory information. Since SOG interneurons have been shown to project axons to or near insulin-producing cells in the fly brain, we also tested whether these flies have altered insulin pathway activity. Indeed, the lifespan of flies lacking labellar taste inputs requires the activity of dFOXO, a transcription factor mediating the effects of insulin signaling.

On the other hand, we found that loss of additional taste inputs from other parts of the fly body suppressed the extended lifespan phenotype of labellar taste-impaired flies and further shortened the lifespan of dFOXO mutants. This suggests that (1) the gustatory influence on Drosophila lifespan involves both positive and negative sensory inputs, as in C. elegans, and (2) some taste inputs affect lifespan through a second pathway acting in parallel to insulin signaling.

Our data and recent work of the group of Scott Pletcher, which shows that olfactory perception also influences fly lifespan, raise the possibility that the mammalian sensory system also affects lifespan. Since both gustatory and olfactory information are relayed to the hypothalamus, a region in the mammalian brain that controls behavior and physiology, the processing of this information by the hypothalamus may in turn modulate lifespan.

Finally, because the sensory influence on lifespan is mediated by peptides that could act as hormones, whose homologues in several animals have been shown to affect homeostatic set points, it is possible that the sensory influence on lifespan involves alterations in homeostasis. Thus, identification of set points that may be altered bi-directionally by lifespan-influencing sensory neurons could yield insight into mechanisms regulating the rate and/or onset of aging in response to the changing quality of the animal’s environment.

Selected publications


INTRODUCTION

Each year, breast cancer is diagnosed in over one million women worldwide and more than 400,000 lives are lost to this disease. Although some patients do well after surgery and initial treatment, drug resistance often occurs and tumors relapse. Metastasis is the ultimate, usually fatal step in cancer progression and its underlying cellular and molecular mechanisms are still poorly understood. Improved understanding of breast cancer initiation and progression, drug resistance and the factors determining metastatic spread are urgently needed. A more thorough understanding of the differentiation program of cells in which cancer originates, of the genetic and epigenetic alterations that transform these cells, and of the cross-talk between cancer cells and the stromal environment may lead to new therapies.

Our group focuses on signaling networks governing breast development and the pathophysiology of breast cancer, with special emphasis on protein-tyrosine phosphatases (PTPs) and the phosphatidylinositol 3-kinase (PI3K) pathway. We also investigate the molecular mechanisms underlying the cross-talk between normal and neoplastic stem/progenitor cells and their niches. We aim ultimately to identify and validate novel targets for therapy.
Signaling and cancer

MOLECULAR MECHANISMS OF MAMMARY GLAND DEVELOPMENT
E. Milani, F. Meier-Abt

The breast epithelium is embedded in a mixture of connective tissue and fat (the stroma) comprising a large network of ducts radiating from the nipple and leading to the lobules (Figure 1A). The lobules are the secretory unit of the breast (parenchyma) and consist of a cluster of ductules (a.k.a. acini or alveoli). Each duct and acinus is composed of a continuous layer of luminal epithelial cells surrounded by contractile myoepithelial cells and a basement membrane. Adult human breast epithelial stem cells from which the luminal epithelial and myoepithelial lineages derive were isolated recently (Figure 1B).

Protein-tyrosine phosphorylation, regulated by protein-tyrosine kinases (PTKs) that add and protein tyrosine phosphatases (PTPs) that remove the phosphate, plays vital roles in proliferation, differentiation, apoptosis and cell migration/invasion and is critical for normal breast development and differentiation. For example, tyrosine phosphorylation regulates the prolactin receptor pathway in breast differentiation but the roles of specific PTPs in this pathway are not well understood. We study PTPs in mammary gland differentiation and ask whether deregulation of specific PTPs affects breast carcinogenesis.

Early menarche, late menopause and late age of first pregnancy are all known risk factors for sporadic breast cancer. Conversely, early full-term pregnancy (<24 years) decreases lifetime breast cancer risk by up to 50%. Clearly the hormonal milieu and breast development cycles, possibly through changes in the differentiation state of breast stem/progenitor cells and the stroma, affect the susceptibility of the breast to oncogenic transformation. Moreover, almost all the signaling pathways governing normal breast development are subverted in breast cancer. We examine changes in mouse mammary gland cells and aim to understand the molecular mechanisms underlying the protective effect of early full-term pregnancy.

ROLES OF PROTEIN-TYROSINE PHOSPHATASES IN BREAST CANCER
N. Aceto, K. K. Balavenkatraman, A. Britschgi, A. Alajati, N. Sausgruber

Abnormal tyrosyl phosphorylation underlies various diseases of deregulated proliferation, apoptosis and differentiation, including cancer. Several PTKs enhance tumorigenesis (e.g., the human oncogene ErbB2, a.k.a. HER2/Neu, in breast cancer) and some of these kinases are the targets of rationally designed therapies (e.g., Trastuzumab). Although the roles of particular PTKs in breast carcinogenesis have been studied extensively, the role of specific PTPs is poorly understood and represents a major focus of our group.

Initially, the PTP superfamily was thought to...
play predominantly signal-attenuating (negative) “housekeeping” roles. Accordingly, it was expected that PTPs function as tumor suppressors and are thus intractable drug targets. However, recent studies have shown that PTPs have both positive and negative functions in cell signaling. For example, we identified two PTPs, SHP2 (downstream of GAB2) and PTP1B, that play positive, signal-enhancing roles in breast cancer progression and could, therefore, be appropriate therapeutic targets. We have generated and validated tetracycline-inducible shRNA against PTPs and aim to identify and validate PTPs involved in breast cancer initiation and metastasis, with special emphasis on PTP1B and SHP2.

In 3D cultures, where cells grow in a matrix rich in laminin and collagen IV (Matrigel), immortalized but non-transformed MCF10A cells show features of normal mammary cells, forming growth-arrested, polarized acinar structures with a hollow lumen. By infecting MCF10A cells with different combinations of human oncogenes, we generated ex vivo models that mimic changes seen in the course of neoplastic progression, such as increased epithelial proliferation, loss of acinar organization, migration/invasion, luminal filling (that characterize ductal carcinoma in situ) and invasion of the extracellular matrix (Figure 2A).

Tumorigenesis and progression to metastasis are complex, multi-step processes of primary tumor growth, local invasion, migration of the primary tumor into blood and/or lymphatic vessels, extravasation and invasion of secondary sites, and tumor cell proliferation at ectopic locales. To address whether candidate PTPs play a role in tumor progression, we use several in vivo approaches. For example, to study the role of candidate PTPs in the transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma, we inject cancer cells expressing shRNA against specific PTPs into the principal duct of the mammary gland and monitor the effect of knockdown on their capacity to invade the surrounding stroma (Figure 2B).

**ROLES OF PIK3CA MUTATIONS IN BREAST CANCER**

The phosphatidylinositol 3-kinase (PI3K) pathway plays a central role in diverse cellular functions, including proliferation, growth, survival, differentiation and migration. Class IA PI3K lipid kinases phosphorylate membrane phosphatidylinositol-4,5-bisphosphate (PIP2) generating phosphatidylinositol-3,4,5-trisphosphate (PIP3). This reaction is reversed by the tumor suppressor phosphatase PTEN.

The PI3K pathway can be subverted during neoplastic transformation and aberrations of the PI3K pathway are common in cancer. By decreasing cell death, increasing cell proliferation, migration, invasion, metabolism, angiogenesis and resistance to chemotherapy, an aberrant PI3K pathway results in cancer cells with a competitive advantage. Hyper-activation of upstream growth factor receptors (e.g., HER2/3) and gain- or loss-of-function of components of the PI3K pathway account for the frequent aberration of the pathway in breast cancer.

**PIK3CA**, the gene encoding the alpha catalytic subunit (p110α) of PI3K, has been found to be mutated with high frequency in several cancers, including endometrial (40 %), colon (30 %), and breast cancer (30 %). p110α is composed of an N-terminal domain (the adaptor-binding domain, ABD), that binds to p85α, a Ras-binding domain (RBD), a C2, a helical, and a kinase catalytic domain. Over 85 % of all mutations occur in two hotspots within the gene: E545K in the helical domain and, most common, H1047R in the kinase domain. These mutations result in constitutive activity of the enzyme, transformation of cells in vitro and increased tumorigenicity in xenograft models. The increase in the lipid kinase activity of PIK3CA makes it a druggable target. We have generated ex vivo and in vivo models of PIK3CA mutations and begun to investigate their biochemical and cell biological consequences. The models are used to test the effects of PI3K inhibitors, some of which are currently in clinical trials.

**HUMAN BREAST PROGENITOR/STEM CELLS AND THEIR Niches**
S. Duss, H. Brinkhaus, A. Britschgi

Stem cells are defined by their capacities for self renewal and differentiation into mature cell types. The balance between these two processes, critical...
Signaling and cancer

For tissue homeostasis, is maintained by cross-talk between stem cells and their niche, the microenvironment within which stemness is maintained. It is no surprise that deregulation of stem cell/niche interaction is found in carcinogenesis but the nature of this deregulation remains ill-defined.

To reconstitute a human breast stem cell niche ex vivo and study the effects of transforming events on stem cell/niche interactions, we have defined culture conditions allowing breast progenitor cells to maintain proliferation and differentiation ex vivo (Figure 3). Using these culture conditions, we will develop ex vivo and in vivo models of breast cancer subtypes and study their interactions with the niche.

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**Selected publications**


INTRODUCTION

Multicellular organisms have a range of cell types constituting different organs with distinct functions. We investigate the importance of cell-cell and cell-extracellular matrix (ECM) interactions for development and for tissue homeostasis in the adult. We focus on the occurrence of degenerative diseases as well as on cancer progression and metastasis caused by disturbed cellular interactions.

Epithelial tissues depend on the presence of stromal cells and the basement membrane for homeostasis, as does the growth of solid cancers. The stromal compartment is the site of angiogenesis, bringing blood that nurtures cancer cells and provides an escape route for metastatic cells. Furthermore, the stroma may provide a favorable environment for cancer stem cells. For these reasons, we focus on proteins expressed in tumor stroma, such as tenasin-C and tenasin-W, and investigate their function in cancer progression.

In connective tissues, mechanical stresses are transmitted from the ECM by physical connections between structural components across the cell membrane to the cytoskeleton and from there to the nucleus. This is exemplified in muscle, where mutations in ECM, cytoskeleton or transmembrane proteins linking the cytoskeleton to the ECM or the nuclear lamina can cause muscular dystrophies. We investigate the importance of this link from the ECM to the nucleus in mechanotransduction and the regulation of tissue-specific gene expression.

Teneurins are transmembrane proteins with a cytoplasmic domain involved in signal transduction and a large extracellular part involved in cell-cell interactions. In the model organism C. elegans, we discovered that teneurin is required for the structural maintenance of basement membranes and that it may represent a novel basement membrane receptor. Since many basic functions of proteins and pathways are conserved between worms and man, we postulate a similar important function for teneurins in cell-ECM interactions in vertebrates.
NOTCH SIGNALING INDUCES TENASCIN-C EXPRESSION IN GLIOBLASTOMAS

M. Degen, in collaboration with M.M. Lino and A. Merlo (University Hospital Basel)

Tenascin-C expression is known to correlate with malignancy in glioblastoma (GBM), a highly invasive and aggressive brain tumor that shows limited response to conventional therapies. We found Notch2 protein to be strongly expressed in these malignant gliomas as well as in GBM cell lines. In a GBM tumor tissue microarray, RBPJk protein, a Notch2 cofactor for transcription, was found to be significantly co-expressed with tenascin-C. We showed that the tenascin-C gene is transactivated by Notch2 in an RBPJk-dependent manner, mediated by an RBPJk binding element in the tenascin-C promoter. The transactivation is abrogated by a Notch2 mutation we detected in the glioma cell line Hs683 that does not express tenascin-C. This L1711M mutation resides in the RAM domain, the site of interaction between Notch2 and RBPJk. In addition, transfection of constructs encoding activated Notch2 or Notch1 increased endogenous tenascin-C expression, identifying tenascin-C as a novel Notch target gene. Overexpression of a dominant negative form of the transcriptional coactivator MAML1 or knocking down RBPJk in LN319 cells led to a dramatic decrease in tenascin-C protein levels accompanied by a significant reduction in cell migration. As the addition of purified tenascin-C stimulated glioma cell migration, this represents a mechanism for the invasive properties of glioma cells controlled by Notch signaling and defines a novel oncogenic pathway in gliomagenesis that could be targeted for therapeutic intervention in GBM patients.

