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BIENNIAL REPORT 2004 – 2005

BIOZENTRUM
UNIVERSITY OF BASEL

BIOZENTRUM
Universität Basel

BIENNIAL REPORT **2004–2005**

BIOZENTRUM
UNIVERSITY OF BASEL

Biozentrum University of Basel
Klingelbergstrasse 50/70
CH–4056 Basel

Phone +41 61 267 21 11
Fax +41 61 267 17 12

info@biozentrum.unibas.ch
www.biozentrum.unibas.ch

Imprint

Coordination
Alexandra Weber
Public Relations, Biozentrum

Design & Layout Concept
Werner Indlekofer, Margrit Jaeggi
Photo & Web Design, Biozentrum

Layout Editing
Alexandra Weber
Public Relations, Biozentrum

Editing
Alexandra Weber, Sarah Güthe, Biozentrum

Portraits
Verena Grieder
Photo & Web Design, Biozentrum

Proof-Reading
Rose Marie Suter-Fritsche, Biozentrum

eBook Progamming
Werner Indlekofer
Photo & Web Design, Biozentrum

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Preface

The balanced growth of the Biozentrum in terms of new members and research groups continued in 2005: During the last 7 years, 20 new professors have been hired expanding the faculty to a total of 33 research groups, increasing the female faculty fraction to 25%. Molecular Biology, structural and dynamic Biophysics, and Bioinformatics remain the core activities of the Biozentrum with the research being centered on three „Focal Areas“:

Cell Growth & Development
Infection Biology
Neurobiology

Additionally, two method-oriented „Core Programs“ have been developed as scientific „glue“. These are:

Structural Biology and Biophysics
Bioinformatics & Genome Scale Biology

Our undergraduate/graduate program in „Molecular Biology“ has been redesigned to correspond to the Bologna system, leading to a Bachelor diploma after 3 years, followed by a fast-track Master thesis/program for an additional 1-1.5 years. The program is well received by our students and their appraisal is reproduced on the following page.

It was very pleasing to see the Biozentrum with its strong post-graduate program ranked #7 in a world-wide competition of best post-doc places outside the US (cf. The Scientist, Vol. 20, No. 3, 2006). It is our continuing goal to build a partnership with the scientific community in industry in Basel and the wider BioValley Basel area. Sponsored by Novartis Pharma International, we have organized, on behalf of the University of Basel a series of lectures of Nobel Laureates, which were widely advertised and usually attracted an audience of 300-500 participants. So far, we had the privilege to receive Prof. Pierre-Gilles de Gennes (Nobel Laureate in Physics, 1991), Thomas R. Cech (Nobel Laureate in Chemistry 1989), Günter Blobel (Nobel Laureate in Medicine 1999), and Stanley B. Prusiner (Nobel Laureate in Medicine 1997).

A further major event of the year 2005 was the visit of Jeb Bush, Governor of the State of Florida, with a high-ranking scientific delegation to the Biozentrum. Governor Bush gave a keynote lecture on “Life Sciences in Florida and Basel”. This visit was organized together with the Basel Chamber of Commerce, F. Hoffmann-La Roche, and the “Wirtschaftsförderung beider Basel”.

The Federal Chancellor Pascal Couchepin was the main speaker in a public discussion on the “Gene-Moratorium”. Co-organizer of this event was the Basel Chamber of Commerce, HKBB. This public debate attracted more than 200 visitors to the Biozentrum.

The Biozentrum is also intensely engaged in the SystemsX initiative: The ETH Zurich, the University of Zurich and the University of Basel will collaborate in the latest highly interactive scientific approach to biological research: Systems Biology. At least one new department of this cross-disciplinary faculty will be located in Basel. In addition, the Biozentrum will participate in the SystemsX program by contributing several scientific programs. 2006 will be the decisive year for launching this joint endeavor.

J. Seelig

Joachim Seelig
Chairman Biozentrum
April 2006

Teaching Activities

The Biozentrum educates scientists at the bachelor, master, doctoral and postdoctoral level. The particular advantage of the 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 basic 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 the 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 block courses lasting several weeks each, determines the Major of the Biology Bachelor. The block courses provide theoretical information which the students then turn 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 1 1/2 years. The students work on their Master thesis and take additional courses in molecular biology. The Master thesis replaced the current Diploma thesis as of 2004 and 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. A complete list of the Master & Diploma theses completed during the report period can be found on pp 5-6.

