BIOZENTRUM

Universität Basel
The Center for
Molecular Life Sciences

Annual Report 2012

Biozentrum

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BIOZENTRUM ANNUAL REPORT 2012

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RESEARCH AT A GLANCE

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.

COMPUTATIONAL & SYSTEMS BIOLOGY

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.

GROWTH & DEVELOPMENT

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.

INFECTION BIOLOGY

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 host-microbe 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 investigate host-pathogen interactions and collaborate closely with their colleagues from other research areas, in particular from Structural Biology & Biophysics and Computational & Systems Biology.

NEUROBIOLOGY

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.

STRUCTURAL BIOLOGY & BIOPHYSICS

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.

RESEARCH GROUP MARKUS AFFOLTER

Cell signalling and cell rearrangement during organ morphogenesis

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 a 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 socalled 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.

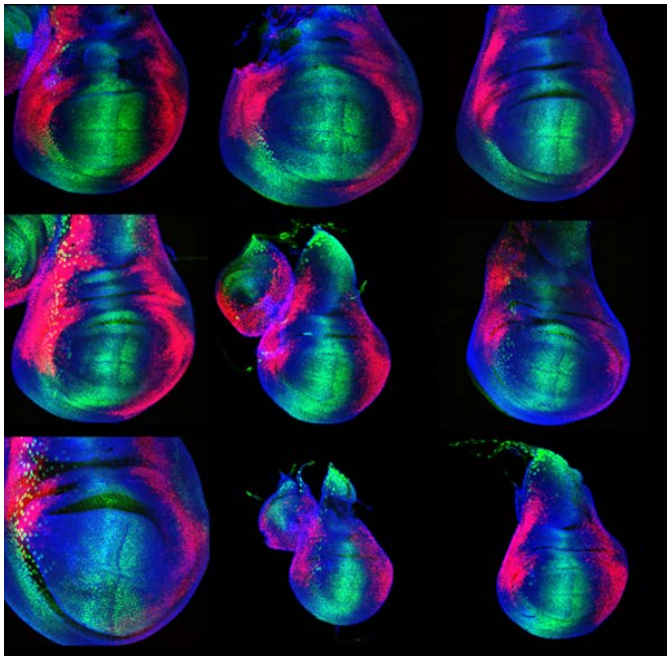

Fig. 1: Dpp gradient readout in wing imaginal discs. Wing imaginal discs of different sizes showing Brinker protein expression (red) and pMad distribution (green). Note that the pMad domain increases in size with increasing disc size, and thus scales with tissue size.

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.

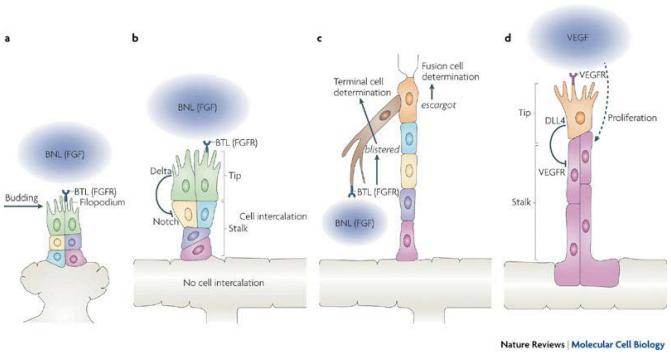

Fig. 2: Drosophila melanogaster trachea and vertebrate vasculature branching. Branchless (BNL), a fibroblast growth factor (FGF), acts at the top of the hierarchy of cellular events that orchestrate tracheal branching in Drosophila melanogaster (a to c). During vertebrate angiogenesis, vascular endothelial 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.

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 re-define 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 (*Fig. 3*). 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.

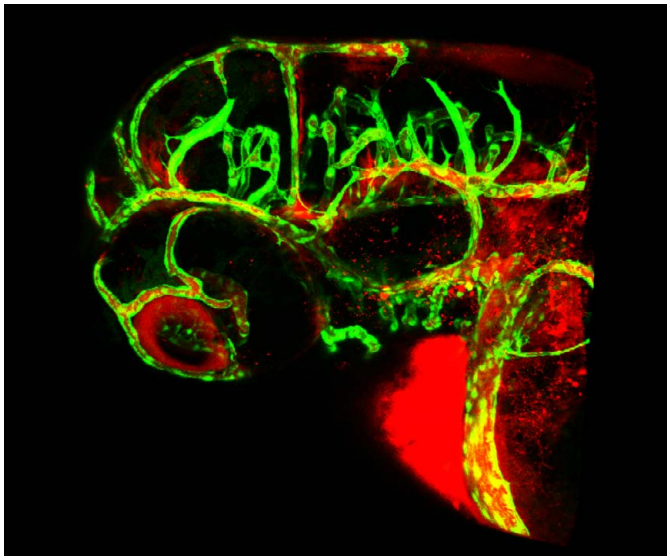

Fig. 3: Blood vessels in the zebrafish embryo. Blood vessels express Green Fluorescent Protein.

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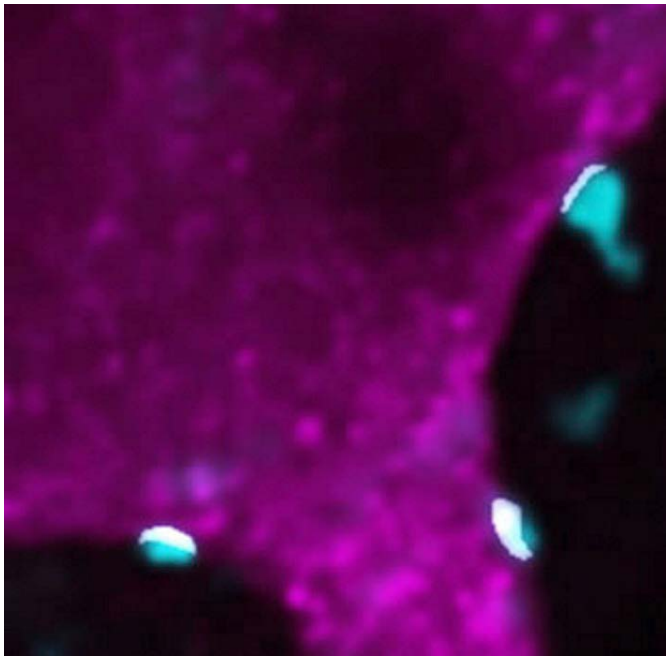
RESEARCH GROUP SILVIA ARBER

Function and assembly of motor circuits

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 multifacetted approaches combining many technologies. These include stateof- 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.



Three-dimensional reconstruction of synapses (turquoise) establishing contact (white) with motor neuron (purple) in the spinal cord.

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.

RESEARCH GROUP SILVIA ARBER

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RESEARCH GROUP CÉCILE ARRIEUMERLOU

Host signaling during bacterial infection of epithelial cells

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-mesodiaminopimelic 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 signal-

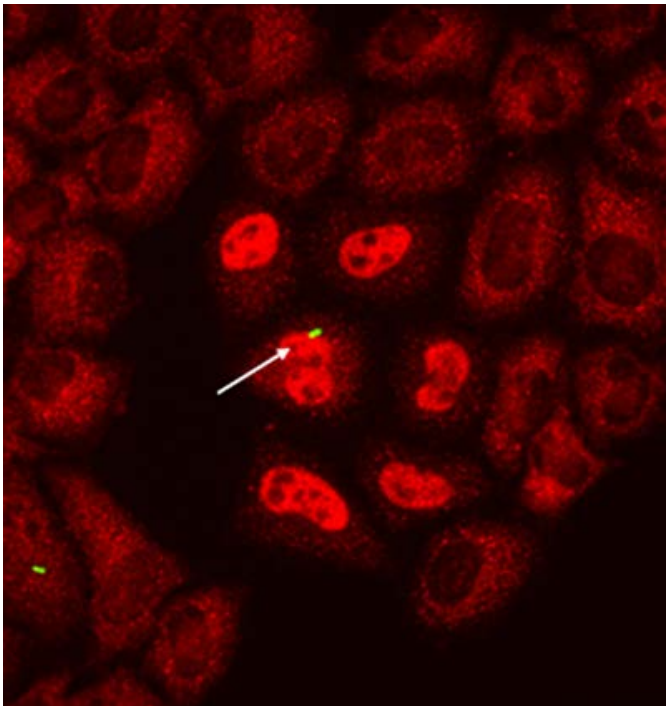

Fig. 1: NF- κB is activated in bystander cells of Shigella flexneri infection. Bacteria are in green, NF- κB p65 in red; White arrow indicates an infected epithelial cell.

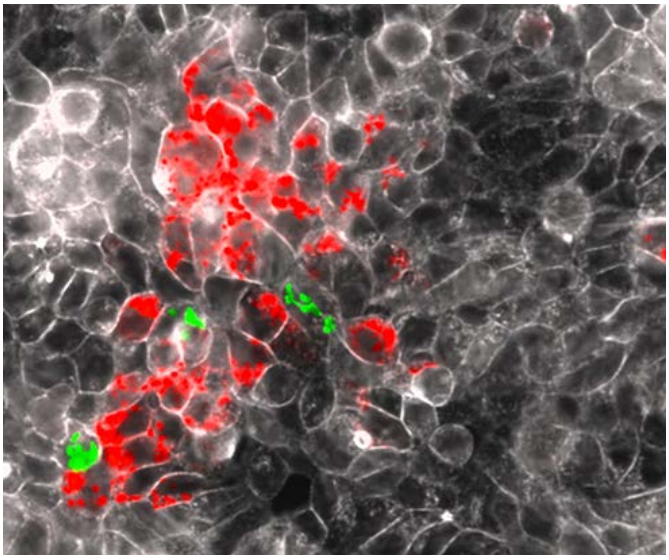

Fig. 2: IL-8 is produced by bystandercells of Shigella flexneri infection. Bacteria are in green, IL-8 in red, F-actin in gray.

ing 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.

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RESEARCH GROUP YVES-ALAIN BARDE

Stem cells and neural development in health and disease

Much of our work focuses on a small family of signaling molecules designated neurotrophins. These growth factors have been identified in the genome of long-lived species, but not in short-lived invertebrates typically used by geneticists. This is unusual and explains why forward genetics could not contribute to this field of research. Long-lived species may benefit from the multiple roles played by neurotrophins in adaptive phenomena, such as the ability of an organism to detect, store and interpret external stimuli, including those that are potentially damaging.

Neurotrophins in health and disease

The neurotrophin brain-derived neurotrophic factor (BDNF) is encoded by a gene that is positively regulated by neuronal activity. Our research concentrates on BDNF as together with its receptor, it is much more widely expressed in the central nervous system than the other genes of the family. In collaboration with the group of Michael Frotscher (then in Freiburg, Germany) we could show that BDNF is stored in pre-synaptic, large dense core vesicles. When activity is chronically increased as observed in a mouse mutant developing epileptic seizures in the adult, BDNF is expressed at much higher levels than in control animals, while its processing and sub-cellular distribution remain as in wild-type animals (see Dieni et al., 2012). Remarkably, the brain of these animals markedly increases in size, in parallel with a postnatal increase in BDNF levels. As we previously showed that conversely, the lack of BDNF leads to smaller adult brains (Rauskolb et al., 2010), we are now exploring how BDNF regulates brain growth. In a mouse model of Rett syndrome, the levels of BDNF are decreased in parallel with a reduction in brain size and we found that BDNF levels can be restored close to normal following the administration of fingolimod (Deogracias et al., 2012). This treatment also increases the size of some of the brain areas affected by the lack of the gene causing Rett syndrome, *MECP*$_2$ (Deogracias et al., 2012). Fingolimod is a drug of special interest as it has been recently introduced for the treatment of multiple sclerosis. It is closely related to the endogenous lipid sphingosine and diffuses into the brain. Our work suggests that it activates receptors present on brain neurons, leading to an increase synaptic activity which in turns increases BDNF levels. We hope that beyond Rett syndrome, these results will also be useful in the context of other diseases of the nervous system that may be ameliorated by increasing BDNF levels.

Embryonic stem cells

The discovery that mouse ES cells can be used to generate essentially pure populations of neurons has greatly facilitated the understanding of genes expressed in neurons. Some of these genes have been very difficult to study in the past given the lack of relevant cell culture systems. The uniform population of Pax6-positive radial glial cells generated by our

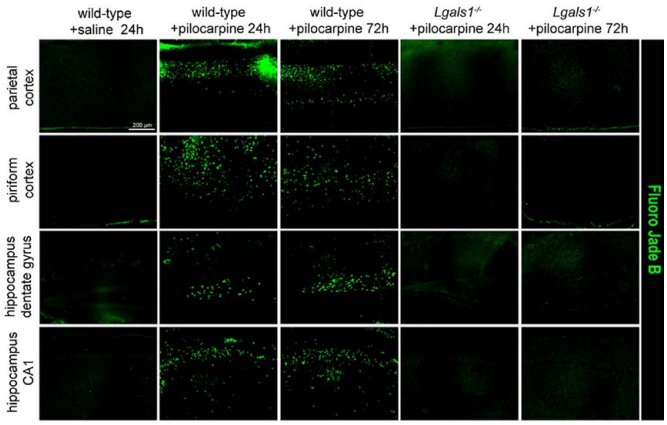

Fig. 1: Fluoro Jade B labels dying cells (green dots) following ilocarpine injection. Pilocarpine is a drug that induces seizure episodes and causes the death of neurons. (Note that in animals lacking the Galectin-1 gene (Lgal1 -/-), pilocarpine fails to cause cell death even though it also induces seizure like in wild-type animals. For additional details see Bischoff et al. 2012 in Publications.)

method under well-defined tissue culture conditions goes on to generate glutamatergic neurons with the functional characteristics of the brain neurons. This extraordinarily powerful system allowed us to uncover new roles for the transcription factor Pax6 (Nikoletopoulou et al., 2007), the amyloid precursor protein APP (Schrenk-Siemens et al., 2008), the neurotrophin receptors p75 (Plachta et al., 2007), TrkA and TrkC (Nikoletopoulou et al., 2010) and *MECP*$_2$. The gene most frequently mutated in Rett syndrome (Yazdani et al., 2012, see Publications for detailed accounts of these findings). This system also allowed the discovery of novel downstream targets of p75 including the endogenous lectin Galectin-1, which turned out to play an essential role in seizure-induced neuronal death in vivo (Bischoff et al., 2012).

RESEARCH GROUP YVES-ALAIN BARDE

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RESEARCH GROUP ATTILA BECSKEI

Epigenetic control and evolutionary optimization of cell decisions

Cell differentiation can be achieved by specifying cell fate through deterministic instructive signals or by stochastic transitions to various epigenetic states. 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 defense. 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 (*Fig. 1*). The logic behind chromosomal epigenetic processes has been unclear. Our recent work unraveled spatial aspects in control of silencing in yeast cells (Kelemen et. al. (2010), PLoS Biology). 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 (Hsu et al. (2012) Nature Communications). During evolution, genetic networks are rewired through strengthening or weakening their interactions to develop new regulatory schemes. In the galactose network, the GAL1/GAL3 paralogues and the GAL2 gene enhance their own expression mediated by the Gal4p transcriptional activator. The wiring strength in these feedback loops is set by the number of Gal4p binding sites. Here we show using synthetic circuits that multiplying the binding sites increases the expression of a gene under the direct control of an activator, but this enhancement is not fed back in the circuit. The feedback loops are rather activated by genes that have frequent stochastic bursts and fast RNA decay rates (*Fig. 2*). In this way, rapid adaptation to galactose can be triggered even by weakly expressed genes. Our results indicate that nonlinear stochastic transcriptional responses enable feedback loops to function autonomously, or contrary to what is dictated by the strength of interactions enclosing the circuit.

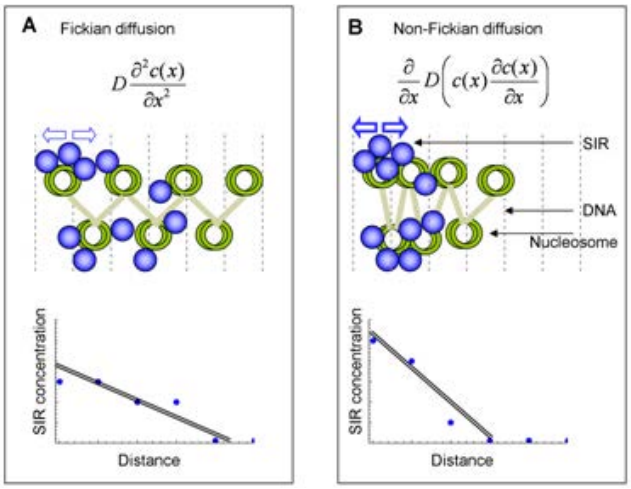

Fig. 1: Diffusion of silencing proteins (SIR) along the chromatin (Transcription, 2011).

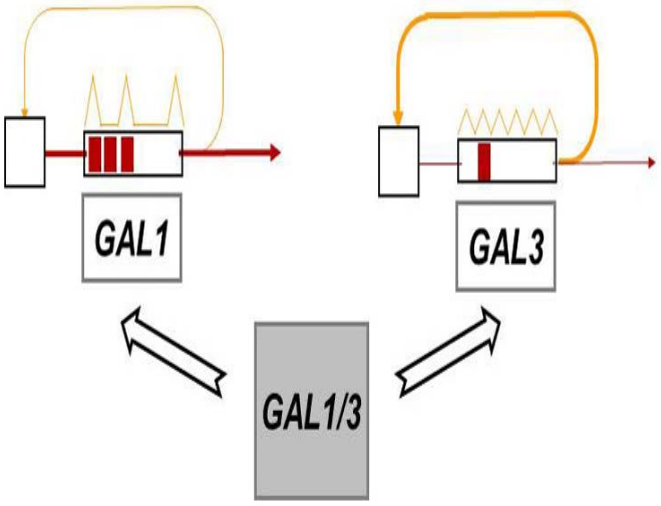

Fig. 2: The number of activator binding sites determines the strength of direct response of the gene (red arrows), while stochastic gene expression determines the activation of feedback response (orange arrows).

RESEARCH GROUP ATTILA BECSKEI

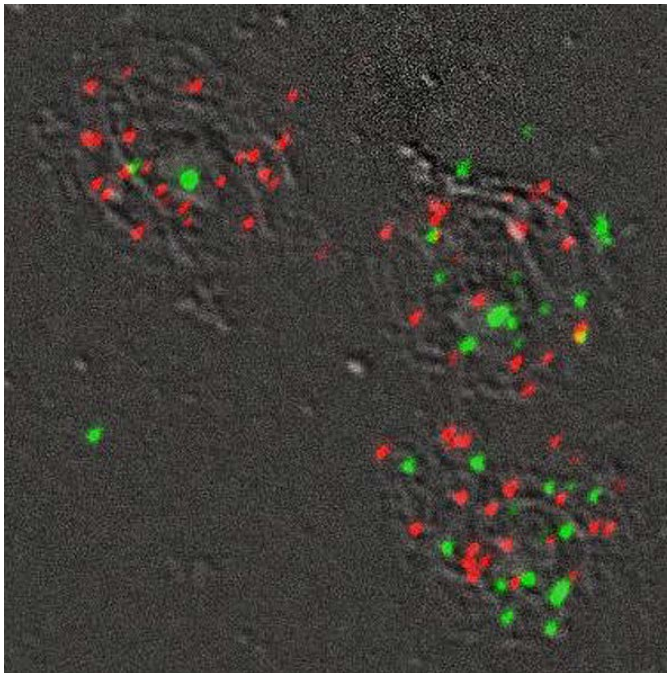

Fig. 3: Single molecule detection of mRNAs by FISH. GAL3 mRNA (red), ILS1 mRNA (green).

Since the decay rate of RNAs is fast, measurements of gene expression in the high-frequency domain is necessary. Therefore, we have started measuring stochastic gene expression at the level of single molecule RNAs (*Fig. 3*).

Publications 2012

Hsu, Chieh; Scherrer, Simone; Buetti-Dinh, Antoine; Ratna, Prasuna; Pizzolato, Julia; Jaquet, Vincent; Becskei, Attila (2012). Stochastic signalling rewires the interaction map of a multiple feedback network during yeast evolution. *Nature Communications, 3, 682.*

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RESEARCH GROUP SIMON BERNÈCHE

Structure-function relationship of ion channels and membrane transporters

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 hierarchal 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.

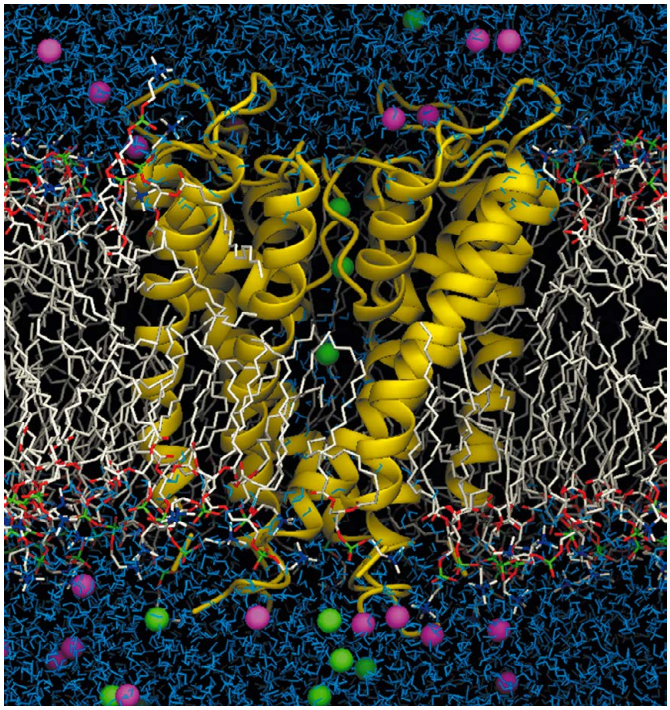

Fig. 1: Molecular graphics representation of the atomic model of the KcsA K+ channel embedded in an explicit DPPC phospholipid membrane bathed by a 150 mM KCl aqueous salt solution.

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). To 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.

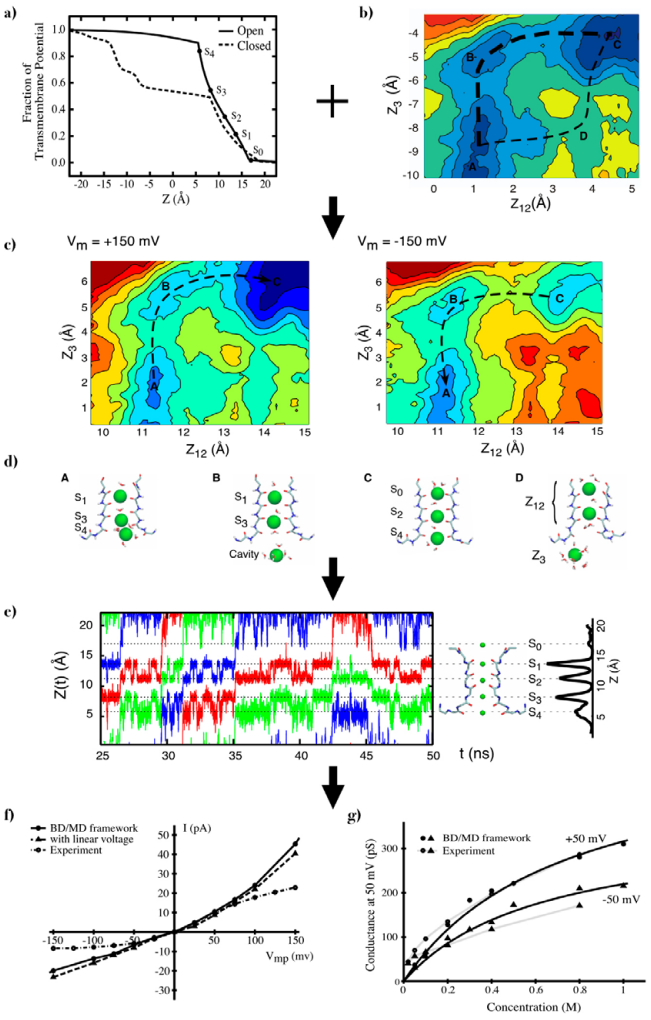

Fig. 2: Stochastic simulation framework a) Transmembrane potential profile along the pore of the KcsA channel b) Equilibrium PMF describing ion permeation in the selectivity filter of KcsA. Each color level corresponds to a free energy of 1 kcal/mol. c) The total multi-ion free-energy profile Wtot(Z1,Z2,Z3) 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).

RESEARCH GROUP SIMONE BERNÈCHE

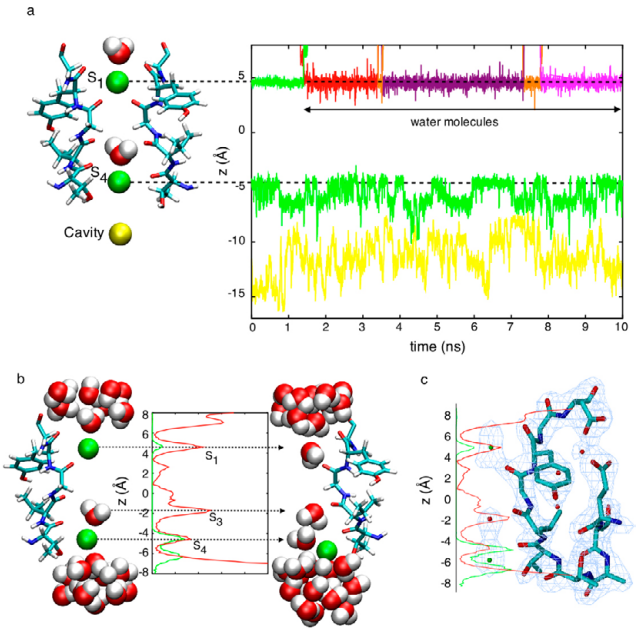

Fig. 3: Simulation of a putatively inactivated K^+ channel with K^+ ions (green spheres) in binding sites S1 and S4 and a Na^+ ion (yellow sphere) in the cavity. a) The time series analysis shows that the K^+ ion in S1 (green line) leaves the selectivity filter after about 250 ps of simulation and is 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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RESEARCH GROUP DIRK BUMANN

Systems biology of *Salmonella* and *Shigella* infection

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 can not 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.

