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Research at the Biozentrum embraces a wide range of topics, however one main focus all the research groups share is a strong interest to understand the molecular organization of living organisms. The major areas of research are concerned with the structure and function of macromolecules, the wiring of regulatory circuits, and the general principles underlying complex biological systems and their dynamic interactions.

Currently, the research groups of the Biozentrum are grouped into five major areas of investigation: Growth & Development, Infection Biology, Neurobiology, Structural Biology & Biophysics and Computational & Systems Biology. These research areas are not strictly separated from each but rather share concepts and technologies. Furthermore, new and relevant questions often arise at the overlap between the research areas, while innovative solutions can be found arising from the expertise of each respective area.

Modern research increasingly depends on sophisticated technologies, notably in the fields of genomics, proteomics, imaging, and data analysis. To meet this challenge, the Biozentrum has established a number of so-called Technology Platforms focusing on recent developments, for example, in the fields of electron microscopy and light microscopy, proteomics, FACS (Fluorescence Activated Cell Sorting) and Research IT. The Biozentrum shares further Technology Platforms (microarray technology, next-generation sequencing, etc.) with other regional research institutes.
The advent of quantitative high-throughput methods in genomics, transcriptomics, proteomics, and imaging has led to a growing need for automated analysis of large volumes of data. As a growing number of molecular cell components continue to be characterized, increasing numbers of scientists are beginning to analyze how much of the behavior of biological systems is determined by the complex dynamic interactions between these molecular components. Such developments are paralleled by an increasing demand on mathematical models and computational approaches.

The research groups involved in Computational & Systems Biology are addressing a wide range of subjects including the computer simulation of the dynamical behavior of proteins at the molecular level, methods for the inference of structure and function of proteins, to the analysis of gene regulatory networks and genome evolution. Many of these projects are undertaken in collaboration with other research groups at the Biozentrum and often also with large international consortia.

Some of the groups have access to a wet laboratory in which experiments are carried out. All group leaders from Computational & Systems Biology are also involved as research group leaders at the Swiss Institute of Bioinformatics (SIB) and support, in collaboration with the SIB, a competitive IT infrastructure that incorporates application-, database-, and web servers, large scale storage and backup facilities.
The spatial and temporal regulation of interactions between molecules is fundamental to life. Growth & Development is dedicated to understanding how these coordinated interactions lead to cell growth, cell division and the development of living organisms.

Life is more complicated than a binary interaction of two factors and its regulation; various processes need to occur in parallel for a cell to function normally. For this reason, this research area covers a broad range of aspects from signal transduction, gene regulatory networks, cell division and cell cycle control to membrane transport, protein and mRNA transport, in a variety of experimental organisms such as bacteria, yeasts, worms, flies, fish and mammals.

This broad spectrum of experimental systems and regulatory processes makes it possible to investigate the basis of organ development, cancer and muscle function. In these endeavors, collaborative arrangements with other groups at the Biozentrum, in particular those from the research areas Structural Biology & Biophysics and Computational & Systems Biology are sought. The research groups involved in Growth & Development are also involved in initiatives within the University and beyond, such as the Basel Stem Cell Network, the Basel Signaling Alliance and SystemsX.ch.
The main objective of Infection Biology is to understand infectious diseases at both a cellular and molecular level in order to better control them in the future. The research groups at the Biozentrum involved in this field complement each other with their specialized skills and interests. The diversity of approaches to a common theme - the hostmicrobe interaction - generates a synergistic effect based on an intense exchange of scientific and technological expertise and experience.

The major research focus is on bacterial infections, in which currently nine pathogens are being studied. These represent archetypes for a range of virulence mechanisms such as intracellular replication, immune evasion, the injection of bacterial effectors into host cells, biofilm formation and persistence. The impact of this research goes beyond the field of microbial pathogenesis, addressing also basic principles in cell and molecular biology including pro-inflammatory signaling, intracellular traffic, regulation in bacteria, nanomachines, and in vivo microbial metabolism.

Several groups within Infection Biology have introduced systems biology as a new approach to investigating host-pathogen interactions and collaborate closely with their colleagues from other research areas, in particular from Structural Biology & Biophysics and Computational & Systems Biology.
One of the major challenges in biology is to understand how the nervous system forms, enabling it to appropriately respond to a broad spectrum of stimuli and to have control over complex functions, such as behavior and emotions. The nervous system must be capable of storing information, integrating it into the already existing memory and be able to retrieve it again.

The mechanisms employed are still poorly understood. The way in which neurons are generated and form meaningful functional circuits is not only of interest to developmental neurobiologists but also of great significance in regard to diseases, such as Alzheimer’s disease and epilepsy, injuries to the nervous system or disturbances in body weight regulation.

The various research groups belonging to Neurobiology are concerned with neurogenesis and cell specification, the use of stem cell-based models of neural function and dysfunction, the assembly and elimination of neurons and synapses, synaptic signaling and the function of neuronal circuits.

Currently, an area of focus in this field of research is neuromuscular disorders, obesity and autism spectrum disorders – all areas involving important translational aspects, which are being further investigated in cooperation with industry. From a scientific point of view, these activities are being supported and further promoted by the Neuroscience Network Basel (NNB), a network bringing together scientists from the Biozentrum and affiliated institutions as well as partners in industry.
The understanding of biological functions depends ultimately on an accurate account of biomolecular interactions in regard to structure, physical forces and their resulting dynamics. Enormous technical advances have been made in visualizing the threedimensional structures and in quantifying the dynamics of cellular components down to the atomic level. The research groups working in Structural Biology & Biophysics aim to apply and further develop these structural and biophysical techniques.

The structural techniques range from light microscopy, electron and scanning microscopy to X-ray crystallography and NMR spectroscopy. Biophysical methods encompass many different time-resolved spectroscopic techniques such as laser-flash spectroscopy and FRET, thermodynamic analytical methods such as ITC and DSC microcalorimetry, NMR imaging and in vivo spectroscopy. The use of rigorous combinations of these techniques enables the structure of biological matter to be determined at all magnifications; from details at the atomic level to entire cells up to small organisms.

In this way, dynamic changes can also be analyzed and quantified over a period of picoseconds or very long periods of time and the energetics and thermodynamics of biomolecular interactions can be investigated with very high precision. There is close teamwork between this branch of research and other areas of science at the Biozentrum, since many of the investigations involve projects which are carried out together with other research groups with a biochemical or biological orientation.
Structure-based functional analysis of cellular nanomachines

Our lab aims to structurally dissect cellular nanomachines and supramolecular assemblies at molecular detail and beyond with a combination of experimental approaches, including light, electron and scanning probe microscopies, X-ray crystallography and protein engineering. Our research focuses on (1) cytoskeleton structure, assembly and mechanics; (2) the nuclear pore complex and nucleocytoplasmic transport; and (3) developing novel optical and mechanical nano-sensors and -actuators for local diagnostics and therapy by minimally invasive interventions.

Characterization of human lamin assemblies

Half-minilamins, representing amino- and carboxy-terminal fragments of human lamin A, B1 and B2 with a truncated central rod domain, were investigated for their ability to form distinct head-to-tailtype dimer complexes. This mode of interaction is an essential step in the assembly reaction shown by full-length lamin dimers. Analytical ultracentrifugation studies indicate that the amino- and carboxyterminal coiled-coil dimers interact to form distinct oligomers. A corresponding interaction occurred also between heterotypic pairs of A and B-type lamin fragments. Hence, A-type lamin dimers may interact with B-type lamin dimers head-to-tail to yield linear polymers. These findings indicate that a lamin dimer has the freedom for a “combinatorial” head-to-tail association with all types of lamins, a property that might be important for the assembly of the nuclear lamina. We suggest that the head-to-tail interaction of the rod end domains represents a principal step in the assembly of cytoplasmic intermediate filament proteins too (Kapinos et al., 2010, J Mol Biol 396, 719-31). More recently, we have also solved the crystal structure of a coil2 fragment of human lamin A that simultaneously forms right- and left-handed anti-parallel coiled-coil interfaces. Based on this novel observation we speculate that the C-terminus of coil2 might unzip, thereby allowing for a right-handed coiled-coil interface to form between two laterally aligned lamin dimers. Such an interface, in turn, might co-exist with a heterotetrameric left-handed coiled coil assembly (Kapinos et al., 2011, J Mol Biol 408, 135-46).

Vimentin organization modulates the formation of lamellipodia

Vimentin intermediate filaments (VIF) extend throughout the rear and perinuclear regions of migrating fibroblasts, but only nonfilamentous vimentin particles are present in lamellipodial regions. In contrast, VIF networks extend to the entire cell periphery in serum-starved or nonmotile fibroblasts. Upon serum addition or activation of Rac1, VIF are rapidly phosphorylated at Ser-38, a p21-activated kinase phosphorylation site. This phosphorylation of vimentin is coincident with VIF disassembly at and retraction from the cell surface where lamellipodia form. Furthermore, local induction of photoactivatable Rac1 or the microinjection of a vimentin mimetic peptide (2B2) disassembles VIF at sites where lamellipodia subsequently form. When vimentin organization is disrupted by a dominant-negative mutant or by silencing, there is a loss of polarity, as evidenced by the formation of lamellipodia encircling the entire cell, as well as reduced cell motility. These findings demonstrate an antagonistic relationship between VIF and the formation of lamellipodia (Helfand et al., 2010, MBoC 22, 1274-89).

The nanomechanical properties of rat fibroblasts are modulated by interfering with the vimentin intermediate filament system

The contribution of the intermediate filament (IF) network to the mechanical response of cells has so far received little attention, possibly because the assembly and regulation of IFs are not as well understood as that of the actin cytoskeleton or of microtubules. The mechanical role of IFs has been mostly inferred from measurements performed on individual filaments or gels in vitro. In this study, we employed atomic force microscopy (AFM) to examine the contribution of vimentin IFs to the nanomechanical properties of living cells under native conditions. To specifically target and modulate the vimentin network, Rat-2 fibroblasts were transfected with GFP-desmin variants. Cells expressing desmin variants
were identified by the fluorescence microscopy extension of the AFM instrument. This allowed us to directly compare the nano-mechanical response of transfected and untransfected cells at high spatial resolution by means of AFM. Depending on the variant desmin, transfectants were either softer or stiffer than untransfected fibroblasts. Expression of the non-filament forming GFP-DesL345P mutant led to a collapse of the endogenous vimentin network in the perinuclear region that was accompanied by localized stiffening. Correlative confocal microscopy indicates that the expression of desmin variants specifically targets the endogenous vimentin IF network without major rearrangements of other cytoskeletal components. By measuring functional changes caused by IF rearrangements in intact cells, we showed that IFs play a crucial role in the mechanical behavior not only at large deformations but also in the nanomechanical response of individual cells (Plodinec et al., 2011, J Struct Biol 174, 476-84).

Cryo-electron tomographic reconstruction of the nuclear pore complex

Nuclear pore complexes (NPCs) are quasi-8-fold symmetric molecular assemblies that fuse the inner and outer nuclear membranes to form aqueous translocation channels. Due to its sheer size and complexity, dissecting the high-resolution 3D structure of the NPC in its hydrated state is a formidable task. We applied cryo-electron tomography to spread nuclear envelopes from Xenopus oocytes and performed asymmetric unit averaging of 3D tomographic NPC volumes to yield a refined model at 6 nm resolution. Novel structural features, particularly in the spoke-ring complex and luminal domains, were apparent. A fused concentric ring architecture of the spoke-ring complex was found along the translocation channel. A comparison of the refined Xenopus model to that of its Dictyostelium homologue yielded similar pore diameters at the level of the three canonical rings, although the Xenopus NPC was found to be 30% taller than the Dictyostelium pore. This discrepancy is attributed primarily to the relatively low homology and different organization of some nucleoporins in the Dictyostelium genome as compared to that of vertebrates (Frenkiel-Krispin et al., 2010, J Mol Biol 395, 578-86).

Analysis of articular cartilage by indentation-type atomic force microscopy

As documented previously (cf. Stolz et al., 2009, Nature Nanotechnology 4, 186-92), articular cartilage exhibits a scale-dependent dynamic stiffness when probed by indentation-type atomic force microscopy. A micrometer-size spherical tip revealed a unimodal stiffness distribution, whereas probing articular cartilage with a nanometer-size pyramidal tip resulted in a bimodal nanostiffness distribution. We concluded that indentation of the cartilage’s soft proteoglycan gel gave rise to the lower nanostiffness peak, whereas deformation of its collagen fibrils yielded the higher nanostiffness peak. The hypothesis was tested with a synthetic gel-microfiber composite. In analogy with the articular cartilage, the microstiffness distribution of the synthetic composite was unimodal, whereas its nanostiffness exhibited a bimodal distribution. Thus, the nanoscale lower peak is a measure of the soft proteoglycan gel, whereas the nanoscale higher peak measures collagen fibril stiffness. In contrast, the micrometer scale measurements failed to resolve separate stiffness values for the proteoglycan and collagen fibril moieties. We propose to use nanostiffness as a new biomarker to analyze structure-function relationships in normal, diseased and engineered cartilage (Loparik et al., 2010, Biophys J 98, 2731-40).

A nonadjuvanted polypeptide nanoparticle vaccine against rodent malaria

We have designed a prototypic malaria vaccine based on a highly versatile self-assembling polypeptide nanoparticle (SAPN) platform that can repetitively display antigenic epitopes. We used this platform to display a tandem repeat of the B cell immunodominant repeat epitope (DPPPPNPN)\(_D\) of the malaria parasite Plasmodium berghei circumsporozoite protein. Without an adjuvant, the SAPN construct P4c-Mal conferred a long-lived, protective immune response to mice with a broad range of genetically distinct immune backgrounds. Immunized mice were protected against an initial challenge of parasites up to six months after the last immunization or for up to 15 months against a second challenge after an initial challenge of parasites had been cleared. Furthermore, we demonstrate that the SAPN platform not only functions to deliver an ordered repetitive array of B cell peptide epitopes but operates as a classical immunological carrier to provide cognate help to the P4c-Mal-specific B cells (Kaba et al., 2009, J Immunol 183, 7268-77).
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The organization of body pattern in developing multi-cellular organisms is controlled to a large extent by cell-cell signaling. In the past two decades, the molecular components of relatively small number of diverse developmental signaling cascades conserved throughout evolution have been identified. We have been studying two important developmental signals (Dpp/BMP and Fgf), and our efforts concentrated firstly on characterizing the signaling pathways in detail and deciphering their molecular logic, and secondly on understanding how these pathways control exquisite cellular behavior during development, both in Drosophila and in zebrafish. Our most intense research efforts are directed towards a profound understanding of cell behavior in branching morphogenesis, a process that leads to the ramification of epithelial structures such as seen in the lung, the kidney, many internal glands as well as the vascular system.

Cell signaling in organ formation

It has been proposed more than a century ago that the organization of body pattern might be controlled by so-called morphogen gradients. Only recently has it been possible to demonstrate that secreted proteins of the Transforming Growth Factor β (TGFβ), wnt and Hedgehog families specify positional information by this mechanism. Drosophila Dpp is a member of the TGFβ superfamily and was the first secreted protein for which a morphogen function has been clearly demonstrated. Over the past ten years we have characterized the Dpp signaling pathway in detail, in collaboration with the group of Konrad Basler in Zurich. Our studies provide the molecular framework for a mechanism by which the extracellular Dpp morphogen establishes a finely tuned, graded read-out of a transcriptional repressor complex including Smad proteins and the zinc-finger protein Schnurri. Targets of this repressor complex include transcriptional regulators as well as secreted proteins involved in morphogen transport. Other morphogens, which pattern the nervous system or the limb fields in higher vertebrates, might use similar mechanisms. Our current efforts are devoted to a systems biology approach and are done in the framework of the WingX project of the Swiss initiative in Systems Biology.

The experiments we concentrate on involve genome-wide target gene identification, real-time analysis of morphogen gradient readout, and computer modelling to better understand the dynamics of the Dpp morphogen system. Just recently, we have identified a novel feedback regulator of the Dpp system which controls the spreading of the Dpp molecule and might be involved in the adaptation of the morphogen gradient to tissue size. Our studies will eventually lead to a comprehensive understanding of morphogen function in tissue growth and patterning, a key issue in modern developmental biology (Fig. 1).

Cell rearrangement in organ formation

To gain insight into how signaling pathways control more complex cellular decisions during the process of organ morphogenesis, we investigate the formation of the Drosophila tracheal system, an epithelial branched network similar to the lung, the kidney or the vasculature. Tracheal development serves as a paradigm to understand how epithelial cell sheets can be transformed by cell signaling and cell-cell or cell-matrix interactions into complex three dimensional networks, a process generally referred to as branching morphogenesis. Our approach has been to identify genes involved in the process by genetic analysis, and the characterization of relevant gene products by in vivo and in vitro analysis. In addition, we have devoted major efforts to characterize branching morphogenesis at the cellular level, using avant-garde, live imaging technology.

Over the past decade, these studies have provided a framework for understanding complex processes involved in the architectural design of developing organs, including the control and integration of cell migration and cell rearrangement via cell-cell signaling and extracellular matrix components (Fig. 2).

Studies on the development of blood vessels in higher organisms suggest strong parallels between tracheal development in insects and tube formation in the growing vasculature (see Fig. 2). Interested by this possible developmental similarity,
we have initiated studies aimed at a better understanding of blood vessel development in zebrafish, one of the most promising animal systems in the study of angiogenesis available at the moment. We have indeed found that our approach to studying cell rearrangement during tracheal development provides a novel insight into how cells behave during angiogenesis when applied to zebrafish. We have recently proposed a novel model for the architecture of the first vessels formed via angiogenesis, a model which is strikingly different to the one previously described. Our studies redefine the cellular routines involved in angiogenesis, and provide the basis for all future studies in the zebrafish regarding angiogenesis. We have now strengthened our efforts to study angiogenesis using live imaging combined with novel transgenic lines and strategies. Particular emphasis is devoted to the study of blood vessel fusion, a process that has not been studied in the past in vivo at the cellular level.

**Fig. 2:** Drosophila melanogaster trachea and vertebrate vasculature branching. Branchless (BNL), a fibroblast growth factor (FGF), acts at the top of the hierarchy of cellular events that orchestrate tracheal branching in Drosophila melanogaster (a to c). During vertebrate angiogenesis, vascular endothelial growth factor (VEGF) signalling determines the formation of angiogenic sprouts and controls tip cell and stalk cell identity through Delta Notch signalling. Taken from Affolter et al. (2009) Nat Rev Mol Cell Biol 10, 831-42.

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Motor behavior represents the ultimate output of nearly all nervous system activity. Our studies aim at identifying the principles that allow neuronal circuits to orchestrate accurate and timely control of motor output behavior in response to a variety of stimuli such as sensory cues or voluntary initiation of movement. To decipher how motor circuits engage in the control of movement, we elucidate the organization and function of neuronal circuits by studying synaptic connectivity, genetic and molecular identity as well as functional properties.

We address these questions using multifaceted approaches combining many technologies. These include state-of-the-art mouse genetics, development and implementation of viral technologies for transsynaptic circuit tracing and functional manipulation, gene expression profiling of identified neuronal subpopulations, electrophysiology and behavioral analysis. The combination of these approaches allows us to assess connectivity and manipulate function in order to determine the role of defined circuit elements in animal behavior. Furthermore, it puts us in a position to uncover the mechanisms involved in the assembly of motor circuits during development as well as circuit reorganization upon disease or injury.

Anatomical organization of motor circuits reflects functional antagonism

Walking represents one of the most fundamental manifestations of motor behavior and is based on the selective control of functionally antagonistic muscles. An important entry point to understand differential regulation of motor output has been the comparative analysis of antagonistic motor neuron pool function. For example, extensor and flexor motor neuron pools in the spinal cord innervate distinct limb muscles, which are generally active in alternation between an “on-ground” stance and an “off-ground” swing phase during walking. Even though functional antagonism can be observed at the level of individual neurons in many cases, an overall anatomical assessment of organizational principles of neuronal circuits relaying information to functionally distinct motor neuron pools is currently lacking.

In recent work, we used monosynaptically-restricted transsynaptic viruses to elucidate premotor anatomical substrates for extensor-flexor regulation in mice (Tripodi et al., 2011). We observe a medio-lateral spatial segregation between extensor and flexor premotor interneurons in the dorsal spinal cord. These premotor interneuron populations are derived from common progenitor domains, but segregate by timing of neurogenesis. We found that proprioceptive sensory feedback from the periphery is targeted to medial extensor premotor populations and required for extensor-specific connectivity profiles during development. Our findings provide evidence for a discriminating anatomical basis of antagonistic circuits at the level of premotor interneurons, and point to synaptic input and developmental ontogeny as key factors in the establishment of circuits regulating motor behavioral dichotomy.

Studying motor circuit organization using transsynaptic viral tools

Motor neurons in the spinal cord are grouped into motor neuron pools, which represent the functional units innervating individual muscles. While much is known about the specificity of peripheral motor neuron pool trajectories, information on diversity, distribution and connectivity of central neurons monosynaptically connected to motor neuron pools and instrumental in controlling motor output is sparse. Since individual motor neurons receive synaptic input from many thousands of presynaptic neurons mostly located at distant sites, a global assessment of connectivity has not been possible up to now. We therefore recently developed a virus-based anatomical connectivity assay allowing us to visualize location and identity of neurons premotor to functionally defined motor neuron pools in three-dimensional space (Stepien et al., 2010).

We employed this method (1) to determine and probe the reproducibility of three-dimensional premotor interneuron distributions connected to motor neuron pools, (2) to visualize the local or distributed nature of defined premotor interneuron subpopulations, and (3) to determine synaptic connectivity rules of cholinergic premotor partition cells, known to regulate motor neuron excitability through Cbouton synapses.
with motor neurons. We found that virally-labeled premotor spinal interneurons exhibit highly reproducible and segmentally widespread distribution patterns differing for functionally distinct muscles, but show specific distributions for defined interneuron populations. Analysis of connectivity between cholinergic partition cells and motor neurons reveals the existence of a bilaterally projecting subpopulation with widespread rostro-caudal segmental origin and preferential connectivity to functionally equivalent motor neuron pools.

Our study establishes the use of monosynaptically restricted rabies viruses in vivo, determines the connectivity matrix in the motor output system at high resolution and makes use of this method to reveal rules of synaptic specificity of one defined premotor interneuron population.