The interaction between students and the lecturers is additionally supported by the so-called career mentoring: at the beginning of their studies, every student is assigned to a certain lecturer, which implies 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 also offers a Graduate Teaching Program. Besides the work on his/her dissertation, the student must attend lectures that take place in regular cycles within a time frame of 2 to 3 years. The cycles of the new Graduate Teaching Program cover infection biology, neuroscience, cell biology, integrated biological systems, structure and function of macromolecules, biomolecular interactions and structure, genomics and bioinformatics, plant sciences, molecular biology, and molecular medicine. After approval of the dissertation by the Natural Science Faculty of the University of Basel, the studies are terminated with a detailed oral examination. A complete list of the dissertations completed during the report period can be found on pp 7-9.

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 on short notice or because of scheduling conflicts. We call these "Informelle Seminare", 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 while sharing a post-seminar drink with the speaker.

VSB
Students' Council Report
Verein Studierender der Biologie

Studying Biology at the Biozentrum in Basel – an enriching and challenging experience

The opportunity to accomplish our Bachelor's degree in either "Molecular Biology", "Integrative Biology" or "Animal and Plant Sciences" enables us students of biology at the University of Basel to take a first step towards our area of preference during undergraduate education. This basic concept of the curriculum is highly appreciated since different students hold interests as rich and various as the enormous diversity of subtopics in biology.

Thus, a certain separation of organismic and molecular biology, which is mirrored in the independence of the Biozentrum as a separate department, appeals to most students. Nonetheless, a vivid exchange between organismic and molecular orientation remains highly important. This is ensured by the opportunity to study "Integrative Biology" – a mixture of the two mentioned orientations – and by the fact that we all attend the same basic lectures in the initial four semesters of our education.

During the first two years, we are trained in basic scientific disciplines adjacent to biology such as math, physics and chemistry, including some practical work. This provides the opportunity to acquire the basic concepts and tools of scientific thinking. In addition, students gather a solid and broad theoretical background knowledge in biology, as lectures cover fields reaching from animal and plant sciences or ecology to neurobiology, structural biology or biochemistry.

The third year is organised in practical block courses, a concept that seems highly popular among nearly all students. It has been repeatedly pointed out that the block courses provide an excellent opportunity to engage in a particular subject much more thoroughly than any lecture would allow: The combination of practical and theoretical work seems to play a key role in understanding biology far beyond the profoundness which theoretical teaching can mediate alone. But apart from helping to understand complex biological mechanisms and pathways, the numerous experiments in the block courses also enable students to develop valuable practical skills. Furthermore, a factor always important to students is the assistant-to-student ratio, which reaches the value of 1:3 during some block courses, allowing almost everyone to have his or her own personal trainer. In addition, the professors do not seem to be too shy either to show up in the teaching labs and give advice on tricky experiments. Although block courses mean very hard work for many students, they are all in all perceived as well-designed and intelligent establishments, connecting theoretical and practical aspects and giving students a valuable first insight into the actual world of science and research.

The basic background taught in the four initial semesters and the block courses complement each other in preparing us for the Master's degree. During graduate studies, students have to prove their abilities to work as scientists in one of the various Biozentrum labs. Supervision by advanced students provides guidance through the whole year to ensure good results in the Master thesis. Generally, graduate students seem to draw a positive personal conclusion from their academic education at the Biozentrum.

The well-organised curriculum seems to logically guide us step by step to our academic goals: The lectures on various fields of biology during the initial semesters build on overall scientific thinking, the block courses build on the theory of the mentioned biology lectures and the graduate studies on many practical aspects taught in the block courses. This shows how important it is to ensure a high quality of teaching not only on the advanced level of graduate studies, but also on an entirely basic level from the very beginning of every academic education. Therefore, we must strongly emphasise the importance of didactics in teaching and would like to encourage the staff at the Biozentrum to continuously give careful consideration to this aspect so important to students. A didactically well-organised lecture may open doors which books alone sometimes fail to do.

All in all, we conclude that studying biology at the Biozentrum in Basel stands for a solid, interesting and challenging academic education of high quality. In this context, the uniqueness of the block courses should be emphasised once more: In addition to their valuable academic function, they mediate a strong practical background leading to advantages for graduate studies and, as a consequence, possibly in the job market.

