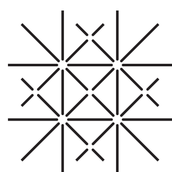
Biennial Report 2008/2009
Biozentrum
University of Basel



Biennial Report 2008/2009
Biozentrum
University of Basel

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Preface

The past two years have seen several major advances at the Biozentrum. Probably the most important development concerns a change in leadership, a concomitant implementation of new governance and a major boost in the financial contributions from the University. In the fall of 2008, I have in fact been appointed by the University Council as Director of the Biozentrum and Professor of Cell Biology. In the former function I followed on Joachim Seelig who had been at the helm of the Biozentrum since 2000.

Research at the Biozentrum, the largest Life Sciences department of the University of Basel, aims at understanding the molecular basis of life. The excellent quality of the Biozentrum's research is widely recognized at an international level, and so is the special appeal of the research-oriented teaching. Current research activities at the Biozentrum encompass three theme-oriented focal areas – Cell Growth & Development, Infection Biology, and Neurobiology – and two technology-driven core programs – Computational & Systems Biology and Structural Biology & Biophysics. As described in the following pages, this research collectively aims at elucidating the structure and function of macromolecules, the wiring of regulatory circuits and signaling systems, as well as the general principles that underpin complex and dynamic biological systems and their interactions.

In 2008, the 'Swiss Foundation for Excellence and Talent in Biomedical Research,' was established at the Biozentrum, with Joachim Seelig as its president. The aim of this foundation is to promote excellent scientific research in the area of biomedicine as well as increasing the attractiveness of Switzerland as a research centre for young talent. To this end, the foundation supports highly qualified Postdocs during lecture tours to Swiss Universities and Research Institutes. Also in 2008, Neurex, the Neuroscience network of the Upper Rhine Valley, which includes Neurobiology researchers of the Biozentrum, obtained a major financial boost. With more than 100 laboratories and 1000 researchers Neurex has become the most important European network in the field of basic, clinical and applied neuroscience.

Prominent amongst the highlights of 2009 is the award of the prestigious Louis-Jeantet Prize for Medicine to Mike Hall, for his discovery of TOR (Target of Rapamycin), a key regulator of cell growth. Several other members of the Biozentrum also received special recognition through the awards of prizes or membership in prestigious scientific organizations. In addition, several groups were highly successful in increasing the Biozentrum's financial endowment through the acquisition of competitive grants. Silvia Arber was awarded an important grant from the ERC (European Research Council), and three of six new projects supported through the Swiss Initiative in Systems Biology will be carried out at the Biozentrum. These include projects entitled 'BattleX – Manipulating the fight between human host cells and intracellular pathogens' (coordinated by Dirk Bumann), 'InfectX – Systems Biology of pathogen entry into human cells' (Christoph Dehio), and 'C-CINA – Center for Cellular Imaging and Nanoanalytics' (Andreas Engel and Henning Stahlberg). Furthermore, several Biozentrum scientists successfully acquired grants through Sinergia, a new initiative by the Swiss National Science Foundation that aims at supporting collaborative research.

With a scientific symposium the C-CINA laboratory was inaugurated in August, 2009. Equipped with state-of-the-art instrumentation this laboratory allows researchers to visualize proteins, protein complexes and cellular architecture through use of cryo-transmission electron microscopy, automated single particle analysis, 2D electron crystallography and dual-axis tomography. Also in 2009, the Biozentrum launched a new series of (public) lectures, the Biozentrum Lectures. These aim at honoring the work of distinguished scientists by highlighting their outstanding contributions to the field of Life Sciences. Most importantly, these lectures bring together researchers from the entire community in Basel and its surroundings.

To end on a personal note, I would like to take this opportunity to thank the University Council and the Rectorate for their support of the Biozentrum and their expression of confidence in the future development of this landmark institution at the University of Basel. I would also like to thank both my fellow scientists and all the highly dedicated people in the Biozentrum's technical and administrative facilities for much generous and competent support and for making me and my collaborators feel welcome. After 10 years at the Max-Planck Institute for Biochemistry in Martinsried/Munich, Germany, my laboratory moved to Basel in the late summer of 2009. Thanks to generous help by many people, our research (on cell cycle regulation in human cells) continued with only minimal disruption. As a newcomer to the Biozentrum, I have been extremely impressed by the very high level of motivation that is tangible at all levels, both amongst scientists and support personnel. Thus, I look very much forward to playing an active role in shaping the future development of this prestigious institution.

Erich A. Nigg, Director Biozentrum

Teaching Activities

Teaching Activities

The Biozentrum educates scientists at the bachelor, master, doctoral and postdoctoral levels. The particular advantage of education at the Biozentrum is its direct integration into research. All lecturers are active, grant holding scientists. The ratio of lecturers to students is ideal and ensures optimal support for every student.

Bachelor in Biology

In 2003 the current biology curriculum was adjusted to the Bachelor/Master system according to the Bologna Convention. The Bachelor curriculum takes three years to complete and is taught by lecturers of the Biozentrum and the Department of Integrative Biology.

During the first two years of studies, the students receive the essential basics in mathematics/statistics, physics, and chemistry, as well as introductory courses in biology. In addition, they are trained in ethics and attend optional courses in biology and non-biological subjects. After having completed their basic studies, the students must choose either: molecular biology, organismic biology or integrative biology to be the main focus of their further studies. The content of the third year, which consists of practical courses lasting several weeks each, determines the Major of the Biology Bachelor. The block courses provide theoretical information which the students can then in turn put into practice in the laboratory or in the field.

Master in Molecular Biology

Students with a Bachelor in Biology, Major Molecular Biology, are admitted to the fast track Master program that takes 1 to 1½ years. The students work primarily on their Master thesis and take additional courses in molecular biology in their particular field of specialization. The Master thesis is the students' real introduction to research.

At the Biozentrum, the students can specialize in Biochemistry, Bioinformatics, Biophysics, Cell Biology, Developmental Biology, Genetics, Immunology, Infection Biology, Microbiology, Neurobiology, Pharmacology, and Structural Biology. The interaction between students and the lecturers is additionally supported by so-called career mentoring: at the beginning of their studies; every student is assigned to a certain lecturer, which denotes an early contact between researchers and students. This lecturer, the career tutor, is the student's contact person and personal advisor during the entire duration of his/her studies.

The PhD Program

The Biozentrum offers an international Graduate Teaching Program in different areas of Molecular Life Sciences. Students accepted to the program conduct their own research project and attend scientific and soft skill lectures offered by the Biozentrum Graduate Teaching Program. The new teaching program features lecture cycles covering infection biology, neuroscience, cell biology, development, structural biology and biophysics, computational and systems biology, plant sciences, molecular biology, and molecular medicine.

Students are expected to finish their PhD within 3 – 4 years. After approval of the dissertation by the Natural Science Faculty of the University of Basel, the studies are concluded with a detailed oral examination. In order to promote excellent young scientists, in 2007 the Biozentrum, together with the Werner Siemens-Foundation, launched the graduate study program „Opportunities for Excellence". The program offers ten PhD fellowships per year on a competitive basis with direct access to the PhD program, generous financial support, a rotation-based selection of the research group and support for attendance at scientific meetings and courses during the dissertation period.

Seminars at the Biozentrum

The Biozentrum has several seminar series, organized by the focal areas and the core programs, planned several months in advance and generally given by a senior scientist. Many additional seminars are given that cannot be fitted into the formal series; either because they are arranged at short notice or because of scheduling conflicts. We call these "Informal Seminars". They are, however, just as widely announced as the formal ones and many speakers are surprised to find themselves giving their informal seminar to a packed auditorium. These seminars play an important role in the life of the Biozentrum. They are perhaps the most important post-graduate educational activity that we offer, and many a graduate student has secured his first postdoctoral position whilst sharing a post-seminar drink with the speaker.

Master Theses

*Abanto, Michael: *Cellular processes and molecular mechanisms underlying agrin-induced acetylcholine receptor clustering.* (P. Caroni, S. Arber)

*Amsler, Philipp: *Structural studies of the interaction between the RecQ helicase Sgs1 and the single-stranded DNA binding protein RFA.* (N. Thomä, T. Schirmer)

*Badertscher, Lukas: *Molecular and cytological characterization of GW182 proteins.* (W. Filipowicz, M. Zavolan)

Balmer, Jasmin: *Amplifying brain neuroblasts contribute to central complex development in Drosophila.* (H. Reichert, M. A. Rüegg)

Barth, Lydia: *Characterisation of immature neurons derived from embryonic stem cells.* (K. Vogt)

Biasini, Marco: *Tools for automated 2D crystallisation.* (A. Engel)

*Biedert, Stefan: *Interactions of tenascin-W.* (R. Chiquet, M. A. Rüegg)

Bobby, Romel: *Characterization of two salt bridges in ubiquitin and structural characterization of HES1.* (S. Grzesiek)

*Bosch, Angela: *Functional analysis of regulatory T cell subpopulations.* (G. Holländer, U. Jenal)

Bosshart, Patrick: *High-throughput single molecule force spectroscopy applied to the bacterial L-arginine/agmatine antiporter AdiC.* (A. Engel)

*Broggi, Maria Anna Sofia: *Characterization of invariant natural killer T cells in atherosclerotic patients.* (G. De Libero, J. Pieters)

Brun, Catherine: *Characterization of the S. cerevisiae Rheb homolog RHB1 and its involvement in the TOR pathway.* (M. Hall)

*Burch, Nathalie: *Development of a new cell culture model to study the role of PGC-1alpha in exercise.* (C. Handschin, U. Boutellier)

*Buschle, Markus: *Class IB phosphoinositide 3-kinase.* (M. Wymann, M. N. Hall)

Casanova, Alain: *Molecular mechanisms controlling poly-GlcNAc dependent biofilm induction in Escherichia coli.* (U. Jenal, A. Böhm)

Cattin, Cédric: *Stoichiometry analysis of type III secretion components by fluorescent labeling.* (G. R. Cornelis)

*Champion, Lysie: *Functions of the mir-35 microRNA family during C. elegans embryogenesis.* (H. Grosshans, M. Zavolan)

Cohen, Yaniv: *Identification of the LcrV secretion signal.* (G. R. Cornelis)

*Despont, Alain: *PN-1 as a new role-player in medulloblastomas: analysis of the double mutant Ptch -/+ PN-1 -/+.* (R. Zeller, M. Affolter)

Golfieri, Giacomo: *Purification and preliminary characterization of a putative pitrilysin-like protein from Capnocythophaga canimorsus.* (A. Danielli, G. R. Cornelis)

*Gordon, Oliver: *The antimicrobial mechanism of silver and its potential use for implant coating.* (R. Landmann, U. Jenal)

Grassinger, Franziska: *Identification and characterization of novel cargo protein of the Chs5p-dependent pathway in Saccharomyces cerevisiae.* (A. Spang)

*Gsell, Andreas: *Evaluation of eukaryotic expression systems for large scale production of the intracellular kinasse domain of vascular endothelial growth factor receptor 2 (VEGFR-2).* (K. Ballmer-Hofer, P. Jenö)

Hauert, Barbara: *Identification of cellular protein targets for Bartonella effector proteins.* (C. Dehio)

Hauri, Simon: *Molecular and functional characterization of the yeast Npr1 kinase.* (P. Jenö)

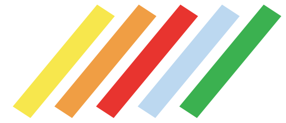
Hirschmann, David: *Analyzing the role of Rab4, Rab11, and Rab14 in endosomal recycling.* (M. Spiess)

*Holzer, Christina: *The role of protein tyrosine phosphatases in breast cancer.* (M. Bentires-Aij, M. N. Hall)

Hornstein, Meret: *AFM studies on 3D importin β crystals.* (R. Lim)

Hügi, Ilona: *Characterization of nucleoporin Nup88 interaction with the human intermediate filament protein lamin A.* (B. Fahrenkrog)

Hyötylä, Janne: *Modeling selective gating by nanopores with poly(ethylene glycol) and PEG-binding antibodies.* (R. Lim)



*Imhof, Fabia: *Characterization of new targeted mutations in the Gli3 locus: Gli3Δ and Gli3fneo.* (R. Zeller, M. Affolter)

Jüttner, Josephine: *Dissecting the interplay between Bir1p and Nma111p during apoptosis.* (B. Fahrenkrog)

Kälin, Simone: *Role of rabaptin-5 in endosomal recycling.* (M. Spiess)

*Kant, Jessica: *Analysis of tenascin-C - induced angiogenesis.* (G. Orend, C. Dehio)

Kasper, Christoph: *Propagation of inflammatory signals upon bacterial infection of epithelial cells.* (C. Arrieumerlou)

*Keller, Claudia: *Analysis of covalent modifications of Copine III by mass spectrometry.* (J. Hofsteenge, M. N. Hall)

Keller, Miyako: *AgDyn2, AgJnm1 and AgNdl1, three proteins important for dynein function in the filamentous fungus A. gossypii.* (S. Grava, P. Philippsen)

Kemmerling, Simon: *Mechanical characterization of the bacterial cyclic-nucleotide modulated potassium channel mlCNG.* (A. Engel)

*Köhli, Sabrina: *Titration of immunosuppressive drugs and its impact on IFN-gamma release of polyomavirus BK- and cytomegalovirus-specific T-cell response in vitro.* (H. Hirsch, C. Arrieumerlou)

*Krawczyk, Claudia: *Dissection of specific functions of the two DNA helicases Mph1 and Sgs1 in the stabilization of stalled replication forks.* (P. Schär, M. N. Hall)

*Kucsera, Stefan: *Analysis of the expression pattern of the immediate early gene arc in the amygdala after fear conditioning and extinction.* (A. Lüthi, M. A. Rüegg)

Kuert, Philipp: *Expression and functional analysis of the homeotic gene labial in post-embryonic brain development of Drosophila melanogaster.* (H. Reichert, W. Salzburger)

Lang, Simon: *Immunosuppressive drugs and their interaction with the efflux transporter P-glycoprotein.* (A. Seelig)

Lehner, Roman: *Purification, crystallization and homology model of the KdgM porin from the plant pathogen "Dickeya dadantii".* (T. Schirmer, S. Dames)

Liesch, Marius: *Bartonella schoenbuchensis genome assembly and functional characterization of the VirB-homologous (Vbh) type IV secretion system.* (C. Dehio)

Luginbühl, Joachim: *Unraveling genetic control mechanisms of motor circuitry in mouse.* (S. Arber)

*Lüthi, Marco: *An in vitro pharmacodynamic model as a tool to determine the pharmacokinetic/pharmacodynamic index and to predict the in vivo pharmacodynamics of a novel antibiotic compound.* (W. Keck, U. Jenal)

Matter, Anja: *A role for histone modifications in yeast apoptosis.* (B. Fahrenkrog)

Metzger, Lisa: *Length control of the Yersinia enterocolitica injectisome needle.* (G. R. Cornelis)

Meury, Cedric: *Role of the E. coli tRNA-specific editing enzyme TadA in protein translation efficiency.* (W. Keller)

Meury, Marcel: *Investigation into putative oligonucleotide binding to BepA from B. henselae.* (T. Schirmer, C. Dehio)

Nicollier, Micael: *The influence of the bacterial second messenger on cell cycle and pole development in Caulobacter crescentus.* (U. Jenal, S. Abel)

*Pugin, Fiona: *Is the period3 polymorphism associated with alterations in the rest-activity cycles in older individuals?* (M. A. Rüegg, C. Cajochen)

Reinhard, Judith: *The role of Copine family members in postsynaptic structures of the central nervous system.* (M. A. Rüegg)

*Ribi, Sebastian Tobias: *MicroRNAs in the dark and light adapted mouse retina.* (W. Filipowicz, M. Zavolan)

Romanino, Klaas: *The role of mTOR complex1 and mTOR complex2 in skeletal muscle.* (M. A. Rüegg)

Sauder, Reto: *Membrane permeation as caused by amphipathic and non-amphipathic cell-penetrating peptides.* (J. Seelig)

Schleicher, Kai: *The impact of nucleoporin Nup88 overexpression on the tumor suppressor p53.* (B. Fahrenkrog)

*Schmid, Daniela: *The influence of MAL on Schwann cell differentiation.* (N. Schären-Wiemers, M. A. Rüegg)

Schoch, Rafael: *Monitoring structural changes in biological molecules with surface plasmon resonance.* (R. Lim)

*Spalinger, Marianne: *The effect of BAFF over-expression on the immune system.* (A. G. Rolink, C. Arrieumerlou)

Sprunger, Joëlle: *Proteolytic processing of endogenous proBDNF in neurons.* (Y.-A. Barde, M. A. Rüegg)

*Stauffer, Andrea: *Towards the molecular target of two novel HDL-raising agents: a transcriptional analysis using oligonucleotide microarray techniques.* (R. G. Clerc, U. A. Meyer)

Steiner, Samuel: *Molecular mechanisms of Escherichia coli biofilm induction by translational inhibitors.* (U. Jenal, A. Böhm)

*Stiefvater, Adeline: *Generation of recombinant TRANCE and characterization of the immune function of TRANCE.* (D. Finke, J. Pieters)

*Trojer, Dominic: I. *Characterization of arc-EGFP and cfos-EGFP transgenic reporter mice by analysis of the expression pattern in the amygdala after Pavlovian fear conditioning. II. Analysis of defined amygdala neuronal subpopulations using an anatomical approach.* (A. Lüthi, H. Reichert)

Tschon-Müller, Therese: *Characterization of the functional role of BRE1p in yeast apoptosis.* (B. Fahrenkrog, U. Aebi)

Urbani, Raphael: *Multi-color approach to track Salmonella during infection.* (D. Bumann)

*Weis, Stefan: *Identification and characterization of CA4, NPY1R and IFITM1 as marker genes for aberrant promoter methylation in colorectal cancer.* (P. Schär, J. Pieters)

Wenger, Daniel: The grammar of the transcription factor binding site regulatory code in proximal promoters. (E. van Nimwegen)

*Ziegler, Reto M.: *Involvement of Notch signaling in T and dendritic cell development in vitro.* (A. G. Rolink, Y.-A. Barde)

*Zietzling, Svenja: *Neuroprotective mechanisms in multiple sclerosis: The role of oligodendrocytes in innate immunity.* (N. Schären-Wiemers, Y.-A. Barde)

Zollinger, Barbara: *Characterization of the role of DARS2 in tracheal cell migration.* (M. Affolter, H. Chanut, M. Baer)

Zumthor, Ludwig: *Structural studies on PopA, a c-di-GMP binding protein from Caulobacter crescentus.* (T. Schirmer, U. Jenal)

Doctoral Dissertations

Abel, Sören: *Analysis of c-di-GMP mediated cell fate determination in Caulobacter crescentus.* (U. Jenal)

Al-Haboubi, Teiba: *Characterisation of the molecular links between the nuclear pore complex and the nuclear lamins and reconstitution of the Xenopus oocytes lamin assembly in vitro.* (B. Fahrenkrog, U. Aebi)

*Aziz, Said Abel: *Identification of serine 867 as new phosphorylation site on the GABAB receptor: Characterization of physiological effects.* (B. Bettler, M. A. Rüegg)

Battey, James: *Modelling the effects of single point mutations on the structure and function of proteins.* (T. Schwede)

Bentzinger, C. Florian: *Function of mTOR complex 1 and 2 in skeletal muscle.* (M. A. Rüegg, F. Zorzato)

Berninger, Philipp: *Computational methods for analyzing small RNAs and their interaction partners with large-scale techniques.* (M. Zavolan)

Birk, Julia: *Fibrillar aggregations of pathogenic pro-vasopressin mutants.* (M. Spiess)

Birrer-Lang, Claudia: *The organization of the microtubule cytoskeleton and its role on nuclear dynamics in the multinucleate hyphae of Ashbya gossypii revealed by live cell imaging and electron microscopy.* (P. Philippsen, S. Jaspersen)

*Bonnici, Brenda: *Axonal regeneration in hippocampal and spinal cord organotypic slice cultures.* (M. A. Rüegg, J. Kapfhammer, C. Nitsch)

*Bozulic, Lana: *Regulation of PKB by upstream kinases.* (B. Hemmings, M. N. Hall)

*Brosig, Michaela: *Meachanotransduction in fibroblasts.* (R. Chiquet-Ehrismann, M. A. Rüegg)

Burger, Lukas: *Inference of biomolecular interactions from sequence data.* (E. van Nimwegen)

*Buser, Andres: *The septin cytoskeleton is associated with distinct myelin structures of the central and peripheral nervous system.* (M. A. Rüegg, N. Schaeren-Wiemers, T. Meier)

Casagrande, Fabio: *Structure and function of amino acid and peptide transport proteins.* (A. Engel, D. Fotiadis)

Casutt-Meyer, Salome: *Capnocytophaga canimorsus: Interaction with the innate immune system.* (G. R. Cornelis)

*Chattopadhyay, Arundhati: *Structural and biophysical analysis of important biomedical enzymes and nano-architectures.* (B. Burkhard, U. Aebi)

Chern, Tzu-Ming: *A trio of unique alternative splicing patterns: The splicing of tandem NAGNAG acceptors, transcription-start-site-dependent and mutually dependent cassette exons.* (M. Zavolan)

*Christen, Verena: *Interferon alpha signaling in viral hepatitis.* (M. Heim, G. Pluschke, M. N. Hall)

*Ciocchi, Stephane: *Role of cellular inhibition in the amygdala during extinction of conditioned fear.* (A. Lüthi, S. Arber)

*Clavaguera, Florence: *Initiation and spreading of Tau pathology: Isb-amyloid the only key?* (M. A. Rüegg, M. Tolnay, M. Staufenbiel)

Cloëtta, Dimitri Y. R.: *A conditional mouse model for the characterization of mTORC1 function in muscle and brain.* (M. A. Rüegg, B. Bettler)

Combaluzier, Benoit: *Studies on the relation between antigen presentation and mycobacterial trafficking.* (J. Pieters, D. Finke)

Corázar, Daniel: *Identification of a possible role of thymine DNA glycosylase (TDG) in epigenome maintenance.* (P. Schär, M. Spiess)

*Costa, Luigi: *Crosstalk between long acting β2-adrenergic receptor agonists and tiotropium: interaction of β2-adrenergic and muscarinic receptors.* (M. Tamm, M. Roth, M. A. Rüegg)

Cybulski, Nadine: *New insights into the in vivo and in vitro functions of mammalian TOR complex 2.* (M. N. Hall, M. A. Rüegg)

Dalla Torre di Sanguinetto, Simon: *Identification of motor neuron pool marker genes and analysis of their roles in motor circuit assembly.* (S. Arber)

Dürig, Anna: *Second messenger mediated spatiotemporal control of cell cycle and development.* (U. Jenal)

*Egler, Viviane: *A combinatorial preclinical in vitro strategy against human glioblastoma cells - specific targeting of protein kinases, histone deacetylases and glycolysis.* (M. A. Rüegg, A. Merlo)

Eickhorst, Angela: *Mechanism of neuroligin-neurexin mediated VDCC recruitment to presynaptic terminals.* (P. Scheiffele)

*Fan, Alex Xiu-Cheng: *Investigation of quantitative and qualitative MtDNA alteration in breast cancer.* (R. Lindberg, X. Y. Zhong, M. N. Hall)

Focke, Frauke: *Insights into genotoxic effects of electromagnetic fields.* (P. Schär, M. Spiess)

Friese, Andreas: *Gene profiling of identified neurons to dissect molecular mechanisms involved in spinal reflex assembly.* (S. Arber)

Gaidatzis, Dimos: *Computational discovery of animal small RNA genes and targets.* (M. Zavolan)

*Galimberti, Ivan: *Structural plasticity of synaptic connectivity in the adult central nervous system.* (P. Caroni, S. Arber)

*Germano, Davide: *Identification of a novel population of bone marrow-derived prominin-1/CD133+ lung progenitors with regenerative capacity.* (A. Eberle, S. Arber)

Göttler, Thomas: *Untersuchung der katalytischen Funktion der Gyrase aus Bacillus subtilis mit Einzelmolekül-Fluoreszenzspektroskopie.* (D. Klostermeier)

Graziussi, Daria Frederica: *Conservation of the retinal determination gene cascade in the jellyfish Cladonema radiatum.* (W. J. Gehring, S. Piraino)

*Guetg, Nicole: *GABAB receptor localization and regulation.* (B. Bettler, M. A. Rüegg)

*Heitz, Stéphane: *Neuronal death mechanisms in cerebellar Purkinje cells.* (M. A. Rüegg, J. Kapfhammer, P. Scheiffele)

Hilbert, Manuel: *Einfluss der eIF4A bindenden Domäne aus eIF4G auf Konformation und Aktivität der minimalen DEAD Box Helikase eIF4A aus Saccharomyces cerevisiae.* (D. Klostermeier)

*Holbro, Niklaus: *Structure-function analysis on the level of individual synapses.* (T. Oertner, S. Arber)

Izergina, Natalya: *Postembryonic development of amplifying neuroblast lineages in the Drosophila brain: proliferation, differentiation and projection patterns.* (H. Reichert, M. Affolter)

*Jankovic, Dragana: *Leukemogenic mechanisms and targets of NUP98-fusion genes.* (J. Schwaller, M. N. Hall)

Jungblut, Stefan Patrick: *Coupling of adenine nucleotide binding and hydrolysis to single- and double-stranded DNA binding determines the topoisomerase activity of reverse gyrase from Thermotoga maritima.* (D. Klostermeier)

*Kalender, Adem: *Regulation of mTOR/S6K pathway by cellular energy.* (G. Thomas, M. N. Hall)

*Kalinovsky, Anna: *Developmental regulation of ponto-cerebellar mossy fiber connectivity.* (P. Scheiffele)

*Kenzelmann Brož, Daniela: *Teneurins in development and disease.* (N. Hynes, R. Chiquet Ehrismann, M. A. Rüegg)

Klocek, Gabriela: *Melittin interaction with sulfated sugars and cell membranes.* (J. Seelig)

Kobialka, Szymon: *In vitro reconstitution of trans-Golgi exit and the effect of GAG attachment on protein sorting.* (M. Spiess)

Kögler, Eva Jutta: *Characterization of P28, a novel ERGIC/cis-Golgi protein, required for Golgi ribbon formation. PH measurements in the early secretory pathway in vivo.* (H.-P. Hauri, M. Spiess)

Kriz, Alexander: *Copine 6, a novel calcium translating synaptic activity into spine plasticity.* (M. A. Rüegg, B. Bettler)

Kumar, Abhilasha: *Analysis of the engrailed-expressing neuroblast lineages in Drosophila brain development.* (H. Reichert, M. A. Rüegg, R. Stocker)

Lin, Li: *Clonal analysis of growth behaviors during Drosophila larval tracheal development.* (M. Affolter, R. Zeller, H. Reichert)

Linden, Martin: *Die DEAD-box Helikase Hera aus Thermus thermophilus: Untersuchung der Wechselwirkung mit Nukleinsäuren und der Helikaseaktivität.* (D. Klostermeier)

Maj, Marcin: *Functional characterization of MuSK, receptor tyrosine kinase required for the formation and the maintenance of nerve-muscle synapses. In vivo and in vitro approaches.* (M. A. Rüegg, T. Meier)

Mally, Manuela: *Capnocytophaga canimorsus: Discovery of a deglycosylation mechanism that links metabolism to pathogenesis.* (G. R. Cornelis)

Mariani, Valerio: *Transfer of tilted sample information in transmission electron microscopy.* (A. Engel, H. Stahlberg)

*Metz, Michaela: *Characterization of a novel family of GABAB receptor interacting proteins - KCTD8, -12, -12b and -16.* (M. A. Rüegg, B. Bettler, Y.-A. Barde)

*Miglino, Nicola Romolo: *Novel molecular pathologies in asthma and COPD.* (M. A. Rüegg, M. Tamm, R. Chiquet-Ehrismann)

Molina, Nacho: *Genome evolution and regulatory network structure in bacteria.* (E. van Nimwegen)

Müller, Catherine Ann: *The tip complex of the Yersinia enterolitica injectisome.* (G. R. Cornelis)

Müller, Philipp: *Studies on the role of coronin 1 and the actin cytoskeleton in T cell signaling and survival.* (J. Pieters, C. Arrieumerlou)

*Necker geb. Luz, Judith: *Hereditary colorectal cancer. Assessment of genotype-phenotype correlations and analysis of rare susceptibility genes in familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC).* (M. N. Hall, H. Müller, P. Schär)

Nikolaev, Yaroslav: *Rethinking leucine zipper – ribonuclease activity and structural dynamics of a ubiquitous oligomerization motif.* (K. Pervushin, S. Grzesiek)

Nikoletopoulou, Vassiliki: *Mouse embryonic stem cells as a discovery tool in neurobiology.* (Y.-A. Barde, S. Arber)

Nisius, Lydia: *Expression, purification and characterization of the HIV-1 coreceptor CCR5 and its ligand RANTES and high-pressure NMR investigations of hydrogen bonds in biomolecules.* (S. Grzesiek)

Polak, Pazit: *Regulation of adipogenesis and adipose maintenance by the mammalian-TOR complex 1.* (M. N. Hall, M. A. Rüegg)

Prenosil, George: *Different signaling of type A gamma-aminobutyric acid receptors in the mouse central nervous system.* (K. Vogt, P. Sonderegger, J.-M. Fritschy)

Raman, Senthilkumar: *Design and analysis of peptide based nanoparticles.* (U. Aebi)

Rauskolb, Stefanie: *Brain-derived neurotrophic factor: Generation and characterization of adult mice lacking BDNF in the adult brain.* (Y.-A. Barde, M. A. Rüegg)

*Sarasin-Filipowicz, Magdalena: *Interferon signaling in chronic hepatitis C: Mechanisms and implications for therapy.* (H.-P. Hauri, M. H. Heim, E. Palmer)

Scheidegger, Florine: *The role of the VirB/VirD4/Bep system in Bartonella henselae-triggered vascular proliferation.* (C. Dehio)

Scherr, Nicole: *Studies on the structure and function of protein kinase G, a virulence factor of mycobacterium tuberculosis.* (J. Pieters, P. Philippsen)

Schmutz, Cornelia: *Characterization of the RhoGAP proteins RGA-3 and RGA-4 and the centrosomal protein SAS-5 in the early Caenorhabiditis elegans embryo.* (A. Spang)

Schroeder, Ulrich: *The "lower dimer" and its role in actin patterning: Studying different forms of actin by electron microscopy, biochemistry and tailor-made antibodies.* (U. Aebi, C.-A. Schoenenberger)

Schwarz-Herion, Kyrill: *Structural and functional characterisation of the p62 complex, a subcomplex of the nuclear pore complex.* (B. Fahrenkrog)

Signorell, Giani: *2D crystallization and image processing of membrane proteins.* (A. Engel)

*Sivasankaran, Balasubramanian: *The role of notch2 gene in human malignant glial brain tumours.* (M. A. Rüegg, A. Merlo, R. Chiquet-Ehrismann)

Suri, Gregor: *A cytosolic factor mediating membrane recruitment of AP-1 clathrin adaptors.* (M. Spiess)

Thorsteinsdottir, Holmfridur B.: *Computational analysis of protein-ligand binding: from single continuous trajectories to multiple parallel simulations.* (T. Schwede)

Vajpai, Navratna: *Structural characterization of the leukemia drug target ABL kinase and unfolded polypeptides by novel solution NMR techniques.* (S. Grzesiek)

Wagner, Stefanie: *Length control of the Yersinia injectisome.* (G. R. Cornelis)

Wassmann, Paul: *Elucidation of the regulatory mechanisms of the diguanylate cyclases PleD, DgcA and DgcB by structural and biophysical analysis.* (T. Schirmer, U. Jenal)

Weiss, Alexander: *Regulation of Dpp target genes by Mad/Medea and Brinker.* (M. Affolter, R. Zeller, W. Gehring)

*Weiss, Andreas: *Novel methods and therapeutic approaches for diagnosis and treatment of Huntington's Disease.* (M. A. Rüegg, P. Paganetti, M. Spiess)

*Westendorf, Jens: *Regulation of human Polo-like kinase 4 via phosphorylation and ubiquitin-dependent proteolytic degradation.* (E. A. Nigg)

*Zeis, Thomas: *Characterization of molecular alterations in normal appearing white matter of multiple sclerosis brain tissue and its animal model experimental autoimmune encepaholomyelitis.* (H. Reichert, N. Schaeren-Wiemers, M. A. Rüegg)

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Focal Area
Growth & Development

Research at the Biozentrum in the area of "Growth and Development" is dedicated to understanding the molecular basis of life. How do biomolecules interact to ultimately constitute a living organism? To understand these interactions at a quantitative and atomic level, members of the focal area collaborate extensively with others at the Biozentrum, in particular with members of the core programs "Structural Biology and Biophysics" and "Computational and Systems Biology". Members of the focal area also participate in several research networks outside the Biozentrum, such as Systems.X.ch and the University Center of Excellence in Signaling (Basel Signaling Alliance). As described below, the research programs of the individual groups in the area "Growth and Development" focus on membrane and protein traffic, signal transduction, gene regulatory networks and cell cycle regulation, in a wide variety of experimental organisms including bacteria, yeast, worms, fish, flies and mammals.