**Molecular codes for synaptic specificity**

The assembly of spinal reflex circuits depends on the selectivity of synaptic connections formed between sensory afferents and motor neurons in the spinal cord. The organization of these reflex circuits exhibits several levels of specificity. Only certain classes of proprioceptive sensory neurons make direct, monosynaptic, connections with motor neurons. 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). Changing the profile of Sema3e-PlexinD1 signaling in sensory and motor neurons leads to a functional and anatomical rewiring of monosynaptic connections, but does not alter motor pool specific connectivity.

Our findings indicate that patterns of monosynaptic connectivity in this prototypic CNS circuit are constructed through a recognition program based on repellent signaling. We thus uncover 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 motor neurons (Vrieseling and Arber, 2006; Livet et al., 2002), our findings also demonstrate how transcriptional pathways intersect with cell-surface recognition codes implementing specificity of synaptic connections.
Publications 2011


Ma, Chi H. E; Brenner, Gary J; Omura, Takao; Samad, Omar A; Costigan, Michael; Inquimbert, Perrine; Niederkofler, Vera; Salie, Rishard; Sun, Chia C; Lin, Herbert Y; Arber, Silvia; Coppola, Giovanni; Woolfe, Clifford J; Samad, TA (2011). The BMP Coreceptor RGMb Promotes While the Endogenous BMP Antagonist Noggin Reduces Neurite Outgrowth and Peripheral Nerve Regeneration by Modulating BMP Signaling. *Journal of Neuroscience, 31,* 18391-18400.

Publications 2010


The aim of our research is to gain a molecular understanding of the mechanisms that control bacterial uptake and inflammation during infection of epithelial cells by the enteroinvasive pathogen *Shigella flexneri*. These bacteria invade the colonic epithelium of humans, causing an acute mucosal inflammation called shigellosis. They enter enterocytes by injecting via a type three secretion apparatus multiple effector proteins that manipulate several key components of the host cytoskeletal machinery and promote bacterial engulfment. Once internalized, *S. flexneri* multiplies in the cytoplasm and uses actin-based motility to spread to adjacent intestinal epithelial cells.

During infection, massive mucosal inflammation is observed in the intestine of infected patients. Intestinal epithelial cells play a central role in this process. They sense pathogenic invasion and respond by inducing a transcriptional program whose major function is to stimulate innate immune defense mechanisms. *Shigella* recognition occurs essentially intracellularly via the pattern recognition receptor Nod1 that recognizes the core dipeptide structure, γ-D-glutamyl-meso-diaminopimelic acid found in the peptidoglycan of Gram-negative bacteria. Among the genes up-regulated in infected epithelial cells, the chemokine interleukin-8 (IL-8) plays a central role. Indeed, by attracting polymorphonuclear cells (PMNs) from the peripheral circulation to the infected area, IL-8 secretion limits the spread of *Shigella* invasion.

**Bacterial entry into epithelial cells**
Few host proteins targeted by *Shigella* have been identified. However, we do not have yet a comprehensive model for the host-pathogen interactions that govern bacterial entry into epithelial cells. With this aim, we have set up, in collaboration with Prof. C. Dehio, a screening platform equipped with automated epifluorescence microscopy, liquid handling and image analysis. A high throughput assay of *Shigella* entry into HeLa cells has been developed and will be used for a genome-wide image-based RNAi screen. This screen is part of the InfectX project (SystemsX.ch), which aims at identifying the components of the human infectome for a set of important bacterial and viral pathogens.

**Cell-cell propagation of proinflammatory signals**
*Shigella flexneri* uses multiple secreted effector proteins to weaken interleukin-8 (IL-8) expression in infected intestinal epithelial cells. For instance, the type III secreted effectors OspG and OspF attenuate IL-8 expression by preventing NF-κB nuclear translocation, and reducing its access to chromatin, respectively. Yet, massive IL-8 secretion is observed in shigellosis. We have reconciled these contradictory observations by showing that a host mechanism of cell-cell communication compensates the immuno-suppressive activity of *Shigella* effectors in infected cells. By monitoring signaling at the single cell level in conditions of low multiplicity of infection, we observed that during *Shigella* infection, the activation of important signaling pathways of inflammation including NF-κB, JNK, ERK and p38, propagates from infected to uninfected adjacent cells (*Fig. 1*). We recently showed that this mechanism of bystander activation amplifies inflammation in response to bacterial infection (Kasper et al. *Immunity*, 2010). Indeed, by monitoring IL-8 expression at the single-cell level,
we showed that bystander cells produce large amount of IL-8 during *Shigella* infection (Fig. 2). In addition, we showed that bystander activation can be mediated by gap junctions. Based on these observations, we proposed that the process of bystander activation functions as an efficient host defense mechanism that circumvents the activities of bacterial effectors and ensures inflammation signaling and IL-8 production during bacterial infection. Such mechanism of signaling amplification might explain the massive inflammation observed in the colonic epithelium of patients infected by *Shigella*.

Using mass spectrometry, RNAi screens and phosphoproteomics, we are currently investigating in more detail the molecular basis of bystander activation. In addition, we are investigating the strategies that *Shigella* bacteria have developed to block IL-8 expression in infected cells. Indeed, our data indicate that besides NF-κB and the MAP kinase pathways, additional signaling pathways may be targeted by *Shigella* to inhibit IL-8 expression in infected cells.

**Publications 2011**


**Publications 2010**


Our group investigates molecular pathways controlling neuronal development in higher vertebrates with the goal of better understanding brain function and dysfunction. Our work focuses on neurotrophins and their receptors, using mouse mutants and novel in vitro assays based on the generation of progenitor cells derived from embryonic stem (ES) cells.

**Neurotrophins in health and disease**

The 4 neurotrophin genes found in mammals encode proteins playing critical roles in virtually all aspects of the biology of neurons, including their survival, process elongation and activity-dependent modifications of synaptic function. Mutations and polymorphisms discovered in humans further indicate that the neurotrophin signaling system is involved in dysfunction of the adult central nervous system. In particular, brain-derived neurotrophic factor (BDNF), the neurotrophin expressed at the highest levels in the mature CNS, is thought to be involved in a variety of conditions including dysregulation of food intake, memory consolidation, depression, Huntington’s disease and Rett syndrome. Our work focuses on the cell biology of BDNF in neurons, including its localization, biosynthesis, processing, storage and activity-dependent release from neurons. To explore its role in the adult, we generated a novel mouse line that can live for several months while essentially lacking BDNF in the CNS following the selective deletion of the gene in neurons (see Rauskolb et al., 2010). The phenotype of these mice resemble what is seen in mouse models of Rett Syndrome and in Huntington’s disease and while the brain appears grossly normal and does not show reduction in the number of neurons, some groups of neurons fail to grow normally (see Fig. 1).

**Embryonic stem cells**

Our group found that mouse ES cells can be “domesticated” to generate essentially pure populations of neuronal progenitors corresponding to those present in the developing mouse cortex and identified as Pax6-positive radial glial cells. These progenitors go on to generate in vitro virtually pure population of neurons using glutamate as neurotransmitters, just like they do in vivo during the development of the telencephalon. We extensively use this novel culture system to study the impact of genes thought to be important for the development and function of the CNS, including for example the neurotrophin receptors. We recently showed that in spite of their similarities, the tyrosine kinase receptors TrkA, TrkB and TrkC exert surprisingly different functions during the development of the nervous system. Briefly, TrkA causes the death of neurons in the absence of its ligand nerve growth factor (NGF), while the highly related BDNF receptor TrkB does not do so (see Fig. 2 and Nikoletopoulou et al., 2010). This and related findings help to explain why tumors in children designated neuroblastoma spontaneously regress when they express TrkA, while they have a very bad prognosis when they express TrkB. Given the extraordinary potential of ES cells used as a discovery tool and the possibility to reprogram human somatic cells into pluripotent cells, our group also uses human ES cells to generate human neurons in vitro and to model diseases.

**Fig. 1:** GABA-ergic neurons fail to develop normally in the absence of BDNF. A represent a neuron filled with a dye in the striatum of a normal mouse (WT) at 2-month of age, compared with a neuron in the same region developing in a brain devoid of BDNF (cbdnf KO). Note the size reduction. B Quantification based on large number of neurons of the reduced dendritic growth of neurons lacking BDNF supply (black dots) compared with wild-type neurons (white dots).

**Fig. 2:** Neurons (red) expressing the NGF receptor TrkA die (middle top panel) and this death can be prevented by the addition of NGF (middle bottom panel). Neurons expressing the TrkA-related receptor TrkB (right panels) do not die, even when BDNF is not present (right top panel).

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**b-III tubulin staining**

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Publications 2011
Heyden, Alexandra; Ionescu, Mihai-Constantin S; Romorini, Stefano; Kracht, Bettina; Ghiglieri, Veronica; Calabresi, Paolo; Seidenbecher, Constanze; Angenstein, Frank; Gundelfinger, Eckart D (2011). Hippocampal enlargement in Bassoon-mutant mice is associated with enhanced neurogenesis, reduced apoptosis, and abnormal BDNF levels. Cell and tissue research, 346(1), 11-26.


Publications 2010

Cell differentiation can be achieved by specifying cell fate through deterministic instructive signals or by stochastic transitions to various epigenetic states. Stochastic differentiation offers the advantage that it can generate cellular diversity even in the absence of external clues. This form of phenotypic diversity is advantageous for adaptation and survival in changing environments, as well. For example, random variations in surface antigens increase the chance of a microorganism to escape from the immune defence.

Recent research in our group has focused on two major aspects of cellular differentiation; the control principles of chromosomal epigenetic mechanisms silencing, and the evolutionary optimization of transcriptional positive feedback loops involved in stochastic adaptation.

The first area of our research has focused on the understanding of epigenetic silencing. This is of paramount importance since cellular differentiation in higher eukaryotic organisms often employs silencing to package genes into the inactive heterochromatin. The logic behind chromosomal epigenetic processes has been unclear. Our recent work unravelled spatial aspects in control of silencing in yeast cells. The corresponding reaction-diffusion model revealed that the same reaction mechanism that describes silencing can support both graded monostable and switch-like bistable gene expression, depending on whether recruited repressor proteins generate a single silencing gradient or two interacting gradients that flank a gene.

Our experiments confirmed that chromosomal recruitment of activator and repressor proteins permits a plastic form of control; the stability of gene expression is determined by the spatial distribution of silencing nucleation sites along the chromosome. Our findings in yeast are expected to stimulate further studies to reveal the logic of chromosomal epigenetic regulation in higher eukaryotic organisms and we are starting a research project in this direction.

The second area of our research has focused on the functioning of feedback loops that arise from evolutionary gene duplication. Cellular networks are rewired during evolution by strengthening or weakening the binding interactions in order to develop new regulatory schemes. Feedback loops can display a broad variety of behaviours depending on if they are embedded into enzymatic, transcriptional and protein interaction networks. Since many cellular components are multifunctional, the coordination of distinct functions by feedback control puts a major demand on feedback regulation. Multifunctional proteins often arise after evolutionary gene duplication. Therefore, it is instructive to study control by parallel feedback loops that arise after gene duplication.

The classical galactose regulatory network contains a pair of duplicated genes. Their predecessor, a single bifunctional protein Gal1/3 catalyzed enzymatically the first step of galactose breakdown and also transduced the information on the intracellular galactose concentration to activate the transcriptional circuitry through the Gal4p transcriptional activator. After the whole genome duplication, Gal3p lost its enzymatic activity and has only the signal transmission capacity. On the other hand, Gal1p retained the enzymatic activity to convert galactose but its signal transmission capacity weakened.
Publications 2011


Publications 2010


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My laboratory is mainly investigating on the structure-function relationship of membrane proteins, with a special interest in the gating mechanisms of K⁺ channels. Other protein families that are currently studied are the Cl⁻ channels and Cl⁻/H⁺ exchangers, the ammonium transporters, the multidrug efflux proteins, and the translocon. We are also developing an automated umbrella sampling framework allowing for the calculation of multi-dimension PMFs.

Fundamental physiological mechanisms such as transport and signalling in living cells involve membrane proteins. The regulated diffusion of various substrates through membrane transport proteins allows for a fine control of the cell’s metabolism and signalling. These fundamental functions of membrane proteins rely on three interdependent mechanisms: permeation, selectivity and gating. Agents, such as the transmembrane voltage, the pH, or various ligands, can potentially modulate these properties and thus be used in signalling processes. Our aim is to elucidate the microscopic molecular determinants of these mechanisms underlying important functions in different families of proteins.

Taking advantage of available X-ray structures, we use molecular dynamics (MD) simulations (see Fig. 1) and free energy calculations to characterize key chemical interactions and the mechanical plasticity of the proteins. These calculations provide information that complements the static picture given by the experimental structures and allow for a better interpretation of functional data. A key element in the understanding of a molecular mechanism is the underlying potential of mean force (PMF) that controls the rate of key transitions. To obtain this fundamental property, we extract statistical information from hundreds of independent MD simulations that, once combined through some statistical mechanics rules, yield a multidimensional view of the free energy valleys and barriers (i.e. the PMF) that determines the function of the protein. Based on a hierarchical approach in which the resulting PMF is used as an input to stochastic simulations, one can calculate macroscopic observables, e.g. the current vs voltage relation of an ion channel (see Fig. 2). Using this approach we aim at providing a better understanding of the mechanisms that regulate the function of membrane transport proteins.

**Potassium channels**

Potassium channels are notably involved in the regulation of action potentials in excitable tissues, such as the heart and brain. The bacterial KcsA channel is recognized as a close homolog of the eukaryotic Shaker channel. Because Shaker is the most studied K⁺ channel and because all members of the large K⁺ channel family share many structural features, it is tempting to discuss the structural properties of KcsA as if it represents all K⁺ channels. While biophysical studies of the KcsA channel have revealed the basic principles underlying many essential functions of K⁺ channels (e.g. Bernèche & Roux, Nature 2001; Noskov et al., Nature 2004), there is still little work done to elucidate the structural mechanisms explaining why different K⁺ channels exposed to the same environment would produce current with different magnitudes and activation/inactivation kinetics.

We are thus trying to elucidate the microscopic mechanisms explaining why, for example, some K⁺ channels conduct ions at rates 10 times higher than others, and why some channels inactivate on the millisecond time-scale, while others do not even seem to inactivate. These questions are central to signalling processes in nervous cells since the shape and firing frequency of action potentials are notably controlled by the synchronized activity of many K⁺ channels with different kinetic properties. Our working hypothesis is that the selectivity filter – the only portion of the pore in which permeating ions interact strongly with the protein – is in large part responsible for the variety of observed phenotypes. While the sequence of the selectivity filter itself is extremely well conserved, the residues in its vicinity are not. These variations in sequence seem to determine how the conductivity of the selectivity filter of the different channels is controlled, notably through a mechanism known as C-type inactivation – which consists in the spontaneous, time dependent, closure of the channel’s pore following activation.
Inactivation mechanism of K+ channels
A few years ago, we proposed a detailed microscopic model of a putative gating mechanism in the selectivity filter of K+ channels that provides a synthesis of all signature properties of C-type inactivation (Bernèche and Roux, Structure 2005). The model illustrates how information can propagate across a physiological tissue by using a simple messenger like K+ ions. At the time of the publication of this work, C-type inactivation had not been yet observed for KcsA, it was even thought that KcsA didn’t inactivate. Soon after, Gao et al. (PNAS 2005) showed by patch-clamp electrophysiology that KcsA was also subject to (C-type) inactivation like its eukaryotic counterparts. Others have proposed that a collapsed conformation of the KcsA channel obtained by X-ray crystallization could correspond to the inactivated state of the channel (Zhou et al., Nature 2001; Cuello et al., Nature 2010). Our recent work suggests that, in this conformation, the channel has little affinity for ions and is most probably occupied by water molecules. The absence of high affinity K+ binding sites seems to be incompatible with the known properties of eukaryotic K+ channels and their inactivated state, suggesting that the proposed inactivated structure is not ubiquitous (Boiteux and Bernèche, Structure 2011). Elucidate the C-type inactivation mechanism of eukaryotic channels we are comparing electrophysiological data and simulations of the KcsA, MthK and Kv1.2 channels.

Development of an automated tool for the calculation of multidimensional PMFs
The computational power that is now at our disposition allows us to investigate on mechanisms of great complexity. However, the handling of data has now become the bottleneck in the calculation of multidimensional potential of mean-force (PMF). To address this issue we have automated the complete PMF calculation process based on the umbrella sampling approach which relies on simulations windows that are restraint to a given region of the configurational space. With our tool, one does not have to predefine the position of each window. Instead, the windows are automatically created based on the information gathered from other windows. The sampling can thus be controlled in such a way that only the region of lower free energy are sampled, revealing the possible transition pathways. The tool allows us to tackle complex problems almost routinely. For example, a complete 3D PMF describing ion permeation in the KcsA channel could involve up to 1800 windows which can take many days to set up. By limiting the sampling to the essential parts of the configurational space, the automated tool decreases the number of windows to about 600 without making any compromise on the accuracy of the data. This tool is specially made to characterize the free energy landscape along well defined reaction coordinates.

Fig. 2: Stochastic simulation framework a) Transmembrane potential profile along the pore of the KcsA channel b) Equilibrium PMF describing ion permeation in the selectivity filter of KcsA. Each color level corresponds to a free energy of 1 kcal/mol. c) The total multi-ion free-energy profile Wtot(Z1,Z2,Z3) including the equilibrium PMF calculated from MD and a transmembrane voltage of ±150 mV. d) Principal ion occupancy states identified on the different PMFs by the letters A, B, C, and D. e) Stochastic trajectory generated with an applied membrane potential +50 mV and under symmetric conditions of K+ concentration. The position of the ions along the Z axis (Z(t)) is alternatively plotted in blue, red, and green for the sake of clarity. The relative ion density along the pore is shown in relation to the different binding sites. f) I–V relation calculated from stochastic simulations under symmetric conditions and K+ concentration of 400 mM. g) Conductance of the KcsA at ±50 mV as a function of permeant ion concentration (Bernèche & Roux, Nature 2001, PNAS 2003).
Fig. 3: Simulation of a putatively inactivated K⁺ channel with K⁺ ions (green spheres) in binding sites S1 and S4 and a Na⁺ ion (yellow sphere) in the cavity. a) The time series analysis shows that the K⁺ ion in S1 (green line) leaves the selectivity filter after about 250 ps of simulation and is replaced by water molecules (purple, red and orange lines). The ion in S4 is not tightly bound either, frequently leaving the binding site for excursions of various duration in the cavity. b) Molecular density along the pore axis extracted from the simulation described in a). The red curve corresponds to water molecules and the green one to the combination of K⁺ and Na⁺ ions. The molecular density shows that ions mainly reside in the lower part of the S4 binding site. The molecular representation on the left corresponds to the initial state of the simulation, the one on the right to the conformation after 5 ns of simulation. c) Superimposition of the calculated molecular electron density with the experimental electron density (Boiteux and Bernèche, Structure 2011).

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Infectious diseases represent a major worldwide threat to human health. Novel strategies to combat infectious disease are urgently needed because of rising resistance of pathogens to antimicrobial therapy, an increasing number of immunosuppressed patients that are highly susceptible to infection, increasing travel which enhances transmission and worldwide spread of novel and re-emerging pathogens, and potential bioterrorism threats.

The substantial progress in infection biology research in the last two decades could provide a basis for novel control strategies. However, it has remained difficult to translate this extensive knowledge into effective new control strategies. One potential reason why it is so difficult to translate basic research to effective strategies for combating infectious diseases, could be the prevailing focus on the action of individual pathogen or host components. While this reductionist approach was highly successful to identify and characterize key virulence and immune factors, it cannot explain the course of complex multifactorial infectious diseases involving hundreds of interacting pathogen and host factors. Our goal is therefore to integrate the vast existing knowledge and to develop appropriate methodology to analyze interacting host/pathogen networks using FACS sorting of pathogens from infected host cells and tissues, quantitative proteomics, metabolomics, molecular genetics, animal infection models, and in silico modeling.

For our research we use Salmonella as well as Shigella as model pathogens. Both pathogens cause diarrhea and Salmonella can also cause typhoid fever and nontyphoidal Salmonella (NTS) bacteremia, which together cause over a million deaths each year. In addition to their importance as human pathogens, Salmonella and Shigella are among the best-studied model pathogens.

Fig. 1: Salmonella-infected mouse spleen (yellow, Salmonella; red, neutrophils; blue, red blood cells).

Metabolism

A large number of Salmonella proteins with detectable expression during infection have metabolic functions. Many of these enzymes could represent promising targets for antimicrobial chemotherapy. However, we have previously shown that actually only a very small minority of enzymes is sufficiently relevant for Salmonella virulence to qualify as a potential target. To understand the differential relevance of metabolic enzymes we systematically characterize the entire Salmonella metabolic network during infection by integrating large-scale data on in vivo nutrient availability and enzyme abundance with a genome-scale in silico model that provides a consistent largescale description of Salmonella metabolism during infection. The results revealed a surprisingly large diversity of host nutrients. However each of these nutrients was available in only minute amounts. This paradoxical situation (“starving in paradise”) has two major consequences, i) broad nutrient supplementation buffers many Salmonella metabolic defects thus limiting opportunities for antimicrobials, ii) Salmonella growth in infected mice is rather slow and nutrient-limited. Both findings reiterate the major importance of metabolism for infectious disease outcome.

Within the framework of the SystemsX.ch RTD project BattleX (coordinator: Dirk Bumann) we have recently started to analyze pathogen and host metabolism in Shigella infections together with five collaborating groups across Switzerland. Initial results suggest that Shigella (like Salmonella) has access to diverse host nutrients. However, in this case excess nutrient quantities that support very fast pathogen growth seem to be available. These differences likely reflect differential localization of Sal-
monella in a membrane-delimited vacuole vs. Shigella freely residing in the host cell cytosol with unrestricted access to cytosolic metabolites. Metabolomics data suggest that Shigella infection causes major rearrangements of metabolic fluxes in the host cells. We currently explore such host cell activities as alternative targets for controlling infection.

**Analysis of pathogen subpopulations**

Salmonella reside in several distinct host microenvironments within the same infected tissue. These microenvironments differ in density of host defense cell types such as neutrophils and inflammatory macrophages and likely provide substantially different conditions for Salmonella. We are developing a set of complementary tools to isolate distinct Salmonella subpopulations from various microenvironments for system-level analysis. Current results suggest dramatic differences in stress exposure and growth rate in Salmonella subpopulations.