TENASCIN-W, A MARKER OF GLIOMA-ASSOCIATED BLOOD VESSELS, STIMULATES ANGIOGENESIS

E. Martina, M. Degen and F. Brellier, in collaboration with C.R. Rüegg (CPO, University of Lausanne) and M.M. Lino and A. Merlo (University Hospital Basel)

The microenvironment hosting a tumor actively participates in regulating tumor cell proliferation, migration, and invasion. Among the ECM proteins enriched in the stroma of carcinomas are the tenascin family members tenascin-C and tenascin-W. Tenascin-C overexpression in gliomas is known to correlate with poor prognosis and tenascin-C expression is found throughout tumor sections in glioblastoma but only around blood vessels in oligodendroglioma (Figure 1). Tenascin-W expression was upregulated in 80% of brain tumor samples independent of the tumor type, whereas tenascin-W was not detected in control, non-tumoral brain tissues. Co-localization of tenascin-W and the blood vessel marker von Willebrand factor revealed the presence of tenascin-W in blood vessels. Co-staining of tenascin-C and tenascin-W with desmin showed that tenascin-C is co-localized with desmin-positive pericytes surrounding the vessels, whilst tenascin-W was distinct from desmin-positive cells and appeared to be expressed by endothelial cells (Figure 1). In vitro, the presence of tenascin-W increased the proportion of elongated human umbilical vein endothelial cells (HUVECs) and augmented the mean speed of cell migration. Furthermore, tenascin-W triggered sprouting of HUVEC spheroids to an extent similar to that of the proangiogenic factor tenascin-C. In conclusion, our results identify tenascin-W as a candidate biomarker for brain tumor angiogenesis that could be used as a molecular target for therapy irrespective of the glioma subtype.

Fig. 1. Tenascin-C and tenascin-W expression in brain cancer blood vessels. Oligodendroglioma and glioblastoma sections are stained with H&E, antibodies against tenascin-W and tenascin-C. Tenascin-W (TNW) is expressed in endothelial cells, while tenascin-C (TNC) is secreted by desmin-positive pericytes.
REGULATION OF TENASCIN-C EXPRESSION

M. Asparuhova, J. Ferralli, M. Schmidt, M. Chiquet

Mechanical forces are essential for tissue growth, maintenance and function. Cells react to externally applied forces by remodeling their actin cytoskeleton and by modifying gene expression, thus adapting to the physical environment. Changes in actin dynamics are monitored by the transcriptional coactivator of serum response factor (SRF) MKL1, also known as MRTF-A/MAL/BSAC. Activation of the RhoA-actin signaling pathway triggers nuclear translocation of MKL1 and determines its transcriptional activity. We have shown previously that the induction of the ECM protein tenascin-C by cyclic strain in fibroblasts depends on RhoA-ROCK signaling and the stimulation of actin assembly and contraction. Thus, we considered MKL1 to provide a link between actin dynamics and gene expression in response to mechanical stress. Indeed, we have observed MKL1 translocation in fibroblasts cyclically stretched via their elastic substrate. We tested whether tenascin-C transcription is directly regulated by MKL1 and found that MKL1 transfection of fibroblasts results in elevated tenascin-C mRNA and protein levels, defining tenascin-C as a downstream target of MKL1. Promoter-reporter studies showed that an MKL1-mutant with an impaired binding to SRF still induced a tenascin-C promoter reporter, as well as endogenous tenascin-C expression, while the induction of an SRF-regulated promoter was completely abolished. Moreover, the activity of the tenascin-C promoter was not induced by an MKL1-mutant lacking a potential DNA-binding domain. This suggests that the transcriptional control of tenascin-C by MKL1 is SRF-independent and may require direct binding of MKL1 to elements in the tenascin-C promoter. Finally, cyclic strain applied to MKL1-deficient fibroblasts failed to induce tenascin-C mRNA, whereas FOS mRNA was still induced. Further work will clarify the exact mechanism of this regulation and whether MKL1 is a regulator of other mechanoreponsive genes.

THE ROLE OF THE LINC COMPLEX IN NUCLEAR ROTATION, MECHANOTRANSDUCTION AND MYOGENESIS

M. Brosig, J. Ferralli, L. Gelman, M. Chiquet

Mechanical stress controls a broad range of cellular functions, affecting many aspects of tissue homeostasis, among them muscle regeneration. Thus, mechanical cues are sensed by cells and translated into biochemical signals. External mechanical stress is transmitted to the cytoskeleton via integrin receptors, which trigger various signals in response to mechanical stimuli. The cytoskeleton is physically connected to the nuclear lamina by the LINC complex consisting of the outer nuclear membrane protein nesprin, which binds to sun proteins spanning the inner nuclear membrane (Figure 2). We asked here how disruption of this direct link from the cytoskeleton to nuclear chromatin affects mechanotransduction. Fibroblasts grown on flexible silicone membranes reacted to cyclic stretch by nuclear rotation (Figure 2). This rotation was abrogated by inhibition of actin contraction and by overexpression of dominant negative versions of nesprin or sun proteins that form the LINC complex. In an in vitro model of muscle differentiation, cyclic strain inhibited differentiation and induced proliferation of C2C12 myoblasts. Interference with the LINC complex in these cells abrogated their stretch-induced proliferation, while stretch increased p38 MAPK and NFkB phosphorylation and the transcript levels of myogenic transcription factors MyoD and myogenin. We found that the physical link from the cytoskeleton to the nuclear lamina is crucial for correct mechanotransduction and that disruption of the LINC complex perturbs the mechanical control of cell differentiation.
PERICELLULAR FIBRONECTIN REQUIRED FOR RhoA-DEPENDENT RESPONSES TO CYCLIC STRAIN

R. Lutz, M. Chiquet

To test the hypothesis that the pericellular fibronectin matrix is involved in mechanotransduction, we compared the response of normal and fibronectin-deficient mouse fibroblasts to cyclic substrate strain. Normal fibroblasts seeded on vitronectin in fibronectin-depleted medium deposited their own fibronectin matrix. In cultures exposed to cyclic strain, RhoA was activated, actin stress fibers became more prominent, MKL1 shuttled to the nucleus, and tenascin-C mRNA was induced. In contrast, these RhoA-dependent responses to cyclic strain were suppressed in fibronectin knockdown or knockout fibroblasts grown under identical conditions. On vitronectin substrate, fibronectin-deficient cells lacked 5-integrin containing fibrillar adhesions. However, when fibronectin-deficient fibroblasts were plated on exogenous fibronectin, their defects in adhesion and mechanotransduction were restored. Studies with fragments indicated that both the RGD/synergy site and the adjacent heparin-binding region of fibronectin were required for full activity in mechanotransduction but not ability to self-assemble. In contrast to RhoA-mediated responses, activation of Erk-1/2 and PKB/Akt by cyclic strain was not affected in fibronectin-deficient cells. Our results indicate that pericellular fibronectin secreted by normal fibroblasts is a necessary component of the strain-sensing machinery. Supporting this hypothesis, induction of cellular tenascin-C by cyclic strain was suppressed by addition of exogenous tenascin-C, which interferes with fibronectin-mediated cell spreading.

HUMAN TENEURIN-1 IS A DIRECT TARGET OF HOMEobox TRANSCRIPTION FACTOR EMX2

J. Beckmann, D. Kenzelmann, J. Ferralli and J. Schoeler, in collaboration with A. Vitobello and F. M. Rijli

Many transmembrane proteins mediate cell–cell interactions and thereby regulate key developmental processes. Teneurins are a unique family of type II transmembrane proteins conserved from Drosophila melanogaster and Caenorhabditis elegans to vertebrates, where the four paralogues teneurin 1-4 exist. Recent findings suggest an important role for the teneurin protein family in establishing arealization and patterning in the developing embryo. These processes are regulated by a network of transcription factors that are expressed in gradients in the developing cortex. The homeobox-containing protein Emx2 is the best-studied protein in the network, although direct targets involved in patterning are not yet established. The human teneurin-1 gene resides on the X chromosome at position Xq25, a locus with low gene density. Patients with the syndrome X-linked mental retardation (XLMR) that maps to this locus suffer from severe mental retardation, motor sensory neuropathy, deafness and severely impaired vision. Given its predominant expression in the developing brain and its function in establishing proper connectivity in the brain, teneurin-1 is a potential target gene in XLMR. In order to provide a basis for an investigation of possible deletions and mutations in teneurin-1 of XLMR patients, we decided to delineate the gene locus and determine the transcription start site(s) of human teneurin-1 (ten-1). We identified a novel promoter upstream of the published transcription start that is conserved in chicken and mice. We showed that Emx2 directly binds to and regulates human teneurin-1 expression at this alternative promoter. Thus, ten-1 is a candidate gene mediating Emx2-induced arealization in the developing cortex.

C. ELEGANS TEN-1 GENETICALLY INTERACTS WITH PHY-1 AND MAINTAINS BASEMENT MEMBRANE INTEGRITY

U. Topf

The teneurins are a family of phylogenetically conserved proteins expressed in metazoans during pattern formation and morphogenesis. A single gene, ten-1, encodes the single teneurin orthologue in C. elegans. TEN-1 is important for epidermal morphogenesis, gonad migration, neuronal pathfinding and basement membrane integrity of some tissues. RNAi knock down experiments and double mutant analyses led to the discovery of genetic interactions of ten-1 with dystroglycan dgn-1, integrin ina-1, laminin epi-1, and nidogen mid-1. These results suggested that ten-1 acts in a parallel pathway and has a partly redundant function to dystroglycan and/or integrin receptors.

To identify further genetic interaction partners of ten-1, we performed a genome wide RNAi screen for suppressors and enhancers of the ten-1 phenotypes. We found that depletion of phy-1 function results in significant increase in embryonic lethality in a ten-1 mutant background. Phy-1 encodes the orthologue of prolyl 4-hydroxylase (P4H) alpha subunit in vertebrates. This collagen-modifying enzyme was shown to be essential during vertebrate development. Mice lacking the P4H alpha subunit died during embryogenesis due to loss of basement membrane integrity. The C. elegans genome encodes three paralogs (phy-2, phy-3, phy-4) of phy-1. Double-mutant worms lacking phy-1 and phy-2 are embryonic lethal due to insufficient modification of cuticle collagens. In contrast, the viability of single mutants is similar to wild type.
We found that arrested embryos of ten-1; phy-1 double mutants show disrupted basement membrane in muscle and epidermis. Double-mutant embryos arrest in various stages during late embryonic elongation. This process converts the bean-shaped embryo into the elongated shape of the worm (Figure 3A, B). This results from the elongation and fusion of epidermal cells along the anterior-posterior axis. Epidermal cells in arrested embryos did not fuse and/or are misplaced. Examination of epithelial junctions using the ajm-1::gfp marker showed them to be present but severely disorganized (Figure 3C, D). Visualization of basal lamina using a lam-1::gfp marker revealed frequent breaks or disorganization of basal laminae surrounding the pharynx, in body wall muscles and underlying the epidermis (Figure 3E, F). This was not the case for wild-type worms, phy-1 single mutants or ten-1 single mutants at this stage of development. Collagen IV is essential to the stabilization of basement membranes. Its loss leads to embryonic lethality due to late elongation arrest and detachment of body wall muscles from the epidermis. We suggest that loss of ten-1 function in combination with unstable basement membranes results in developmental arrest of C. elegans.
INTRODUCTION

Regulation of protein kinases temporally, spatially and quantitatively is crucial to the correct maintenance of all aspects of cell biology from metabolism to transcription, cell growth, shape, migration, survival and differentiation. Kinases phosphorylate one-third of all intracellular proteins, making them key regulators in signaling. The mammalian genome encodes about 518 different protein kinases (the largest enzyme family).

We focus on the regulation of these enzymes in order to delineate signaling pathways in novel contexts of normal development as well as in disease, particularly cancer. In cancer cells, mutations in key regulatory enzymes result in permanently upregulated proliferation and survival signaling pathways. Integrating knowledge of these pathways gained from molecular biology, biochemistry, animal models and patient samples may lead to specific therapeutic targets and treatments.

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NDR/MOB SIGNALING COMPLEX

The NDR (nuclear-Dbf2-related) kinase family represents a conserved subclass of the AGC serine/threonine protein kinases from yeast to man. MOB proteins (NDR non-catalytic subunits) are essential components of the signaling pathways complex.