During the period covered by this report, our focal area included eleven research groups, headed by Profs. Markus Affolter, Walter Gehring, Michael Hall, Christoph Handschin, Hans-Peter Hauri, Urs Jenal, Walter Keller, Erich Nigg, Peter Philippsen, Anne Spang, and Martin Spiess. Profs. Walter Keller and Walter Gehring retired in 2008 and 2009, respectively, after long and exceptionally distinguished careers at the Biozentrum as teachers, researchers and leaders. Profs. Christoph Handschin and Erich Nigg joined the Biozentrum in 2009 and are already well integrated. During the last two years, several members of the focal area received special recognition. Prof. Markus Affolter was elected a member of the Leopoldina, the German Academy of Science. Prof. Michael Hall was recognized with the 2009 Louis-Jeantet Prize for Medicine, and was elected a Fellow of the American Association for the Advancement of Science. Prof. Urs Jenal was promoted to Full Professor in 2008. Prof. Anne Spang was elected a member of the European Molecular Biology Organization. Furthermore, the students and postdoctoral fellows within the focal area continue to be successful in their career advancement.

All members of the focal area are heavily involved in teaching at the undergraduate and postgraduate levels, including introductory and advanced lecture series. The graduate teaching program at the Biozentrum consists of nine topic-oriented cycles with a total of almost 50 courses. Peter Philippsen also teaches extensively in the Biotechnology curriculum, organized by the "Upper Rhein Universities" in Basel, Strasbourg, Karlsruhe and Freiburg/Breisgau. In addition, seminars by invited speakers are organized on a regular basis, in particular through the well-attended "Growth and Development" seminar series that hosts internationally prominent scientists.

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Focal Area
Growth & Development

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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 so-called morphogen gradients. Only recently has it been possible to demonstrate that secreted proteins of the Transforming Growth Factor β (TGFβ), Wnt and Hedgehog families specify positional information by this mechanism. Drosophila Dpp is a member of the TGFβ superfamily and was the first secreted protein for which a morphogen function has been clearly demonstrated. Over the past ten years we have characterized the Dpp signaling pathway in detail, in collaboration with the group of Konrad Basler in Zurich.

Our studies provide the molecular framework for a mechanism by which the extracellular Dpp morphogen establishes a finely tuned, graded read-out of a transcriptional repressor complex including Smad proteins and the zinc-finger protein Schnurri. Targets of this repressor complex include transcriptional regulators as well as secreted proteins involved in morphogen transport. Other mor-phogens, 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

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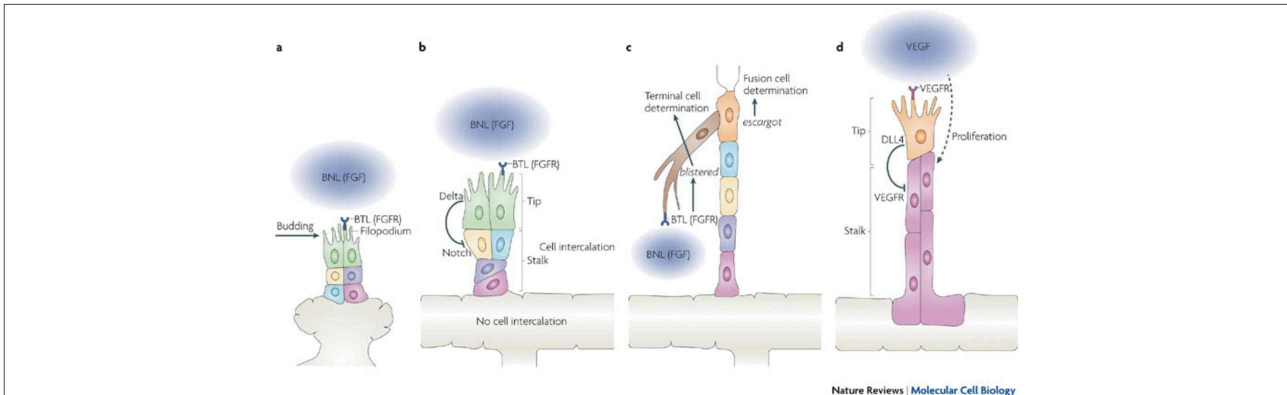


Drosophila melanogaster trachea and vertebrate vasculature branching. Branchless (BNL), a fibroblast growth factor (FGF), acts at the top of the hierarchy of cellular events that orchestrate tracheal branching in Drosophila melanogaster (a to c). During vertebrate angiogenesis, vascular endothelial growth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

morphogen function in tissue growth and patterning, a key issue in modern developmental biology.

Cell rearrangement in organ formation

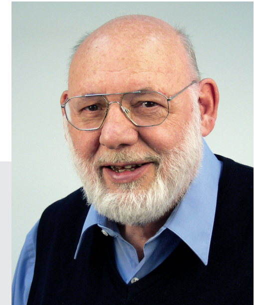
To gain insight into how signaling pathways control more complex cellular decisions during the process of organ morphogenesis, we investigate the formation of the Drosophila tracheal system, an epithelial branched network similar to the lung, the kidney or the vasculature. Tracheal development serves as a paradigm to understand how epithelial cell sheets can be transformed by cell signaling and cell-cell or cell-matrix interactions into complex three-dimensional networks, a process generally referred to as branching morphogenesis. Our approach has been to identify genes involved in the process by genetic analysis, and the characterization of relevant gene products by in vivo and in vitro analysis. In addition, we have devoted major efforts to characterize branching morphogenesis at the cellular level, using avant-garde, live imaging technology. Over the past decade, these studies have provided a framework for understanding complex processes involved in the architectural design of developing organs, including the control and integration of cell migration and cell rearrangement via cell-cell signaling and extracellular matrix components *(see figure).*

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 figure).* 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 (see Blum et al., 2008). 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. 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.

Most important publications 2008-2009

- Affolter, M., Zeller, R. & Caussinus, E. (2009). Tissue remodelling through branching morphogenesis. *Nat Rev Mol Cell Biol* 10, 831-42.
- Affolter, M. & Caussinus, E. (2008). Tracheal branching morphogenesis in Drosophila: new insights into cell behaviour and organ architecture. *Development* 135, 2055-64.
- Blum, Y., Belting, H.-G., Ellertsdottir, E., Herwig, L., Luders, F. & Affolter, M. (2008). Complex cell rearrangements during intersegmental vessel sprouting and vessel fusion in the zebrafish embryo. *Dev Biol* 316, 312-22.
- Caussinus, E., Colombelli, J. & Affolter, M. (2008). Tip-cell migration controls stalk-cell intercalation during Drosophila tracheal tube elongation. *Curr Biol* 18, 1727-34.
- Kleaveland, B., Zheng, X., Liu, J. J., Blum, Y., Tung, J. J., Zou, Z., Sweeney, S. M., Chen, M., Guo, L., Lu, M. M., Zhou, D., Kitajewski, J., Affolter, M., Ginsberg, M. H. & Kahn, M. L. (2009). Regulation of cardiovascular development and integrity by the heart of glass-cerebral cavernous malformation protein pathway. *Nat Med* 15, 169-76.

Hox genes



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Mechanism of action of synthetic Hox genes in Drosophila

Hox genes are master control genes found in all bilaterian animals which specify the body plan along the antero-posterior axis. The molecular mechanisms underlying Hox-mediated transcription and the differential requirements for the specific regulation of the vast number of Hox-target genes are largely unknown.

In order to reduce the complexity of the Hox transcription factors we have generated synthetic Hox genes which retain their homeotic function in vivo in transgenic flies. Using confocal laser scanning microscopy with avalanche photodiodes (APDs), so-called APD imaging, and fluorescence correlation spectroscopy we have established a high-resolution experimental modality that enables non-destructive observation of molecular interactions in live cells with single-molecule sensitivity.

Quantitative analysis of synthetic Hox transcription factor – DNA interactions in live Drosophila cells by single-molecule imaging and fluorescence correlation spectroscopy (FCS)

Genetic studies of synthetic *Sex combs reduced (Scr)* genes consisting of the homeodomain (HD) and the YPWM motif show that they are functional in vivo. They are capable of inducing ectopic salivary glands in the embryo and homeotic transformations of the antenna into tarsal structures. Synthetic YPWM-HD genes were fused to yellow fluorescent protein (YFP) and other fluorescent dyes and expressed in transgenic flies using upstream activating sequences (UAS) of yeast gal 4 and a minimal heat shock promoter in the absence of a gal 4 driver. Using advanced fluorescence imaging and FCS we were able to analyze synthetic Scr-DNA interactions in live salivary gland cells. Our results suggest that the synthetic Scr-HD transcription factors find their specific target sites by multiple association/dissociation events, the rapidity of which is largely due to electrostatic interactions. The dissociation constant for the non-specific DNA-Scr HD complexes is rather low, $K_d^{non\text{-}specific} = 25 \pm 15$ μM, whereas the specific DNA-Scr HD complexes were estimated to be in the nanomolar range, $K_d^{specific} = 7 \pm 5$ nM. These are the first estimated DNA-binding constants in live cell nuclei. (Papadopoulos et al., *PNAS* 107, 4087-4092; Vukojevic et al., *PNAS* 107, 4093-4098).

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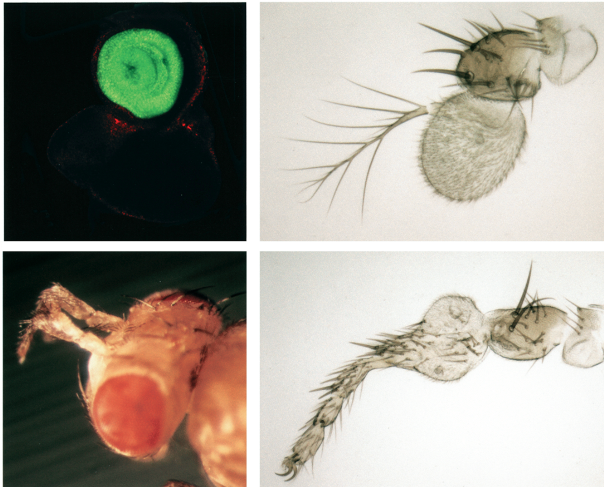

Synthetic Antennapedia gene action. A synthetic Antennapedia gene consisting of the YPWM motif and the Homeodomain (HD) fused to Green Fluorescent Protein (GFP) driven by the yeast transcription factor Gal4 fused to the Distalless (Dll) enhancer. In transgenic flies this construct is expressed in the antennal disc (green circle) and transforms the antenna (upper right) into a foot (below).

Most important publications 2008-2009

- Blanco, J., Seimiya, M., Pauli, T., Reichert, H. & Gehring, W. J. (2009). Wingless and Hedgehog signaling pathways regulate orthodenticle and eyes absent during ocelli development in Drosophila. *Dev Biol* 329, 104-15.
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TOR signaling and control of cell growth

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Introduction

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

The TOR signaling network

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

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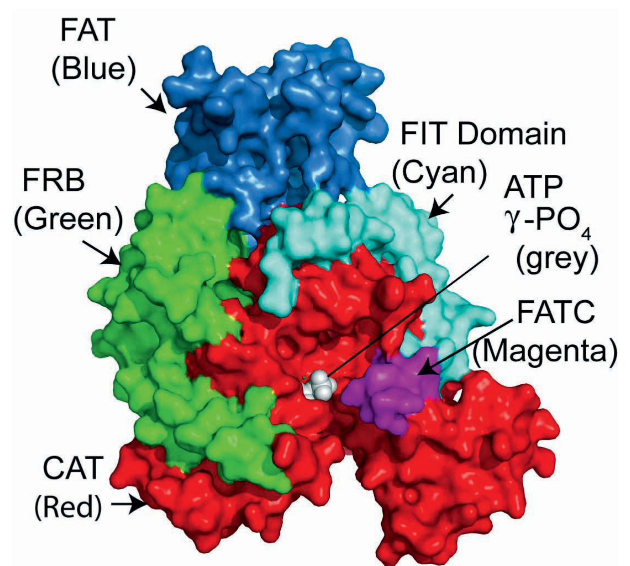
pathway, grafted onto the more ancestral nutrient sensitive TOR pathway, co-evolved with multicellularity. TOR activates cell growth by positively and negatively regulating several anabolic and catabolic process, respectively, that collectively determine mass accumulation and thus cell size. The anabolic 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, and in mice. 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. Examples of our recent studies on the TOR signaling network and growth control in yeast and mammals are described in the selected publications listed below. The figure illustrates our recent efforts to determine the structure of TOR (see Sturgill and Hall, 2009).



Model of the catalytic region of human TOR.

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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 post-translational modifications of the PGC-1α protein. In turn, PGC-1α regulates the adaptations of muscle to endurance training. Accordingly, ectopic expression of PGC-1α in muscle is sufficient to induce a trained phenotype whereas mice with a genetic ablation of the PGC-1α gene in muscle have an impaired endurance capacity.
Our group is studying the mechanisms that control muscle cell plasticity and their physiological consequences. We try to integrate molecular biology, work in muscle cells in culture and observations on mice with different activity levels to obtain a comprehensive picture of the adaptations in the active and the inactive muscle.

Regulation and coordination of metabolic pathways

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

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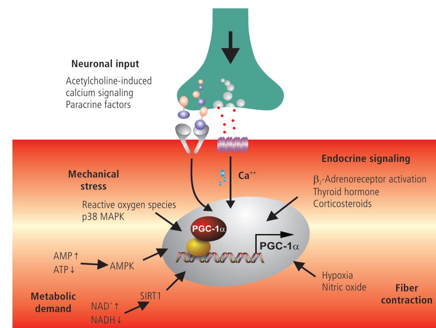
muscle-associated diseases. We study the coordination of anabolic and catabolic pathways in order to pinpoint the differences in substrate fluxes in the healthy and the diseased muscle.

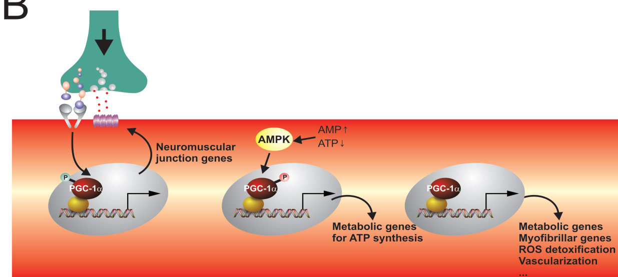
Molecular changes in muscle atrophy and dystrophies
Muscle disuse, induced by a Western life-style or caused by diseases, leads to fiber atrophy, reduced muscle functionality and is ultimately fatal in certain inherited and sporadic muscular dystrophies. Little is known about the etiology of most of 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. In an energy crisis, AMP-dependent protein kinase (AMPK) promotes posttranslational modifications of the PGC-1α in a temporally controlled manner which dedicates PGC-1α to specifically induce genes involved in substrate metabolism and ATP production. We are investigating these effects using systemic approaches to obtain a comprehensive overview on the coordination of the adaptations of muscle to exercise on the molecular level.

A



B



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

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Traffic in the secretory pathway

Understanding the molecular mechanisms underlying the secretory pathway is a major goal of cell biology and biomedical research. In eukaryotic cells one third of all newly synthesized proteins are co-translationally inserted into the endoplasmic reticulum (ER) where they are folded, modified and subjected to quality control prior to secretion or transport to various intracellular organelles. The current challenge is to identify all key molecules that catalyze each of the numerous steps in protein sorting and to integrate their function to understand how organelles are formed and maintained with characteristic structure and function, how itinerant proteins are separated from resident proteins, and how traffic is regulated by cell signaling. We are studying major questions related to these issues in human and other mammalian cells using live cell imaging, protein fragment complementation and high-throughput siRNA-based gene silencing as well as numerous other cell biological, biochemical, biophysical, and molecular approaches. Knowledge of the fundamental processes controlling the function of the secretory pathway may ultimately lead to new strategies for the treatment of inherited and acquired diseases in which protein secretion is impaired.

Control of the architecture of the early secretory pathway

The secretory pathway of higher eukaryotic cells is composed of the three membrane organelles ER, ERGIC (ER-Golgi intermediate compartment) and Golgi. Maintenance of these organelles requires a balance of anterograde (secretory) and retrograde (backward) vesicular traffic, but how this balance is controlled is poorly understood. Our siRNA-based silencing studies of membrane proteins that cycle between ER, ERGIC and Golgi have revealed that the cargo receptors Surf4, ERGIC-53, p25 and p28 are required to maintain the architecture of ERGIC and Golgi (Koegler et al., 2009; Mitrovic et al., 2008). Another determinant that controls organelle morphology is cargo load. Depending on whether more or less protein needs to be handled, the secretory pathway modulates the size of its organelles. We uncovered key factors that determine the adaptation of ER exit sites (ERES) to acute and chronic increases in cargo load (Farhan et al., 2008).

Dynamics of the ERGIC studied in living cells

The ERGIC defined by the mannose-binding membrane lectin ERGIC-53 consists of a few hundred tubulovesicular membrane clusters and is a mandatory intermediate station for proteins moving from ER to Golgi. Our live imaging studies have established that the ERGIC clusters constitute stationary membrane entities rather than mobile carriers. Three vesicular pathways depart form the ERGIC:

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1. to the cis-Golgi in anterograde direction; 2. to the ER in retrograde direction; 3. laterally, connecting individual ERGIC clusters. Likewise, the ERGIC receives vesicles from three sides: the ER, the cis-Golgi, and other ERGIC clusters. In studying the mechanisms controlling tri-directional traffic from and to the ERGIC we are focusing on the Arf proteins and phospholipases.

Signal-mediated export of glycosylphosphatidyl-inositol (GPI)-anchored membrane proteins from the ER

Many secretory proteins leave the ER not just passively by default but in a selective way involving transport signals. We found that transmembrane proteins possess cytosolic ER-export motifs that interact with isoforms of the COPII coat protein subunit Sec24 in a distinctive way. Luminally exposed GPI-anchored membrane proteins cannot bind directly to Sec24. We uncovered that ER-to-Golgi transport of the human GPI-anchored protein CD59 requires Sec24, with preference for the Sec24C and Sec24D isoforms, and the recycling transmembrane protein complex p24/p23 that exhibits the same Sec24 isoform preference for ER export. Our results suggest that the p24/p23 complex acts as a cargo receptor for GPI-anchored proteins by facilitating their ER export in a Sec24-isoform-selective manner involving lipid rafts as early sorting platforms (Bonnon et al., 2010).

Capturing protein interactions in the secretory pathway of living cells to study cargo receptor-mediated traffic

Numerous protein-protein interactions guide newly synthesized proteins through the secretory pathway. Most of these interactions are of low affinity and transient, and therefore difficult to study. We have developed a yellow fluorescent protein (YFP) - based protein fragment complementation assay (PCA) to detect protein-protein interactions in the secretory pathway of living cells and applied it for the screening of human cDNA libraries. By this approach we identified alpha1-antitrypsin as a new cargo protein of ERGIC-53 (Nyfeler et al., 2008). The results provide direct evidence for active receptor-mediated ER export of a soluble secretory protein in higher eukaryotes. The same strategy is currently used to find cargo proteins for the ERGIC-related mannose lectin VIP36.

Control of traffic in the secretory pathway by kinases and phosphatases

Kinases and phosphatases are key enzymes regulating diverse cellular processes. In collaboration with M. Zerial we have conducted a siRNA high throughput screen for human kinases and phosphatases involved in controlling traffic routes in the secretory pathway. This screen uncovered numerous enzymes whose silencing affects ER export, ERES, ERGIC, and the Golgi. Among the pathways identified, the Raf/MEK/ERK cascade controls the number of ERES via Sec16 phosphorylation.

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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-guanosine-monophosphate (c-di-GMP) and its role in bacterial cell signaling and dynamics (Schirmer & Jenal, 2009; *Nature Reviews Microbiol* 7, 724-735). C-di-GMP emerges as an 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. In the past two years we have continued our efforts 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

In *Caulobacter crescentus,* 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, 152-160; Jenal, 2009, *Res Microbiol* 160, 687-695). We recently discovered that c-di-GMP is an integral part of this regulatory network and that the DGC PleD is a key regulatory components of pole development. We showed that during the cell cycle PleD is activated by phosphorylation and that phosphorylation-mediated dimerization serves to sequester activated PleD to the differentiating *Caulobacter* cell pole 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. We have provided *in vitro* and *in vivo* evidence that DivK acts as an allosteric regulator of both DivJ and PleC and that these kinase feedback loops quickly and robustly determine *C. crescentus* cell fate through the activation of the PleD DGC (Paul et al., 2008) *(see figure).*
We could also show that c-di-GMP promotes *C. crescentus* cell cycle progression by mediating S-phase entry through the degradation of the replication initiation inhibitor CtrA (Dürig et al., 2009). Upon S-phase entry CtrA and its protease ClpXP dynamically localize to the old cell pole where CtrA is rapidly degraded. While localization of the protease complex is regulated through the CckA-ChpT-CpdR phosphorylation cascade, sequestration of CtrA to the cell pole depends on PopA, a newly identified c-di-GMP effector protein *(see figure).* This is the first report that links c-di-GMP to protein dynamics and cell cycle control in bacteria. Recent results indicated that PleD plays a major role in PopA activation, establishing the PleC-DivJ-DivK-PleD circuitry as an

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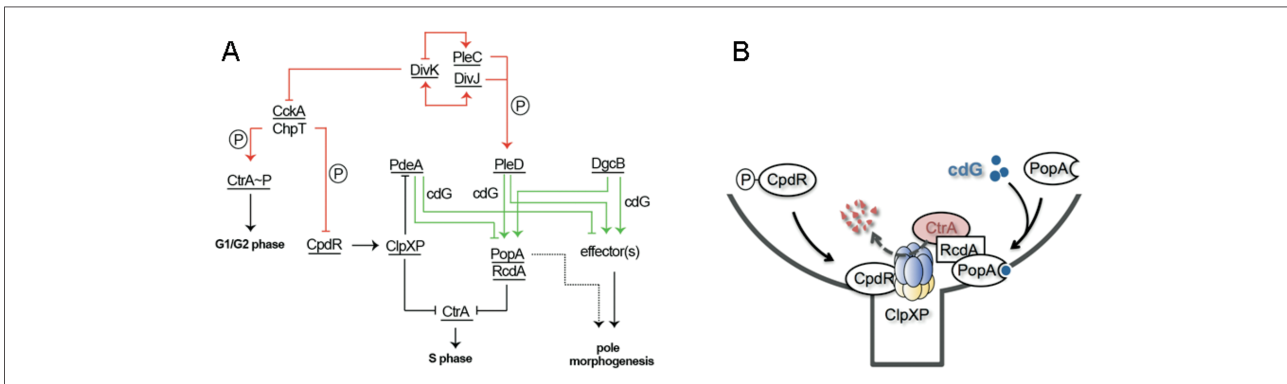

Schematic of the molecular circuitry driving and coordinating cell cycle progression and pole morphogenesis in C. crescentus. Phosphorylation-mediated control is indicated in red, c-di-GMP (cdG) mediated control is shown in green (left). Spatio-dynamic control of selected components of the circuitry is shown on the right.

important pathway to coordinate cell cycle progression and pole development *(see figure).*

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 et al., 2009B). Swimming velocity is controlled by the synergistic action of at least five signaling proteins that adjust the cellular concentration of c-di-GMP. 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 et al., 2009A). The synergistic roles of ppGpp and c-di-GMP in biofilm induction, suggest that interference with bacterial second messenger signaling might represent an effective means for biofilm control during chronic infections.

Chronic *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) patients can be treated with antibiotics, however full clearance is not possible due to the adaptation of infective species to a persistent lifestyle. Adaptive *P. aeruginosa* morphotypes include small colony variants (SCVs), slow growing and strongly adherent variants whose appearance correlates with poor lung function. 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 and drastically reduced growth rates, two hallmarks of SCV behavior. YfiN-mediated SCVs were shown to be highly resistant to macrophage phagocytosis, suggesting a role for the SCV phenotype in immune system evasion. Consistent with this, activation of YfiN significantly increased the persistence of *P. aeruginosa* in long-term mouse infections, establishing a firm causal link between SCV, c-di-GMP, and persistence in chronic *P. aeruginosa* infections.

Most important publications 2008-2009

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3' end processing and quality control of eukaryotic RNAs

We are investigating the protein machinery involved in the processing of messenger RNA precursors (pre-mRNAs) in the nucleus of eukaryotic cells. Pre-mRNAs are synthesized by RNA polymerase II and undergo three processing steps that include the addition of a 7-methyl guanosine "cap" at the 5' end, the removal of intron sequences by RNA splicing and the addition of a poly(A) tail at their 3' ends. The capping, splicing and 3' end processing reactions are coupled to each other and to the transcription process as well. We are studying the composition of the multiprotein complexes and the reaction mechanism of the steps involved in 3' end formation: endonucleolytic cleavage of the pre-mRNA followed by polyadenylation. The overall reaction involves a number of trans-acting protein factors that interact with sequence signals in the pre-mRNA substrates and which each other, forming a network of RNA-protein and protein-protein interactions. The repertoire of the proteins making up the different 3' end processing factors is known. However, except for poly(A) polymerase, the enzyme catalyzing the synthesis of the poly(A) tails, and the putative endonuclease involved in the cleavage step of the reaction, the functions of most other subunits remain to be elucidated. A second topic of our research is the characterization and functional analysis of non-canonical poly(A) polymerases involved in RNA quality control and other processes.

3' end processing of yeast messenger RNA precursors
3' end processing of yeast pre-mRNAs *in vitro* requires cleavage factor IA (CF IA), cleavage factor IB (CF IB) and cleavage and polyadenylation factor (CPF). CF IA consists of four subunits, CF IB is a single polypeptide and CPF is a multiprotein complex of about fifteen polypeptides.

New functions of the CPF subunit Ysh1p

Pre-mRNA 3' end formation is tightly linked to upstream and downstream events of eukaryotic mRNA synthesis. The two-step reaction involves endonucleolytic cleavage of the primary transcript followed by poly(A) addition to the upstream cleavage product. Ysh1p is the yeast homologue of the 73 kd subunit of mammalian CPSF, which most likely is the endonuclease responsible for the cleavage step of pre-mRNA 3' end processing. We have isolated a number of new temperature- and cold-sensitive mutant alleles. We showed that Ysh1p plays a crucial role in 3' end formation and in RNA polymerase II (RNAP II) transcription termination on mRNA genes. Moreover, we observed additional deficiencies in *ysh1* mutant strains, which were partially allele specific. Interestingly, 3' end formation of small nucleolar RNAs (snoRNA) and RNAP II termination of specific snoRNAs were defective in the cold-sensitive *ysh1-12* strain. We also observed the accumulation of several mRNAs including the *NRD1* pre-mRNA in this

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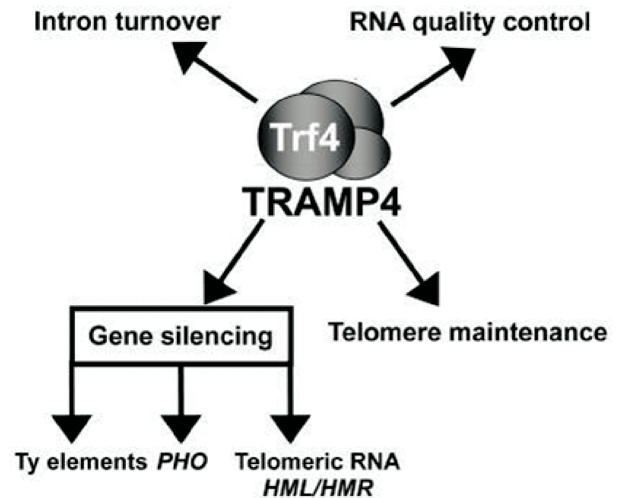
mutant. *NRD1* is one of several proteins required for transcription termination of snoRNA genes. We found that *NRD1* autoregulation is associated with endonucleolytic cleavage and that this process may involve Ysh1p. In addition, the *ysh1-12* strain displayed defects in splicing of pre-mRNAs and pre-snoRNAs, suggesting a functional link between splicing and 3' end formation in yeast.

The function of non-canonical poly(A) polymerases in RNA metabolism

Trf4p and Trf5p are non-canonical poly(A) polymerases of *Saccharomyces cerevisiae* and are part of the heteromeric protein complexes TRAMP4 and TRAMP5 that promote the degradation of aberrant and short-lived RNA substrates by interacting with the nuclear exosome. Work from several laboratories has shown that the polyadenylation activity of Trf4p is required for tagging the 3' ends of aberrant tRNA molecules and that the oligo(A) tails facilitate their interaction with the exosome. To assess the level of functional redundancy between the paralogous Trf4 and Trf5 proteins and to investigate the role of the Trf4-dependent polyadenylation *in vivo,* we used DNA microarrays to compare gene expression of the wild type yeast strain with either that of *trf4D* or *trf5D* mutant strains or the *trf4D* mutant expressing the polyadenylation-defective Trf4(DADA) protein. We found little overlap between the sets of transcripts with altered expression in the *trf4D* or the *trf5D* mutants, suggesting that Trf4p and Trf5p target distinct groups of RNAs for degradation. Surprisingly, most RNAs the expression of which was altered by the trf4 deletion were restored to wild type levels by overexpression of *TRF4(DADA),* showing that the polyadenylation activity of Trf4p is dispensable *in vivo.* Apart from previously reported Trf4p and Trf5p target RNAs, this analysis along with *in vivo* cross-linking and RNA immunopurification-chip experiments revealed that both the TRAMP4 and the TRAMP5 complexes stimulate the degradation of spliced out introns via a mechanism that is independent of the polyadenylation activity of Trf4p. In addition, we showed that disruption of *trf4* causes severe shortening of telomeres suggesting that *TRF4* functions in the maintenance of telomere length. The results demonstrate that paralogous TRAMP complexes have distinct RNA selectivities with functional implications in RNA surveillance as well as other RNA-related processes and suggest widespread and integrative functions of TRAMP complexes in the coordination of gene expression.