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Publications 2011

Leschner, Sara; Deyneco, Igor V; Lienenklaus, Stefan; Wolf, Kathrin; Bloeker, Helmut; Bumann, Dirk; Loessner, Holger; Weiss, Siegfried (2011). Identification of tumor-specific Salmonella Typhimurium promoters and their regulatory logic. Nucleic acids research, Epub ahead of print.


Publications 2010


Asymmetric cell division generates cellular diversity. Cell polarity, spindle orientation and cleavage furrow positioning are cellular mechanisms enabling cells to divide in a molecular and physical asymmetric manner. Stem cells in particular divide asymmetrically in order to self-renew the stem cell yet generate differentiating siblings. Many diseases such as breast cancer susceptibility, acute promyelocytic leukemia, the initiation of colon cancer but also the neurodevelopmental disorders lissencephaly or microcephaly are due to defective asymmetric stem cell division. Thus, understanding the cellular and molecular mechanisms of asymmetric cell division is important to increase our knowledge of basic stem cell biology.

We are using *Drosophila melanogaster* neuroblasts, the precursors of the fly’s central nervous system, to study the mechanism of (1) spindle orientation and (2) cleavage furrow positioning during asymmetric cell division. Neuroblasts are polarized cells and divide in a stem cell-like fashion, undergoing repeated self-renewing asymmetric divisions (Fig. 1). The mitotic spindle invariably orients itself along the neuroblast intrinsic apical-basal polarity axis and asymmetric cleavage furrow positioning results in a physical and molecular asymmetric cell division, generating a large self-renewed apical neuroblast and a smaller differentiating basal ganglion mother cell (GMC). *Drosophila* neuroblasts provide an ideal experimental system because precise genetic manipulations are possible and superb imaging properties are available.

### Mechanism and function of spindle orientation during asymmetric cell division

Asymmetric cell division relies on the correct orientation of the mitotic spindle in relation to an internal or external polarity axis. In *Drosophila* neuroblasts, the mitotic spindle aligns itself along the intrinsically apical-basal polarity axis such that after division only the apical neuroblast inherits the Par proteins, whereas the Mira/Pros complex proteins segregate into the GMC (Fig. 2). Spindle orientation is controlled through a conserved protein complex consisting of the apically localized protein Partner of Inscuteable (Pins; LGN/AGS3 in vertebrates), the coiled-coil protein Mushroom body defect (Mud; NuMA in vertebrates) and the small G-protein Gαi. Mud is a key effector protein in spindle orientation and is providing a physical interaction between the mitotic spindle and the apical cortex.

We are using *Drosophila* larval neuroblasts to specifically address the following questions:

1. **How is centrosome positioning controlled, in order to establish a properly oriented metaphase spindle?**
2. **How is the orientation of the metaphase spindle maintained?**
3. **What are the key proteins involved in spindle orientation and what is their temporal and spatial requirement?**

In order to answer these questions, we utilize precise and powerful genetic tools in combination with high temporal and spatial resolution live imaging (spinning disc). We further use immunoprecipitation mass spectrometry (IPMS) and forward genetics to identify novel proteins and genes involved in centrosome positioning and spindle orientation maintenance.

### Cellular and molecular mechanism of cleavage furrow positioning

Asymmetric cell division can result in the formation of molecularly and physically distinct siblings. We are using *Drosophila* neuroblasts to investigate how cell size differences are generated. In particular, we are focusing on the cellular and molecular mechanism of cleavage furrow positioning. Until recently, it was widely believed that cleavage furrow positioning is solely dependent on cues delivered by the mitotic spindle. However, new results suggest that two cues are used for the correct positioning of the contractile ring: (1) a microtubule-dependent cue and (2) a polarity derived signal. The novel polarity-dependent cleavage furrow positioning pathway is utilizing the two conserved polarity components Pins and Discs large (Dig) (Fig. 3).
We are investigating how cellular polarity is translated into asymmetric Myosin localization and ultimately, asymmetric cleavage furrow positioning. Furthermore, we are also testing the idea whether other polarized cell types utilize the “polarity-dependent” pathway to position the cleavage furrow. We are using forward and reverse genetics, live imaging with high temporal and spatial resolution and biochemistry in order to identify the cellular and molecular mechanism of cleavage furrow positioning in *Drosophila* neuroblasts.

**Publications 2011**

Connell, Marisa; Cabernard, Clemens; Ricketson, Derek; Doe, Chris Q; Prehoda, Kenneth E (2011). Asymmetric cortical extension shift cleavage furrow position in *Drosophila* neuroblasts. *Molecular biology of the cell, 22*(22), 4220-6.


**Publications 2010**


Callan, Matthew A; Cabernard, Clemens; Heck, Jennifer; Louis, Samantha; Doe, Chris Q; Zarnescu, Daniela C (2010). Fragile X protein controls neural stem cell proliferation in the *Drosophila* brain. *Human molecular genetics, 19*(15), 3068-79.
The type III secretion (T3S) injectisome is a nanosyringe allowing bacteria to inject bacterial effector proteins across the cellular or vacuolar membrane of target cells. It is made of 27 Ysc proteins and consists of a cylindrical basal body, made of two rings, anchored to the inner and outer membranes and supporting a hollow needle about 7 nm thick and 67 nm long (Fig. 1). This length is controlled by a molecular ruler that is exported by the apparatus itself, during assembly. The inner-membrane ring surrounds five different integral membrane proteins that represent the translocon allowing the passage of the inner membrane. A third ring encloses the cytosolic part of the apparatus, which consists of a complex ATPase. Presently, our effort is devoted to unravel the atomic structure and the mechanism of assembly, including how the molecular ruler works. As shown in (Fig. 2), formation of the injectisome proceeds from two independent branches. A branch starts with the formation of the secretin ring (YscC) in the outer membrane and proceeds to the proximal side by subsequent discrete attachment steps of YscD (A). These two proteins form a pore through the outer membrane and the peptidoglycan but, at this stage, the pore is closed. A second branch starts with the insertion of YscR, YscS and YscT in the plasma membrane. YscV then oligomerizes around this nucleus and recruits YscU. These five proteins form the complete gated channel through the plasma membrane (B). The subsequent polymerization of the YscJ ring connects the substructures assembled by the two branches (C). After the merge of the two membrane ring structures, the ATPase–C ring complex, consisting of YscN, K, L, and Q assembles at the cytoplasmic side of the injectisome (D), completing the export apparatus. The nascent export apparatus then starts exporting YscF, the subunits of the needle, which open the secretin channel and polymerise at the distal end of the growing structure. When the needle has reached the length corresponding to the molecular ruler YscP (not shown), the apparatus stops exporting YscF subunits and starts exporting LcrV, the subunits forming the tip structure. This completes the assembly (E).

**Capnocytophaga canimorsus** are Gramnegative bacteria from the normal oral flora of dogs and cats. They cause rare but severe infections in humans that have been bitten or simply licked by a dog or cat. Fulminant septicaemia and peripheral gangrene with a high mortality are the most common symptoms. *C. canimorsus* are resistant to phagocytosis by human polymorphonuclear leukocytes and by macrophages. *C. canimorsus* are also resistant to killing by complement by virtue of their LPS. Finally, the LPS is not detected by the TLR4-MD2 complex at the surface of macrophages and dendritic cells.

A surprising feature of these bacteria is their capacity to feed.
by foraging the glycan moieties of glycoproteins from the host, including immunoglobulins and proteins from the surface of phagocytic cells. We presently determine what are the parameters of the LPS that confer these low inflammatory properties. We also unravel the complete protein deglycosylation mechanism (Fig. 3). It is based on several specialized systems that are abundant at the bacterial surface and which evoke the archetypal starch utilization system (Sus) of Bacteroides thetaiotaomicron. The Sus-like systems consist of a set of lipoproteins associated to a specialized porin. They are completed by a sialidase anchored in the inner leaflet of the outer membrane and by an array of periplasmic exoglycosidases that degrade the glycan moieties down to monosaccharides. In the natural niche, the dog’s mouth, these systems allow C. canimorsus to feed on mucine, an abundant glycoprotein from saliva. This observation illustrates how the adaptation of a commensal to its ecological niche in the host, here the dog’s oral cavity, contributes to being a potential pathogen. In parallel to this work, we completed the engineering of a genetic “toolbox” for the study of this microorganism. Finally, we sequenced the genome of several strains and we determined the surface proteome of Cc5, our type strain.

Fig. 3: Functional model of complex N-linked glycan moieties deglycosylation processing by C. canimorsus. Individual glycan processing steps are illustrated. (A) The glycan moiety is bound at the bacterial surface by the Gpd complex. (B) The glycan moiety is endo-cleaved by GpdG and imported into the periplasm through the GpdC pore. (C) Terminal sialic acid is cleaved by sialidase (SiaC). (D) The glycan is further processed by the sequential activity of several periplasmic exoglycosidases. Renzi et al., PLoSPathogens 2011; doi:10.1371/journal.ppat.1002118.g009

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The aim of our studies is to gain a molecular understanding of the function of type IV secretion (T4S) systems in establishing bacterial persistence in the host. T4S systems are ancestrally related to bacterial conjugation systems that mediate interbacterial DNA transfer. Bacterial pathogens targeting eukaryotic host cells have adopted these supramolecular protein assemblies for the intracellular delivery of virulence factors from the bacterial cytoplasm directly into the host cell cytoplasm. Our longstanding research on the vascular tumor-inducing pathogens of the genus Bartonella revealed crucial roles of two distinct T4S systems, VirB and Trw, in the ability of these bacteria to colonize, invade and persist within vascular endothelial cells and erythrocytes, respectively (see Fig. 1, reviewed in Dehio, 2008, Cell. Microbiol.). More recently, we have initiated a new project to study the role of the T4S system VirB in intracellular persistence of the closely related bacterial pathogens of the genus Brucella that represent the etiological agents of brucellosis - the worldwide most important bacterial zoonosis. We are using a multidisciplinary research approach including genetics, genomics, biochemistry, structural biology, cell biology, animal experimentation and bioinformatics in order to reveal the cellular, molecular and evolutionary basis of T4S in bacterial persistence of Bartonella and Brucella. Moreover, we employ a systems biology approach to reveal the host cell signaling network underlying cell entry and intracellular persistence of these pathogens in order to define novel targets for the development of innovative anti-infectiva.

Structure/function analysis of VirB-translocated Bep effector proteins of Bartonella

The cocktail of Bep effectors translocated by the VirB T4S system into vascular endothelial cells mediates multiple cellular effects, including anti-apoptosis, internalization of bacterial aggregates via the Factin-independent invasome structure and proinflammatory activation (Schulein et al., 2005, PNAS). The N-terminal parts of the Beps carry diverse domains or peptide motifs that are considered to mediate effector functions within host cells. For instance, upon translocation the effectors BepD, BepE and BepF become tyrosine-phosphorylated on short N-terminal repeat motifs, thereby interfering with eukaryotic signal transduction processes (Selbach et al., 2009, Cell Host & Microbe). Defining the cellular targets and molecular mechanisms of how individual Bep effectors interfere with eukaryotic signaling processes have become a focus of our recent studies. In the frame of an in-house collaboration with the X-ray crystallography group of Tilman Schirmer particular emphasis is given to the structure/function analysis of the Fic domain - present in multiple Beps - that mediates posttranslational modification of specific host target proteins via covalent transfer of AMP (AMPylation).
Fig. 2 Hela cells infected with GFP-expressing Brucella abortus (green) for 48 h and stained for F-actin (red) and DNA (blue). Intracellular bacteria replicate in an endoplasmic reticulum-associated compartment localizing proximal to the nucleus.

A systems biology approach to Bartonella and Brucella entry and intracellular persistence in human cells

The goal of InfectX (www.infectx.ch) – at the research and development project (RTD) of the Swiss-wide systems biology initiative SystemsX.ch – is to comprehensively identify components of the human infectome for a set of important bacterial and viral pathogens and to develop new mathematical and computational methods with predictive power to reconstruct key signaling pathways controlling pathogen entry into human cells. In the frame of InfectX we use a systems biology approach to reconstruct the host signaling processes underlying Bartonella and Brucella entry of the human model cell line HeLa that lead to the establishment of a persisting intracellular infection. For Bartonella henselae, the VirB T4S effector BepG or the combined activity of the effectors BepC/BepF was found to inhibit endocytic uptake of individual bacteria, thereby redirecting bacterial uptake to the invasome-mediated pathway facilitating the uptake of large bacterial aggregates. This unique cell entry process is mediated by massive F-actin rearrangements that depend on the small GTPases Rac1, the Rac1-effector Scar1, and the Factin organizing complex Arp2/3 (Rhomberg et al., 2009, Cell Microbiol.; Truttmann et al., 2011, Cell Microbiol.) and bidirectional signaling via the integrin pathway (Truttmann et al., 2011, J. Cell Sci.). The uptake process triggered by Brucella abortus is less well defined but considered to depend on lipid rafts and the small GTPase Cdc42. Genome-wide RNA interference screens and related modeling approaches currently performed on the basis of high-content fluorescence microscopy assays for pathogen entry and intracellular replication should facilitate the comprehensive identification of the human infectomes involved in establishing persistent intracellular infection of these pathogens as a first step towards the identification of human targets suitable for the development of a new class of antiinfectives that interfere with the function of host proteins essential for pathogen infection.

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de Paz, Héctor D; Larrea, Delfina; Zunzunegui, Sandra; Dehio, Christoph; de la Cruz, Fernando; Llosa, Matxalen (2010). Functional dissection of the conjugative coupling protein TrwB. Journal of bacteriology, 192(11), 2655-69.
The scientific goal of the group is the application and development of high-resolution Nuclear Magnetic Resonance (NMR) methods for the elucidation of structure, function, and dynamics of biological macromolecules. The structural and functional projects currently encompass the human chemokine receptor CCR5, which is also the HIV1-coreceptor; Abelson kinase, a prime drug target in the treatment of chronic myelogeneous leukemia; endotoxin, the causative agent of endotoxic shock; cadherins; bacterial PilZ domains, which are targets for signaling via cyclic di-GMP; the TipA multidrug resistance protein of S. lividans; and an atomic-detail description of protein folding by new NMR methods.

**CCR5.** The chemokine receptor CCR5 belongs to the class of G-protein coupled receptors. CCR5 is expressed on the surface of T-cells and activated after binding the endogenous chemokines MIP-1α, MIP-1β, and RANTES. CCR5 is the key molecule for HIV entrance into target cells, which proceeds via the sequential interaction of the viral protein gp120 with the host-cell factors CD4 and CCR5. Very promising HIV entry inhibitors are based on CCR5 ligands, comprising the natural ligand RANTES. The structures of CCR5 and of its complexes are unknown. In recent years, we have made progress towards the structure elucidation of CCR5 and its complex with RANTES by (1) characterizing the interaction of the soluble protein RANTES with peptides derived from CCR5 and (2) developing a method to produce pure, active CCR5 in sufficient amounts for structural studies by NMR, electron microscopy and X-ray crystallography. The project is embedded into the EU-FP7 project CHAARM (Combined Highly Active Anti-Retroviral Microbicides), a collaborative effort to develop combinations of new and existing anti-HIV agents, which can be applied topically to reduce transmission of HIV.

**Abelson (Abl) kinase.** Chronic myelogenous leukemia (CML) is caused by an abnormal rearrangement of chromosomes resulting in the aberrant fusion protein Bcr-Abl. The unregulated kinase activity of Bcr-Abl leads to the uncontrolled production of immature blood cells and thus leukemia.

The clinically highly efficacious drugs imatinib, nilotinib and dasatinib have been developed against Bcr-Abl. These inhibitors block the kinase activity by competing against ATP. However, spontaneous mutations of Bcr-Abl in advanced-stage patients render these inhibitors inefficient. This has stimulated the search for new inhibitors that can overcome resistance.

In collaboration with Novartis (Basel) we have been able to determine the hitherto unknown, physiologically relevant solution conformations of Abl kinase in complex with several inhibitors. The data show that the crucial activation loop adopts the inactive conformation for the imatinib and nilotinib inhibitor complexes, whereas dasatinib preserves the active conformation, thus contradicting earlier predictions from molecular modeling. NMR data have also helped to elucidate the mechanism of a new class of allosteric CML inhibitors, which had been discovered by the group of Prof. N. Gray (Harvard). Using NMR, X-ray, mutagenesis and hydrogen exchange data, the binding of the lead compound GNF-2 could be located to the Bcr-Abl myristoyl-binding pocket. This site is remote from the ATP binding pocket. The improved analogue GNF-5 displays in vitro and in vivo efficacy against the important Bcr-Abl T315I resistance mutant. Thus this class of inhibitors opens new therapeutic opportunities via a novel mechanism of action.

**Lipopolysaccharide (LPS, endotoxin)** is a major component of the outer membrane of Gram-negative bacteria, which makes it a prime target for recognition by the innate immune system. In small amounts, LPS provokes a beneficial immune response. However, in larger amounts LPS causes endotoxic shock, which is highly lethal due the lack of effective therapeutic approaches. A detailed molecular description of the recognition events of LPS is of great medical interest and essential for the understanding of pro-inflammatory processes of the innate immune system.
In collaboration with Prof. U. Zähringer (FZ Borstel, Germany) we have been able to make LPS amenable to analysis by solution NMR conditions that mimic the bacterial membrane and to determine a structure that comprises the motif responsible for the endotoxic reaction. Our approach presents a general new methodology for the structural analysis of complex and heterogeneous LPS molecules. Current efforts are directed towards characterizing complexes of LPS with immune system receptors.

Towards an atom-scale description of order in unfolded proteins from new NMR parameters. A detailed, quantitative description of the unfolded state ensemble of proteins is crucial for understanding protein folding, protein misfolding diseases such as Alzheimer’s and Parkinson’s, and function of intrinsically disordered proteins. The astronomical size of the conformational space of an unfolded polypeptide chain makes such a description both experimentally and theoretically very difficult.

Using new NMR experimental parameters comprising residual dipolar couplings and paramagnetic relaxation enhancements, we have been able obtain a highly detailed, quantitative description of unfolded polypeptides. The results show that unfolded states contain considerably more residual, native-like structure than previously anticipated, thereby resolving Levinthal’s paradox that protein folding would need almost infinite times in an unbiased search of all accessible conformations.

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Introduction
Cell growth is highly regulated. Cells respond to nutrients or other appropriate growth stimuli by up-regulating macromolecular synthesis and thereby increasing in size. Conversely, cells respond to nutrient limitation or other types of stress by downregulating macromolecular synthesis and enhancing turnover of excess mass. Thus, the control of cell growth involves balancing positive regulation of anabolic processes with negative regulation of catabolic processes. Growth is also controlled relative to cell division. In proliferating cells, growth is linked to the cell cycle such that most cells precisely double their mass before dividing. In other physiological contexts, such as load-induced muscle hypertrophy or growth factor-induced neuronal growth, cell growth is controlled independently of the cell cycle. Furthermore, in addition to the temporal control of cell growth described above, cell growth can be subject to spatial constraints. For example, budding yeast and neurons grow in a polarized manner as a result of new mass being laid down only at one end of the cell. Finally, in multicellular organisms, growth of individual cells is controlled relative to overall body growth such that the organs and tissues constituting the organism are properly proportioned.

The TOR signaling network
What are the mechanisms that mediate and integrate the many parameters of cell growth? In other words, what determines that a cell grows only at the right time and at the right place? Remarkably, the study of these mechanisms has been largely neglected, despite their clinical relevance and despite cell growth being, along with cell division and cell death, one of the most fundamental (and obvious) features of life. Also remarkable is the finding that cell growth control, regardless of eukaryotic organism or physiological context, seems always to involve the protein kinase TOR (Target Of Rapamycin) and its signaling network. TOR has thus become known as a central controller of cell growth. Indeed, the discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR, originally discovered in our laboratory, is structurally and functionally conserved from yeast to human (including worms, flies, and plants). TOR in mammals (mTOR) controls cell growth and metabolism in response to nutrients (e.g., amino acids), growth factors (e.g., insulin, IGF-1, PDGF), and cellular energy status (ATP). Nutrients are the dominant TOR input as high levels of amino acids can compensate for an absence of the other mTOR inputs but not vice versa, and only nutrients activate TOR in unicellular organisms. The growth factor signaling pathway, grafted onto the more ancestral nutrient sensitive TOR pathway, co-evolved with multicellularity. TOR activates cell growth by positively and negatively regulating several anabolic and catabolic processes, respectively, that collectively determine mass accumulation and thus cell size.

The anabolic processes include transcription, protein synthesis, ribosome biogenesis, nutrient transport, and mitochondrial metabolism. Conversely, TOR negatively regulates catabolic processes such as mRNA degradation, ubiquitin-dependent proteolysis, autophagy and apoptosis. TOR is an atypical serine/threonine kinase that is found in two functionally and structurally distinct multiprotein complexes, TORC1 and TORC2 (mTORC1 and mTORC2 in mammals), each of which signals via a different set of effector pathways. TORC1

![Fig. 1: Model of the catalytic region of human TOR (see Sturgill and Hall., 2009).](image1)

![Fig. 2: The mTOR signaling network (see Zinzalla et al., 2011).](image2)
is rapamycin sensitive whereas TORC2 is rapamycin insensitive. The best-characterized phosphorylation substrates of mTOR are S6K and 4E-BP1 via which mTORC1 controls translation, and Akt/PKB via which mTORC2 controls cell survival and likely other processes. Like TOR itself, the two TOR complexes and the overall architecture of the TOR signaling network appear to be conserved from yeast to human. Thus, the TOR signaling network is a primordial or ancestral signaling network conserved throughout eukaryotic evolution to regulate the fundamental process of cell growth. As a central controller of cell growth and metabolism, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.