MST1 AND HMob1 CONTROL CENTROSOME DUPLICATION THROUGH NDR KINASE

A. Hergovich

We investigated the role of human MOB proteins in centrosome duplication and addressed the regulation of centrosome duplication by Ste20-like (MST) kinases and MOB proteins. Studying the six human MOB proteins and five MST kinases, we found that MST1/hMOB1 signaling controls centrosome duplication through an hMOB1/MST1/NDR1 signaling pathway. Analysis of shRNA-resistant hMOB1 and NDR1 mutants showed a functional NDR/hMOB1 complex to be critical for MST1 to phosphorylate NDR on the hydrophobic motif, which in turn is required for human centrosome duplication. Furthermore, shRNA-resistant MST1 variants revealed that MST1 kinase activity is crucial for centrosome duplication but MST1 binding to the hSAV and RASSF1A tumor suppressor proteins is dispensable. Finally, studying the PLK4/HsSAS-6/CP110 centriole assembly machinery, we also observed that normal daughter centriole formation depends on intact MST1/hMOB1/NDR signaling, although HsSAS-6 centriolar localization is not affected. Our data suggest a novel pathway controlling human centriole duplication after recruitment of HsSAS-6 to centrioles.

hMOB2 NEGATIVELY REGULATES NDR

R. Kohler

The human MOB protein family consists of six distinct members (hMOB1A, 1B, 2, 3A, 3B and 3C), with hMOB1A/B being the best studied due to their putative tumor suppressive functions through regulation of NDR/LATS kinases. We characterized all six human MOB proteins in the context of NDR/LATS binding and activation. Both hMOB2 and hMOB1A bound to the N-terminal region of NDR1 but their binding modes differ significantly. We also showed that hMOB2 competes with hMOB1A for NDR binding and that hMOB2, in contrast to hMOB1A/B, is bound to unphosphorylated NDR. Consistently, hMOB2 expression interfered with the roles of NDR in death-receptor signaling and centrosome overduplication. Our data indicate that hMOB2 is a negative regulator of human NDR kinases.

MAMMALIAN NDR KINASE 1 AND 2 ARE ESSENTIAL FOR EMBRYO DEVELOPMENT

D. Schmitz, D. Hynx

Mammalian NDR1 and NDR2 compensate for each other in single isofrom knock-out settings by upregulating both the total level and activity of the remaining isofrom. However, mating of parents that retain a single allele of either ndr1 or ndr2 did not yield ndr1/2 null embryos. Ndr double-knockout embryos showed first signs of growth retardation at embryonic day (E)8.0-8.25 and died prior to E10.5. Both ndr1 and ndr2 were broadly expressed in wild-type embryos at E8.5. Mutant embryos showed a clear reduction in somite size and number and a delay in turning with respect to their littermates. The posterior part of the embryo was most severely affected. Nevertheless, expression of sonic hedgehog and fta markers for proper posterior patterning was normal. However, tbx6 and fgf8 as factors crucial for proper somitogenesis and subsequent posterior development were clearly reduced. Mutant embryos showed a general decrease in presomitic mesoderm reflected in a narrower tail. We are analyzing expression of genes of the Notch and Wnt pathway involved in the sequential generation of somites. Furthermore, heart development was compromised in embryos lacking both NDR1 and NDR2 and a cardiovascular defect could account for the early lethality of ndr1/2 double knock-out embryos.

ABLATION OF MAMMALIAN NDR1 KINASE PREDISPOSES TO T-CELL LYMPHOMA

H. Cornils, D. Hynx

Defective apoptosis contributes to a variety of human malignancies. NDR1 and NDR2 have been implicated in the regulation of apoptosis downstream of tumor necrosis factor-receptor family member FAS. FAS signaling is crucial for lymphocyte homeostasis with defects linked to lymphoproliferative disorders, autoimmunity and cancer. NDR1-deficient mice generated to analyze the role of NDR1 downstream of FAS were normal and NDR1-deficient T-cells exhibited normal responses to pro-apoptotic stimuli. NDR1 loss was functionally compensated by an increase in NDR2 protein. Despite this compensation, NDR1-deficient and heterozygous mice showed significantly increased T-cell lymphoma development, which was accompanied by a decrease in NDR kinase levels in mice and human cells. Thus, reduction in NDR1 triggers a decrease in total NDR kinase expression and low expression of NDR kinases results in defective responses to pro-apoptotic stimuli, thus facilitating tumor development (Figure 1).
**The PKB signalling pathway in development and disease**

Several proteins of the PI3K/PTEN/PKB signaling pathway are mutated in tumors, making this the most frequently mutated signaling pathway in human cancer.

**PKB phosphorylation of Twist-1 inhibits p53 activity in response to DNA damage**

A. Vichalkovski, E. Gresko, D. Hess, D.F. Restuccia

Cells expressing Twist-1 displayed inefficient p53 upregulation in response to DNA damage by γ-irradiation or a genotoxic drug. This influenced the activation of p53 target genes such as p21Waf1 and Bax and led to aberrant cell cycle regulation and inhibition of apoptosis. Impaired induction of p53 effector molecules may be mediated by PKB-dependent phosphorylation of Twist-1 because, unlike the wild type, the Twist-1 S42A mutant did not confer cell resistance to DNA damage. Significantly, phosphorylation of Twist-1 at Ser42 was found in human cancer tissues, suggesting that this posttranslational modification ensures functional activation of Twist-1 after promotion of survival during carcinogenesis.

**The role of PKB phosphorylation of Twist1 in cancer metastasis**

G. Xue, A. Vichalkovski, D. Restuccia, D. Hynx

The bHLH transcription factor Twist1 plays a vital role in mouse embryonic development. It is important in anti-apoptosis and promotes epithelial-mesenchymal transition as well as tumor metastasis in vivo. Moreover, aberrant overexpression of Twist1 has been reported in 22 types of human tumors. We found that Twist1 phosphorylation positively correlates with invasiveness in breast cancer cell lines. Strong inhibition of Twist1 phosphorylation by the PI3K/PKB pathway was accompanied by decreased cell migration and invasion. Twist1 phosphorylation led to disrupted intercellular junctions and upregulated migration-enhancing proteins, thus triggering full phenotypic transition from epithelial to fibroblastic spindle-like morphology. Compensatory expression of phosphorylatable but not unphosphorylatable Twist1 in 4T1 cells in which endogenous Twist1 was knocked down strongly rescued the metastatic phenotype in lung. Phosphorylated Twist1 was found in several malignant human tumor samples closely correlated with hyperactivation of PKB. PKB-mediated phosphorylation of Twist1 is essential for Twist-induced metastasis.

**Loss of PKBβ predisposes aged mice to ovarian abnormalities**

D. Restuccia, D. Hynx

Luteinizing Hormone (LH) binding to thecal cells stimulates androgen production of progesterone and testosterone, the latter being converted into estrogens by Follicle Stimulating Hormone (FSH)-stimulated granulosa cells. Progesterone and estrogens support the developing egg and control uterine morphology. Disruption of this system can lead to ovarian and uterine abnormalities. Aged PKBβ knockout mice developed severe ovarian and uterine abnormalities, with cysts characterized by thecal and interstitial cell hyperplasia. These cell populations, responsible for production of androgen hormones, increased with time and this was correlated with the size of the ovarian cyst, thus playing an active role in the pathology. The granulosa cell population required for negative feedback control and testosterone conversion was lost and testosterone levels increased in PKBβ null mice. PKBβ loss increased the severity of Polycystic Ovarian Syndrome (PCOS), with more and larger cysts in PKBβ null mice than in wild type. Thus, PKBβ is involved in hormone regulation in the ovary and deregulation of PKBβ contributes in an as yet undefined role to PCOS.

**Mitochondrial dynamics and CTMP proteins**

Carboxy-Terminal Modulator Protein (CTMP) was identified as a negative regulator of PKB activity, diminishing its activation and thus suppressing its pro-survival activities in the normal physiological state and in tumors.
CTMP REGULATES MITOCOCHONDRIAL DYNAMICS AND APOPTOSIS
A. Parcellier, L. A. Tintignac, E. Zhuravleva, P. Cron, D. Hynx

We showed that CTMP1 protein belongs to the mitochondrial proteome with dual sub-mitochondrial localization: both inner-membrane associated and in intra-membrane space fractions. During import into the mitochondria, a mitochondrial translocation signal (MTS) is cleaved to give the mature form of CTMP1. In apoptotic conditions, CTMP1 is released into the cytosol, where it negatively affects PKB activation, thus sensitizing cells to undergo apoptosis.

Both full-length CTMP1 and a CTMP1 mutant refractory to MTS cleavage promote clustering of spherical mitochondria, suggesting a role for CTMP in the fission process. Indeed, cellular depletion of CTMP led to accumulation of swollen and interconnected mitochondria without affecting mitochondrial fusion. In vivo results support the relevance of these findings, as mitochondria from livers of adult CTMP knockout mice had a phenotype similar to that of cells depleted of CTMP1.

THE ROLE OF CTMP1 IN LIVER INJURY AND CANCER DEVELOPMENT
S. M. Schultze, D. Hynx, O. Tschopp

Recent studies show that CTMP1 sensitizes cells to cell death in vitro and in vivo in response to various stimuli such as genotoxic stress and ischemia. This suggests that CTMP1 has a functional role in liver injury. Indeed, our data show that ctmP1-deficient mice have reduced susceptibility to chemical-induced liver injury as shown by reduced cell death.

As liver cancer development is positively correlated to the extent of liver injury and CTMP1 seems to have a critical role in liver injury, we predict that CTMP1 is also involved liver cancer. To test this, we are applying a diethylnitrosamine/phenobarbital-induced liver cancer model to ctmP1-deficient mice.

ROLE OF CTMP2 PROTEIN
E. Zhuravleva, D. Hynx, O. Tschopp, A. Parcellier, S. Schenk, B. Duemmier

CTMP2 and CTMP1 genes probably arose by gene duplication. After confirming the mitochondrial localization of CTMP2 protein via an N-terminal MTS, we studied the involvement of CTMP2 protein in the regulation of mitochondrial morphology and related physiological processes using CTMP2 knockout animals. CTMP2 null mice are insulin hypersensitive and develop age-dependent fatty liver disease. The mitochondrial function of CTMP2 null animals is also affected, suggesting that CTMP2 is involved in the regulation of metabolic processes, possibly through a PI3K/PKB pathway.

SIGNALLING NETWORK IN Glioblastoma Multiforme
Glioblastoma multiforme (GBM) is the most aggressive human brain cancer with extensive neurological destruction. Despite intensive research, the median survival of GBM patients remains at 12 months, where low survival rates have been attributed to high invasion and resistance to standard-of-care treatments. Our transcript analysis of brain tumors, including primary and secondary GBM as well as low-grade astrocytomas and oligodendrogliomas, identified several novel deregulated protein kinases.

TARGETING SIGNALLING PATHWAYS REGULATING TRANSLATION
M. Grzmil, P. Morin

MAP kinase-interacting protein kinase 1 (MNK1) is one of the novel targets identified in our microarray analysis of brain tumors. MNK kinases function downstream of p38 and ERK MAP kinases and are closely associated with translation initiation complexes. They can bind to translation initiation factor eIF4G and phosphorylate the cap-binding protein, translation initiation factor eIF4E. A very recent key finding demonstrates that eIF4E phosphorylation at Ser209 by MNK kinases is absolutely critical for eIF4E action in opposing apoptosis and promoting tumorigenesis in vivo. MNK1 inhibitor CGP57380 reduced GBM cell proliferation, whereas concomitant treatment with CGP57380 and the mTOR inhibitor rapamycin resulted in an additive effect on growth inhibition (Figure 2). Analysis of polysome profiles revealed potent inhibition of global translation in CGP57380- and rapamycin-treated cells. MNK1-
specific knockdown reduced proliferation of cells incubated with rapamycin and the overexpression of full-length MNK1 reduced such rapamycin-induced growth inhibition. To investigate further the role of MNK1-signaling in translation control, we purified and compared polysomal-associated RNA from MNK1-depleted and control-transfected cells. The data indicate an important role for MNK1 in GBM cell growth and survival and support the development of new therapies against human glioblastoma based on the targeting of signaling pathways that regulate translation.

**ROLE OF MER TYROSINE KINASE IN GлиOBlASTOMA**

Y. Wang

Mer tyrosine kinase (MerTK) is a member of the TAM receptor tyrosine kinase (Tyro3, Axl and Mer) family. Overexpression of MerTK has been reported in mantle cell lymphomas, prostate cancer, breast cancer and melanoma. In addition, MerTK has oncogenic potential. We found MerTK to be significantly upregulated in GBM tumors. High levels of MerTK protein were confirmed by Western blot and immunohistochemistry. Double staining of MerTK and GFAP (an astrocyte marker) on GBM tumor sections confirmed that MerTK is expressed by astrocyte-derived tumor cells. Moreover, a MerTK inhibitor significantly inhibited GBM cell proliferation and colony formation on soft agar. Thus, research on the role of MerTK in GBM may provide a new approach to alternative therapeutic strategies for human GBM and possibly other cancers.