Functions of the TRAMP4 complex. (See San Paolo et al. (2009) for details).

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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, spectacular and beautiful stage of the cell cycle. The main goal of our research is to elucidate the mechanisms that underlie the error-free segregation of chromosomes and those that regulate mitosis in time and space. A better understanding of mitosis will hopefully illuminate the origins of the chromosome aberrations (aneuploidy) 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. In addition to studying the composition, regulation and dynamics of the mitotic spindle and kinetochores, we aim at elucidating the function of a surveillance mechanism ('the spindle assembly checkpoint') that monitors the complete attachment of all mitotic chromosomes to the spindle. At the two poles of the spindle apparatus are tiny organelles known as 'centrosomes'. These function to organize microtubule arrays in most animal cells and are present as only one or two copies per cell (depending on cell cycle stage). Deregulation of the centrosome duplication cycle is believed to constitute a major cause of chromosome missegregation during the development of human cancers. Furthermore, mutations in genes coding for centrosome-associated proteins are held responsible for a variety of human diseases, ranging from brain diseases (e.g. microcephaly) to a multitude of ciliopathies. Hence, the second major research interest in our laboratory is focused on the biogenesis, duplication and function of centrosomes.

After 10 years at the Max-Planck-Institute for Biochemistry (Germany), our laboratory moved to the Biozentrum in the course of 2009. We continue to combine reverse genetics, immunocytochemistry and modern biochemical techniques (notably mass spectrometry) to unravel the molecular mechanisms that ensure correct centrosome duplication and chromosome segregation in human cells. A common thread running through our studies is a focus on phosphorylation (a reversible protein modification controlled by kinases and phosphatases). Studying mostly human cells in culture, we take two complementary

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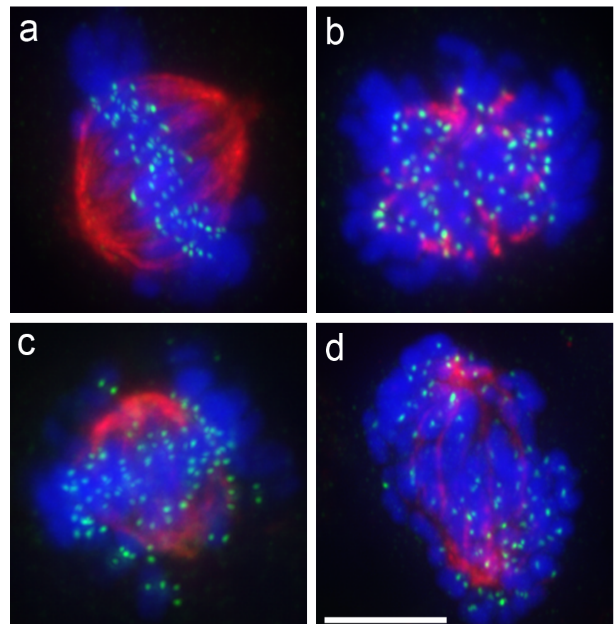
approaches: a 'comprehensive' strategy uses mass spectrometry to establish inventories of proteins and phosphorylation sites in the spindle apparatus, the kinetochore and the centrosome, whereas a second 'in depth' approach aims at elucidating the wiring of key regulatory circuits, as defined by kinases, phosphatases, and selected substrates. We anticipate that the convergence of these two approaches will lead to a clearer picture of the regulation of centrosome duplication and chromosome segregation in normal cells, as well as provide insights into the deregulation of these processes in disease. In the past year, we have successfully completed a large scale phospho-proteomics study that provides information, with unprecedented temporal resolution, on hundreds of mitotic phosphorylation sites. In parallel, we have characterized three novel spindle components, termed Chica, Spindly and Ska3, respectively. Of particular interest is our discovery that a ternary complex of Ska proteins (Ska1, 2 and 3) plays a major role in stabilizing the attachment of spindle microtubules to kinetochores. We anticipate that the correct expression, localization and function of the Ska complex will turn out to be critical for error-free segregation of chromosomes during cell division. Ongoing work also concerns the function and regulation of several cell cycle-regulatory kinases, including Cyclin-dependent kinases, Polo-like kinases, Aurora kinases and spindle checkpoint kinases (Mps1/Bub1/BubR1). With regard to centrosome biology, we continue our functional characterization of Plk4/Sak, a protein kinase that we have previously shown to function as a key regulator of centrosome duplication.

Concerning the future, we believe that one major challenge concerns the development of technologies that will permit the acquisition of *quantitative* information about the abundance, localization and dynamics of cell cycle-regulatory gene products under physiological conditions. We anticipate that such technologies will become increasingly important not only in systems biology but in life science research altogether. There is no shortage of important unresolved questions related to the mechanisms that underlie centrosome duplication and chromosome segregation, and the cell cycle field clearly holds promise for the development of novel therapeutic approaches. In particular, it appears legitimate to hope that new information will contribute to the design of novel strategies to thwart cancer growth.



Chromosome alignment in mitotic Hela S3 cells (a). Chromosome congression defects in the absence of CHICA (b), hSpindly (c) or Ska complex (d) (Hec1: green; α-Tubulin: red; DNA: blue). Scale bar = 10 µm.

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Evolution of biological systems: the transition from uninucleated yeast cells to multinucleated permanently elongating hyphae

The high degree of gene order conservation (synteny) of the budding yeast *S. cerevisiae* and the filamentous ascomycete *A. gossypii* convincingly shows that both organisms derived from a common ancestor (Dietrich et al., *Science* 2004). *S. cerevisiae* proliferates as budding, uninucleated cells and *A. gossypii* as multinucleated, permanently elongating, frequently branching but non-dividing hyphae.

We want to identify main genetic alterations responsible for the cell biological differences between both systems. In the past 14 years, we have uncovered for several proteins new cellular functions unknown to their yeast homologues. Examples are AgRsr1 (polarisome stability), AgCla4 (hyphal growth speed), AgRho3 (prevention of isotropic growth phases), AgCln1/2 (permanent colocalization with growth zone), AgDyn1 (pulling force for maintaining equally distant nuclei), AgKip2 (nuclear oscillation and bypassing), AgRax2 (coordination of cell wall synthesis).

Work published in 2008 and 2009 focused on sustained polar growth of hyphae and the dynamics of nuclei. The maturation from slowly growing hyphae, which form lateral branches (new axes of polarity), to fast growing hyphae which perform tip splitting (symmetric division into two novel axes of polarity) is clearly different from polar growth periods of budding yeast. We found that AgPxl1, the homologue of the yeast paxillin, is essential for symmetric tip splitting but plays no detectable role in laterally branching hyphae (Knechtle et al., *FGB*). The vesicle fusion zone at the tip cortex of slow and fast hyphae was determined from the co-localization of polarity control and exocyst components. Per minute this zone processes about 1'000 vesicles in slow and 10’000 or more vesicles in fast hyphae, however, its cortical area only marginally increases. The permanent presence of the polarity machinery in this confined area, a controlled accumulation of vesicles plus several polarity components in the tip region, and spatial separation of the zones of exocytosis (tip front) and endocytosis (tip rim) are conditions to achieve fast hyphal growth (Köhli et al., *JCS*). AgRho3 and AgBoi1/2 are important for crosstalks between exocytosis and endocytosis. We also found a threefold increase in the intitial rate of surface expansion by deleting AgGic1/2, one of the effectors of the growth-promoting GTPase AgCdc42 (Köhli et al., *submitted*).

Hyphae are compartmentalized by septa. In contrast to budding yeast, formation of septa is uncoupled from nuclear cycles. Septation sites are selected at the growing tips. While septa mature and finally close hyphal elongation continues. The longer the open apical compartments the more secretory vesicles can be produced and transported

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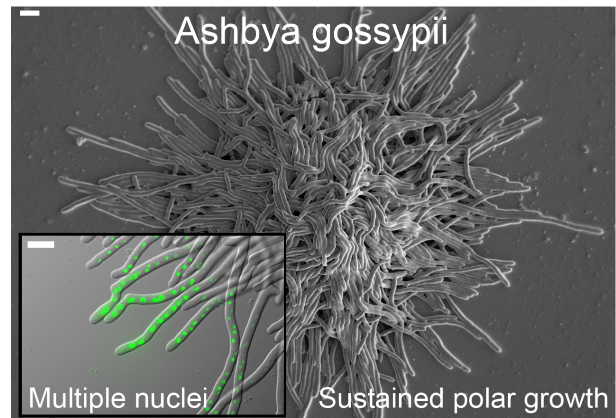
to the growing tip, thus explaining hyphal growth speed acceleration until a maximal exocytic capacity is reached. Cell divisions cannot occur at septa because genes for degradation of the primary septum were lost during evolution (Kaufmann and Philippsen, *MCB*).

In collaboration with Sue Jaspersen, we studied the migration of multiple nuclei in hyphae of *A. gossypii.* Three types of cytoplasmic microtubule (cMT)-dependent nuclear movements were characterized: short range oscillation, rotation, and long-range nuclear bypassing. In the absence of cMTs hyphae continue growing with normal speeds because nuclei are co-transported by the cytoplasmic stream. cMT-dependent nuclear movements are in part controlled by the spindle pole body (SPB) the sole site of microtubule nucleation in *A. gossypii.* Electron microscopy revealed a laminar structure similar to budding yeast SPBs. Up to six perpendicular and tangential cMTs emanated from the outer plaque (OP). The perpendicular and tangential cMTs most likely correspond to short, often cortex-associated cMTs and to long, hyphal growth-axis oriented cMTs, respectively. Each SPB nucleates an independent array of cMTs explaining the autonomous nuclear oscillations and bypassing in *A. gossypii* hyphae (Lang et al., *MBC*). Hyphae lacking the OP components AgSpc72, AgNud1, AgCnm67 or the MT-stabilizing factor AgStu2 grew like wild-type but showed substantial alterations in the number, length and/or nucleation sites of cMTs. Based on residual nuclear dynamics in these mutants we conclude that long tangential cMTs most likely promote nuclear bypassing and short cMTs are important for nuclear oscillation (Lang et al., *MBC*).

We also successfully collaborated with Fred Dietrich (Duke University) in sequencing novel *Ashbya* species isolated from insects (Dietrich et al., submitted), Piet de Groot and Franz Klis (University of Amsterdam) on the *A. gossypii* cell wall proteome (Rischatsch et al., submitted), Carmen Ruiz and Antonio Di Pietro (University of Cordoba) on the coordination of polar growth, nuclear division and septation in the pathogenic fungus *Fusarium oxysporum* (Ruiz et al., submitted) and Anna Chelstowska (Polish Academy of Science) on functional conservation of *S. cerevisiae* and *A. gossypii* SH3 proteins.



Scanning electron microscopy image of a radially expanding A. gossypii mycelium 12 hours after germination of spores (ZMB, Uni Basel). Insert: Fluorescence microscopy image of histone-GFP labelled nuclei in hyphae (Claudia Lang). White bars = 10 µm.

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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 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. Below a part of our ongoing research over the last 2 years is highlighted.

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

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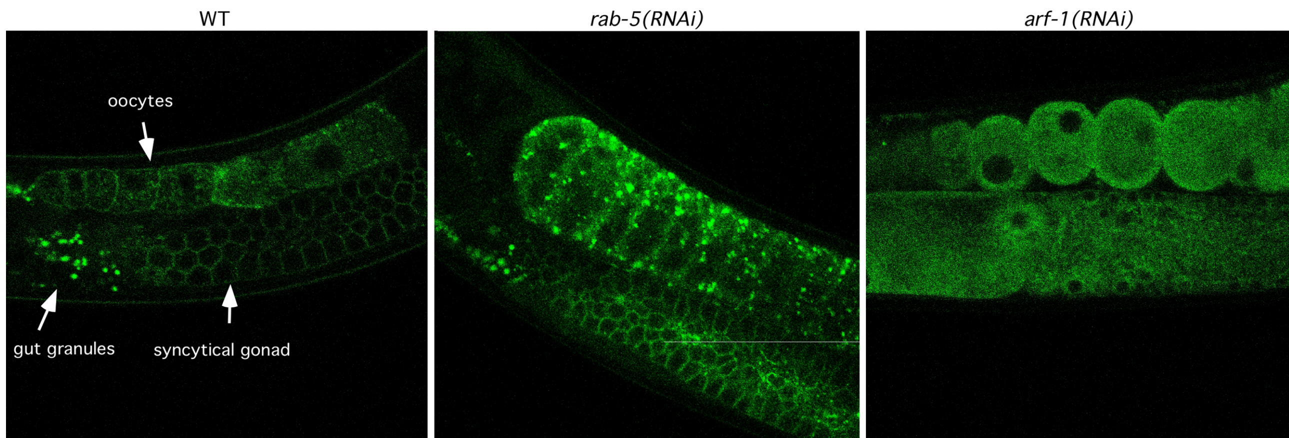

The architecture of the Golgi is disturbed upon knockdown of the small GTPases RAB-5 and ARF-1. Worms expressing the Golgi marker UGTP-1::GFP (green) under the pie-1 promoter, which drives expression in the gonad and in early embryos were subjected to RNAi by feeding. The distribution of UGTP-1::GFP 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.

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, we could recently show in collaboration with Manuel Muñiz that 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 identified 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).

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 a critical switch in endosome maturation. These functions of SAND-1 are also conserved in mammalian cells (Poteryaev et al., 2010).

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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 pro-vasopressin cause dominant *diabetes insipidus*.

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 figure, part A)*. To test their function, we deleted the plug and/or mutated all six ring residues to more hydrophilic or even charged amino acids. The translocon was found to be surprisingly tolerant to these mutations. The plug domain is not essential, but its deletion affects signal recognition and the regulated opening of the translocon required for faithful protein orientation. Most interestingly, ring mutants were found to affect the integration of hydrophobic sequences into the lipid bilayer, indicating that the translocon does not simply catalyze the partitioning of potential transmembrane segments between an aqueous environment and the lipid bilayer, but that it plays an active role in setting the hydrophobicity threshold for membrane integration.

Post-Golgi protein sorting
Transport between organelles is generally mediated by membrane vesicles formed by the recruitment of cytosolic coat proteins. In a permeabilized cell assay, we found the formation of recycling vesicles to return receptors from endosomes back to the plasma membrane to be dependent on clathrin coats with AP-1 adaptors and to be regulated by

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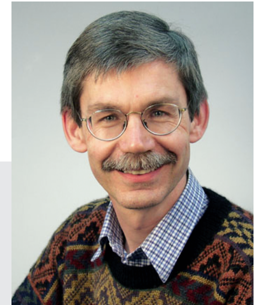

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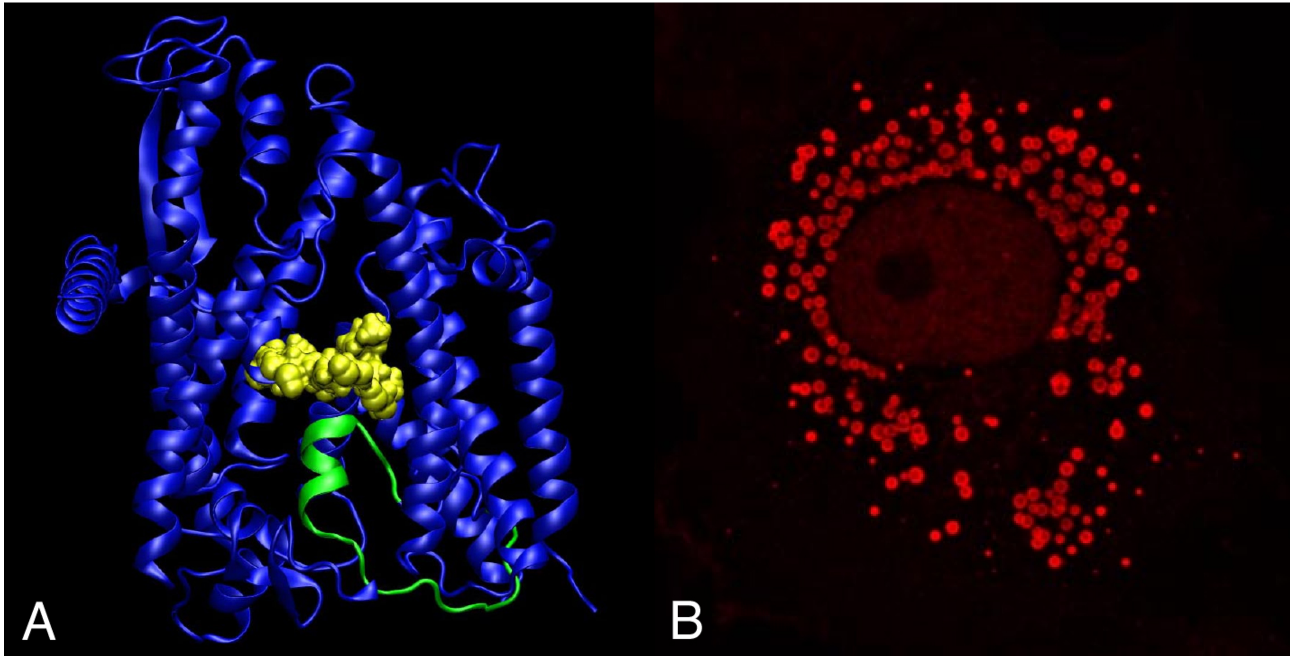

rab4, rab11, and rabaptin-5/rabex-5. We are studying the molecular mechanism by which these proteins mediate and regulate vesicle formation and *in vivo* transferrin recycling.

A) View of the interior of the closed Sec61 translocon with the plug domain in green and the constriction residues in gold (Sefer Baday). B) Pro-vasopressin aggregates in COS-1 cells.

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.

Diabetes insipidus: a trafficking disease

Autosomal dominant neurohypophyseal *diabetes insipidus* results from mutations in the precursor protein of the hormone vasopressin. Mutant precursors are retained in the ER of vasopressinergic neurons and cause cell degeneration. We discovered that pro-vasopressin mutants form disulfide-linked oligomers and develop large, fibrillar aggregations in fibroblast and neuronal cell lines *(see figure, part B)*. 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. The responsible sequence and the mechanism of cell death remain to by elucidated.

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Focal Area Infection Biology

Objective

Infectious diseases represent a serious health problem, worldwide. New pathogens emerge as a consequence of new behavioral, industrial or agricultural practices while ancient plagues represent a recurrent problem in some areas of the world. The objective of the Focal Area Infection Biology is to understand infectious diseases at the very basic cellular and molecular levels in order to better control them in the future. The focus lies on bacterial infections and a number of different pathogens are studied, representing archetypes for different virulence mechanisms such as intracellular replication, immune evasion, injection of bacterial effectors into host cells, biofilm formation and persistence.

The impact of this research goes beyond the specific field of microbial pathogenesis, addressing also basic principles in cell and molecular biology including pro-inflammatory signaling, intracellular traffic, regulation in bacteria, nanomachines, *in vivo* microbial metabolism. Recently, several groups have introduced systems biology as a new approach to address host-pathogen interactions. Molecular and cellular biology are thus at the core of the Focal Area activity anchoring it within the research landscape of the Biozentrum. This is reflected by extensive collaborations with our colleagues from the Biozentrum, especially with those from the Core Programs "Structural Biology and Biophysics" and "Computational & Systems Biology".

Education

The Focal Area Infection Biology organizes two weekly seminars in infection biology and a complete graduate teaching cycle (Cycle A) including the lectures entitled "Major Microbial Diseases and Vaccine Development", "Microbial Cell Structures and Drug Targets", "Signaling in Inflammation" and "Recent Advances in Systems Biology". The Focal Area also contributes to the teaching in the Master program in "Infectious Diseases, Vaccinology and Drug Discovery" jointly organized by the Novartis Institute for Tropical Diseases in Singapore (NITD), The University of Singapore, The Swiss Tropical Institute Basel, and the Biozentrum.

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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-meso-diaminopimelic acid found in the peptidoglycan of Gram-negative bacteria. Among the genes up-regulated in infected epithelial cells, the chemokine interleukin-8 (IL-8) plays a central role. Indeed, by attracting polymorphonuclear cells (PMNs) from the peripheral circulation to the infected area, IL-8 secretion limits the spread of *Shigella* invasion.

Bacterial entry into epithelial cells

Few host proteins targeted by *Shigella* have been identified. However, we do not have yet a comprehensive model for the host-pathogen interactions that govern bacterial entry into epithelial cells. With this aim, we have set up, in collaboration with Pr 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, starting January 2010), which aims at identifying the components of the human infectome for a set of important bacterial and viral pathogens. In parallel, we have done follow-up studies on two proteins found in a pilot RNAi screen: the small GTPase Arf1 and type 3 phosphatidylinositol 4-kinase. We have found that these proteins are required for efficient bacterial uptake by promoting actin polymerization at the entry foci.

Pathogen sensing and NF-κB regulation

The receptor Nod1 is critical for the recognition of intracellular bacteria. Upon ligand recognition, Nod1 homo-dimerizes and initiates a complex cascade of signal transduction that leads to activation of the transcription

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factor NF-κB and the different MAP kinases *(see figure)*. Using an RNAi screening strategy, we have found that the protein TAOK3 was involved in this signaling pathway. Work is ongoing to characterize its exact function during *Shigella* infection. We also found that the protein clathrin heavy chain (CLTC) negatively regulated NF-κB activation.

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 *(see figure)*. Yet, massive IL - 8 secretion is observed in Shigellosis. We have reconciled these contradictory observations by showing that a host mechanism of cell-cell communication compensates the immuno-suppressive activity of *Shigella* effectors in infected cells. By monitoring signaling at the single-cell level in conditions of low multiplicity of infection, we observed that during *Shigella* infection, the activation of important signaling pathways of inflammation, including NF-κB, JNK, ERK and p38, propagates from infected to uninfected adjacent cells. We now have evidence that this mechanism of bystander activation amplifies inflammation in response to bacterial infection. 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. 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*. We are currently investigating in more detail the molecular basis of bystander activation using RNAi and chemical screens. In addition, we are testing whether other proinflammatory pathways can also spread from infected to uninfected cells during *Shigella* infection (i.e. cPLA2 activation, calcium). Finally, we are examining the different strategies that *Shigella* uses to block IL - 8 expression in infected cells. Indeed, our data indicate that besides NF-κB and the MAP kinase, some additional signaling pathways are targeted by *Shigella* to inhibit IL - 8 expression in infected cells.

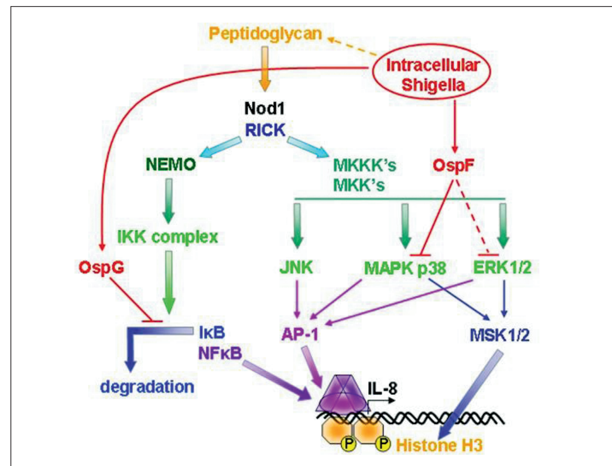

Illustration of the signaling pathways that control IL - 8 expression in infected cells. The effectors OspF and OspG block the activation of the MAP kinases and NF-κB, respectively.

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Systems Biology of *Salmonella* infection

Infectious diseases are a major worldwide health problem. The situation is worsened by increasing bacterial resistance against antibiotics and rising human mobility. Unfortunately, development of new antimicrobial drugs and effective vaccines is rather slow resulting in increasingly unmet medical needs. We try to address some aspects of this complex and difficult issue. In particular, we are aiming at rational approaches to identify protective antigens for efficacious vaccines and suitable targets for development of novel antibiotics.

For our research we use *Salmonella* as a model pathogen. *Salmonella* can cause diarrhea, typhoid fever, or non-typhoidal *Salmonella* (NTS) bacteremia, which together still cause several hundred thousand deaths each year. In addition to its importance as a human pathogen, *Salmonella* is one of the best-studied model pathogens because of its easy genetic manipulation and excellent mouse infection models that closely mimic human diseases.

We use an integrated experimental approach to investigate *Salmonella*-host interactions. Specifically, we purify *Salmonella* from infected tissues using high-speed flow cytometry. We analyze these samples using proteomics to obtain a comprehensive overview of *Salmonella* activities during infection.

Vaccine development
Salmonella proteins with detectable in vivo expression could represent promising antigen candidates for vaccination. However, even among these proteins only a small minority can actually confer protective immunity. To enable more efficient antigen identification, we systematically evaluate additional parameters such as physicochemical properties, recognition by T cells and/or antibodies in immune individuals, etc. Using this approach we have successfully identified highly protective antigens with increasing hit frequencies. Our ultimate goal is to use some of these antigens to develop a subunit vaccine, which is broadly effective against typhoid/paratyphoid fever and NTS bacteremia.

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 reasons for high and low relevance of metabolic enzymes we systematically characterize the entire *Salmonella* metabolic network during infection. We also analyze what nutrients cross the

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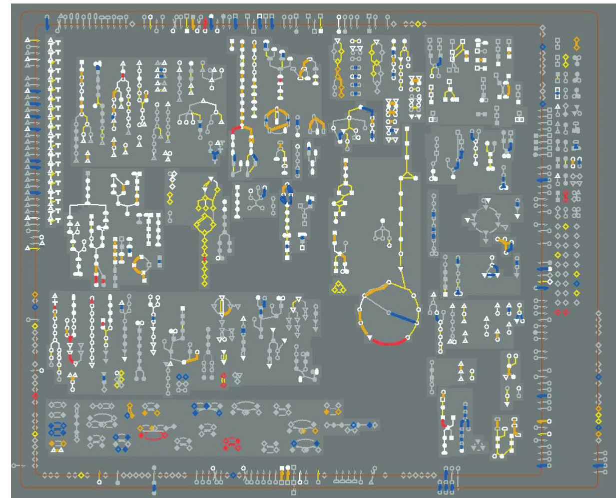
interface between the host and *Salmonella*. We integrate these large-scale data sets in a genome-scale in silico model of *Salmonella* metabolism during infection. The model correctly predicts more than 94% of some 1000 *Salmonella* mutant phenotypes that have been experimentally determined in *in vivo* infection models. Systematic in silico perturbation of the model revealed that diverse nutrients available from the host tissue are largely responsible for the limited importance of most metabolic enzymes in *Salmonella*.

The severity limited number of promising targets in *Salmonella* impairs development of urgently needed novel antibiotics. A potential alternative to targeting single enzymes could be simultaneous inhibition of two different enzymes (combination chemotherapy). This strategy has been successfully used to treat certain infectious diseases, and is also actively pursued for many other medical indications. However, because of the combinatorial explosion there is a massive number of potential combination targets. Comprehensive experimental testing of all these possibilities is unfeasible. Our in silico model enabled us to predict virulence phenotypes for all possible double enzyme defects. We are currently validating several of these predictions and have already identified four novel enzyme combinations that are absolutely essential for *Salmonella* virulence. Some such combination targets might enable development of new antimicrobial chemotherapies.

Heterogeneous host microenvironments

Examination of infected tissues using immunohistochemistry with various cell-type specific antibodies revealed that *Salmonella* reside in several distinct host microenvironments within the same infected tissue that greatly differ in density of host defense cell types such as neutrophils and inflammatory macrophages. It is likely that *Salmonella* faces substantially different conditions in these distinct microenvironments and this could have major impacts on the course of disease and success of control strategies. Unfortunately, current technology does not permit detailed experimental analysis of such pathogen subpopulations *in vivo*. We are developing a set of tools to assess local phenotypes of *Salmonella* defects in each microenvironment. In particular, we generated a series of strains expressing various variants of the green fluorescent protein. Optimized flow cytometry and confocal microscopy methods enable us to distinguish the various strains even in mixed infections and to quantify bacterial tissue loads for each strain. Moreover, we can directly compare local mutant phenotypes in inflamed and non-inflamed tissue microenvironments. Our ultimate goal in these studies is to understand differential host-*Salmonella* interactions in distinct tissue microenvironments.



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.

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

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The type III secretion (T3S) injectisome is a nanosyringe allowing bacteria to inject bacterial effector proteins across the cellular or vacuolar membrane. It is made of 27 Ysc proteins and consists of a cylindrical basal body, made of three rings, anchored to the inner and outer membranes, joined by a central rod and supporting a hollow needle about 7 nm thick and 67 nm long. This scaffold is completed by five different integral membrane proteins and an ATPase complex forming the core of the translocon. In 2008-2009, we pursued our effort to unravel the mechanism of needle length control by the molecular ruler protein YscP. We analyzed the correlation between the size of YscP and the needle length in seven wt *Y. enterocolitica* strains and found a linear correlation (as expected) but the behavior of two strains hinted that the secondary structure of YscP might influence needle length. Hence, eleven variants of YscP were generated by multiple Pro or Gly substitutions. The needle length changed in inverse function of the helical content, indicating that not only the number of residues but also their structure controls length. Taking the secondary motifs into account, Pro/Gly-variants were subjected to in silico modeling by molecular dynamics (coll. M. Dal Peraro, EPFL) to simulate the extension of YscP upon needle growth. The calculated lengths when the helical content is preserved correlated strikingly with the measured needle length, with a constant difference of ~29 nm, which corresponds approximately to the size of the basal body. These data support the ruler model and show that the functional ruler has a helical structure. We then addressed the question of how many rulers control the length of one needle. We monitored the needle length distribution in bacteria encoding simultaneously a long and a short ruler and we compared this distribution to the distribution predicted by mathematical modelling of the different possible scenarios (coll. M. Dal Peraro, EPFL). The results showed unambiguously that only one ruler controls the length of one needle.

In order to decipher the different steps of the assembly of the injectisome, we have grafted the green fluorescent protein (GFP) and the mCherry protein onto four different structural components. The injectisomes were functional and the fluorophores appeared as spots at the bacterial surface, indicating that they corresponded to assembled injectisomes. We then combined these hybrid proteins with an array of mutations in other genes, which allowed us to unravel the sequential assembly order. We came to the conclusion that the assembly starts with the most distal ring and proceeds inwards to the cytosolic part.