We are studying the TOR signaling network in the yeast *Saccharomyces cerevisiae*, in mammalian cells, in mice, and in human tumors. A major finding in our laboratory in recent years was the fact that TOR controls cell growth via two major signaling branches. Furthermore, we discovered the two TOR complexes and demonstrated that these two complexes correspond to the two previously described TOR signaling branches. More recently, in collaboration with our in-house colleague Markus Rüegg, we introduced the mouse as an experimental system to study the role of mTOR in regulating whole body growth and metabolism. In collaboration with the clinician Markus Heim, we have initiated a translational research project aimed at defining signaling pathways that allow tumors to evade therapy. The overall goal of our studies is to elucidate how growth and metabolism are regulated in health and disease. Examples of our recent studies on the TOR signaling network in yeast and mammals are described in the selected publications listed on the next pages.
Publications 2011


Publications 2010


Colombi, M; Molle, K D; Benjamin, D; Rattenbacher-Kiser, K; Schaefer, C; Betz, C; Thiemeeyer, A; Regnass, U; Hall, M N; Moroni, C (2011). Genome-wide shRNA screen reveals increased mitochondrial dependence upon mTORC2 addiction. *Oncogene*, 30(13), 1551-65.
Skeletal muscle has an enormous capacity to adapt to external stimuli including physical activity, oxygen, temperature, nutrient availability and composition. Inadequate muscle function is linked to an increased risk for many chronic diseases such as obesity, type 2 diabetes, cardiovascular disorders, osteoporosis, neurodegenerative events, mood disorders, age-related muscle wasting, and certain cancers. Inversely, regular exercise is an excellent prevention and therapeutic intervention for many of these pathologies and improves life quality and expectancy.

Skeletal muscle cell plasticity in exercise is a complex process: even a single endurance exercise bout alters the transcription of more than 900 genes in muscle. Chronic exercise leads to a metabolic and myofibrillar remodeling, increase in tissue vascularization, adaptation of the neuromuscular junction, a shift in the balance between protein degradation and biosynthesis rates, elevated heme biosynthesis, improved reactive oxygen species detoxification and a resetting of the peripheral circadian clock. Due to this complexity, it is not surprising that our knowledge about the molecular mechanisms that underlie muscle cell plasticity remains rudimentary.

The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is one of the key factors in muscle adaptation to exercise. Muscle activity induces PGC-1α gene expression and promotes posttranslational modifications of the PGC-1α protein. In turn, PGC-1α regulates the adaptations of muscle to endurance training. Accordingly, ectopic expression of PGC-1α in muscle is sufficient to induce a trained phenotype whereas mice with a genetic ablation of the PGC-1α gene in muscle have an impaired endurance capacity.

Our group is studying the mechanisms that control muscle cell plasticity and their physiological consequences. We try to integrate molecular biology, work in muscle cells in culture and observations on mice with different activity levels to obtain a comprehensive picture of the adaptations in the active and the inactive muscle.

**Regulation and coordination of metabolic pathways**

Endurance exercise is a strong promoter of mitochondrial biogenesis and oxidative metabolism of lipids. At the same time, skeletal muscle of endurance athletes exhibits increased storage of intramyocellular lipids, similar to what is observed in muscle of type 2 diabetic patients (the “athlete’s paradox”). Furthermore, the boost in mitochondrial function potentially augments the generation in harmful side-products, e.g. incomplete fatty acid oxidation products or reactive oxygen species. However, neither the lipid accumulation nor the oxidative metabolism in the exercised muscle exert detrimental effects, in stark contrast to the pathologies that
develop under seemingly similar conditions in type 2 diabetes and other muscle-associated diseases. We study the coordination of anabolic and catabolic pathways in order to pinpoint the differences in substrate fluxes in the healthy and the diseased muscle.

**Molecular changes in muscle atrophy and dystrophies**

Muscle disuse, induced by a Western life-style or caused by diseases, leads to fiber atrophy, reduced muscle functionality and is ultimately fatal in certain inherited and sporadic muscular dystrophies. Little is known about the etiology of most of these diseases and as a result, no efficacious therapy exists for these devastating disorders. However, the induction of a trained phenotype ameliorates many of the symptoms of muscle wasting and thereby improves muscle function. For example, we have shown that using a genetic model for endurance training, PGC-1α muscle-specific transgenic mice, helps to ameliorate disuse-induced muscle fiber atrophy and Duchenne muscular dystrophy. Other groups have demonstrated that ectopically expressed PGC-1α also improves a mitochondrial myopathy, blunts muscle damage by the statin drugs and reduces sarcopenia, muscle wasting in aging in the respective animal models. We are currently studying how PGC-1α mediates this broad spectrum, health-beneficial effect on muscle and how this could be exploited therapeutically.

**Integration of signaling pathways and spatiotemporal control of gene expression**

In exercise, PGC-1α transcription, protein levels and activity are modulated by different signaling pathways. While all of the major signaling pathways in the trained muscle converge on PGC-1α (figure part A), the consequences, the integration and the temporal coordination of these signals are not clear. Upon activation, PGC-1α controls the transcription of many different gene families in muscle to promote a trained phenotype. However, the specificity of gene regulation by PGC-1α varies according to the cellular context (figure part B). For example, the regulation of postsynaptic neuromuscular junction genes by PGC-1α is spatially restricted to subsynaptic nuclei in the muscle fiber.

Histological visualization of neuromuscular junctions in mouse muscles. The motor neuron is depicted in red (anti-neurofilament immunohistochemistry) and the acetylcholine receptor clusters on the muscle fiber membrane in green (using fluorescently labeled alphabungarotoxin). Image by Anne-Sophie Arnold.
Publications 2011


Publications 2010


Membrane proteins comprise about one third of typical proteomes. They are responsible for a wide range of vital cellular functions, including signal transduction, catalysis, respiration, and transport. The function of a protein is dependent on its three-dimensional structure. Whereas tens of thousands of high-resolution structures of soluble proteins are known with only about 250 unique membrane protein structures, our knowledge of this class of proteins is still relatively sparse. We use solution nuclear magnetic resonance (NMR) spectroscopy as the main experimental method to understand structure, function and folding of integral membrane proteins and their complexes at atomic resolution. Our research focuses on selected protein systems with high biological relevance. Since solution NMR spectroscopy of integral membrane proteins and their interactions is technically still highly challenging, a substantial part of our activities is the development of new NMR experiments and biochemical and biophysical protocols for studies of membrane proteins.

Our biological focus is the outer mitochondrial membrane. In terms of structural biology, this membrane is largely a “terra incognita.” The mitochondrial outer membrane features essential biological roles in the eukaryote, such as regulation of metabolism, apoptosis and cancer. Still, so far the atomic resolution structure of just a single integral outer membrane protein is known, the voltage-dependent anion channel VDAC. We have been able to determine the structure of VDAC by solution NMR and are now addressing further proteins from the mitochondrial outer membrane.

On the other hand, we study the function of the voltage-dependent anion channel VDAC and its complexes, which play crucial roles in the regulation of the cellular metabolism. By characterizing the complexes of VDAC with its natural ligands and its protein interaction partners at atomic resolution, we attempt to elucidate the structural bases for these functions.

A second line of research addresses the folding mechanism of β-barrel outer membrane proteins. These proteins simultaneously fold and insert into their target membrane by a biophysically intriguing process that is only poorly understood. We are developing new approaches to study this process at atomic resolution by solution NMR spectroscopy in combination with additional spectroscopic techniques. The combination of ensemble averaged and single-molecule techniques will allow a description of the folding process resolved for individual atomic sites.

On the NMR technical side, we develop improved methods for NMR studies of integral membrane proteins and other proteins and large protein assemblies. These include sparse data sampling and multidimensional processing of NMR spectra of large molecular weight complexes, including automated approaches for sequence-specific resonance assignments and spectral analysis.
Publications 2011


Publications 2010


Our studies aim at understanding the molecular and cellular principles involved in the propagation and differentiation of bacterial cells. We focus on the nucleotide second messenger, cyclic di-guanosinemonophosphate (c-di-GMP) and its role in bacterial cell signaling and dynamics (Schirmer & Jenal, 2009, *Nature Reviews Microbiol* 7, 724). C-di-GMP emerges as a ubiquitous signaling molecule that modulates multiple aspects of bacterial growth and behavior, including the formation of a sedentary, community-based lifestyle and its association with chronic forms of bacterial infections (Schirmer & Jenal, 2009, *Nature Reviews Microbiol* 7, 724). Our aims are to identify and characterize c-di-GMP control modules in different bacterial model organisms, to uncover and exploit the basic molecular and mechanistic principles of c-di-GMP signaling, and to probe its role in bacterial growth and persistence.

**Fig. 1**: Schematic of c-di-GMP mediated signaling in bacteria.

**Role of c-di-GMP in cell cycle progression and cell fate determination**

We investigate the role of c-di-GMP in the *Caulobacter crescentus* asymmetric life cycle. In this organism, cell polarity and cell cycle progression are implemented by oscillating global transcriptional regulators and by spatially dynamic phosphosignaling and proteolysis pathways (Jenal & Galperin 2009, *Curr Opin Microbiol* 12, 152; Jenal 2009, *Res Microbiology* 160, 687). We have shown that periodic fluctuations of c-di-GMP are an integral part of the *C. crescentus* cell cycle clock and serve to control pole development in time and space and to coordinate these processes with the underlying cell cycle. Several DGCs and PDEs contribute to the characteristic bimodal distribution of the second messenger during the *Caulobacter* life cycle. This includes PleD, a DGC that upon activation by phosphorylation sequesters to the differentiating *Caulobacter* cell pole during G1-S transition, where it orchestrates pole morphogenesis. PleD is regulated by the localized activities of the DivJ kinase and the PleC phosphatase and by the single domain response regulator DivK, which dynamically positions to both the PleC and DivJ occupied poles (Paul 2004, *Genes Dev* 18, 715; Paul 2007, *J Biol Chem*, 282, 29170). DivK acts as an allosteric regulator of PleC and DivJ to produce kinase feedback loops that quickly and robustly determine *C. crescentus* cell fate through the activation of PleD (Paul 2008, Cell 133, 452).

**Fig. 2**: Network controlling cell cycle progression and pole development in *C. crescentus*. Phosphorylation reactions (blue), c-di-GMP network (green), and protein degradation pathways (orange) are indicated. Unidentified c-di-GMP effector proteins (E) regulating pole morphogenesis are indicated.

The *Caulobacter* G1-to-S transition is mediated by a second DGC, DgcB. In the G1 swarmer cell DgcB is “neutralized” by its specific and dominant antagonist PdeA, which reduces c-di-GMP in this cell type and keeps it in the motile, non-reproductive phase. Upon entry into S-phase, when cells make the decision to settle down, PdeA is specifically degraded leaving DgcB unopposed. PdeA dynamically localizes to the old cell pole where it is degraded by the polar protease complex ClpXP. This cell cycle dependent process is orchestrated by the single domain response regulator CpdR, which itself localizes to the old cell pole in response to its phosphorylation status, where it recruits both PdeA and ClpXP and mediates substrate delivery. Through this process DgcB is “unleashed” coincident with PleD activation, thereby triggering pole morphogenesis and S-phase entry through a rapid and robust upshift of c-di-GMP (Abel 2011, *Mol Cell* 43, 550).
C-di-GMP controls all aspects of Caulobacter polarity, including flagellar motility, pili biogenesis as well as holdfast and stalk formation. The mechanisms and regulatory components of polar organelle formation are one main focus of our current research (Christen 2007, PNAS 104, 4112; Christen 2006, J Biol Chem 281, 32015; Christen 2005, J Biol Chem 280: 30829). In addition to driving the motile-sessile switch, the c-di-GMP up-shift at the onset of S-phase contributes to replication and cell division control through the controlled destruction of the replication initiation inhibitor CtrA and the cell division inhibitor KidO by the ClpXP protease complex. Cell cycle dependent degradation of these proteins entails a specific spatial arrangement where both protease and substrates transiently localize to the incipient stalked cell pole during the G1-S transition. While ClpXP localization requires CpdR, substrate delivery to the same pole requires PopA, a protein that sequesters to the incipient stalked pole upon binding of c-di-GMP (Duerig 2009, Genes Dev 23, 93). PopA activation and localization at the onset of S-phase are coordinately driven by PteD phosphorylation and PdeA degradation (see above). Our recent studies demonstrate how phosphosignaling, protein degradation, and c-di-GMP mediated regulatory processes are tightly interconnected to coordinately drive the Caulobacter life cycle (Figure 2) (Abel 2011, Mol Cell 43, 550).

Role of c-di-GMP in biofilm formation and persistence
We have used Escherichia coli as a genetically versatile model organism to analyze the molecular basis of the inverse regulation of cell motility and biofilm formation by c-di-GMP. Our studies revealed that E. coli can fine-tune its swimming speed with the help of a molecular brake (YcgR) that, upon binding of c-di-GMP, interacts with the motor protein MotA to curb flagellar motor output (Böhm 2010 Cell, 141, 107). These experiments demonstrate that bacteria can modulate motor output in response to environmental cues. Our studies also led to identify c-di-GMP and ppGpp as key regulatory factors of poly-ß-1,6-N-acetyl-glucosamine (poly-GlcNAc) synthesis, a polysaccharide adhesin secreted by E. coli as response to sub-inhibitory concentrations of antibiotics targeting the ribosome (Böhm 2009, Mol Microbiol. 72, 1500). The synergistic roles of ppGpp and c-di-GMP in biofilm induction, suggested that interference with bacterial second messenger signaling might represent an effective means for biofilm control during chronic infections.

Chronic Pseudomonas aeruginosa infections in cystic fibrosis (CF) patients can be treated with antibiotics, however full clearance is not possible due to the adaptation of infective species to a persistent lifestyle. Adaptive P. aeruginosa morphotypes include small colony variants (SCVs), slow growing and strongly adherent variants whose appearance correlates with poor lung function (Fig. 3). Our research on P. aeruginosa SCVs suggests that SCV-mediated persistence might be a novel target for antimicrobial chemotherapy. We characterized a tripartite signaling system called YfiBNR, mutations in which lead to the generation of SCV variants. YfiN was shown to be a membrane-bound cyclic di-GMP synthase, whose activity is tightly controlled by YfiR and YfiB. Activation of YfiN resulted in increased levels of c-di-GMP, which in turn triggered massive production of exopolysaccharides, drastically reduced growth rates, and resistance to macrophage phagocytosis. Consistent with a role for the SCV phenotype in immune system evasion, activation of YfiN significantly increased the persistence of P. aeruginosa in long-term mouse infections (Malone 2010, PLOS Pathogens, 6(3), e1000804). These studies establish a firm causal link between SCV, c-di-GMP, and chronic P. aeruginosa infections.

Fig. 3: Characteristic morphologies of normal “smooth” (large colony) and SCV morphotypes (small colonies) of Pseudomonas aeruginosa. Image by Anne-Sophie Arnold.
Publications 2011


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Nature has developed sophisticated biological machines that are fundamentally intriguing and technologically unprecedented. Here, we use quantitative nanoscience techniques and develop new biophysical methods to obtain deep insight into the interactions that drive biological functionality. In return, we realize and implement biomimetic concepts for novel non-physiological applications.

**Selective transport through the nuclear pore complex**
We want to know how the nuclear pore complex (NPC) facilitates the rapid and selective exchange of specific cargo proteins into and out of the cell nucleus. Each 50 nm-diameter NPC functions as a gate that is open or closed depending on whether it recognizes the "identity" of its molecular "guest". Our objective is to decipher the biophysical mechanisms that govern NPC transport selectivity. To underscore this point, viruses hijack the very same mechanisms to infiltrate the nucleus.

**Molecular mechanics of the nuclear lamina**
The nuclear lamina (NL) is a structural scaffold that provides the mechanical integrity of the nucleus. Mutations in the NL proteins are responsible for diseases known as laminopathies. Here, we study the structural and biochemical interactions that underlie the organization of the NL and how this regulates the mechanobiology of the nucleus.

**Mechanobiology and the diagnostics of disease**
We use atomic force microscope (AFM)-based technologies developed in our lab to study the mechanobiology of cells within tissues with subnano Newton precision. We anticipate that such information can be relevant to understanding diseases such as breast cancer and cartilage degeneration i.e. osteoarthritis.

**Building biomimetic devices inspired by nature**
"Protein targeting" refers to how proteins are delivered to the precise spatial location within the biologically complex environment of the cell. Based on our understanding of molecular transport and selectivity, we aim to replicate the same biochemical selectivity and protein targeting control in biomimetic molecular transport systems with potential applications in water purification and bioseparations.
Publications 2011


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Lipid biosynthesis and degradation are essential and tightly regulated cellular processes in all organisms and they are closely linked to human health. Lipids are an important source of natural chemical diversity and integrate the metabolic state with cellular processes such as inflammatory response, transmembrane signalling, and trafficking. Impaired lipid and fatty acid metabolism plays a significant role in the pathogenesis of some of the most common threats to human health, including type 2 diabetes, fatty liver disease, atherosclerosis and cancer. The aim of our work is to improve our understanding of eukaryotic lipid metabolism and its regulation at the molecular level by elucidating the structure and functional principles of key proteins involved. The results of this work may ultimately guide the development of novel metabolism based intervention in the treatment of cancers or other diseases linked to lipid metabolism.

Recent developments in lipid metabolomics allow quantitative studies of the cellular lipidome, real-time cellular imaging of lipid dynamics and systems biology studies on, which are providing new insights into lipid networks and their regulation at the cellular scale. However, eukaryotic lipid and fatty acid metabolism and its regulation remains a critical challenge for studies at the molecular and atomic scale: in contrast to simple prokaryotic systems, fatty acid metabolism in eukaryotes builds upon huge multifunctional enzymatic complexes more than all other metabolic pathways. Later steps of lipid metabolism are in large parts occurring in the membrane space and rely for regulation and catalysis on membrane asso-

Our work is focussed on eukaryotic multienzymes and molecular machines involved in key steps of lipid metabolism and homeostasis. Particular interests are to understand regulatory mechanisms with implications for human health as well as basic principles of multienzyme architecture, highly relevant for pathway reconstruction in synthetic biology. Additional studies on polyketide synthase molecular assembly lines serve to obtain complementary information on the mechanism of the related enzymes of animal fatty acid synthesis. They will also provide important insights for combinatorial biosynthesis of novel polyketide drug candidates. Our approach builds on X-ray crystallography as a key method to obtain insights at atomic resolution in combination with chemical biology approaches to the stabilization, labelling and trapping of macromolecules, aided by biophysical characterization of macromolecular interactions and biochemical analysis of biological function in in vitro systems.

We have moved to the Biozentrum in February 2011 from the ETH Zürich, Switzerland. The lab is well set for all aspects of modern protein production, crystallization and X-ray crystallographic analysis. Facilities are available for large-scale protein expression in bacterial, yeast and mammalian cell based systems. Crystallization is aided by microfluidic screen
preparation and nanoliter robotics for crystallization setup. Crystallographic data collection is carried out on an in-house microfocus source or at the nearby Swiss Light Source. Excellent support is available for biophysical characterization of molecular interactions. Besides a longstanding experience with proteins of lipid metabolism our key expertise is the structural analysis of very large and flexible macromolecules at intermediate to high resolution. A landmark example is the previous success in the structure determination of the giant eukaryotic fatty acid synthases, which was a breakthrough for the understanding of a classic and essential metabolic pathway after more than 50 years of research.

For the future, we are building our work on efficient medium-throughput expression screening of large eukaryotic multienzymes, multisubunit-complexes or parts thereof in bacterial and eukaryotic expression systems and high quality protein purification. Conditions for conformational stabilization are screened using biophysical techniques and guide high-throughput crystallization. We are keeping our focus on large multienzymes and regulatory complexes particularly relevant for the control of lipid and fatty acid metabolism. The targeted structural and mechanistic insights would advance our notion of the concepts of multifunctional assemblies characteristic for higher eukaryotes. They would also provide key insights into the mechanism and regulation of lipid metabolism, which has a central role for human health.
Cell proliferation depends on passage of cells through a series of biochemical reactions that are collectively termed ‘cell cycle’. This fundamental process is indispensable for the development of an entire organism from a single cell (fertilized egg), as well as the constant renewal of most cells throughout adult life. Key events during cell cycle progression include the duplication of the chromosomes (the genome) and their subsequent segregation to two nascent daughter cells. Chromosome segregation occurs during a cell cycle phase known as ‘mitosis’, a highly dynamic and spectacular stage of the cell cycle (Fig. 1). The main goal of our research is to elucidate the mechanisms that regulate mitosis in time and space and thereby ensure the error-free segregation of chromosomes. A better understanding of mitosis will hopefully illuminate the origins of the chromosome aberrations (aneuploidies) that give rise to birth defects and constitute hallmarks of aggressive human tumors.

Central to mitosis is the spindle apparatus, a complex and highly dynamic microtubule-based structure that captures chromosomes through specialized protein structures termed kinetochores (Fig. 2). In addition to studying the composition, regulation and dynamics of the mitotic spindle and kinetochores, we aim at elucidating the function of a surveillance mechanism - the spindle assembly checkpoint - that monitors the complete attachment of all mitotic chromosomes to the spindle. At the two poles of the spindle apparatus are tiny organelles known as ‘centrosomes’ (Fig. 3).

These function to organize microtubule arrays in most animal cells and are present as only one or two copies per cell, depending on cell cycle stage. At the core of each centrosome are two microtubule-based cylindrical structures called ‘centrioles’, embedded in a matrix of pericentriolar proteins. Importantly, centrioles function as basal bodies for the formation of cilia and flagella in quiescent cells. Deregulation of the centrosome/centriole duplication cycle is believed to constitute a major cause of chromosome miss-segregation during the development of human cancers. Furthermore, mutations in genes coding for centrosome- and centriole/basal body-associated proteins are held responsible for a variety of human diseases, ranging from brain diseases (notably microcephaly) to a multitude of syndromes that reflect the absence or malfunction of the basal-body/ciliary apparatus (ciliopathies). Hence, the second major research interest in our laboratory is focused on the biogenesis, duplication and function of centrosomes/centrioles.

Our laboratory combines reverse genetics (e.g. RNA interference), immunocytochemistry and multiple biochemical techniques (notably mass spectrometry) to unravel the molecular mechanisms that ensure correct centrosome duplication and chromosome segregation in human cells. A common thread running through our studies is a focus on phosphorylation (a reversible protein modification controlled by kinases and...
phosphatases). Studying mostly human cells in culture, we take two complementary approaches: a ‘comprehensive’ strategy uses mass spectrometry to establish inventories of proteins and phosphorylation sites in the spindle apparatus, the kinetochore and the centrosome, whereas a second ‘in depth’ approach aims at elucidating the wiring of key regulatory circuits, as defined by kinases, phosphatases, and selected substrates. We anticipate that the convergence of these two approaches will lead to a better understanding of the regulation of centrosome duplication and chromosome segregation in normal cells, as well as provide insights into the deregulation of these processes in disease.