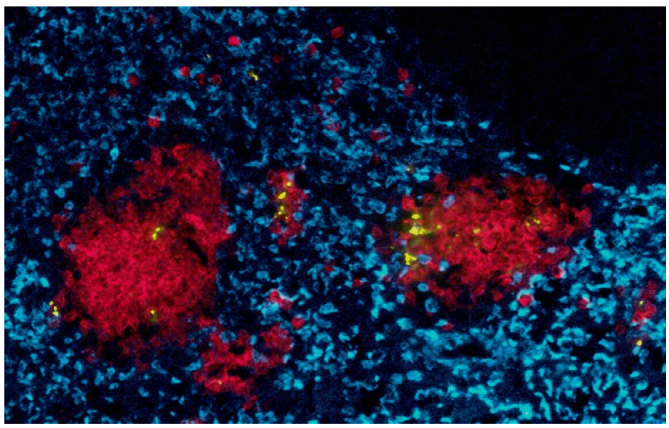

Fig. 1: Salmonella-infected mouse spleen (yellow, Salmonella; red, neutrophils; blue, red blood cells).

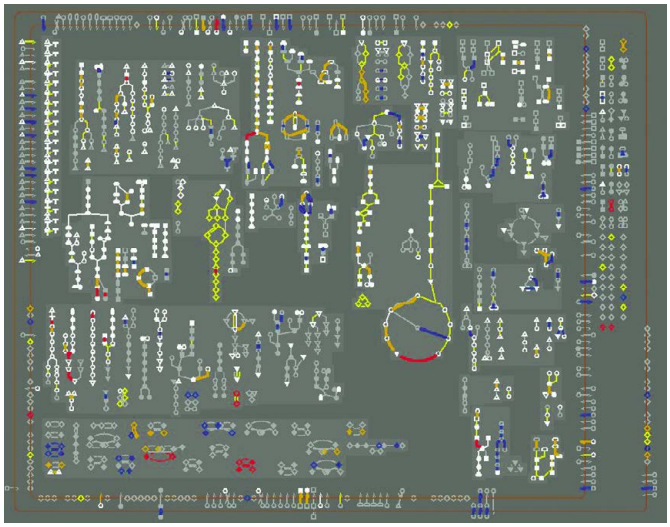

Fig. 2: Schematic overview of Salmonella metabolism during infection. Enzymes with detectable in vivo expression (yellow) and enzymes with experimentally determined relevance for virulence (red, essential; orange, contributing; blue, dispensable) are shown.

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 opportunites 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 differ-

ential localization of *Salmonella* 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 systemlevel analysis. Current results suggest dramatic differences in stress exposure and growth rate in *Salmonella* subpopulations.

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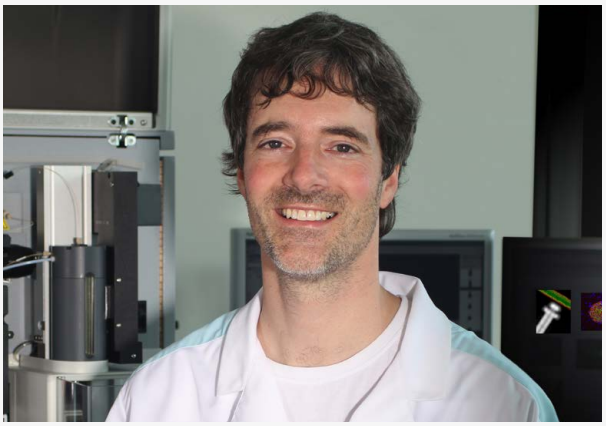

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Molecular and cellular mechanism of asymmetric stem cell division

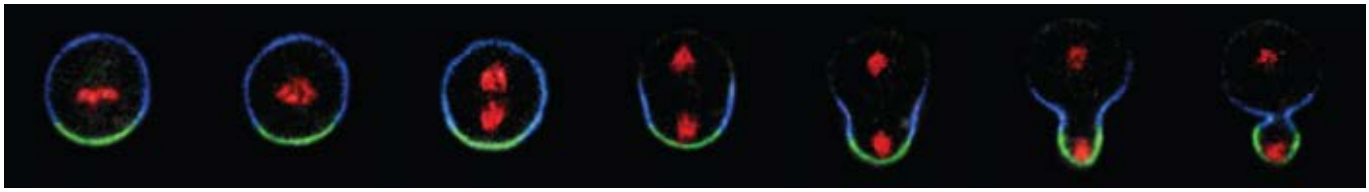

Fig. 1: Asymmetric cell division generates molecularly and physically distinct siblings.

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.

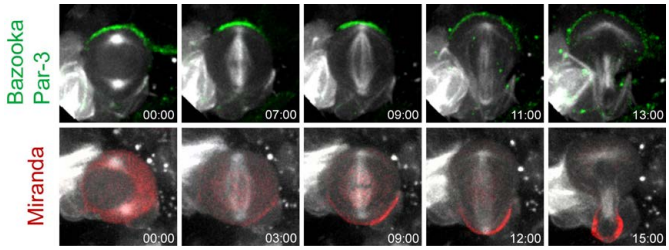

Fig. 2: Neuroblast spindles are aligned along the cell intrinsic polarity axis.

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 intrinsic 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.

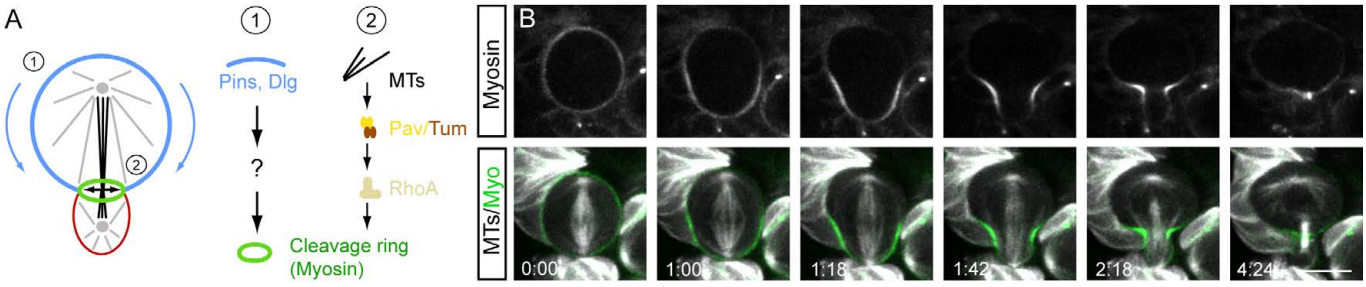

Fig. 3 Two pathways provide positional information for the placement of the cleavage furrow.

RESEARCH GROUP CLEMENS CABERNARD

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. microtubule-dependent cue
2. polarity derived signal

The novel polarity-dependent cleavage furrow positioning pathway is utilizing the two conserved polarity components Pins and Discs large (Dlg) (*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 2012

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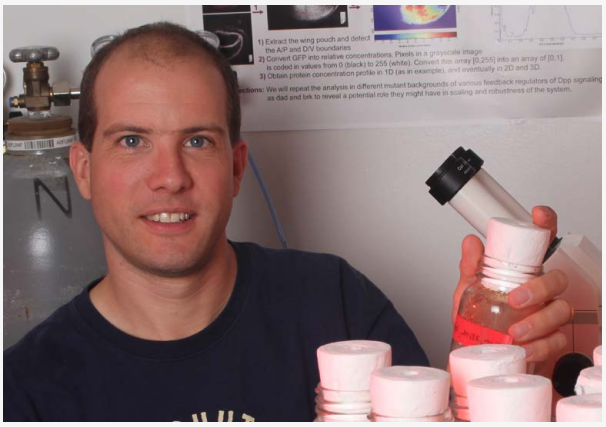

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RESEARCH GROUP GUY R. CORNELIS

Study of the type III secretion injectisome from *Yersinia* and the pathogenesis of *Capnocytophaga canimorsus*

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

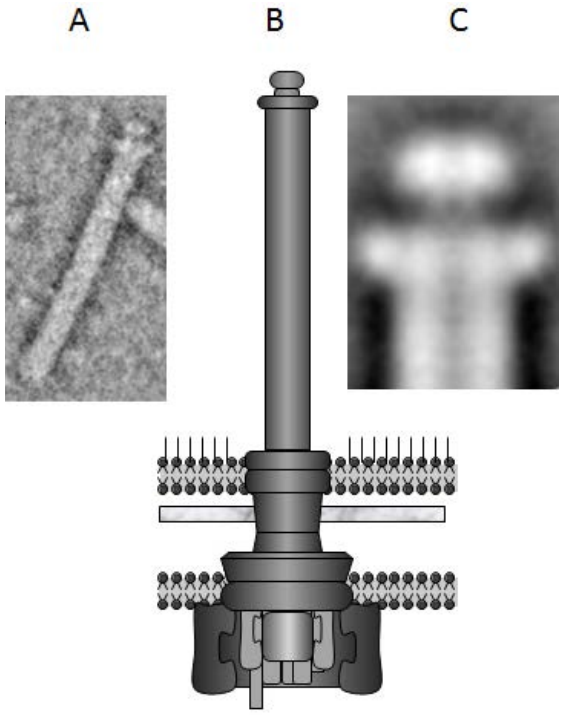

Fig. 1: The type III secretion injectisome. (A) Scanning-Transmission Electron Microscopy images of negatively stained needles (CA Mueller et al., Science 310:674-6, 2005); (B) Schematic representation of the whole apparatus, inserted into the outermembrane (top), the peptidoglycan layer (center) and the plasma membrane (below). The lower part of the basal body is located in the cytosol while the needle protrudes outside from the bacterium. The needle terminates with a "tip"structure (C) Projection averages of the tip structures (resolution 1.5 nm; ref as in A).

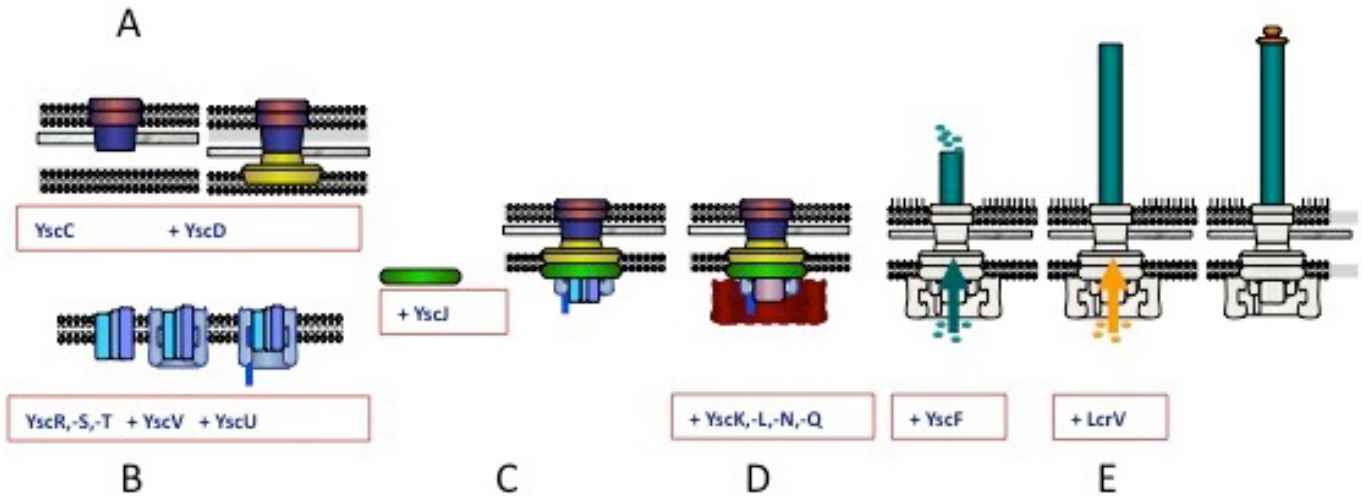

Fig. 2: Model of assembly of the Yersinia injectisome (Diepold et al., EMBO J29:1928-40; 2010 and Diepold et al., Mol. Microbiol, in press).

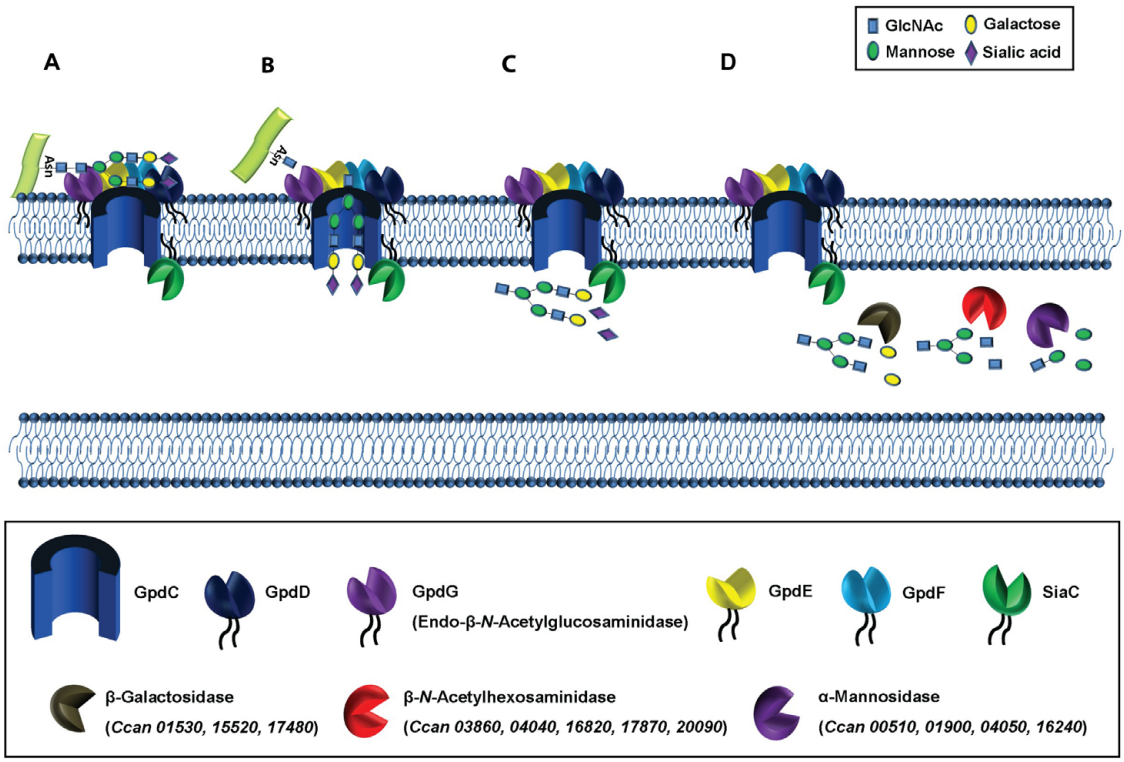

Fig. 3: Functional model of complex N-linked glycan moieties deglycosylation processing by C. canimorsus. Individual glycan processing steps are illustrated. (A) The glycan moiety is bound at the bacterial surface by the Gpd complex. (B) The glycan moiety is endo-cleaved by GpdG and imported into the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

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 tethaiotaomicron*. 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.

Prof. Dr. Guy Cornelis retired in summer 2012. His group has gradually decreased.

RESEARCH GROUP GUY R. CORNELIS

Publications 2012

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Prof. Dr. Guy R. Cornelis

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RESEARCH GROUP CHRISTOPH DEHIO

Role of type IV secretion systems in the establishment of persistent bacterial infections

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.; and Harms and Dehio, 2012, Clin. Microbiol. Rev.). 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 multi-disciplinary 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.

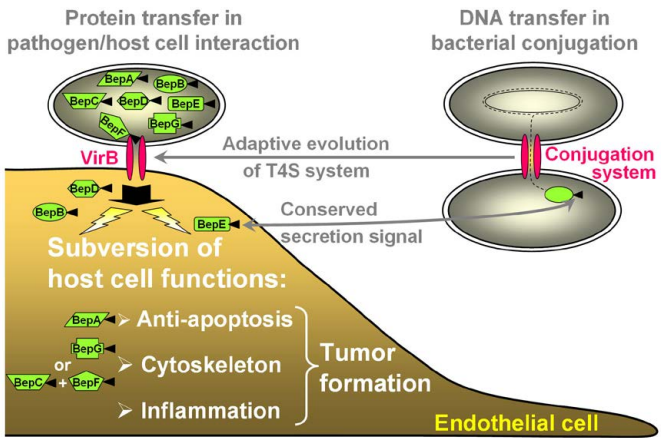

Fig. 1: Effector proteins translocated by the Bartonella T4S systemVirB/VirD4 subvert human endothelial cell functions.

T4S systems play diverse roles in *Bartonella*-host interaction: They are essential for establishing persistent infection and contribute to host adaptability

A functional and comparative genomics approach allowed us to demonstrate that both the VirB and Trw T4S systems of *Bartonella* represent essential virulence factors for establishing persistent infection in mammals. Further, these virulence devices must have played major roles during evolution in facilitating adaptation of these pathogens to their specific mammalian reservoirs (Saenz et al., 2007, Nat. Genet.; Engel et al., 2011, PLoS Genetics). Genetic and cell biological analysis of Trw has shown that this T4S system mediates the host-restricted adhesion to erythrocytes (Vayssier et al., 2010). Important to note, during adoption of this dedicated role in host interaction this T4S system has lost its ancestral substrate transfer capability. In contrast, we have recently shown that the VirB T4S is capable of translocating DNA into endothelial target host cells in a process similar to the interbacterial DNA transfer mediated by the ancestral conjugation systems (Schroeder et al., 2011, PNAS; reviewed in Llosa et al., 2012, Trends Microbiol.). However, the physiological role of the VirB T4S system is to translocate a cocktail of *Bartonella* effector proteins (Beps) into vascular endothelial cells that subvert cellular functions to the benefit of the pathogen (Schulein et al., 2005, PNAS). A recent evolutionary genomics study allowed us to propose that the horizontally acquired VirB T4S system and its translocated Bep effectors facilitated adaptations to novel hosts via two parallel adaptive radiations (Engel et al., 2011, PLoS Genet.). We showed that the functional versatility and adaptive potential of the VirB T4S system evolved convergently – prior to the radiations – by consecutive rounds of lineage-specific gene duplication events followed by functional diversification. This resulted in two diverse arrays of Bep effector proteins in the two radiating lineages of *Bartonella*. Together, we established *Bartonella* as a bacterial paradigm of adaptive radiation, allowing for the first time to study the molecular and evolutionary basis of this fundamental evolutionary process for the generation of organismic diversity in bacteria.

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 F-actindependent invasome structure and proinflammatory activation (Schulein et al., 2005, PNAS). Defining the cellular targets and molecular mechanisms of how these Beps interfere with eukaryotic signaling processes have become a focus of our recent studies. The C-terminal parts of the Beps carry the Bep intracellular delivery (BID) domain that serves as T4S signal, but has in several instances adopted additional effector function within

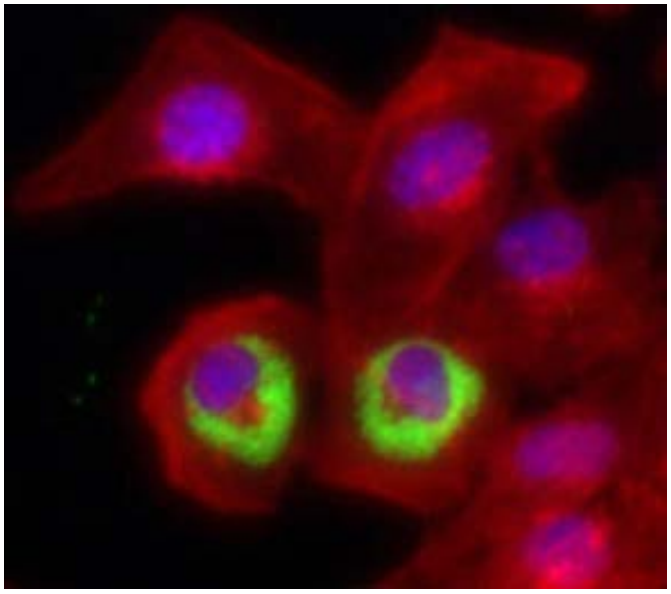

Fig. 2: Hela cells infected with GFP-expressing Brucella abortus (green) for 48 h and stained for F-actin (red) and DNA (blue). Intracellular bacteria replicate in an endoplasic reticulum-associated compartment localizing proximal to the nucleus.

host cells. A prominent example is the BID domain of BepA that binds adenylate cyclase to potentiate $G\alpha_s$dependent cAMP production, which leads to inhibition of apoptosis in vascular endothelial cells (Pulliainen et al., 2012, PNAS). The N-terminal parts of the Beps carry diverse domains or peptide motifs considered to mediate effector functions within host cells. For instance, upon translocation the effectors BepD, BepE and BepF become tyrosinephosphorylated on short N-terminal repeat motifs, thereby interfering with eukaryotic signal transduction processes (Selbach et al., 2009, Cell Host & Microbe). Together with the Schirmer group (Biozentrum) we study the structure/function relationship of the Fic domains that are present in the N-terminus of multiple Beps and mediate posttranslational modifications of specific host target proteins via covalent transfer of AMP (AMPylation) (Palanivelu et al., 2011, Protein Sci.). A particular focus of these studies is the identification of target proteins and the regulation of the AMPylation activity, i.e. via binding of the Fic domain to an inhibitory protein termed antitoxin (Engel et al., 2012, Nature).

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

The goal of InfectX (www.infectx.ch) – a 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 into 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 (reviewed in Eicher and Dehio, 2012, Cell. Microbiol.). 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 F-actin organizing complex Arp2/3 (Rhomberg et al., 2009, Cell Microbiol.; Truttmann et al., 2011, Cell Microbiol.) and bi-directional 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 anti-infectives that interfere with the function of host proteins essential for pathogen infection.

RESEARCH GROUP CHRISTOPH DEHIO

Publications 2012

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RESEARCH GROUP STEPHAN GRZESIEK

Nuclear magnetic resonance spectroscopy of biomolecules

We apply and develop high-resolution Nuclear Magnetic Resonance (NMR) methods to elucidate 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; bacterial PilZ domains, which are targets for signaling via cyclic di-GMP; endotoxin, the causative agent of endotoxic shock; cadherins; 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 characterizing the interaction of the soluble protein RANTES with peptides derived from CCR5 and 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.

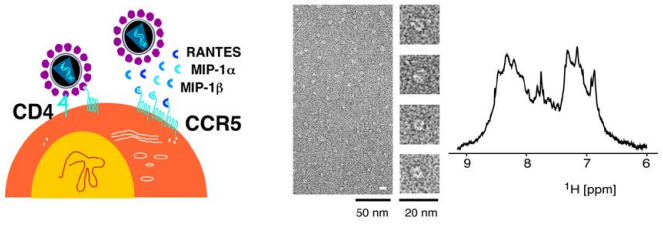

Fig. 1: Left: docking of HIV-1 to the receptors CD4 and CCR5 on T-cell surface. Middle: electron micrographshowing detergent micellescontaining CCR5. Right: 1H-15N spectrum of CCR5 in detergent micelles [Wiktor et al. J. Biomol. NMR 2012 Dec 11; Van den Bergh et al. PLoS One. 2012;7:e35074; Nisius et al. Protein Expr Purif 61, 155 (2008)].

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. 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. NMR data have also helped to elucidate the mechanism of a new class of allosteric CML inhibitors discovered by the group of Prof. N. Gray (Harvard), which presents new therapeutic opportunities via a novel mechanism of action.

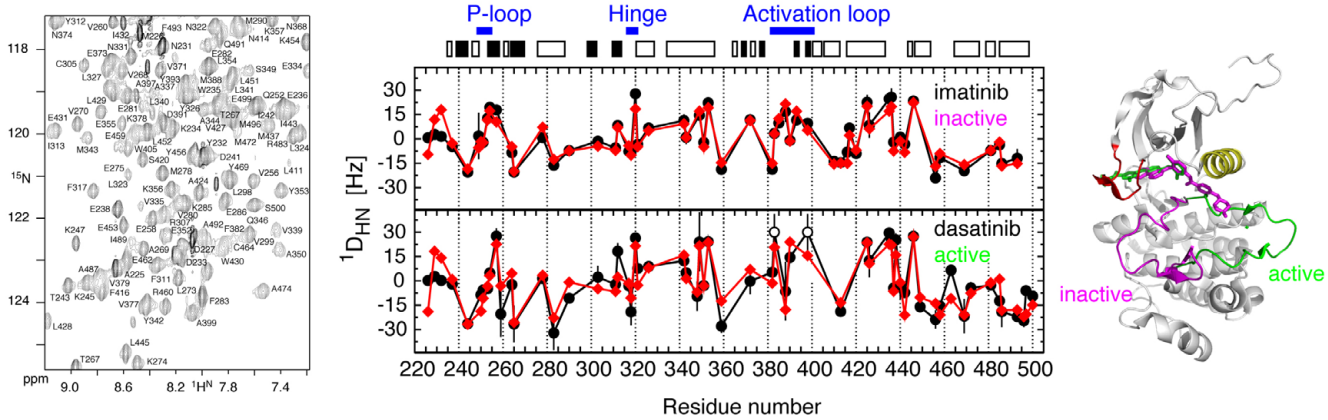

Fig. 2: NMR analysis of Ablkinase, the key proteinresponsible for thedevelopment of chronicmyelogeneous leukemia. Left:part of assigned 1H-15Nspectrum. Middle: detected(black) and predicted (red)RDCs for imatinib- anddasatinib-bound forms. Right: inactive (imatinib) and active(dasatinib) solutionconformations of activationloop derived from RDC data [Vajpai, N. et al. J Biol Chem 283, 18292 (2008); Zhang, J. et al. Nature 463, 501 (2010)].

c-di-GMP signaling

Cyclic di-guanosinemonophosphate (c-di-GMP) is a bacterial signaling molecule that triggers a switch from motile to sessile bacterial lifestyles. This mechanism is of considerable pharmaceutical interest, since it is related to bacterial virulence, biofilm formation and persistence of infection. Understanding this mechanism may offer new routes to treatment of bacterial infections. We have recently solved the structure of the PilZ homolog PA4608 in complex with cyclic di-GMP. This complex shows large structural changes relative to the apo form. As a result of the rearrangements of N- and C-termini, a highly negative surface is created on one side of the protein complex. We propose that this movement of the termini and the resulting negative surface forms the basis for downstream signaling. We have recently also determined the exchange kinetics and equilibrium constants of various oligomeric forms of c-di- GMP. These projects are in collaboration with the groups of Prof. Urs Jenal and Prof. Tilman Schirmer (Biozentrum).