We pursued the study of the interaction between *C. canimorsus* and the human innate immune system. Bacteria from the archetype strain Cc5 were found to be

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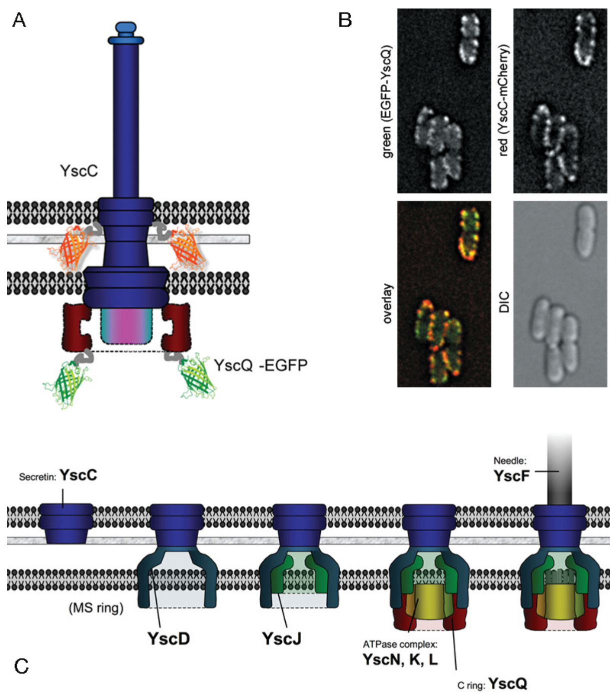

Study of the assembly of the Y. enterocolitica injectisome by fluorescence microscopy. A) Schematic representation of the injectisome tagged simultaneously with mCherry (red basket) grafted onto secretin YscC and with EGFP (green basket) grafted onto YscQ, the component of the cytosolic ring. B) Fluorescence images of Y. enterocolica expressing this dual tagged injectisome. Injectisomes appear as fluorescent spots at the bacterial surface. The overlay of pictures taken in the red and green channels shows that the green and red spots co-localize and hence correspond to assembled nanomachines. C) The combination of four different tagged components with an array of deletions in all the components allowed to decipher the order of assembly of the components. Assembly starts with the insertion of the secretin ring in the outer membrane and then proceeds inwards. (A. Diepold, M. Amstutz).

totally resistant to killing by complement. A hypersensitive transposon mutant turned out to be affected in its LPS structure. *C. canimorsus* were also found to be resistant to phagocytosis by human polymorphonuclear leukocytes and by macrophages. Interestingly, infection of J774.1 cells with live Cc5 bacteria led to the release of a soluble factor, which interfered with the ability of macrophages to kill phagocytosed *E. coli*. Our most striking observation was that *C. canimorsus* feeds on surface-exposed glycoproteins from cultured animal cells. This property was found to depend on a bacterial surface-exposed sialidase. It means that, in the dog's oral cavity, *C. canimorsus* most likely feeds on the dog's mucosal cells. Interestingly however, *C. canimorsus* can also feed on phagocytes and sialidase contributes to persistence in a mouse model. 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. It consists of transposon Tn4351, derived from *Bacteroides fragilis* and a series of *E. coli – Capnocytophaga* shuttle and expression vectors based on the replicon of a naturally occurring plasmid. Finally, we carried out a survey of the presence of *C. canimorsus* in dogs and found a prevalence of about 60 %.

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Cellular, molecular and evoluationary basis of type IV secretion in bacterial pathogenesis

The aim of our studies is to gain a molecular understanding of the function of type IV secretion (T4S) systems in bacterial pathogenesis. T4S systems are ancestrally related to bacterial conjugation systems. Bacterial pathogens targeting eukaryotic host cells have adopted these supramolecular protein assemblies for intracellular delivery of virulence factors from the bacterial cytoplasm directly into the host cell cytoplasm. Our previous work on the pathogenesis of the vascular tumor-inducing bacteria of the genus *Bartonella* revealed crucial roles of two distinct T4S systems, VirB and Trw, in the ability of bacteria to colonize, invade and persist within vascular endothelial cells and erythrocytes, respectively (*see figure,* reviewed in Dehio, 2008). We have shown that the VirB system of *Bartonella henselae* mediates subversion of endothelial cell functions related to vascular tumor formation, including (i) inhibition of apoptotic cell death, (ii) F-actin rearrangements resulting in uptake of large bacterial aggregates via a specialized structure, the invasome, and (iii) NFκB-dependent pro-inflammatory signaling. Further, we have shown that all these effects are mediated by the VirB-dependent injection of seven bacterial effector proteins termed BepA-BepG (Bep = *Bartonella*-translocated effector protein). These effectors carry a bipartite secretion signal at their C-terminus, which is composed of the BID (Bep intracellular delivery) domain and a short positively-charged tail sequence. The N-terminal parts of the Beps carry diverse domains or peptide motifs that are considered to mediate effector functions within host cells. For instance, upon translocation the effectors BepD, BepE and BepF become tyrosine-phosphorylated on short N-terminal repeat motifs, thereby interfering with eukaryotic signal transduction processes (Selbach et al., 2009). Defining the cellular targets and molecular mechanisms of how individual Bep effectors interfere with eukaryotic signaling processes have become a focus of our recent studies.

Opposing roles of BepA and BepG in controlling *in vitro* angiogenesis

B. henselae-triggered vascular proliferation is a well-described clinical manifestation. However, up to date no appropriate model of pathological angiogenesis was available to study the underlying molecular mechanism of vascular proliferation. We have established an *in vitro* model of bacteria-triggered vascular proliferation using spheroids formed by human umbilical vein endothelial cells (HUVECs). Embedded in 3-dimensional collagen gels, these spheroids respond to pro-angiogenic signals by forming characteristic sprouts representing a quantitative read-out of *in vitro* angiogenesis. Using this assay we demonstrated that BepA, previously shown to inhibit apoptosis of endothelial cells, strongly stimulates *in vitro* angiogenesis. In contrast, BepG, involved in

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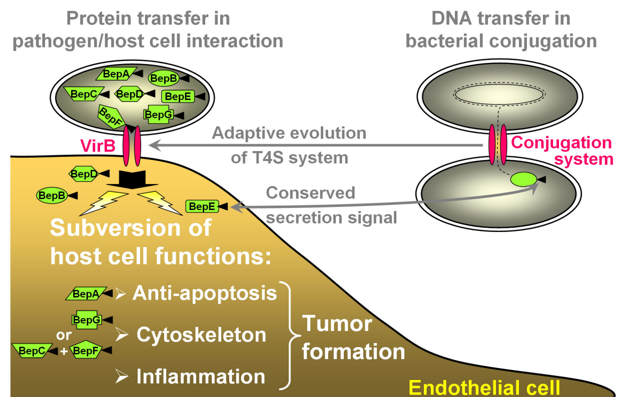
F-actin-dependent cell entry, was found to inhibit *in vitro* angiogenesis (Scheidegger et al., 2009). Together, the established in vitro model of *Bartonella*-induced angiogenesis revealed opposing activities for individual Bep effectors, which together with T4S-independent effects may control the angiogenic actitity of *B. henselae* during chronic infection of the vasculature.

BepG or BepC/BepF trigger F-actin dependent bacterial uptake into host cells

The effector BepG was found to inhibit endocytic uptake of *B. henselae* by endothelial cells, thereby redirecting bacterial uptake to the invasome-mediated pathway. This unique bacterial uptake process is facilitated by massive F-actin rearrangements that depend on the small GTPase Rac1, the Rac1-effector Scar1, and the F-actin organizing complex Arp2/3 (Rhomberg et al., 2009). While BepG is the only T4S effector sufficient to trigger invasome-mediated uptake, the synergistic action of BepC and BepF have a similar effect. As BepC, BepF and BepG have diverse domain structures we assume that the redundancy in triggering this specific mode of bacterial uptake is the result of different, yet converging signal transduction pathways.

Evolutionary genomics identifies T4S systems as host adaptability factors

Based on our published genomic analysis of host adaptation in *Bartonella* (Saenz et al., 2007, *Nat. Genet.* 39, 1469-1476) we have performed an extensive evolutionary genomic analysis of the role of T4S systems in host adaptation (Engel et al., submitted). We could demonstrate that the horizontally acquired VirB system and its translocated Bep effectors represent an evolutionary key innovation that facilitated adaptations to novel hosts via two parallel adaptive radiations. We further showed that the functional versatility and adaptive potential of the VirB system evolved convergently – prior to the radiations – by consecutive rounds of lineage-specific gene duplication followed by functional diversification. This resulted in two diverse arrays of Bep effector proteins in the two radiating lineages of *Bartonella*. Together, we establish *Bartonella* as a bacterial paradigm of adaptive radiation, allowing for the first time in bacteria to study the molecular and evolutionary basis of this fundamental evolutionary process for the generation of organismic diversity.



Effector proteins translocated by the Bartonella T4S system VirB/VirD4 subvert human endothelial cell functions.

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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-guanosine-monophosphate (c-di-GMP) and its role in bacterial cell signaling and dynamics (Schirmer & Jenal, 2009; *Nature Reviews Microbiol* 7, 724-735). C-di-GMP emerges as an 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. In the past two years we have continued our efforts 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

In *Caulobacter crescentus,* 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, 152-160; Jenal, 2009, *Res Microbiol* 160, 687-695). We recently discovered that c-di-GMP is an integral part of this regulatory network and that the DGC PleD is a key regulatory components of pole development. We showed that during the cell cycle PleD is activated by phosphorylation and that phosphorylation-mediated dimerization serves to sequester activated PleD to the differentiating *Caulobacter* cell pole 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. We have provided *in vitro* and *in vivo* evidence that DivK acts as an allosteric regulator of both DivJ and PleC and that these kinase feedback loops quickly and robustly determine *C. crescentus* cell fate through the activation of the PleD DGC (Paul et al., 2008) *(see figure).*
We could also show that c-di-GMP promotes *C. crescentus* cell cycle progression by mediating S-phase entry through the degradation of the replication initiation inhibitor CtrA (Dürig et al., 2009). Upon S-phase entry CtrA and its protease ClpXP dynamically localize to the old cell pole where CtrA is rapidly degraded. While localization of the protease complex is regulated through the CckA-ChpT-CpdR phosphorylation cascade, sequestration of CtrA to the cell pole depends on PopA, a newly identified c-di-GMP effector protein *(see figure).* This is the first report that links c-di-GMP to protein dynamics and cell cycle control in bacteria. Recent results indicated that PleD plays a major role in PopA activation, establishing the PleC-DivJ-DivK-PleD circuitry as an

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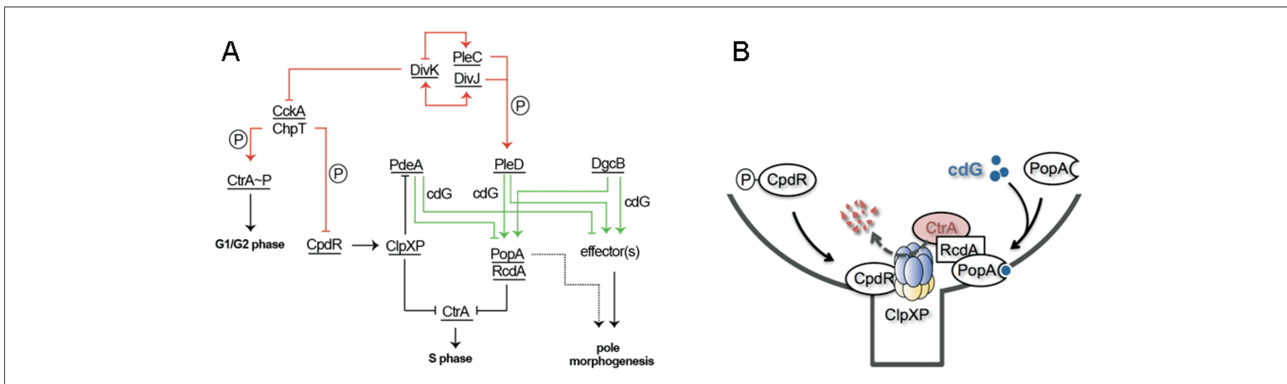

Schematic of the molecular circuitry driving and coordinating cell cycle progression and pole morphogenesis in C. crescentus. Phosphorylation-mediated control is indicated in red, c-di-GMP (cdG) mediated control is shown in green (left). Spatio-dynamic control of selected components of the circuitry is shown on the right.

important pathway to coordinate cell cycle progression and pole development *(see figure).*

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 et al., 2009B). Swimming velocity is controlled by the synergistic action of at least five signaling proteins that adjust the cellular concentration of c-di-GMP. 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 et al., 2009A). The synergistic roles of ppGpp and c-di-GMP in biofilm induction, suggest that interference with bacterial second messenger signaling might represent an effective means for biofilm control during chronic infections.

Chronic *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) patients can be treated with antibiotics, however full clearance is not possible due to the adaptation of infective species to a persistent lifestyle. Adaptive *P. aeruginosa* morphotypes include small colony variants (SCVs), slow growing and strongly adherent variants whose appearance correlates with poor lung function. 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 and drastically reduced growth rates, two hallmarks of SCV behavior. YfiN-mediated SCVs were shown to be highly resistant to macrophage phagocytosis, suggesting a role for the SCV phenotype in immune system evasion. Consistent with this, activation of YfiN significantly increased the persistence of *P. aeruginosa* in long-term mouse infections, establishing a firm causal link between SCV, c-di-GMP, and persistence in chronic *P. aeruginosa* infections.

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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) 304:5378; *PNAS* (2007) 29:12151). 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 mycobacterium (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 identified some time ago as a host protein utilized by *M. tuberculosis* to survive within macrophages (*Cell* (1999) 97:435; *Science* (2000) 288:5471). To understand the mechanisms of action of coronin 1 as well as its normal function in leukocytes, we generated coronin 1 deficient mice, which allowed us to

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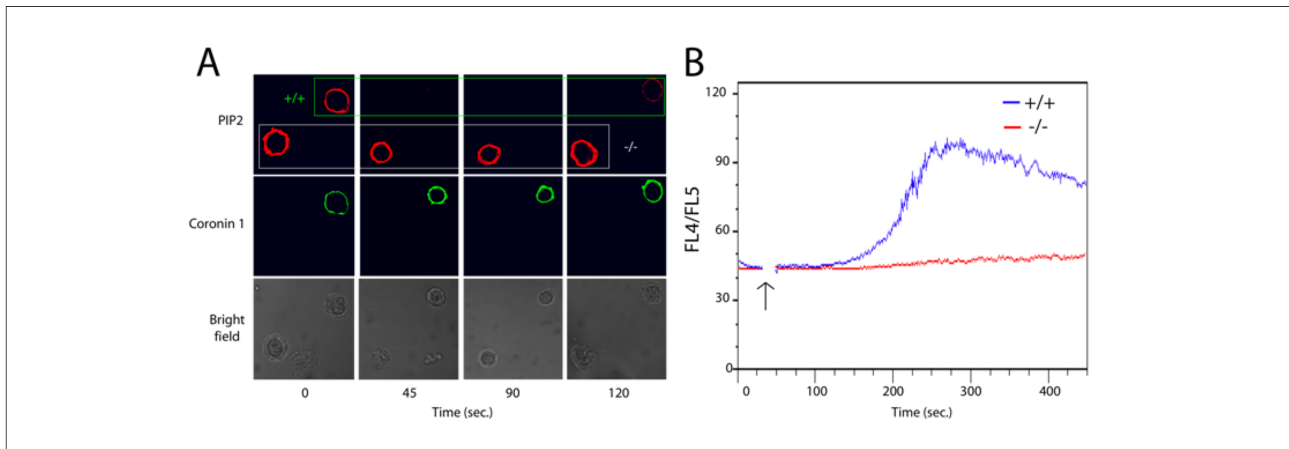

Coronin 1-dependent PIP2 hydrolysis and Ca^{2+} mobilization in T cells. A) Activation of wild type T cells (+/+, upper right corner cell) but not coronin 1-deficient (-/-, lower left corner cell) T cells results in a rapid hydrolysis of PIP2, as suggested by the disappearance of the PIP2-specific fluorescence 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 Ca^{2+}-sensitive probes. Arrow indicates the time of stimulus. For details see Mueller et al., 2008.

delineate the molecular mechanisms whereby coronin 1 modulates the survival of *M. tuberculosis* inside macrophages: upon infection by *M. tuberculosis,* coronin 1 is responsible for the activation of the Ca^{2+}-dependent phosphatase calcineurin, thereby preventing mycobacterial killing within lysosomes (*Cell* (2007) 130:37). In addition, we could show that coronin 1 is required for the maintenance of T lymphocytes in peripheral lymphoid organs (Mueller et al., 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 figure).*

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

Together our recent work has uncovered a role for coronin 1 in Ca^{2+}-dependent signaling in leukocytes. We are currently aiming to understand the molecular interactions of coronin 1 as well as analyze the importance of coronin 1-deficient signaling for the generation of immunity.

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Focal Area Neurobiology

Mechanisms controlling the development and function of the central nervous system

A major challenge in biology is to understand how the nervous system is assembled to appropriately respond to a large range of stimuli and control complex functions such as behavior and emotions. The nervous system must be capable of storing, integrating and retrieving information using mechanisms that are still poorly understood, but surely dependent on the establishment of precise connections. How neurons are generated to then form meaningful functional circuits is an intriguing question that is not only of interest to developmental neurobiologists, but also of great significance in the context of diseases, injury of the nervous system or perturbations of body weight regulation. There is little growth of axons in the central nervous system (brain and spinal cord) after injury in the adult, and when neurons die or fail to efficiently contact other cells, the brain is unable to store or retrieve memories, as illustrated for example in the disease of Alzheimer, a condition that affects a large proportion of the aging population in industrialized countries. The fine-tuning of synaptic connections and the homeostasis between excitatory and inhibitory inputs is also frequently perturbed in neurological diseases, including epilepsy in particular. A better knowledge of the mechanisms controlling the assembly and function of neuronal networks is thus desirable as it will significantly contribute to the understanding of diseases of the nervous system.

Education

The members of the Focal Area Neurobiology organize and teach a graduate and post-graduate program in the biology of the nervous system. This includes a series of formal introductory lectures in Neurobiology, a 4-week intense program including practical work, as well as a Journal Club series both at the FMI and at the Biozentrum. In addition, a weekly, 2-h lecture series is organized on Current Topics in Neurobiology and weekly seminars with invited speakers. This teaching program includes the active participation of a large number of members of the Basel Neuroscience Program, in particular neurobiologists working at the FMI and for pharmaceutical companies. More information on the program at www.biozentrum.unibas.ch/neuro.

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Assembly and function of motor circuits

Motor behavior represents the ultimate output of nearly all nervous system activity. The overall goal of our studies is to identify principles on how - at the neuronal circuit level - specificity of neuronal connections in the motor system can explain behavior as complex as motor behavior. The organization and function of the mature nervous system relies on the precision with which defined neuronal circuits assemble into functional units during development. Hierarchies of genetic programs allow diversification of neuronal subtypes, differentiation, and connectivity into precise neuronal circuits.

Our research aims to identify the molecular and mechanistic basis involved in the establishment of specific connections within a defined circuit of interconnected neurons, with a particular focus on the establishment of circuits involved in motor behavior. We have shown that precise signaling interactions between neurons and their target tissues as well as the action of several classes of transcription factors and cell surface molecules play important roles at sequential steps of motor circuit assembly and function (reviewed in Dalla Torre et al., 2008). We address these questions using multi-facetted approaches combining many technologies, including gain- and loss-of-function mouse genetics, high-end microscopy analysis of connectivity and synapses, gene expression profiling of identified neuronal subpopulations, viral techniques, electrophysiology and behavioral analysis.

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

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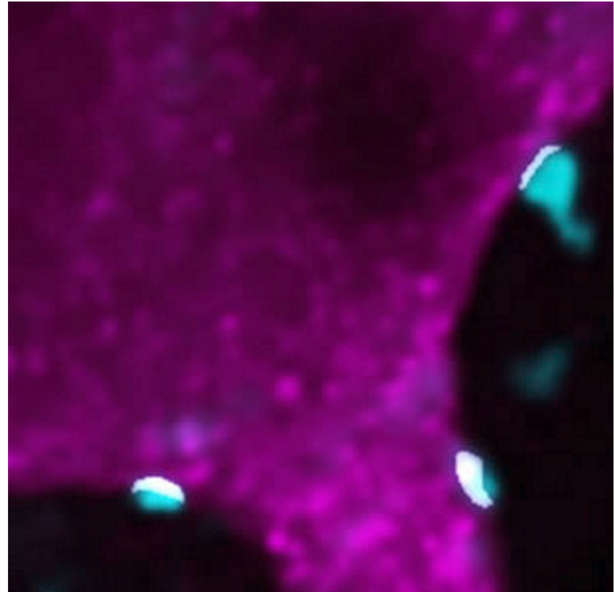
neuron pools, and its high-affinity receptor PlexinD1 by proprioceptive sensory neurons, is a critical determinant of synaptic specificity in sensory-motor circuits (Pecho-Vrieseling et al., 2009). Changing the profile of Sema3e-PlexinD1 signaling in sensory and motor neurons leads to a functional and 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.

Identifying functionally distinct motor neuron subclasses by transcription factors

Spinal motor neurons are specified to innervate different muscle targets through combinatorial programs of transcription factor expression (Dalla Torre et al., 2008). Whether transcriptional programs also establish finer aspects of motor neuron subtype identity, notably the prominent functional distinction between alpha and gamma motor neurons, remains unclear.

In this study, we set out to determine whether gamma and alpha motor neurons in the mouse spinal cord are distinguishable on the basis of their profile transcription factors expression and other molecular markers. We uncovered two genes with complementary expression profiles in gamma and alpha motor neurons (Friese et al., 2009). The transcription factor Err3 is expressed at high levels in gamma but not alpha motor neurons, whereas the neuronal DNA binding protein NeuN marks alpha but not gamma motor neurons. Signals from muscle spindles are needed to support the differentiation of $Err3^{on}/NeuN^{off}$ gamma motor neurons, whereas direct proprioceptive sensory input to a motor neuron pool is apparently dispensable. These findings establish that gamma and alpha motor neurons are molecularly distinct, and extend the principle that spinal motor neuron subtype identity has its origins in hierarchical programs of transcription factor expression, even within a single anatomically coherent motor neuron pool.



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

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Stem cells and neural development in health and disease

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

Neurotrophins in health and disease
The 4 neurotrophin genes found in mammals encode proteins playing critical roles in virtually all aspects of the biology of neurons, including their survival, process elongation and activity-dependent modifications of synaptic function. Mutations and polymorphisms discovered in humans further indicate that the neurotrophin signalling system is involved in dysfunction of the adult central nervous system. In particular, brain-derived neurotrophic factor (BDNF), the neurotrophin expressed at the highest levels in the mature CNS, is thought to be involved in a variety of conditions including dysregulation of food intake, memory consolidation, depression, Huntington's disease and Rett syndrome. Our work focuses on the cell biology of BDNF in neurons, including its localisation, biosynthesis, processing, storage and activity-dependent release from neurons. To explore its role in the adult, we generated a novel mouse line that can live for several months while essentially lacking BDNF in the CNS following the selective deletion of the gene in neurons (see Rauskolb et al., 2010). We also extensively use ES cells (see below) lacking genes such as MeCP2, the cause of most cases of Rett Syndrome, with the goal of understanding the links between MeCP2 and BDNF and how increasing BDNF levels could contribute to treating the disease. Our group also explores the link between neurotrophin receptors and endogenous lectins designated galectins in animal models of epilepsy following our discovery that Galectin-1 is up-regulated by the neurotrophin receptor p75 and causes axonal degeneration.

Embryonic stem cells
As a result of a lucky observation, we are now in a position to complement our *in vivo* work with cellular assays of unique relevance. We found that mouse ES cells can be "domesticated" to generate essentially pure populations of neuronal progenitors corresponding to those present in the developing mouse cortex and identified as Pax6-positive radial glial cells. These progenitors go on to generate *in vitro* virtually pure population of neurons using glutamate as neurotransmitters, just like they do *in vivo* during the development of the telencephalon. We extensively use this novel culture system to study the impact of genes thought to be important for the development and function of the CNS, including the transcription factor Pax6 and MeCP2. Thus, ES cells lacking both functional copies of Pax6 generate misspecified progenitors that go on to produce

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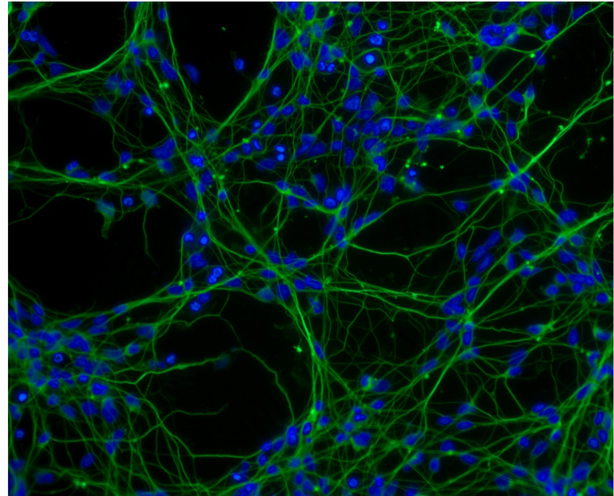
neurons synthesizing the inhibitory neurotransmitter GABA, as opposed to excitatory neurotransmitter glutamate. Progenitors lacking MeCP2 generate fewer neurons and their nuclei are smaller than those of wild-type neurons. They also express BDNF at lower levels, in line with in vivo experiments indicating that BDNF levels are decreased in the brain of mice lacking MeCP2. As increasing BDNF levels is thought to prolong the lifespan of mice lacking MeCP2 we use our *in vitro,* ES cell-based system to explore the potential of substances increasing the transcription of BDNF, both in wild-type and in cells lacking MeCP2.

Given the extraordinary potential of using ES cells as a discovery tool and the possibility to reprogram human somatic cells intro pluripotent cells, our group is also using human ES cells to explore whether results similar to those obtained with mouse ES cells can also obtained with human ES cells. While our differentiation protocol has not yet reach the stability of what we developed with mouse ES cells, we already know that in principle, similar results can be obtained *(see figure).*



Human neurons generated from human embryonic stem cells. Based on a previous success using mouse cells our laboratory is exploring the possibility to generate similarly homogeneous populations of neurons. The location of the cell bodies are indicated by a dye staining nuclei (blue) and the axons covering the dish with antibodies reacting with a tubulin variant found in neurons (green).

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New concepts for the diagnosis and treatment of obesity and cachexia

During 2008/09 our research focused on the validation of new concepts for the diagnosis and treatment of obesity or cachexia by modulation of the activity of the hypothalamic melanocortin-4 receptor (MC4R) and its downstream pathways. Our pharmacological approach consisted in the generation and characterization of functionally active antibodies against extracellular sequences of the MC4R, the MC3R and the TrkB receptor.

Our previous studies in rats revealed an obese phenotype after immunization against the N-terminal domain of the MC4R. In subsequent experiments we generated monoclonal anti-MC4R antibodies as pharmacological tools. One of the antibodies was further characterized as an inverse agonist with non-competitive antagonistic activity. Since this monoclonal antibody (mAb) and its recombinant single chain fragment variable (scFv) showed protective effects in an acute model of lipopolysaccharide-induced anorexia a priority claim for its therapeutic use has been filed in collaboration with the Technology Transfer Unit of the University of Basel.

In collaboration with William Banks, University of St. Louis, MO, USA, it was demonstrated that the scFv passed the blood-brain barrier after systemic administration and reached its presumed site of action in the hypothalamus in rats. We complemented these findings by showing an increased food intake after intravenous injection of the scFv in rats. These results have been published in the Journal of Pharmacology and Experimental Therapeutics in January 2010.

Our observation that immunization against the MC4R resulted in an obese phenotype in rats prompted us to explore whether anti-MC4R autoantibodies (autoAbs) can be detected in the plasma of obese patients. In a first clinical study more than 200 plasma samples from patients with a wide range of body mass indices (BMI) were screened in our laboratory with an ELISA assay using the N-terminal peptide sequence of the MC4R. Positive samples were further assessed by flow cytometry and cAMP measurements in HEK293 cells which overexpressed the human MC4R. We found that inhibitory anti-MC4R autoAbs were present in a small subgroup of patients with overweight or obesity but not in normal-weight subjects. A patent application for diagnostic and therapeutic applications based on our findings has been filed in collaboration with the Technology Transfer Unit of the University of Basel.

These results have been published in the Journal of Clinical Endocrinology and Metabolism in March 2009. Our article was accompanied by an Editorial entitled "Melanocortin-4-receptor autoAbs: a new player in obesity" and received

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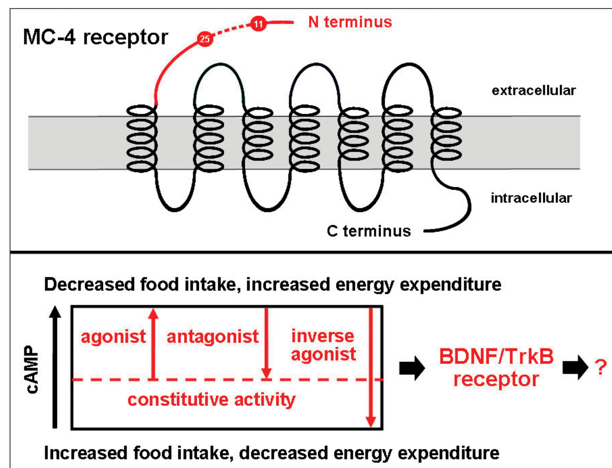
widespread interest in the lay press and professional journals. In May 2009 Jean-Christophe Peter presented these findings at the meeting of the European Association for the Study of Obesity in Amsterdam and received the Young Investigators Award in Clinical Science for his contribution.
We have now implemented an improved flowchart for the detection of such autoAbs and completed another cross-sectional study in obese patients from London (GB) and Lille (F). We are currently evaluating plasma samples from four additional sources: two cross-sectional studies are being performed on patients undergoing bariatric surgery (Vienna, A) and on obese children (Strasbourg, F). Two longitudinal studies are being performed on obese patients before and after dietary or surgical treatment (both in Strasbourg, F). It is expected that the correlation between the presence of anti-MC4R autoAbs and the course of body weight changes in the longitudinal studies should provide more information on the clinical relevance of anti-MC4R autoAbs.

Our previous studies on immunization against the MC4R in rats were complemented with a new series of experiments on immunization against extracellular sequences of the MC3R. These experiments were initiated because presynaptic MC3Rs appear to modulate the release of the MC4R agonist alpha-melanocyte stimulating hormone from nerve terminals. Our studies with active immunization against the MC3R provided evidence for direct and indirect effects of the MC3R on food intake. However, the effects were much smaller than those observed after immunization against the MC4R.

Our studies on brain derived neurotrophic factor (BDNF), a downstream mediator of the MC4R, and the corresponding TrkB receptor were continued. After raising polyclonal antibodies against the TrkB receptor we started the generation of mAbs against this target by the immunization of mice. The possible therapeutic efficacy of these mAbs and their derivatives will be validated in animal models of cachexia and anorexia nervosa. The latter indication may be particularly interesting because several experimental and clinical findings suggest a role of the BDNF/TrkB system in the pathogenesis of this disorder. In 2009 our research in this field has been supported by a grant from the Swiss Anorexia Nervosa Foundation.