Apart from the mentioned benefits which the Biozentrum at the University of Basel holds in contrast to other Swiss universities, prospective students should not forget either that Basel is an interesting place to live: As a culturally rich international hotspot, just a few minutes away from the French and German border, it is not surprising that one meets people from different countries in Basel and international students of various background at the Biozentrum. The exchange with a foreign student is often an experience of both personal and scientific enrichment.

For questions or comments: Simon.Staehli@stud.unibas.ch

Diploma Theses & Masters

Altermatt, Patrick: *Functional Analysis of the Yeast Protein Kinase Npr1p.* (P. Jenö)

Basler, Andrea: *Characterisation of BatR, a regulator of the VirB type IV secretion system in Bartonella henselae.* (C. Dehio)

*Benoit, Roger: *Universal, seamless and directional integration of DNA fragments into plasmids by RecA independent homologous recombination in E.coli.* (O. Mayans)

Bentzinger, C. Florian, *Overexpression of mini-agrin in skeletal muscle increases muscle integrity and regenerative capacity in laminin-a2 deficient mice.* (M.A. Rüegg)

Birrer, Claudia: *Proteins influencing nuclear distribution in the filamentous fungus Ashbya gossypii.* (P. Philippsen)

Casagrande, Fabio: *Recombinant MIP: expression, purification and two-dimensional crystallization.* (A. Engel)

Engel, Philipp: *The genome sequence of Bartonella tribocorum and comparative genomics of the genus Bartonella.* (C. Dehio)

Falk, Sven: *In vitro and in vivo analysis of the repressive activity of the integral Dpp-signalling pathway component Shn.* (M. Affolter)

Finnbogason, Gudmundur: *Comparison of global gene expression in human primary hepatocytes and hepatoma derived cell lines*. (U.A. Meyer)

Fünfschilling, Raphael: *A Functional Dissection of the Antennapedia Protein of Drosophila melanogaster*. (W. Gehring)

Gander, Stefan: *Topogenesis of Membrane Proteins.* (M. Spiess)

Geissbühler, Rina: *Kationische Peptide als Transportmoleküle über Membranbarrieren.* (A. Seelig & FHBB)

Halbeisen, Regula: *Proteomics of ERGIC membranes identifies CGI-100 as a new p24 family member.* (H.P. Hauri)

Huber, Sybille: *The role of zf-memo in zebrafish development.* (M. Affolter)

Hurschler, Benjamin A.: *The Role of Sorting Signals in COPII Coat Recruitment.* (M. Spiess)

Joder, Karin: *Dynamics if Intermolecular Interactions Measured by Triplet-Triplet Energy Transfer Experiments.* (T.Kiefhaber)

Kübler, Robert C.: *Influence of Salt Bridges on the Dynamics of Unfolded Polypeptide Chains.* (T. Kiefhaber)

Längle, Celia: C*onsequences of Premature EWS-Pea3 Expression in Chick and Mouse Spinal Cord.* (S. Arber)

Ley, Serej: *Identification of drug responsive elements in the 5'flanking region of the mouse Cytochrome P450 3a11 gene.* (U.A. Meyer)

Lussi, Yvonne: *YscU and the substrate specificity switch in the Yersinia enterocolitica injectisome.* (G.R. Cornelis)

Lutz, Roman: *The Molecular Mechanism of Rabaptin-5/Rabex-5 Complex in Recycling Vesicle Formation.* (M. Spiess)

Maylandt, Kerstin: *Analysis of small exported Ysc proteins in the type three secretion system of Y. enterocolitica*. (G.R. Cornelis)

Melone, Anna: *Establishment of an Experimental System for the In Vivo Analysis of Lysosomal Trafficking.* (J. Pieters)

Mertz, Ann: *Pib2p, a novel vacuolar membrane and endosome localized FYVE protein, mediates exocytosis of the general amino acid permease, Gap1p.* (S. Helliwell, &M. Hall)

Meyer, Dominique: *Characterization of a gene cluster involved in holdfast formation and timing during development.* (U. Jenal).