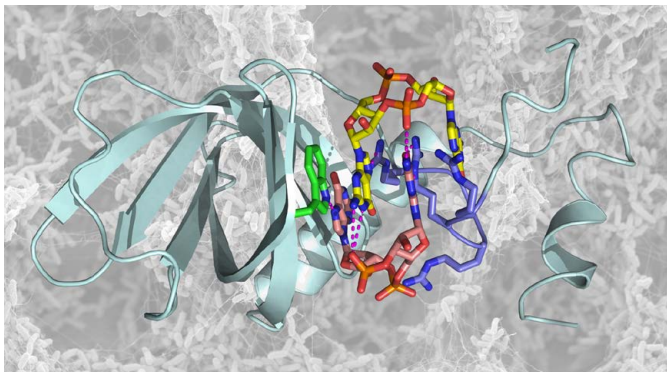

Fig. 3: NMR structure of the cyclic di-GMP receptor PA4608 from Pseudomonas aeruginosa in complex with its cyclic di-GMP ligand on the background of a typical bacterial biofilm [Habazettl et al. J Biol Chem 286, 14304 (2011); Gentner et al. J Am Chem Soc 134, 1019 (2012)].

Lipopolysaccharide (LPS, endotoxin)

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.

Key stabilizing elements of protein structure identified through pressure and temperature perturbation of its hydrogen bond network

Hydrogen bonds are key constituents of biomolecular structures, and their response to external perturbations may reveal important insights about the most stable components of a structure. NMR spectroscopy can probe hydrogen bond deformations at very high resolution through hydrogen bond scalar couplings (HBCs). However, the small size of HBCs has so far prevented a comprehensive quantitative characterization of protein hydrogen bonds as a function of the basic thermodynamic parameters of pressure and temperature. Using a newly developed pressure cell, we have now mapped pressure- and temperature-dependent changes of 31 hydrogen bonds in ubiquitin by measuring HBCs with very high precision. Short-range hydrogen bonds are only moderately perturbed, but many hydrogen bonds with large sequence separations (high contact order) show greater changes. In contrast, other high-contact-order hydrogen bonds remain virtually unaffected. The specific stabilization of such topologically important connections may present a general principle with which to achieve protein stability and to preserve structural integrity during protein function.

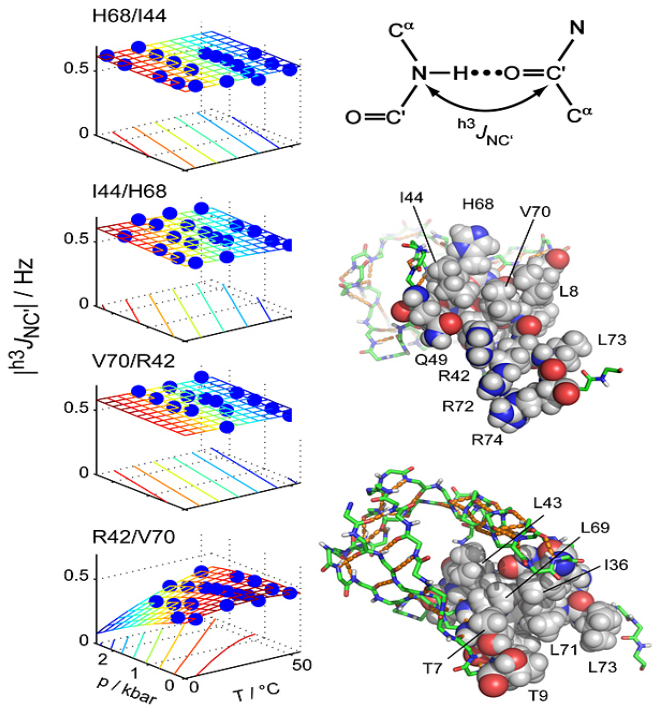

Fig. 4: Pressure and temperature perturbation of protein hydrogen bonds reveals key stabilizing elements of protein structure. Highly precise measurements of h3JNC' scalar couplings across hydrogen bonds as a function of temperature and pressure show that the functionally important Cterminal part of ubiquitin is particularly stabilized against perturbations [Nisius and Grzesiek Nat Chem 4, 711– 717 (2012)].

High pressure NMR reveals close similarity between cold and alcohol protein denaturation

Proteins denature not only at high, but also at low temperature as well as high pressure. These denatured states are not easily accessible for experiment, since usually heat denaturation causes aggregation, whereas cold or pressure denaturation occur at temperatures well below the freezing point of water or pressures above 5 kbar, respectively. We have obtained atomic details of the pressureassisted, cold-denatured state of ubiquitin at 2500 bar and 258 K by high-resolution NMR techniques. This contains on the order of 20 % native-like and non-native secondary structure elements. These structural propensities are very similar to the previously described alcohol-denatured (A-) state. The close similarity of pressure-assisted, colddenatured and alcohol-denatured state supports a hierarchical mechanism of folding and the notion that similar to alcohol, pressure and cold reduce the hydrophobic effect. Indeed, at non-denaturing concentrations of methanol, a complete transition from the native to the A-state can be achieved at ambient temperature by varying the pressure from 1 to 2500 bar. This method should allow highly detailed studies of protein folding transitions in a continuous and reversible manner.

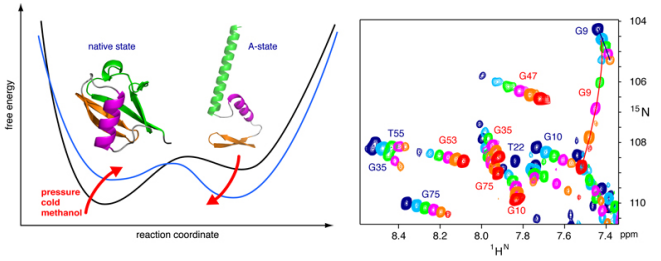

Fig. 5: High pressure NMR reveals close similarity between cold and alcohol protein denaturation. Application of high pressure and low temperature makes it possible to observe the cold denatured state of ubiquitin. This state contains native and non-native secondary structure elements that are every similar to the alcohol denatured state. This provides a method to study protein unfolding at atomic resolution in a completely reversible manner [Vajpai et al. PNAS, in press, (2012)].

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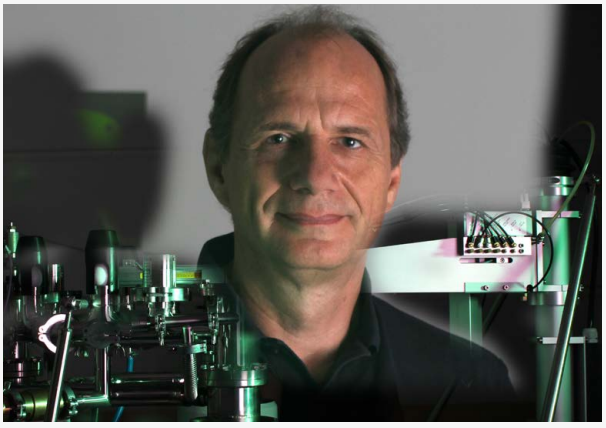

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TOR signaling and control of cell growth

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 factorinduced 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.

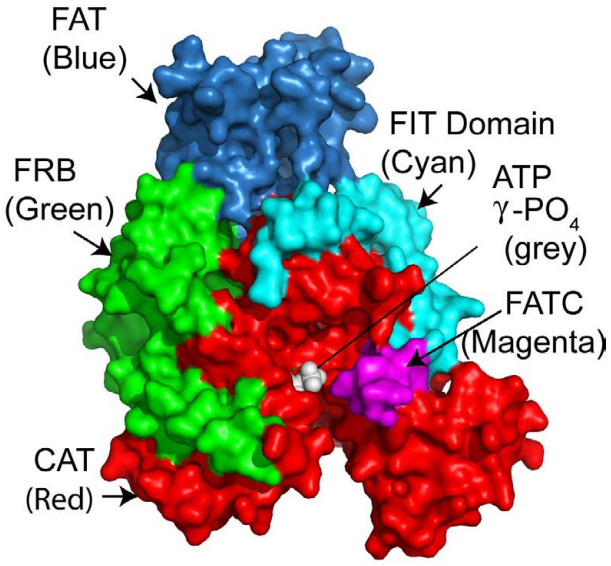

Fig. 1: Model of the catalytic region of human TOR .

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

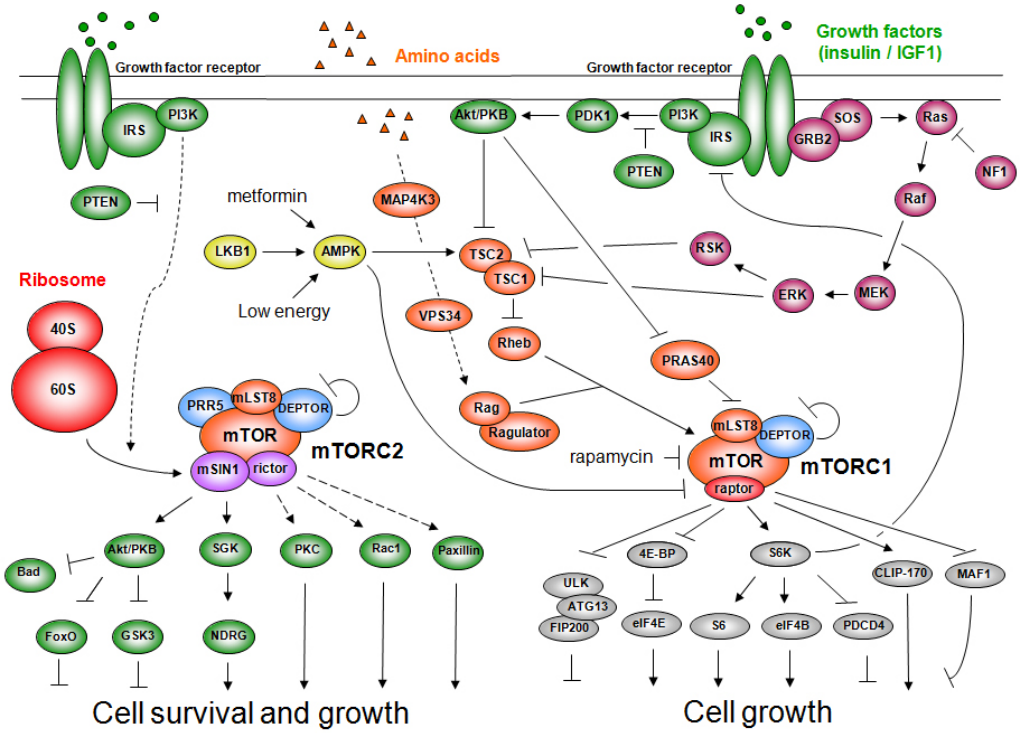

Fig. 2: The mTOR signaling network.

(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 process, 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 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.

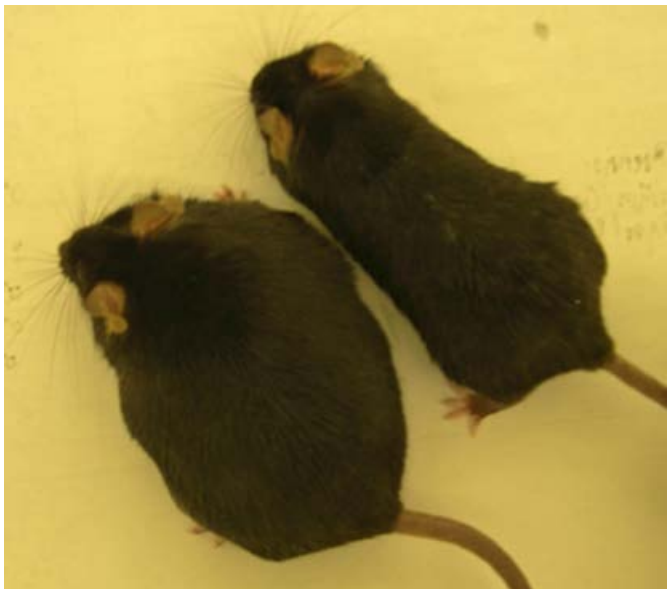

Fig. 3: Adipose-specific mTORC1 knockout mice are resistant to diet-induced obesity.

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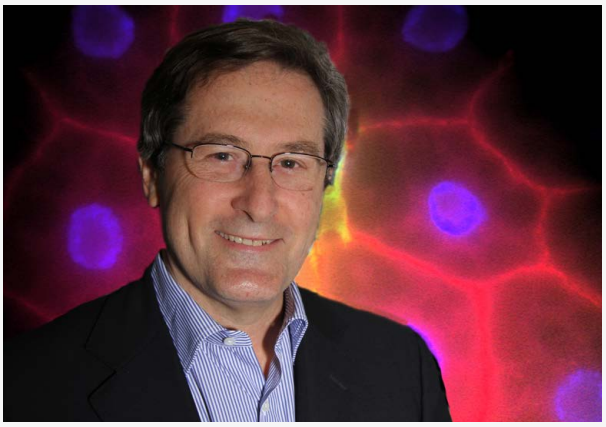

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RESEARCH GROUP CHRISTOPH HANDSCHIN

Regulation of skeletal muscle cell plasticity in health and disease

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.

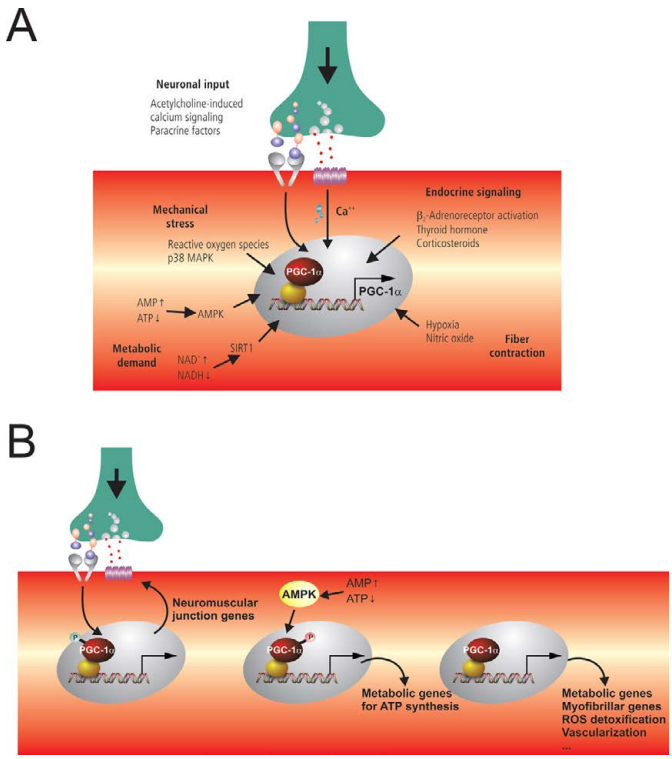
Skeletal muscle cell plasticity in exercise is a complex process: even a single endurance exercise bout alters the transcription of more than 900 genes in muscle. Chronic exercise leads to a metabolic and myofibrillar remodeling, increase in tissue vascularization, adaptation of the neuromuscular junction, 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.

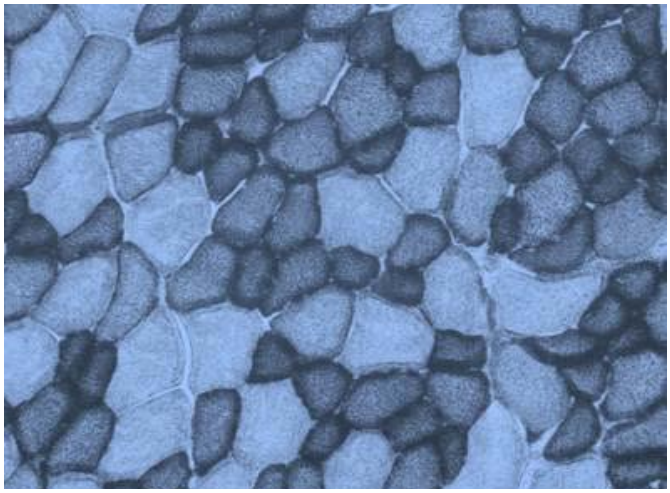
Our group is studying the mechanisms that control muscle cell plasticity and their physiological consequences. We try to integrate molecular biology, work in muscle cells in culture and observations on mice with different activity levels to obtain a comprehensive picture of the adaptations in the active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



PGC-1 α controls skeleta muscle plasticity in exercise. A) Every major signaling pathway in the trained muscle converges on PGC-1 α by inducing PGC-1 α gene expression, posttranslationally modifying the PGC-1 α protein, or by doing both. B) Spatiotemporal control of the specificity of the response to PGC-1 α activation in muscle depending on the cellular context. Abbreviations: AMPK, AMP-dependent protein kinase; p38 MAPK, p38 mitogenactivated protein kinase; PGC-1 α, peroxisome proliferatoractivated receptor γ coactivator 1 α; ROS, reactive oxygen species; SIRT1, sirtuin 1.



Visualization of fast (light blue) and slow (dark blue) muscle fibers in histological muscle sections with an NADH staining. Image by Joaquín Perez Schindler.

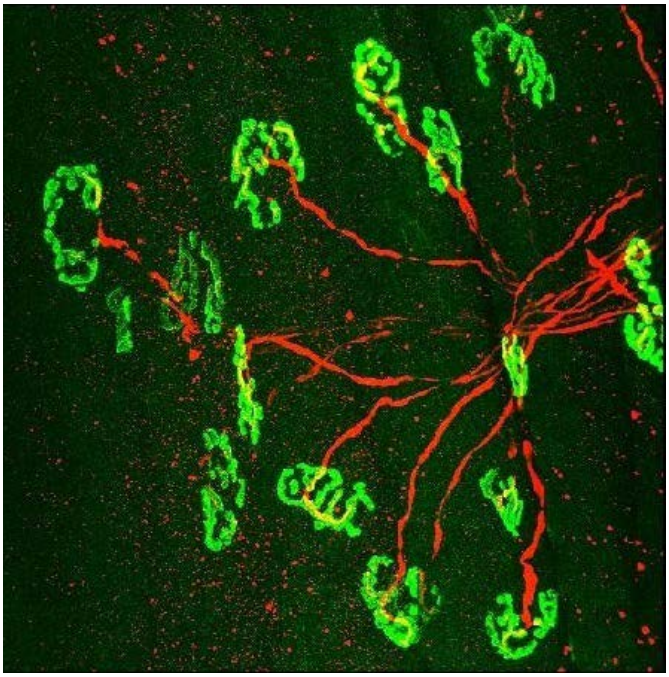
develop under seemingly similar conditions in type 2 diabetes and other muscle-associated diseases. We study the coordination of anabolic and catabolic pathways in order to pinpoint the differences in substrate fluxes in the healthy and the diseased muscle.

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.

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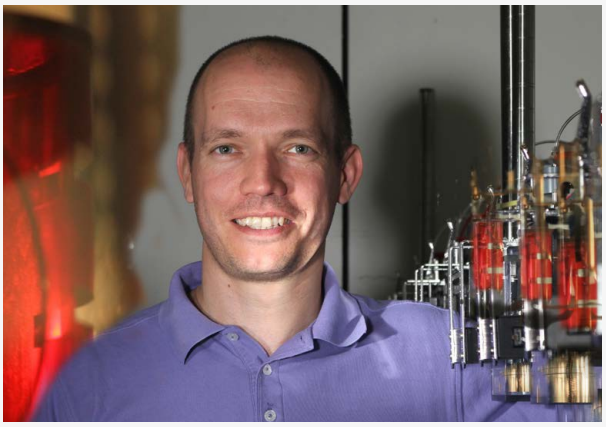

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Structure, Function and Folding of Membrane Proteins and their complexes

Membrane proteins comprise about a 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 and whereas tens of thousands of high-resolution structures of soluble proteins are known, with only about 250 unique membrane protein structures our knowledge on 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 processes 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 ensembleaveraged and single-molecule techniques will allow a description of the folding process resolved for individual atomic sites.

Thirdly, we are interesting in understanding the structural and functional aspects of membrane protein-chaperone complexes, which are part of the biosynthesis pathways of the outer membranes of bacteria and mitochondria. A natural assembly line of chaperones is responsible for the transport of the unfolded membrane protein polypeptides prior to the final folding event. We employ high-resolution NMR studies of large 70-100 kDa membrane protein-chaperone complexes to provide an atomic resolution description of the underlying molecular mechanisms. We want to know the details how the polypeptide transport is accomplished, how the substrates are recognized and how the final folding and insertion step is catalyzed.

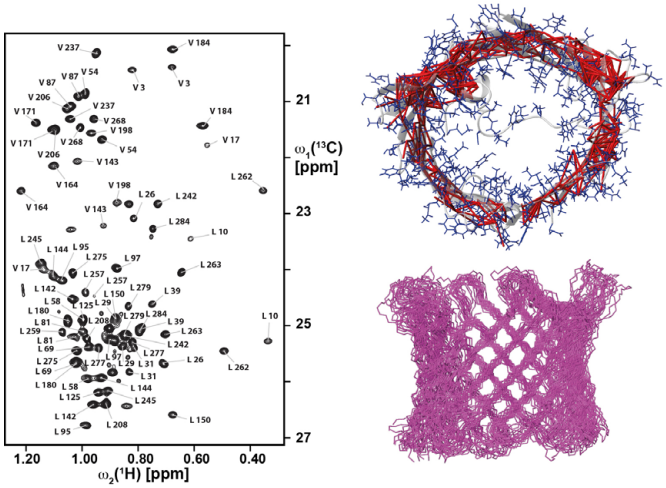

Fig. 1: 2D Methyl-TROSY NMR of VDAC in LDAO micelles for high-resolution structure determination.

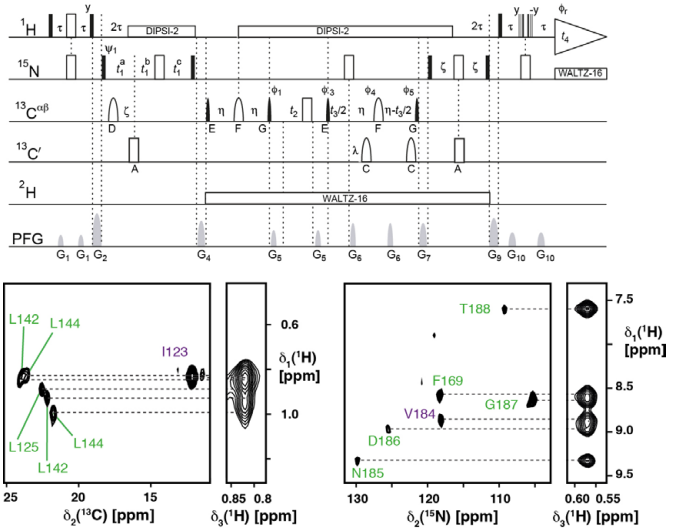

Fig. 2: 4D methyl-methyl and amide-methyl NOESY spectroscopy with coupled multidimensional processing of the integral membrane protein VDAC.

RESEARCH GROUP SEBASTIAN HILLER

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 2012

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Hiller, S.; Wider, G. (2012). Automated projection spectroscopy and its applications. *Top. Curr. Chem., 316,* 21-47.

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Cell signaling and dynamics in bacterial growth, adaptation, and persistence

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 (*Fig. 1*) (Böhm 2009, *Mol Microbiol*. 72, 1500; Böhm 2010 *Cell*, 141, 107). 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.

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

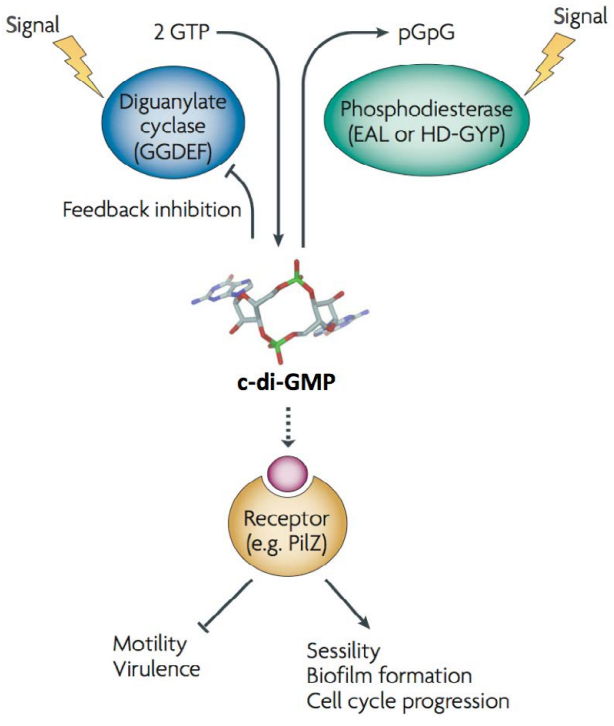

Fig. 1: Schematic of c-di-GMP mediated signaling in bacteria.