Based on our previous patent applications for diagnostic and therapeutic applications of anti-MC4R antibodies and autoAbs a start-up company was founded in August 2009. The main objective of Obexia AG is the development of a diagnostic test for anti-MC4R autoAbs and of anti-MC4R mAb derivatives as therapeutic agents for the treatment of cachexia and anorexia nervosa.



In the upper panel a schematic illustration of the structure of the MC4R is given. The N terminus is thought to interact with other extracellular domains to maintain the constitutive activity of this receptor. It was therefore chosen as the target for our immunization experiments. In the lower panel the functional states of the MC4R are schematically illustrated. The MC4R is unique in having an endogenous inverse agonist, Agouti-related protein. It is likely that inverse agonists have a higher efficacy than antagonists because they not only reverse the activation of the receptor by an agonist but also reduce its constitutive activity. The activation of the MC4R mediates a decrease in food intake and an increase in energy expenditure. Thus blockers of the MC4R may be useful agents for the treatment of anorexia and cachexia. Brain-derived neurotrophic factor (BDNF) acting on TrkB receptors appears to be a downstream mediator of MC4R-induced effects. For this reason the TrkB receptor may be regarded as another possible pharmacological target.

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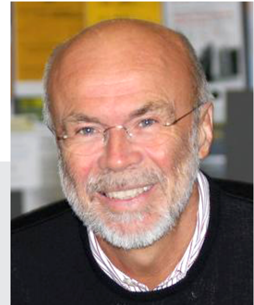
Drosophila brain development: from neural stem cells to neural circuits

The vast arrays of different neural cell types that characterize the complex circuits of the brain are generated by neural stem cells. During brain development, these neural stem cells produce defined sets of neural progeny composed of specific neural cell types which interconnect to form functional circuitry. Understanding the molecular mechanisms that underlie this process and give rise to the astonishing number and diversity of precisely defined cell types in the brain is one of the most challenging problems in biology.

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

In studies focused on neuroblast proliferation, we have discovered and analyzed a novel mode of neurogenesis in *Drosophila,* in which a striking amplification of neuronal proliferation is achieved by specific brain neuroblasts through the generation of intermediate progenitor cells (Bello et al., 2008; Izergina et al., 2009). Using cell lineage-specific tracing and marker analysis we have shown that remarkably large neuroblast lineages, which contribute interneurons to the central complex, develop in the dorsomedial area of the larval brain. These neuroblast lineages contain a pool of cells which do not express neuronal differentiation markers, are engaged in the cell cycle, and show mitotic activity. While some of these mitotically active cells are committed ganglion mother cells, the others express neuroblast-specific molecular markers and divide repeatedly to produce neural progeny implying that they are intermediate progenitors. These intermediate progenitors are generated by a specific set of neuroblasts that do not segregate Prospero to their smaller daughter cell thereby allowing this cell to retain proliferative capacity instead of undergoing its final neurogenic division. This amplification of neuroblast proliferation through intermediate progenitors in *Drosophila* bears remarkable similarities to mammalian neurogenesis, where neural stem cells as primary progenitors amplify the number of progeny they generate via symmetrically dividing secondary progenitors.



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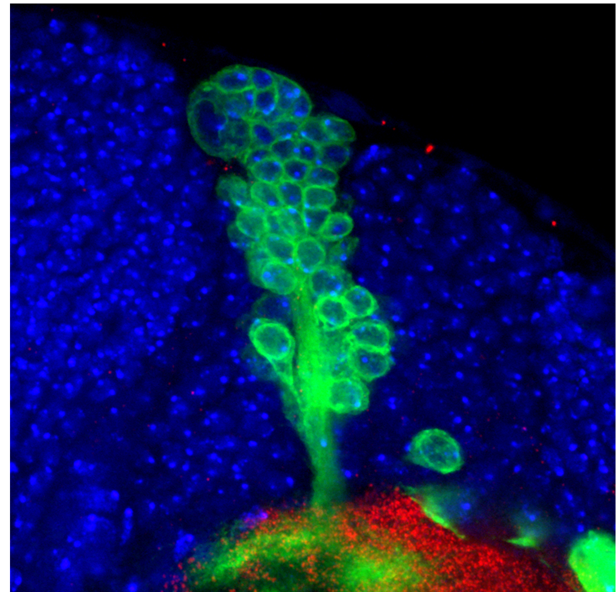
Heinrich Reichert

In studies focused on the specification of the neuronal features of neuroblast lineages, we have analysed the role of the cephalic gap gene *empty spiracles (ems)* in olfactory interneuron development and demonstrated that *ems* is expressed postembryonically in the progenitors of the two major olfactory interneuron lineages in *Drosophila* (Lichtneckert et al., 2008; Das et al., 2008). Moreover, we have shown that *ems* has cell lineage-specific functions in postembryonic olfactory interneuron development. Thus in the lateral olfactory interneuron lineage, transient *ems* expression is essential for development of the correct number of cells; in *ems* mutants the number of interneurons in the lineage is dramatically reduced due to apoptosis. In contrast, in the anterodorsal olfactory interneuron lineage, transient ems expression is necessary for precise targeting of neuronal dendrites to appropriate glomeruli; in *ems* mutants the interneurons fail to innervate correct glomeruli, innervate inappropriate glomeruli, or mistarget dendrites to other brain regions. Furthermore, in the anterodorsal lineage, *ems* controls the expression of the POU domain transcription factors in approximately half of the cells and, in at least one glomerulus, *ems* function in dendritic targeting is mediated through these transcription factors. The finding that *Drosophila ems,* like its murine homologues *Emx1/Emx2,* is required for the formation of olfactory circuitry implies that conserved genetic programs control olfactory system development in insects and mammals.

Finally, in a molecular genetic analysis of the role of programmed cell death in neural proliferation and specification we focused on four neuroblast lineages in the central brain of *Drosophila* that express the homeobox gene *engrailed (en)* and demonstrated that programmed cell death eliminates up to 50 % of the neuroblast progeny (Kumar et al., 2009). Moreover, we showed that programmed cell death selectively affects *en*-positive versus *en*-negative cells in a lineage-specific manner and, thus, controls the number of *en*-expressing neurons in each lineage. Furthermore, we provided evidence that Notch/Numb signalling is involved in binary cell fate decision processes that result in the hemilineage-specific regulation of en expression. These findings demonstrate that binary cell fate decisions combined with lineage-specific programmed cell death plays a prominent role in the generation of appropriate neuronal number and diversity in normally proliferating neuroblast lineages.



An individual neuroblast lineage in the developing brain of Drosophila visualized by a clonal mosaic labeling technique (membrane marker; green).

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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 in the brain *(figure parts A and B)*. Pathological changes at the NMJ, as for example seen in myasthenia gravis *(C)* or muscle dystrophies *(D)*, impair muscle function and can be life-threatening. Our group investigates several different pathways to elucidate their contribution to the function of synapses. We aim 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 ask 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. However, brain size is strongly decreased and the mice display behavioral abnormalities. In some areas, neurons show deficits in dendritic arborization and the synaptic circuitry. We are currently investigating the detailed molecular mechanisms underlying these phenotypes as well as the function of mTORC1 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 muscle dystrophy and early 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. In addition, we are testing whether activation mTORC1 can prevent muscle wasting diseases such as muscle dystrophies, which are characterized by the massive loss of muscle fibers.

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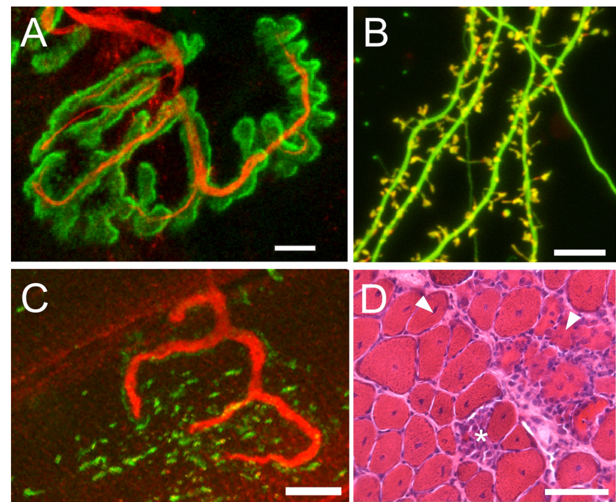
Copine 6, a novel calcium sensor involved in synapse structure

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 lead us to hypothesize that Copine 6 could serve as a calcium sensor that links high neuronal activity with the subsequent changes in synaptic structure. We are now investigating the function of Copine 6 *in vivo* and 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. Thus, 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 these two subgroups. We are currently testing whether the phenotypic difference between these subtypes can also be reiterated in a murine model of experimental autoimmune myasthenia gravis (EAMG). Indeed, EAMG mice induced in mice by MuSK antibodies shows a phenotype (kyphosis, weight loss and sign of neuromuscular hyperactivity) that differs from EAMG induced by acetylcholine receptor antibodies. In particular, MuSK-EAMG causes fragmentation of the NMJ and some loss of innervation *(C)*. We now examine whether these pathological changes differ between individual muscles and whether we can interfere with the course of the disease.

Another set of NMDs are genetic diseases such as muscle dystrophies. The common feature of muscle dystrophies is the severe loss of muscle mass due to the degeneration of muscle fibers. As a consequence, muscle becomes replaced by fibrotic tissue *(D)*. In one project we develop new methods to treat some of these muscle dystrophies in experimental mouse models. For example, we have shown that transgenic expression of the structurally distinct extracellular matrix molecule agrin can substantially compensate for the loss of laminin-211, which is essential



Structure of synapses and muscle in health and disease. (A) Neuronal, presynaptic elements (red) perfectly match postsynaptic structures (green) at the NMJ of a wild-type sternomastoid muscle. (B) Actin (yellow) is highly enriched at postsynaptic spines on the dendrites of cultured hippocampal neurons (green). (C) Experimentally induced myasthenia gravis causes a severe fragmentation of postsynaptic structures (green) in sternomastoid *muscle so that neuromuscular transmission initiated by the presynaptic nerve terminal (red) is abrogated. (D) Cross-section of skeletal muscle from a mouse model for Duchenne muscle dystrophy after hematoxilin-eosin staining shows signs of degeneration (white arrow) and fibrosis (asterisk). Note also the big difference in size between NMJ and synapses in the brain. Scale bars 10 µm (A, B, C) and 100 µm (D).*

to warrant muscle function. In current projects we test whether treatment with pharmacological agents can also ameliorate the disease.

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Neuronal circuit assembly and synapse formation

The long-term goal of research in the Scheiffele Lab is to understand molecular mechanisms underlying the formation of neuronal circuits in health and disease. Synapses are the key processing units in neuronal circuits. Therefore, we are examining mechanisms of synapse formation and synaptic re-arrangements in the central nervous system. We are exploring the 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

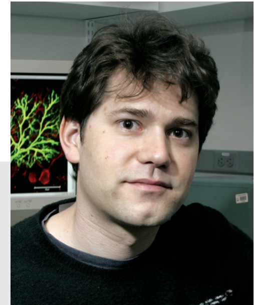
Synaptic adhesion molecules have important roles in organizing synaptic structures. In the past years we have focused on one pair of synaptic adhesion molecules called the neuroligin-neurexin complex which spans the synapse and contributes to the organization of pre- and postsynaptic membrane compartments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

Regulation of alternative splicing of the neurexin gene family

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

Synaptic defects in autism-spectrum disorders

Autism-spectrum disorders are amongst the most heritable neurodevelopmental disorders known to date. Human genetic studies conducted over the past 5-10 years have led



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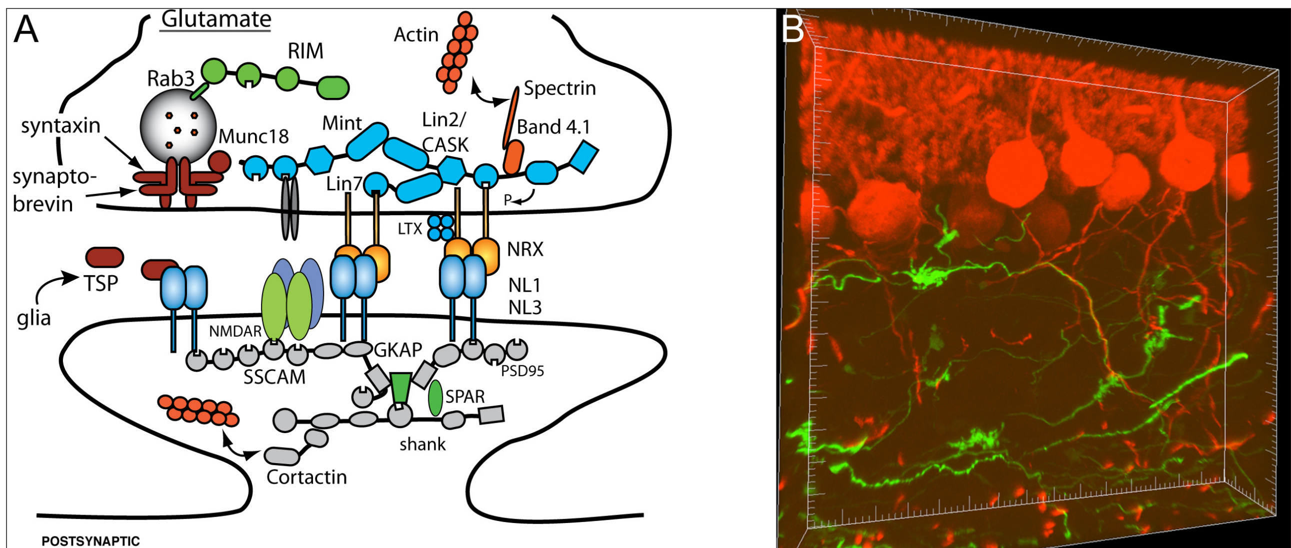

to the identification of several candidate genes that may confer susceptibility to autism but also environmental risk factors might exist. One group of autism candidate genes, including FMRP1 and MeCP2, is involved in the regulation of gene expression. A second, recently identified group relates directly to synaptic function. Importantly, the synaptic neuroligin-neurexin complex has emerged as a central player in this group of synaptic targets for monogenic forms of autism. Mouse models recapitulating mutations in this complex exhibit defects in synaptic physiology and animal behavior, thereby further supporting an important role for these mutations in the etiology of autism. The goal of our ongoing work is to test the hypothesis that a common "synaptic core pathway" is affected in autism which results in perturbation of neuronal network assembly and/or function.

Emergence of synaptic specificity in the ponto-cerebellar projection system

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

A) Schematic model of the neuroligin-neurexin complex at glutamatergic synapses. Interactions between presynaptic NRX and postsynaptic NL1/NL3 span the synaptic cleft and organize cytoplasmic scaffolding molecules, release machinery and neurotransmitter receptors. Cartoon: Stéphane Baudouin. B) Pontine mossy fibers (green) elaborate large multi-synaptic glomeruli in the cerebellum. In the mature cerebellum, these structures are positioned below the Purkinje cells (red). Image: Fatiha Boukhtouche.

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Excitation and inhibition in neural networks

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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 signal-to-noise ratios that allow the study of virtually all electrical dendritic events at a spatial resolution of sometimes less than 1 µm.
Our results confirm that dendrites can generate extremely complex, localized signals and harness different signaling cascades depending on the exact location within the dendritic tree. We are just beginning to explore the local interaction between excitatory and inhibitory signals, which may provide dendrites with additional highly dynamic signaling capabilities.

Imaging GABAergic signals

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

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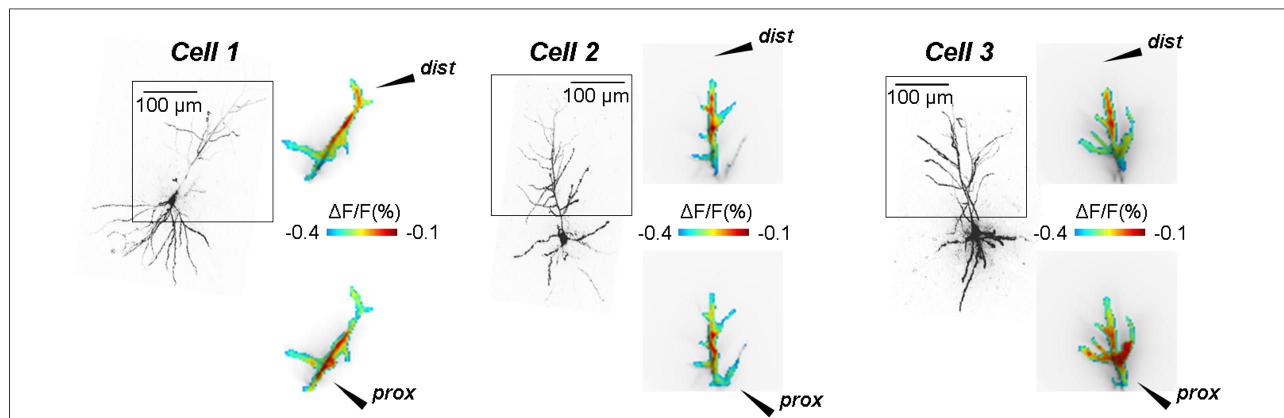

Voltage response of three different CA1 pyramidal cells to different GABAergic inputs. A distal (dist) and proximal (prox) stimulus electrode were placed in the vicinity of three CA1 pyramidal cells. The cells were filled with voltage-sensitive dye and then imaged. The black images show the morphology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

Imaging excitatory signals

Excitatory synaptic signals are often accompanied by calcium transients – either through the opening of voltage-gated calcium channels or through calcium-permeant receptors. These calcium signals are important second messengers that can induce synaptic plasticity. To better elucidate the role of different calcium sources during synaptic activity and -plasticity we have imaged both calcium signals and the membrane potential in cerebellar Purkinje cells after parallel and climbing fiber stimulation. We have found that brief bursts of parallel fiber activity can produce supralinear calcium signals. By testing the effect of exogenous calcium buffers we demonstrated that the calcium entering during the burst saturated the endogenous buffer system. This is the first time postsynaptic calcium buffer saturation has been found to play a role in synaptic plasticity.

Characterizing neuronal connectivity through optogenetics

Together with the group of Peter Scheiffele here at the 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 in-vitro 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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Core Program Computational & Systems Biology and SIB – Swiss Institute of Bioinformatics

A number of developments in molecular biology research over the last decades have lead to an ever increasing demand for mathematical and computational approaches. First, the advent of quantitative high-throughput measurement approaches in genomics, transcriptomics, proteomics, and imaging has lead to an increasing need for automated analysis of large-scale data. Second, as more and more of the molecular components of cells are characterized, researchers are increasingly turning toward analyzing how the complex dynamic interactions between molecular components determine the behavior of biological systems. Given the complexity of biological systems, such systems-level analyses inherently require a formal approach in which hypotheses are formulated mathematically and both existing biological knowledge and experimental data are described in a rigorous quantitative manner. Moreover, to investigate what behaviors different hypotheses imply, or to infer which hypotheses are consistent with available experimental data, one generally turns to computational modeling and simulation, and this typically requires the development of new computational methods and algorithms specifically designed for the analysis of biological systems and data.

Computational & Systems Biology is thus a relatively new interdisciplinary field where sophisticated methodologies from applied mathematics, computer science, physics, and chemistry are applied to the study of biological systems. The field spans a large range of topics from the development of algorithms for the processing of large-scale data, through development of new simulation and probabilistic inference methods, to the general investigation of the organizational and functional design principles of biological systems. The "Core Program Computational & Systems Biology" of the Biozentrum Basel consists of research groups headed by Simon Bernèche (Computational Biophysics), Torsten Schwede (Computational Structural Biology), Erik van Nimwegen (Genome Systems Biology), and Mihaela Zavolan (RNA Regulatory Networks). The research topics of the groups range from computational simulation of the dynamical behavior of proteins at the molecular level, through methods for the inference of structure and function of proteins, to the analysis of gene regulatory networks and genome evolution.

Scientific collaborations and infrastructure

Many of the research projects of the Core Program Computational & Systems Biology involve collaborations with experimental groups that obtain high-throughput experimental data, sometimes involving large international consortia. For example, apart from in-house collaborations with colleagues at the Biozentrum, the Core Program is

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Core Program Computational & Systems Biology and SIB – Swiss Institute of Bioinformatics

involved in several research projects of the Swiss SystemsX initiative in systems biology, in the FANTOM project of the Riken institute (Japan), the Research Collaboration for Structural Bioinformatics hosting the Protein Data Bank, the European Bioinformatics Institute (EBI), the Novartis Institute for Tropical Diseases in Singapore (NITD), and the European Alternative Splicing Network (EURASNET).
All group leaders are also research group leaders at the Swiss Institute of Bioinformatics (SIB) and our groups host and maintain several important web-resources in bioinformatics. To support the computational work, the Core Program supports a competitive IT infrastructure in collaboration with the SIB that includes application-, database-, and web-servers, large scale storage and backup facilities for the Core Program's research groups, and various services for the entire Biozentrum.

Three of the four groups of the Core Program's research groups now also include a wet-lab in which experimental projects are being pursued that are largely motivated by questions that arose through the computational analysis, e.g. for validation of predictions of the computational modeling, and for the measurement of crucial parameters of computational models. Several high-throughput experiments are also pursued such as genome-wide characterization of protein-RNA interactions and genome-wide measurements of single-cell variations in gene expression.

Teaching and scientific events

The members of the Core Program Computational & Systems Biology are involved in a variety of teaching and training activities at the Biozentrum. They take part in a B. Sc. teaching program in Computational Biology as part of the Computational Sciences curriculum, and have just introduced a new program for Bachelor of Science in Molecular Biology: specializing in Computational Sciences. They also organize the graduate teaching program Cycle F: Genomics and Bioinformatics.

The Basel Computational Biology Conference [BC]2 is an annual interdisciplinary symposium which is now established as the major Computational Biology event in Switzerland. It provides a platform for exchange of ideas among scientists from academic research institutes, the pharmaceutical industry and biotech companies. The theme of the 6th [BC]2 conference in March 2008 was "Computational Structural Biology", while the 7th [BC]2 conference, was organized in June 2009 with the motto "Molecular Evolution" celebrating the bicentenary of Charles Darwin.

Membrane transport mechanisms

The regulated diffusion of various substrates through the pore of membrane transport proteins allow the cell to control the concentration of essential compounds used for its metabolism, as well as for intra- and extra-cellular signalling. These fundamental functions of membrane proteins rely on three elementary mechanisms: permeation, selectivity and gating. Agents, such as the transmembrane voltage, the pH, or different ligands, can potentially modulate each of them. Our aim is to elucidate the microscopic molecular determinants of these mechanisms in different families of proteins. We are particularly interested in ion channels and proton co-transporters (K^+ channel, ClC Cl^-/H^+ exchanger, AmtB ammonium transporter, ASIC Na^+ channel), and have recently initiated work on transporters of larger compounds like the SecY translocon and the NanC transporter.

The determination of high-resolution three-dimensional structures of members of these different membrane protein families has brought new impetus in the study of transport mechanisms. However, without information on the dynamics of the proteins, it remains difficult to establish a clear and reliable connection between their structure and their function. Taking advantage of available X-ray structures, we use molecular dynamics (MD) simulations and free energy calculations to characterize key chemical interactions and the protein mechanical plasticity. These calculations thus provide information that complement the static picture given by the experimental X-ray crystallography structures.
A key element in the understanding of a molecular mechanism is the underlying potential of mean force (PMF) that controls the rate of any 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 membrane system. Using 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 a given ion channel.

The potassium channel family
We are specifically interested in gating mechanisms in the ubiquitous K^+ channel family. We notably try to elucidate the basis of C-type inactivation, which consists in the spontaneous, time dependent, closure of the channel's pore following activation. This physiologically important mechanism is a key factor in the regulation of cardiac and cerebral rhythmicity. It has been proposed that a conformation of the prokaryotic KcsA channel obtained by crystallization in low K^+ concentration (pdb

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entry 1K4D; Zhou et al., *Nature,* 2001) could correspond to the inactivated state of the channel. Our simulations suggest that, in this conformation, the selectivity filter 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 K^+ channels and their inactivated state, thus raising some doubts on the physiological relevance of this so-called low-K^+ structure (submitted for publication).

Co-transport of H^+ and pH gated channel

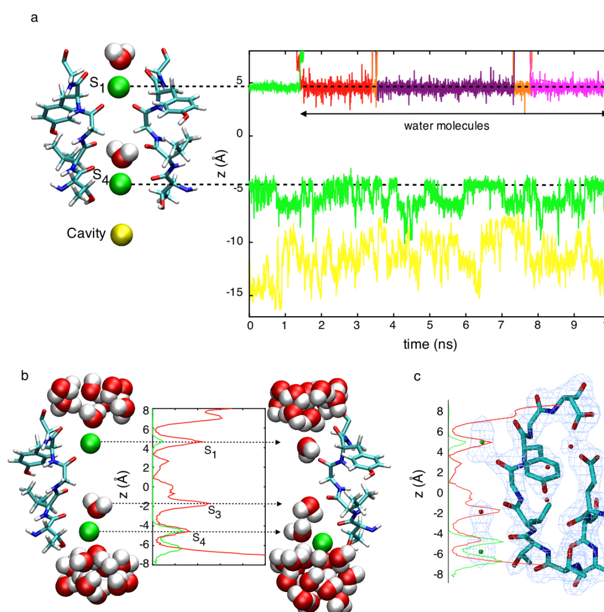
Different transport processes involve coupling with H^+, either through gating or co-transport mechanisms. We pursue our investigation on the possible co-transport of NH_3 and H^+ in the AmtB ammonium transporter of E. coli. Also, together with the group of Stephan Kellenberger at the University of Lausanne, we try to elucidate the pH dependent gating mechanism of the ASIC acid sensitive Na^+ channel. We performed pKa calculations to provide a subset of residues that were afterward characterized by mutagenesis electrophysiological experiments. This approach has allowed us to identify three key residues involved in the activation and inactivation of this pH gated channel (Liechti et al., *JBC,* 2010).

Inhouse collaborations

In collaboration with Martin Spiess from the Growth & Development focal area, we have initiated simulation work on the SecY translocon. Our preliminary analysis has lead to a reassessment of the mechanism by which the translocon pore favors the translocation or membrane integration of its substrate. This pore is generally described as hydrophilic, but the simulations show a more complex reality suggesting that the pore can potentially accommodate hydrophilic as well as hydrophobic substrates.

Peneff and co-workers from the Schirmer's lab have recently solved the structure of NanC, an outer-membrane protein from E.coli. Following the elucidation of this structure, questions remained on the actual and most likely substrate of NanC. We have performed modelling and simulations to provide an initial hypothesis on the key interactions sustaining possible permeation of sialic acid (Wirth et al., *JMB,* 2009).

Simulations of pure phospholipid membranes are currently being performed and analysed to support the interpretation of neutron diffraction experiments performed in the laboratory of Joachim Seelig. It can be observed, by both the experimental and simulation approaches, that lipid polar heads preferentially adopt a limited number of conformations. This intriguing structural feature is likely to explain some well known functional properties of biological membranes that are still not well understood.



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.

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

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

Molecular modeling of protein-ligand interactions

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

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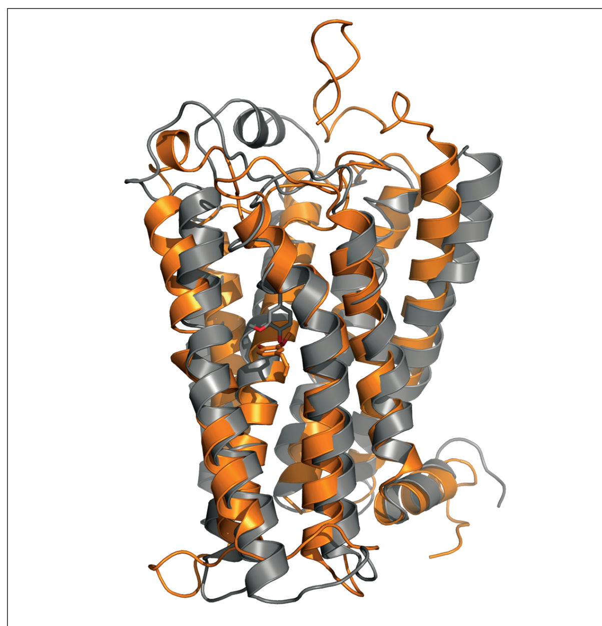
the insect vector, more recent estimates suggest this number has risen to 50–100 million, and dengue fever is now seen as one of the most important emerging infectious diseases in many areas of the world. We have used a structure based virtual screening approach to identify novel inhibitors of Dengue virus RNA methyltransferase (MTase), which is necessary for virus replication. In a multistage molecular docking approach in the MTase crystal structure, we screened a library of more than 5 million commercially available compounds against the two binding sites of this enzyme. In 263 compounds selected for experimental verification at the Novartis Institute for Tropical Diseases in Singapore, 10 inhibitors with IC50 values of <100 µM were identified, of which four exhibited IC50 values of <10 µM in *in vitro* assays.

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



Comparative models of the mouse Eugenol olfactory receptor based on β2-adrenergic receptor and bovine rhodopsin template structures. (Photo courtesy of Morena Speafico).

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Structure and dynamics of gene regulatory networks

There are two main lines of research in our group. On the one hand we develop computational and theoretical methods for inferring the structure and dynamics of genome-wide regulatory networks. This work involves the development of probabilistic methods that integrate various high-throughput biological data sets such as genome sequences, genome-wide expression data, genome-wide protein binding data, etc. The other main line of research involves the investigation of whole genome evolution, especially in bacteria, and the identification of quantitative laws that govern genome evolution.

Before moving to a post-doc at the EPFL (Lausanne), PhD student Nacho Molina finished two projects in my lab, the most important of which is the development of a general theory for the evolution of gene-content in prokaryotic genomes that can explain the scaling laws that we have observed previously. In particular, we show that, using only an assumption of time invariance, a rather explicit evolutionary model can be inferred from the observed scaling laws. Moreover, this model predicts a quantitative connection between the scaling exponents (which relate to the growth of the number of genes in particular functional classes with genome size) and the relative rates of duplication and deletion of genes in different functional classes. In this work we demonstrate that this theoretically predicted relationship is supported by available complete genome data. We believe that this is an important step toward a more quantitative understanding of the evolution of bacterial genomes. Our previously discovered observation that the number of transcription factors in prokaryotic genomes grows quadratically with the total number of genes in the genome has important implications for the large-scale structure of their transcription regulatory networks. Using a comprehensive comparative genomic analysis of the evolution of intergenic regions in clades of related bacteria, we showed that, as genome size increases, the transcription regulatory network goes from having very few regulators that each regulate many genes, to having many regulators each of which regulate only a few genes, whereas the number of regulatory inputs per gene stays constant. This result has far reaching consequences for our understanding of the evolution of regulatory networks.