**ONCOGENIC MECHANISMS OF HEMATOPOIETIC PROTEIN KINASES DURING GLIMAGENESIS**

G. Moncayo

Two cytoplasmic tyrosine kinases normally found only in hematopoietic cells are highly overexpressed in GBM tumors. This is not surprising since GBM tumors show a high level of lymphocyte infiltration. These kinases were expressed by the GBM tumor cells and not only in infiltrating leukocytes. We confirmed overexpression in GBM cell lines and, strikingly, treatment with two specific small molecule inhibitors strongly blocked basal and EGFR-mediated proliferation and migration of GBM cells (EGFR is hyperactivated in most GBM). Given the known oncogenic roles of these kinases in leukemia, we will investigate their transformation potential and their functions in EGFR-mediated malignancy, and will identify putative upstream receptors, hematopoietic adaptors and downstream pathways that could activate and synergize in GBM.

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**Selected publications**


INTRODUCTION

Cancer results from cumulative alterations in the genetic make-up of somatic cells that lead to aberrant expression, mutation or deletion of proteins modulating cellular proliferation, differentiation and survival. Through deregulation of intracellular pathways, cancer cells are no longer responsive to signals that control normal proliferation and survival. Signals emanating from cancer cells to their surroundings are also abnormal, resulting for example in the stimulation of neovascular growth, which increases nourishment of the tumor and dispersion of metastatic cells.

Receptor tyrosine kinases are often aberrantly activated in cancer. We have demonstrated the constitutive activation of epidermal growth factor receptor and ErbB2 as well as fibroblast growth factor receptors and the Ret receptor in breast cancer. We also found that the canonical Wnt pathway is constitutively active in breast cancer cells and that interference with autocrine Wnt signaling reduces proliferation of breast tumor cells.

A major goal of our research is to progress from a description of the molecular alterations in breast cancer cells to an understanding of how these altered signal transduction proteins contribute to the metastatic cancer process. In this context, we have identified two new ErbB2-interacting proteins, Memo and Copine-III, with roles in tumor cell migration, which is fundamental to the metastatic process. We also found that the activity of PN-1, a serine protease inhibitor, contributes to metastasis in a breast cancer model. Such increased understanding of the molecular basis of cancer is essential for the further rational development and clinical application of new classes of targeted signal transduction inhibitors, some of which are already in use for cancer therapy.
Fibroblast Growth Factor Receptors in Breast Cancer

J. Dey and A. Issa, in collaboration with F. Bianchi (IFOM, Milan) and D. Graus-Porta (Novartis)

Members of the fibroblast growth factor/fibroblast growth factor receptor (FGFR) family and their ligands, the FGFs, have multiple essential developmental roles and have also been implicated in different types of human tumors, including breast cancer. FGF ligands bind specific FGFR isoforms, inducing receptor dimerization, kinase activation and autophosphorylation of intracellular tyrosine residues. In addition to phosphorylation of tyrosine residues on receptors and on PLCγ the adaptor protein FRS2 that links FGFRs to the MAPK and PI3K pathways is phosphorylated on multiple tyrosine residues in response to receptor activation (Figure 1A, left). Different types of FGF/FGFR alterations have been described in cancers, including abnormal expression levels, single nucleotide polymorphisms (SNPs), mutations and amplifications. Based on increasing evidence supporting the relevance of FGFRs in human breast cancer, we have explored the role of this receptor in the 4T1 breast cancer model. 4T1 mammary tumor cells co-express multiple FGFRs and ligands and display autocrine FGFR activity and constitutive activity of many targets of FGFR signaling. Treatment of these tumor cells in vitro with TKI258, a small molecule kinase inhibitor that blocks FGFR, decreased the activity of the signal transduction pathways and blocked cell proliferation (Figure 1A, right). Treatment of 4T1 tumor-bearing mice with TKI258 led to a strong reduction in mammary tumor growth, blocked tumor-induced angiogenesis (Figure 1B) and led to a decrease in lung metastasis. Moreover, we have shown that FGFR blockade downregulates key players in the metastatic process, in particular protease MMP-9 and transcription factor Twist, which are major regulators of metastatic spread. In conclusion, our results strengthen the idea that targeting FGFRs in certain sub-types of breast cancer with aberrant activation of these receptors is a valuable approach for the future.

Ret Receptor Tyrosine Kinase Function in Breast Cancer

A. Gattelli and A. Boulay, in collaboration with C. Brisken (ISREC, EPFL, Lausanne) and M. Fische (CHUV, Lausanne)

Ret is the receptor for the glial-derived neurotrophic factor (GDNF) family of peptides and a single Ret gene encodes this member of the receptor tyrosine kinase (RTK) superfamily. These peptides bind Ret in conjunction with glycoposphatidylinositol (GPI)-anchored co-receptors of the GFRα family. Activating mutations in Ret, which allow the receptor to signal independently of GFRα and GDNF ligands, promote a spectrum of endocrine neoplasias including multiple endocrine neoplasia type 2a (MEN2A), type 2b (MEN2B) and familiar medullary thyroid carcinoma. Ret expression has not been consistently reported in other human cancers. We found Ret expression in breast cancer cell lines and primary tumors, in particular in estrogen receptor (ER) positive cancers and have investigated the contribution of Ret to breast cancer biology using ER-positive breast tumor models. GDNF treatment of these cell lines caused increased oncogenicity and Ret-dependent anchorage-independent proliferation, showing that Ret is functional in breast tumor cells. Ret and GFRα1 mRNA levels were induced by estrogens and Ret signaling enhanced estrogen-driven proliferation, highlighting the functional interaction of the Ret and ER pathways. Furthermore, Ret was detected in primary breast tumors and higher Ret levels found in ER-positive cancers. We are now examining in vivo breast cancer models for expression of Ret, its co-receptors and ligands and studying the importance of this network in tumor cell proliferation and metastasis.

c-Myc Has a Role in Mammary Gland Development

T. Stoelzl, in collaboration with A. Trumpp (DKFZ, Heidelberg) and P. Schwab and S. Bichet (FMI)

c-Myc has been intensely studied in human breast cancer and in mouse mammary tumor models, but relatively little is known about the normal physiological role of c-Myc in the mammary gland. The gland of a mature virgin female mouse has two compartments, a ductal epithelial network and the stroma, also referred to as the mammary fat pad. Upon hormonal stimulation in pregnancy, bursts of proliferation followed by differentiation convert the gland into a milk-synthesizing machine. We employed a conditional approach using the Cre-loxP system to study the role of c-Myc in the mammary gland. Whey acidic protein (WAP)iCre transgenic mice were used to recombine the LoxP-flanked c-myc locus in luminal alveolar cells, starting at mid-pregnancy and throughout lactation. In c-myc<sup>fl/fl</sup> mice carrying the WAP<i>iCre</i> transgene, c-Myc was lost in mammary alveolar epithelial cells starting in mid-pregnancy and three major phenotypes observed in glands of mutant mice. First, c-Myc-deficient alveolar cells had a slower proliferative response at the start of pregnancy and a resulting delay but not a block in alveolar development. Second, while milk composition was comparable between wild-type and mutant animals, milk production was reduced in mutant glands, leading to a slower increase in pup weight.
Electron microscopy and polysome fractionation revealed a general decrease in translational efficiency. Furthermore, analysis of mRNA distribution along the polysome gradient showed the effect to be specific for mRNAs whose protein products are involved in milk synthesis. Moreover, quantitative RT-PCR analysis revealed decreased levels of ribosomal RNAs and ribosomal protein-encoding mRNAs in mutant glands. Third, using the mammary transplantation technique to functionally identify alveolar progenitor cells, we observed that mutant epithelium has a reduced ability to repopulate the gland when transplanted into NOD/SCID recipients. These results suggest that c-Myc has multiple roles in the mammary gland, affecting proliferation, biosynthetic capacity and progenitor cell proliferation and/or survival.

WNT SIGNALING IN BREAST CANCER

Y. Matsuda and I. Samarzija, in collaboration with E. J. Oakeley (Novartis) and T. Schlange (Bayer Schering Pharma)

The WNT pathway has important roles in the biology of multiple cell types. In breast cancer, deregulation of the WNT signaling pathway occurs by autocrine mechanisms. WNT ligands and Frizzled receptors are co-expressed in primary breast tumors and breast cancer cell lines. Moreover, many breast tumors show hypermethylation of the promoter region of secreted Frizzled-related protein 1 (sFRP1), causing low expression of this WNT antagonist. We have shown that interference with WNT signaling in breast cancer cell lines reduces their proliferative ability. We also found that WNT stimulates the migratory ability of breast tumor cells and that ectopic expression of sFRP1 blocks canonical WNT signaling and decreases the migratory potential of these cells. Moreover, the ability of sFRP1-expressing breast tumor cells to grow as xenografts in mammary glands and to form lung metastases was dramatically impaired compared with controls. Our results show that the WNT pathway influences multiple biological properties of breast cancer cells, suggesting that interference with WNT signaling may be a valid therapeutic approach in breast cancer.

STUDIES ON Memo IN MODEL SYSTEMS

B. Haenzi, S. Kondo, I. Schlatter, A. Frei, J. Zmajkovic, S. Lienhard and F. Maurer, in collaboration with R. Mowa (Novartis)

We identified Memo as an ErbB2-interacting protein required for ErbB2-dependent migration. A single Memo protein is encoded in the human genome and Memo homologues are found in all branches of life. In situ analyses revealed that Memo is widely expressed during embryogenesis and present in all examined organs of adult mice. After conventional Memo knockout, progeny from crosses of Memo<sup>−/−</sup> mice were screened but no Memo null pups identified in > 150 offspring. Closer examination revealed that Memo null embryos start to die at E13.5 and none are alive by E14.5. The exact cause of embryonic lethality is under investigation but hemorrhaging and pericardial cavity edema were already noted in the Memo null embryos. We are employing conditional approaches to ablate Memo in order to examine the effects of Memo deletion on adult physiology and tumor development. The in vivo work on Memo is complemented by analyses using Memo null embryonic fibroblasts.

To position Memo within a genetic network, experiments are being carried out in Saccharomyces cerevisiae. Genetic approaches in model organisms have been important in the past for gaining insight into the function of evolutionarily conserved proteins. A S. cerevisiae memo<sup>Δ</sup> strain generated by standard technology is viable and we are examining this strain for alterations in pathways involved in microtubule and actin cytoskeleton as well as other physiological processes. A major goal is to discover whether Memo could be an attractive target for cancer therapy, i.e., whether blocking Memo will have an impact on in vivo tumor development and metastasis.
STUDIES ON Memo IN TUMOR CELL MIGRATION AND METASTASIS

G. MacDonald, M. Meira and S. Lienhard, in collaboration with S. Bichet (FMI) and A. Doelemeyer (Novartis)

We have identified Memo, a 33-kDa protein that interacts with the HRG-activated ErbB2 receptor and is required for the migration of breast cancer cells in response to ligands that activate the ErbB RTK family. Using the yeast two-hybrid approach to gain insight into Memo function, an interaction was uncovered between Memo and coflin, a regulator of actin dynamics. The interaction was confirmed in vitro using recombinant proteins and in vivo in co-immunoprecipitation experiments, where Memo was found to be complexed with ErbB2, PLCγ and coflin (Figure 2A). By tracing HRG-induced cell migration in Dunn Chambers, we found that Memo or PLCγ knock-down strongly impairs cell directionality.

Memo is also required for FGFR-induced migration, suggesting a broad role for Memo downstream of RTK-induced cell motility. To clarify whether Memo’s role in migration is important in metastasis, we used specific shRNAs to knock down expression of Memo in a metastatic breast cancer model. In vitro studies showed that loss of Memo results in decreased cellular motility but does not affect proliferation. In xenograft studies using these lines, knockdown of Memo did not affect tumor growth but appeared to reduce the number of lung metastases from the primary tumor. These results suggest that Memo is important for tumor cell migration and the formation of metastases.