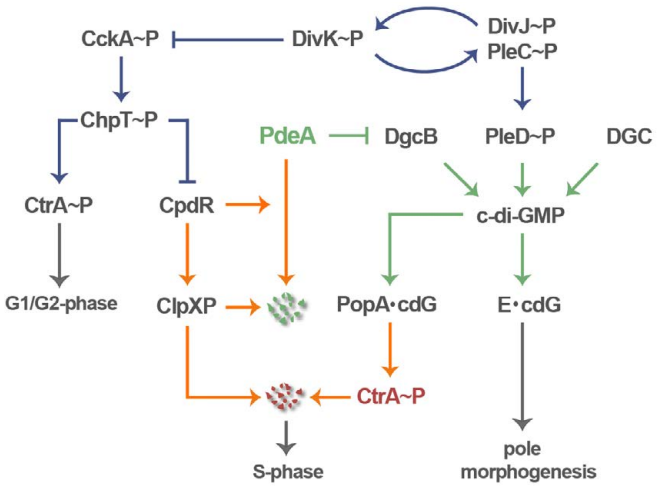

Fig. 2: Network controlling cell cycle progression and pole development in C. crescentus. Phosphorylation reactions (blue), c-di-GMP network (green), and protein degradation pathways (orange) are indicated. Unidentified c-di-GMP effector proteins (E) regulating pole morphogenesis are indicated.

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 (*Fig. 2*) (Paul 2008, *Cell* 133, 452).

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 PleD 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 (*Fig. 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-β-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.

Fig. 3: Characteristic morphologies of normal "smooth" (large colony) and SCV morphotypes (small colonies) of Pseudomonas aeruginosa.

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.

RESEARCH GROUP URS JENAL

Publications 2012

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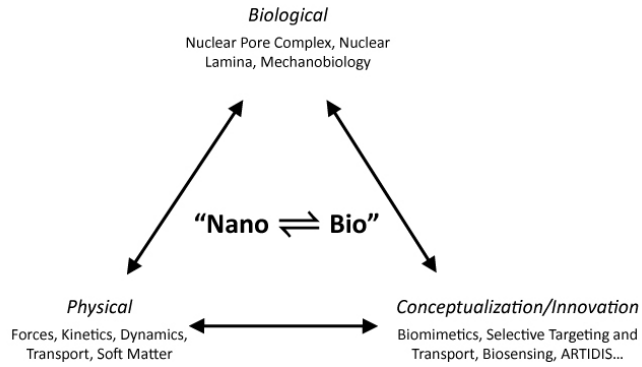
Nature's nanomachines

Biological nanomachines exhibit an exquisite functional sophistication within living cells that is fundamentally intriguing and technologically unprecedented. To unravel their inner workings, not only is it necessary to know the structure of the protein machinery and how this is altered in response to biochemical signals, but also how the separate components interact collectively over time to carry out a particular function. Importantly, the inter-dependence of these dynamic nanomechanical movements on the kinetics of intra- and intermolecular interactions - as governed by geometric and other contextual factors – necessitates new approaches that surpass macroscopic "bulk" concepts and reasoning.



This represents the overarching mission of our lab where we strive to obtain a holistic understanding of biological complexes and machines from the "bottom-up". Far from being confined to any one particular technique, we are inclined towards casting a wide net covering and correlating across different aspects of the biological problem(s). Having such fundamental questions at the fore challenges us to innovate beyond established methods in order to tackle a problem(s) in a meaningful way.

Naturally, the functional significance of Nature's nanomachines further underscores the potential benefits of replicating their mechanisms synthetically. Hence, the deep insight we obtain allows us to apply this knowledge in novel ways with potential technological impact.

The Nuclear Pore Complex (NPC)

Our fascination with the NPC lies in its ability to regulate macromolecular traffic between the nucleus and the cytoplasm in a highly selective manner. As a physical pore ~50 nm in diameter, the NPC nanomachinery functions to restrict or promote cargo translocation via biochemical selectivity and not size exclusion per se. Moreover, unlike engineered nanopores, the NPC does not clog *in vivo* – in spite of the molecular complexity of the cellular environment.

Simply put, nothing like it exists in technology.

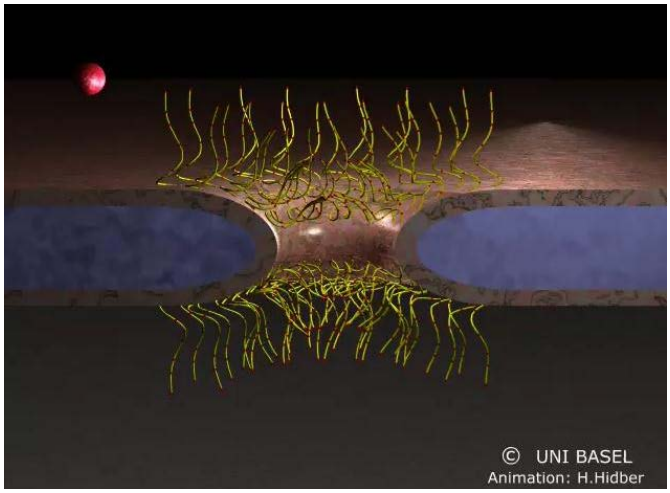


Fig. 2: Polymer Brush Model of the NPC: Non-specific cargo (red) is repelled by a brush-like barrier consisting of natively unfolded FG-Nups (chains). Specific cargo (green) in complex with a transport receptor (grey with red spots) breaches the barrier via exclusive receptor-FG interactions. Transport is a success when RanGTP (grey sphere) sequesters the transport receptor thereby releasing the specific cargo into the nucleus.

Owing to the complex make-up of the NPC, studies directed at resolving its selective gating mechanism have provided only limited insight *in vivo* and *in vitro*. Here, our efforts have been centered on correlating the structural nanomechanics and molecular biophysics of the key NPC proteins (i.e., natively unfolded phenylalanine-glycine (FG)-rich nucleoporins or FG-Nups) to kinetic binding interactions of transport receptors that accompany cargoes through the NPC.

These include:
(I) applying the atomic force microscope (AFM) to studying the conformational changes of the FG-Nups due to receptorbinding on pore-like nanostructures;
(II) correlating receptor-FG binding affinities to conformational changes in the FG-Nups using surface plasmon resonance (SPR); and,
(III) constructing biomimetic nanopores that reproduce the single molecule transport selectivity of the NPC.

Our findings have thus far contributed considerable insight into the underlying principles that govern molecular mechanics, selectivity and transport in the NPC. See News and Views by Jovanovic-Talisman and Zilman, Building a Basic Nanomachine, *Nature Nanotechnology*, 6 397 (2011).

From biological machines to molecular devices of the future

We anticipate that the knowledge gained from the NPC can have considerable impact in technology. By replicating the NPC protein machinery with synthetic polymers, we are interested in targeting specific proteins from authentic biological environments to siteselective locations with nanoscale precision. In this way, we envision that complex molecular transport processes can one day be orchestrated as elegantly in technological systems as they occur in the living cell.

From molecules to cells and tissues

On a more technological note, we are applying our interdisciplinary knowledge of nanotechnology towards medical tissue diagnostics. Known as "ARTIDIS" (Automated and Reliable Tissue Diagnostics), we are building on the exquisite nanomechanical sensitivity of the AFM to detect and differentiate between the various stages of disease in soft human tissues. With federal funding provided by the Commission for Technology and Innovation (CTI) and in partnership with local Swiss AFM company Nanosurf, we anticipate that ARTIDIS will have key applications in the rapid diagnosis of diseases such as breast cancer and osteoarthritis.

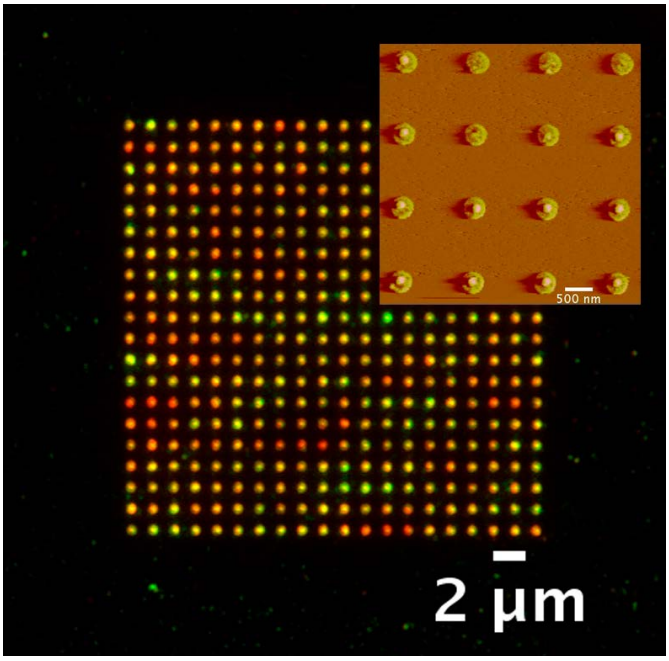


Fig. 3: The functional principles of the NPC applied to sorting proteins from complex biological environments (i.e. serum) to synthetic targets with both, spatial and biochemical precision as resolved by total internal reflection fluorescence microscopy (TIRF; main image) and atomic force microscopy (AFM; inset).

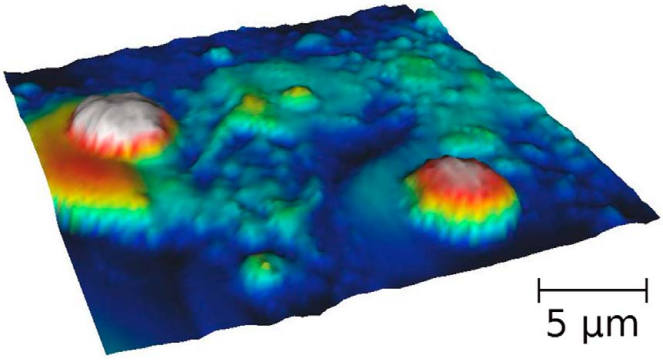


Fig. 4: Nanomechanical "heat" map of human breast tissue reveals individual epithelial cells embedded within the extracellular matrix.

RESEARCH GROUP RODERICK LIM

Publications 2012

Henrich, P.B.; Monnier, C.A.; Halfter, W.; Haritoglou, C.; Strauss, R.W.; Lim, R.Y.H.; Loparic, M. (2012). Nanoscale topographic and biomechanical studies of the human internal limiting membrane. *Investigative Ophthalmology & Visual Science, 53,* 2561-2570.

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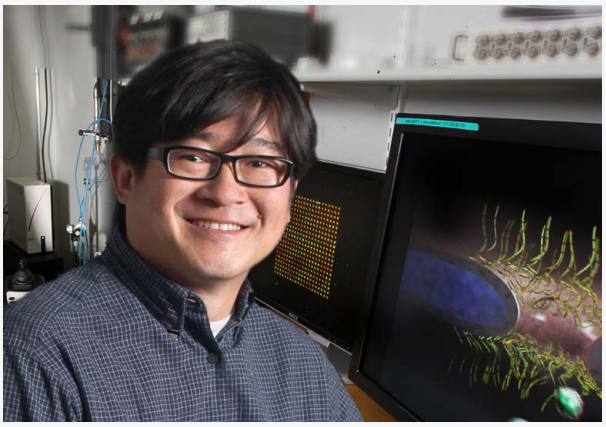

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Multienzymes and the regulation of eukaryotic lipid metabolism

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.

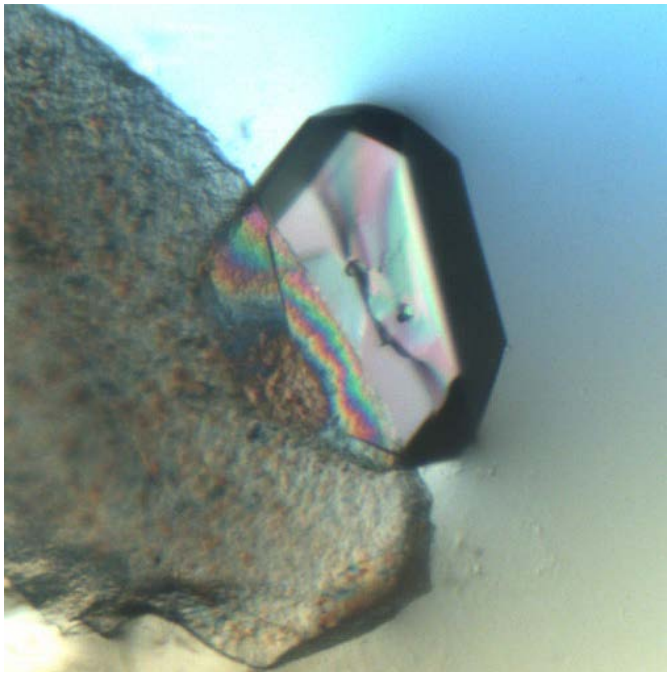

Fig. 1: Growth of a protein crystal from a porous bioglass seed.

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 associated and integral membrane proteins, very few of which

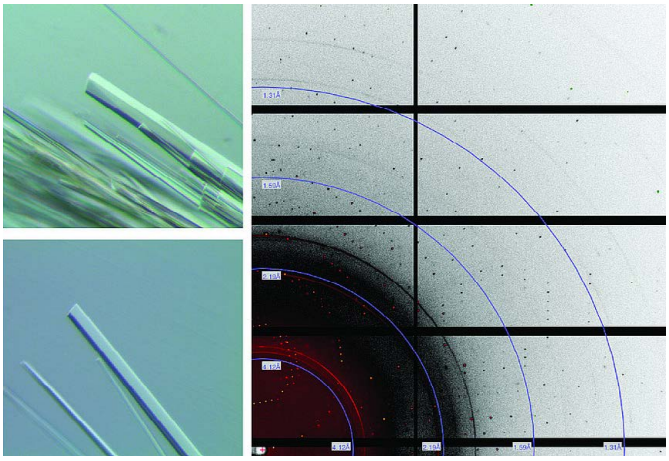

Fig. 2: Left side: Crystals of different lectin-ligand complexes. Right side: Atomic resolution diffraction pattern of a lectin-ligand complex collected on a PILATUS detector at SLS (Villigen).

have been structurally characterized. Also the regulation of lipid homeostasis involves dynamic multi-subunit assemblies or membrane bound sensor systems for lipid or sterol composition.

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.

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. In collaboration with Sebastian Hiller (Biozentrum, Universität Basel) we are studying pro- and eukaryotic beta-barrel membrane transporters and proteins involved in their biosynthesis in a joint effort combining NMR and X-ray crystallography. Together with Beat Ernst (Pharmazentrum, Universität Basel) and Rudi Glockshuber (ETH Zürich) we study domain interactions in pro- and eukaryotic lectin proteins and their crosstalk with ligand interactions.

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. 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

RESEARCH GROUP TIMM MAIER

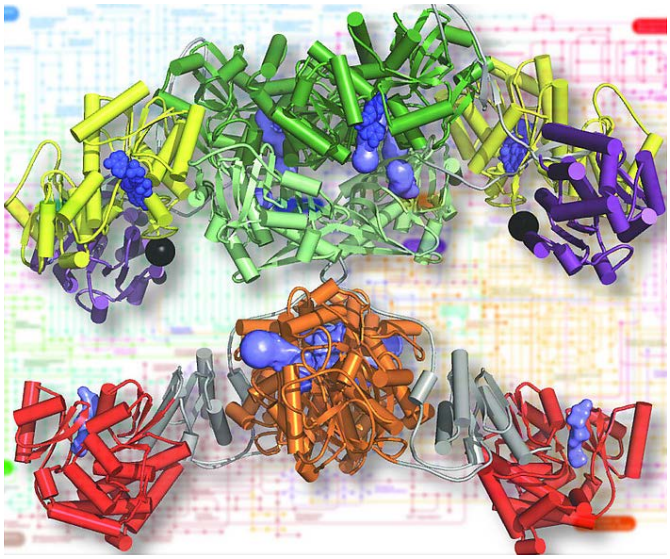

Fig. 3: Detailed structural models uncover the functional organization of complex biomolecular machines such as the animal fatty acid synthase.

and eukaryotic cell based systems. Crystallization is aided by microfluidic screen preparation and nanoliter robotics for crystallization setup and automated seeding. 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. The focal area Structural Biology & Biophysics provides a highly collaborative environment between groups with expertise in all major techniques in structural biology. 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 mediumthroughput 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.

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RESEARCH GROUP ERICH NIGG

Control of chromosome segregation and centrosome duplication in human cells

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*). Hence, we study the composition, regulation and dynamics of the mitotic spindle and kinetochores. In addition, 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.

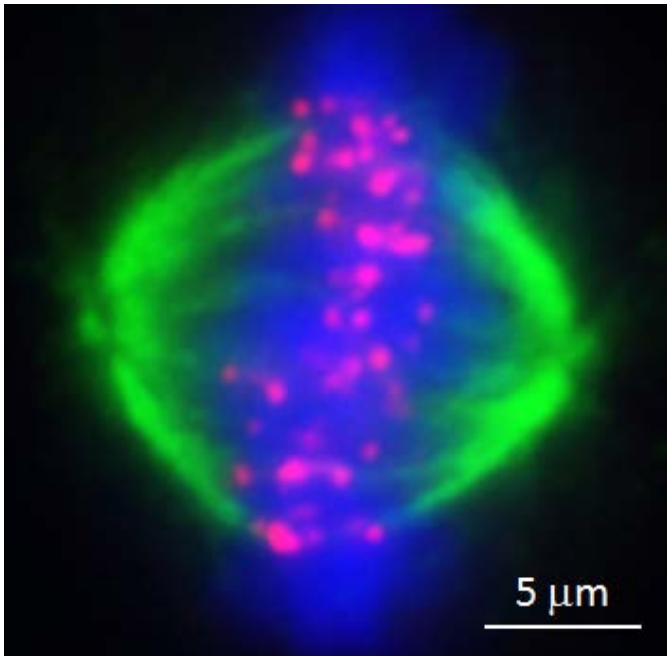

Fig. 1: A dividing human cell was stained with antibodies against tubulin (green) and a kinetochore marker (red); condensed chromosomes are visualized by staining with DAPI (4',6-diamidino-2- phenylindole; blue). Size bar: 5 µm.

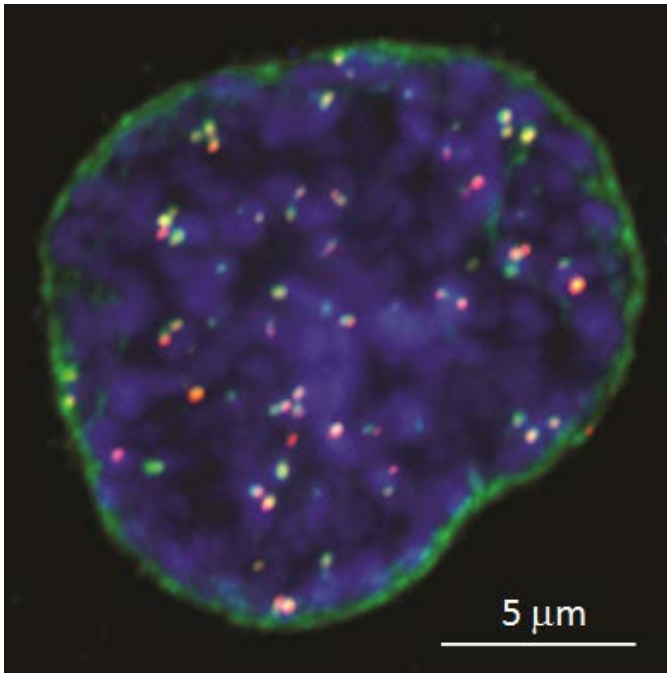

Fig. 2: A cultured human cell was stained with antibodies against two kinetochore components, Mad1 (green) and Mad2 (red); DNA was stained by the dye DAPI (4',6-diamidino-2- phenylindole; blue). Also visible is the nuclear envelope (stained by anti- Mad1 antibodies, green). Size bar: 5 µm.

At the two poles of the spindle apparatus are tiny organelles known as 'centrosomes' (*Fig. 3*). The biogenesis, duplication and function of centrosomes (and their constituent centrioles) constitute a second major research focus of our laboratory. Centrosomes 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. Deregulation of the centrosome/centriole duplication cycle is believed to constitute a major cause of chromosome mis-segregation during the development of human cancers. Furthermore, certain brain diseases (notably microcephaly) and some forms of dwarfism have been causally linked to mutations in specific centrosomal proteins. Importantly, centrioles function also as basal bodies for the formation of cilia and flagella in quiescent cells, and mutations in genes coding for centriole/basal body proteins contribute to a multitude of diseases and syndromes (ciliopathies) that reflect the absence or malfunction of the basal-body/ciliary apparatus.

Our laboratory combines reverse genetics (e.g. RNA interference), immunocytochemistry (including structured illumination superresolution microscopy) and multiple biochemical techniques (notably mass spectrometry) to unravel the molecular mechanisms that ensure correct centrosome duplica-

tion 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 have used mass spectrometry to establish inventories of proteins and phosphorylation sites in the spindle apparatus, the kinetochore and the centrosome. More recently, we focus on the wiring of key regulatory circuits, as defined by kinases, phosphatases, and selected substrates. We anticipate that our work will lead to a better understanding of the regulation of chromosome segregation and centrosome duplication in normal cells, as well as provide insights into the deregulation of these processes in disease.

In the recent past, we have successfully completed several large scale phospho-proteomics studies that provide information, with unprecedented temporal resolution, on hundreds of mitotic phosphorylation sites. In parallel, we have discovered and characterized several novel spindle components and 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 and Plk4), Aurora kinases and spindle checkpoint kinases (Mps1 and Bub1).

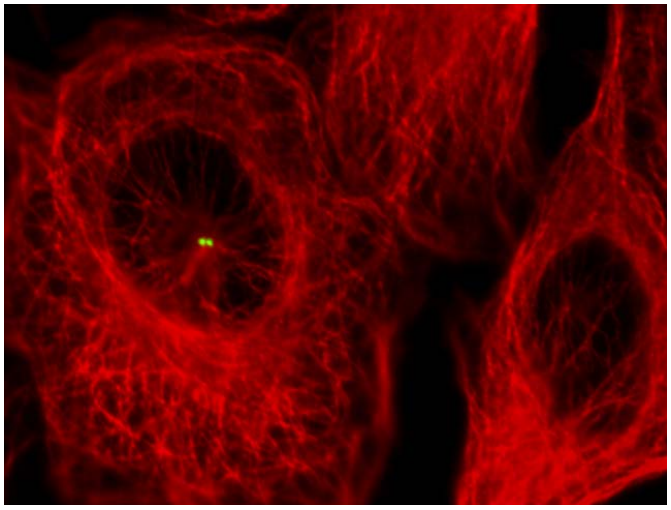

Fig. 3: Centrosomes organize microtubule arrays. A cultured human cell was costained with antibodies against the protein kinase Plk4, a key regulator of centriole duplication (green), and antibodies against the cytoskeletal component tubulin (red). Size bar: 5 μm.

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 have optimized massspectrometry based procedures (selected reaction monitoring) that allow us to monitor, in quantitative terms, the abundance of key components involved in both centrosome duplication and chromosome segregation. In parallel, we have begun to use somatic gene targeting approaches that should allow us to visualize and quantify a subset of these very same components in time and space.

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.

RESEARCH GROUP ERICH NIGG

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RESEARCH GROUP JEAN PIETERS

Role of signal transduction in pathogen persistence and lymphocyte survival introduction

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.

We have previously identified a serine/threonine protein kinase, termed protein kinase G (PknG) from *M. tuberculosis*, as an essential virulence factor (*Science* (2004) ; *PNAS* (2007). More recently, we demonstrated that expression of PknG in different mycobacterial species is dictated by regulatory elements present upstream of PknG. Our data suggest that changes in expression levels may underlie evolution of PknG and other pathogenicity genes in Mycobacteria (Houben et al., 2009). Second, we mapped the autophosphorylation sites in PknG, demonstrated that autophosphorylation is dispensable for kinase activity, but crucial for the capacity of PknG to promote mycobacterial survival within macrophages (Scherr et al., 2009). Ongoing work aims to define the intracellular targets modulated by protein kinase G, to further understand the mechanisms of virulence employed by *M. tuberculosis*.

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) ; *Science* (2000)). 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)). In addition, we could show that coronin 1 is required for the maintenance of T lymphocytes in peripheral lymphoid organs (Nature Immunol. (2008)). 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*).

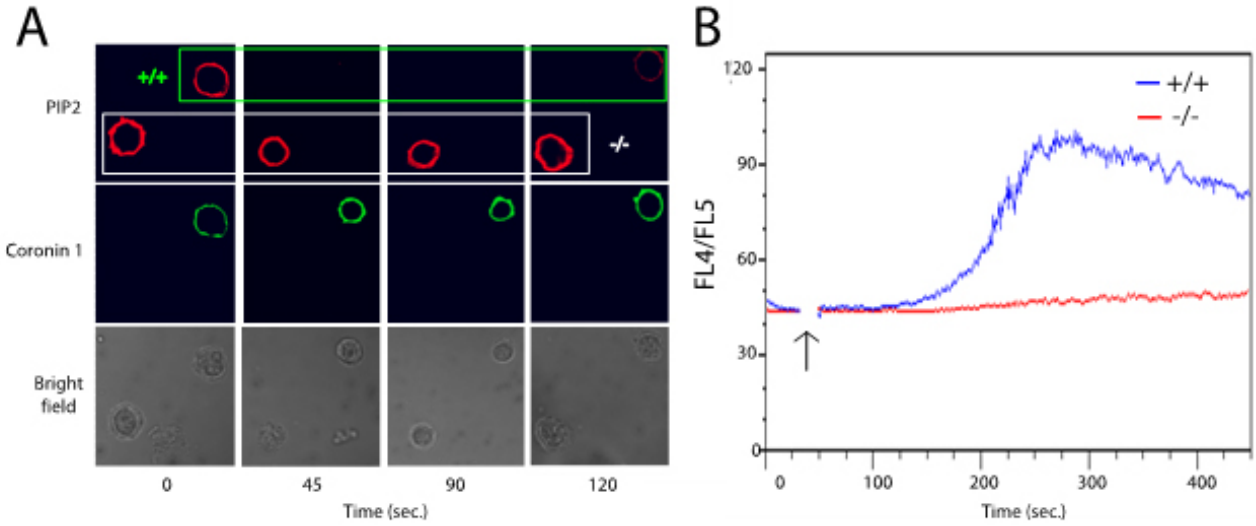

Fig. 1: Coronin 1- dependent PIP2 hydrolysis and Ca2+ mobilization in T cells. A) Activation of wild type T cells (+/+, upper right corner cell) but not coronin 1- deficient (- /-, lower left corner cell) T cells results in a rapid hydrolysis of PIP2, as suggested by the disappearance of the PIP2- specific fluorescence signal (upper panels). B) Coronin 1 is required for calcium mobilization upon stimulation of the T cell receptor. The graph depicts the fluorescence signal of wild type (blue) or coronin 1- deficient (red) T cells loaded with Ca2+-sensitive probes. Arrow indicates the time of stimulus. For details see Mueller et al., 2008.