In the recent past, we have successfully completed a large scale phospho-proteomics study that provides information, with unprecedented temporal resolution, on hundreds of mitotic phosphorylation sites. In parallel, we have characterized several novel spindle components as well as proteins implicated in centriole duplication. Of particular interest is our discovery of Plk4 as a key regulator of centriole biogenesis and the demonstration that a ternary complex of Ska proteins (Ska1, 2 and 3) plays a major role in stabilizing the attachment of spindle microtubules to kinetochores. Ongoing work also concerns the function and regulation of several cell cycleregulatory kinases, including Polo-like kinases (notably Plk1), Aurora kinases and spindle checkpoint kinases (Mps1 and Bub1).

One major challenge in contemporary biological and biomedical research concerns the development of technologies that will permit the acquisition of quantitative information about the abundance, localization and dynamics of proteins and protein modifications under physiological conditions. We anticipate that such technologies will become increasingly important not only in systems biology but in life science research altogether. Hence, we are currently optimizing mass-spectrometry based procedures (selected reaction monitoring) and somatic gene targeting approaches that will hopefully allow us to monitor, in quantitative terms, key components involved in both centrosome duplication and chromosome segregation.

The cell cycle field holds considerable promise for the development of novel therapeutic approaches. In particular, it appears legitimate to hope that new information on the mechanisms that govern chromosome segregation and cell division will contribute to the design of novel strategies to thwart cancer growth. This has been widely recognized not only in Academia, but also in the Pharmaceutical and Biotechnology industry, providing ample opportunities for collaboration and translational research.
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Publications 2011


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Our laboratory is investigating signal transduction processes that are involved in the body’s immune defense. We are pursuing two lines of research: On the one hand, we aim to understand the mechanisms of immune cell activation in order to control pathogen invasion. On the other hand, we are interested in elucidating how pathogens cause disease despite the presence of a functioning immune system. Together this work may allow a better understanding of the host immune system as well as delineate strategies utilized by pathogens to survive and cause disease.

Interaction of Mycobacterium tuberculosis with immune cells

Many pathogenic microorganisms have gained the capacity to circumvent the effectiveness of the immune response at several levels, and one project within the laboratory aims to decipher the mechanisms that are used by pathogens to escape immune recognition. In particular, we are studying the survival mechanisms of the important pathogen Mycobacterium tuberculosis. Mycobacteria have the ability to survive within eukaryotic cells, by preventing phagosome-lysosome fusion. We are interested in both the host as well as the mycobacterial factors contributing to mycobacterial survival.

Coronin 1 signaling in leukocytes

A major focus in the laboratory concerns the analysis of coronin 1-dependent signaling. Coronin 1 is a leukocyte specific protein, that our laboratory has identified as a host protein utilized by M. tuberculosis to survive within macrophages (Cell (1999) 97:435; Science (2000) 288:5471). To understand the mechanisms of action of coronin 1 as well as its normal function in leukocytes, we generated coronin 1 deficient mice, which allowed us to delineate the molecular mechanisms whereby coronin 1 modulates the survival of M. tuberculosis inside macrophages: upon infection by M. tuberculosis, coronin 1 is responsible for the activation of the Ca^2+-dependent phosphatase calcineurin, thereby preventing mycobacterial killing within lysosomes (Cell (2007) 130:37). In addition, we could show that coronin 1 is required for the maintenance of T lymphocytes in peripheral lymphoid organs (Nature Immunol. (2008) 9:424). We uncovered that coronin 1 is essential for the generation of the second messenger inositol-1,4,5-trisphosphate (IP3) following T cell activation thereby regulating Ca^2+-dependent signaling reactions (see Fig. 1).

Besides macrophages and T cells, all other leukocytes express coronin 1, but a role for this molecule in these other leukocytes has remained unknown. By analyzing B cells as well as neutrophils from coronin 1-deficient mice, we found that in B cells, like in T cells, coronin 1 is essential for intracellular Ca^2+ mobilization and proliferation upon triggering of the B cell receptor. However, the presence of costimulatory signals rendered coronin 1 dispensable for B cell signaling, consistent with the generation of normal immune responses against a variety of antigens in coronin 1-deficient mice. Thus, coronin 1, while being essential for T cell function and survival, is dispensable for B cell function in vivo (Combaluzier et al., 2009). Furthermore, we showed that in mice lacking coronin 1, neutrophil populations developed normally, and that coronin 1-deficient neutrophils are fully functional with respect to adherence, membrane dynamics, migration, phagocytosis and the oxidative burst. These data therefore suggest that coronin 1 is dispensable for neutrophil functioning (Combaluzier and Pieters, 2009).

Together our recent work has uncovered a role for coronin 1 in Ca^2+-dependent signaling in leukocytes. We are currently aiming to understand the molecular interactions of coronin 1 as well as analyze the importance of coronin 1-deficient signaling for the generation of immunity.
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The vast arrays of different neural cell types that characterize the complex circuits of the brain are generated by neural stem cells. During normal brain development, these neural stem cells produce defined sets of neural progeny composed of specific cell types which interconnect to form functional circuitry. Understanding the molecular mechanisms that underlie this process and give rise to the astonishing number and diversity of precisely defined cell types in the brain is one of the most challenging problems in biology.

In *Drosophila*, the neural stem cells, called neuroblasts, are similar to vertebrate neural stem cells in their ability to self-renew and to produce many different types of neurons and glial cells. Recent work has shown that the numerous cell types that make up the central brain of *Drosophila* derive from a set of approximately 100 neuroblast pairs, each of which generates its own lineage-specific unit of neural progeny. The goal of our lab is to analyze the developmental mechanisms by which these proliferating brain neuroblasts generate the lineage-specific units of the brain and specify the number and diversity of cell types in each of these units. In doing so, we also aim to analyze the cellular and molecular mechanisms by which deregulated neuroblast proliferation leads to the formation of brain tumors.

In studies focused on normal neuroblast proliferation, we have discovered and analyzed a novel mode of neurogenesis in *Drosophila*, in which a striking amplification of neuronal proliferation is achieved by specific brain neuroblasts through the generation of intermediate progenitor cells (Bello et al., 2008; Reichert, 2011). Using cell lineagespecific tracing and marker analysis we have shown that remarkably large neuroblast lineages develop in the dorsomedial area of the larval brain. These neuroblast lineages contain a pool of cells which do not express neuronal differentiation markers, are engaged in the cell cycle, and show mitotic activity. While some of these mitotically active cells are committed ganglion mother cells, the others express neuroblast-specific molecular markers and divide repeatedly to produce neural progeny implying that they are intermediate progenitors. These intermediate progenitors are generated by neuroblasts that do not segregate Prospero to their smaller daughter cell thereby allowing this cell to retain proliferative capacity instead of undergoing its final neurogenic division. This amplification of neuroblast proliferation through intermediate progenitors in *Drosophila* bears remarkable similarities to mammalian neurogenesis, where neural stem cells as primary progenitors amplify the number of progeny they generate via symmetrically dividing secondary progenitors. Our current data indicates that these novel amplifying neuroblast lineages in *Drosophila* are highly vulnerable to tumorigenesis caused by the impairment of tumor suppressor genes that control cell fate determination. We have studied the cellular and molecular mechanisms that are responsible for the high oncogenic vulnerability of these neural stem cell lineages and, based on this analysis, plan to design and test molecular genetic strategies to prevent tumorigenesis of these progenitors in vivo, in vitro and in a host environment. Given the remarkable evolutionary conservation of fundamental mechanisms in stem cell biology, this research may be useful for designing, testing and implementing novel strategies aimed at preventing neural stem cell-derived brain tumors in stem cell transplantation therapy.

In studies focused on the developmental mechanisms by which brain neuroblasts generate the circuitry of the brain, we have characterized several key cellular and molecular features that operate in the *Drosophila* brain (Kumar et al., 2009a, b; Pereau et al., 2010; Colonques et al., 2011; Reichert and Bello, 2010). Importantly, we have carried out a detailed analysis of the lineage-specific organization of the central complex, a major sensorimotor integration center in the *Drosophila* brain. Our work shows that the neurons comprising this complex brain circuitry are generated by a surprisingly small number of neural stem cells during a remarkably short developmental period. Lineage-specific genetic labeling techniques show that four identified neuroblast lineages give rise to the numerous columnar small field neurons that project to and interconnect the protocerebral bridge, fan-shaped body, ellipsoid body and noduli of the central complex (Izergina et al., 2009). Remarkably, the corresponding neuroblasts, which belong to the class of neural stem cells that amplify prolifera-
tion through intermediate progenitors, also produce the glial cells that ensheathe the neuropile of the developing central complex (Viktorin et al., 2011). Hence the neuroblasts that give rise to these lineages represent novel pluripotent neuroglioblasts that are programmed to generate both the neurons and the glia of one and the same complex brain circuitry (Boyan and Reichert, 2011).

In further studies on the specification of the neuronal features in neural progenitor lineages, we have identified novel olfactory interneuron lineages, explored the role of Notch signaling and programmed cell death in these lineages, and analyzed the role of the cephalic gap gene empty spiracles (ems) in olfactory sensory and interneuron development in Drosophila (Lichtneckert et al., 2008; Das et al., 2008, 2010, 2011; Sen et al., 2010). Moreover, we have shown that ems has cell lineagespecific functions in olfactory interneuron development. Thus in the lateral olfactory interneuron lineage, ems expression is essential for development of the correct number of cells; in ems mutants the number of interneurons in the lineage is dramatically reduced due to apoptosis. In contrast, in the anterodorsal olfactory interneuron lineage, ems expression is necessary for precise targeting of neuronal dendrites to appropriate glomeruli; in ems mutants the interneurons fail to innervate correct glomeruli, innervate inappropriate glomeruli, or mistarget dendrites to other brain regions. Our finding that Drosophila ems, like its murine homologues Emx1/Emx2, is required for the formation of both peripheral and central olfactory circuitry implies that conserved genetic programs control olfactory system development in insects and mammals. Finally, based on our experimental and methodological insight into the development of the Drosophila brain, we have successfully initiated work on brain development in a related dipteran fly, the biomedically important yellow fever mosquito Aedes (Mysore et al., 2011).

Fig. 2: Neural stem cell lineages are the basic building blocks of complex brain circuitry in Drosophila (arrows show site of developing central complex primordia).
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The overall research topic concerns the molecular mechanisms important for the function and dysfunction of synapses at the neuromuscular junction (NMJ) and between neurons in the brain (Fig. 1). Pathological changes at the NMJ, as for example seen in myasthenia gravis (Fig. 2) or muscle dystrophies (Fig. 3), impair muscle function and can be life-threatening. Our group investigates different signaling pathways important for the establishment and function of synapses. Using this knowledge, we also try to find new ways to treat pathological alterations at the NMJ and in skeletal muscle. All these projects synergize with each other as we are using the same systems to answer related questions.

**Fig. 1:** Comparison of neuromuscular junctions and synapses in the brain. (A) At the NMJ, the presynaptic motor nerve, visualized by the staining with antibodies to neurofilament and synaptophysin (red), perfectly matches the postsynaptic structure, stained with an agent that binds to acetylcholine receptors. (B) At neuron-to-neuron synapses, actin (yellow) is highly enriched at postsynaptic spines on the dendrites of cultured hippocampal neurons (green). Thus, the structure of synapses is similar at the NMJ and at neuron-to-neuron synapses but there is a big difference in size. Scale bar = 10 μm.

**Fig. 2:** Experimentally induced myasthenia gravis causes a severe fragmentation of postsynaptic structures Size (green) in sternomastoid muscle so that neuromuscular transmission initiated by the presynaptic nerve terminal (red) is largely abrogated. Scale bar = 10 μm.

The role of mTORC1 and mTORC2 in brain and skeletal muscle

Both, neurons and skeletal muscle fibers are postmitotic and thus their growth largely depends on changes in cell size and not cell number. The mammalian target of rapamycin (mTOR), which was discovered in yeast by Michael Hall and collaborators, assembles into two multiprotein complexes called mTOR complex 1 (mTORC1) and mTORC2. The two complexes are characterized by the presence of particular proteins that are necessary for their function, such as raptor (mTORC1) and rictor (mTORC2). While mTORC1 is inhibited by the immunosuppressant rapamycin, mTORC2 is not inhibited by this drug. We are investigating the role of mTORC1 and mTORC2 in brain and skeletal muscle in mice using the Cre/loxP technology. In both tissues, deletion of mTORC1 causes the organ to be smaller. In the brain, mTORC1 is essential for brain development while mTORC2 mutants survive. In the brain, mTORC2 deficiency causes a strong decrease in its size and the mice display behavioral abnormalities. Neurons show deficits in dendritic arborization and synaptic circuitry. We are currently investigating the detailed molecular mechanisms underlying these phenotypes as well as the function of mTORC1 and mTORC2 at adult synapses.

Like in the brain, mTORC1 deletion in skeletal muscle results in a more severe phenotype than mTORC2 deletion. Here, mTORC2 deletion does not cause any overt phenotype whereas mTORC1 mutation affects the metabolism and function of skeletal muscle. Importantly, mTORC1 deficiency causes a severe myopathy and leads to the death of the mice at the age of 4 to 6 months. Moreover, the mice display a general wasting syndrome that is not restricted to skeletal muscle. Current projects study the mechanisms that trigger this wasting and investigate the contribution of mitochondrial biogenesis to the overall phenotype. In addition, we examine the effect of activating mTORC1 in skeletal muscle by the deletion of its inhibitor TSC1.

**Copine 6, a novel calcium sensor involved in synapse structure**

Changes in synaptic activity alter synaptic transmission and ultimately change neuronal network dynamics. Structural changes induced by electrical activity are often mediated by calcium-dependent processes. Initiated by gene expression studies at the NMJ, we focus on the function of Copine 6, a member of a small family of calcium-binding proteins. We have shown that Copine 6 affects spine formation and maintenance in hippocampal neurons. Copine 6 shuttles from the cytosol to postsynaptic sites upon NMDA receptor-dependent calcium influx. It binds to the Rho GTPase Rac1 and mediates...
its translocation to membranes upon calcium influx. These results strongly suggest that Copine 6 serves as a calcium sensor that links neuronal activity to the subsequent changes in synaptic structure. In the current projects, we investigate the function of Copine 6 and additional family members in vivo and we try to identify additional binding partners.

**NMJ and disease**

There are sporadic and genetic neuromuscular diseases (NMDs). Although they are often severe, they affect only a small proportion of the human population and most of the diseases are still not treatable. One of the acquired NMDs is myasthenia gravis that is caused by auto-immune antibodies directed to components of the NMJ. While most of the antibodies are directed against the acetylcholine receptor, in about 10% of the cases antibodies are directed against the receptor tyrosine kinase MuSK. Interestingly, the clinical symptoms are clearly distinct between the two subgroups. We have recently shown that the phenotypic difference between the two subtypes of myasthenia gravis can also be reiterated in a murine model of experimental autoimmune myasthenia gravis (EAMG). The symptoms in MuSK-EAMG mice are a severe kyphosis, weight loss and signs of neuromuscular hyperactivity, which are all distinct from the symptoms in EAMG induced by acetylcholine receptor antibodies. At the NMJs, MuSK-EAMG causes fragmentation and often loss of innervation (Figure 2). Interestingly, like in human patients, the pathological changes observed at NMJs differ between muscles. Current studies aim at elucidating the molecular mechanisms that are responsible for this difference between muscles.

Another set of NMDs are muscular dystrophies, where the skeletal muscle fibers and not the NMJs are affected. Although the mutations causing muscular dystrophies are distinct, the severe loss of muscle mass due to the degeneration of muscle fibers is common to all diseases. As a consequence, muscle becomes replaced by fibrotic tissue (Figure 3A). In one project we develop new methods to treat some of the muscular dystrophies in experimental mouse models. For example, we have recently shown that transgenic expression of a miniaturized form of the extracellular matrix molecule agrin (mini-agrin) can substantially compensate for the loss of laminin-211. This compensation is even more complete when cell death of muscle fibers is prevented by additionally expressing the anti-apoptotic protein Bcl2 (Figure 3B) or treating mice with the anti-apoptotic agent omigapil. Such combination therapy restores the structure of the muscle to control levels (Figure 3C). In current projects we test additional pharmacological agents for their potential to ameliorate the disease.
RESEARCH GROUP MARKUS RÜEGG

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The long-term goal of research in the Scheiffele Lab is to understand molecular mechanisms underlying the formation of neuronal circuits in health and disease. Synapses are the key processing units in neuronal circuits. Therefore, we are examining mechanisms of synapse formation and synaptic re-arrangements in the central nervous system. We are exploring the transsynaptic signals that coordinate the choice of synaptic partners, assembly of synaptic junctions and stabilization of appropriate contacts.

**Coupling of postsynaptic neurotransmitter complexes to synaptic adhesion molecules**

Synaptic adhesion molecules have important roles in organizing synaptic structures. In the past years we have focused on one pair of synaptic adhesion molecules called the neuroligin-neurexin complex which spans the synapse and contributes to the organization of pre- and postsynaptic membrane compartments (figure part A). While the postsynaptic neulrigins play important roles in the function of neurotransmitter receptors at central synapses, the mechanisms underlying coupling between the neurotransmitter receptors and adhesion complexes are not understood. Using biochemical and cell biological approaches we could demonstrate that the extracellular domain of the neuroligin-1 isoform recruits NMDA receptors through direct interactions via the extracellular domains of the protein. Similarly, the neuroligin-2 isoform recruits GABA receptors via the extracellular domain. These studies identified a mechanism for coupling of neurotransmitter receptors complexes to the neuroligin-neurexin adhesion complex and highlighted the possibility that neurotransmitter receptors may have structural roles at central synapses.

**Regulation of alternative splicing of the neurexin gene family Neurexins**

Neurexins are a family of highly polymorphic synaptic cell surface receptors. Alternative splicing controls the interaction of neurexins with their synaptic neurexin receptors and other binding partners and may underlie an adhesive code at central synapses. To understand how molecular diversity of neurexins is regulated we have explored the molecular mechanisms controlling neurexin alternative splicing. We identified a family of KH-domain RNA-binding proteins that are required for regulation of one key splicing decision in the neurexin pre-mRNA. In ongoing experiments we are examining the regulation of the KHdomain proteins by neuronal activity and the synaptic connectivity in knock-out mice.

**Synaptic defects in autism-spectrum disorders**

Autism-spectrum disorders are amongst the most heritable neurodevelopmental disorders known to date. Human genetic studies conducted over the past 5-10 years have led to the identification of several candidate genes that may confer susceptibility to autism but also environmental risk factors might exist. One group of autism candidate genes, including FMRP1 and MeCP2, is involved in the regulation of gene expression. A second, recently identified group relates directly to synaptic function. Importantly, the synaptic neuroligin-neurexin complex has emerged as a central player in this group of synaptic targets for monogenic forms of autism. Mouse models recapitulating mutations in this complex exhibit defects in synaptic physiology and animal behavior, thereby further supporting an important role for these mutations in the etiology of autism. The goal of our ongoing work is to test the hypothesis that a common “synaptic core pathway” is affected in autism which results in perturbation of neuronal network assembly and/or function.

**Emergence of synaptic specificity in the pontocerebellar projection system**

A key question in neural development is how axons choose their appropriate synaptic partners. We performed a detailed anatomical analysis to unravel how target specificity of ponto-cerebellar mossy fiber projections emerges during development (figure part B). We observed that mossy fibers form transient synapses with Purkinje cells (an “inappropriate target”) before precise connectivity with granule cells is established. We discovered that Purkinje cell-derived bone morphogenetic protein 4 (BMP4) acts as a retrograde signal that drives the destabilization of mossy fiber contacts. Using mouse genetics and in utero electroporation approaches we introduced mutant BMP-receptors into the pontocerebellar afferents and are now dissecting the molecular mechanisms that transduce target-derived BMP signals in mossy fiber growth cones.
Publications 2011


Publications 2010


Molecular mechanisms of c-di-GMP signal transduction and AMP transferases

We are employing crystallographic and biochemical/biophysical techniques to reveal the structural basis for the catalysis and regulation of c-di-GMP related proteins. Our second focus is on bacterial type IV secretion system (T4SS) effector proteins with AMP transferase activity.

**Diguanylate cyclases and regulation of c-di-GMP synthesis**

Recent discoveries show that a novel second messenger, c-di-GMP, is extensively used by bacteria to control multicellular behavior, such as biofilm formation. Condensation of two GTP to the dinucleotide is catalyzed by GGDEF domains that usually occur in combination with sensory and/or regulatory modules. The opposing phosphodiesterase activity is provided by EAL domains that are also regulated.

In collaboration with the Jenal group (Biozentrum) and based on crystallographic studies we have elucidated the catalytic and regulatory mechanisms of PleD, an essential part of the signaling pathway regulating the developmental cycle of *Caulobacter crescentus*. More recently, we have determined the structure of the putative c-di-GMP specific phosphodiesterase YkuI in complex with c-di-GMP, which allowed us to propose the catalytic mechanism of EAL domains. Moreover, the structure provided clues about how this class of enzymes may be regulated in a modular and universal fashion by sensory domains.

**Effector proteins of the type IV secretion system**

Type IV secretion systems (T4SS) are utilized by many bacterial pathogens for the delivery of virulence proteins or protein-DNA complexes into their eukaryotic target cells. Together with the Behio group (Biozentrum) we are working on a class of effector proteins that are composed of a Fic and a BID domain responsible for pathogenic action in the host cell and translocation, respectively.

Only recently, it has become apparent that the Fic domain catalyzes AMP transfer onto host target protein(s) to subvert cellular function. From a Fic crystal structure (truncated BepA from *Bartonella henselae*) we were able to deduce the mechanisms of catalysis and target positioning. Currently, we are investigating Fic inhibition that - depending on the protein - is caused by an α-helix that interferes with productive binding of the ATP substrate or, inter-molecularly, by complex formation with an anti-toxin. Interestingly, both inhibition mechanisms are structurally related. This knowledge may be utilized for drug development to target Fic proteins of bacterial pathogens.
Porins
Porins are integral membrane proteins from the outer membrane of Gram-negative bacteria. They allow the uptake of nutrients by passive diffusion through an intrinsic pore that extends along the axis of the transmembrane β-barrel structure. After extensive work on the general trimeric porins OmpF and OmpC from *E. coli*, we have recently determined the high-resolution 12-stranded β-barrel structures of NanC from *E. coli* and KdgM from *Dickeya dadantii*, representatives of a porin family that is specific for the translocation of negatively charged poly-saccharides. We are now studying the molecular details of translocation of oligogalacturonate, the degradation product of pectin, through KdgM.
**Publications 2011**


**Publications 2010**


The ability of the cell to change its shape and move is a fundamental requirement for development and differentiation but also plays a role in disease, particularly neoplasia. Cellular plasticity and mechanical function of cells and tissues in response to environmental, genetic and epigenetic signals involve the dynamic remodeling of the cytoskeleton. Our work aims at understanding the molecular mechanisms underlying cellular plasticity in tumorigenesis.