Copine-III INTERACTS WITH ErbB2 AND PROMOTES TUMOR CELL MIGRATION

C. Heinrich and S. Lienhard, in collaboration with J. Hofsteenge, R. Sack and D. Hess (FMI) and M. Vecchi and M. Bianchi (IFOM, Milan)

ErbB2 amplification and overexpression in breast cancer correlates with aggressive disease and poor prognosis. To identify ErbB2-interacting proteins, we have used P-peptides encompassing specific ErbB2 autophosphorylation sites to uncover novel proteins that interact with the active tyrosine phosphorylated receptor. Copine-III, a member of a Ca2+-dependent phospholipid binding protein family, was shown to bind to phosphorylated Tyr1248 of ErbB2. In breast cancer cells, Copine-III requires Ca2+ for binding to the plasma membrane, where it interacts with ErbB2 upon receptor stimulation, an interaction dependent on receptor activity (Figure 2A). Copine-III also binds RACK1 (receptor of activated C kinase 1) and co-localizes with phosphorylated focal adhesion kinase at the leading edge of migrating cells. Importantly, knockdown of Copine-III in T47D breast cancer cells caused a decrease in Src kinase activation and ErbB2-dependent wound healing. Our data suggest that Copine-III is a novel player in the regulation of ErbB2-dependent cancer cell motility. In primary breast tumors, high CPNE3 RNA levels significantly correlated with ErbB2 amplification. Moreover, in an in situ tissue microarray analysis, we detected differential protein expression of Copine-III in normal versus breast, prostate and ovarian tumors, suggesting a more general role for Copine-III in carcinogenesis (Figure 2B, C).
THE SERINE PROTEASE INHIBITOR PN-1 AND BREAST CANCER METASTASIS
B. Fayard, in collaboration with D. Monard (FMI) and F. Bianchi (IFOM, Milan)

Through their ability to degrade the extracellular matrix, proteases mediate cancer cell invasion and metastasis. Paradoxically, some serine protease inhibitors (serpins) are often overexpressed in human tumors. Using computational analysis, we found that the RNA level of Protease Nexin-1 (PN-1), a serpin that blocks numerous proteases, is significantly elevated in ER-negative and in high-grade breast cancers. The in silico approach was complemented by mechanistic studies of two mammary cancer cell lines, PN-1-negative 168FARN cells and PN-1-positive 4T1 cells, both of which form primary mammary tumors, but only 4T1 tumors metastasize to the lungs. We found that treatment of 168FARN cells with PN-1 stimulated Erk activation via low density lipoprotein receptor-related protein (LRP-1) binding, resulting in increased MMP-9 RNA, protein and secreted activity (Figure 3). PN-1-silenced 4T1 cells expressed low MMP-9 levels. Moreover, injection of PN-1-silenced cells into mice did not affect 4T1 primary mammary tumor outgrowth; however, the tumors had impaired metastatic potential that was restored by re-expressing MMP-9 in the PN-1-silenced 4T1 cells.

Thus, using mammary tumor models, we describe a novel pathway whereby the serpin PN-1 by binding LRP-1 stimulates Erk signaling, MMP-9 expression and metastatic spread of mammary tumors. Importantly, of 126 breast cancer patients examined, those whose tumors had elevated PN-1 levels had a significantly higher probability of lung metastasis upon relapse but not to other sites. This suggests that PN-1 could become a prognostic marker in breast cancer.

Selected publications


Fig. 3. A Silencing PN-1 in tumor cells decreases Erk signaling, MMP-9 expression levels, and the metastatic properties of the mammary tumors. B A complex formed from cancer cell-secreted PN-1 and proteases binds with high affinity to the LRP-1 receptor and activates LRP1 signaling. The resulting Erk activation and MMP-9 upregulation contributes to the metastatic potential of cancer cells.
INTRODUCTION

The maintenance of genetic and epigenetic information is an important aspect of every cell division. Genomic instability is the major driving force in the conversion of precancerous lesions into tumors and the genome is most frequently compromised during replication and by endogenous and exogenous forms of DNA damage. The progression of the replication fork may be halted by DNA-bound proteins, lesions and by DNA secondary structure. Completion of replication depends upon the activation of cell cycle checkpoints that halt cell cycle progression and resolve these obstacles. Failure to do so contributes to genomic instability. Following replication, the genetic code remains continuously compromised through endogenous and exogenous DNA damage ranging from oxidative damage and UV photolesions to single- or double-strand breaks.

Defects in DNA repair or replication result in mutations, deletions and amplifications. These are counteracted by gatekeeper or antimutator genes that survey the genome and coordinate a repair response. Mutation of these genes often leads to pronounced cancer predisposition in patients.

In addition to the genetic code, the cellular epigenetic code must also be established and maintained. Epigenetic signatures play important roles in cellular identity and proliferation and, not surprisingly, deregulation of the epigenetic state is emerging as an important driver in a number of diseases, including cancer.

My laboratory works on the structure and function of the macromolecular machines involved in genome and epigenome maintenance, focusing on the molecular workings of multi-protein complexes involved in DNA repair, replication fork restart and histone methylation. We combine X-ray crystallography with biochemical, biophysical and cell biological techniques.
Persistent DNA damage increases the risk of mutations and drives the oncogenic transformation process. The DNA repair machinery counteracts this process and safeguards the genome. Lesions induced by UV-radiation such as cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts are repaired by the nucleotide excision repair (NER) pathway. There are two NER repair branches (Figure 1): in global genome repair (GGR), UV-damaged nucleotides are recognized by specific damage recognition proteins in the non-transcribed strand. In transcription coupled repair (TCR), damage recognition is indirectly mediated by stalling RNA polymerase II. Both pathways subsequently converge and trigger a common nucleotide excision response.

Mutations in GGR result in Xeroderma pigmentosum (XP) complementation groups A to G and manifest themselves by defects at various stages of the nucleotide excision repair pathway. Xeroderma pigmentosum patients show severe UV sensitivity, a mild neurological phenotype and an up to 2000-fold increase in a variety of skin cancers. In Cockayne syndrome, a premature aging disease characterized by dwarfism and severe neurological defects, damage detection via stalling of the RNA polymerase II is disrupted. The Cockayne syndrome proteins CSA and CSB are essential for coupling RNA polymerase stalling to TCR. CSA forms a complex with DDB1, which unlike the DDB1/DDB2 complex does not bind to DNA.

In GGR, the DNA is scanned for UV-induced lesions by the XP-C/Rad23 complex and by the DDB1/DDB2 complex also known as UV-DDB; DDB2 is also known as XP-E. After damage is detected in either GGR or TCR, the NER pathway proceeds by unwinding of the duplex by the two helicases XP-B and XP-D as part of the basal transcription factor TFIIH. The unwound region is stabilized and proofread by XP-A, followed by excision via XP-G nuclease (3’ excision and the XP-F/ERCC1 heterodimer (5’ cleavage). Fragments of 27-29 nucleotides are then removed. The entire protein complex required for nucleotide excision repair is referred to as the repairosome. We are interested in the initial damage-detection step, focusing on (1) how UV-lesions are detected in naked DNA and in chromatin, and (2) how these damage-detection proteins coordinate the downstream repair response.

UV-DDB has the highest affinity for 6-4 photoproducts and CPDs of all known human damage-detection proteins. Patients with mutations in DDB2 show defects in CPD repair in the GGR branch of NER. The DDB1/DDB2 system also appears to be involved in triggering apoptosis once the level of DNA damage has been deemed irreparable. The UV-DDB complex contains two principal subunits DDB1 (p127) and DDB2 (p48). Mutations in DDB2 give rise to XP complementation group E. Several lines of evidence indicate that UV-DDB is the GG-NER factor specialized for the detection of UV-induced lesions in chromatin. In vitro, UV-DDB binds to pyrimidine dimers, including isomers of CPD and 6-4 pyrimidine-pyrimidone photodimer (6-4PP) with the highest reported affinity and specificity of all NER proteins. XPC, in contrast, has substantially lower affinity and specificity for UV-lesions. In vivo, DDB2 localizes ahead of XPC to CPD and 6-4PP lesions. In the absence of DDB2, XPC still localizes to 6-4PP and to a lesser extent to CPDs, although with substantially delayed kinetics. In DDB2-deficient XPE cells, CPD repair is largely abolished, whilst 6-4PP repair is affected to a lesser extent.

The DDB1 subunit associates tightly with the CUL4-RBX1 complex and forms a cullin family ubiquitin ligase. Following UV exposure, the DDB1-DDB2-CUL4A-RBX1 complex (DDBCUL4) localizes to the site of damage and ubiquitinates XPC and DDB2. Polyubiquitination of DDB2 reduces its affinity for damage while the affinity of polyubiquitinated XPC to DNA remains unaffected. This is thought to facilitate the handover of the lesion from DDB2 to XPC. Additional DDBCUL4 substrates include histones H2A, H3 and H4 around the site of damage. H3 and H4 ubiquitination has been shown to loosen nucleosome binding in vitro, providing a pathway to assemble the NER repairosome in the otherwise inaccessible chromatin environment.

We have solved the structure of the DDB1-DDB2 complex bound to a DNA fragment containing a 6-4PP (Scrima et al. 2008), revealing that the lesion is bound exclusively by the DDB2 WD40 domain. A DDB2 hairpin protrudes into the duplex, kinking the DNA and flipping-out the photodimer. The extruded photodimer binds to...
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The Polycomb Repressive Complex
F. Schmitges, M. Lingaraju

The Polycomb repressive complex 2 (PRC2) methylates lysine 27 of histone H3, an important epigenetic mark in the development of multicellular organisms. Amplification of PRC2 complex components is observed in a variety of tumors. We are studying the molecular basis of target recognition and activation of the PRC2 methyltransferase complex.

The Drosophila PRC2 contains the three PcG proteins E(z) (Enhancer of zeste), SUZ12 (Suppressor of zeste 12) and Esc (Extra sex combs), as well as Nurf55. The core complex has an apparent molecular weight of 600 kDa. The stoichiometry of individual subunits is currently unknown. The PRC2 core complex functions as a histone methyltransferase (HMTase) catalyzing the mono-, di- and tri-methylation of lysine 27 of histone H3 (H3K27) in vitro. The SET domain located in E(z) is responsible for the PRC2 histone methyltransferase activity (HMTase). The E(z) HMTase is only poorly active by itself and requires the remainder of the PRC2 subunits and nucleosomes for activity. The non-catalytic subunits SUZ12 and Nurf55 are required for stabilization of E(z) and the presence of ESC further boosts E(z) activity. The non-catalytic residues in PRC2 are largely conserved and comprise a substantial part of the core complex (2200 out of a total 2500 residues). The only additional folds recognized in the PRC2 complex is a WD40 sequence motif in Esc and Nurf55. This type of β-propeller domain has previously been shown to play a role in recognizing a wide range of epitopes, from phosphorylated peptides and methyl-lysine residues to protein-protein interactions and DNA.

Which epitopes are recognized by Nurf55 and ESC and the role they play in the architecture and function of the PRC2 complex are part of the focus of this project. We set out to examine the structure of the PRC2 core complex and to study how external cues, epigenetic marks and the presence of nucleosomes modulate E(z) HMTase activity.

Selected publications

Crystal structures of human cardiac beta-myosin II S2-Delta provide insight into the functional role of the S2 subfragment. Proc Natl Acad Sci USA 103:17713-17717


Structural basis of UV DNA-damage recognition by the DDB1-DDB2 complex. Cell 135:1213-1223


BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science 297:1837-1848

Fig. 2. UV-induced DNA-damage recognition by UV-DDB
Administration and services

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C. elegans facility
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Eva Marina Libralato
Matthias Müller*
Susanne Ottiger
Gieu Sanh Trinh

Microscopy and imaging
Electron microscopy
Christel Genoud
Image analysis
Patrick Schward
Aaron Ponti
Microscope facility
Laurent Gelman
Steven Bourke
Special applications
Jens Rietdorf

Monoclonal antibodies
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Susan Thomas

Protein analysis
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Dominique Klein
Ragna Sack

Protein structure
Heinz Gut
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Jeremy Keusch

Radiation safety, biosafety, transport
Patrick King

Transgenic mice production
Jean-François Spetz
Patrick Kopp
Bernard Kuchemann

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Acknowledgements

The scientific achievements of the FMI described in this report were made possible by the generous financial support of the Novartis Research Foundation. In addition, the following organizations have awarded research grants to group leaders and personal fellowships to students and postdoctoral fellows during 2009-2010.