RESEARCH GROUP JEAN PIETERS

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.

Publications 2012

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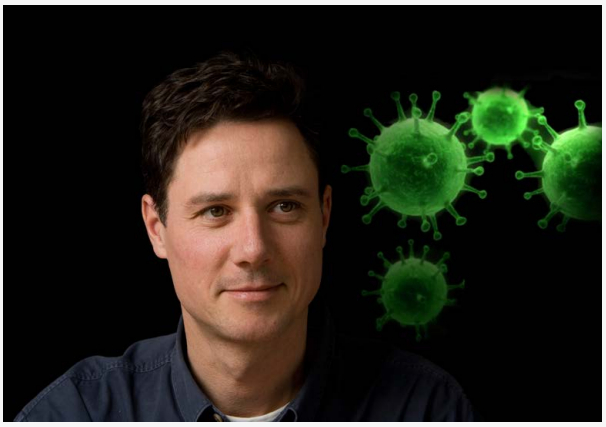

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RESEARCH GROUP HEINRICH REICHERT

Drosophila neuroblasts: neural stem cells in normal brain development and in brain tumor formation

The vast arrays of different neural cell types that characterize the complex circuits of the brain are generated by neural stem cells. In Drosophila, 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. The numerous cell types that make up the brain of Drosophila derive from a set of approximately 200 neuroblasts, each of which generates its own lineage-specific unit of neural progeny during embryonic and postembryonic development. The goals of our lab are to analyze the developmental mechanisms by which these proliferating brain neuroblasts generate the lineage-specific units of the brain and to analyze the cellular and molecular mechanisms by which the deregulated proliferation of these neural stem cells leads to the formation of brain tumors.

Previous work on Drosophila neuroblasts indicates a causative link between impaired neural stem cell proliferation and brain tumor formation in this genetic model system and supports the hypothesis that impaired cellfate determination is a major cause of cancerous overgrowth (reviewed in Reichert, 2011; Saini and Reichert, 2012), Notably the amplifying neural stem cells that produce the neural cells of higher brain centres in the fly are prone to tumorigenic dysregulation, and this makes them excellent models for neural stem cell-derived tumors (reviewed in Boyan and Reichert, 2011). We focus on these neural stem cells in the Drosophila central brain and use a comprehensive set of genetic, genomic and transgenic methods to investigate how the balance between stem cell self-renewal, neural differentiation and programmed cell death is precisely controlled to ensure normal brain development as well as to study how dysregulation of this balance leads to neural stem cell derived tumorigenesis and formation of metastasis.

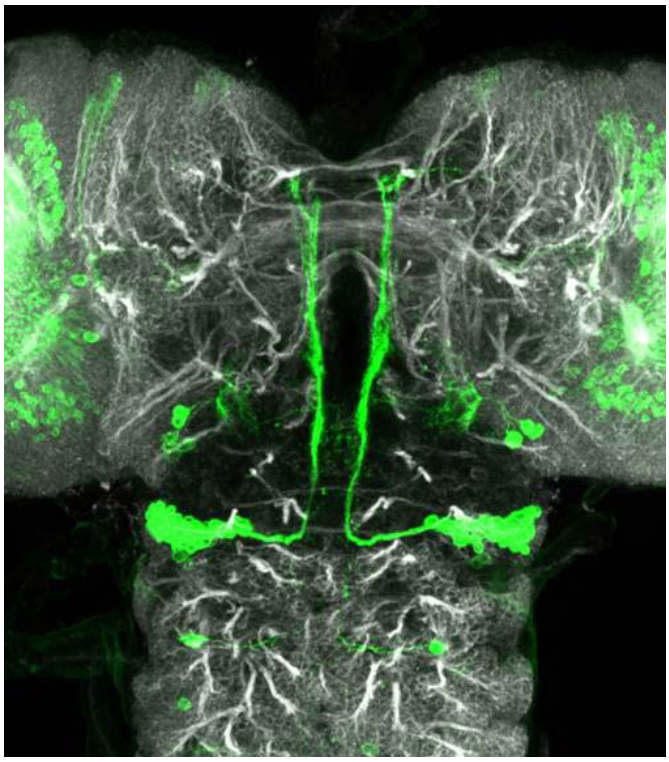

Fig. 1: Neural stem cell lineages in the developing brain of Drosophila.

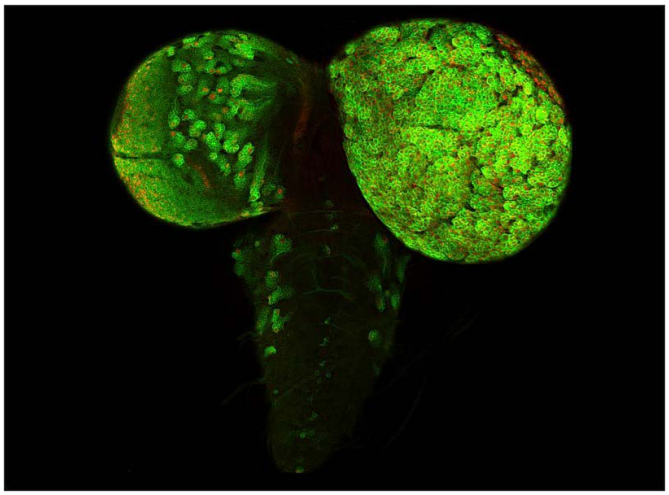

Fig. 2: Neural stem cellderived brain tumor in Drosophila brain (right hemisphere).

An important advance in the analysis of neural stem cell biology in Drosophila is based on the availability of methods for isolating large numbers of pure neural stem cells and differentiating neurons. With the development and implementation of a novel FACS-based method for isolating neuroblasts and differentiating neurons that retain both cell-cycle and lineage characteristics from the Drosophila brain, this has been achieved (Berger et al., 2012). This FACS purification method does not affect viability, proliferation properties, or lineage characteristics of the isolated neuroblasts and of their progeny in vitro or in vivo. Importantly, with this methodological advance, it is now possible to obtain high-quality neural stem cell-specific gene expression data. Based on transcriptional profiles derived by mRNA sequencing of pure populations of isolated cells a total of 28 predicted neural stem cell-specific transcription factors are identified and arranged into a transcriptional network which reveals hubs for Notch signalling, growth control, and chromatin regulation. In addition to their identification, the functional relevance of these individual transcription factors in brain tumor formation is tested in genetic gain-of-function and loss-of-function studies.

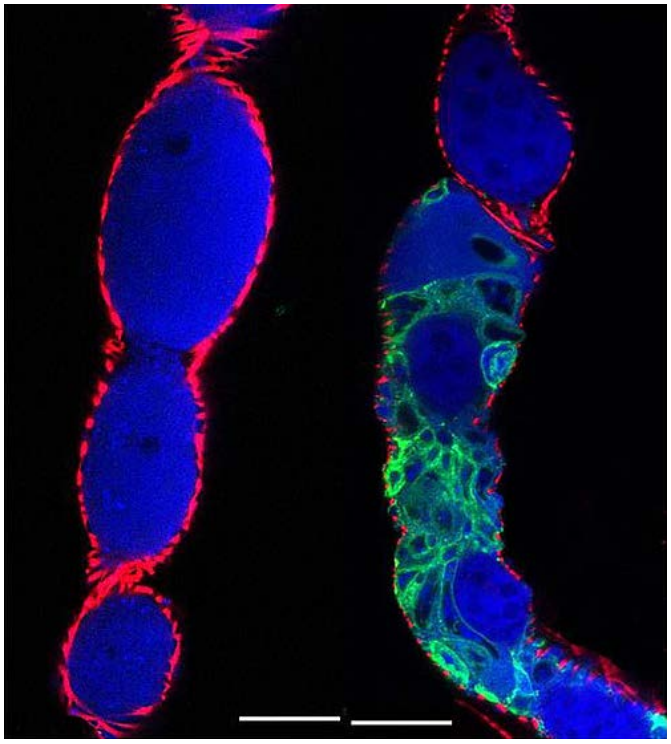

Fig. 3: Fig. 3. Metastasis in ovariole (right; green cells) after transplantation of stem cell tumor tissue.

In addition to previously known factors, this functional analysis uncovers the Klumpfuss transcription factor, a member of the EGR family of transcriptional regulators which has been connected to human cancers, as a novel regulator of self-renewal. Targeted overexpression of Klumpfuss in neural stem cells results in tumorigenesis and metastasis after transplantation into a host environment. This demonstrates that the obtained transcriptional data provides a useful basis for a more targeted search for functional interactions between redundantly acting factors. Thus, both the method for isolation of pure neural stem cells and the data on the transcription factor network that underlies self-renewal in these stem cells will be valuable resources for further work.

Previous studies based on genome-wide RNAi analysis have uncovered additional candidate genes with potential tumor suppressor function. The tumor suppressor function of these candidate genes is analysed in terms of stem cell tumor formation potential using an in vivo transplantation model (Laurenson et al., 2012). These investigations comprise transplantation of transgenic brain tissue, in which RNAi knockdown is targeted to genetically labeled neural stem cells, qualitative and quantitative assays of tumor formation after transplantation into wildtype host, and investigation of the metastatic potential of the transplanted tissue using an ovarian micrometastasis assay. These experiments demonstrate that loss-of-function of candidate genes such as alpha-Adaptin, ap2-sigma, Brahma, Moira, and Daughterless in neural stem cells results in (transplantable) tumor formation as well as in metastases which are invariably lethal. Continuing work on additional candidate tumor suppressor genes and oncogenes is revealing insight into the genetic network that underlies brain tumor formation in the Drosophila model system.

Insight into the neural stem-cell dependent mechanisms that operate during normal brain development is obtained for the class of Drosophila brain neuroblasts that amplify proliferation through intermediate neural progenitors (Jiang and Reichert, 2012a; Viktorin et al., 2011). These studies show that substantial neural overproliferation occurs normally in these neural stem cell lineages and that elimination of excess neurons through programmed cell death during postembryonic development is required for the formation of correct innervation in the developing brain. Thus, amplification of proliferation through intermediate progenitors is counterbalanced by reduction of neuronal number through programmed cell death, and both operate during the normal development of these neural stem cell lineages. While the triggering mechanisms for programmed cell death in the amplifying neuroblast lineages remains currently unknown, key molecular signals for initiation of programmed cell death in other identified lineages are now revealed, and a novel neuroblast-specific role of Hox genes during normal brain development is documented (Kuert et al., 2012). This work focuses on identified lineages in the tritocerebral and subesophageal brain neuromeres, which represent the postembryonic expression domains of

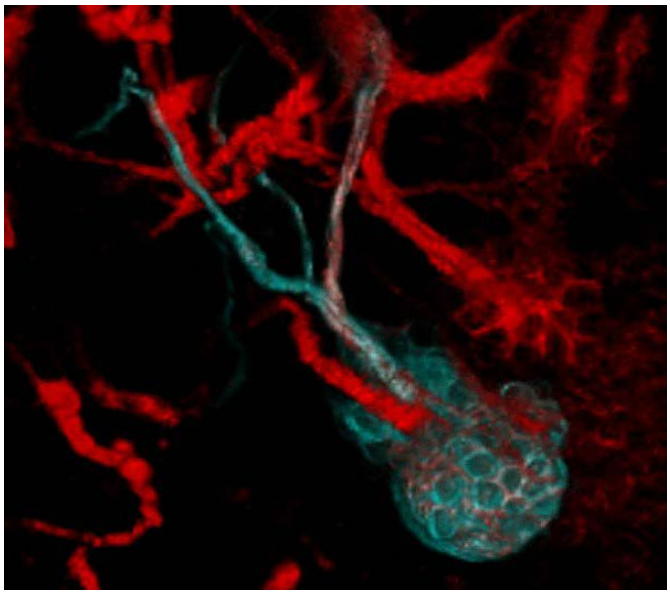

Fig. 4: Ectopic neuroblast lineage (grey cells) caused by Hox gene inactivation.

the Antp-class Hox genes. Thus, for example, the Hox gene labial is required in two identified neuroblast lineages of the tritocerebral brain neuromere for correct and cell autonomous termination of proliferation through programmed cell death. This Hox-dependent programmed cell death is stage- and lineage-specific, and in its absence identified ectopic neuroblast lineages are formed which never occur in the normal wildtype brain. Remarkably similar findings obtained in the developing mammalian hindbrain for the murine homolog Hoxa1 gene support the notion of highly conserved mechanisms of brain development.

In addition, a comprehensive study of the neuroblast lineages generated during normal embryogenesis in the larval olfactory system is carried out, which sheds light on the lineal relationship between larval and adult neurons of the brain (Das et al., 2012). This work reveals an unexpected level of complexity of the different projection neurons and local interneurons in the simple larval olfactory system. Moreover, it establishes the lineages of origin for the large diversity of larval neuron cell types in the olfactory system and, in doing so, identifies a novel fifth neuroblast lineage of olfactory interneurons. Remarkably, in this system each different cell type appears to be represented by a small number of neural cells, and in many cases, by a single identified neuron. Thus, although the larval olfactory circuitry may be reduced in terms of neuronal number, it shows a surprising diversity of interneuronal cell types that is comparable to that of the highly complex adult olfactory system which derives from the same set of neuroblasts.

Finally, we are pioneering novel investigations into the diversity and evolution of brain development by studying non-model systems organisms that belong to so called lesser phyla. For this we are analysing the central nervous systems in Cycliophora, Loricifera, Kinorhyncha, and Tardigrada using both state of the art imaging and 3D ultrastructural techniques as well as RNA-seq based gene expression studies.

The general significance of these and related findings is the subject of several invited reviews (Saini and Reichert, 2012; Jiang and Reichert, 2012b; Bailly et al., 2012).

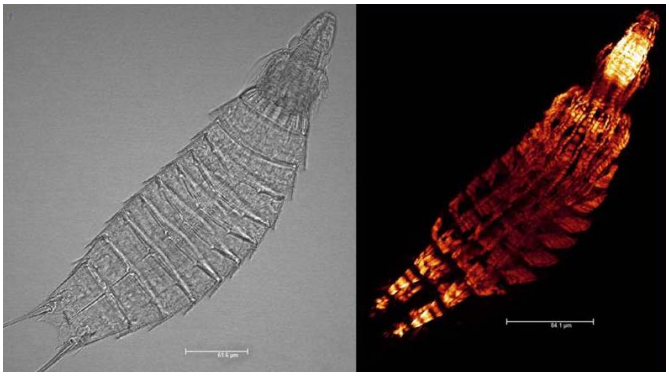

Fig. 5: Kinorhynch external morphology (left) and internal myoanatomy (right).

RESEARCH GROUP HEINRICH REICHERT

Publications 2012

Das, Abhijit; Gupta, Tripti; Davla, Sejal; Prieto Godino, Laura Lucia; Diegelmann, Sören; Reddy, O Venkateswara; Raghavan, K Vijay; Reichert, Heinrich; Lovick, Jennifer; Hartenstein, Volker (2012). Neuroblast lineage-specific origin of the neurons of the Drosophila larval olfactory system. *Developmental Biology,* http://dx.doi.org/10.1016/j.ydbio.2012.11.003.

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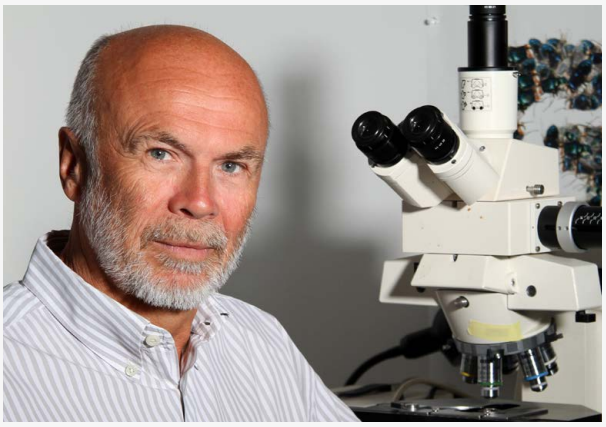

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Molecular mechanisms involved in synapse formation and neuromuscular disease

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.

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.

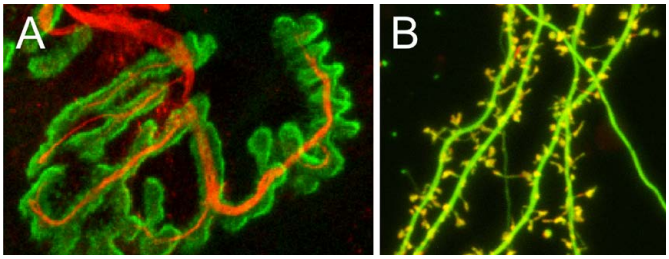

Fig. 1: Comparison of neuromuscular junctions and synapses in the brain. (A) At the NMJ, the presynaptic motor nerve, visualized by the staining with antibodies to neurofilament and synaptophysin (red), perfectly matches the postsynaptic structure, stained with an agent that binds to acetylcholine receptors. (B) At neuron-toneuron 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.

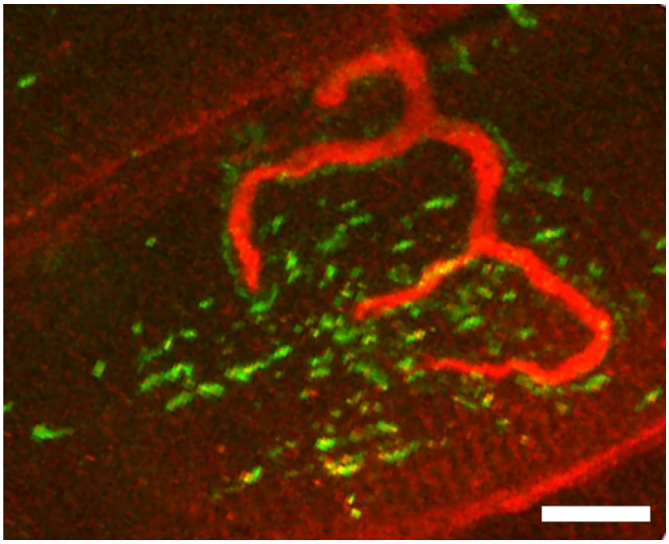

Fig. 2: Experimentally induced myasthenia gravis causes a severe fragmentation of postsynaptic structures Size (green) in sternomastoid muscle so that neuromuscular transmission initiated by the presynaptic nerve terminal (red) is largely abrogated. Scale bar = 10 μm.

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 (*Fig. 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.

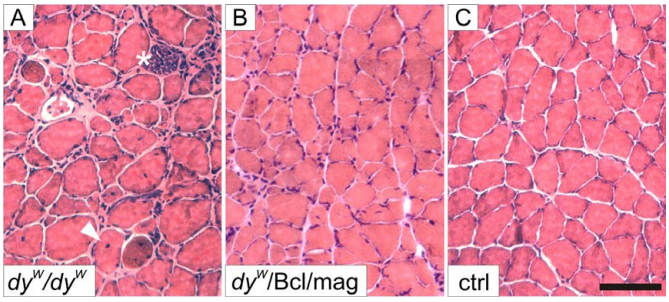

Fig. 3: Cross-sections of skeletal muscle from mice stained with hematoxilin & eosin to visualize their structure. (A) Muscle from mice suffering from a severe muscular dystrophy due to loss of laminin-211 (dyW/dyW) with signs of degeneration (white arrowhead) and fibrosis (asterisk). (B) The muscle from mice with the same muscular dystrophy that also express Bcl2 and mini-agrin (dyW/Bcl/mag) is much improved and looks like muscle from healthy, wildtype mice (ctrl; C). Scale bar = 100 μm.

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 (*Fig. 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 (*Fig. 3B*) or treating mice with the anti-apoptotic agent omigapil. Such combination therapy restores the structure of the muscle to control levels (*Fig. 3C*). In current projects we test additional pharmacological agents for their potential to ameliorate the disease.

RESEARCH GROUP MARKUS RÜEGG

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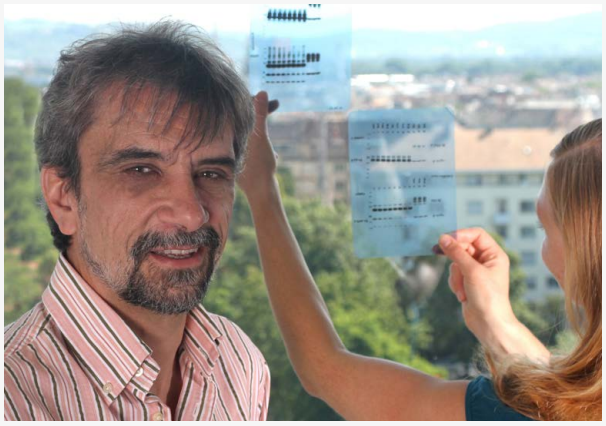

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RESEARCH GROUP PETER SCHEIFFELE

Neuronal circuit assembly and synapse formation

The 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 trans-synaptic 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. In cell biological studies we identified a novel mode of lateral coupling between neuroligins and neurotransmitter receptors in the postsynaptic membrane. We demonstrated that neuroligin-1 recruits NMDA-type glutamate receptors through interactions via the extracellular domains of the protein. These interactions are critical for physical retention of a pool of NMDA-receptors at glutamatergic synapses in vivo and regulate NMDA-receptor-dependent synaptic plasticity in the mouse hippocampus (Budreck et al., *PNAS*, 2013). These findings highlight the possibility that neurotransmitter receptors and adhesion molecules assemble into complexes that have structural roles at central synapses.

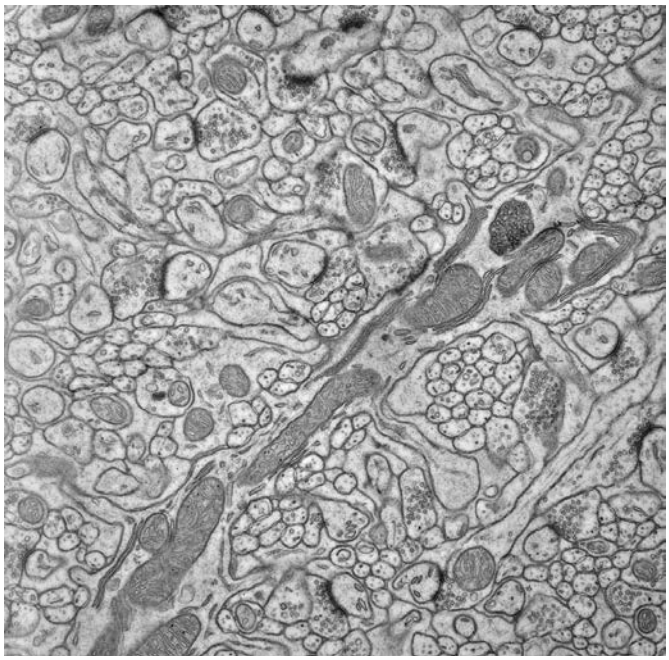

Fig. 1: Synapses in the mouse cerebellum.

Molecular diversification of recognition molecules by alternative splicing

Neuronal networks in the mammalian brain represent one of the most complex examples of a highly organized biological system. The finite number of protein-coding genes in the human genome severely limits the genetic resources that can be employed for generating molecular diversity. Therefore, highly polymorphic cell surface receptor families arising from extensive alternative splicing provide attractive candidates for neuronal recognition. Neurexins are highly polymorphic synaptic cell surface receptors that are extensively modified by alternative splicing. Alternative splice variants of neurexins differ in biochemical interactions with neuroligins and other binding partners and may underlie an adhesive code at central synapses. We discovered that neurexin alternative splicing is regulated by neuronal activity. The KH-domain RNA-binding protein SAM68 binds directly to the neurexin-1 pre-mRNA and is essential for activity-dependent splicing regulation (Iijima et al., *Cell*, 2011). SAM68-like proteins (SLM1 and SLM2) exhibit highly selective expression patterns in interneuron populations in the mouse brain. These findings provide an entry point to unraveling the cell type-specific neurexin repertoires and their contribution to neuronal connectivity.

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 10 years have led to the identification of several candidate genes that may confer susceptibility to autism but also environmental risk factors might exist. The study of neuronal circuit alterations in autism has been most advanced for monogenic forms of syndromic autism, such as Fragile X and Rett's Syndrome, where specific alterations in synaptic transmission have been identified. We focused our studies on a mouse model of a non-syndromic form of autism, carrying a mutation in the synaptic adhesion molecule neuroligin-3. Using a combination of electrophysiological, anatomical, and behavioral studies we identified a remarkable convergence in the synaptic pathophysiology in neuroligin-3 knock-out mice and a rodent model of Fragile X, characterized by a defect in metabotropic glutamate receptor-dependent synaptic plasticity. Importantly, the synaptic defects could be reversed by re-expression of neuroligin-3 in adult animals highlighting a substantial reversibility of the neuronal phenotypes in this model (Baudouin et al., *Science*, 2012). In ongoing studies we are now testing pharmacological interventions in transgenic mouse and rat models of autism to identify treatment strategies for the disorder.