PhD student Lukas Burger has developed a rigorous and easily practically applicable Bayesian network algorithm for inferring protein-protein interactions from sequence alignments. The algorithm models joint alignments of sets of homologous protein sequences in terms of all possible models of pair-wise interactions between residues directly from first principles and without any tuneable parameters. We demonstrated the power of this method by reconstructing two-component signalling networks in all

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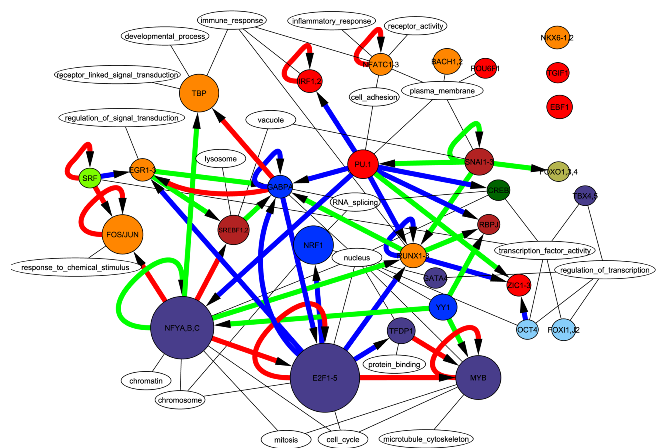
bacterial genomes. Very recently we have also extended this method for predicting residue-contact in proteins from amino-acid alignments and shown that it dramatically improves on previous bioinformatic methods.

One of the main areas over the last two years concerned the FANTOM4 project, lead by the RIKEN Institute in Yokohama, Japan, of which our group was part. In this work we, for the first time, derive a mammalian transcriptional regulatory network (for a differentiating human cell line) *ab initio* using a combination of high-throughput experimental time course measurements, theoretical inference, and large-scale validation experiments. The experimental work for this project, particularly the deep-sequencing of CAGE tags (5' ends of mRNAs), and the large-scale siRNA knock-down experiments were done at RIKEN, and our group was responsible for essentially all of the analysis and modeling.

The methodologies that we developed in this project are already being employed in a number of other collaborations, in particular in the large SystemsX collaboration, called CellPlasticity, where our group is responsible for the analysis and modeling. The main new method, called Motif-Activity-Response-Analysis (MARA), models observed genome-wide expression patterns in terms of genome-wide computational predictions of transcription factor binding sites, in order to infer *ab initio,* what the key transcription regulatory factors are, how these factors change their activities in the process under study, and what sets of genes are targeted by each factor. We are now also offering MARA as an online tool to which researchers can upload their own genome-wide expression data for automated analysis.

Two additional publications this year result from the FANTOM4 collaboration. One of these, which is the work of Piotr Balwierz and myself, involves the development of a suite of analytical methods for the normalization, and noise analysis of expression data obtained with so-called deep-sequencing technologies. These methods are the first rigorous methods for analysis of deep sequencing expression data to be published and, as the number of laboratories that are using deep sequencing rather than micro-array technology for expression analysis is expanding rapidly, we foresee that our methods will be of great use to the community in the coming years.



The predicted core regulatory network controlling differentiation of the human THP-1 cell line. Each colored node corresponds to one of the core regulatory factors inferred by the computational analysis. The size of the node reflects its significance in controlling gene expression changes and its color reflects its activity profile; upregulated factors are in orange and red, downregulated factors are in light and dark blue, and transiently regulated factors are in green. Edges X->Y are drawn whenever the promoter of regulatory factor Y had a predicted regulatory input from factor X, and the edge has independent experimental support. Edges confirmed by literature, by ChIP or by siRNA are shown in red, blue and green, respectively. Gene Ontology terms significantly enriched among target genes are shown as white nodes.

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Uncovering principles of post-transcriptional gene regulation

Although for many years transcription factors held the center stage in the regulation of gene expression, a very complex post-transcriptional regulatory layer implemented by microRNAs (miRNAs) and RNA-binding proteins (RBPs) and frequently acting precisely on the mRNAs encoding transcription factors, has been uncovered. Far from passively carrying the genetic information to the ribosomal translation machines, messenger RNAs engage in multiple interactions with proteins and ribonucleoprotein complexes that regulate mRNA lifetime and translation rate. Although the miRNAs have initially been described as regulators of the mRNA translation rate, it is now clear that an important mechanism behind the miRNA-based regulation is target mRNA degradation. The activity of miRNA-containing ribonucleoprotein complexes can itself be modulated, i.e. by RBPs that recognize sites in the vicinity of miRNA-binding sites and act as competitors for miRNA binding. Much effort in the past has been devoted to identifying new players in post-transcriptional control.

Many groups, including our own, contributed to the catalog of miRNA genes in species ranging from viruses to human. They contributed through computational or experimental approaches to the list of putative miRNA-target interactions. Yet although it is clear that a miRNA targets hundreds of genes, much remains to be done to understand the determinants of miRNA targeting specificity. For this reason, we have undertook a computational study of a large number of experimental data sets of mRNA expression changes upon miRNA transfection or knockdown, and found that the sequence composition and the RNA secondary structure in the region of the putative miRNA target sites have a substantial impact on the effect that the binding of miRNAs to these sites has.

We have further determined that miRNA target sites that are functional in target mRNA degradation have also been under evolutionary selection pressure, indicating that mRNA degradation is an important mechanism of miRNA-dependent regulation. Through computational analyses and experimental studies, we have identified an apparent feedback loop that an abundantly expressed miRNA, miR-30, exerts on the effector miRNA pathway by targeting the GW repeat-containing protein TNRC-6A. Much about the principles of miRNA-based regulation has been discovered through computational analyses of predicted target sites. A commonly used approach is to manipulate the expression of a single miRNA and then assess the effects of this manipulation through mRNA profiling studies. This approach was used by the group of Witek Filipowicz from the Friedrich Miescher Institute, together with whom we characterized a function for the embryonically-expressed miRNAs of the miR-290 sequence family. These

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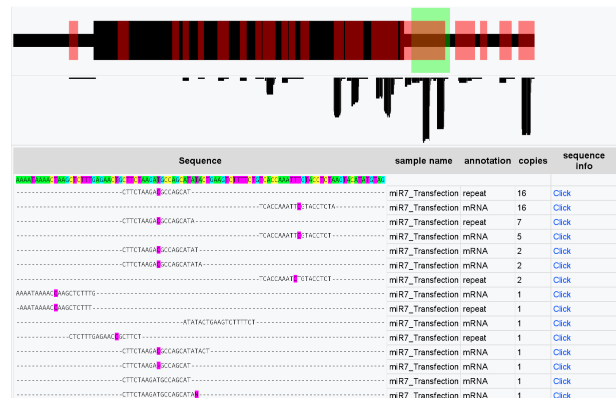
miRNAs target the transcript of a transcriptional repressor, the retinoblastoma-like 2 gene, which in turn regulates the expression of *de novo* DNA methylases. The miR-290 miRNAs thus contribute to the establishment of the appropriate methylation patterns during embryonic development.

In collaboration with the group of Markus Stoffel (ETH Zurich), we studied with a similar approach the function of miR-375. We found that this pancreas-specific miRNA has an important function in maintaining a normal mass of the pancreatic alpha- and beta-cells. High-throughput methods are heavily used in order to identify novel genes, particularly for non-protein-coding RNAs, and also to determine, at the genome or transcriptome level, binding sites of various regulators. These technologies bring computational challenges, because the data resulting from even a single experiment cannot be analyzed without the use of significant computational resources. For this reason, we developed a variety of computational tools that enable us to automate much of the preliminary computational analyses related to a few experimental methods that we used heavily in our studies.

In collaboration with the Tuschl group (The Rockefeller University) we worked on the development of a very efficient and general method to isolate targets of RNA-binding proteins via crosslinking, immunoprecipitation and deep sequencing. The method takes advantage of photoreactive nucleotides, such as 4-thio-uridine (4-thio-U), which, when fed to cells, are readily incorporated into mRNAs. 4-thio-U appears to have little toxicity, while specifically crosslinking at 365 nm (as opposed to 254 nm, which is what other CLIP methods use) UV. Our computational analysis of sequence data sets obtained with this method established that the site of the crosslink can be readily identified by a high frequency of T-to-C changes in the cDNAs prepared from the cells. This observation enables us to pinpoint the location of RBP binding sites in RNAs with very high resolution. The method was applied to a variety of RNA-binding proteins, such as the Argonautes 1-4 that participate in miRNA-dependent silencing, the Insulin growth factor binding proteins 2 isoforms 1-3, Quaking and Pumilio 1-2, for which we generated transcriptome-wide maps of binding sites. In future, such maps will be generated for more RBPs that regulate all aspects of mRNA metabolism. These data will enable us to more accurately predict the response of mRNAs to signals during normal and pathological cell behavior, as well as to better interpret the mechanism by which mutations in the DNA lead to genetic diseases.



Location of miRNA binding sites in the transcript for proliferating cell nuclear antigen. The binding sites were determined by crosslinking the Argonaute protein to mRNAs with UV light, isolation and deep sequencing of RNA fragments that were bound by the Argonaute protein after transfection with miR-7. 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.

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Core Program
Structural Biology & Biophysics

Introduction
The ultimate understanding of biological function rests on the detailed description of biomolecular interactions in terms of structure, physical forces, and the resulting dynamics. Enormous advances have been made during the last decades in the techniques that visualize the three-dimensional structure and quantify the dynamics of cellular components down to the atomic level. It is the goal of the Core Program 'Structural Biology and Biophysics' to apply and where possible further develop these structural and biophysical techniques in order to understand at all levels the interactions that make up the dynamical network of a living system.

The structural techniques used range from light, electron and scanning microscopy over X-ray crystallography to NMR spectroscopy. Biophysical methods encompass many different time-resolved spectroscopic techniques such as laser-flash spectroscopy and single molecule FRET, thermodynamic analytical methods such as ITC and DSC microcalorimetry, NMR imaging and in vivo spectroscopy. Using rigorous combinations of these techniques, we are in a unique position to determine the structure of biological matter at all length scales, from atomic detail over entire cells to small animals, to analyze and quantify dynamical changes starting from picosecond time scales to very long periods, and to determine the energetics and thermodynamics of biomolecular interactions at very high precision.

In all of the research areas described below, a very strong interplay exists between structure determination and biophysics that is being used for the characterization of the dynamics and stability of proteins and their interactions. Strong synergisms also exist with all the other focal areas of the Biozentrum since many of the systems are shared projects with other biochemically or biologically oriented groups.

Research groups
The core program comprises six senior research groups headed by Ueli Aebi (electron and force microscopy), Andreas Engel (electron and force microscopy), Stephan Grzesiek (NMR spectroscopy), Tilman Schirmer (x-ray diffraction), Joachim Seelig (membrane biophysics and NMR) and Henning Stahlberg (electron and force microscopy). Additional junior or independent project groups are headed by tenure track assistant professors Dagmar Klostermeier (single molecule fluorescence) and Roderick Lim (force microscopy), Titular professor Anna Seelig (selectivity and kinetics of membrane transport), guest scientist Prof. Konstantin Pervushin (NMR spectroscopy) and PD Cora-Ann Schoenenberger (structural

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Core Program
Structural Biology & Biophysics

biology of actin). PD Birthe Fahrenkrog (structural biology of the nuclear pore complex, until September 2009). The core program receives generous support by the M. E. Müller Foundation via the fully integrated M. E. Müller Institute for Structural Biology (research groups Ueli Aebi and Andreas Engel).

Highlights
2008: The Engel group moved to its new location at the ETHZ D-BSSE institute in the Mattenstrasse as a contribution of the Biozentrum to the Swiss SystemsX.ch initiative. A new frontier transmission electron microscope (FEI Titan KRIOS) and a serial block-face scanning electron microscope could be acquired with funds from the University Basel, SystemsX.ch, Roche Pharmaceuticals and the Friedrich Miescher Institute. Anna Seelig received the STK Award for "Applied Chemical Thermodynamics" of the Swiss Society for Thermal Analysis and Calorimetry. Joachim Seelig received the Julian Sturtevant Award for Outstanding Contributions to Experimental Thermodynamics. Roderick Lim received the Pierre-Gilles de Gennes Prize for his work on the nuclear pore complex. Stephan Grzesiek was elected Member of the Swiss National Research Council of the Swiss National Science Foundation. Sonja Dames and Konstantin Pervushin successfully passed their habilitation.

2009: Henning Stahlberg was elected as the successor of Andreas Engel and took up the position in April 2009. He is now co-directing C-CINA together with Andreas Engel until Engel's retirement at the end of 2010. Roderick Lim has accepted the position of a tenure track assistant professor for Nanobiology, which was newly created in collaboration with the Swiss Nanoscience Institute (SNI) and is jointly funded by the Argovia program (a collaboration between the Cantons Basel and Aargau for nanosciences) and the Biozentrum. Birthe Fahrenkrog accepted a position as Assistant Professor at the Free University of Brussels, Belgium.

Teaching
The knowledge of structural principles of biomacromolecules and the understanding and quantitative application of biophysical methods are prerequisites for success in all areas of biomolecular research. In addition to the specific research, the Core Program's teaching contributes fundamentally to the Biozentrum's Biology curriculum. Structural biology and biophysical theories together with the relevant techniques are being taught to all students of the Biozentrum at various levels by all members of the Core Program. Undergraduate lectures are in part also attended by students of Pharmaceutical Sciences, Chemistry, Physics and Nano-Science.

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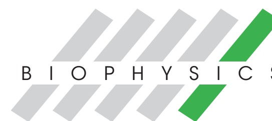

Structure-based functional analysis of cellular nanomachines

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

Characterization of human lamin assemblies

Half-minilamins, representing amino- and carboxy-terminal fragments of human lamins A, B1 and B2 with a truncated central rod domain, were investigated for their ability to form distinct head-to-tail-type dimer complexes. This mode of interaction is an essential step in the assembly reaction shown by full-length lamin dimers. Analytical ultracentrifugation studies indicate that the amino- and carboxy-terminal coiled-coil dimers interact to form distinct oligomers. A corresponding interaction occurred also between heterotypic pairs of A- and B-type lamin fragments. Hence, A-type lamin dimers may interact with B-type lamin dimers head-to-tail to yield linear polymers. These findings indicate that a lamin dimer has the freedom for a "combinatorial" head-to-tail association with all types of lamins, a property that might be important for the assembly of the nuclear lamina. We suggest that the head-to-tail interaction of the rod end domains represents a principal step in the assembly of cytoplasmic intermediate filament proteins too (Kapinos et al., 2010, *J Mol Biol* 396, 719-31).

Cryo-electron tomographic reconstruction of the nuclear pore complex

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

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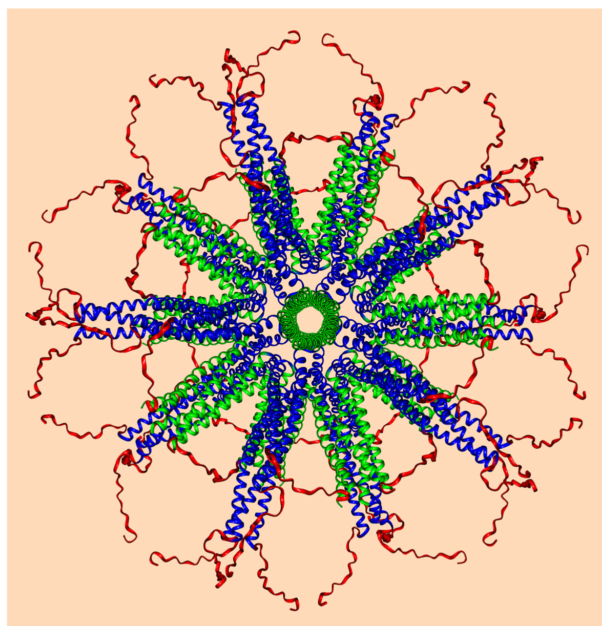
in the *Dictyostelium* genome as compared to that of vertebrates (Frenkiel-Krispin et al., 2010, *J Mol Biol* 395, 578-86).

Analysis of articular cartilage by indentation-type atomic force microscopy

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

A nonadjuvanted polypeptide nanoparticle vaccine against rodent malaria

We have designed a prototypic malaria vaccine based on a highly versatile self-assembling polypeptide nanoparticle (SAPN) platform that can repetitively display antigenic epitopes. We used this platform to display a tandem repeat of the B cell immunodominant repeat epitope (DPPPPNPN)$_2$D of the malaria parasite Plasmodium berghei circumsporozoite protein. Without an adjuvant, the SAPN construct P4c-Mal conferred a long-lived, protective immune response to mice with a broad range of genetically distinct immune backgrounds. Immunized mice were protected against an initial challenge of parasites up to six months after the last immunization or for up to 15 months against a second challenge after an initial challenge of parasites had been cleared. Furthermore, we demonstrate that the SAPN platform not only functions to deliver an ordered repetitive array of B cell peptide epitopes but operates as a classical immunological carrier to provide cognate help to the P4c-Mal-specific B cells (Kaba et al., 2009, *J Immunol* 183, 7268-77).



Model of an icosahedral SAPN functionalized with a repetitively displayed tandem repeat of the B cell immunodominant epitope (DPPPPNPN)$_2$D of the malaria parasite Plasmodium berghei circumsporozoite protein. The view is down a five-fold symmetry axis of the icosahedron. The B cell epitope is presented on the SAPN's surface (Kaba et al., 2009, J Immunol 183, 7268-77).

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- Herrmann, H., Strelkov, S. V., Burkhard, P. & Aebi, U. (2009). Intermediate filaments: primary determinants of cell architecture and plasticity. *J Clin Inves* 119, 1772-83.
- Kreplak, L., Herrmann, H. & Aebi, U. (2008). Tension mechanics of single desmin intermediate filaments. *Biophys J* 94, 2790-9.
- Meier, M., Padilla, G. P., Herrmann, H., Wedig, T., Hergt, M., Patel, T. R., Stetefeld, J., Aebi, U. & Burkhard, P. (2009). Vimentin coil 1A – A molecular switch involved in the initiation of filament elongation. *J Mol Biol* 390, 245-61.
- Wiesel, N., Mattout, A., Melcer, S., Melamed-Book, N., Herrmann, H., Medalia, O., Aebi, U. & Gruenbaum, Y. (2008). Laminopathic mutations interfere with the assembly, localization and dynamics of nuclear lamins. *Proc Natl Acad Sci USA* 105, 180-5.

Nuclear magnetic resonance spectroscopy applied to study the structure and interactions of signaling proteins and transcription regulators

1) The protein target of rapamycin (TOR) is a central controller of cellular growth that intercepts different signaling cascades. Recently, I determined the structure of the FATC domain, which revealed a regulatory disulfide bond that influences the cellular stability of TOR (Dames et al., *JBC,* 2005). To better understand the factors that govern the association of TOR with different cellular membranes, the interactions of the FATC domain with different lipids and the structures of the oxidized and the reduced forms bound to membrane-mimetic DPC micelles were characterized. The data suggests that TOR cannot only associate with biological membranes via protein-protein interactions or the interaction of the FRB domain with membrane-embedded phosphatidic acid but further by a membrane anchor in the redox-sensitive TOR FATC domain (Dames, *JBC,* epub 2009).
July 2009, I employed Dr. Nina Link, who finished her Ph.D. thesis at the university of Duisburg Essen in May 2009. Dr. Link works on the characterization of the interaction of the FRB domain of TOR with phosphatidic acid embedded in membrane-mimetic systems and on the interaction with the small GTPase Rheb that regulates TOR function.

2) Recently, I characterized the structure, calcium binding, and homoassociation of the N-terminal extracellular domain of human T-cadherin (Tcad1, residues 1-105) in collaboration with Prof. Grzesiek and Prof. Engel. The respective data support a role for T-cadherin as signaling protein in cell-cell recognition processes that rather involve cell detachment than adhesion (Dames et al., *JBC,* 2008).

3) The protein Hexim1 and the small nuclear RNA 7SK form an inhibitor for the positive transcription elongation factor b (P-TEFb). In its active form P-TEFb consists of Cyclin T1, T2, or K and the cyclin dependent kinase 9 (CDK9). The structure of the C-terminal Cyclin T-binding domain (TBD) of human Hexim1 consists of two consecutive coiled-coil regions and a short α-helix (Dames et al., *PNAS,* 2007). In order to better understand how deviations from the coiled-coil sequence in Hexim1 impair the affinity and stoichiometry of its interaction with Cyclin T1, we characterized the structural features and dynamic behavior of different TBD mutants. Part of the results together with additional biophysical data from PD Dr. M. Geyer have been published in the journal Biochemistry ('A flexible bipartite coiled-coil structure is required for the interaction of Hexim1 with the P-TEFb subnit Cyclin T1' web publication ahead of print March 8, 2010). A second publication is in preparation. With PD Dr. Geyer I collaborate further on the structure determination of a novel potential GTPase-binding protein from *Dictyostelium discoideum.*

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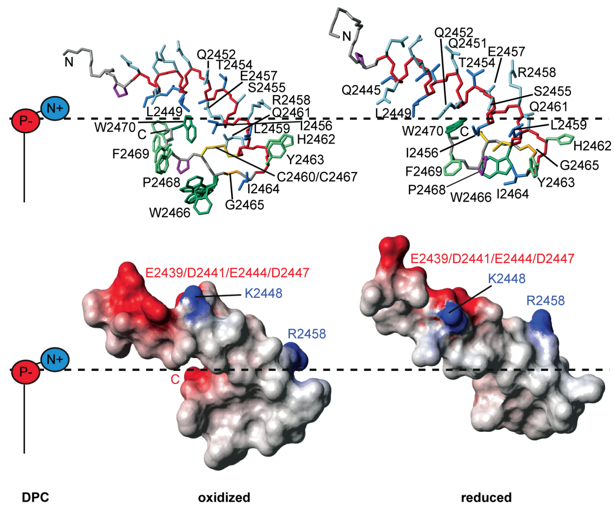
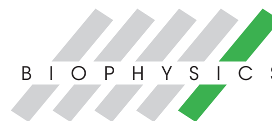


Model for the membrane-association of the TOR FATC domain.

Most important publications 2008-2009

- Dames, S. A. (2008). A fast and simple method to prepare the FKBP-rapamycin binding domain of human TOR for NMR binding assays. *Protein Expr Purif* 59, 31-7.
- Dames, S. A. (2010). Structural basis for the association of the redox-sensitive TOR FATC domain with membrane-mimetic DPC micelles. *J Biol Chem* 285, 7766-75.
- Dames, S. A., Bang, E., Ahrens, T., Häussinger, D., Engel, J. & Grzesiek, S. (2008). Insights into the low adhesive capacity of human T-cadherin from the structure of its N-terminal domain. *J Biol Chem* 283, 23485-95.



Membrane protein structure and function

The projection structure of DtpD a prokaryotic peptide transporter *(in collaboration with H. Daniel, Germany)*

We have cloned, overexpressed, purified, and biochemically characterized DtpD from E. coli. Its homologues in mammals, PEPT1 and PEPT2, not only transport peptides but also are of relevance for uptake of drugs. Uptake experiments indicated that DtpD functions as a canonical peptide transporter and is thus a valid model for structural studies of this family of proteins. Blue native polyacrylamide gel electrophoresis, gel filtration, and transmission electron microscopy of single-DtpD particles suggest that the transporter exists in a monomeric form when solubilized in detergent. Two-dimensional crystallization of DtpD yielded first tubular crystals that allowed the determination of a projection structure at better than 19 Å resolution. This structure of DtpD represents the first structural view of a member of the peptide transporter family.

Membrane protein function
(in collaboration with R. Kaldenhoff, Darmstadt, Germany)

We have finalized extended stopped flow spectrophotometry experiments, and demonstrate that wild type and different PIP2 mutants from spinach and tobacco plants functionally expressed in yeast do not exhibit phosphorylation induced gating. Osmotic water permeability measurements and freeze-fracture electron microscopy of purified SoPIP2 reconstituted into proteoliposomes resulted in pf = 1.2 10-13 cm^3/s, comparable to the water permeability of aquaporin-1 (pf = 5.4 10-14 cm^3/s). Our results contradict the phospho-induced gating hypothesis proposed to be essential for water homeostasis in plants. They also indicate that the S274→D mutant traffics more prominently to the PM of P. pastoris than the S74→A mutant. Since the former emulates the phosphorylated state and the latter the de-phosphorylated state our observation may suggest that phosphorylation induces trafficking to PM and hence regulates water flux.

The 20 Å structure of PDE6
(in collaboration with K. Palczewski, Cleveland, USA)

Rod cGMP phosphodiesterase 6 (PDE6) is a key enzyme of the phototransduction cascade, consisting of PDE6α, PDE6β and two regulatory PDE6γ subunits. We have developed a rapid purification method for PDE6-PrBP/δ from bovine rod outer segments (ROS) utilizing recombinant GST-PrBP/δ. We found that PDE6αβγ2-PrBP/δ forms a heterohexamer with a subunit stoichiometry of 1:1:2:2. Transmission electron microscopy of negatively stained samples reveals the location of PrBP/δ and thus where the carboxyl-termini of PDE6α and PDE6β must be situated.

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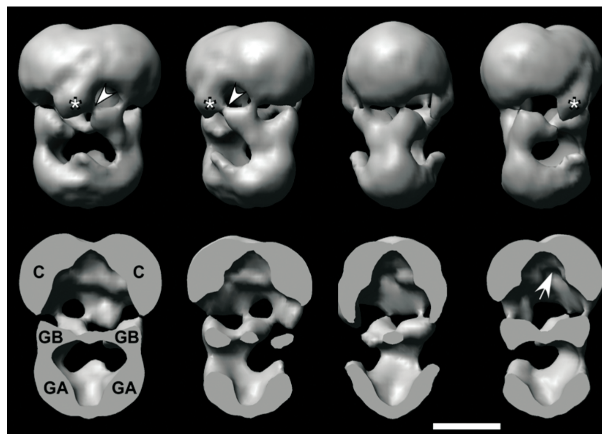
Andreas Engel

The three-dimensional structure of the PDE6αβγ complex was determined to 18 Å resolution from single particle projections. The PDE6 complex appears to have two pronounced cavities, the larger being formed by the catalytic domains at its top and the GAF-B domains at its bottom. The C-terminal cavity is partially closed by an elongated density that emerges from the right GAF-B domain, at roughly 45° with respect to the long axis of the complex, to reach up and over to the catalytic domain on the left. The smaller cavity at the N-terminal end of the complex is more open and is closed off at its bottom by the GAF-A domains.



3D structure of PDE6 at 18 Å resolution. A. The skull-like shape of the PDE6 complex houses two major cavities, one enclosed at the top by the catalytic domains (C) and an oblate 30 Å wide density (*), the other by the GAF-A domains at the bottom (GA). The oblate density is connected to the GAF-B domain (GB) by a slender linker (arrowheads). GAF-B forms a shelf-like structure separating the two cavities. The cut-away representations show the hemispherical shape of the catalytic domains. The active site is on the interior surface of the top cavity (arrow). The map was rendered at 100% mass, and each view is rotated wrt to its left neighbor by 45° in counter-clock direction. Scale bar: 50 Å.

Scanning Transmission Electron Microscopy (STEM)

(in collaboration with H. Saibil, M. Steinmetz, R. Rapuoli and H. Hebert)

The molecular architecture of the S. pneumoniae pilus: Although many Gram-negative pili have been studied in detail, relatively little is known about Gram-positive pili and most structural data are based on classical, low-resolution, immuno-electron microscopy studies. S. pneumoniae pili have one major (RrgB) and two minor components (RrgA and RrgC). A combination of TEM, antibody labeling and STEM was used to study their structure and localize the minor components. In particular, the high signal-to-noise of the STEM and its ability to make quantitative mass measurements was exploited to show that that the up to 1 µm long, flexible pili are formed by a single string of monomeric RrgB subunits. Further, the STEM images revealed a distinct nose-like structure, which gave the pilus shaft a clear polarity indicating that the RrgB monomers are linked head-to-tail. Primary antibodies, without gold tags, were directly visualized by TEM. Together with the pilus polarity, this showed that the minor components RrgA and RrgC are only present at opposite ends of the pilus shaft. Finally, STEM analysis of the unassembled pilus components revealed their domain structures and the aggregation state of RrgA-His.

High-throughput single molecule force spectroscopy

The atomic force microscope (AFM) allows single membrane proteins to be addressed, grabbed and pulled out from the membrane. During this process the protein is unfolded, which is monitored by measuring the force acting on the AFM cantilever during its retraction from the membrane surface. These force-distance curves provide valuable information on the membrane protein's fold, its interaction with adjacent proteins, and the binding of ligands. To obtain statistically relevant information, many (ideally thousands) force-distance curves need to be acquired and quantitatively interpreted – a task requiring automation. We have first solved the automated data acquisition, and have recently developed a fully automated strategy for the quantitative analysis of force-distance curves.

Most important publications 2008-2009

- Casagrande, F., Harder, D., Schenk, A., Meury, M., Ucurum, Z., Engel, A., Weitz, D., Daniel, H. & Fotiadis, D. (2009). Projection structure of DtpD (YbgH), a prokaryotic member of the peptide transporter family. *J Mol Biol* 394, 708-17.
- Frederix, P. L., Bosshart, P. D. & Engel, A. (2009). Atomic force microscopy of biological membranes. *Biophys J* 96, 329-38.
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- Iacovache, I., Biasini, M., Kowal, J., Kukulski, W., Chami, M., van der Goot, F. G., Engel, A. & Remigy, H. W. (2009). The 2DX robot: A membrane protein 2D crystallization Swiss Army knife. *J Struct Biol* 169, 370-378.
- White, H. E., Hodgkinson, J. L., Jahn, T. R., Cohen-Krausz, S., Gosal, W. S., Muller, S., Orlova, E. V., Radford, S. E. & Saibil, H. R. (2009). Globular tetramers of beta(2)-microglobulin assemble into elaborate amyloid fibrils. *J Mol Biol* 389, 48-57.

Analysis of nuclear pore complex proteins in nuclear transport and other cellular processes

The nucleoporin Nup153 and its multiple functions

Nuclear pore complexes (NPCs) are the sole sites for macromolecular trafficking between the nucleus and cytoplasm of eukaryotic cells. NPCs are among the largest supra-molecular assemblies in cells and their composition is fairly well characterised. While NPCs primarily control nucleocytoplasmic transport during interphase, evidence is accumulating that individual nucleoporins control distinct aspects of cell cycle progression during mitosis. In this context, several nucleoporins were found to localise to kinetochores, where they have roles in spindle assembly and chromosome segregation. The spindle assembly checkpoint (SAC) proteins Mad1 and Mad2, on the other hand, localise to NPCs in interphase and relocalise to kinetochores in mitosis. Our studies on the nucleoporin Nup153 have now revealed that the interaction of Nup153 with the protein Mad1 is important in the regulation of the spindle checkpoint. Overexpression of human Nup153 in HeLa cells leads to abnormal mitoses, including inactivation of the spindle checkpoint. Depletion of Nup153 using RNA interference results in the decline of Mad1 at NPCs during interphase and causes a delayed dissociation of Mad1 from kinetochores in metaphase, while the spindle checkpoint remains active. *In vitro* studies revealed a direct binding of Mad1 to the N-terminal domain of Nup153. Our data suggest that Nup153 levels regulate the localisation of Mad1 during the metaphase/anaphase transition thereby affecting spindle checkpoint activity and mitotic exit. We are currently further analysing the implication of Nup153 in SAC regulation.