**Structural plasticity in actin dynamics**

The actin cytoskeleton, a complex filamentous network, undergoes rapid cycles of assembly and disassembly in a spatially and temporally controlled fashion. This structural plasticity, illustrated in Fig. 1, provides the basis for changes of cell architecture and function in response to signals from the environment. In vitro studies have revealed an actin dimer at the onset of polymerization with subunits arranged in an antiparallel orientation. Because of its transient nature, this so-called ‘lower dimer’ (LD) has until now escaped detection in vivo. To address the functional significance of LD in actin patterning, we have raised antibodies that specifically react with LD. Immunelectron microscopy studies using synthetic actin structures showed that the LD antibody predominantly decorated sites where subunits contact each other in an antiparallel orientation. More importantly, immunofluorescence revealed the presence of LD in intact cells where it partially colocalizes with the endosomal compartment. The LD antibody will be helpful in addressing the role of LD-related actin structures in intracellular transport. LD-related epitopes were also detected at the cell periphery and in the nucleus, notably in association with heterochromatic regions at the inner nuclear membrane.

Despite actin’s acknowledged role in chromatin organization and gene expression, it remains largely unknown how it is earmarked for its diverse nuclear functions. We have previously identified distinct nuclear actin assemblies by different antibodies. Recent data from cells that overexpress actin in the nucleus reveal a filamentous fraction localized to the inner regions of the nucleus, whereas an unpolymerized population resides at the periphery (Fig. 2). To elucidate the role of actin’s structural plasticity in transcription, we are currently testing antibodies that label distinct actin populations in the nucleus for their association with regions of different transcriptional activity.

**Mechanical plasticity of cells as marker for transformation**

Cells within tissues continuously encounter a dynamic range of mechanical forces to which they respond by remodeling their cytoskeleton. Tumorigenic transformation frequently changes the nature of the forces experienced by cells and the cellular response is modified accordingly. We are employing atomic force microscopy (AFM) in combination with light/fluorescence microscopy to quantitatively probe with high sensitivity and spatial resolution the nanomechanical properties of cells and tissues. Our aim is to understand the spe-
cific contributions of cytoskeletal components to mechanical function. In particular, we are investigating correlations between changes in cellular elasticity and the cytoarchitecture that are associated with tumorigenic transformation.

To study the contribution of the intermediate filament (IF) network to the nanomechanical properties of cells, we compared elasticity maps of fibroblasts with an unmodified network or a network that was specifically modulated by transfected GFP-desmin variants. Expression of the nonfilament forming GFP-DesL345P mutant led to a collapse of the endogenous vimentin network in the perinuclear region that was accompanied by localized stiffening. Correlative confocal microscopy indicates that the expression of desmin variants specifically targets the endogenous vimentin IF network without major rearrangements of other cytoskeletal components. By measuring functional changes caused by IF rearrangements in intact cells we showed that IFs play a crucial role not only at large deformations but also determine the nanomechanical properties at a single cell level (Plodinec et al., 2011).

Because the characteristics of cancer are more appropriately reflected by a threedimensional (3D) tissue organization we use cultured cell spheroids and native tumor tissue from a transgenic mouse and human patient biopsies to investigate nanomechanical changes associated with tumorigenic transformation. Consistent with the mechanical response of tumor spheroids, preliminary data revealed a gradual centripetal softening from the periphery to the core in early breast carcinoma tissue from MMTV-PyMT mice. The cellular stiffness of the stromal tumor tissue at the periphery of the malignant lesion was comparable to that measured in normal mammary gland tissue. Ongoing genetic profiling studies will hopefully provide information on the molecular mechanisms that underlie the differential nanomechanical response.

The nanomechanical AFM testing of human breast biopsies showed that malignant lesions are not characterized by a specific stiffness but rather a characteristic stiffness distribution that is related to the malignant phenotype. At high resolution, stiffness maps reveal individual features at the cellular level (Fig. 3). In contrast, benign lesions typically show a uniform stiffness that is consistent with fairly homogenous tissue morphology. We are currently testing whether a correlation between tissue softening and increasing hypoxia exists in breast tumors.

Fig. 3: The nanomechanical signature of human breast cancer. High resolution AFM stiffness maps (left) of breast biopsies reveal distinct cancer phenotypes that are confirmed by histology (right).
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Publications 2010
Protein structure modeling and evaluation

The main interest of my group is the development of methods and algorithms for molecular modeling and simulations of threedimensional protein structures and their interactions. One of the major limitations for using structure-based methods in biomedical research is the limited availability of experimentally determined protein structures. Prediction of the 3D structure of a protein from its amino acid sequence remains a fundamental scientific problem, and it is considered as one of the grand challenges in computational biology. Comparative or homology modeling, which uses experimentally elucidated structures of related protein family members as templates, is currently the most accurate approach to model the structure of the protein of interest. Template-based protein modeling techniques exploit the evolutionary relationship between a target protein and templates with known experimental structures, based on the observation that evolutionarily related sequences generally have similar 3D structures. The SWISS-MODEL expert system developed by our group is a fully automated web-based workbench, which greatly facilitates the process of computing of protein structure homology models.

Estimating the expected quality of predicted structural models is a vital step in homology modeling. Especially when the sequence identity between target and template is low, individual models may contain considerable errors. To identify such inaccuracies, scoring functions have been developed which analyze different structural features of the protein models in order to derive a quality estimate. To this end, we have introduced the composite scoring function QMEAN, which consists of four statistical potential terms and two components describing the agreement between predicted and observed secondary structure and solvent accessibility. We have shown that QMEAN can not only be used to assess the quality of theoretical protein models, but also to identify experimental structures of poor quality. Ultimately, the quality of a model determines its usefulness for different biomedical applications such as planning mutagenesis experiments for functional analysis, or studying protein-ligand interactions, e.g. for structure based drug design. In the following, three exemplar projects involving molecular modeling of protein-ligand interactions at different levels of model resolution are briefly presented.

Molecular modeling of protein-ligand interactions

Dengue fever is a viral disease that is transmitted between human hosts by Aedes mosquitoes, particularly Aedes aegypti. In 1997, 20 million cases of dengue fever were estimated to occur annually. Partially because of increased urbanization and failure to effectively control the spread of the insect vector, more recent estimates suggest this number has risen to 50 –100 million, and dengue fever is now seen as one of the most important emerging infectious diseases in many areas of the world. We have used a structure based virtual screening approach to identify novel inhibitors of Dengue virus RNA methyltransferase (MTase), which is necessary for virus replication. In a multistage molecular docking approach in the MTase crystal structure, we screened a library of more than 5 million commercially available compounds against the two binding sites of this enzyme. In 263 compounds selected for experimental verification at the Novartis Institute for Tropical Diseases in Singapore, 10 inhibitors with IC50 values of <100 μM were identified, of which four exhibited IC50 values of <10 μM in in vitro assays.

Olfaction refers to the sense of smell which is mediated by specialized sensory cells in the nasal cavity of vertebrates and in the antennae of invertebrates. Activated olfactory receptors are the initial player in a signal transduction cascade which ultimately produces a nerve impulse which is transmitted to the brain. These receptors are members of the class A rhodopsin-like family of G protein-coupled receptors (GPCRs), which can detect a limited range of different odorant substances. In a collaborative project with the group of Horst Vogel (Ecole Polytechnique Federale de Lausanne, CH), we aim to explore the molecular determinants of specific olfactory receptors. We have modeled the mouse Eugenol olfactory receptor based on the crystal structure of β2-adrenergic receptor.
receptor. Based on this model, we have designed a series of site directed mutagenesis experiments to study the structural determinants of receptor specificity on various chemically diverse odorant molecules.

Second messengers control a wide range of important cellular functions in eukaryotes and prokaryotes. Cyclic di-GMP is a ubiquitous second messenger that regulates cell surface-associated traits in bacteria. Genome sequencing data revealed several large and near-ubiquitous families of bacterial c-di-GMP related signaling proteins. In pathogenic bacteria, this switch is often accompanied by the transition from an acute to a chronic phase of infection. This makes c-di-GMP signal transduction an attractive target for novel antibiotics that interfere with bacterial persistence. We are collaborating in-house with the groups of Urs Jenal, Tilman Schirmer and Dagmar Klostermeier in a Sinergia project aiming to discover novel components of the c-di-GMP signaling network and to uncover their molecular mechanisms.
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Publications 2010


ATP binding cassette (ABC) transporters are expressed in all phyla of life and constitute one of the largest protein superfamilies. They translocate very diverse substrates across cellular membranes at the expense of ATP hydrolysis. The best-investigated ABC transporter is P-glycoprotein (ABCB1, MDR1). It prevents drug and toxin uptake at absorptive membranes such as the intestinal (IB) and the blood-brain barrier (BBB), respectively, and enhances metabolite efflux at excretory barriers in proximal tubules of the kidney and biliary ducts of the liver. Cells can be induced to overexpress ABCB1 by the exposure to a single agent (e.g. anticancer drugs, certain antibiotics, or food components) or to physical stress, such as X-ray, UV light irradiation or heat shock. Over-expression of ABCB1 leads to multidrug resistance (MDR), that is, to a resistance towards all drugs that are substrates forABCB1. The expression level of ABCB1 depends (among other factors) on the exposure of cells to various stimuli. The same type of stimuli that induce MDR due to ABCB1 overexpression in humans can also induce MDR in bacteria, parasites, and fungi by promoting the expression of related ABC transporters. MDR is detrimental not only for the treatment of many cancers, but also for the treatment of bacterial, parasitic, and fungal diseases and can be considered as a general problem for pharmacotherapy.

Although ABCB1 is known for more than 30 years, its substrate specificity and transport mechanism have long remained enigmatic. This is due on one hand to the unusual location of substrate binding site and on the other hand to the polyspecificity of the transporter. ABCB1 binds its substrates in the cytosolic membrane leaflet and moves them to the extracellular membrane leaflet or directly to the extracellular medium, depending on the hydrophobicity of the substrate, quite in contrast most well characterized transporters that move substrates from the aqueous phase at one side of the membrane to the aqueous phase at the other side of the membrane. Substrate binding in the lipid membrane is thus preceded by a lipid-water partitioning step. We have shown that lipid-water partitioning is dominated by hydrophobic groups of the substrate, whereas substrate binding to the transporter in the lipid membrane is due exclusively to hydrogen bond acceptor groups of the substrate. To be transported by ABCB1 a compound has to carry minimally one binding module composed of two hydrogen bond acceptors. Up to ten binding modules have been observed whereby the binding affinity of the compound to the transporter in the lipid membrane increases linearly with the number of binding modules. Hydrogen bond acceptors recognized by ABCB1 are carbonyl, ether, or tertiary amino groups, halogen substituents and p-electron systems. The hydrogen bond acceptor groups in binding modules most likely form hydrogen bonds with the numerous hydrogen bond donor groups in the transmembrane domains of the transporter. In the lipid environment of the cytosolic membrane leaflet (exhibiting a low dielectric constant) hydrogen bonds are more specific and stronger than van der Waals interactions. As soon as the substrate reaches the extracellular leaflet, where water can approach, hydrogen bonds with the transporter vanish and the substrate is released either into the lipid or the aqueous phase.

The rate of substrate transport by ABCB1 is directly proportional to the rate of ATP hydrolysis. However, the fact that substrates have to partition into the cytosolic membrane leaflet to bind to ABCB1 implies that many substrates partially escape to the cytosol before being caught by the transporter which complicates the analysis of substrate transport. Quantitatively comparing passive influx and active efflux of drugs revealed that the net flux of drugs across membranes protected by ABCB1 results from the sum of the two processes. For different compounds and a given membrane passive influx exponentially decreases with the size and the charge of the molecule and changes by several orders of magnitude for different compounds whereas active efflux is more constant and changes only by about one order of magnitude. Large and/or highly charged compounds diffuse slowly and are therefore prone to being completely effluxed by ABCB1. The diffusion step from the extracellular to the cytosolic leaflet is crucial for the fate of the molecule. If it is fast compared to efflux (flipping) compounds will reach the cytosol even though they are partially exported, however, if it is slow compounds will not reach the cytosol (see figure A-D). Our analyses revealed that the lipid bilayer membrane plays an important role in the transport process.
synergistic role in substrate binding as well as in substrate transport by ABCB1. By taking into account the membrane contribution allowed unraveling substrate recognition and transport by ABCB1. Ongoing projects in our laboratory are dedicated to elucidating the substrate specificity and function of other ABC transporters such as the Staphylococcus aureus transporter Sav1866, the cystic fibrosis transduction regulator (CFTR, ABCC7) and the cholesterol transporter ABCA1.

**Publications 2010**


Cell penetrating peptides (CPP)
CPPs are highly charged cationic peptides of varying length and composition which have received much attention because they can facilitate the transport of a large variety of “cargos” into the living cell. Most recent are the attempts of pharma development departments to package si-RNA and miRNA with CPPs for selective and specific target addressing. We showed by a variety of physical chemical techniques as early as 2003 that CPPs bind to anionic lipid vesicles but cannot enter the vesicle interior.

We could exclude the still popular model that the induction of nonbilayer structures plays a role in CPP membrane translocation. We observed however that CPPs bind with high affinity to extracellular domains of sulfated glycosaminoglycans such as heparin sulfate, heparin and others. We quantitated this interaction for a large variety of CPPs and glycosaminosulfates using high-sensitivity titration calorimetry (ITC), dynamic light scattering (DLS) and fluorescence spectroscopy. Most important was the application of this physical chemical knowledge to living cells. We synthesized a fluorescent derivative of the HIV-1 TAT protein transduction domain and observed its uptake into non-fixated living fibroblasts with time-lapse confocal microscopy, eliminating the need of fixation. Depending on the concentration, the fluorescent CPP entered the cell within seconds. Several observations suggested that the CPP binding leads to an aggregation or “capping” of sulfated glycosaminoglycans, inducing finally endocytosis.

Lipid membranes as catalysts for protein folding
Amphipathic peptides or proteins such as the bee venom melittin, the antibacterial peptide magainin 2 or the lipoprotein Apo-A1 are mainly random coil in solution but adopt an α-helical structure when bound to membranes. Likewise, a membrane-induced random coil-to-β-structure transition has been found for Alzheimer peptides such as Aβ(1-40) or fragments thereof. Melittin and related amphipathic compounds insert into the lipid membrane and modify the lipid structure. In contrast, the Aβ peptides remain on the surface of the bilayer. In a series of publications we have studied in detail the thermodynamics of the membrane-induced random-coil-to-β-structure transition. This work is widely cited and the results have been confirmed by other groups. Most recently we succeeded in a related analysis for the membrane-induced random-coil-to-β-structure transition. Indeed, our work appears to be the first quantitative analysis of a random coil β-structure transition. In collaboration with Y. Shai, Israel, we have applied this knowledge to a modified melittin which is β-structured on the membrane surface. The thermodynamics of this system ideally confirms the results obtained with other model systems for the rc β-structure transition.

Related projects
We are strongly interested in the physical chemical properties of Alzheimer peptides Aβ(1-40,42). The major obstacle for thermodynamic and kinetic studies is the low solubility of Aβ(1-40,42) in aqueous media. Nevertheless, we succeeded to provide a systematic thermodynamic analysis using ITC of the interaction of antibodies, specifically designed against different Aβ(1-40) segments, with Aβ(1-40). We have continued our work on detergent membrane investigations by analyzing in detail the biologically relevant lipopeptide surfactin. In ad-
dition, we have selectively deuterated two trans-membrane helices, WALP-19 and glycophorin A72-97, and have incorporated them into model membranes selectively deuterated at various segments. Using ITC we have studied in collaboration with the groups of M. Steinmetz, Paul-Scherrer-Institut, the interaction of different phosphorylated statmins with tubulin. Finally, we have initiated together with the group of A. Seelig thermodynamic studies on the interaction of P-glycoprotein with its substrates.

**Fig. 3: P-NMR as a method to study membrane structure.**

**Publications 2011**


**Publications 2010**


Asymmetry is an inherent property of most cells. Proteins and mRNA have to be distributed at specific cellular locales to perform their proper function or to be translated in a spatially and temporally regulated manner. Although the localization of the mRNAs is restricted to the cytoplasmic face of intracellular organelles or the plasma membrane, proteins and lipids have to be localized to these organelles to provide a platform on which mRNAs and/or proteins can be recruited and restricted. In general this compartmentalization is achieved by intracellular transport through exocytic (secretory pathway) and endocytic avenues. Communication between different organelles is maintained in large part by transport vesicles that are covered with a proteinaceous coat, which polymerizes and which helps to recruit cargo proteins into the nascent transport vesicle. One class of small GTPases - the family of Arf and Sar GTPases - is essential for the generation of transport carriers, while another class - Rab GTPases - is involved in the consumption of transport carriers and seems to play an essential role in the maintenance of organelar identity.

Our research interests center around questions like how intracellular traffic contributes to cellular asymmetry and how intracellular processes are regulated by small GTPases of the Arf and Rab families. We use the unicellular yeast Saccharomyces cerevisiae and the roundworm Caenorhabditis elegans for our studies as both organisms are particularly well suited to answer the kind of questions we like to address.

The regulation of Arf family proteins

In recent times, we have investigated the role of GTPase-activating proteins for Arf1p. We could show that the yeast homologues of ArfGAP1 and ArfGAP2/3, Gcs1p and Glo3p have overlapping functions in retrograde transport from the Golgi apparatus to the ER (Poon et al., 1999), and that Glo3p is an integral part of the COPI coat, which mediates this transport step (Lewis et al., 2004). The finding that ArfGAPs can induce a conformational change in SNARE proteins, which are essential components in membrane fusion processes (Rein et al., 2002, Robinson et al., 2006, Schindler and Spang, 2007), prompted us to investigate more closely the role of the ArfGAP2/3 Glo3p in transport vesicle formation. We identified a region in Glo3p, which binds to SNAREs, coatomer and cargo (BoCCS) (Schindler et al., 2009). Moreover, the C-terminal Glo3 regulatory motif, GRM appears to transmit the Arf1p nucleotide state via the GAP domain to the BoCCS region. Upon stimulation of the GTPase activity, SNAREs, coatomer and cargo could be released from the BoCCS region. We are currently trying to understand the molecular rearrangements in Glo3p and to identify interaction partners to gain further insights in the regulation of Glo3p. We also returned recently again to the analysis of the function of different Arf guanine nucleotide exchange factors (ArfGEFs) (Spang et al., 2001) and investigate their roles in Caenorhabditis elegans.

The regulation of cargo sorting and transport

In our quest to understand the life cycle of a transport vesicle, we realized that cargo, which needs to be transported in vesicles, is not just a passive bystander, but plays a more active role. Overexpression of cargo proteins with a coatomer-binding sequence (-KKXX) can rescue coatomer mutants in the –KKXX recognizing subunit (Sandmann et al., 2003). Furthermore, in the absence of the ArfGAP Glo3p, the p24 family proteins, which cycle between the ER and the Golgi apparatus, are required to bud efficiently vesicles from the Golgi (Aguliera et al., 2008). Moreover, in collaboration with Blanche Schwappach, we identified a novel bi-partite cargo recognition motif in coatomer (Michelsen et al., 2007). These results strongly indicate that cargo-coat interaction stabilize the priming complexes suggested by Springer et al. (1999) and that the formation of coat-cargo complexes is an essential integral part of vesicle biogenesis. We also demonstrated that Ypt1p is the Rab-GTPase responsible for anterograde and retrograde transport in the ER-Golgi shuttle as well as for Golgi maintenance in S. cerevisiae (Kamena et al., 2008). Finally, we have identified a novel trans-Golgi localized complex, exomer, which is required for the sorting and transport of specific cargo to the plasma membrane (Trautwein et al., 2006). We have found more cargo proteins that follow this pathway and are in the process of investigating the exomer interaction interface and decipher the transport mechanisms.

The regulation of early-to-late endosomal transport

Recently, we cloned a C. elegans mutant, sand-1(or552) that shows a defect in endocytosis. While initial uptake of material was normal in oocytes and coelomocytes, the transport from early-to-late endosomes seemed to be blocked (Poteryaev and Spang, 2005; Poteryaev et al., 2007). sand-1(or552) mutants had strongly enlarged early endosomes, which were positive for the small GTPase RAB-5. In contrast, RAB-7, the Rab protein normally found on late endosomes was mislocal-
ized to the cytoplasm. This finding opened the possibility that SAND-1 was a regulator of early-to-late endosome transition. We followed up on this hypothesis and could show that in coelomocytes early-to-late endosome transport is performed through Rab conversion, and not through vesicle transport. We went on to demonstrate that SAND-1 actively interrupts the activation of RAB-5 by displacing the guanine nucleotide exchange factor of RAB-5, RABX-5 from early endosomes (Poteryaev et al., 2010). At the same time SAND-1 helps to recruit RAB-7 to endosomes to drive Rab conversion, indicating that SAND-1 acts as a critical switch in endosome maturation.

These functions of SAND-1 are also conserved in mammalian cells (Poteryaev et al., 2010). We are now investigating the regulation of SAND-1 function and how multi-vesicular body formation, recycling pathways and endosome maturation are coordinated.

The regulation of mRNA metabolism and transport
This research direction was inspired by our finding that the poly A binding protein, Pab1p, associates with Arf1p and COPI vesicles in an mRNA-dependent manner and that Arf1p is required for ASH1 mRNA localization to the bud tip of yeast cells (Trautwein et al., 2004). The subsequent analysis allowed us to identify the first distal pole-localized mRNA in yeast (Kilchert and Spang, 2011) and to identify a novel pathway by which mRNAs are sequestered in processing bodies (P-bodies) for their degradation (Kilchert et al., 2010).
We have started a new international collaborative program with Prof. Chris Brown (University of Otago, Dunedin, New Zealand) and Prof. Ian Macara (University of Virginia, Charlottesville, USA) to identify mRNA localization codes in yeast and mammalian cells and to test their conservation in function.