Research grants to group leaders

Agence Nationale de la Recherche
Association for International Cancer Research
Austrian Science Foundation
Cancer League Basel-Baseliland
Centre Européen de Recherche en Biologie et en Médecine – GIE
Deutsche Forschungsgemeinschaft – Sonderforschungsbereich
EMBO Young Investigator
European Commission FP6
European Commission FP7
European Research Council StG
European Research Council AdG
Fonds zur Förderung der Wissenschaftlichen Forschung
Gebert Rüf Stiftung
Heinemann Prize
Indo-Suisse (SNF)
Komen Stiftung
Leukemia and Lymphoma Society
Marie Curie Excellence Grant
Marie Curie IRG
Marie Curie ITN
National Ataxia Foundation
Neurex
Novartis Institutes for Biomedical Research Inc.
Schlumberger Foundation
Stiftung Swiss Bridge
Swiss Cancer League/Oncosuisse/ Swiss Cancer Research
Swiss Foundation for Research into Muscle Diseases
Swiss Japanese Collaboration (SNF)
Swiss National Science Foundation
SystemsX (SNF)
The Amytrophic Lateral Sclerosis Association
US Office of Naval Research
Volkswagen Stiftung

Personal fellowships to students and postdocs

Boehringer Ingelheim Fund
Erwin Schrödinger Stipendium
European Molecular Biology Organisation
German Research Council
Human Research Council
Marie Curie International Incoming Fellowship (IIF)
Marie Curie Intra-European Fellowship (IEF)
Muscle Foundation
National Center of Competence in Research
Schering Stiftung
Swiss National Science Foundation
Terry Fox Foundation

Collaboration with the University of Basel

We are very grateful to the University of Basel, in particular the faculty of the Biozentrum, for their continued cooperation in research and in our International PhD Program.
Group Joy Alcedo


Group Silvia Arber


Group Mohamed Bentires-Alj


Group Marc Bühler


Publications and dissertations

Publications and dissertations


Group Pico Caroni


Group Ruth Chiquet-Ehrismann


Group Susan Gasser


Group Helge Grosshans


Hurschler BA, Ding XC, Grosshans H (2010) 
Translational control of endogenous microRNA target genes in C. elegans. 
In: Rhoads RE (ed) miRNA regulation of the translational machinery. 
Prog Mol Subcell Biol 50:21-40

Group Brian A. Hemmings

Prog Mol Subcell Biol 50:21-40

Hemmings BA, Restuccia D, Tonks N (2009) 
Targeting the kinase II (editorial overview). 
Curr Opin Cell Biol 21:135-139

Hervgoh A, Hemmings BA (2010) 
TAZ mediated crosstalk between Wnt and Hippo signaling. 
Dev Cell 18:508-509

Hervgoh A, Hemmings BA (2009) 
Mammalian NDR/LATS protein kinases in hippo tumor suppressor signaling. 
Biofactors 35:338-345

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Curr Biol 19:1692-1702

Differential NDR/LATS interactions with the human MOB family reveal a negative role for hMOB2 in the regulation of human NDR kinase. 
Mol Cell Biol 2010 Jul 12 (Epub ahead of print)

GSK3beta regulates differentiation and growth arrest in glioblastoma. 
PLOS One 4:e7443

The endogenous inhibitor of Akt, CTMP, is critical to ischemia-induced neuronal death. 
Nature Neurosci 12:618-626

Carboxy-terminal modulator protein (CTMP) is a mitochondrial protein that sensitizes cells to apoptosis. 
Cell Signal 21:639-650

The Carboxy-Terminal Modulator Protein (CTMP) regulates mitochondrial dynamics. 
PloS One 4:e5471

Park J, Feng J, Li Y, Hammarsten O, Brazil DP, Hemmings BA (2009) 
DNA-PK phosphorylation of PKB requires a specific recognition sequence in the C-terminal hydrophobic motif. 
J Biol Chem 284:6169-6174

Association of LETM1 and MRPL36 contributes to the regulation of mitochondrial ATP production and necrotic cell death. 
Cancer Res 69:3397-3404

Regulation of OPA1-mediated mitochondrial fusion by Leucine zipper/EF-hand-containing transmembrane protein-1 plays a role in apoptosis. 
Cell Signal 21:767-777

Heat shock protein 70-mediated sensitization of cells to apoptosis by Carboxyl-Termina. Modulator Protein. 
BMC Cell Biol 10:53

Restuccia DF, Hemmings BA (2010) 
From man to mouse and back again: in vivo advances defining tumor AKTivities. 
In: Siegel V (ed) Disease models and mechanisms. The Company of Biologists (in press)

Restuccia DF, Hemmings BA (2009) 
Cell Signaling. Blocking Akt-ivity. 
Science 325:1083-1084

In vivo analysis of protein kinase B (PKB)/Akt regulation in DNA PKcs-null mice reveals a role for PKB/Akt in DNA damage response and tumorigenesis. 
J Biol Chem 283:30025-30033

Bone vascularization and trabecular bone formation are mediated by PKBAlph/Akt1 in a gene dosage dependent manner: in vivo and ex vivo MRI. 
Magn Reson Med 64:54-64

NDR kinase is activated by RASSF1A/MS1 in response to Fas receptor stimulation and promotes apoptosis. 
Curr Biol 18:1889-1895
Publications and dissertations


Group Nancy Hynes


Group Andreas Lüthi


Group Patrick Matthias


Group Thomas Oertner


Group Antoine Peters


Group Jan Pielage


Group Filippo Rijli


Group Botond Roska


Group Dirk Schübeler


Dissertations

FMI students successfully defended the following dissertations since the last FMI Report in 2008:

**Group Joy Alcedo**

Adilov B (2010)
The sensory system acts with a neurenomed U signaling pathway to mediate food type-dependent effects on lifespan. PhD thesis, University of Basel

Cornils A (2010)
Insulin-like peptides encode sensory information to regulate *C. elegans* development. PhD thesis, University of Basel

Octojic I (2010)
The gustatory system influences *Drosophila* lifespan. PhD thesis, University of Basel

**Group Silvia Arber**

Dalla Torre di Sanguinetto S (2009)

Friese A (2008)
Gene profiling of identified neurons to dissect molecular mechanisms involved in spinal reflex assembly. PhD thesis, University of Basel

Gürler S (2010)
Premotor neurons of the rectus femoris motor pool in the mouse spinal cord and mapping of semaphorin3E in the mouse hindbrain. MSc thesis, University of Basel

**Group Momo Bentires-Alj**

Holzer C (2009)
The role of protein tyrosine phosphatases in breast cancer. MSc thesis, University of Basel

**Group Pico Caroni**

Deguchi Y (2009)
Genetic diversity of principal neurons in the hippocampus. PhD thesis, University of Basel

Galimberti I (2008)
Structural plasticity of synaptic connectivity in the adult central nervous system. PhD thesis, University of Basel

Kramvis I (2010)
Spatial and temporal distribution of microglia clusters in the spinal cord of a murine model of FALS. MSc thesis, University of Basel

**Group Ruth Chiquet-Ehrismann**

Brosig M (2009)
Mechanotransduction in fibroblasts. PhD thesis, University of Basel

Kenzelmann D (2008)
Teneurins in development and disease. PhD thesis, University of Basel

Lutz R (2010)

**Group Rafal Ciosk**

Biedermann B (2009)
Regulation of totipotency in the *Caenorhabditis elegans* germ line. PhD thesis, University of Basel

**Group Witold Filipowicz**

Badertscher L (2009)
Molecular and cytological characterization of GW182 proteins. MSc thesis, University of Basel

Ribi S (2009)
MicroRNAs in the dark and light adapted mouse retina. MSc thesis, University of Basel

Sinkkonen L (2008)
MicroRNAs regulate “de novo” DNA methylation and histone mRNA 3’ end formation in mammalian cells. PhD thesis, University of Basel

**Group Rainer Friedrich**

Von Saint Paul F (2009)
Integration of olfactory bulb output in the zebrafish telencephalon analyzed by electrophysiology and 2-photon Ca^{2+} imaging. PhD thesis, University of Heidelberg

**Group Susan Gasser**

Friedel AM (2010)
The role of the Sgs1-RPA interaction in stabilizing stalled replication forks. PhD thesis, University of Basel

Gehlen L (2009)
Martino F (2008)

Nagai S (2009)
Roles for nuclear organization in the maintenance of genome stability. PhD thesis, University of Geneva

Group Helge Grosshans
Champion L (2009)
Role of the mir-35 microRNA family during C. elegans embryogenesis. MSc thesis, University of Basel

Ding XC (2009)

Group Brian A. Hemmings
Bozulic L (2009)
Regulation and functions of protein kinase B in DNA damage signaling. PhD thesis, University of Basel

Cornils H (2010)

Surucu B (2008)
Regulation of protein kinase B (PKB/Akt) by DNA-dependent protein kinase (DNA-PK) under physiological conditions. PhD thesis, University of Basel

Group Jan Hofsteenge
Heinrich C (2009)
Copine-III interacts with ErbB2 and promotes tumor cell migration. PhD thesis, University of Basel 
(together with Jan Hofsteenge)

Matsuda Y (2009)

Meira M (2008)
Studies on Memo, an important ErbB2 receptor-mediated component of the cellular migratory machinery. PhD thesis, University of Basel

Stölzle T (2009)

Group Andreas Lüthi
Ciocchi S (2009)
Fear conditioning and extinction-induced plasticity in the mouse amygdala. PhD thesis, University of Basel

Senn V (2009)
Differential activation of anatomically defined neuronal subpopulations in the amygdala during fear conditioning and extinction. PhD thesis, University of Basel

Group Patrick Matthias
Bordon A (2008)
Dissecting the roles of the different isoforms of the lymphoid-specific transcriptional coactivator OBF-1. PhD thesis, University of Basel

Yamaguchi T (2008)
Dissecting the roles of histone deacetylase 1 and 2 in the hematopoietic system. PhD thesis, University of Basel

Group Yoshikuni Nagamine
Chalupnikova K (2008)

Group Thomas Oertner
Holbro N (2009)
Structure-function analysis at the level of individual synapses. PhD thesis, University of Basel

Group Antoine Peters
Bryczynska U (2009)
Role of histone methylation in paternal transmission of epigenetic information. PhD thesis, University of Basel

Posfai E (2010)
Epigenetic regulation of germ cell and early embryonic development by Polycomb group proteins. PhD thesis, University of Basel

Group Jan Pielage
Bulat V (2010)
Identification of kinases and phosphatases controlling synapse formation and stability. MSc thesis, University of Basel

Group Botond Roska
Viney T (2010)
The diverse roles of inhibition in identified neural circuits. PhD thesis, University of Basel

Group Dirk Schübeler
Mohn F (2009)

Schwaiger M (2008)
Genome organization of DNA replication timing and its link to chromatin and transcription. PhD thesis, University of Basel

Group Nicolas Thomä
Amsler P (2009)
Role of RPA-Sgs1 interaction in stabilizing stalled replication forks. MSc thesis, University of Basel

Fischer E (2010)
Structural studies of the DDB1-DDB2-Cul4-Rbx1 complex. MSc thesis, University of Basel
Lectures and seminars

The following lectures and seminars were given by visitors to the FMI since the last FMI Report in 2008:

**Friedrich Miescher Lectures**

Richard Benton  
*University of Lausanne*  
*Lausanne, Switzerland*  
The molecular biology of *Drosophila* olfaction

Jack Greenblatt  
*University of Toronto*  
*Toronto, Canada*  
Protein complexes and functional pathways in yeast, bacteria and mammalian cells

Cynthia Kenyon  
*University of California*  
*San Francisco, USA*  
Genes and cells that influence the lifespan of *C. elegans*

Tak W. Mak  
*University of Toronto*  
*Toronto, Canada*  
The PI3 kinase pathway in metabolic stress

**40th Anniversary Seminar Series 2010**  
*Medical Applications of Basic Research*  
*Organized by the FMI postdocs*

Kari Alitalo  
*University of Helsinki*  
*Helsinki, Finland*  
VEGFs, a family portrait with the youngest members

Mina Bissell  
*Lawrence Berkeley National Laboratory*  
*Berkeley, USA*  
Microenvironment and tissue architecture in the development of breast cancer

Volker Brinkmann  
*Novartis Institutes for Biomedical Research Inc.*  
*Basel, Switzerland*  
Neurological diseases and the immune system

John Donoghue  
*Brown University*  
*Providence, USA*  
Neural prosthetics – to restore movement in humans with paralysis

**Gideon Dreyfuss**  
*University of Pennsylvania*  
*Philadelphia, USA*  
RNPs: instruments of global transcriptome regulation and therapeutic targets in neurodegenerative diseases

**Rudolf Jaenisch**  
*Whitehead Institute of Biomedical Research*  
*Cambridge, USA*  
Stem cells, the molecular control of reprogramming and the potential for personalized medicine

En Li  
*Novartis Institutes for Biomedical Research Inc.*  
*Shanghai, China*  
Epigenetics: from basic research to drug discovery

Huda Zoghbi  
*Baylor College of Medicine*  
*Houston, USA*  
Neurodevelopmental disorders and neurodegenerative diseases

**40th Anniversary Symposium**  
*From Basic Research to Pharmaceutical Breakthrough*  
*March 16, 2010*

Guest speakers:  
Patrick Aebischer  
*EPFL*  
*Lausanne, Switzerland*  
A new academia-industry partnership for the info-nano-bio convergence?