RESEARCH GROUP PETER SCHEIFFELE

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. 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 (Kalinovsky et al., *PLoS Biology*, 2011). Interestingly, the bone morphogenetic protein signaling pathway continues to be active in the adult cerebellum. Therefore, we are now examining functions of this signaling system in learning-dependent plasticity in mature cerebellar circuits.

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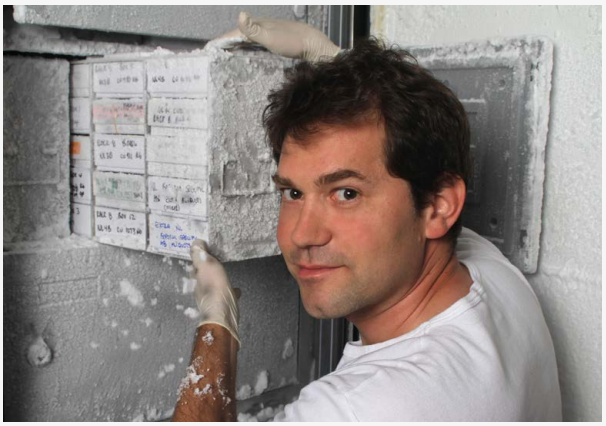

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RESEARCH GROUP TILMAN SCHIRMER

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 Dehio 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.

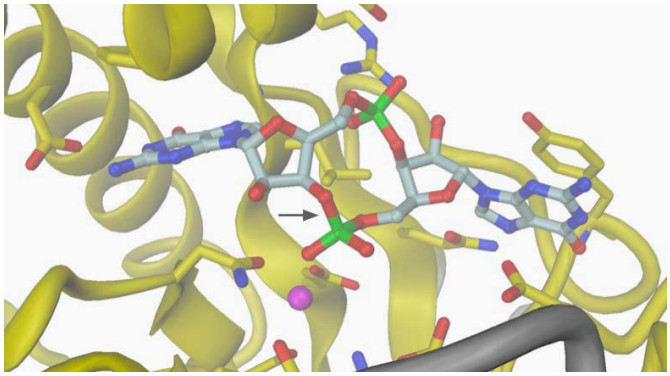

Fig. 1: Dinucleotide c-di-GMP bound to phosphodiesterase YkuI from B. subtilis. The scissile bond is indicated by the arrow, the catalytic magnesium ion (magenta) is found at the bottom of the binding site.

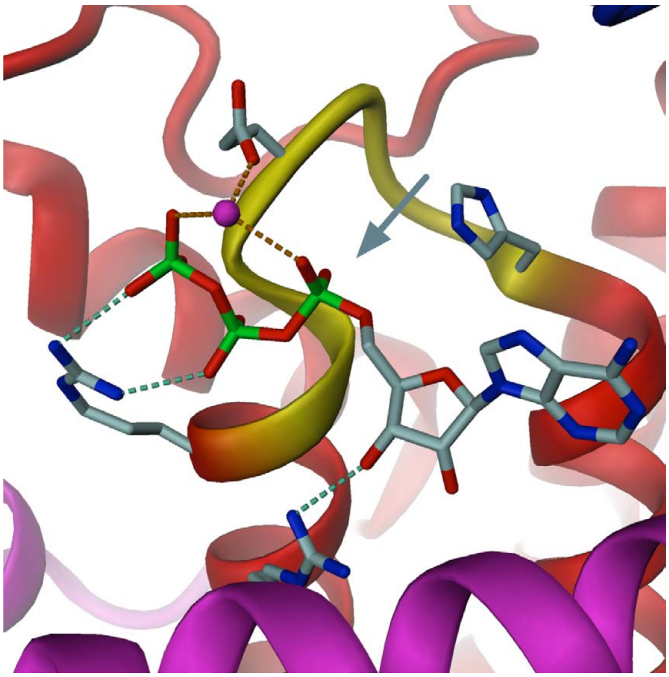

Fig. 2: An AMP transferase with Fic fold. Also shown, the ATP/ Mg++ in the putative active site. Adopted rom Palanivelu et al. (2011).

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.

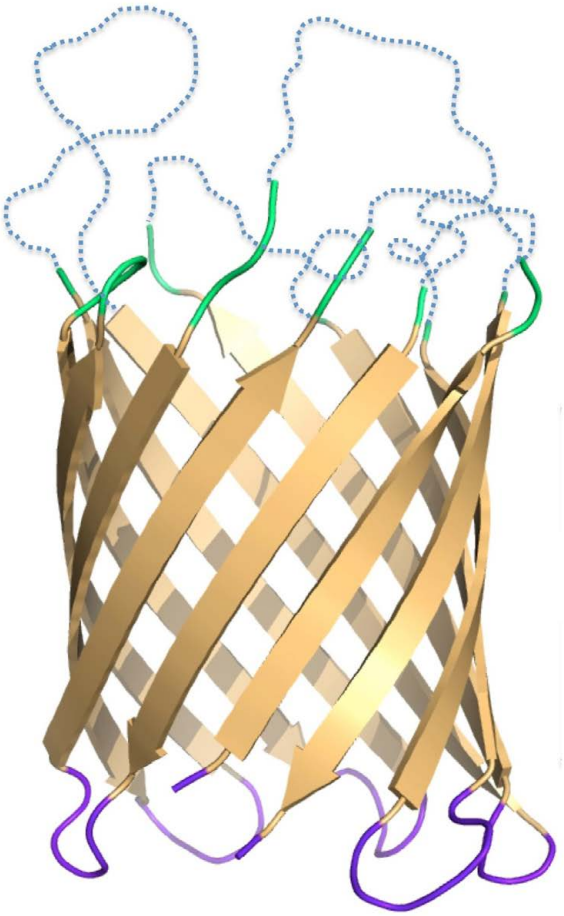

Fig. 3: KdgM porin folded to a small 12-stranded hollow ß- barrel.

Publications 2012

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RESEARCH GROUP CORA-ANN SCHOENENBERGER

Cytoskeletal proteins as mediators of structural and nanomechanical cell plasticity in health and disease

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 supramolecular actin assembly

A cellular machinery that is based on the reversible polymerization of globular nucleotide-bound actin protomers into polar microfilaments is a persistent feature from prokaryotes to higher vertebrates. While in bacteria actin-like proteins have evolved into a large family with divergent sequences and polymeric structures, eukaryotes express only a small number of highly conserved actins. It is all the more astounding that these closely related actins are able to carry out so many different functions at distinct cellular sites. A sheer endless variety of supramolecular actin structures is required to change cell architecture and function in response to signals from the environment. More than two hundred actin-binding proteins orchestrate the patterning of actin in space and time. These interactions, together with its inherent plasticity render actin one of the most versatile proteins in the eukaryotic cell, both with respect to structure and function. We strive to gain insight into less known forms of actin and their involvement in functional diversity.

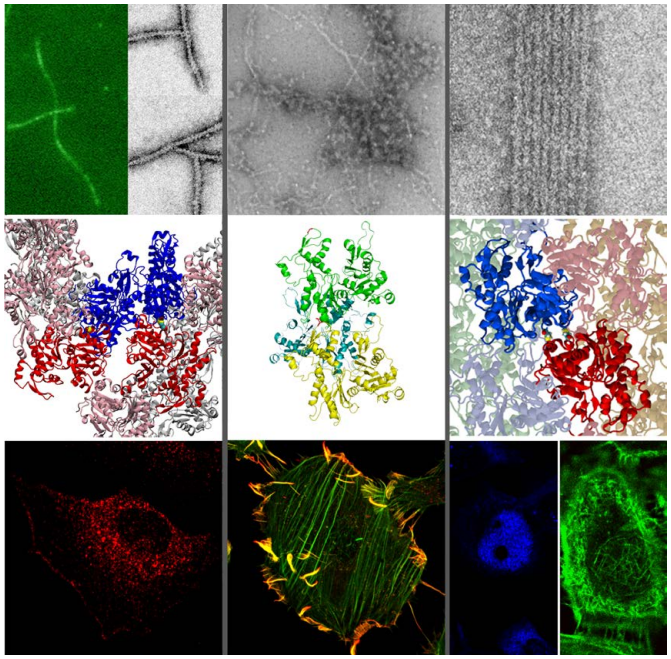

Fig. 1: Unconventional configura-tions and mutations confer structural plasticity to actin. Both contribute to the assembly of distinct supramolecular structures at different cellular sites. The forms of actin in the nucleus (bottom right) remain an enigma.

The vast majority of studies on cellular actin functions consider mainly two structural states, monomeric G-actin and F-actin filaments. However, figure 1 shows actin configurations that differ from monomeric G-actin and supramolecular actin structures that extend beyond classical F-actin. Such unconventional configurations are increasingly recognized as key factors in actin patterning. Biochemical crosslinking has revealed an actin dimer at the onset of polymerization in vitro with subunits arranged in an antiparallel orientation. At first sight, this configuration seems to be incompatible with the subunit interactions defining the geometry of mature F-actin. The 'lower dimer' (or LD), named for its relative migration on an SDS-polyacrylamide gel, is transient and with ongoing polymerization is replaced by the 'upper dimer' (UD) whose subunits are arranged like those in the F-actin filament.

To address the functional significance of the unconventional configuration, we have raised antibodies that specifically react with LD. Immunoelectron microscopy studies using synthetic actin structures revealed LD at sites where subunits contact each other in an antiparallel orientation. More importantly, immunofluorescence revealed the presence of LD in intact cells where it is associated with the endosomal compartment, the cell periphery and the nucleus.
To further elucidate the molecular mechanism by which the LD mediates actin patterning, we are monitoring the effects of LD on actin polymerization in real time by TIRF microscopy. We now have evidence that LD initiates the formation of branched actin structures.

Transformation of cellular architecture by an actin mutation

Mutation of Gly245 to Asp (actin-Asp) in β- actin renders fibroblasts tumorigenic. To understand how this unconventional actin modifies cellular architecture in the context of tumorigenic transformation, we examine sm9 fibroblasts, a Rat-2 derivative that expresses similar amounts of actin-Asp and normal actin, in 2D monolayers and in scaffold-free 3D cell culture models. Although the mutation interferes with polymerization properties in vitro, the ratio of G- to F-actin in sm9 cells is unchanged. Typical for malignant cells, sm9 exhibit extensive membrane ruffling in monolayers (*Fig. 1*) and in 3D spheroids (*Fig. 2*). Normal and actin-Asp accumulate in theses ruffles, which indicates altered cortical actin dynamics. In our current studies we address the effects of the modified actin structures on cellular function. In particular, we are interested in the relationship between actin-Asp and the migratory behavior of tumorigenic sm9 versus normal fibroblasts on flat substrates and in 3D constructs.

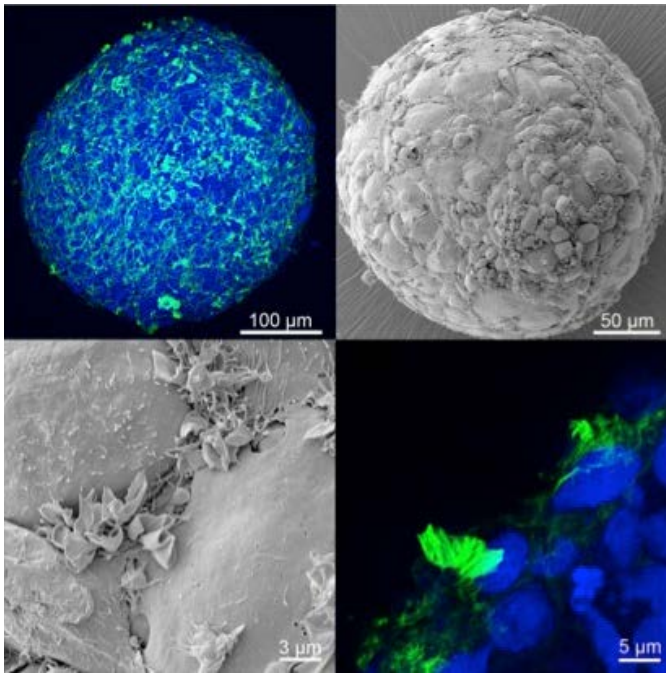

Fig. 2: Filamentous actin (green) is prominent in membrane ruffles of tumorigenic Rat-2-sm9 spheroids.

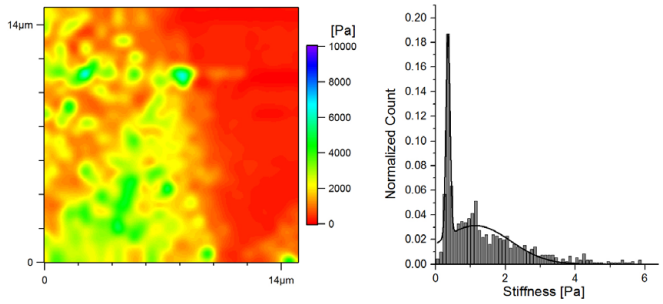

Fig. 3: Nanomechanical properties of tumors associated with a mutant actin-Asp. High resolution AFM stiffness maps (left) and corresponding histograms reveal (right) regions of distinct stiffness in Rat-2-sm9 tumor tissue.

Mechanical plasticity as indicator of tumor progression

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. Together with the Lim group, we use indentation-type atomic force microscopy (AFM) to quantitatively probe mechanical properties of living cells and tissues at unprecedented subcellular resolution. We have recently shown that AFM stiffness mapping of human and mouse breast biopsies reveal unique mechanical fingerprints that help define the stages of cancer progression. Our focus lies in studying specific contributions of cytoskeletal components to mechanical function. Sm9 cells represent an excellent model system since tumorigenic transformation is related to mutant actin-Asp and nanomechanical consequences are revealed by comparison with parental Rat-2 stiffness profiles. Because the characteristics of cancer are more appropriately reflected by a 3D tissue organization, we use cultured sm9 spheroids shown in figure 2 and sm9 tumors grown as xenografts in nude mice to investigate nanomechanical changes associated with tumorigenic transformation. At high resolution, stiffness maps of sm9 tumor tissues reveal individual features at the cellular level (*Fig. 3*). One topic we are intensely pursuing is the correlation between stiffness and increasing hypoxia in tumorigenic transformation.

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Publications 2012

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RESEARCH GROUP TORSTEN SCHWEDE

Computational structure biology

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 SWISSMODEL 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.

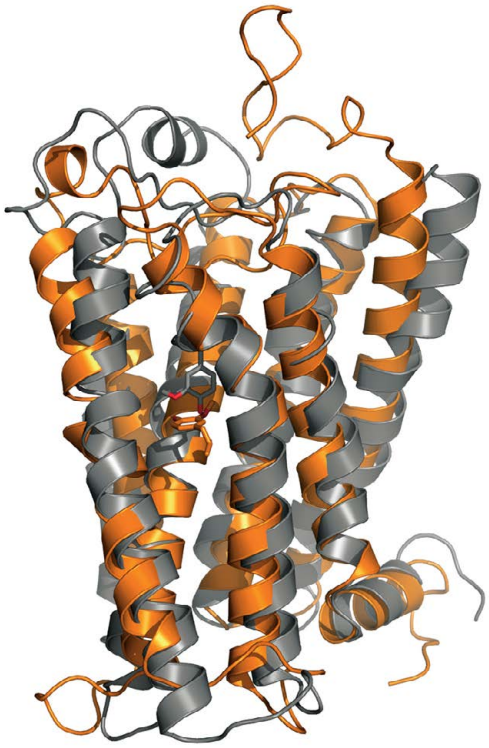

Fig. 1: Comparative models of the mouse Eugenol olfactory receptor based on ß2-adrenergic receptor and bovine rhodopsin template structures. (Photo courtesy of Morena Speafico).

Molecular modeling of protein-ligand interactions

Dengue fever is a viral disease that is transmitted between human hosts by *Aedes mosquitoes*, particularly *Aedes aegyptii*. 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.

RESEARCH GROUP TORSTEN SCHWEDE

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. 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.

Publications 2012

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RESEARCH GROUP ANNA SEELIG-LÖFFLER

Unraveling substrate recognition and transport by ATP binding cassette (ABC) transporters

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. Overexpression of ABCB1 leads to multidrug resistance (MDR), that is, to a resistance towards all drugs that are substrates for ABCB1. 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 π-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.

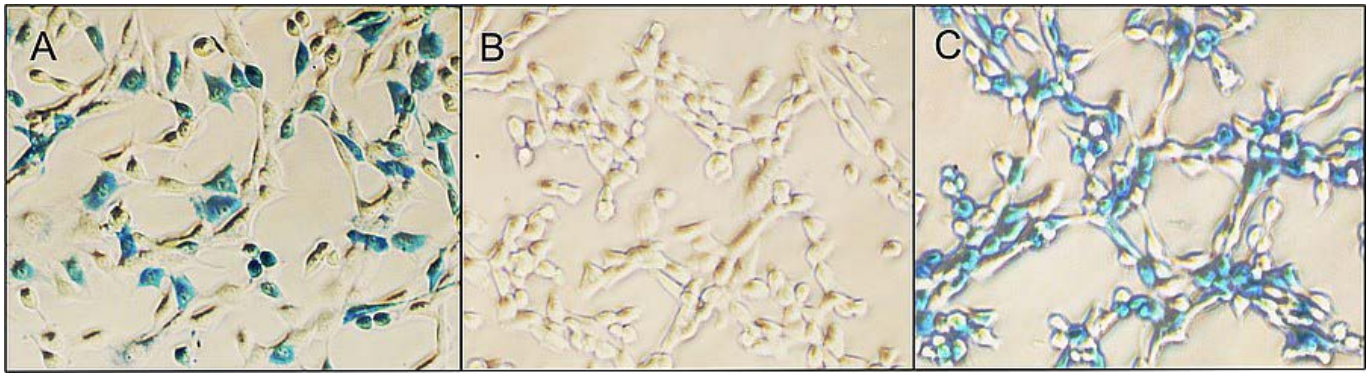

Fig. 1: A: Intracellular uptake of methylene blue (MB) by wildtype mouse embryo fibroblasts (NIH3T3). B: P-glycoprotein in MDR1-transfected embryo fibroblasts (NIH-MDR1-G185) prevents the intracellular uptake of MB. C: Cyclosporin A inhibits P-glycoprotein in MDR1-transfected cells and the intracellular uptake of MB is again possible. (Light micrographs.

RESEARCH GROUP ANNA SEELIG-LÖFFLER

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 Fig. A-C*). Our analyses revealed that the lipid bilayer membrane plays an important 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.

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RESEARCH GROUP JOACHIM SEELIG

Cell penetrating peptides, Alzheimer peptides and membrane-induced protein folding

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 mi-RNA with CPPs for selective and specific target addressing. We showed by a variety of physicalchemical 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 glycosamionosulfates using high-sensitivity titration calorimetry (ITC), dynamic light scattering (DLS) and fluorescence spectroscopy. Most important was the application of this physicalchemical 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.

We further showed that the HIV-1 TAT protein transduction domain has a high affinity for double stranded DNA. The binding of this CPP leads to DNA condensation and, in parallel, a distinct reduction of fluorescence intensity is observed. This change in fluorescence quantum yield impedes the identification of uptake routes and makes the quantitative comparison of uptake efficiency by fluorescence microscopy rather difficult. As the aggregation of glycosaminoglycans on the cell surface could be the starting point of endocytosis we have studied the binding and clustering of various mono-and multivalent cell penetrating peptides and non-peptidic compounds to heparin with ITC and DLS. Finally, we were interested if antimicrobial peptides may also take advantage of sulfated glycosaminoglycans for cell entry. We investigated in detail

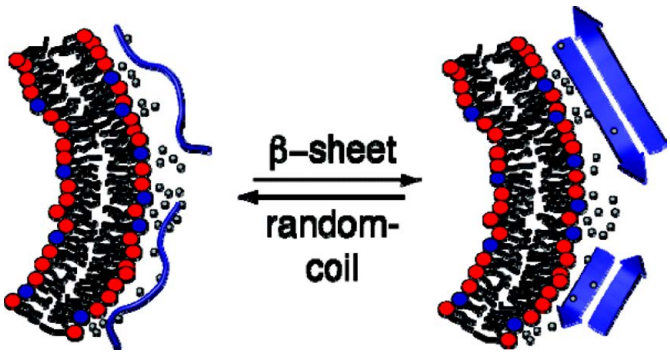

Fig. 2: Aggregation of Alzheimer peptides on the surface of a membrane.

melittin and melittin-analogs and found that melittin binds strongly to sulfated glycosamins. However, melittin appears to an exception among the amphipathic antimicrobial peptides as other peptides such as magainin 2 or nisin Z do not show such an interaction.

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-coilto-α-helix 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 membraneinduced *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.

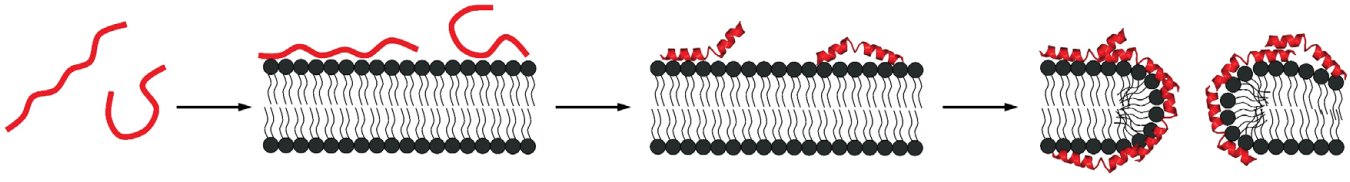

Fig. 1: Model of melittin interaction on the lipid membranes.

RESEARCH GROUP JOACHIM SEELIG

Related projects

We are strongly interested in the physicalchemical 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 addition, 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.

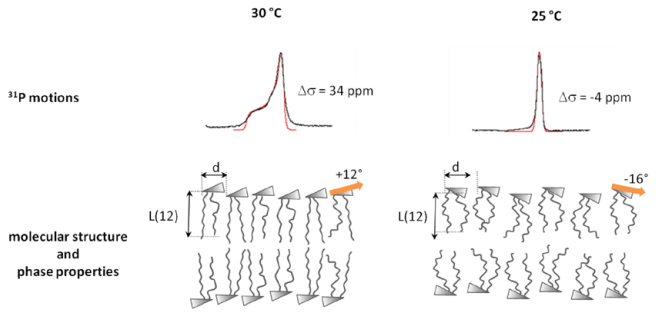

Fig. 3: ***P-NMR as a method to study membrane structure.***

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RESEARCH GROUP ANNE SPANG

Intracellular transport in yeast and worms

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 organellar 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

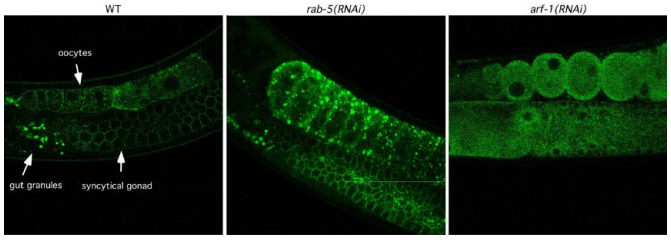

Fig. 1: The architecture of the Golgi is disturbed upon knockdown of the small GTPases RAB-5 and ARF-1. Worms expressing the Golgi marker UGTP-1: GFP (green) under the pie-1 promoter, which drives expression in the gonad and in early embryos were subjected to RNAi by feeding. The distribution of UGTP-1::GFP was analyzed by confocal microscopy. The Golgi morphology was greatly altered upon RNAi against RAB-5 and ARF-1. In particular, arf-1(RNAi) let to a dispersal of Golgi structures.

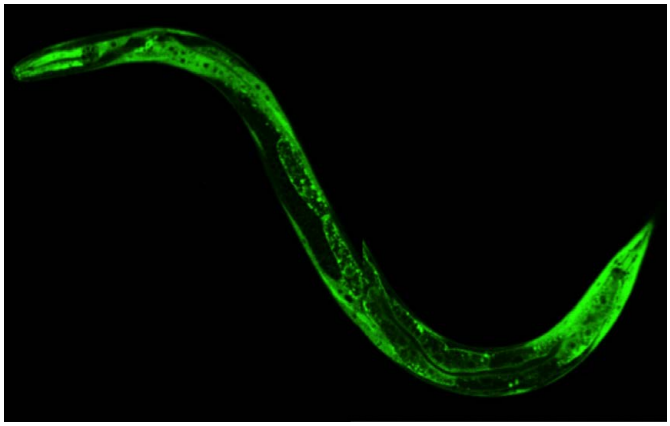

Fig. 2: Adult worm expressing SAND-1:GFP.

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 (Aguilera 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, Zanolari et al., 2010, Rockenbauch et. al., 2012). We have found more cargo proteins that follow this pathway and are in the process of investigating the cargo-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 mislocalized 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 multivesicular 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 performed screens to identify mRNAs that are restricted to certain sites and are currently investigating the mechanism of the localization.

Prof. Dr. Anne Spang
» **further information**

Publications 2012

Rockenbauch, U.; Ritz, A.M.; Sacristan, C.; Roncero, C.; Spang, A. (2012). The complex interactions of Chs5p, the ChAPs, and the cargo Chs3p. *Molecular Biology of the Cell, 23*(22), 4402-15.

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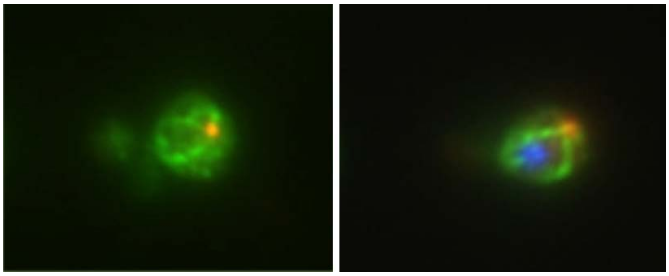

Fig. 3: Asymmetrically localized mRNA to the distal pole of yeast cells. FISH/IF picture of 2 yeast cells. mRNA is in red, actin in green and DNA in blue.

RESEARCH GROUP ANNE SPANG

Human Frontier Science Program: In search of conserved mRNA localization and anchoring mechanisms

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.