Proteins, lipids and mRNA are distributed in the cell in a non-random manner. While protein localization is already a widely studied subject, much less is known about specific mRNA localization. Recent studies indicate, however, that a large portion of mRNAs is restricted to distinct places in the cell, and that these localization patterns may change over the cell cycle or upon external cues. The mechanism of mRNA localization is still poorly understood and even less is known about its regulation. One reason for the lack of knowledge is that very often mRNA localization signals – so-called zip codes – are hard to decipher since they are not linear and they involve secondary and tertiary structure elements on the mRNA. Therefore a major aim of the project is to develop robust algorithms that can identify such zip codes. The datasets required for the development and testing of the algorithms are derived from genome-wide mRNA expression/localization studies.

We will try to determine how many different zip codes exist and whether those codes are conserved from yeast to man. Are these zip codes modular? Are separate codes used for mRNA transport and for anchoring? How do external cues and the cell cycle regulate mRNA localization?

Our data so far indicate that intracellular transport pathways and in particular small GTPases play a pivotal role in mRNA localization in yeast. In addition, genome-wide studies in mammalian cells demonstrated that specific mRNAs localize with APC to cell protrusions and axonal growth cones. Based on these and other data we will develop new algorithms and the predicted zip codes will then be tested in yeast and mammalian cells.
**Publications 2011**


**Publications 2010**


Proteins synthesized on cytosolic ribosomes must be sorted to the specific compartment(s) in which they perform their function. Proteins of the endoplasmic reticulum (ER), the Golgi apparatus, the plasma membrane, the endosomal/lysosomal system and the cell exterior are all first targeted to the ER, translocated across the membrane or inserted into the lipid bilayer, and then distributed via the secretory pathway. Our research focuses on (1) how membrane proteins are inserted into the ER membrane and acquire a defined topology, and (2) how transport vesicles are formed at the trans-Golgi or endosomes, or in vitro from purified components and liposomes. In close collaboration with Jonas Rutishauser, we furthermore study the mechanism by which trafficking mutants of provasopressin cause dominant diabetes insipidus.

**Fig. 1:** "One ring to rule them all": View of the interior of the closed Sec61 translocon with the plug domain in green and the constriction ring in gold (Sefer Baday).

**Topogenesis of membrane proteins**

Hydrophobic signal or signal-anchor sequences target newly synthesized proteins to the translocon in the ER membrane. By systematic mutation of substrate proteins, we analyze the determinants that define their orientation in the membrane and the mechanism by which topogenesis occurs. Signal hydrophobicity, flanking charges, and the position of the signal within the protein determine the mode of insertion as well as the final orientation in the membrane.

The translocon is a compact helix bundle that forms a pore for the translocation of hydrophobic sequences and a lateral gate for the integration of transmembrane segments. In its empty state, the pore is closed by a lumenal plug domain and a hydrophobic constriction ring (see Fig. 1). To test their function, we deleted the plug and/or mutated all six ring residues to more hydrophilic or even charged amino acids. The translocon was found to be surprisingly tolerant to these mutations. The plug domain is not essential, but its deletion affects signal recognition and the regulated opening of the translocon required for faithful protein orientation. Most interestingly, ring mutants were found to affect the integration of hydrophobic sequences into the lipid bilayer, indicating that the translocon does not simply catalyze the partitioning of potential transmembrane segments between an aqueous environment and the lipid bilayer, but that it plays an active role in setting the hydrophobicity threshold for membrane integration.

**Post-Golgi protein sorting**

Transport between organelles is generally mediated by membrane vesicles formed by the recruitment of cytosolic coat proteins. In a permeabilized cell assay, we found the formation of recycling vesicles to return receptors from endosomes back to the plasma membrane to be dependent on clathrin coats with AP-1 adaptors and to be regulated by rab4, rab11, and rabaptin-5/rabex-5. We are studying the molecular mechanism by which these proteins mediate and regulate vesicle formation and in vivo transferring recycling.

Sulfation is a trans-Golgi-specific modification useful to study post-Golgi trafficking. To introduce sulfation sites, we have tagged proteins with short sequences for the attachment of (heavily sulfated) glycosaminoglycans (GAG). Interestingly, GAG attachment was found to affect protein traffic by inhibiting endocytosis and by accelerating trans-Golgi-to-cell surface transport both for secretory and membrane proteins. We are analyzing the mechanistic and physiological implications for proteoglycan sorting. In endocrine cells, prohormones and granins are sorted at the trans-Golgi network into dense-core secretory granules by an entirely different mechanism. We found expression of granule cargo to be sufficient to generate granule-like structures in nonendocrine cells. Deletion analysis of chromogranin A showed that the same segments that are required for granule sorting in endocrine cells produce granule-like structures in fibroblasts. The results support the notion that self-aggregation is at the core of granule formation and sorting into the regulated pathway.

**Diabetes insipidus: a trafficking disease**

Autosomal dominant neurohypophyseal diabetes insipidus results from mutations in the precursor protein of the hormone vasopressin. Mutant precursors are retained in the ER of vasopressinergic neurons and cause cell degeneration. We discovered that pro-vasopressin mutants form disulfide-linked oligomers and develop large, fibrillar aggregations in fibroblast and neuronal cell lines (see Fig. 2). Purified mu-
tant pro-vasopressin spontaneously formed fibrils in vitro. Dominant diabetes insipidus thus belongs to the group of neurodegenerative diseases associated with fibrillar protein aggregates. The responsible sequence and the mechanism of cell death remain to be elucidated.

Fig. 2: Pro-vasopressin aggregates in COS-1 cells.

Publications 2010

The Center for Cellular Imaging and Nano Analytics (C-CINA) is studying membrane protein systems at different size and length scales with a combination of various light and electron microscopy methods. C-CINA is located in the D-BSSE building in the northern part of Basel, and is supported by the Swiss systems biology initiative SystemsX.ch.

The Stahlberg group studies biological membranes and the contained membrane proteins at several length scales and resolution levels. We use fluorescence light microscopy, and combine the obtained localization information about fluorescently labeled protein complexes or viruses with Serial Blockface Scanning Electron Microscopy (SBF-SEM), in collaboration with the Friedrich Miescher Institute. SBF-SEM can characterize the 3D structure at 20nm resolution of large specimen areas of thousands of human cells at a time, thereby extending light microscopy to higher resolution. We also employ electron tomography (ET) in a transmission electron microscope, to study small specimens like individual bacteria at even higher resolution.

To this end, C-CINA operates an FEI Titan Krios transmission electron microscope (TEM), which is one of the worlds most advanced highresolution electron microscopes for the study of biological specimens. Further instruments in C-CINA include atomic force microscopes and a scanning transmission electron microscope (STEM). The latter is used to determine the mass-distributions of biological particles, which are adsorbed to ultra-thin carbon films and freeze-dried. We apply these different methods to the same specimens, enabling correlative light and electron microscopy (CLEM). The study of biological specimens at different levels of resolution and scale allows understanding the biological system at the cellular, molecular and submolecular level.

Visual proteomics
As part of the SystemsX.ch funded project, we develop a visual proteomics platform to study the 3D structure, and size and mass distribution of the proteome of a biological cell. In collaboration with the Hierlemann group at the D-BSSE, we develop a microfluidics setup to pick individual cells, lyse and fractionate them, and cross-link the cytosolic content of a single cell, which is then stained and deposited on a TEM grid for automated 3D imaging to obtain structural information about the entirety of the proteome. Samples can also be freeze-dried and their mass analyzed by the scanning transmission electron microscope (STEM). This platform will also be combined with mass spectrometry in collaboration with the Zenobi laboratory at the ETHZ, and with the multiarray optical tweezers system developed in the Vogel laboratory at the EPFL.

Membrane proteins
Membrane proteins are of central importance for health and disease. We study the highresolution structure of membrane proteins by electron crystallography, and also characterize the arrangement of larger membrane protein complexes or the dynamic conformation of certain membrane protein systems in the biological membrane by multiresolution microscopy, including electron tomography. In collaboration with Crina Nimigean, Cornell University, NY, USA, Joe Mindell, NIH Bethesda, USA, and Horst Vogel, EPFL, Lausanne, Switzerland, we study the structure and function of gated ion channels, transporters, and receptors by single particle EM and electron crystallography.
Software development
We are also developing software for the computer evaluation of the recorded data. We distribute a software package called 2dx for the computer image processing of 2D crystal images of membrane proteins (available at http://2dx.org). This MRC-based software is now used by over 400 external users, and features a user-friendly graphical user interface, and optionally fully automatic image processing, merging, and 3D structure reconstruction. In collaboration with Niko Grigorieff, Brandeis University, MA, we have developed a maximum-likelihood module, so that high-resolution structures of membrane proteins can also be determined in the absence of large well-ordered 2D crystals. We have developed a software algorithm for projective constraint optimization, to improve the resolution of the reconstruction, also in the direction perpendicular to the viewing direction of the microscope (effectively filling the so-called missing cone). We are also developing software solutions for the structure analysis of in-vivo membrane protein systems by electron tomography, by enabling user-friendly tomographic high-contrast reconstructions and tomographic molecular structure averaging.

The visual proteomics imaging platform has as its central part an automated sample staining and desalting module for micropatterning of electron microscopy grids. (a) Schematic representation of the main components and the meander-type writing pattern. (b) Nozzle positioned above an EM grid (enlarged inset, arrow indicates the nozzle tip) on the xyz-stage. (c) TEM image of a micropatterned grid showing a section of the six 200-300-μm-wide lines of a stained sample (dark grey) diagonally to the EM grid (black) with empty carbon film in between (bright grey). Scale bar, 200 μm.

Publications 2011


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RESEARCH GROUP ERIK VAN NIMWEGEN
Inferring and modeling genome-wide regulatory networks and genome evolution

There are two main lines of research in our group. On the one hand we develop computational and theoretical methods for inferring the structure and dynamics of genome-wide regulatory networks. This work involves the development of probabilistic methods that integrate various high-throughput biological data sets such as genome sequences, genome-wide expression data, genome-wide protein binding data, etc. The other main line of research involves the investigation of whole genome evolution, especially in bacteria, and the identification of quantitative laws that govern genome evolution. Since 2010 the group also has a wet-lab component which focuses on the evolution of genomes and gene regulatory systems in E. coli.

\[
n_c = A_c n_c^{\beta_c} \Leftrightarrow \log(n_c) = \beta_c + \alpha_c \log(n)
\]

**Fig 1:** Scaling laws in the functional composition of bacterial genomes. The numbers of genes in different functional categories grow as power-laws of the total number of genes in the genome. The red, blue, and green dots in the left panel show the estimated numbers of transcription regulators, metabolic genes, and genes involved in translation, as a function of the total number of genes in the genome, with each dot corresponding to a sequenced bacterial genome. Both axes are shown on a logarithmic scale. Such scaling laws are observed for essentially all high-level functional categories. The table on the right shows the inferred scaling exponents for several example categories.

**Laws of Genome Evolution**

Although there is almost 100 years of work in evolutionary theory and mathematical population genetics, it is still exceedingly rare for evolutionary theorists to attempt to develop a quantitative theory for what is actually observed in the ultimate substrates of natural selection: complete genomes. With the advent of whole genome sequencing it has become possible to identify 'laws' of genome evolution not from general theoretical considerations, but empirically, from quantitative analysis of the available genome data. Indeed, over the last decade we and other groups have uncovered a number of quantitative laws in several genomic features including the distributions of evolutionary rates and gene family sizes, and the distribution of genes across different functional categories. In particular, we have found that the numbers of genes in different functional categories grow as power-laws in the total number of genes in the genome, with the scaling exponent varying across categories from close to zero for genes functioning in translation, to approximately quadratic scaling for genes involved in signalling and transcription regulation. Part of the work in our group concerns the development of models explaining the origin of these scaling laws and their implications for organism design and functioning.

**Genome-wide annotation of regulatory sites**

A key interest of our group is the design, functioning, and evolution of the regulatory networks that control genome-wide gene expression patterns. A crucial first step in the reconstructing of regulatory networks is the identification, genome-wide, of the functional binding sites that are recognized by specific regulatory factors. Regulatory factors. To this end we have developed a collection of probabilistic methods that combine comparative sequence analysis with analysis of next-generation sequencing data. For example, our algorithms PhyloGibbs and MotEvo predict regulatory motifs and regulatory sites, respectively, in multiple alignments of orthologous intergenic regions using explicit models of the evolution of regulatory sites. Using comprehensive data of 5' ends of mRNAs (CAGE-tags) from the RIKEN Omics Science Center on 5' ends we have reconstructed the 'promoteromes' of human and mouse and predicted regulatory sites for roughly 200 mammalian regulatory motifs in all proximal promoters. We also developed methods for inferring accurate regulatory motifs from ChIP-seq binding data. All our genome-wide annotations of regulatory sites, in model organisms ranging from E. coli to human and mouse, are available through a easy-to-use web-interface at our SwissRegulon website. Several current projects involve extending and refining these methods and include methods for predicting distal cis-regulatory modules by incorporation of relevant chromatin modification data, extending models of the sequence specificity of regulators to include dependencies between different positions in sites, and methods for taking dynamics changes in and methods for taking dynamics changes in DNA accessibility into account.

**From regulatory sequence to gene expression dynamics**

Currently very little is understood about how constellations of regulatory sites are 'read out' by regulatory proteins to determine the expression levels of target genes. Several of our research projects aim to understand how regulatory signals in DNA determine gene expression dynamics. For example, starting from random DNA sequences we are using inlab evolution to evolve promoters in E. coli and are studying how local sequence determines expression level, condition-dependent regulation, and expression noise in these relatively simple systems.
A major project involving several members of the group is the automated modeling of gene expression dynamics in terms of genomewide predicted regulatory sites. Our main area of example here is cellular differentiation processes in model organisms ranging from flies to human.

The methods that we have developed so far allow us to, starting from genome-wide expression patterns, infer ab initio which regulatory factors are the main drivers of expression changes in a particular regulatory program, infer how these factors change their activities through time, and predict the sets of target genes of each regulatory factor.

Our current work in this area focuses on extending these methods to take into account the interplay between the actions of transcription factors and epi-genetic changes to chromatin state. In particular, we are developing methods for predicting which transcription factors are involved in recruiting specific chromatin changes, analyzing how local chromatin state affects the accessibility of target sites. We are also extending our modeling methods to incorporate the interplay between regulation at the level of transcription initiation and post-transcriptional regulation which is guided by small regulatory RNAs. Our efforts in these area are taking place mainly in the context of the SystemsX.ch CellPlasticity. The goal of Cell Plasticity is to develop a systems-level understanding of the gene regulatory networks responsible for cellular differentiation in mice and involves the systematic tracking of chromatin state and expression dynamics across a number of cellular differentiation systems in mouse.


In the last two years we have intensified our focus on imaging dendritic signals. Several technical advances in voltage sensitive dye imaging – to which the lab has contributed – have made it possible to achieve signal-to-noise ratios that allow the study of virtually all electrical dendritic events at a spatial resolution of sometimes less than 1 μm.

Our results confirm that dendrites can generate extremely complex, localized signals and harness different signaling cascades depending on the exact location within the dendritic tree. We are just beginning to explore the local interaction between excitatory and inhibitory signals, which may provide dendrites with additional highly dynamic signaling capabilities.

Imaging GABAergic signals

Many areas of the brain are built up of large numbers of repetitive, specialized circuits. The characterization of the composition and connectivity of such circuits is crucial for a proper understanding of the function of the central nervous system. We are mostly interested in the function of GABAergic interneurons, which are an integral part of almost all such circuits. In contrast to the rather homogeneous properties of excitatory cells, GABAergic inhibitory interneurons occur in many varieties, characterized by their anatomy, connectivity, physiological and cytochemical characteristics.

Fast synaptic signals from these interneurons are mediated by postsynaptic GABA receptors; ligand-gated ion channels that are chiefly permeant for chloride ions. The effect of their opening therefore depends on the relationship between the chloride reversal potential and the membrane potential of the target neuron. There is considerable uncertainty over the exact chloride concentration in neurons and especially its homogeneity in different compartments.

By using voltage-sensitive dye imaging we were able to demonstrate that neurons can return to a physiological chloride level in a short time after a disturbance. This demonstrates that the neurons possess a surprisingly effective and robust chloride handling system.

We have started to stimulate different inhibitory circuits using extracellular stimulus electrodes and investigated the effect on pyramidal cell dendrites (see Fig. 1). Different patterns of dendritic hyperpolarization could clearly be observed after stimulation. To further refine these experiments we will be stimulating individual identified interneurons, either through patch-clamp recordings or by using optogenetic methods. Optogenetics – the expression of the light sensitive ion channel channelrhodopsin in select neurons greatly simplifies network analysis. We will shortly receive a mouse line in which all interneurons are expressing channelrhodopsin.

Fig. 1: Voltage response of three different CA1 pyramidal cells to different GABAergic inputs. A distal (dist) and proximal (prox) stimulus electrode were placed in the vicinity of three CA1 pyramidal cells. The cells were filled with voltage-sensitive dye and then imaged. The black images show the morphology of the whole cells. The rectangle indicates the area imaged in the functional tests. The two adjacent false-color images show the stimulus-induced change in fluorescence and therefore membrane potential – red colors show strong and blue colors weak hyperpolarization.

Imaging excitatory signals

Excitatory synaptic signals are often accompanied by calcium transients – either through the opening of voltage-gated calcium channels or through calcium-permeant receptors. These calcium signals are important second messengers that can induce synaptic plasticity. To better elucidate the role of different calcium sources during synaptic activity and plasticity we have imaged both calcium signals and the membrane potential in cerebellar Purkinje cells after parallel and climbing fiber stimulation.

We have found that brief bursts of parallel fiber activity can produce supralinear calcium signals. By testing the effect of exogenous calcium buffers we demonstrated that the calcium entering during the burst saturated the endogenous buffer system. This is the first time postsynaptic calcium buffer saturation has been found to play a role in synaptic plasticity.

Characterizing neuronal connectivity through optogenetics

Together with the group of Peter Scheiffele here at the Biocentrum, we are examining an important input into the cerebellum, namely the mossy fiber inputs to the cerebellar cortex. Morphological data indicates a transient connection of mossy fibers and Purkinje cells early in postnatal development. Using selectively labeled mossy fibers we will study the possible functional connection between mossy fiber and Purkinje cells. Preliminary experiments have been conducted to evaluate different methods to express and stimulate channelrhodopsin.
Stem-cell derived neurons and their early development

Functioning neuronal networks depend on a balance between excitation and inhibition. In order to understand this balance and its control we are using stem-cell derived neuronal cultures, in which such a balance is established during the differentiation of the neurons and their network formation. As a first step we characterized the functional properties of developing neurons. We found that their intrinsic signaling capabilities developed gradually and relatively slowly. We observed a transient loss of the resting membrane potential in the first day of invitro differentiation, which then recovered over the next few days in culture. This transient loss parallels a trend in the expression pattern of genes in screens done on the same types of cells in the laboratory of Yves Barde at the Biozentrum.

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For many years transcription factors held the center stage in the regulation of gene expression. This paradigm has changed with the discovery of Piwi-protein-associated small RNAs that regulate gene expression at either transcriptional or post-transcriptional level. Among these, the microRNAs (miRNAs) have initially been discovered in the worm *Caenorhabditis elegans*, but in recent years they have been found in the genomes of organisms as varied as viruses, plants and humans. miRNAs play essential roles in development, metabolism, immune responses, and they can either suppress or enhance specific pathogenic processes such as infections and cancer.

Fig. 1: Location of binding sites of Argonaute 2 and HuR proteins in transcripts of the p27 cell cycle regulator. The binding sites were determined by crosslinking the Argonaute 2 and HuR proteins to mRNAs with UV light, isolation and deep sequencing of RNA fragments that were bound by these proteins. The alignment shows the transcript sequence at the top, with each following track representing a unique sequenced fragment. The number of times each fragment was observed in the sample is indicated on the right of the corresponding track in the alignment. Differences between the sequenced reads and the genomic sequence are indicated by the color boxes. They represent crosslinking-induced mutations.

By combining high-throughput experimental approaches with data analysis and computational modeling, the group of Mihaela Zavolan aims to uncover posttranscriptional regulatory circuits that control cellular differentiation. The vast volumes of data that are obtained with current technologies such as deep sequencing can only be interpreted with the help of computational tools. By developing such tools, the Zavolan group has contributed to the discovery of many miRNAs in animals, as well as in viruses. Because the function of most of the miRNAs that have been discovered is unknown, computational prediction of miRNA targets remains essential for guiding the experiments. The Zavolan group used a comparative genomics approach to develop ElMMo, which is one of the most accurate miRNA target prediction programs currently available. They further studied target sites that are identified based on various types of measurements (evolutionary conservation, mRNA degradation or translational inhibition upon miRNA transfection or depletion) and uncovered several properties that are predictive for functional miRNA target sites. These properties are common to evolutionarily conserved miRNA target sites and to target sites that are associated with the degradation of target mRNAs, indicating that mRNA degradation is a common, important outcome of miRNA-target interaction. One of the most intriguing features of miRNA-dependent regulation is that most miRNAs that carry highly conserved miRNA target sites respond only mildly to changes in miRNA concentrations. It is therefore believed that miRNAs mostly “fine-tune” gene expression. Understanding the mechanisms behind this fine-tuning function is one of the current projects of the group.

Given the still limited understanding of what constitutes a miRNA target, it is important to have an experimental method that allows identification of a large number of miRNA targets in a manner that is as unbiased as possible. The Zavolan group contributed to the development of a photoreactive nucleoside-enhanced crosslinking and immunoprecipitation (CLIP) method that enables isolation of miRNA targets on the basis of their being bound by miRNA-guided Argonaute proteins (collaboration with the group of Tom Tuschl, The Rockefeller University). Through further computational analyses of such Argonaute CLIP data the group aims to uncover miRNA-target interactions that are relevant for the progression of cancers.
Mammalian transcriptomes are extremely complex. Generation of a mature mRNA involves many steps (transcription initiation, splicing, 3’ end processing) that can be independently regulated to give rise to multiple transcripts with different properties. In different phases of their cycle, cells can for e.g. express transcript forms that translate into the same protein, but have different susceptibilities to post-transcriptional regulation. In collaboration with Walter Keller, professor emeritus at the Biozentrum, the Zavolan group is mapping binding sites of 3’ end processing factors transcriptomewide in order to understand what configurations of interactions determine the choice of 3’ end processing site. This study will provide further insights into the interplay between post-transcriptional processes that regulate gene expression.