Müller, Borna Ivan: *RNA interference directed against components of the Pre-mRNA 3'end processing machinery.* (W.Keller)

Müller, Catherine: *Identification of minor components associated with the Yersinia entero-colitica injectisome needle.* (G.R. Cornelis)

Christina Nef: *Genetic and molecular characterization of the querulant gene in Drosophila melanogaster: A gene coding for a ZP domain protein involved in wing formation*. (M. Affolter)

Vassiliki Nikoletopoulou: *The role of Pax6 in neuronal specification: Studies using embryonic stem cells*. (Y.-A. Barde)

Rost, Florian: *Aufbau eines in vivo NMR-Experimentes mit Erythrozyten.* (J. Seelig & FHBB)

Rüdiger, Sarah: *Analysis of intersectin 1 and bromodomain-containing protein2 gene function in primary hippocampal neurons.* (M.A. Rüegg)

Sasselli, Clelia: *Establishment of a DNA vector-based RNAi technique to investigate the Role of BRF1 in the maintenance of ES cel pluripotency. (*C. Moroni & M. Spiess)

Schaffner, Thierry Oliver: *Functional analysis of CdpA, a novel substrate of the ClpXP protease in Caulobacter crescentus.* (U. Jenal)

Scheidegger, Florine: *Functional characterization of the type IV secretion substrates BepD1 and BepD2 of Bartonella tribocorum.* (C. Dehio)

Schlatter, Ivan: *Function of the four homologues of yeast Dynamins in the filamentous Ascomycete Ashbya gossypii*. (P. Philippsen)

Schmid, Michael: *Subversion of endothelial cell functions by the human pathogen Bartonella henselae.* (C. Dehio)

*Scott, Rachel: *Intestinal gene expression analysis of wild type and vitamin D receptor knock out mice challenged with 1*a*,25(OH)2Vitamin D3 or 24R,25(OH)2Vitamin D3.* (W. Hunziger & U.A. Meyer)

Stadler, Andreas: *Characterization of dmemo/CG 8031 in Drosophila melanogaster.* (M. Affolter)

Stebler, Michael: *Gene expression induced during postsynaptic differentiation in rat soleus muscle: Verification of candidate genes by real-time PCR.* (M.A. Rüegg & U. Suter)

Stöckli, Michèle Claudia: *Identification and characterization of avirulent mutants in Bartonella tribocorum.* (C. Dehio)

Suri, Gregor: *The Topology of Proprotein Convertase 1/3.* (M. Spiess)

Sustreanu, Nicoleta: *Cell cycle regulation: Evaluation of the general function of the Anaphase promoting complex in the filamentous fungus A. gossypii*. (P. Philippsen)

Tschopp, Patrick: *Eye development and evolution in the jellyfish Cladonema radiatum*. (W. Gehring)S

Toller, Isabella: *Characterization of ChvD, a regulator of Bartonella henselae virulence.* (C. Dehio)

*Vath, Frederik: *The TGIF KO mouse links TGF-b signaling to the nuclear hormone receptors RAR/RXR in the retinoid signaling pathways.* (R.G. Clerc, P. Matthias, & U.A. Meyer)

von Castelmur, Eleonore: *Structural characterisation of I-band spring elements from the muscle filament titin.* (O. Mayans)

Waldmeier, Lorenz: *Characterization of the novel p24 family member CGI-100.* (H.P. Hauri)

*Wang, Xueya: *Interferon alpha induced Jak-STAT signalling in mouse liver during repeated injection.* (M. Heim & U.A. Meyer)

* work performed outside of the Biozentrum

Doctoral Dissertations

Albrecht, Imke: *Studies on the role of cholesterol and coronin 1 in antigen-presenting cells.* (J. Pieters & A. Rolink)

Agrain, Céline: *YscP, a key player in the type three secretion system of Yersinia enterocolitica.* (G.R. Cornelis)

Appenzeller, Christian Andreas: *Molecular insights into the transport lectin function of ERGIC-53.* (H.-P. Hauri & M. Spiess)

*Babochkina, Tatiana Ivanovna: *Fetal cells in maternal circulation: fetal cell spearation and FISH analysis.* (S. Hahn, U.A. Meyer, H.-J. Müller & T. Bickle)

Ben, Tekaya Houchaima: *The ER-Golgi-intermediate compartment: dynamics and cargo sorting studied by time-lapse video microscopy.* (H.-P. Hauri & M. Spiess)

Bissière, Stephanie: *Dopamine modulation of synaptic transmission and plasticity in the lateral amygdala.* (A. Lüthi, M.A. Rüegg & D. Monard)

Bittcher, Godela: *The role of transmembrane agrin in reorganizing the cytoskeleton in neurons and non-neuronal cells.* (M.A. Rüegg & S. Kröger)