Nup153 has previously been shown to interact with B-type lamins. Lamins are type-V nuclear intermediate filament proteins and the major constituent of the nuclear lamina. They are essential for nuclear architecture and basically implicated in all nuclear functions, such as DNA replication, transcription, NE stability and NPC positioning. On the basis of their biochemical properties and their behaviour during mitosis they are grouped in A- and B-type lamins. B-type lamins are essential for cell viability and are expressed in all cells during development, whereas A-type lamins are expressed in a tissue-specific manner, and mutations in the *LAMIN A* gene lead to a set of distinct human diseases known as laminopathies. NPC organisation and distribution within the NE is often affected in laminopathic diseases, suggesting a direct interaction between NPCs and lamin A. However, previously Nup153 as well as Nup35/Nup53 were found to interact with B-type lamins only. We have performed a systematic biochemical approach and found now that Nup153 interacts with both A- and B-type lamins. Nup153 has multiple bindings sites in its N- and C-terminal domains, respectively, for the so-called Ig-fold in the tail domain of the lamins. Interestingly, A- and B-type lamins differ in their binding properties to

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Nup153, indicating a functional difference between Nup153-lamin A and Nup153-lamin B complexes.

Elucidating the molecular mechanism underlying yeast apoptosis

A second research interest of our group is apoptosis in yeast. Apoptosis is a form of programmed cell death that is crucial for the development and maintenance of multicellular organisms and in the past few years it became evident that such a death program also exists in the yeast *Saccharomyces cerevisiae.* A number of orthologues of key mammalian apoptosis regulators have been found in yeast and we have previously identified the pro-apoptotic nuclear serine protease Nma111p and its anti-apoptotic substrate Bir1p. Both proteins are nuclear proteins and we have now shown that Nma111p is not able to shuttle between the nucleus and the cytoplasm and that consequently its nuclear localisation is a prerequisite for its pro-apoptotic activity. This is in clear contrast to Nma111p's human homologue Omi/HtrA2, which is a mitochondrial protein that is released into the cytoplasm upon apoptosis activation.

Histone proteins are well-known substrates for numerous covalent posttranslational modifications, which are known to regulate a number of cellular processes including apoptosis. Histone H2B is mono-ubiquitinated at Lys 123 by the ubiquitin conjugase Rad6p and the E3 ligase Bre1p, and only recently H2B ubiquitination has been implicated in DNA repair and DNA damage response. DNA damage response is closely linked to apoptosis in yeast and higher eukaryotes, which prompted us to study a putative role of H2B ubiquitination in apoptosis. We found that enhanced levels of Bre1p protect yeast cells from hydrogen peroxide-induced cell death, whereas deletion of *BRE1* enhances cell death. Apoptosis resistance is conferred by histone H2B ubiquitination mediated by the E3 ligase activity of Bre1p. Furthermore, Δbre1 cells exhibit increased caspase activity when compared to wild type cells and deletion of the yeast metacaspase *YCA1* leads to reduced apoptosis sensitivity of cells lacking Bre1p, indicating that Bre1p's activity is caspase-dependent. Further experiments to dissect the role of nuclear events in apoptosis signalling in yeast are under way.

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Nuclear magnetic resonance spectroscopy of biomolecules

The scientific goal of the group is the application and development of high-resolution Nuclear Magnetic Resonance (NMR) methods for the elucidation of structure, function, and dynamics of biological macromolecules.
The structural and functional projects currently encompass the human chemokine receptor CCR5, which is also the HIV1-coreceptor; Abelson kinase, a prime drug target in the treatment of chronic myelogeneous leukemia; endotoxin, the causative agent of endotoxic shock; cadherins; the TipA multidrug resistance protein of *S. lividans;* bacterial PilZ domains, which are targets for signaling via cyclic di-GMP; domains of the cell growth regulator TOR; minicollagen cysteine-rich domains from *Hydra* and an atomic-detail description of protein folding by new NMR methods. We describe a few salient results:

Abelson (Abl) kinase. Chronic myelogenous leukemia (CML) is caused by an abnormal rearrangement of chromosomes resulting in the aberrant fusion protein Bcr-Abl. The unregulated kinase activity of Bcr-Abl leads to the uncontrolled production of immature blood cells and thus leukemia. The clinically highly efficacious drugs imatinib, nilotinib and dasatinib have been developed against Bcr-Abl. These inhibitors block the kinase activity by competing against ATP. However, spontaneous mutations of Bcr-Abl in advanced-stage patients render these inhibitors inefficient. This has stimulated the search for new inhibitors that can overcome resistance.
In collaboration with Novartis (Basel) we have been able to determine the hitherto unknown, physiologically relevant solution conformations of Abl kinase in complex with several inhibitors. The data show that the crucial activation loop adopts the inactive conformation for the imatinib and nilotinib inhibitor complexes, whereas dasatinib preserves the active conformation, thus contradicting earlier predictions from molecular modeling.
NMR data have also helped to elucidate the mechanism of a new class of allosteric CML inhibitors, which had been discovered by the group of Prof. N. Gray (Harvard). Using NMR, X-ray, mutagenesis and hydrogen exchange data, the binding of the lead compound GNF-2 could be located to the Bcr-Abl myristoyl-binding pocket. This site is remote from the ATP binding pocket. The improved analogue GNF-5 displays *in vitro* and *in vivo* efficacy against the important Bcr-AblT315I resistance mutant. Thus this class of inhibitors opens new therapeutic opportunities via a novel mechanism of action.

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

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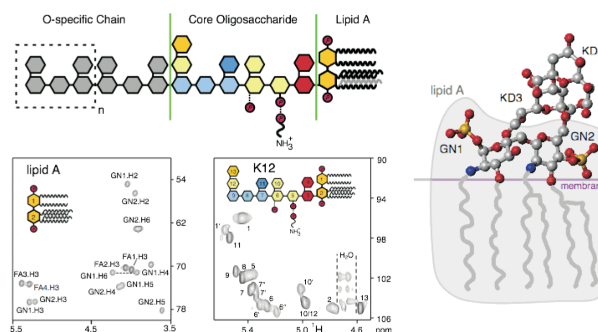
proceeds via the sequential interaction of the viral protein gp120 with the host-cell factors CD4 and CCR5. Very promising HIV entry inhibitors are based on CCR5 ligands, comprising the natural ligand RANTES. The structures of CCR5 and of its complexes are unknown. In recent years, we have made progress towards the structure elucidation of CCR5 and of its complex with RANTES by (1) characterizing the interaction of the soluble protein RANTES with peptides derived from CCR5 and (2) developing a method to produce pure, active CCR5 in sufficient amounts for structural studies by NMR, electron microscopy and X-ray crystallography.

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.



NMR analysis of LPS in detergent micelles. Top: schematics of LPS overall structure. Bottom left: assigned finger print spectra of lipid A and K12 forms of LPS. Right: sugar arrangement of LPS derived from NMR data.

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Conformational dynamics of ATP-driven molecular machines

We study the dynamics of molecular machines that convert the energy of ATP hydrolysis into structural changes of nucleic acid substrates. Large conformational changes during their catalytic cycles have been inferred, but the role of these conformational changes for enzyme activity remains elusive.

RNA helicases
DEAD box helicases catalyze the ATP-dependent structural rearrangement of RNA and RNA/protein complexes. Their helicase core comprises two flexibly linked RecA-domains, and N- and C-terminal extensions mediate interactions with RNA substrates or with proteins. We have shown that the cooperative binding of ATP and RNA leads to the closure of the cleft between the RecA domains. In this closed conformation, both the catalytic site for ATP-hydrolysis and a continuous RNA binding site are formed by residues from both domains. Structural data indicate that formation of the closed conformer is coupled to a local distortion of the RNA, and this distortion has been suggested as a first step of RNA unwinding. In single molecule FRET experiments, we have shown that mutations preventing closure of the helicase core lead to unwinding deficiency. Strikingly, an ATPase-deficient mutant still adopts the closed conformation, but is unable to catalyze RNA unwinding. The closure of the inter-domain cleft is thus necessary, but not sufficient for RNA unwinding. We have followed the conformation of the helicase core through all functionally relevant states in the nucleotide cycle using ADP BeF_x and ADP MgF_x as substrate and product state analogs. The helicase core maintains the closed conformation during ATP hydrolysis. Subsequent to ATP hydrolysis, phosphate release triggers re-opening the inter-domain cleft, and the concomitant reduction in RNA affinity leads to RNA release.

Our data provide a model for the mechanism of RNA unwinding by DEAD box helicases that links nucleotide-driven conformational changes with structural changes in the RNA, and rationalizes the effect of mutations, nucleotide analogs, and the stability of the RNA substrate on the unwinding reaction *(see figure).*

Hera from *Thermus thermophilus* is the first DEAD box helicase that forms a stable dimer in the absence of ligands. We have shown that Hera binds to ribosomal and RNase P RNA via its C-terminal domain and may be involved in RNase P and ribosome assembly. We have assembled the structure of the complete Hera dimer from overlapping fragments. The Hera helicase core is followed by a bipartite C-terminal domain that consists of a novel dimerization motif, and an RNA binding domain that folds into a modified RNA recognition motif. The RNA binding sites on the two

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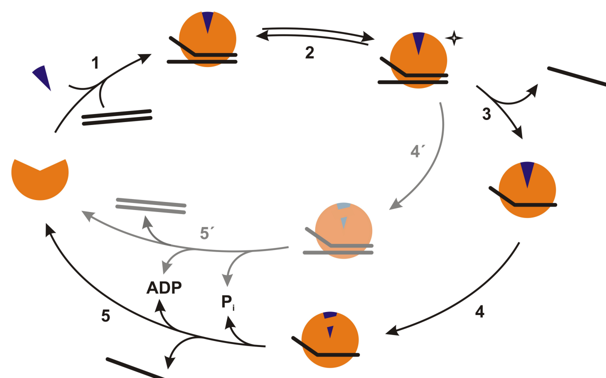
helicase cores in the dimer face towards each other, possibly allowing for their concerted action on one large RNA molecule.

DNA topoisomerases

DNA gyrase introduces negative supercoils into DNA at the expense of ATP hydrolysis. The active enzyme is an A_2B_2 heterotetramer of two GyrA and two GyrB subunits. The GyrA subunit mediates DNA binding and harbors the catalytic tyrosine for transesterification reactions, and the GyrB subunits contain the ATPase sites. The ATP-induced dimerization of GyrB provides a nucleotide-regulated gate (N-gate). In a current model for the mechanism of DNA supercoiling by gyrases, a first DNA segment binds to the center of the heterotetramer, the DNA-gate, and is cleaved. A second DNA segment enters through the N-gate, is passed through the gap of the cleaved segment (DNA-gate), and exits the gyrase *via* the C-gate. Religation of the first DNA segment ends one catalytic cycle.

Directed strand-passage towards negative DNA supercoiling requires the regulated opening and closing of the N-gate, the DNA-gate, and the C-gate, and may be guided by the C-terminal domains (CTDs) that wrap DNA. We have shown that DNA bound to the DNA-gate is distorted in a process coupled to cleavage. Our results demonstrate that DNA binding, distortion and cleavage, and gate-opening are mechanistically distinct events. Presumably, distortion of the gate-DNA unlatches the DNA-gate, and prepares it for opening. During the relaxation and supercoiling reactions, the DNA-gate is predominantly in the closed conformation, consistent with gate-opening as a very rare event that is strictly coupled to strand passage. To dissect the individual conformational changes and to gain insight into their coordination, we now focus on the conformational dynamics of the N-gate and the role of the CTDs. Preliminary experiments point towards an active role of the CTDs in presenting the T-DNA and in coordinating N-gate closure.

Reverse gyrase catalyzes the ATP-dependent introduction of positive supercoils into DNA. It consists of a helicase-like module, fused to a topoisomerase domain. We have shown that positive DNA supercoiling by reverse gyrase can be powered by hydrolysis of the "non-hydrolyzable" ATP analog ATPγS. The helicase-like domain is a nucleotide-dependent switch that is attenuated in reverse gyrase. This inhibitory effect of the topoisomerase domain decelerates the progression of reverse gyrase through the nucleotide cycle, possibly providing optimal coordination of ATP hydrolysis with the complex reaction of DNA supercoiling.



DEAD box proteins bind double-stranded RNA (line pair) and ATP (triangle, step 1). In complex with nucleotide and RNA, the DEAD box protein adopts a closed conformation. One strand of the bound RNA is kinked, leading to the local destabilization of (terminal or internal) base pairs. The initial complex undergoes a rearrangement towards an activated, hydrolysis- and unwinding competent conformation (star, step 2). The structural difference between these two complexes is currently unclear. From the activated complex, the first RNA strand can dissociate (step 3) before ATP is hydrolyzed (step 4). Product release is coupled to the dissociation of the second RNA strand (step 5), thereby re-setting the enzyme for further cycles. For longer RNA duplexes, destabilization by the kink does not lead to the same probability for the complete strand separation and dissociation of the first RNA strand from the activated complex. In this case, ATP is hydrolyzed before the first RNA strand dissociates (step 4'). Product release and dissociation of the RNA (step 5') reset the enzyme for further cycles. The partitioning between dissociation of the first strand and ATP hydrolysis rationalizes the higher amount of ATP required for unwinding longer duplexes.

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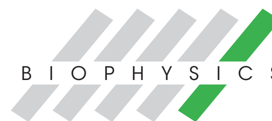


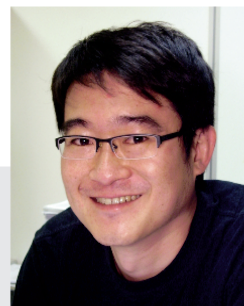
Deconstructing nuclear pore complex function by bio-synthetic reconstruction

Our mission is to resolve and understand biological mechanisms as they occur at the molecular-nanometer length scale i.e., nanobiology. We more affectionately refer to this as the "cartoon" length scale, which challenges us to innovate beyond conventional bio-physical/-chemical and molecular biology approaches because the physicochemical properties of biomolecules (and their interactions) can be unexpectedly novel at the nanoscale.

The success of such an endeavor entails a bottom-up, multi-disciplinary effort. Having just started in Jan 2009, the group is now seven-member strong, with expertise spanning the physical and biological sciences. In addition to the requisite know-how in biochemistry/molecular biology and surface chemistry, our key expertise lies in developing and integrating high-resolution microscopies (i.e. total internal reflection fluorescence, atomic force microscopy and optical tweezing), with nanofabrication, functional assays, bio-sensing techniques (e.g. Biacore) and computer simulations.

Importantly, our research is problem-driven with an emphasis on biological nanomachines. Not least for revealing important insights into biological functional, the functional significance of biological nanomachines underscores the potential benefits of harnessing their mechanisms outside the biological context. Hence, by combining the key biological molecules with synthetic nanostructures *(see figure),* our strategic blueprint is to create biomimetic reconstructions that can replicate aspects of biological functionality. In this way, we can rationalize plausible mechanisms from the bottom-up and heuristically validate if similar effects are physiologically relevant.

Our approach has thus far contributed considerable insight into the functional mechanism of how the nuclear pore complex (NPC) regulates nucleocytoplasmic transport (Lim et al., *Science,* 2007). Besides bringing fundamental questions to the fore, we are now applying such operational principles so as to harness NPC-like functionality using synthetic polymers. On a more technological note, we envision that such bio-synthetic devices may have key applications ranging from molecular sieving and drug delivery to the miniaturization of array-based bio-sensing technologies.



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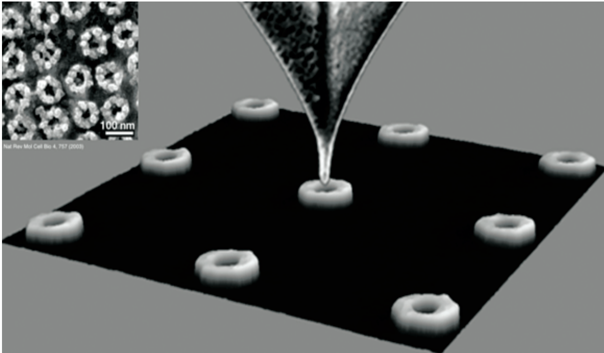

An AFM tip probes a bio-synthetic "NPC" consisting of FG-nucleoporins tethered onto gold nanorings. Physiological NPCs (of the same size) are shown in the inset for comparison.

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NMR methods and applications for challenging biological systems

Our group focuses on development and application of Nuclear Magnetic Resonance (NMR) methodology in the inter-disciplinary context, enhancing NMR toolkit with latest Information Technology tools and applying NMR to answer challenging questions on the structure and function of biological macromolecules.

Beyond addressing fundamental problems in NMR and structural biology, our long term goals include establishment of an intelligent electronic infrastructure for accumulation and dissemination of NMR knowledge and expertise. With the first components already in development, this software framework will automate complex NMR routines, making advanced NMR technology accessible to a wider range of Life Science specialists.

Leucine zipper transcription factors as biological catalysts

Adding to the growing number of proteins with multiple functionalities, we have recently shown that leucine zipper (LZ) motifs of basic-region leucine zipper (bZIP) transcription factors GCN4 and c-Jun are capable of catalyzing degradation of RNA (collaboration with B. Gutte, University Zurich). This observation is especially intriguing given the tightness of RNA turnover control and the antiquity and prevalence of bZIP factors. Our NMR studies show that LZ peptides are more catalytically potent towards the single-stranded RNAs and that RNA binding is attained by the dimeric (coiled coil) conformation of LZ. NMR data on the active site composition indicates that catalysis follows the acid-base mechanism of the phosphoester bond transesterification *(see figure)*. We propose that in vivo catalytic activity is associated with the DNA-bound bZIP factors and may serve as a negative feedback loop for the transcription activation process (Nikolaev et al., submitted). This work paves the way for the structural and in vivo studies of a diverse family of LZ transcription factors as biological catalysts.

Structure and inhibition of coronavirus envelope protein ion channel

Coronaviruses cause a variety of lethal diseases in birds and mammals, and common colds in humans. In 2003 an animal coronavirus, termed SARS-CoV, was able to infect humans and caused a near pandemic with 774 deaths worldwide. Such events are likely to reoccur in the future, making new antiviral strategies a necessity. The envelope (E) protein from coronaviruses is a small peptide that contains at least one α-helical transmembrane domain. Inactivation of E protein results in attenuated viruses. Previous studies show that the transmembrane domain of protein E forms pentameric bundles that are likely responsible for the membrane-permeabilizing

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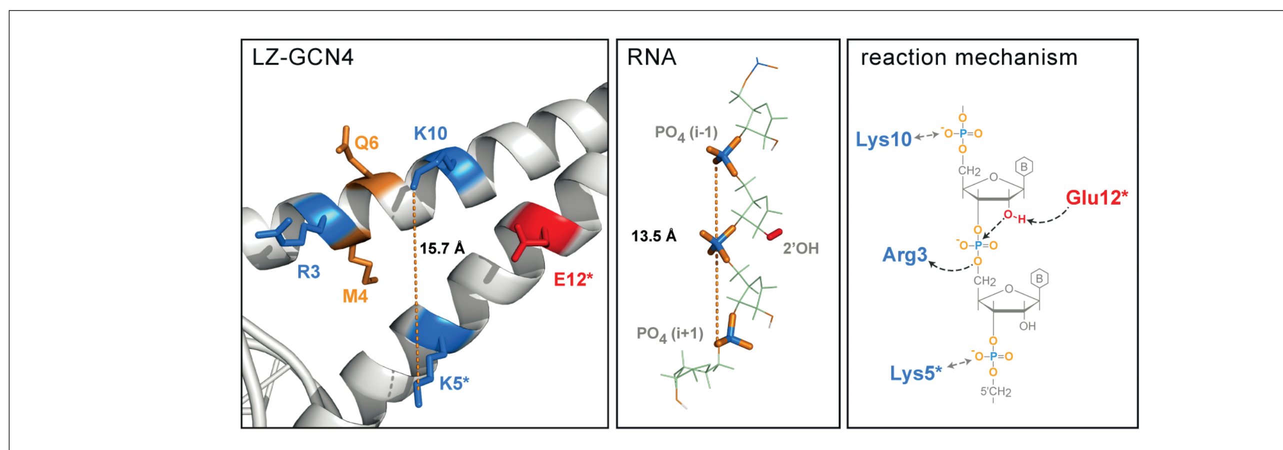

Structure of LZ-GCN4 active site and the proposed mechanism of the ribonuclease reaction. Glu12 and Arg3 act as a catalytic base and catalytic acid respectively.

properties of the protein. Using solution NMR in dodecylphosphatidylcholine micelles we have obtained a model of SARS-CoV channel which features regular α-helices that form a pentameric left-handed parallel bundle. This model provides a valuable insight into envelope protein ion channel activity, and could serve as a platform for the development of novel antiviral drugs. (In collaboration with J. Torres, NTU Singapore).

Simulation and benchmarking of NMR experiments

Systematic benchmarking of multi-dimensional NMR experiments is a critical prerequisite for optimal allocation of NMR resources for structural analysis of challenging proteins, e.g. large proteins with limited solubility or proteins prone to aggregation. We developed a set of benchmarking parameters for essential protein NMR experiments organized into a relational database, which includes all the necessary auxiliaries (waveforms, decoupling sequences, calibration tables and setup algorithms). The database is interfaced to the Spinach library (http://spindynamics.org), which enables accurate simulation and benchmarking of NMR experiments on large spin systems. A key feature is the ability to use a single user-specified spin system to simulate the majority of solution state NMR experiments, thus providing the (hitherto unavailable) unified framework for pulse sequence evaluation. This development enables predicting relative sensitivity of different implementations of NMR experiments, thus providing a basis for comparison, optimization and, eventually, automation of NMR analysis. (In collaboration with I. Kuprov, University of Oxford).

Web portal for NMR experiment setup and knowledge accumulation

Modern NMR techniques are heavily dependent on the software and hardware infrastructure employed for particular experimental setup. With more than 500 research groups worldwide, NMR community lacks an efficient mechanism for experimental knowledge dissemination in the platform-independent format. As a result, interoperability between different research laboratories is greatly reduced. Swiss-NMR web portal (http://nmrplus.com) is aimed to solve this problem by providing an integrated environment for NMR development and applications. Its core functionality includes (1) online repository of NMR pulse-sequence templates for accumulation of experimental knowledge; (2) desktop client software for automated setup of NMR experiments; and (3) a set of "Web 2.0" tools to aid efficiency of collaborative projects. Furthermore, open architecture of the service will allow third parties to embed Swiss-NMR components in their own workflows, add new functionality to the portal and perform knowledge extraction using latest Semantic Web and Linked Data technologies.

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Molecular mechanisms of c-di-GMP signal transduction and solute translocation across membranes

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 membrane proteins facilitating or catalyzing solute translocation across bacterial membranes.

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 group of Urs Jenal (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 *(see figure)*.

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. 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. In collaboration with the Dehio group (Biozentrum), we have revealed the structure of the Fic domain of BepA from *Bartonella henselae* and, most recently, got evidence for auto-AMPylation activity of this domain.

Sugar transporters of the bacterial phosphoryl-transfer-system
The carbohydrate: phosphotransferase system (PTS) couples solute translocation across the inner membrane to solute phosphorylation. The PTS is ubiquitous in bacteria, but not found in eukaryotes rendering it an attractive target for novel antibiotics. The PTS consists of general proteins (enzyme I and HPr) and sugar-specific components (enzymes II). The latter are comprised of several structural domains or subunits: IIA and IIB are hydrophilic cytoplasmic

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components catalyzing the phosphorelay, whereas IIC is integrated in the plasma membrane and catalyzes translocation. We are working on sucrose permease IIBC from *S. typhimurium.* IIBC is composed of two domains: a membrane-spanning IIC domain with a covalently linked cytosolic IIB domain, which may, in our experiments, facilitate crystal formation by providing additional hydrophilic surface. Active IIBC has been successfully expressed in *E. coli* and purified. The homogeneity of the sample was verified by ultracentrifugation and electron microscopy. Since extensive crystallization trials did not yield suitable crystals, we are now focusing on the structure determination of IIBC in complex with monoclonal antibody Fab fragments.

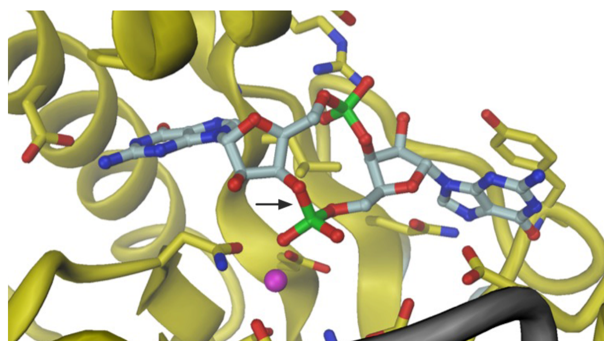
Porins

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 beta-barrel structure of NanC from *E. coli,* providing a model for members of the KdgM family of small monomeric acid-sugar specific porins. NanC is an N-acetylneuraminic acid-inducible outer membrane channel allowing the translocation of sialic acid. In collaboration with S. Bernèche (Biozentrum), we are now studying the molecular details of substrate translocation.

Plant and insect allergen and allergen – antibody complex

Type I allergy is a hypersensitivity disease affecting more than 25% of the population. It is characterized by an increased production of IgE antibodies against otherwise harmless antigens, i.e. allergens. The objective of this research is to determine the structural and immunological characteristics of major allergenic proteins from insect and plants. Crystal structures of allergens define the surface exposed residues which are most likely to interact with antibodies, whereas structures of allergens in complex with specific antibody reveal the detailed molecular interactions and may reveal features that may discriminate allergens from common antigens.

Particularly important was the crystal structure determination of the major grass pollen allergen Phl p 2 in complex with a specific human IgE-derived Fab domain. The antibody recognizes, quite unusually, the large flat surface of a β-sheet of the allergen. We have also determined the structures of the major fire ant allergen Sol i 3 and the major carrot allergen Dau c 1 which is almost identical to celery allergen Api g 1, explaining the observed cross-reactivity. The identified epitope of Phl p 2 and predicted epitopes of Sol i 3 and Dau c 1 may now be subjected to site-directed mutagenesis to produce hypoallergens for therapeutic use.



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.*

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

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

Structural plasticity in actin dynamics

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

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

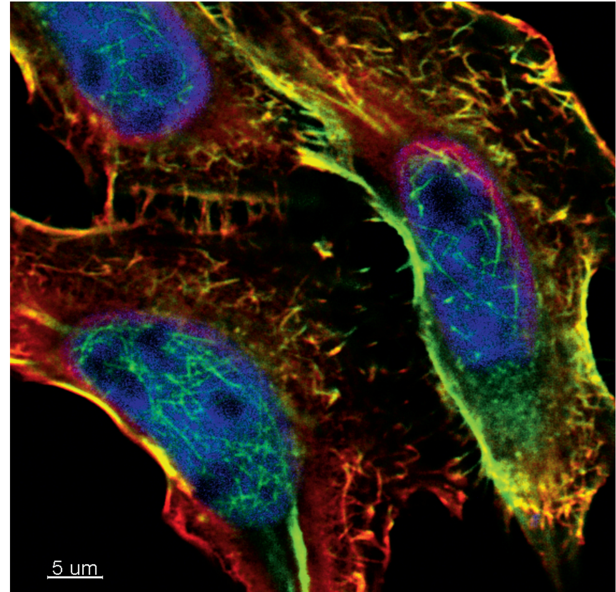
Mechanical plasticity of cells as marker for transformation

Cells within tissues continuously encounter a dynamic range of mechanical forces to which they respond by remodeling their cytoskeleton. Tumorigenic transformation

frequently changes the nature of the forces experienced by cells and the cellular response is modified accordingly. We are employing atomic force microscopy (AFM) in combination with light/fluorescence microscopy to quantitatively probe with high sensitivity and spatial resolution the nanomechanical properties of cells and tissues. Our aim is to understand the specific contributions of cytoskeletal components to mechanical function. In particular, we are investigating correlations between changes in cellular elasticity and the cytoarchitecture that are associated with tumorigenic transformation.

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

Because the characteristics of cancer are more appropriately reflected by a three-dimensional (3D) tissue organization we use cultured cell spheroids and native tumor tissue from a transgenic mouse and human patient biopsies to investigate nanomechanical changes associated with tumorigenic transformation. Consistent with the mechanical response of tumor spheroids, preliminary data revealed a gradual centripetal softening from the periphery to the core in early breast carcinoma tissue from MMTV-PyMT mice. The cellular stiffness of the stromal tumor tissue at the periphery of the malignant lesion was comparable to that measured in normal mammary gland tissue. The nanomechanical AFM testing of human biopsies showed that malignant lesions are not characterized by a specific stiffness but rather a radial gradient of stiffness that is related to the malignant phenotype. In contrast, benign lesions typically show a uniform stiffness which is consistent with fairly homogenous tissue morphology. We are currently testing whether a correlation between tissue softening and increasing hypoxia exists in breast tumors.



Different actin populations in the nucleus. Filamentous actin (green) and non-filamentous actin (red) is present in the nucleus (blue) of HeLa cells that overexpress actin tagged with a nuclear localization signal (NLS).

Most important publications 2008-2009

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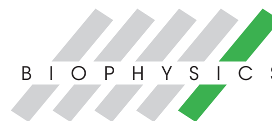

Unraveling substrate recognition and transport by ATP binding cassette (ABC) transporters

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 singl e 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

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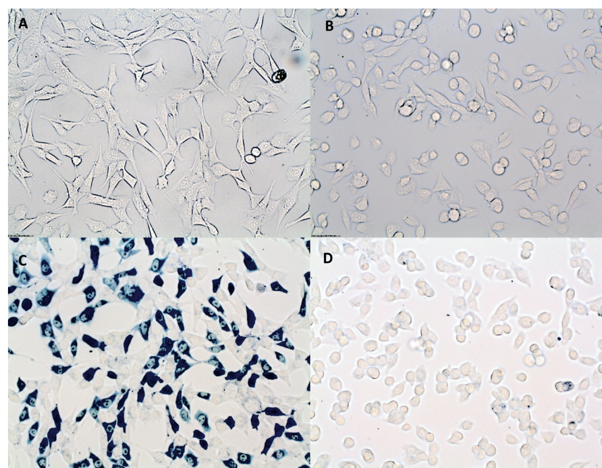
hydrogen bonds with the numerous hydrogen bond donor groups in the transmembrane domains of the transporter. In the lipid environment of the cytosolic membrane leaflet (exhibiting a low dielectric constant) hydrogen bonds are more specific and stronger than van der Waals interactions. As soon as the substrate reaches the extracellular leaflet, where water can approach, hydrogen bonds with the transporter vanish and the substrate is released either into the lipid or the aqueous phase.

The rate of substrate transport by ABCB1 is directly proportional to the rate of ATP hydrolysis. However, the fact that substrates have to partition into the cytosolic membrane leaflet to bind to ABCB1 implies that many substrates partially escape to the cytosol before being caught by the transporter which complicates the analysis of substrate transport. Quantitatively comparing passive influx and active efflux of drugs revealed that the net flux of drugs across membranes protected by ABCB1 results from the sum of the two processes. For different compounds and a given membrane passive influx exponentially decreases with the size and the charge of the molecule and changes by several orders of magnitude for different compounds whereas active efflux is more constant and changes only by about one order of magnitude. Large and/or highly charged compounds diffuse slowly and are therefore prone to being completely effluxed by ABCB1. The diffusion step from the extracellular to the cytosolic leaflet is crucial for the fate of the molecule. If it is fast compared to efflux (flipping) compounds will reach the cytosol even though they are partially exported, however, if it is slow compounds will not reach the cytosol *(see figure A-D)*. Our analyses revealed that the lipid bilayer membrane plays an important 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.