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RESEARCH GROUP MARTIN SPIESS

Topogenesis and intracellular sorting of membrane proteins

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*.

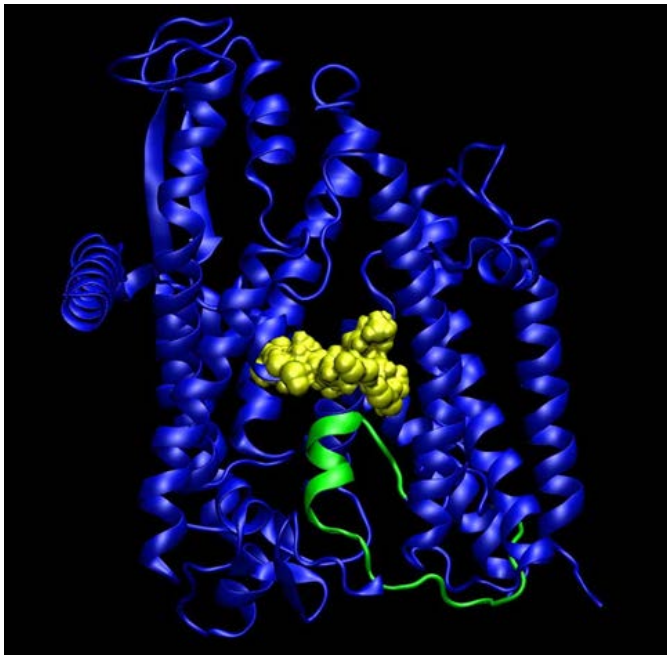

Fig. 1: "One ring to rule them all": View of the interior of the closed Sec61 translocon with the plug domain in green and the constriction ring in gold (Sefer Baday).

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 protein translocation 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*). By random or targeted mutagenesis, we explore the contributions of the translocon to signal acceptance and orientation, as well as to transmembrane domain integration. The plug domain stabilizes the closed state of the translocon and defines the stringency of signal recognition, whereas the constriction ring defines the hydrophobicity threshold for membrane integration. In collaboration with Dominic Höpfner (Novartis), we identified novel fungal translocation inhibitors by chemogenomic profiling and selected for resistant translocon mutants to study the inhibitors' mode of action.

Post-Golgi protein sorting

Endosome identity, morphology, and transport are regulated by rab GTPases and their effectors. We are studying the role of rabaptin-5, an effector of rab4 and rab5, that associates with rabex5, the exchange factor of rab5. Based on mutational analysis, rabaptin-5 is found to control endosome morphology without affecting transferrin transport (determined by automated microscopy) in a manner that is incompatible with the prevailing model of rab5 feed-forward loop.

Little is known about how proteins exit the *trans*-Golgi. We use sulfation, a *trans*-Golgi-specific modification, to characterize the exit pathway and kinetics to the cell surface. If necessary, proteins of interest are tagged to introduce tyrosine-sulfation sites or short sequences for the attachment of (heavily sulfated) glycosaminoglycans (GAG). In this manner, we found GAG-attachment to accelerate exit kinetics and to change the exit pathway of model proteins. Similarly, the proteoglycan form of the amyloid precursor protein exits in a manner distinct from that of GAG-free splice variants.

Sulfation is a *trans*-Golgi-specific modification useful to study post-Golgi traffic. 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.

RESEARCH GROUP MARTIN SPIESS

Diabetes insipidus: a degenerative 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 mutant pro-vasopressin spontaneously formed fibrils *in vitro*. Dominant *Diabetes insipidus* thus belongs to the group of neurodegenerative diseases associated with fibrillar protein aggregates. We identified the vasopressin nonapeptide in the precursor sequence to be primarily responsible for aggregation in the ER, i.e. the same sequence that had been proposed to be responsible for amyloid aggregation into secretory granules at the *trans*-Golgi. The sequence physiologically important for cargo aggregation into the regulated secretory pathway thus is responsible for pathological aggregation of mutant precursors in the ER.

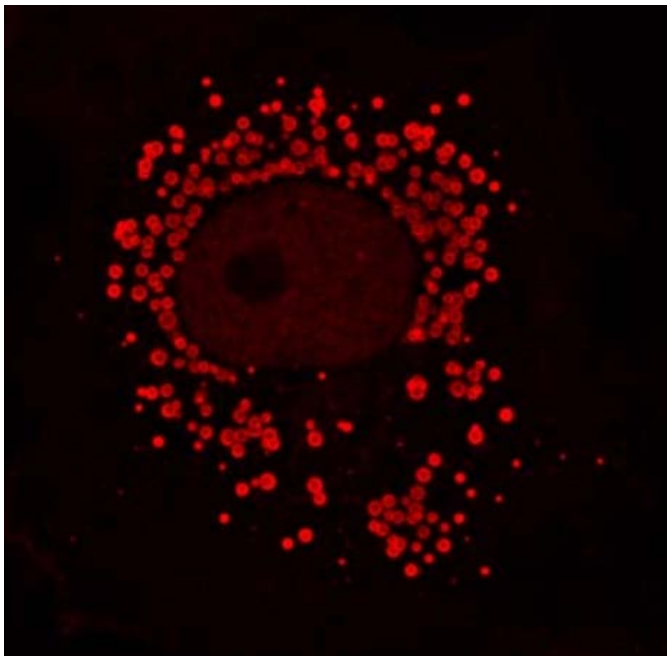

Fig. 2: Pro-vasopressin aggregates in COS-1 cells.

Prof. Dr. Martin Spiess
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Publications 2012

Kocik, Lucyna; Junne, Tina; Spiess, Martin (2012). Orientation of Internal Signal-Anchor Sequences at the Sec61 Translocon. *J Mol Biol, 424*(5), 368-378.

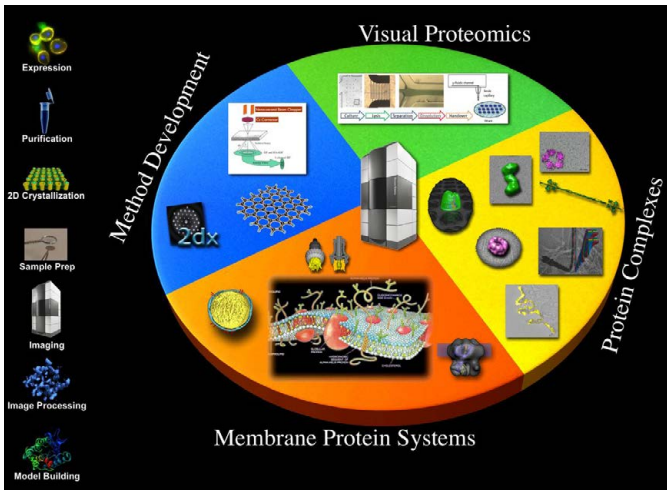
RESEARCH GROUP HENNING STAHLBERG

The Center for Cellular Imaging and Nano Analytics (C-CINA)

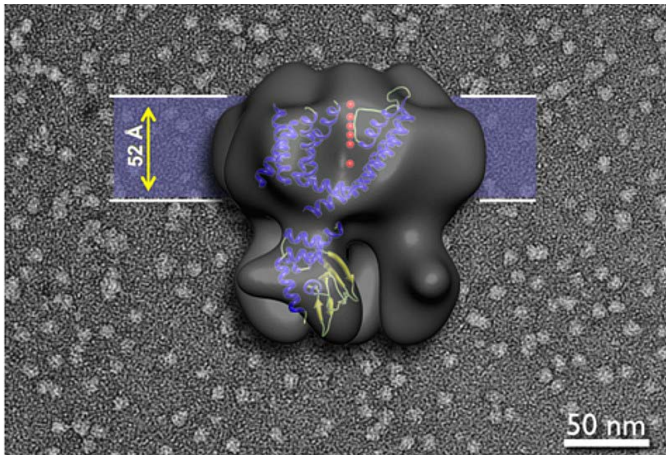
The Center for Cellular Imaging and Nano Analytics (C-CINA) is studying membrane protein systems at different size and length scales with a combination of various light and electron microscopy methods. C-CINA is located in the D-BSSE building in the northern part of Basel, and is supported by the Swiss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



C-CINA studies Membrane Protein Systems and also other protein complexes. We develop methods for sample preparation, microscopy hardware, and image analysis software. In a systems-biology project for Visual Proteomics we are developing a tool for the cellular total content analysis by microscopy and ther methods.



The cyclic nucleotide gated potassium channel MloK1 is studied in C-CINA by single particle electron microscopy and by cryo-EM of twodimensional membrane crystals.

Visual proteomics

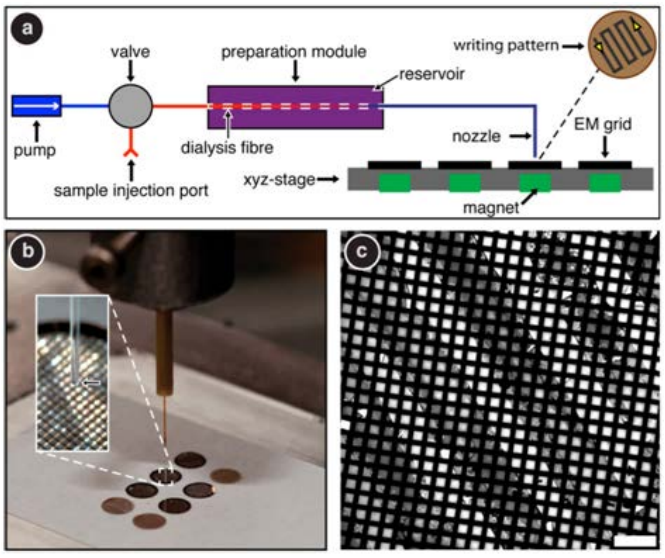
As part of the SystemsX.ch funded project, we develop a visual proteomics platform to study the 3D structure, and size and mass distribution of the proteome of a biological cell. In collaboration with the Hierlemann group at the D-BSSE, we develop a microfluidics setup to pick individual cells, lyse 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 high-resolution 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 mico-patterning 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.

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» **further information**

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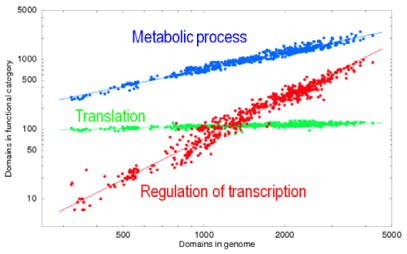
RESEARCH GROUP ERIK VAN NIMWEGEN

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 highthroughput biological data sets such as genome sequences, genome-wide expression data, genome-wide protein binding data, etcetera. 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 functioning and evolution of gene regulatory systems in *E. coli*, with a particular emphasis on the analysis of the role of noise in gene expression regulation.



GO term	Exponent α_c
translation	0.08 ± 0.01
DNA replication	0.41 ± 0.04
DNA repair	0.61 ± 0.04
metabolic process	0.80 ± 0.01
carbohydrate metabolic process	1.10 ± 0.06
transport	1.30 ± 0.06
porphyrin metabolic process	1.66 ± 0.16
regulation of transcription	1.94 ± 0.04
secretion	1.98 ± 0.20
DNA recombination	2.23 ± 0.23

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.

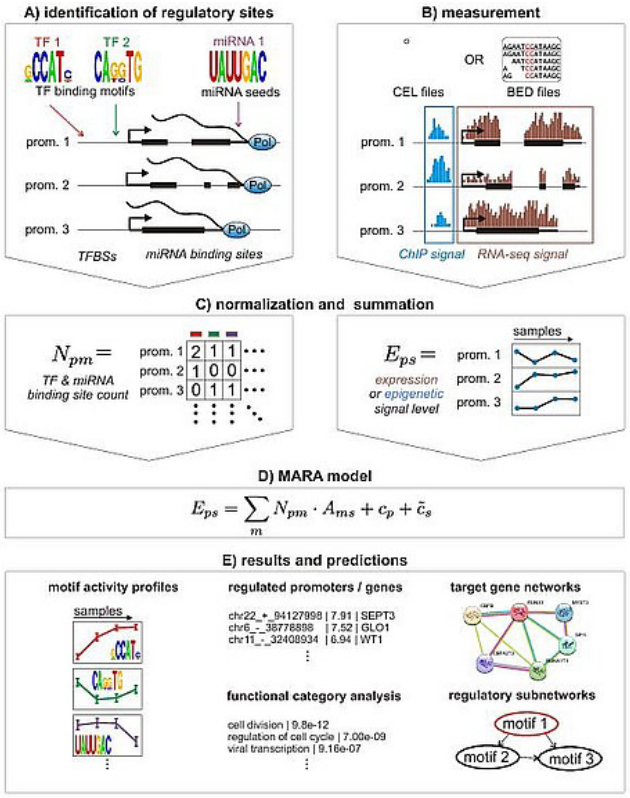


Fig. 2: Outline of Integrated System for Motif Activity Response Analyis (ISMARA). ISMARA uses computationally predicted regulatory sites for hundreds of transcription factors and miRNAs (panel A) to explain observed gene expression or chromatin state data across a set of biological conditions (panel B) using a linear model (panels C and D). As a consequence, ISMARA automatically predicts the key regulators acting in the system under study, their genome-wide target genes, functional categories enriched among these target genes, and direct interactions between the key regulatory motifs (panel E). The system is entirely automated and allows experimental users to simply upload their data and obtain predictions on the key regulatory interactions acting in their system of interest.

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 ongoing projects we are aiming to understand the origins of these laws on the one hand, and to connect the genomic evolution to evolution at the phenotypic level on the other hand. For the latter we are currently focusing on the analysis of joint genomic and phenotypic evolution observed in wild strains *E. coli*. Our final aim is to uncover statistical laws describing the way natural selection shapes evolution in bacteria.

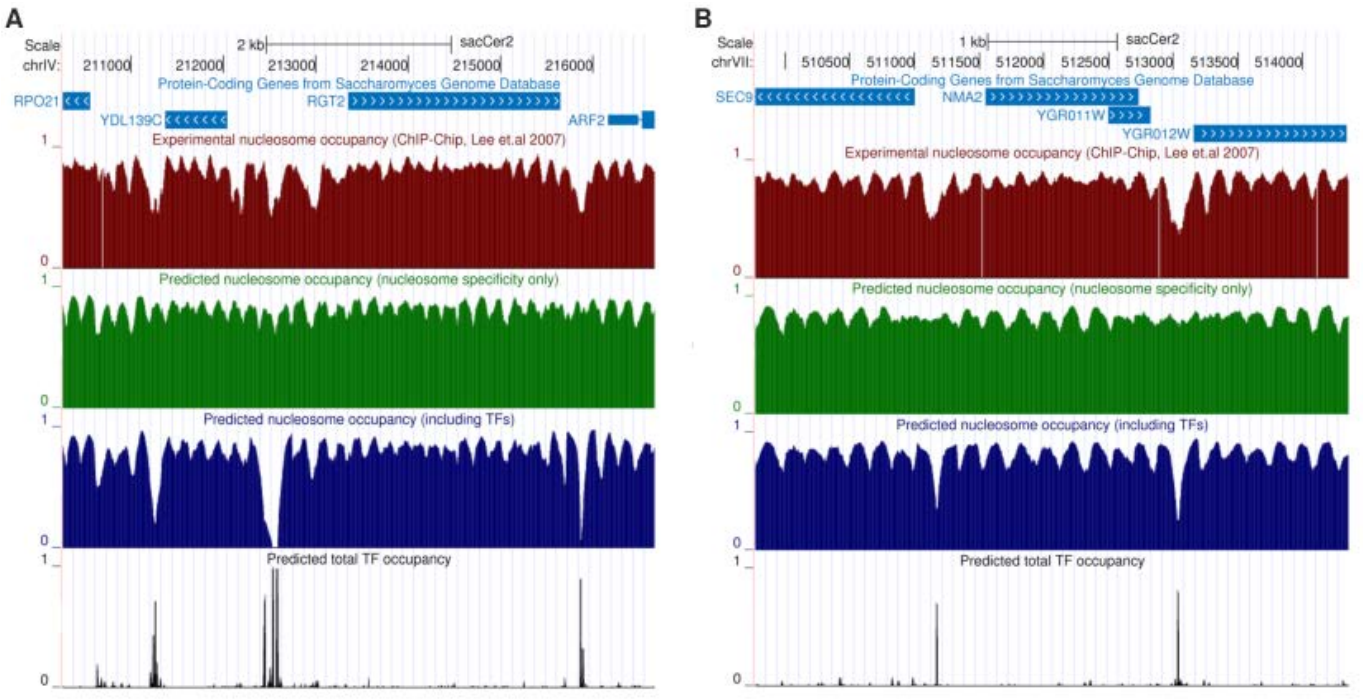


Fig. 2: Transcription factor binding determines nucleosome-free regions in Saccharomyces cerevisiae. Shown are the measured (red) and computationally predicted (green and blue) nucleosome coverage as well as transcription factor binding (black) in two regions of the yeast genome. The green profile corresponds to a model which takes into account the sequence preferences of nucleosomes but does not include transcription factors whereas the blue profile corresponds to a model that incorporates the effects of transcription factor binding. Only the model including transcription factors can explain regions with significant reduction in nucleosome coverage.

Structure, function, and evolution of genome-wide regulatory networks

A core interest of our group is the design, functioning, and evolution of the regulatory networks that control genome-wide gene expression patterns. Over the years our group has developed a collection of probabilistic methods that combine comparative sequence analysis with analysis of next-generation sequencing data to predict functional regulatory sites on a genome-wide scale. Among the methods we developed are the PhyloGibbs and MotEvo algorithms for the annotation of transcription factor binding sites. We have also developed algorithms for predicting target sites or miRNA, including the recent MIRZA algorithm which explicitly models the biophysics of the interaction between miRNAs and their mRNA target sites. All our genome-wide annotations of regulatory sites, in model organisms ranging from *E. coli* to human and mouse, are available through an easy-to-use webinterface at our SwissRegulon website (swissregulon.unibas.ch).

From regulatory sequence to gene expression dynamics to attractors of the regulatory network

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. Gene expression is an inherently stochastic process and several wet-lab projects aim at understanding how the DNA sequences of individual promoters, together with environmental conditions, determine the kinetic parameters of this stochastic process, and how this in turn determines expression dynamics across a population of cells. These projects involve quantitation of expression dynamics within individual cells using microfluidics devices in combination with time lapse microscopy, as well as FACS measurements.

RESEARCH GROUP ERIK VAN NIMWEGEN

From regulatory sequence to gene expression dynamics

One of the main areas of research, involving the majority of the theoretical researchers in the group, is the the automated modeling of gene expression and chromatin state dynamics in terms of genome-wide predicted regulatory sites. In recent years we have found that, combining our genome-wide regulatory site predictions, with a simple linear modeling approach, and applying this to gene expression or chromatin state data, we are able to *ab initio* predict the key regulatory factors active in the system under study, how these regulators change their activities across conditions, and the sets of genes targeted by each regulator. In collaborations with other experimental groups we have successfully applied this methodology to a number of model systems, and we have recently completely automated the methodology into a web service (ISMARA) which allows researchers to automatically analyze their high-throughput data.

Research from our group has suggested that one of the key differences between the regulatory networks of prokaryotic and eukaryotic cells, is that whereas promoters in prokaryotes are limited to being regulated by a small number of regulatory factors, in complex eukaryotes a single gene can be regulated by many different regulatory factors. We believe that chromatin structure, which is unique to eukaryotes, plays a key role in allowing the much more complex structures of regulatory networks in eukaryotes. Consequently, one of our research interests is elucidating the principles of interplay between transcription factors and chromatin structure in eukaryotic gene regulation. Our efforts in these area are taking place mainly in the context of the SystemsX.ch CellPlasticity project. For example, we have recently shown that our MARA methodology can be extended to predict transcription factors that recruit specific chromatin modifications in a dynamic fashion. Another major area of interests is studying how the competition between nucleosomes and transcription factors for binding to DNA, determines genome-wide DNA accessibility patterns. A comprehensive theoretical study of the role of transcription factors in determining nucleosomefree regions in the yeast *Saccharomyce cerevisiae* has shown that transcription factors play a key role in causing nucleosomes to be expelled from specific promoter regions. Moreover, our study strongly suggests that not all transcription factors contribute to nucleosome eviction, but that this activity is limited to a specific class of transcription factors which recruit chromatin modifiers. Finally, given the highly stochastic nature of gene expression, and the small number of regulatory factors involved in individual cells, it seems puzzling that single cells can stably maintain cellular phenotypes. One area of more theoretical study in our group is analysis of models of networks of mutual interactions between regulatory factors that aim to elucidate the mechanisms behind the stability of cellular phenotypes in the face of the strong stochasticity of the underlying gene expression mechanism.

RESEARCH GROUP ERIK VAN NIMWEGEN

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RESEARCH GROUP KASPAR VOGT

Excitation and inhibition in neural networks imaging dendritic signals

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 signaltonoise 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 GABAA 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.

Imaging excitatory signals

Excitatory synaptic signals are often accompanied by calcium transients – either through the opening of voltagegated 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.

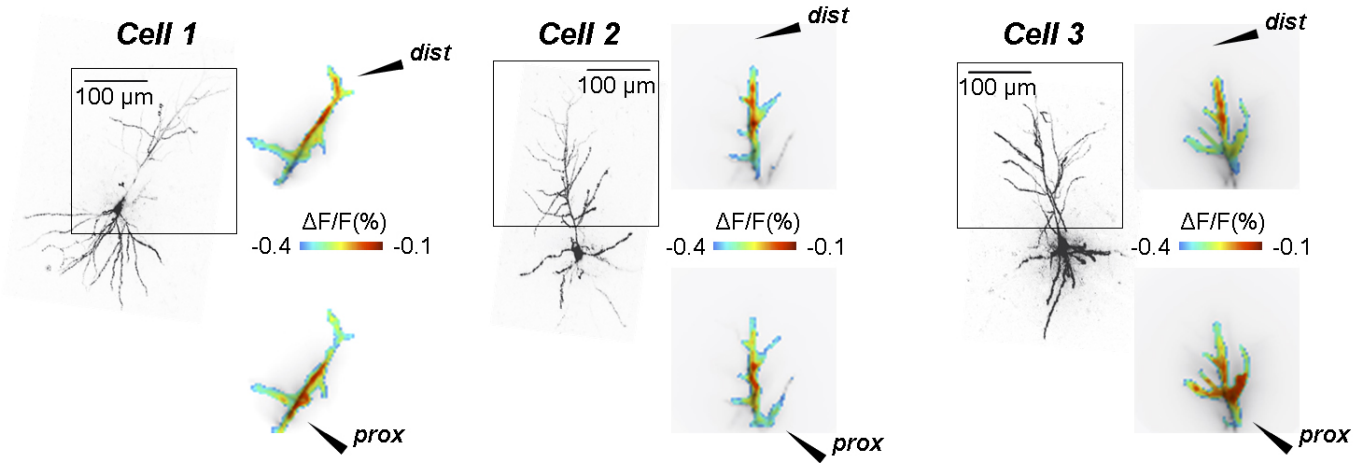

Fig. 1: Voltage response of three different CA1 pyramidal cells to different GABAergic inputs. A distal (dist) and proximal (prox) stimulus electrode were placed in the vicinity of three CA1 pyramidal cells. The cells were filled with voltagesensitive dye and then imaged. The black images show the morphology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

RESEARCH GROUP KASPAR VOGT

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 Biozentrum, 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 of 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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RESEARCH GROUP MIHAELA ZAVOLAN

The role of small regulatory RNAs in regulating gene expression

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.

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 EIMMo, 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 mRNAs 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.

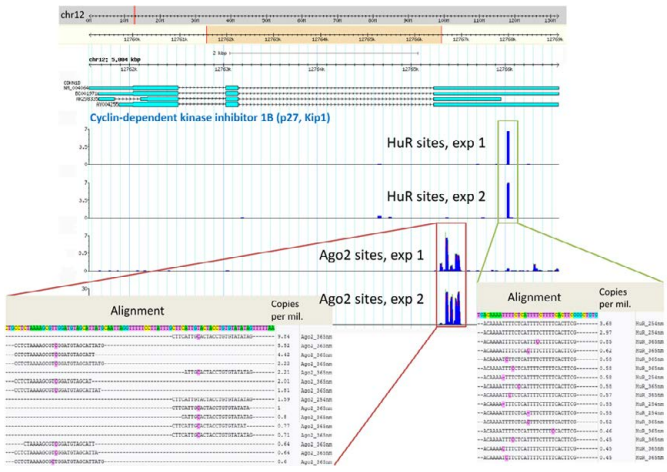

Fig. 1: Location of binding sites of Argonaute 2 and HuR proteins in transcripts of the p27 cell cycle regulator. The binding sites were determined by crosslinking the Argonaute 2 and HuR proteins to mRNAs with UV light, isolation and deep sequencing of RNA fragments that were bound by these proteins. 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. Crosslinking induces diagnostic T-to-C mutations.

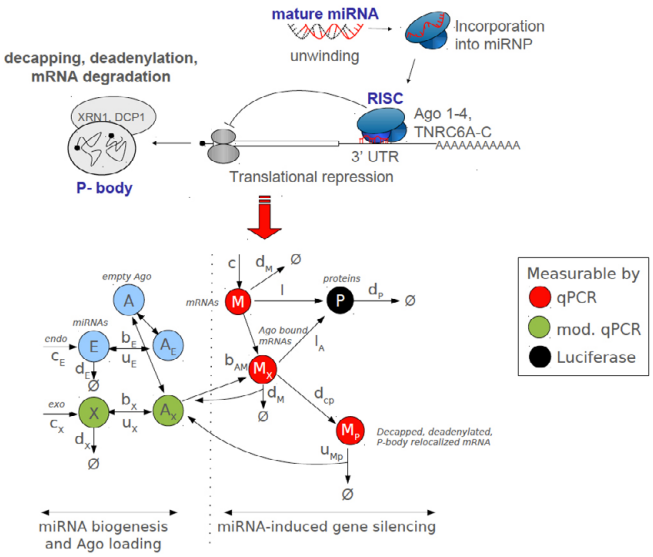

Fig. 2: A detailed model of miRNA-dependent regulation. It serves as a working model in our attempt to quantify the magnitude of the effects that miRNAs exert at different steps of mRNA processing and translation.