Börries, Melanie: *Dissecting novel regulatory mechanisms of S100A41 on cardiac function.* (U. Aebi)

Boudier, Kamila: *The role of AgRax1p, AgRax2p, AgBud7p and AgBud10p in mycelial development of the filamentous fungus Ashbya gossypii.* (P. Philippsen)

*Butz, Silvia: *Protecting the ischemic heart. Pharmacological protection against myocardial ischemia in rats in vivo and in vitro.* (C. Zaugg & K.G. Hofbauer)

Cabernard, Clemens: *Studying Fibroblast growth factor (FGF) mediated cell migration in Drosophila larval air sacs.* (M. Affolter)

Carnejac, Soizic: *Search for molecules involved in the formation of the nerve-muscle synapse.* (H.-R. Brenner, M.A. Rüegg & H. Reichert)

Chan, Carmen: *Structural elucidation of the multidomain response regulator PleD using X-ray crystallography.* (T. Schirmer)

Coers, Jörn: *Regulation of thrombopoietin receptor expression and function.* (M. Spiess, R. Skoda & A. Rolink)

Di Cara, Alessandro: *Disruption of ribosome biogenesis triggers a p21/p53 mediated cell cycle checkpoint.* (M. Hall, G. Thomas & B. Amati)

Di Scala, Franck: *Analysis of skeletal muscle in amyotrophic lateral sclerosis: etiological, diagnostic and therapeutic aspects.* (M. Primig, J.-P. Loeffler, M.A. Rüegg, M. Schwab, S. Potier & S. Braun)

Dossenbach, Caroline: *Functional dissection of the Drosophila melanogaster Fibroblast Growth Factor signalling pathway in branching morphogenesis of the developing tracheal system.* (M. Affolter)

*Driamov, Sergey: *The self-maintaining nature of ventricular fibrillation. Contribution of L-type Ca2+ channels and Na+/ Ca2+ exchange to cardiomyocyte Ca2+ overload in ventricular fibrillation. Surface fluorescence study in isolated perfused rat hearts.* (C. Zaugg & K.G. Hofbauer)

Egler, Viviane: *A combinatorial preclinical in vitro strategy against human glioblastoma cells – specific targeting of protein kinases, histone deacetylases and glycolysi.* (A. Merlo, M.A. Rüegg, K. Takeda & B. Hemmings)

Emter, Roger: *Functional dissection of the C-terminal part of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator alpha (PGC-1).* (M. Hall, A. Kralli & R. Clerc)

*Failly, Mike: *Combinatorial strategy using protein kinase inhibitors and a cytotoxic compound for highly resistant glioblastoma cells-in vitro studies*. (A. Merlo, M.A. Rüegg & N. Hynes)

Feder-Mengus, Chantal: I*mmungenic capacities of recombinant vaccinia virus expressing CD154: Effects on CTL priming.* (J. Pieters, G. Spagnoli & G. De Libero)

Frère, Samuel: *GABA signaling in the thalamus.* (A. Lüthi, M.A. Rüegg & H.-R. Brenner)

Friberg, Michael Andrea: *Degradation of vasopressin precursor and pathogenic mutants in diabetes insipidus.* (M. Spiess & H.P. Hauri)

Gasser, Olivier: *Microparticles released by Ectocytosis from human neutrophils: characterisation, properties and functions.* (M. Spiess, J. Schifferli & C. Moroni)

Golub, Tamara: *Regulation of the leading edge motility by PI(4,5)P2-dependent lipid microdomains.* (P. Caroni, M.A. Rüegg & S. Arber)

*Grange, Susan: *Assessment of Pharmacokinetic Interaction or L-Dopa and Benserazide.* (T.W. Günthert, N.H.G. Holford & U.A. Meyer)

Güthe, Sarah: *Mechanisms of Evolutionary Optimized Complex Folding Reactions.* (T. Kiefhaber)

*Gupta, Anurag Kumar: *Immune-modulation by the placenta and it's dysregulation in preeclampsia: role of syncytiotrophoblast microparticles and cytokines.* (S. Hahn, M.A. Rüegg & P. Erb)

Hangartner, Christoph: *Pharmacological blockade of g-protein-coupled receptors: Interventions to alter expression or internalization.* (U.A. Meyer, K.G. Hofbauer & A.N. Eberle)