Much of the work in the Zavolan group is collaborative, involving application of the computational tools developed in the group to various experimental data sets. One example is the study of the function of the embryonically-expressed miR-290 family of miRNAs in mouse development (collaboration with the group of Witek Filipowic, Friedrich Miescher Institute). The study found that miR-290 miRNAs target transcripts of the retinoblastoma-like 2 gene, which encodes a transcriptional repressor of de novo DNA methylases. miR-290 miRNAs thus contribute to the establishment of the appropriate methylation patterns during embryonic development. The focus of the collaboration with the group of Markus Stoffel (ETH Zurich) is the function of miRNAs in metabolism. The study found that miR-375, a pancreas-specific miRNA, has an important function in maintaining a normal mass of the pancreatic alpha- and beta-cells.

Fig. 3: Sketch of the components of the core 3’ end processing complex. By crosslinking and immunoprecipitation of these components together with the RNA fragments that are bound to them, we are attempting to unravel the grammar of 3’ end recognition and processing in mammalian cells.
RESEARCH GROUP MIHAELA ZAVOLAN

Publications 2011

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Publications 2010


During the past decade the importance of light microscopy increased tremendously in all types of biological research. In addition to the image acquisition at various types of microscopes, data handling and image analysis is becoming more and more important.

**Microscopes and support for image analysis is available for all research groups of the Biozentrum**

The Imaging Core Facility (IMCF) provides infrastructure for all microscopy techniques currently used in life sciences. The microscopes in the facility span the entire range from standard wide-field microscopy to confocal microscopy and in the near future) super-resolution microscopy. The newly established facility will start to operate in autumn 2011. Initially there will be 4 point scanning confocal microscopes, 2 spinning disk confocal microscopes, and 2 widefield microscopes available. The aim of the IMCF is not only to provide the facility users with well-maintained modern microscopy systems as well as with theoretical and technical support but also to develop and adapt new microscopy techniques and image analysis routines.

**Areas of interest**

Besides the microscopy service for the Biozentrum, research at the IMCF focuses on the development of (i) optimal system performance in terms of image acquisition speed and experimental flexibility thereby enabling users to do all types of experiments under optimal conditions, (ii) image analysis and automation solutions, and (iii) a data storage and database solution (in collaboration with Research IT).

**Areas of activity**

The IMCF supports projects requiring specific light and fluorescence microscopy setups. Preferably, the IMCF should be involved at every stage: in the planning of a new imaging / microscopy project (selection of fluorescent dyes, choice of microscopy system), during the initial phase (helping with the actual experimental setup including the soft- and hardware settings), and in image analysis and quantification (advice on usage of image analysis software such as Imaris and ImageJ). Only this allows the IMCF to provide the best support at all stages of the project and to adapt the experimental settings to the specific needs.
Specific services and resources
In detail, we will provide the following state of the art microscopy systems for research groups:

Point Scanning Confocal Microscopes
- Inverted Leica SP5 I system with resonance scanner for fast scanning (live cell imaging), multiphoton laser, and incubation chamber.
- Inverted Leica SP5 II system with resonance scanner for fast scanning (live cell imaging), high sensitivity HyD-detectors, Matrix screening software, and incubation chamber.
- Zeiss LSM 700 upright
- Zeiss LSM 700 inverted with incubation chamber for live cell imaging

Spinning Disk Confocal Microscopes
- PerkinElmer Ultraview with dual camera setup for
- 3i Spinning Disk with highly sensitive Photometrics Evolve EM-CCD camera

Wide-field live cell imaging systems (coming soon)
- Applied Precision DeltaVision system

Standard wide-field system
- Leica DM 6000, upright microscope with color camera for histology images

Data visualization and image analysis software
- For 3D-rendering, tracking, and animations of microscopy data: Imaris, Volocity, ImageJ
- For image analysis: Matlab, CellProfiler, In-house plugin or macro development for specific image analysis solutions

Steering committee
To coordinate the Imaging Core Facility activities at the Biozentrum a steering committee has been established in 2011. The steering committee is composed of five group leaders from different focal areas of the Biozentrum that are heavily involved in microscopy, and representatives from Research IT and from the Biozentrum coordination office.

Outlook
Light microscopy is developing at a tremendous pace and several new microscopy techniques have been developed to bypass Abbé’s diffraction limit of light. These new super resolution technologies are either based on structured illumination, nonlinear fluorophore responses, or on the precise localization of single molecules. Depending on the type of method used, it is now possible to improve the maximal resolution from 200nm to values of 50 to 100nm.
Proteomics rapidly evolves from a discovery-oriented technique to a robust and sensitive quantitative tool in biological research to study changes in protein expression and protein modifications in a high-throughput manner.

Proteomics service is available for all research groups of the Biozentrum
The Proteomics Core Facility (PCF) provides infrastructure for the identification and quantification of proteins and their modifications. This includes profound expertise in phosphopeptide enrichment strategies, various platforms for protein and peptide separations, state-of-the-art mass spectrometry (MS) for discovery based MS and LC-MS/MS experiments as well as directed and targeted MS workflows for sensitive and consistent quantitative monitoring of pre-selected sets of proteins. The PCF continuously develops and adapts new sample preparation techniques, MS approaches and software tools to provide optimal analytical services for the individual research groups and their projects.

Fig. 1: Quantitative analysis of the rapamycin-sensitive phosphoproteome by SILAC. A) Two yeast cultures are metabolically labeled with normal or isotopically labeled Lysine and Arginine (heavy culture). The heavy culture is treated for 15 minutes with rapamycin. Cell lysates mixed in a ratio of 1:1 are separated by preparative SDS-PAGE, sliced into horizontal bands and proteins are digested. Phosphopeptides are enriched via IMAC and measured in an LTQOrbitrap. B) Four independent experiments yielded 972 phosphoproteins, corresponding to 2,383 unique phosphopeptides. C) Motif analysis with Motif-X of all downregulated and upregulated phosphopeptide sequences. Motifs are ranked from top to bottom according to their score.

Fig. 2: Global protein profiling workflow. In the first phase of the study (discovery phase), the peptide samples representing different cell states were mixed and analyzed by data-dependent acquisition (DDA) followed by directed one-dimensional LCMS/MS. To achieve comprehensive proteome coverage, all detectable precursor ions, referred to as features, were extracted, sequenced in sequential directed LC-MS/MS analyses and identified by database searching. All identified peptide sequences were stored in a 1D-PeptideAtlas together with their precursor on signal intensity, elution times and mass-to-charge ratio. For each protein, mass and time coordinates from the 5 most suitable peptides (PTPs) for quantification were extracted from the PeptideAtlas and stored in an inclusion list. Additionally, a spectral library was generated from the identified spectra to improve both, the sensitivity and speed of spectral matching in the quantification phase. In this phase (scoring phase), LCMS/MS analysis was focused on the pre-selected PTPs as well as a set of heavy labeled reference peptides that were added to each sample. This determined the concentrations of the corresponding proteins in the sample, which could be used as anchor points to translate the MS-response of each identified protein into its concentration. After spectral matching, label-free quantification was employed to extract and align identified features and monitor their corresponding protein abundances redundantly over all samples.
Areas of interest
Besides the analytical service for the Biozentrum, research at the PCF focuses on the development and application of (i) quantitative phosphoproteomics for tracking complex cellular phosphorylation events (Jenoe lab) and (ii) directed and targeted MS workflows for proteome-wide quantitative studies of microbes and the specific monitoring of proteins and their modifications in complex systems such as human cell lines (Schmidt lab).

Specifically, large quantitative phosphoproteome sets are currently being acquired in yeast, mammalian cells containing specific knockout/knockdown systems, and in surgical biopsies of patients undergoing various clinical treatments. Additionally, directed proteomewide studies of various human pathogens and yeast strains at multiple states are carried out, including time-resolved comparison of proteome and mRNA abundances on a molecules-per-cell level. Furthermore, quantitative datasets of selected sets of proteins involved in mitosis and neuronal synapse formation and synaptic specificity are currently acquired by targeted proteomics.

Areas of activity
The PCF supports projects requiring the identification and quantification of proteins and protein modifications. Preferably, the PCF should be involved at every stage: in the planning of new research projects, during the initial phase and while the project progresses. Only this allows the PCF to provide the best analytical tools at all stages of the project and to adapt the analytical strategies to the specific needs. Furthermore, this facilitates the interpretation of the data and its communication in a user-friendly and plain manner.

Specific services and resources
In detail, we provide the following state of the art MS instrumentation and methods for the research groups:

LC-MS/MS platforms
- High-resolution hybrid LTQ Orbitrap- Velos coupled online to an Easy-nLC system (both from Thermo-Fisher Scientific) for discovery-driven workflows
- TSQ Vantage Triple Stage Quadrupole Mass Spectrometer coupled online to an Easy-nLC system (both from Thermo-Fisher Scientific) for hypothesis-driven workflows using selected-reaction monitoring for protein quantification
- High-resolution hybrid Orbitrap-LTQ (Thermo-Fisher Scientific) coupled online to a nano 1200 LC-system (Agilent) for discovery-driven workflows

Sample preparation and fractionation instruments
- 3100 OFFGEL Fractionsator for peptide separation using isoelectric focusing (Agilent)
- Capillary liquid chromatograph for peptide separation and fractionation (Agilent)

Software
- Database search tools: Mascot, Sequest and XTandem for tandem mass spectra interpretation, also in combination with the trans proteomic pipeline
- Scaffold (Proteome software) for communicating proteomics results in a user-friendly format
- Progenesis LC-MS label-free quantification software (Nonlinear Dynamics)
- MaxQuant for quantification of isotopically labeled samples Skyline and Pinpoint for the generation and analysis of targeted protein quantification experiments of preselected protein sets
- In-house software tools for absolute protein quantification and statistical analysis of large quantitative datasets

Methods
- Protein identification, including posttranslational modifications
- Absolute and differential protein quantification (label-free or isotope labeling-based)
- Enrichment and quantification of phosphopeptides
- Targeted protein quantification by selected reaction monitoring

Steering board
To coordinate Proteomics activities at the Biozentrum an IT steering board was established in 2010. The steering board committee is composed of four group leaders who are strongly committed to proteomics and the two co-directors of the PCF.

Outlook
With the continuing advances in MS instrumentation and methodology, proteomics is well suited to meet the requirements for biological projects on a system-wide level. Recent studies have uncovered the majority of the proteins expressed in human cell lines using state of the art MS approaches as provided by the PCF. Although this clearly marks a major leap forward, we are still far from being able to fully characterize a whole proteome with all its modifications and interactions. However, the consistent screening of microbial proteomes or specific sub-proteomes, like phosphoproteomes of selected pathways, is already possible and it is to be expected that ever-larger parts of biological systems will be accessible for MS in the near future.
PROTEOMICS CORE FACILITY (PCF)

**Group Members**

**Research Associates**
- Dr. Erik Lennart Ahnè
- Dr. Timo Glatter

**Master Student**
- Steve Kunnakatt

**Administrative Office**
- Nadine Iberl

**Technical Associate**
- Suzanne Moes

- **Dr. Alexander Schmidt**
  » further Information

- **Dr. Paul Jenö**
  » further Information
Molecular biology research continues to evolve into a quantitative discipline, relying more and more on large-scale, automated acquisition and subsequent evaluation of biological data. At the same time, bioinformatics tools are commonly used in the laboratory, but often not well integrated. This creates a need to solve complex methodological and IT infrastructure issues, as these questions go beyond the typical scope of a research group. The Research IT technology platform provides consulting and project support for research projects with increased computational demands. Our goal is to accompany projects from their inception to a stable productive solution.

New science and technologies...
Large-scale functional screening: Functional screens of large arrays of compounds, siRNAs, etc. can be performed in medium to high-throughput manner. Read-out data is large and complex to interpret.

Advanced microscopy techniques: Recent advances in imaging technology allow the capture of cellular phenomena resolved in detail along spatial and temporal dimensions. These data easily challenge existing analysis and storage infrastructure.

Massively parallel sequencing technology: Next-generation sequencing generates large volumes of raw and first-pass interpreted data at an unprecedented rate.

Simulation of molecular behavior: For instance, structure-based simulations of protein-ligand interactions yield new candidates for modulators of protein action.

... are creating new challenges.

Rapid increase of data rates: Instrumentation becomes increasingly more automated. The temporal and spatial resolution of data acquisition is increasing. This requires efficient transfer and storage of large acquired data sets.

Rapid increase of data volumes: Acquired data needs to be appropriately stored and backed up to meet disaster recovery and long-time archiving requirements.

High Performance Computing: Analytical processes (image analyses, statistical analyses, simulations, etc.) are computationally intense and routinely applied to large data sets. As large-scale experiments become common-place, HPC resources are no longer only needed only by computational research groups, but by all types of research groups.

Collaboration requires access to integrated data and their transfer
Systems approaches (e.g. SystemsX.ch) emphasize the collaborative nature of projects. This requires timely and reliable access to shared data and their annotation.

Documentation requirements: Persistent annotation of experimental data and metadata about projects and individual experiments. Explore the use of collaborative documentation tools (Wiki, etc)

Areas of interest
Challenges for IT in biology research generally present themselves in three areas: 1) The ability to efficiently store, annotate and retrieve large and rapidly growing biological data sets, 2) to allow biologist users to perform analyses of datasets requiring significant computational power, and 3) satisfying specific application needs by custom software development. We are working together with the Biozentrum’s technology platforms and research groups to address these challenges.

Project support and collaborations
Research IT supports in-house projects with activities related to the above three areas, ranging from infrastructure coordination to software evaluation and development. At best, our involvement begins in the planning phase of new research projects and continues along the project’s progress. The goal is to align tools and infrastructure to the specific project requirements. In particular, we collaborate with the other Technology Platforms (e.g. Proteomics Core Facility, Imaging Core Facility) to capture requirements for data storage, annotation and processing at the point where data sets are generated. As the complexity of projects rapidly exceeds the scope of what can be achieved on a user’s workstation in terms of data storage and processing, Research IT also draws upon the resources and expertise of partners in-house: IT Support
and bioinformatics system administration, of local partners within the university (URZ) and outside, and on a national level (SystemsX.ch SyBIT).

**Services and resources**
Beyond project-based work, Research IT also develops and maintains a portfolio of services as common infrastructure to all research groups. Our projects and services can be divided into three categories:

**Scientific Research Projects** where we actively and specifically collaborate with a research group on tackling a specific research question.

**Research Core Projects** aim to provide tools that are used by more than one research group.

**Core Infrastructure Projects** are projects that are auxiliary in the sense that they enable the functioning of the institute and its groups, or allow us to perform the abovementioned activities in the best possible way.

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**Group Members**

**Research Associates**
Dr. Rainer Pöhlmann
Dr. Vincent Rouilly

**Technical Staff**
Eva Pujadas
The Center for Cell Imaging and Nano Analytics (C-CINA) examines biological specimens at various magnifications. Different types of light and electron microscope are used to produce three-dimensional images of proteins at different magnifications and from various aspects.

**Combined use of different microscopes**
Researchers in the C-CINA use the serial block face scanning electron microscope to determine the rough three-dimensional structure of biological tissues. They then examine individual cells from interesting areas of the specimen using high-resolution electron tomography. And, finally, the atomic structure of individual proteins in the cells can be reconstructed. The microscopes function at different magnifications, ranging from hundreds of micrometers to less than a fraction of a nanometer. The key element of C-CINA’s equipment is the very high resolution microscope called “Titan”. This machine is 4.5 meters high and operated exclusively by computerized remote control.

**Computer image processing gives us insight into 3D**
Two-dimensional images from the various microscopes are collated into three-dimensional data by computer image processing. Just like Google Maps, the computer allows the combination of different magnifications. C-CINA also uses many different methods and types of equipment to prepare biological specimens for examination under the electron microscope.

**Method development at C-CINA**
C-CINA is also active in developing methods for specimen preparation, the microscopy itself, and computer-based evaluation of the images.

C-CINA is active in research into the structure of membrane protein systems and protein complexes, the development of methods for microscopy, and visual proteomics.
Publications 2011


Publications 2010

Schenk, Andreas D; Castaño-Díez, Daniel; Gipson, Bryant; Arheit, Marcel; Zeng, Xiangyan; Stahlberg, Henning (2010). 3D reconstruction from 2D crystal image and diffraction data. Methods in enzymology, 482, 101-29.


Buban, James P; Ramasse, Quentin; Gipson, Bryant; Browning, Nigel D; Stahlberg, Henning (2010). High-resolution low-dose scanning transmission electron microscopy. Journal of electron microscopy, 59(2), 103-12.
The Next Generation Sequencing Core Facility (NGS) is a central research and service facility located in the Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich in Basel, supported and run jointly with the University of Basel and the Friedrich Miescher Institute (FMI) for Biomedical Research. The NGS team provides technical support for next generation sequencing applications in genomics and epigenomics, including high-throughput data management and analysis.

In parallel with the human genome sequencing effort, several new technologies have emerged that allow sequencing at unprecedented throughput and low cost. These technologies are generally referred to as ‘Next Generation Sequencing (NGS)’. They have enabled a large diversity of applications from genome resequencing to identify variations within populations to quantification of mRNA and small RNA expression and the abundance of various epigenetic marks.

In order to take advantage of these powerful technologies, scientists from the Department of Biosystems Science and Engineering (DBSSE), University of Basel (DBM, Biozentrum) and FMI established a NGS unit, which is housed by the D-BSSE. It currently comprises an Illumina GAIIx and a HiSeq2000 sequencing machine as well as storage and a data analysis pipeline.

Chromatin-IP combined with NGS (ChiP-Seq) to identify binding sites of proteins on DNA or specific histone modifications is one type of application frequently making use of the NGS facility. Another comes from the new field of metagenomics that emerged due to the ability to sequence DNA from diverse biological communities in ecosystems or in infectious diseases. Sequencing of hundreds of cancer genomes is yielding an unprecedented wealth of information about how this deadly disease restructures the genome. It has become evident that NGS technologies will revolutionize many areas of biology and medicine.
The LSTF is an academic facility that provides access to microarray and deep-sequencing technologies and contributes to the identification of novel molecular pathways in health and disease.

Until recently, researchers were able to study only single or just few genes related to the biological question they were interested in. Novel genome-wide methods now allow for studying all genes of an organism simultaneously and pave the way towards new discoveries related to the regulation and function of genes. The Life Sciences Training Facility (LSTF) provides researchers in Basel and throughout Switzerland a unique platform to perform their microarray and deepsequencing experiments.

Access to latest DNA microarrays technologies
The methods of DNA microarrays allow researchers to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. The LSTF use the Affymetrix microarray technology to conduct projects in various organisms ranging from worms to humans. These projects have led to the identification of novel genes and molecules, and confirm the notion that the genome is far more complex than originally thought. Indeed, there is a lot to be discovered and understood understood in the context of gene expression and gene regulation.

Novel deep-sequencing methods – fast at no-frills
Recently, the LSTF moved an important step forward and now provides support for deep sequencing. With this new technology it is possible to determine the complete sequence of an organism’s genome, or selected regions thereof, at accessible prices and fast. This method bears a huge potential for new discoveries in biological and biomedical research and can be also used for diagnostic purposes.

With both types of technologies we offer a broad panel of tools to get a complete, genome-wide picture of biological systems. The LSTF is well equipped to narrow the gap between genotype and trait.
The Center for Microscopy (ZMB) of the University of Basel provides electron microscope equipment of every description for research group projects and also plays a key role in education.

The ZMB accepts microscopy commissions from all disciplines in Life Sciences and Medicine, supporting the research groups in their projects. The ZMB also carries out its own research projects to develop and refine methods of preparation, imaging techniques, and image processing software. The most important thing is to keep up with state-of-the-art microscopy and make the necessary new methods available to research.

**ZMB for teaching purposes**

Another of the ZMB’s tasks is training laboratory staff and students on the electron microscopes. We also offer courses for students, so that they can acquire basic knowledge in microscopy. These courses are organized as part of the curriculum in Biology and Nanosciences. The head of the ZMB, Dr. Markus Dürrenberger, has a contract with the Faculty of Philosophy and Natural Sciences, University of Basel, to run the courses.

**History of the Center**

The Center for Microscopy of the University of Basel has been in existence since September 2001. It resulted from the merger of the Biozentrum’s Interdepartmental Electron Microscopy (IEM) unit and the Scanning Electron Microscope (SEM) Laboratory of the University of Basel, at the time when Prof. Richard Guggenheim was appointed emeritus professor. The goal of the merger was to create a central platform for services related to electron microscopy, to be provided to researchers as well as for teaching purposes.

Administration of the ZMB is integrated into the University of Basel’s Biozentrum. The president of the ZMB Users’ Board, the professor of Microscopy at the Biozentrum, is at the same time the scientific director of the ZMB. Prof. Ueli Aebi of the Biozentrum’s M.E. Müller Institute was the scientific director until 2010. Prof. Henning Stahlberg, the successor to Prof. Andreas Engel, was appointed head of the CCINA and awarded the chair in Microscopy at the Biozentrum in 2010. He is currently the Scientific Director of the ZMB and President of the Users’ Board.
MANAGEMENT, ADMINISTRATION & SERVICES

**Directorate**
Erich Nigg (Director)
Urs Jenal (First Deputy)
Peter Scheiffele (Second Deputy)
Bettina Delbridge (Secretary to the Director)

**Administrative Divisions**
Marion Bollmann (Head Fi&HR since 01.11.11)
Monika Gessler (Head Scientific Affairs)
Roger Jenni (Head Operation & Logistics)
Urs Fürstenberger (Head Fi&HR until 31.10.11)

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Jessica Berchtold
Urs Berglas
Jérôme Bürki
Monika Furrer
Roland Geiser
Patricia Lanzi
Torsten Stauffer
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Werner Indlekofer (Webmaster)
Heike Sacher

**Electronics Workshop**
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Paul Henz

**Facility Services**
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Bruno Marioni
David Schaub
Christine Widmer (Room Coordination)

**Floor Staff**
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