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). Very recently, the CLIP data was used to infer a biophysical model of miRNAtarget interaction that can be used to infer target sites of individual miRNAs from Ago-CLIP data (collaboration with Erik van Nimwegen). By modeling the combined effects of transcription factors and miRNAs on the transcriptome of various cells, the group aims to uncover regulatory cascades that are triggered by miRNAs in the context of various differentiation processes.

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 has mapped binding sites of 3′ end processing factors transcriptomewide and related these to 3′ end processing sites that were identified in the same cell type as well as in cells in which various 3′ end processing factors underwent siRNAmediated knockdown. With this approach, two components of the mammalian cleavage factor I (CFIm) have been found to be important for the regulation of 3′UTR lengths. The underlying mechanism and its consequences for the cell function are currently under study.

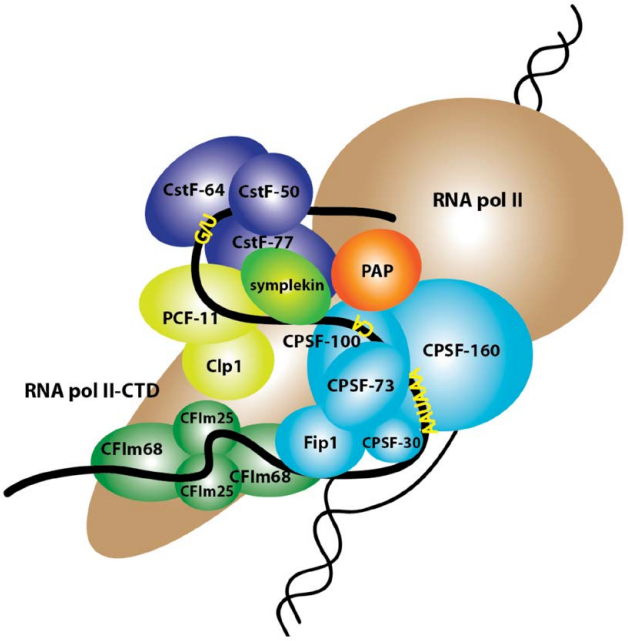

Fig. 3: Sketch of the components of the core 3′ end processing complex. By crosslinking and immunoprecipitation of these components together with the RNA fragments that are bound to them, we are attempting to unravel the grammar of 3′ end recognition and processing in mammalian cells.

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.

RESEARCH GROUP MIHAELA ZAVOLAN

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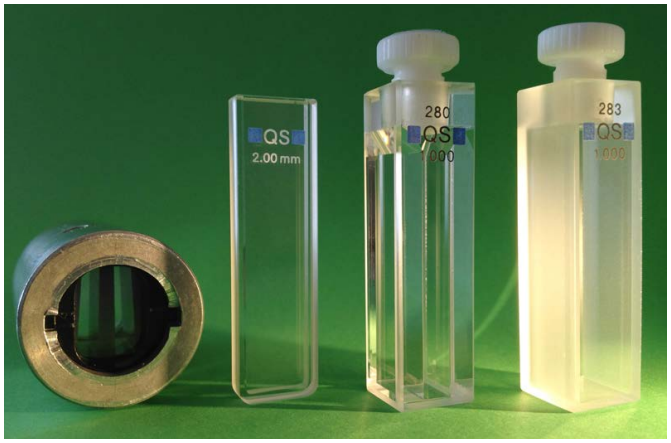
BIOPHYSICS FACILITY (BF)

Biophysical answers to biological questions

The Biophysics Facility supports researchers in the use of sophisticated instrumentation to measure the interactions, reactions, stability and size of biological molecules with accuracy and precision.

A growing range of techniques has been developed to characterise the physical properties of biological macromolecules such as proteins and nucleic acids. These biophysical techniques enable us to investigate how those molecules gain and retain structure, perform reactions, and interact when they assemble into larger structures, in order to play their essential roles in living cells.



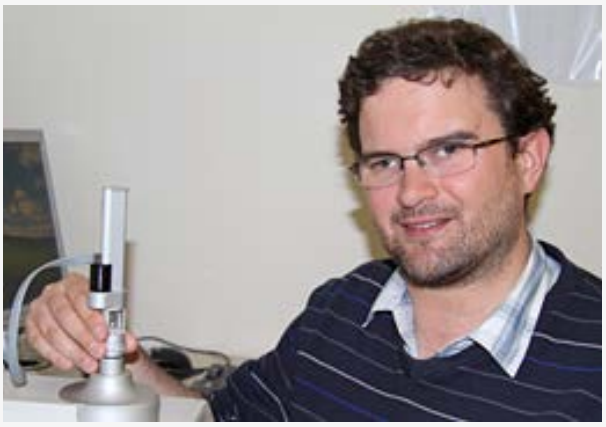
Cells made of quartz glass are used to hold biological samples for absorbance, circular dichroism and fluorescence measurements.

Choosing the best methods to answer your question

We identify the best combination of methods to answer each question. The facility has instruments for micro-calorimetry, circular-dichroism and fluorescence spectroscopy, surface plasmon resonance, analytical ultracentrifugation and light-scattering measurements. A few examples of typical applications are:

- Measuring the energies of macromolecular interactions
- Determining the size and shape of complexes
- Assessing the impact of mutations on protein structure and stability
- Screening small molecules libraries to identify chemical probes that bind to a specific macromolecular target

We support researchers who wish to use these techniques, from the first stages of experimental design to the final stages of data interpretation and presentation. Depending on individual requirements, we can perform experiments for users or train them to operate instruments and to collect high-quality data. We are also responsible for maintaining and improving the instruments to meet the future needs of the Biozentrum.



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Publications 2012

Adams, Cassandra J; Pike, Ashley C W; Maniam, Sandra; Sharpe, Timothy D; Coutts, Amanda S; Knapp, Stefan; La Thangue, Nicholas B; Bullock, Alex N (2012). The p53 cofactor Strap exhibits an unexpected TPR motif and oligonucleotide-binding (OB)-fold structure. *Proceedings of the National Academy of Sciences of the United States of America*, (10), 3778-83.

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IMAGING CORE FACILITY (IMCF)

Imaging Core Facility at the Biozentrum

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.

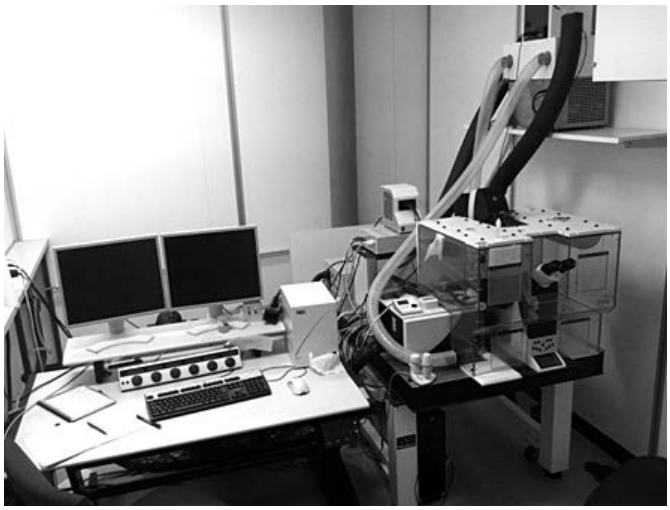

Fig. 1: Inverted Leica SP5 I system with resonance scanner for fast scanning (live cell imaging), multiphoton laser, and incubation chamber.

Fig. 2: PerkinElmer Ultraview with dual camera setup for high speed imaging.

IMAGING CORE FACILITY (IMCF)

Imaging Core Facility at the Biozentrum

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 high speed imaging
- 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
- FiJi/ImageJ

For reconstruction of 3D multi-position stacks (registration, stitching):

- XuvTools

For image analysis:

- Python / SciPy / NumPy
- 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.

After careful evaluation of the different techniques, the IMCF of the Biozentrum is planning to provide super-resolution techniques at the facility. Super-resolution microscopy will open up a new dimension for research at the cellular and subcellular level. Hitherto non-resolvable small subcellular structures such as centrioles, lipid rafts, neuronal dendrite spines, nuclear pore complexes, bacterial polar complexes, and many other macromolecular structures with less than 200nm in diameter - so far exclusively accessible by electron microscopy - will be visualized by light microscopy, and eventually live cell imaging.

Publications 2012

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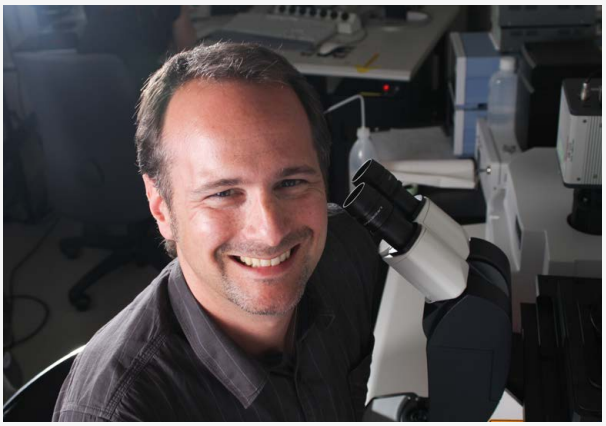
Nitschké, Maximilian; Aebischer, David; Abadier, Michael; Haener, Simone; Lucic, Matije; Vigl, Benjamin; Luche, Hervé; Fehling, Hans Jörg; Biehlmaier, Oliver; Lyck, Ruth; Halin, Cornelia (2012). Differential requirement for ROCK in dendritic cell migration within lymphatic capillaries in steady-state and inflammation. *Blood*, *120*(11), 2249-58.



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PROTEOMICS CORE FACILITY (PCF)

Proteomics at the Biozentrum

Proteomics rapidly evolves from a discoveryoriented 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.

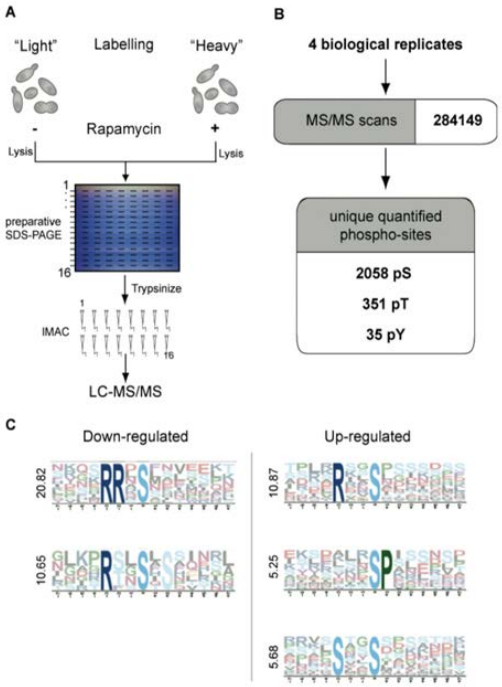

Fig. 1: Quantitative analysis of the rapamycin-sensitive phosphoproteome by SILAC. A) Two yeast cultures are metabolically labeled with normal or isotopically labeled Lysine and Arginine (heavy culture). The heavy culture is treated for 15 minutes with rapamycin. Cell lysates mixed in a ratio of 1:1 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 down-regulated and upregulated phosphopeptide sequences. Motifs are ranked from top to bottom according to their score.

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 (Jenö 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-nLCsystem (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

PROTEOMICS CORE FACILITY (PCF)

Sample preparation and fractionation instruments

- 3100 OFFGEL Fractionator 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.

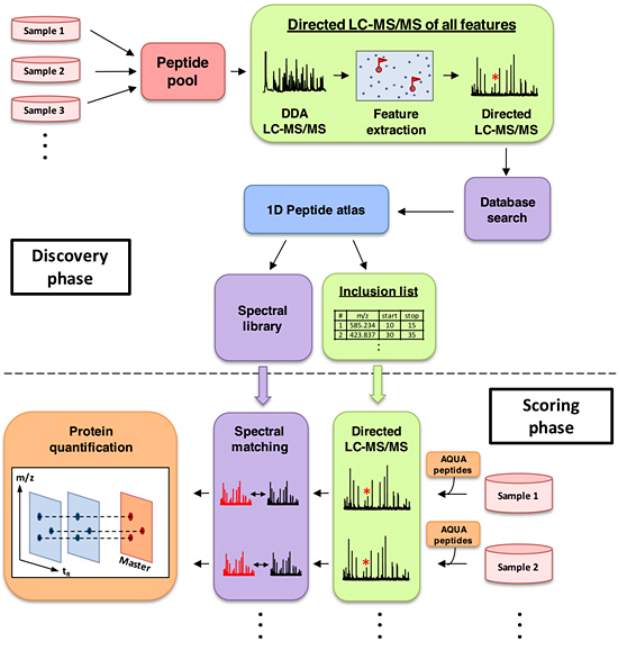

Fig. 2: Global protein profiling workflow. In the first phase of the study (discovery phase), the peptide samples representing different cell states were mixed and analyzed by data-dependent acquisition (DDA) followed by directed one-dimensional LCMS/MS. To achieve comprehensive proteome coverage, all 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.

PROTEOMICS CORE FACILITY (PCF)

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Barat, Somedutta; Willer, Yvonne; Rizos, Konstantin; Claudi, Beatrice; Mazé, Alain; Schemmer, Anne K; Kirchhoff, Dennis; Schmidt, Alexander; Burton, Neil; Bumann, Dirk (2012). Immunity to Intracellular Salmonella Depends on Surface-associated Antigens. *PLoS pathogens*, 8(10), e1002966.

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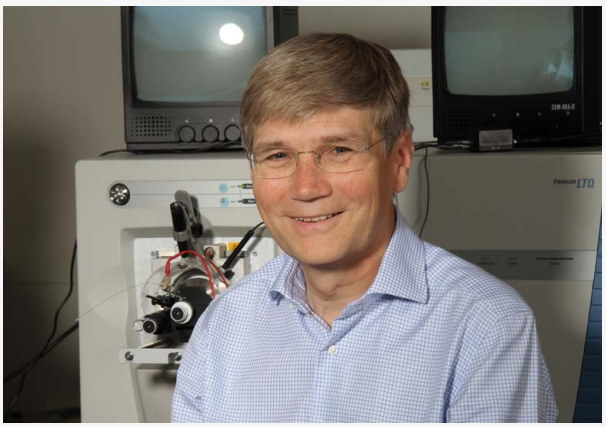
Dr. Alexander Schmidt
» **further information**



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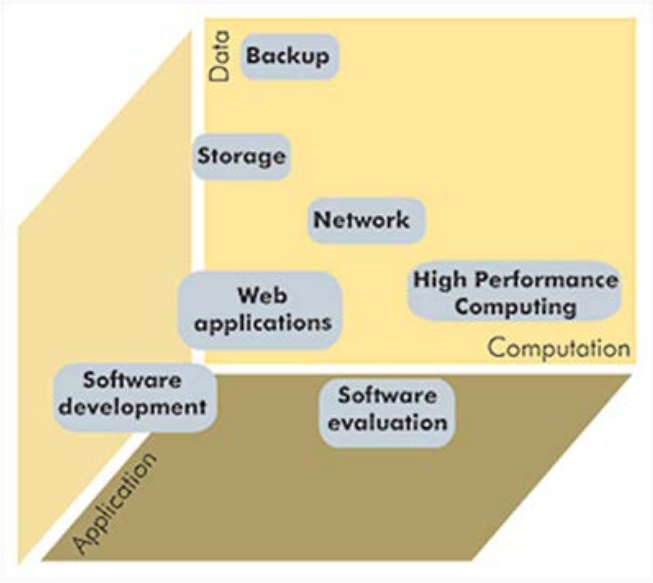
RESEARCH IT

Research IT support for the Biozentrum

Our group provides and integrates IT tools that help our institute's members organize and handle their daily work as efficiently and simply as possible in scientific and organizational aspects.

In particular, we support the Biozentrum's research groups and technology platforms in dealing with the large and complex data sets arising from modern analytical approaches.

The Research IT technology platform provides consulting and project support for research and administration projects with increased computational demands. Our goal is to accompany projects from their inception to a stable productive solution.



Research IT Solution Space.

Areas of interest

In the past years, we have led projects that cover the whole spectrum from scientific research projects (with direct involvement in the science and the analysis of data) to core infrastructure projects (where we develop a tool or technology for widespread use in the institute). We are working with the Biozentrum's technology platforms, research groups and administration to address these challenges.

Common to our projects is the focus on the management of information. A few examples of recent projects are useful to explain the breadth of scope:

Platforms for external and internal information exchange

- Customization/extension of a content-management system for the operation of the institute's web site. This system is extensively integrated with pre-existing databases, so that information does not need to be maintained in many places.
- Adaptation of a corporate-level wiki platform as an Intranet to efficiently structure and publish internal and administrative information to internal user groups, and enabling these groups to provide their own content easily.

Research data storage and archiving

Biological data sets are growing exponentially. These data need to be efficiently stored, annotated and retrieved. We are aligning scientists' needs and technical implementation of storage systems, in partnership with the University central IT services. In this way, we can provide for the storage of large scientific datasets with appropriate disaster recovery strategies, as well as develop processes for archiving to slower, cheaper storage media. At the same time, systems to manage these large datasets are indispensible, given the complexity of data sets. We currently employ Imagic, OpenBIS and Omero as scientific data management systems.

Automated data processing

Increased automation in the production of large-scale data (e.g. siRNA screens) require the development of automated systems to capture, validate, and store the data, as well as trigger automated analysis on high-performance computing infrastructure. This allows biologist users to perform analyses of data sets requiring significant computational power without the significant learning curve associated with setting up intensive calculations on large datasets.

New science and technologies...

Large-scale functional screening

Functional screens of large arrays of compounds, siRNAs, etc. can be performed in medium to highthroughput 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.

RESEARCH IT

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)

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.

Dr. Michael Podvinec
» **further information**

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Dr. Rainer Pöhlmann

Technical Staff
Manuel Kammermann
Eva Pujadas

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, local partners within the university (URZ) and outside.

Services and resources
Beyond project-based work, Research IT also develops and maintains a portfolio of services that are provided as common infrastructure to all members of the institute.

C-CINA

Center for Cell Imaging and Nano Analytics (C-CINA)

The Center for Cell Imaging and Nano Analytics (C-CINA) combines a wide range of microscopy equipment, methods and software tools to investigate biological specimens in three dimensions.

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 highresolution 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.

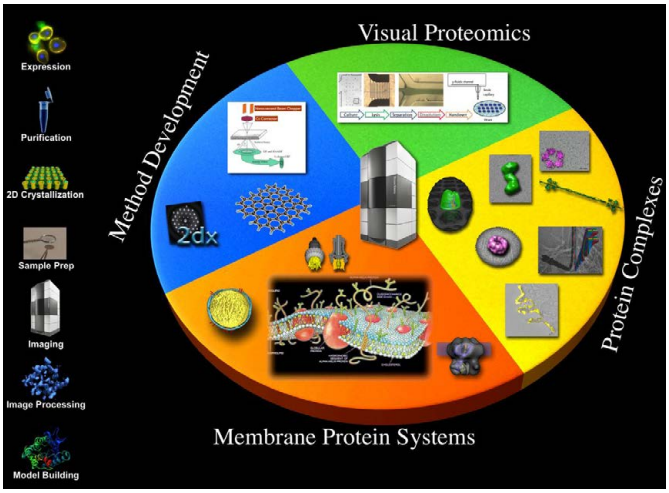
Computer image processing gives us insight into 3D

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.

Prof. Dr. Henning Stahlberg
» **further information**



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.

FACS CORE FACILITY (FCF)

FACS Facility Staining, Analysis and Sorting of Cells

Fluorescence Activated Cell Sorting (FACS) enables a qualitative and quantitative analysis of mixtures of cells, as well as the sorting of individual particles using a flow cytometer. The application of this methodology is available to all research groups at the FACS Facility at the Biozentrum.

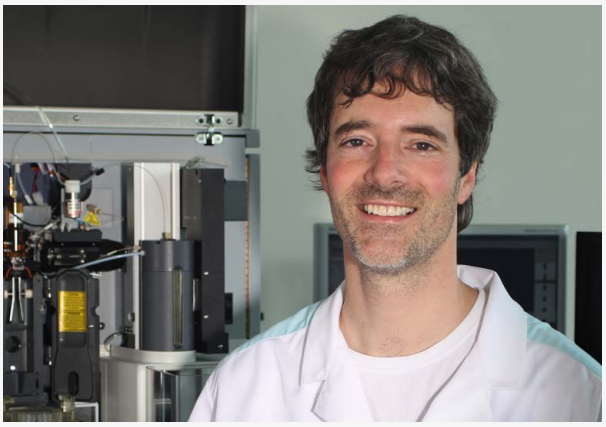
Fluorescence activated cell sorting (FACS) is a technology with which mixtures of cells can be analyzed, counted and separated with a special instrument, the flow cytometer. This procedure is already being routinely applied in medical diagnostics in hematology and immunology, in order to identify specific cell types in samples. Furthermore, in various research fields such as cell biology, neurobiology and infection biology FACS is being used more and more frequently and offers completely new possibilities for the analysis and purification of cells and cell organelles.

The Procedure

The analysis can be carried out on suspended cells with a size range between 0.2 und 100 micrometers, which are first stained with different fluorescent markers such as fluorescent proteins like GFP and RFP, fluorescently labeled antibodies and/or many other stains. In the flow cytometer, these particles/cells pass a laser at up to 130km/h. The scattered light reveals the size and internal structure of the cell, while the fluorescence indicates which stain the cell contains. Cells displaying the desired characteristics are identified and counted. Finally, the fluid stream is divided into many miniscule droplets. Droplets which contain a desired cell are electrically charged and diverted by an electric field into different collecting tubes. The various separated cells can subsequently be investigated using microscopicy, biochemistry and functional experiments.

The Service

All research groups at the Biozentrum are invited to use the services of the facility and receive advice for upcoming experiments. They will be supported and guided in the planning and carrying out of experiments as well as the subsequent analysis. Besides support and teaching, the service facility FACS is concerned with further development and optimization of various aspects of the FACS methodology.



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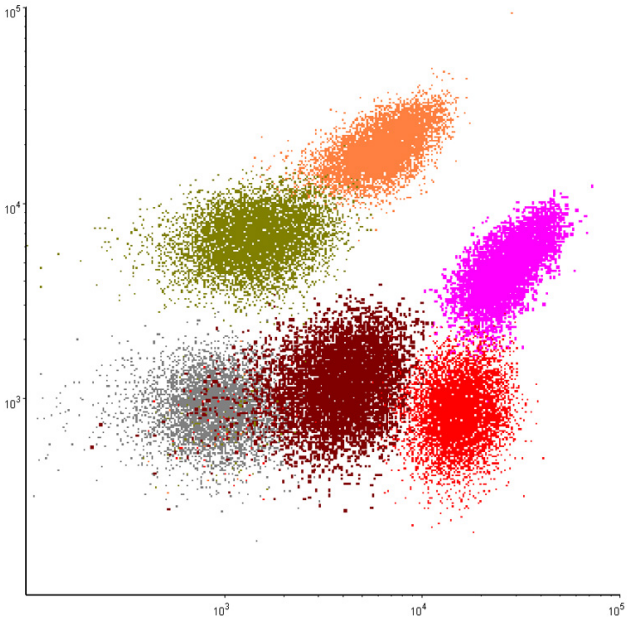

Detection of 6 subpopulations in a mixture of various Salmonella strains.

LIFE SCIENCES TRAINING FACILITY

A genome-wide dimension of research: the Life Sciences Training Facility (LSTF)

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 deep-sequencing 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 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.

Prof. Dr. Andreas Papassotiropoulos
» **further information**

Microarrays for the analysis of the human genome processed at the LSTF

MICROSCOPY CENTER

Electron microscopy for research and teaching purposes

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.

Dr. Markus Dürrenberger
» **further information**

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Daniel Mathys
Gianni Morson
Vesna Olivieri
Ursula Sauder

The greatest achievements of the ZMB were put on display at the façade of the Biozentrum on the occasion of the 2nd University-night; a compound eye of a fruit fly sized 6 by 9 meters (1 million final magnification).

QUANTITATIVE GENOMICS FACILITY

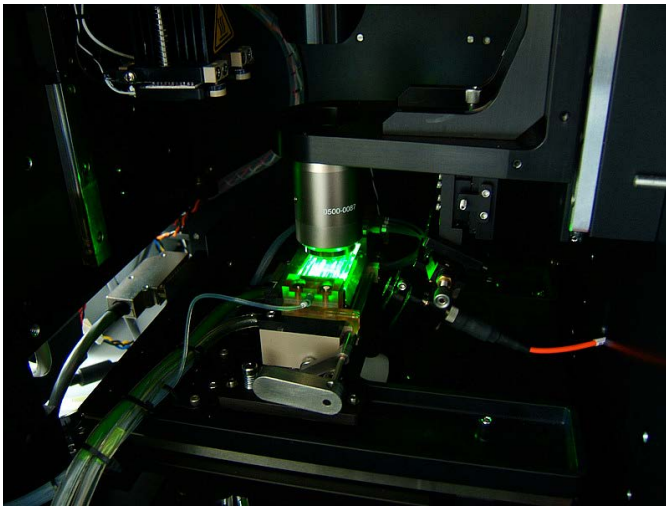
Next Generation Sequencing

The Quantitative Genomics Facility (QGF) 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 QGF team provides technical support for next generation sequencing applications in genomics and epigenomics, including highthroughput 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 (D-BSSE), 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 QGF 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 Interior of a Genome Analyzer, located in the Quantitative Genomics Facility at D-BSSE.

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Mihaela Zavolan (Second Deputy)
Bettina Delbridge (Secretary to the Director)

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Monika Gessler (Head Scientific Affairs)
Roger Jenni (Head Operation & Logistics)

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