Mark C. Fishman  
*Novartis Institutes for Biomedical Research Inc.*  
*Cambridge, USA*  
Changing the grammar of drug discovery

Heidi Lane  
*Basilea AG*  
*Basel, Switzerland*  
mTOR: a validated target for the treatment of human cancer

Michel Maira  
*Novartis Pharma AG*  
*Basel, Switzerland*  
Clinical development of everolimus in oncology: Afinitor is born

Paul Nurse  
*Rockefeller University*  
*New York, USA*  
How discovery research works and why it matters
40th Anniversary Symposium
Frontiers in Biomedical Research
September 20/21, 2010

Guest speakers:

Thomas Boller
University of Basel
Basel, Switzerland

Gerhard Christofori
University of Basel
Basel, Switzerland

Barry Dickson
Research Institute of Molecular Pathology
Vienna, Austria

Catherine Dulac
Harvard University
Boston, USA

Amanda Fisher
MRC Clinical Sciences Centre
London, UK

Tony Hunter
Salk Institute
San Diego, USA

Josef Jiricny
University of Zurich
Zurich, Switzerland

Erich Nigg
University of Basel
Basel, Switzerland

Ueli Schibler
University of Geneva
Geneva, Switzerland

William Sellers
Novartis Institutes for Biomedical Research Inc.
Cambridge, USA

Francoise Stutz
University of Geneva
Geneva, Switzerland

George Thomas
University of Cincinnati
Cincinnati, USA

Susemil Tonegawa
Massachusetts Institute of Technology
Cambridge, USA

Jürg Tschopp
University of Lausanne
Lausanne, Switzerland

Student Science Colloquia*
*Organized by the Student Representatives

Judith Kimble
University of Wisconsin
Madison, USA

Stem cell controls: lessons from the C. elegans germline

Barbara Meyer
University of California
Berkeley, USA

Chromosome-wide control: regulating the repression, segregation, and genetic exchange of chromosomes

Terry Orr-Weaver
Massachusetts Institute of Technology
Cambridge, USA

Developmental dynamics of DNA replication: mechanisms leading to gene copy number variation

Robert Roeder
Rockefeller University
New York, USA

Transcriptional regulatory mechanisms in animal cells

Michael Rosbash
Howard Hughes Medical Institute
Waltham, USA

Circadian rhythms and sleep in Drosophila, neurons, circuits and molecules

Eran Segal
Weizmann Institute of Science
Rehovot, Israel

Reading the genome from DNA sequence to expression

Research Seminars

Reuven Agami
The Netherlands Cancer Institute
Amsterdam, The Netherlands

Cancerous miRNAs and regulatory RNA binding proteins

Julie Ahhinger
The Gurdon Institute
Cambridge, UK

Cell polarity transduction in C. elegans, and a bit about chromatin

Genevieve Almouzni
Institut Curie
Paris, France

Histone chaperones, centromeres and cell cycle

Adam Antebi
Baylor College of Medicine
Houston, USA

Life stages, aging and the nature of biological time

Suneel Apte
Cleveland Clinic Foundation
Cleveland, USA

Singular and cooperative developmental roles of ADAMTS proteases

Alan Ashworth
The Institute of Cancer Research
London, UK

Synthetic lethal approaches to the development of new therapies targeting DNA repair deficiencies in cancer

Peter Askjaer
Pablo de Olavide University
Seville, Spain

Essential roles of the protein kinase VRK-1 in nuclear envelope dynamics and organogenesis

Ted Baker
University of Auckland
Auckland, New Zealand

Serendipity and discovery: structure, assembly and stability of Gram-positive bacterial pilus

Frances Balkwill
Barts & London School of Medicine
London, UK

Autocrine cytokine networks in ovarian cancer growth and spread

Ernst Bamberg
Max Planck Institute of Biophysics
Frankfurt, Germany

Molecular properties of the light-gated ion channel channelrhodopsin

Nuno L. Barbosa-Morais
Cambridge Research Institute
Cambridge, UK

The Illumina Code: are microarrays telling you the whole truth about breast cancer?

Alberto Bardelli
Institute for Cancer Research and Treatment
Candiolo/Torino, Italy

Cancer mutations and targeted therapies in cells, mice and patients

Alison Barth
Carnegie Mellon University
Pittsburgh, USA

Activity-dependent control of BK channel trafficking

Charaf Benarafa
Harvard Medical School
Boston, USA

Role of SerpinB1 in inflammation and neutrophil homeostasis

Daniel Besser
Max Delbrück Center
Berlin, Germany

Signaling mechanisms in pluripotency and reprogramming
Stefano Biffo  
University of Eastern Piedmont  
Milan, Italy  
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Keith Blackwell  
Harvard Medical School  
Boston, USA  
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Frank-Dietmar Boehmer  
Friedrich Schiller University  
Jena, Germany  
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Giuseppina Bonizzi  
IFOM-IEO Campus  
Milan, Italy  
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Anne-Lise Borresen-Dale  
Institute for Cancer Research  
Oslo, Norway  
Exploring the systems biology of breast cancer

Jérôme Boulanger  
Johann Radon Institute  
Linz, Austria  
ND-Safir: non-parametric regression for patch-based fluorescence microscopy image sequence denoising

Jean-Pierre Bourquin  
University Childrens Hospital  
Zurich, Switzerland  
A xenograft model of drug-resistant childhood ALL for translational research

Derek P. Brazil  
University College Dublin  
Dublin, Ireland  
Insulin signaling in development and disease

Kevin Briggman  
MPI for Medical Research  
Heidelberg, Germany  
Neuronal circuit reconstruction using serial block-face scanning electron microscopy

Judith Campbell  
California Institute of Technology  
Pasadena, USA  
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Valérie Castellani  
University of Lyon  
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Constance Cejko  
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Boston, USA  
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Alain Charriot  
University of Liege  
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NIBR  
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Paterson Institute for Cancer Research  
Manchester, UK  
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Jennifer A. Cobb  
Southern Alberta Cancer Research Institute  
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Sigma-Aldrich Corporation  
St. Louis, USA  
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Ludwig Maximilians University  
Munich, Germany  
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Centre de Recherche de l’Hôtel-Dieu de Quebec  
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Munich, Germany  
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Yale University  
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University of Sussex  
Brighton, UK  
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Oregon Health & Science University  
Portland, USA  
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Jean-Marc Egly  
Institut de Génétique et de Biologie Moléculaire et Cellulaire  
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Bioinformatics Institute  
Singapore  
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Medical University of Vienna  
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Edinburgh, UK
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The Hebrew University
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Commissariat à l’Energie Atomique
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Kassel, Germany
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Magdeburg, Germany
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Braunschweig, Germany
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Biotech Research and Innovation Center
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Basel, Switzerland
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Madrid, Spain  
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Centre de Regulació Genòmica  
Barcelona, Spain  
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Cornelius Murre  
University of California San Diego  
San Diego, USA  
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University of Zurich  
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Northwestern University  
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Roger Wepf  
ETH Zurich (EMEZ)  
Zurich, Switzerland  
Zoom-in beyond light microscopy: a new approach for biological structure research – correlative light and electron microscopy on one and the same sample

Dale Wigley  
Cancer Research UK  
London, UK  
How do helicases know which way to go?

Alexander Wlodawer  
National Cancer Institute Frederick, USA  
Molecular puzzles: learning about function and mechanism of enzymes from their structures

Gregory Wulczyn  
Charité University Hospital  
Berlin, Germany  
let-7 and Lin-28: a feedback loop controlling neural stem cell maturation

Zhongzhou Yang  
Nanjing University  
Nanjing, PR China  
Homecoming with a heart of Aktive signaling – PKB’s function in cardiac development and diseases

Yimin Zou  
University of California  
San Diego, USA  
Wnts and cell polarity signaling in axon guidance
Teaching

FMI laboratories are home to approximately 100 PhD and MSc students registered at nearby universities, in particular the University of Basel, and carrying out their dissertation studies under the supervision of FMI group leaders. There are also students visiting from abroad, for example under the IAESTE scheme. In addition, FMI group leaders present lecture and laboratory courses at universities in Switzerland and other countries. Since the last FMI Report in 2008, the following lecture series and courses were given by FMI staff at the University of Basel (unless indicated otherwise):

Professor

Silvia Arber
Seminars in neurobiology
Basel Seminars in Neuroscience
Advanced seminars in neurobiology
Genes, brain and behavior
Foundation course: Cell biology and neurobiology
Introductory lectures in neurobiology
Developmental neuroscience

Marc Bühler
Chromatin and epigenetics
Structure, processing, and function of RNA
Chromatin and epigenetics
Postgraduate course on RNA (EPFL Lausanne)
6th course on epigenetics (Institute Curie, Paris)
Eukaryotic gene expression (University of Bern)

Pico Caroni
Genes, brain and behavior
Seminars in neurobiology
Basel Seminars in Neuroscience
Developmental neuroscience

Ruth Chiquet-Ehrismann
Cell interactions in development and disease
Literature on cell adhesion
FMI Seminars on growth control
Seminars in growth control
Literature on cell adhesion and cancer

Witold Filipowicz
Structure, processing and function of RNA
Recent literature in epigenetics
Advanced seminar in epigenetics
Post-translational protein modification

Susan Gasser
Recent literature in epigenetics
Seminars on genomic instability
Functional organization of the nucleus
Chromatin and epigenetics
Advanced seminars in epigenetics
Cytoskeleton
Dynamics and maintenance of the genome

Jan Hofsteenge
FMI Seminars on growth control
Translational control, protein modification
Seminars in growth control
Advanced seminars in epigenetics

Nancy Hynes
Literature on cell adhesion
FMI Seminars on growth control
Cellular signaling
Seminars in growth control
Literature on cell adhesion and cancer

Andreas Lüthi
Genes, brain and behavior
Seminars in neurobiology
New literature in neurobiology
Seminars: Neurobiology literature
Block course: Cell biology and neurobiology

Patrick Matthias
Recent literature in epigenetics
Advanced seminars in epigenetics
Advanced immunology
Mechanisms of eukaryotic transcription
Transcription regulation and gene expression in eukaryotes

Privatdozent

Rainer Friedrich
Genes, brain and behavior
Seminars: Neurobiology literature
Developmental neuroscience
New literature in neurobiology
Signaling in the nervous system

Filippo Rijli
Developmental neuroscience
Development of the somatosensory system

Dirk Schübeler
Genomics and drug discovery
Chromatin and epigenetics
Dynamics and maintenance of the genome

Non-faculty

Mohamed Bentires-Alj
Cell interactions in development and disease
Cellular signaling

Rafal Ciosk
Structure, processing and function of RNA

Helge Grosshans
Structure, processing and function of RNA

Brian Hemmings
FMI Seminars on growth control
Post-translational protein modification

Antoine Peters
Chromatin and epigenetics

Jan Pielage
Cell interactions in development and disease
Signaling in the nervous system

Botond Roska
Block course: Cell biology and neurobiology

Michael Stadler
Protein bioinformatics
Introduction to bioinformatics

Nicolas Thomä
Biophysics and structural biology
Structural biology
Replication, recombination and repair
Technology platforms

The FMI offers a wide range of central technical services that are continually updated to include the latest developments.

The broad-based approach at the FMI to the regulation of the human genome and proteome, organized loosely into the areas of signaling and cancer, epigenetics and neurobiology, has always been fertile ground for comparative studies at different structural levels, in different model systems, using multiple techniques. What has now crystallized in many institutes into "systems biology" is dependent upon high-throughput and sensitive techniques to both quantify changes in the genome and proteome and to integrate and analyze large amounts of resulting data in order to model the function of particular biological systems. Equally important is the availability of dedicated, highly trained technical staff who operate these facilities.

The FMI offers a wide range of central services that are continually updated to include the latest developments. The technology platforms available at the FMI include analytical and preparative fluorescence activated cell sorting (FACS), functional genomics (gene sequence analysis and DNA microarray technology), DNA deep sequencing, bioinformatics and computational biology support, mass spectrometric protein and peptide analysis, protein X-ray crystallography, tissue preparation and histology, monoclonal antibody production, photo-activated light microscopy (PALM), structured illumination microscopy (SIM) and three-dimension serial block face scanning EM (3D-EM). An extensive animal facility maintains and produces new strains of transgenic and genomic mice lines and stem cell technology under approved conditions. Yeast, Drosophila and Caenorhabditis elegans genetics are also well supported. Our information technology group provides high speed networked computer services and a dedicated facility for computer-based imaging and printing. The FMI has an extensive and expanding online library.

Picture: Heinz Gut, Protein Structure Facility
Biological systems are determined and deeply influenced by their anatomical organization. As biological units are without exception organized in three-dimensional space, a flat two-dimensional image of a structure is only a partial view of the reality. Therefore, a lot of effort in research is put into the visualization of anatomical structures at every size level from the organism to the molecule.

For a long time, biological research has tried to bring the third dimension into imaging techniques, with partial success. For example, MRI is now a valuable diagnostic tool in medicine. In cell biology, confocal microscopy and multiphoton microscopy were crucial developments, but following the distribution of a given component in an organism requires a view in depth, looking at each cell.