The protective effect of ABCB1 demonstrated with methylene blue in MDR1 transfected mouse embryo fibroblasts. Light microscopic pictures (20 x magnification) of wild-type (NIH-WT) (A and C) and MDR1-transfected mouse embryo fibroblasts (NIH-MDR1-G185) (B and D) before (top row) and after 1h incubation with methylene blue (64.5 µM) (bottom row). Wild-type cells get stained (C), whereas MDR1-transfected cells remain unstained (D) because the efflux transporter, ABCB1 (MDR1) is able to prevent intrusion of methylene blue into the cell due to the methylthioninium ion (Päivi Äänismaa, Thesis, Univ. Basel, 2007).

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

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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 physical-chemical techniques as early as 2003 that CPPs bind to anionic lipid vesicles but cannot enter the vesicle interior. We could exclude the still popular model that the induction of non-bilayer 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 physical-chemical knowledge to living cells. We synthesized a fluorescent derivative of the HIV-1 TAT protein transduction domain and observed its uptake into non-fixated living fibroblasts with time-lapse confocal microscopy, eliminating the need of fixation. Depending on the concentration, the fluorescent CPP entered the cell within seconds. Several observations suggested that the CPP binding leads to an aggregation or "capping" of sulfated glycosaminoglycans, inducing finally endocytosis. 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 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

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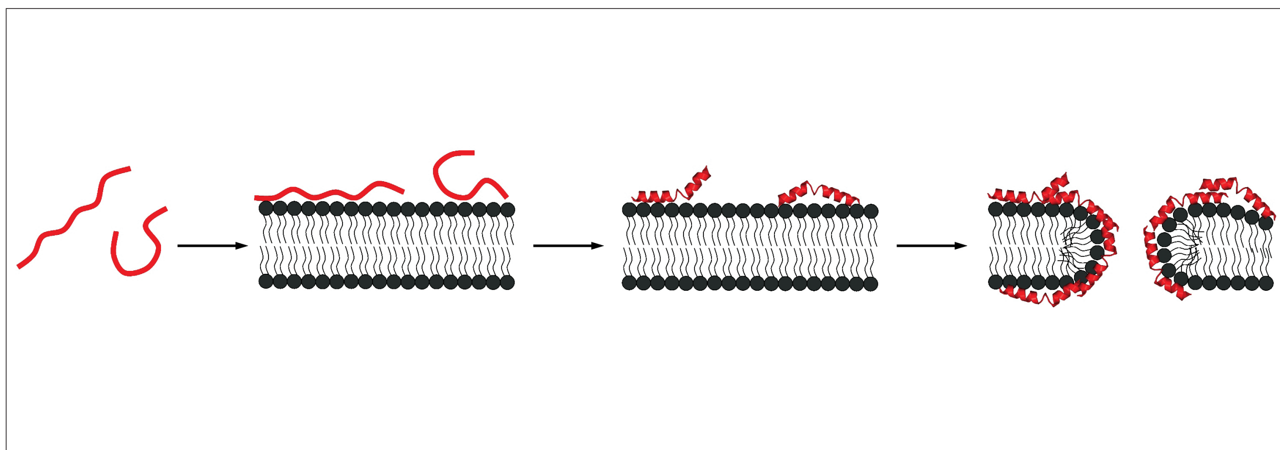

Model of melittin interaction on the lipid membranes.

adopt an α-helical structure when bound to membranes. Likewise, a membrane-induced *random coil-to-β-structure transition* has been found for Alzheimer peptides such as Aβ(1-40) or fragments thereof. Melittin and related amphipathic compounds insert into the lipid membrane and modify the lipid structure. In contrast, the Aβ peptides remain on the surface of the bilayer.

In a series of publications we have studied in detail the thermodynamics of the membrane-induced *random-coil-to-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 membrane-induced *random coil-to-β-structure transition.* Indeed, our work appears to be the first quantitative analysis of a random coil β-structure transition. In collaboration with Y. Shai, Israel, we have applied this knowledge to a modified melittin which is β-structured on the membrane surface. The thermodynamics of this system ideally confirms the results obtained with other model systems for the rc β-structure transition.

Related projects

We are strongly interested in the physical-chemical properties of Alzheimer peptides Aβ(1-40,42). The major obstacle for thermodynamic and kinetic studies is the low solubility of Aβ(1-40,42) in aqueous media. Nevertheless, we succeeded to provide a systematic thermodynamic analysis using ITC of the interaction of antibodies, specifically designed against different Aβ(1-40) segments, with Aβ(1-40). We have continued our work on detergent membrane investigations by analyzing in detail the biologically relevant lipopeptide surfactin. In addition, we have selectively deuterated two trans-membrane helices, WALP-19 and glycophorin A_{72-97}, 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.

Most important publications 2008-2009

- Hayley, M., Perspicace, S., Schulthess, T. & Seelig, J. (2009). Calcium enhances the proteolytic activity of BACE1: An in vitro biophysical and biochemical characterization of the BACE1-calcium interaction. *Biochim Biophys Acta* 1788, 1933-8.
- Klocek, G., Schulthess, T., Shai, Y. & Seelig, J. (2009). Thermodynamics of melittin binding to lipid bilayers. Aggregation and pore formation. *Biochemistry* 48, 2586-96.
- Klocek, G. & Seelig, J. (2008). Melittin interaction with sulfated cell surface sugars. *Biochemistry* 47, 2841-9.
- Meier, M. & Seelig, J. (2008). Length dependence of the coil <–> beta-sheet transition in a membrane environment. *J Am Chem Soc* 130, 1017-24.
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Multi-resolution microscopy from cells to atoms

Center for Cellular Imaging and Nano Analytics (C-CINA)
The Center for Cellular Imaging and Nano Analytics (C-CINA) was established in 2008 - 2009 by Andreas Engel and his team in the D-BSSE building in the northern part of Basel. Henning Stahlberg joined the C-CINA in April 2009. C-CINA is supported by the Swiss systems biology initiative SystemsX.ch, and is applying various microscopy methods to study biological specimens at different size and length scales.

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.

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

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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 multi-resolution microscopy, including electron tomography. In collaboration with Crina Nimigean, Cornell University, NY, USA, we study the cyclic-nucleotide gated potassium channel MloK1 by single particle EM and electron crystallography and obtained a first direct characterization of the orientation of the putative voltage sensor "paddles" of this protein in the membrane embedded conformation. In collaboration with Joe Mindell, NIH Bethesda, USA, we study the E. coli chloride/proton antiporter ClC-ec1, of which we obtained highly-ordered two-dimensional membrane protein crystals at different pH values. In collaboration with Horst Vogel, EPFL, Switzerland, we study the 5HT3-R serotonin receptor membrane protein. We also obtained highly-ordered 2D crystals of a bacterial porin in complex with viral attachment protein, in collaboration with Cecile Breyton, IBS, Grenoble, France. The high-resolution structure determination of these membrane proteins under different buffer conditions is under way.

Software development

The Stahlberg group has developed 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 2D maximum-likelihood module, which we are now extending for 3D capabilities, so that high-resolution structures of membrane proteins can also be determined in the absence of large well-ordered 2D crystals. We have also developed a module for projective constraint optimization, to significantly improve the resolution of the reconstruction, also in the direction perpendicular to the membrane plane, where structural information was previously missing due to sample tilt limitations (the so-called missing cone problem). These modules enable 2dx to automatically determine the high-resolution structure of membrane proteins also from badly ordered 2D crystals.

The fold of α-Synuclein, a protein involved in Parkinson's disease, was studied by a combination of high-resolution cryo-electron microscopy and NMR, in collaboration with the group of R. Riek.

Most important publications 2008-2009

- DeVay, R. M., Domingues-Ramirez, L., Lackner, L. L., Hoppins, S., Stahlberg, H. & Nunnari, J. (2009). Coassembly of Mgm1 isoforms requires cardiolipid and mediates mitochondrial inner membrane fusion. *J Cell Biol* 186, 793-803.
- Evans, J. E., Hetherington, C., Kirkland, A., Chang, L.-Y., Stahlberg, H. & Browning, N. (2008). Low-dose aberration corrected cryo-electron microscopy of organic/biological specimens. *Ultramicroscopy* 108, 1636-44.
- Harrington, J. M., Chou, H.-T., Gutsmann, T., Gelhaus, C., Stahlberg, H., Leippe, M. & Armstrong, P. B. (2009). Membrane activity of C-reactive protein. *FEBS Letters* 583, 1001-5.
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- Vilar, M., Chou, H.-T., Luehr, T., Maji, S.K., Riek-Loher, D., Verel, R., Manning, G., Stahlberg, H. & Riek, R. (2008). The fold of α-Synuclein fibrils. *Proc Nat Acad Sci USA* 105, 8637-42.

Molecular evolution and public perception of scientific knowledge

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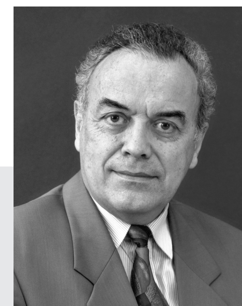

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As one of the founding members and since 1996 emeritus professor of the Biozentrum I see my task in science communication and debate with scientific colleagues as well as in the dialogue with the general public on basic scientific knowledge and on its technological applications. Depending on the envisaged mission, this can be at a local, a national or an international level. I expect that this can represent valid contributions to the wide radiation of the Biozentrum. This shall be illustrated in the following sections that report on some of my activities during the last two years.

Major events at the Biozentrum

In the context of the annual national days of genetic research in May 2009 I supplied the contribution of the Biozentrum by giving a public lecture with extended discussion on molecular mechanisms of spontaneous and bioengineered genetic variations and their impact on biological evolution. A month later a public celebration of my 80th birthday was organized by the University at the Biozentrum with a lecture on synthetic biology given by Hamilton Smith, co-recipient of the Nobel Prize Medicine 1978. Both of these events had full house.

The Darwin year 2009

The international scientific community celebrated in 2009 Charles Darwin's 200th birthday and the 150th anniversary of the publication of his seminal book on biological evolution. This stimulated me to give many lectures on various aspects of molecular evolution, some of which shall be mentioned below. Numerous interviews for the press, the radio and the TV were also given in this context, and several reviewing papers on experimental and conceptual aspects of molecular evolution were published during the period of this report. These topics were covered in invited lectures, among others at the following occasions:

- In March 2008 in Mexico City at the Auditorium Inauguration Ceremony of the Mexican Academy of Sciences
- In June 2008 in Gran Canaria, Spain at the Health Forum of the Campus of Excellence
- In September 2008 at the Siena Meeting "From Genome to Proteome"
- In October 2008 in Dalian, China, at the closing session of the International Biotechnology Symposium
- In October 2008 in Shanghai, China: Xu Guangqi Lecture
- In November 2008 at the Plenary Session of the Pontifical Academy of Sciences, Vatican
- In December 2008 at the University of Kassel
- In February 2009 in Basel: Novartis Campus Lecture
- In March 2009 in Rome, STOQ International Conference on Evolution at the Pontifical University Gregoriana

- In May 2009 at the study week on transgenic plants for food security, Pontifical Academy of Sciences, Vatican
- In May 2009 at the University of Freiburg i.Br., Germany
- In July 2009 in Lindau, Germany, at the meeting of Nobel Laureates
- In October 2009 in Heidelberg, Germany, at the Conference "Biotechnology and its Impact on Society" of the International Journalists Programme Alumni
- In October 2009 in Munich, Germany, at the Symposium "New Frontiers in Science" of the International Max Planck Research School
- In November 2009 in Budapest, Hungary, at the Hungarian Academy of Sciences at the occasion of the World Science Forum
- In November 2009 in Florence, Italy, at the Conference "Evolutionism and Religion" of the International Academy for Philosophy of Science

Science and spirituality

The search for consensus opinions between science and spirituality is of primary importance for our civilization. I contribute to this endeavour since many years. I am an active member since 1981 of the Pontifical Academy of Sciences and a Council member of this Academy since 1996. In the latter function, I co-organize conferences and study weeks at the seat of the Academy in the Vatican and I act also as editor for some of the proceedings of these conferences. As a protestant Christian I am pleased to observe that the Vatican maintains very good relations with the sciences and that the dialogue is steady and constructive. In the context of the Pontifical Council for Culture, I serve as a member of the Program Development Committee of the STOQ projects, where STOQ stands for Science, Theology and the Ontological Quest. These activities turn out to be quite fruitful, in particular in the framework of Pontifical Universities.

Science and society

In more general contexts I also contribute to debates on the impact of science and technology on society. For example I am vice-president of the Steering Committee for the biennial World Science Forum held in Budapest, Hungary. I also deal with this interdisciplinary topic at discussions with groups of students and of the general public such as organized by the church or by Rotary or other clubs and organizations.

Honors

In the period of this report I became honorary member of the Hungarian Academy of Sciences in recognition of my activities as Co-president of the World Conference on Science held in 1999 in Budapest and of the above mentioned contributions to the World Science Forum. In 2009 I was attributed the Lennart Bernadotte Medal in recognition of my activities as member since 1991 of the Council for the Lindau Nobel Laureates Meetings.

Most important publications 2008-2009

- Arber, W. (2008). Molecular mechanisms driving Darwinian evolution. *Math Computer Modelling* 47, 666-74.
- Arber, W. (2008). Stochastic genetic variations and their role in biological evolution. In: Arber, W., Cabibbo, N. & Sanchez Sorondo, M. (eds.) Predictability in Science: Accuracy and Limitations, *The Pontifical Academy of Sciences,* Acta 19, 126-40.
- Arber, W. (2009). The impact of science and technology on the civilization. *Biotech Adv* 27, 940-4.
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- Arber, W. (2009). From microbial genetics to molecular Darwinism and beyond. In: Arber, W., Cabibbo, N. & Sanchez Sorondo, M. (eds.) Scientific insights into the evolution of the universe and of life, *The Pontifical Academy of Sciences,* Acta 20, 259-72.

Assembly of the extracellular matrix

The following work was done in collaboration with the Research Department of Shriners Hospital for Children in Portland , Oregon, in which I was working as a visiting scientist. This department is connected to the Health and Sciences University of Oregon (OHSU). It is a leading institution working on diseases related to the extracellular matrix. Many of these diseases are caused by inherited defects of matrix components and effect children at an early age.

In the last two years I was involved with the assembly domains in collagens, which serve to link and register the three polypeptide chains of collagens for correct folding to a collagen triple helix. In particularly we were investigating the assembly domain of collagen XVIII, which is located near the C-terminus as an interruption of the regular collagenous region. It was found to be a novel type of fold with a very strong potential to trimerize. We also showed that a non-collagenous domain of a different type, namely an unusual variant of an α-helical coiled-coil structure serves a similar function in collagen XIX.

Many collagens contain a set of interchain disulfide bonds commonly called disulfide knot, which help to keep the chains in proper register and stabilize the collagen triple helix. A well known example is collagen III. The C-terminal fragment of collagen III was crystallized to investigate the pattern of disulfide linkage. Unfortunately the resolution of the crystal structure was not sufficient in the region of the knot to decide on the mode of linkage. On the other hand the remaining structure of the fragment was of excellent resolution. Our structure is at present the most detailed structure of a natural collagen and allowed interesting conclusions on inter- and intramolecular interactions.

In addition to the described published work I was engaged in a long-lasting project dealing with a quantitative description of the hysteresis of collagen folding. A publication was recently accepted by Biophysical Journal and the results will be explained in the next research report. The mathematical algorithm to describe the hysteresis is rather general and can be applied to other systems. In a collaboration with Michael Kümin and Helma Wennemers from the Chemisty Department we evaluated the hysteresis of polyproline from helical form I to helical form II. A publication was submitted to Journal of Peptide Science

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Most important publications 2008-2009

- Boudko, S. P., Engel, J. & Bächinger, H. P. (2008). Trimerization and triple helix stabilization of the collagen XIX NC2 domain. *J Biol Chem* 283, 34345-51.
- Boudko, S. P., Engel, J., Okuyama, K., Mizuno, K., Bächinger, H. P. & Schumacher, M. A. (2008). Crystal structure of human type III collagen Gly991-Gly1032 cystine knot-containing peptide shows both 7/2 and 10/3 triple helical symmetries. *J Biol Chem* 283, 32580-9.
- Boudko, S. P., Sasaki, T., Engel, J., Lerch, T. F., Nix, J., Chapman, M. S. & Bächinger, H. P. (2009). Crystal structure of human collagen XVIII trimerization domain: A novel collagen trimerization fold. *J Mol Biol* 392, 787-802.
- Mizuno, K., Peyton, D. H., Hayashi, T., Engel, J. & Bächinger, H. P. (2008). Effect of the -Gly-3(S)-hydroxyprolyl-4(R)-hydroxyprolyl- tripeptide unit on the stability of collagen model peptides. *FEBS J* 275, 5830-40.

ZMB
Center of Microscopy of the University of Basel

The ZMB is a central service unit for microscopy that provides imaging support and training. The service covers Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and specialized light microscopy, such as Confocal Laser Scanning Microscopy (CLSM), Video- and Fluorescence Microscopy. Administratively, the ZMB is integrated into the Biozentrum. Nevertheless, its service covers the microscopy requirements of all the faculties of the University of Basel. Historically, it resulted from a fusion of the Interdepartmental Electron Microscopy unit of the Biozentrum and the REM-Laboratory of the University of Basel.

Services

The ZMB facilities comprise a Preparation Laboratory, two Transmission Electron Microscopes and three Scanning Electron Microscopes. It not only offers full microscopy service but also training for individuals to use the instruments independently in collaboration with the Biozentrum, that provides certain additional instruments, the ZMB service also includes training and technical support for Fluorescence - and Confocal Light Microscopy. Additionally, a Confocal Laser Scanning Microscope (CLSM) with image processing hard- and software is available on on request.

Courses

The ZMB organizes following training courses in microscopy:

- One week workshops in "Applied Microscopy for Molecular Biologists"
- One week workshops in "Applied Microscopy for Organismic Biologists"
- Two three-week workshops in microscopy for students in nanotechnology

Research

The ZMB collaborates with researchers and offers to take over the imaging part of their work. In addition, it continously works on the development of new preparation methods and innovative instrumentation.

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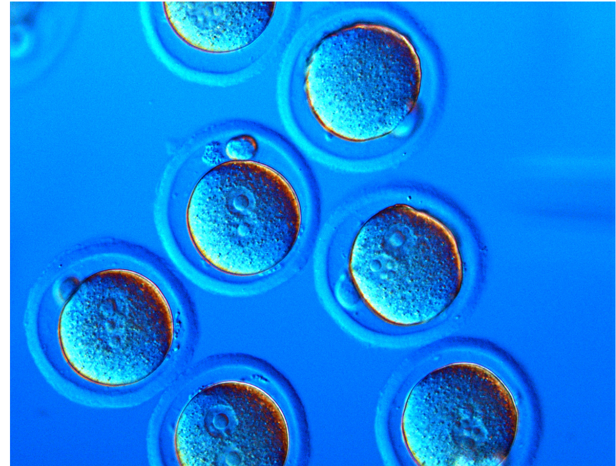
TMCF
Transgenic Mouse Core Facility

The Transgenic Mouse Core Facility (TMCF) of the University of Basel provides transgenic and transgenic related techniques as a service to the researchers of the University of Basel.



Oocytes before fertilisation is completed.

Service
TMCF offers the injection of DNA constructs and BAC's into the nuclei of fertilised oocytes of various strains, and carries out the injection of embryonic stem (ES) cells into blastocysts to generate chimeras. As mouse line rederivation by embryo transfer has become increasingly important, TMCF supports the research groups of the University of Basel in the handling of frozen or fresh mouse pre-implantation embryos.

As a further important service, TMCF is offering cryo-preservation of mouse embryos including storage. TMCF provides gene targeting of mouse ES cells and supports research groups with tested material and methods for ES cell work. In addition, TMCF offers de novo rederivation of mouse ES cells and embryonic fibroblasts from transgenic mouse lines. The technique of aggregating diploid and tetraploid embryos with mouse ES cells is currently being established. The aim is to work closely with the researchers and provide technical support during their experiments. TMCF continuously evaluates the technical needs and requirements in order to extend their services.

Set up
The facility consists of an injection suite, a cryo-preservation and mouse embryo thawing work station, lab space for general mouse embryo work, animal rooms, and a lab to carry out surgical procedures under sterile conditions, a molecular biology lab for quality control and sample preparation, a mouse stem cell lab, and a workstation for primary mouse cell culture.

Staff
TMCF consists of one head of facility and four technical staff members, two of whom are employed half-time. TMCF staff members are funded by the founding institutions, the Biozentrum, the University of Basel and the Department of Biomedicine.

Advisory board
The TMCF advisory board is composed of professors of the Biozentrum and the Department of Biomedicine with equal representation.

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Insights into TOR signalling by quantitative phosphoproteomics

While the main focus of the laboratory is to provide mass spectrometric services to the Biozentrum of the University of Basel, in the recent years particular emphasis has been given to developing methods suitable for the tracking of complex cellular phosphorylation events. Protein phosphorylation regulates a vast number of biological processes. Traditional biochemical and genetic analyses of phosphoproteins, and of the protein kinases and phosphatases that modify them, have yielded a wealth of information about signalling pathways. The availability of modern analytical technology has made it possible to study a large number of phosphorylation sites at once. In our lab, the focus in phosphoproteomics lies mainly on two levels. Firstly, phosphorylation usually occurs substoichiometrically and therefore efficient selection methods are being constantly improved for collecting as many phosphopeptides as possible from cellular extracts. Secondly, to track changes in the extent of phosphorylation that occur by a given treatment, we heavily rely on the SILAC technology to track changes in phosphorylation in extracts of yeast and HeLa cells. In a collaborative effort for finding novel targets for the cellular growth regulators mTORC1 and mTORC2 we set out to analyze the mTOR phosphoproteome in yeast and in MEF cells with quantitative SILAC labeling (stable isotope labeling with amino acids in cell culture, see Ong et al., 2002).

For yeast, we streamlined a procedure consisting of separating extracts from untreated and rapamycin-treated cells by preparative SDS gel electrophoresis, in-gel digestion and selective isolation of phosphopeptides followed by mass spectrometric analysis. In this way, we were able to monitor the phosphorylation levels after rapamycin treatment of more than 2,400 sites from about 1,000 proteins of which more than 100 phosphoproteins were affected by rapamycin *(see figure, part A and B).* The phosphorylation of many of them was up regulated, which was often caused by increased protein abundance. Among the regulated phosphoproteins we found protein kinases (Sch9, Npr1, Rim15, Gcn2) and transcription factors (Maf1, Rtg3) but also a number of uncharacterized proteins, which could play a novel role in TORC1 signalling. Interestingly we found that many down regulated phosphopeptides contained RRXpS motifs, which suggests a link between the PKA and the TORC1 pathways *(see figure, part C).* For the mammalian TOR phosphoproteome, a tamoxifen inducible knockout system in mouse embryonic fibroblasts (MEFs) is being used. Upon induction, protein levels of raptor (TORC1) or rictor (TORC2) are reduced to less than 95% after three days when compared to control cells. In the MEFs, we used triple SILAC labelling to compare the phosphoproteomes of mTORC1 (raptor) depletion with mTORC2 (rictor) depletion and control cells treated with tamoxifen. Phosphopeptides are isolated and enriched

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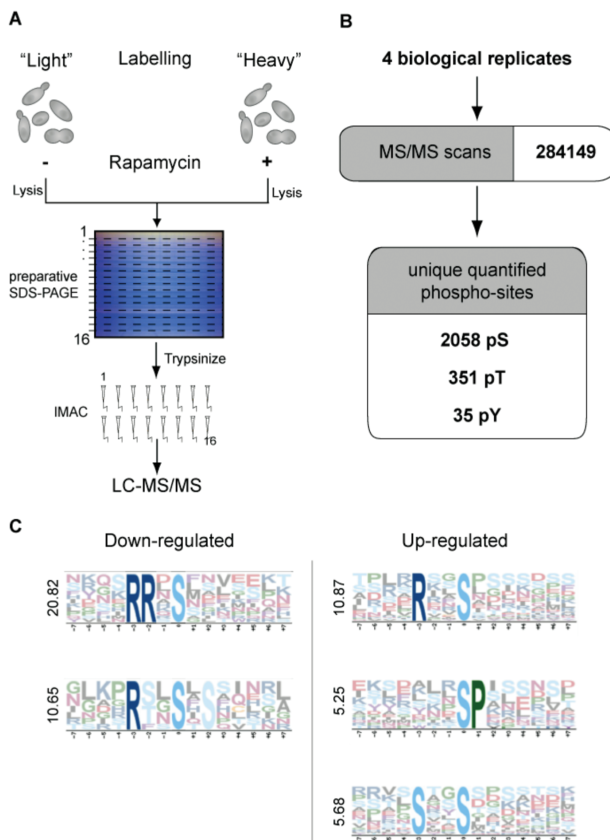
using the IMAC/TiO2 Gel-LC-MS/MS protocol established in yeast. We have generated a preliminary dataset identifying 410 phosphopeptides and 27 mTOR-regulated phosphopeptides, including previously identified mTOR-regulated phosphopeptides. We are currently working to expand this preliminary data set to reach deeper into the phosphoproteome so that novel substrates of mTORC1 and mTORC2 can be discovered with novel biological processes involved in the regulation of mTOR.



Quantitative analysis of the rapamycin-sensitive phosphoproteome by SILAC. A) Two yeast cultures are metabolically labelled with normal or isotopically labelled 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 LTQ-Orbitrap. B) Four independent experiments yielded 972 phosphoproteins, corresponding to 2,383 unique phosphopeptides. C) Motif analysis with Motif-X of all down-regulated and up-regulated phosphopeptide sequences. Motifs are ranked from top to bottom according to their score.

Most important publications 2008-2009

- Gander, S., Bonenfant, D., Altermatt, P., Martin, D. E., Hauri, S., Moes, S., Hall, M. N. & Jenoe, P. (2008). Identification of the rapamycin-sensitive phosphorylation sites within the Ser/Thr-rich domain of the yeast Npr1 protein kinase. *Rapid Commun Mass Spectrom* 22, 3743-53.
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- Jiang, H., Schiffer, E., Song, Z., Wang, J., Zurbig, P., Thedieck, K., Moes, S., Bantel, H., Saal, N., Jantos, J., Brecht, M., Jeno, P., Hall, M. N., Hager, K., Manns, M. P., Hecker, H., Ganser, A., Dohner, K., Bartke, A., Meissner, C., Mischak, H., Ju, Z. & Rudolph, K. L. (2008). Proteins induced by telomere dysfunction and DNA damage represent biomarkers of human aging and disease. *Proc Natl Acad Sci USA* 105, 11299-304.
- Kolla, V., Jenö, P., Moes, S., Tercanli, S., Lapaire, O., Choolani, M. & Hahn, S. (2009). Quantitative proteomics analysis of maternal plasma in Down syndrome pregnancies using isobaric tagging reagent (iTRAQ). *J Biomed Biotechnol* 952047, Epub 2009 Nov 5.
- Scherr, N., Müller, P., Perisa, D., Combaluzier, B., Jenö, P. & Pieters, J. (2009). Survival of pathogenic mycobacteria in macrophages is mediated through autophosphorylation of protein kinase G. *J Bacteriol* 191, 4546-54.

IT at the Biozentrum

Molecular biology research continues to evolve into a quantitative discipline, relying more and more on large-scale, automated acquisition and subsequent evaluation of biological data.

IT support according to each project's demands
The current trends in biological research demand an IT infrastructure and support organization that can flexibly react to the researchers' requirements. Therefore, IT infrastructure and support at the Biozentrum is composed of different layers:
Firstly, BioPhIT (Biozentrum/Pharmazentrum IT) administers and supports all desktop workstations throughout the Biozentrum, centrally managed standardized desktop systems as well as specialized workstations. Together with the University Computing Centre (URZ), BioPhIT also provides central Home and Group drive storage available to all research and service groups of the Biozentrum.
Bioinformatics system administration manages the working environment of the CP "Computational & Systems Biology". Among other resources, this includes a high-performance computing cluster with more than 80 TB of storage available on a parallel high-performance file-system.
Finally, Research IT integrates and coordinates the various IT aspects of research projects from their inception to a stable productive solution. They solve the complex methodological and IT infrastructure issues arising from large and complex datasets.

Areas of interest
Challenges for IT in biology research generally present themselves in three areas: 1) The ability to efficiently store, annotate and retrieve large and rapidly growing biological datasets, 2) to allow biologist users to perform analyses of datasets requiring significant computational power, and 3) satisfying specific application needs by custom software development.

Project support
Research IT supports 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.

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 (BioPhIT and Bioinformatics system administration), of local partners within (URZ) and outside of the University, and on a national

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level (SystemsX.ch). Since 2009, we take part in SyBIT (Systems Biology IT), the life science IT research and technology development program of the SystemsX.ch initiative. Its aim is to provide the IT infrastructure and tools to enable systems biology research in Switzerland.

Services and resources

Beyond project-based work, Research IT also develops and maintains a large portfolio as common infrastructure to all research groups. Within the reporting period, notable developments were:

- Proteomics analysis software and data storage array – maintenance and installation of hard- and software for protein mass spectrometry analysis
- Animal facility management software – customization, set-up, and scale-up of a software solution
- Biozentrum Wiki - enterprise-level Wiki available to all users of the Biozentrum to manage data in collaborations and research projects.
- Issue and Project Tracking software for the Biozentrum
- Publication database – customized open-source software for internal management and external display of publication data from the Biozentrum
- Safety lectures and a self-service test for biological and chemical safety have been developed as web applications
- Biozentrum Web server – maintenance of the official Biozentrum web presence and associated sites
- International PhD Program Evaluation Database – development of a web-based application to handle portfolios of PhD applicants
- Virtualization infrastructure – jointly (Research IT/ BioPhIT) operated high-availability virtualization cluster
- PC-Grid infrastructure – harvesting of computer power from desktop PCs for scientific applications
- Oracle Collaboration Suite – maintenance of groupware server

Steering boards

To coordinate IT activities at the Biozentrum and to communicate developments to the group leader conference two IT steering boards were established in 2009:

- Steuerungsausschuss IT (SIT) for BioPhIT, composed of: head of central services, two group leaders, one student/ postdoc representative, one representative of the secretary's offices and central/lab services each, head of course organisation, head of Research IT, team leader BioPhIT.
- Steuerungsausschuss Wissenschaftliche IT (SWIT) for Research IT, composed of: three group leaders with elevated IT requirements in science, head of central services, team leader BioPhIT, one representative of the proteomics core and imaging facility each, head of Research IT.

Outlook

The continuing increase in the volume of acquired data necessitates the development of infrastructure, software and policies to archive research data. This process has been initiated by gathering requirements and an initial project plan for the implementation of an archiving infrastructure. Another key future goal is the tight integration with application/domain IT specialists from the core facilities, which are currently being established. This has been successfully initiated with the proteomics core facility.

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