In the last 10 years, the demand for three-dimensional data has also grown in electron microscopy but constraints of electron microscopy have made the development of 3D techniques particularly difficult. The use of an electron beam provides a higher resolution but the sample has to be stained with heavy metals and located in a vacuum chamber. Furthermore, the electron beam cannot penetrate thick samples. Techniques such as tomography and serial sections for transmission electron microscopy have been around for quite a long time already. However, tomography is concerned only with small volumes and serial sectioning is a laborious and time-consuming nightmare for electron microscopists.

In 2004, an article by Horstman and Denk described a new technique termed “serial block face scanning electron microscopy” (SBFSEM) that allows the cutting and imaging of thousands of sections at the ultrastructural level. This technique is based on the following procedure: an entire piece of fixed tissue embedded in a resin is inserted into a scanning electron microscope. The microscope scans the surface of the block with an electron beam, generating a signal based on the presence of heavy metals in the sample that backscatter electrons. Backscattered electrons are detected and an image of the surface of the block is generated by a computer. A microtome within the microscope then removes a (typically 50-nm) slice and the freshly cut surface is scanned to produce the second image. The cycle is repeated automatically as long as required. This technique does not require the electron beam to go through the sample and avoids the collection of thin sections on a grid. The sections themselves are in fact discarded and the block itself is constantly imaged. A further advantage is that the field of view can be enlarged by tiling techniques without the distortion produced in transmission electron microscopy.

Following its commercialization, the FMI in 2008 acquired the second such unit in Europe at

Picture: Christel Genoud, 3D Electron Microscopy Facility
the instigation of Rainer Friedrich (FMI) and the C-CINA of the Biocenter, University of Basel, and established a 3D-EM Facility. The C-CINA and FMI are ideal partners for the possession of such a tool. The C-CINA provides unique expertise and trained researchers in electron microscopy, with the microscope housed in a perfect environment of controlled temperature, vibration and electromagnetic fields. On the other hand, the FMI provides expertise for sample preparation, image processing and many exciting projects linked to research in the neurosciences, epigenetics and signaling and cancer fields at the FMI. Research projects initiated in the last 2 years have ranged from the connectomic of the zebrafish olfactory bulb to the nuclear organization of the chromatin.

Furthermore, some projects at the FMI now cover the complete size scale with the SBFSEM used to locate a protein of interest in a cell and its effects on the cells, whilst the protein structure facility examines the 3D structure of the protein itself.

A further synergy is also now possible at the FMI with correlative light and electron microscopy studies. Filling the gap between light and electron microscopy is only possible because the light microscopy and imaging platforms of the FMI are at the cutting edges of these techniques. Thus, we can plan projects where 3D light images of a fluorescent-labeled molecule are acquired prior to an EM study of the same area of the tissue to examine the location of this molecule at much higher resolution in 3D. With this unique environment in Basel, the SBFSEM is finding its place, helping researchers who need to cross the bridge to electron microscopy.

Christel Genoud

PROTEIN STRUCTURE FACILITY

Structural biology has become an invaluable tool for gaining insight into complex cellular mechanisms and is also widely used in modern structure-based drug discovery. At present, structural biology methods include cryo electron microscopy, small-angle X-ray scattering, nuclear magnetic resonance and X-ray crystallography, with the latter having contributed to the vast majority of high-resolution structures in the Protein Data Bank.

A Protein Structure Facility has been established at the FMI to meet the increasing demand by research groups for structural biology data. The Facility focuses on protein X-ray crystallography, offering both advice and hands-on support at all stages from target analysis to the final three-dimensional structure. X-ray crystallography yields not only unrivaled near-atomic resolution of protein molecules complexed with small-molecule ligands or drug candidates but also crucial understanding of large protein-protein and protein-nucleic acid complexes.

The method relies on the production of micrometer-sized crystals containing the protein molecules of interest. By exposing these crystals to a highly focused and intense X-ray beam and recording with sensitive detectors the X-rays that are diffracted by the crystal, the 3D structure of the molecules in the crystal can be calculated. The FMI facility uses state-of-the-art structural bioinformatics and modeling tools to design expression constructs. These are then cloned into a versatile vector suite that is used for protein expression in bacterial, mammalian or insect cell systems. Once protein expression is achieved, the Facility provides advice and practical help with protein purification, using state-of-the-art crystallization robotics to produce high-quality crystals. The crystals are then tested and optimized for diffraction using our in-house microfocus Rigaku X-ray generator.

High-resolution data collection and demanding phasing experiments are carried out at the Swiss Light Source (SLS) synchrotron of the Paul Scherrer Institute in Villigen, to which we have regular access. The FMI Protein Structure Facility gives expert advice on the production of seleno-methionine labeled proteins for multi-wavelength anomalous dispersion (MAD) experiments and on heavy-atom derivatization of protein crystals for the multiple isomorphous replacement (MIR) method to solve crystal structures. The Facility has computing capacity for diffraction data analysis consisting of 3D-graphics workstations for model building and multiple-CPU servers for fast scientific computing, running the latest macromolecular crystallography software packages.

Besides guiding FMI scientists through the data collection and structure solution process, Facility experts offer support to investigators in the analysis of the final 3D-structures and may suggest further experiments or assist in detailed biophysical characterization of certain protein features. The Facility offers a very flexible mode of interaction with FMI collaborators, helping scientists to solve a particular problem or guiding them through the many steps of the complex structure determination process.

Understanding proteins and their complexes at the molecular level will give FMI scientists important insight into complex biological processes such as protein-protein and protein-DNA (RNA) interactions, catalytic activity and the mechanism of mutations involved in disease, and can also guide the development of potent inhibitors as therapeutic agents.

Heinz Gut
Students and postdocs Our students and postdoctoral fellows are not only exposed to the latest molecular approaches but are also immersed in a culture that takes delight in relating knowledge to biomedical application.

OUR GOALS
In 2010, the FMI celebrates 40 years as an internationally recognized center for fundamental biomedical research. Why do scientists continue to explore the basic molecular mechanisms that lead to healthy growth or to dysfunctional degeneration? Despite our ever-expanding understanding of cells and their genetic information, we simply do not know enough about the molecular mechanisms of human disease to be able to design treatments for those who suffer. Thus, enhancing our grasp on the fundamental mechanisms at work within living cells is a goal of vital interest both to society at large and to the pharmaceutical industry. Now more than ever, it is clear that basic biological research efforts can and will have a major impact on the quality of our lives.

RESEARCH AT THE FMI
The FMI is situated at the interface of academic research and pharmaceutical application and provides an open, collegial environment that encourages scientists to explore new research areas with daring ideas. Our research focuses on signaling and cancer, epigenetics and neurobiology. By understanding the control of cell division, the patterning of gene expression during cell differentiation, or the formation and maintenance of neuronal circuitry, we hope to forge new means to combat cancer, to correct degenerative states, and/or to suppress disease correlated with neuronal dysfunction. With an array of cutting-edge techniques, including genetic approaches in model organisms, as well as detailed proteomic and genomic analyses and live imaging, FMI scientists hope that their discoveries will help explain human disease and thereby contribute to novel treatments.

FMI INTERNATIONAL PhD PROGRAM
The FMI is also dedicated to the training of young scientists and fosters a lively educational atmosphere, having trained hundreds of young scientists at the postgraduate and postdoctoral levels. We offer dedicated programs for MSc and PhD students as well as research experience to visiting students from abroad. The FMI is affiliated with the University of Basel, where we also contribute to the teaching program. Of the 22 current FMI research group leaders, 15 hold professorships at various levels at the University of Basel and 3 are Privatdozent, all actively involved in various teaching activities. Students and postdocs are an extremely important component of the Institute. Today, out of a total of 338 members, the FMI is home to 100 students and 86 postdocs, from many different countries. For details of the FMI International PhD Program, consult www.fmi.ch/training/PhD/.

FINANCIAL SUPPORT
Students are selected competitively from countries the world over. The FMI provides financial support to graduate students in accordance with the scale of the Swiss National Science Foundation. The income is generous relative to international standards for PhD students.
STUDENT ACTIVITIES
Steffen Wolff and Sylvia Tippmann

Since PhD students make up about one-third of the people at the FMI, they are an active and influential component in all aspects of life at the Institute. This includes science education, career guidance, networking and, of course, social life.

A very important aspect is the Student Science Colloquia series. Here, students have the opportunity to invite highly recognized scientists for formal seminars and discussions at the FMI. This special series allows much closer interactions between FMI students and famous scientists than at normal seminars, both on a scientific and a career guidance level. In the last 2 years, the students invited Prof. Judith Kimble (University of Wisconsin), Dr. Eran Segal (Weizmann Institute), Prof. Barbara Meyer (University of California), Prof. Anthony Hyman (MPI, Dresden), Prof. Robert Roeder (The Rockefeller University) and Prof. Terry Orr-Weaver (MIT).

With the emphasis on career guidance and to provide information about well-known and also surprising career opportunities, speakers from different fields were invited to a career day and to a series of “career seminars”. Some of the career opportunities presented were in the fields of scientific administration, scientific writing, science politics, the pharmaceutical industry, scientific journalism, academia and science consulting. In parallel, the student body organizes language courses and a tutoring program for new PhD students.

FMI students are also active in networking with other PhD students, both in Switzerland and around the world. In 2009, the FMI student representatives organized a joint meeting with PhD students from MRC/LMBC (London). For three days on Lake Lucerne in Switzerland, 30 students of each institute discussed science and formed valuable contacts. Further such PhD Meetings will become an important part of the PhD life at the FMI. Networking locally in Basel is also promoted, with numerous social events organized in collaboration with the Biocenter and other institutes of the University of Basel. Notable among these is the annual football match between the FMI and the Biocenter. In-house social events include Friday Happy Hours, movie nights and guided city tours.

A complete overview of life as an FMI student can be found on the student website at www.fmi-students.ch.

POSTDOCTORAL TRAINING
Sandra Ziegler Handschin

The Friedrich Miescher Institute for Biomedical Research is an excellent place to obtain a comprehensive postdoctoral training. It offers a well-developed scientific infrastructure, good living conditions and an English-speaking international work force.

In 2006, the FMI was voted “Best Place to Work for Postdocs” outside the USA by The Scientist, an American magazine of the life sciences.

Postdoctoral investigators are hired by group leaders and are normally required to conduct research and studies in areas directly relevant to a particular research group. Normally, appointments are initially for one year, renewable each year up to four years.

Postdoctoral investigators are supported by five postdoc representatives. The representatives provide useful information to new and current postdocs and serve as mediators between postdoctoral fellows and the Director of the Institute. They also organize a variety of events and seminar series.

Applications
Please consult www.fmi.ch for full details of the graduate studies program and of openings for postdoctoral fellows.
Visiting the FMI

The FMI is located close to the center of Basel on the north bank of the river Rhine, just a few minutes from the borders of France and Germany. It is easily accessible by public transport from the two main railway stations and the airport.

From the Swiss/French railway station SBB/SNCF
Trams stop in front of the station. Take tram #1 or #2 from platform #3 (direction Bad. Bahnhof). Trams leave every 5 minutes. Get off at the stop Gewerbeschule at Mattenstrasse. Cross the tram tracks and follow Mattenstrasse for about 200 m, then turn right into Maulbeerstrasse. You will find the FMI about 150 m along Maulbeerstrasse on the right. Journey time is 10 minutes.

From the Swiss/German railway station Basel Bad. Bahnhof
The FMI is a short distance from the front entrance of this railway station. Cross the main road Schwarzwaldallee using the subway. Turn right, and 150 m on your left turn into Maulbeerstrasse. The FMI is about 100 m down the street on the left. Journey time is 3 minutes.

From the airport (EuroAirport Basel/Mulhouse/Freiburg)
Leave the baggage claim hall by the Swiss exit!
Bus: Take bus #30 to the Swiss/French railway station SBB/SNCF. Buses leave every 15 minutes (more frequent at peak times). Trams stop in front of the railway station. Take tram #1 or #2 from platform #3 (direction Bad. Bahnhof). Trams leave every 5 minutes. Get off at the stop Gewerbeschule at Mattenstrasse. Cross the tram tracks and follow Mattenstrasse for about 200 m, then turn right into Maulbeerstrasse. You will find the FMI about 150 m along Maulbeerstrasse on the right. Total journey time is 35 minutes.
Taxi: The FMI is a short ride from the airport by taxi. Journey time about 15 minutes.

By car
There is no parking space at the FMI entrance area on Maulbeerstrasse. Parking is possible on the adjacent Syngenta site. Enter the Syngenta site at gate 1047 on Mattenstrasse (see map). Register at the gate office. You will receive an ID badge and be allocated a parking space. Park the car and walk to the FMI (building 1066).

FMI telephone number for enquiries:
+41 61 697 66 51

Street map
www.geo-bs.ch/stadtplan_stadtplan_karte.cfm