Hartmann, Britta: *Characterization of Schnurri, an integral component of the Dpp-signaling pathway in Drosophila melanogaster.* (M. Affolter)

Higy, Marie: *Dynamic insertion of membrane proteins at the endoplasmic reticulum.* (M. Spiess & H.-P. Hauri)

Hippenmeyer, Simon: *Molecular Mechanisms of Neuronal Circuit Assembly in the Vertebrate Spinal Cord.* (S. Arber)

Kramer, Ina: *Molecular Pathways of Proprioceptive Dorsal Root Ganglion (DRG) Sensory Neuron Specification.* (S. Arber)

Krieger, Florian: *Dynamics in Unfolded Polypeptide Chains as Model for Elementary Steps in Protein Folding.* (T. Kiefhaber)

Ksiazek, Iwona: *Role of agrin in the brain and kidney of adult mice.* (M.A. Rüegg & T. Meier)

Laissue, Philippe: *Morphogenesis of a filamentous fungus: Dynamics of the actin cytoskeleton and control of hyphal integrity in Ashbya gossypii.* (P. Philippsen)

Li, Weimin: *Responses to hypoxia via mTOR. Role in endothelial cell proliferation and HIF-1a stabilization.* (M. Hall, E. Battegay & G. Christofori)

*Luttringer, Olivier: *Physiologically-based pharmacokinetic modeling of active transport processes in liver.* (T.W. Guentert, T. Lavé & U.A. Meyer)

Meier, Karsten Daniel: *Mechanisms of sphingolipid functions during heat stress in Saccharomyces cerevisiae.* (H. Riezman & M. Hall)

Meier, Markus: *Implication of molecular interactions for protein structure, function and design.* (U. Aebi)

Meier, Sebastian: *Novel weak alignment techniques for nuclear magnetic resonance spectroscopy and applications to biomolecular structure determination.* (S. Grzesiek)

Meinen, Sarina: *Artificial restoration of the linkage between laminin and dystroglycan ameliorates the disease progression of MDC1A muscular dystrophy at all stages.* (M.A. Rüegg & T. Meier)

*Meury, Thomas: *Endothelialized tissue engineered 3D-construct for bone repair.* (U. Aebi)

Meyer, Daniel: *Recruitment of AP-1 clathrin adaptors to liposomal membranes.* (M. Spiess & J. Pieters)

Middendrop, Oliver: *Yeast growth selection system for the identification of cell-active inhibitors of beta–secretase.* (H. Riezman & M. Rüegg)

Möglich, Andreas: *Structure and Dynamics of Unfolded Polypeptide Chains.* (T. Kiefhaber)

*Monigatti, Flavio: *Algorithms for the analysis of MALDI peptide mass fingerprint spectra for proteomics.* (H. Langen & T. Schwede)

Morales Johansson, Helena: *Phosphatidylinositol (4,5)-bisphosphate turnover by INP51 regulates the cell wall integrity pathway in saccharomyces cerevisiae.* (M. Hall & H.-P. Hauri)

*Munk, Veronica: *Angiotensin II-induced angiogenesis of the heart in vitro.* (K.G. Hofbauer & E. Battegay)

Nair, Prashant: *Signals involved in protein intracellular sorting.* (M. Spiess, J. Rohrer & J. Pieters)

Neumann, Marc: *Epithelial cell rearrangements during tubular organ formation.* (M. Affolter)

*Neysari, Shiva: *The angiogenic response to Bradykinin in vitro. The role of Bradykinin receptors in hypoxic hearts and tumors.* (E. Battegay & K.G. Hofbauer)

Niederkofler, Vera: *Identification and Functional Characterization of the RGM Family in Mouse.* (S. Arber)

Pokidysheva, Elena: *Structural Study of Hydra Nematocyst Wall Assembly.* (J. Engel)

Sadhu, Anirban: *Role of the neuronal protein Cap23 in the maturation and maintenance of dendritic arbors in-vivo.* (Y.-A. Barde, C. Caroni & I. Mansuy)

Saenz, Henri: *Large-scale identification of pathogenicity factors in Bartonella.* (C. Dehio)

Salie, Rishard: *Mouse RGMs: A Three Protein Family with Diverse Function and Localization.* (S. Arber)

Schätzle, Manuela: *Properties of the Free Energy Barriers for Folding of the α-Amylase Inhibitor Tendamistat.* (T. Kiefhaber)

Schmidt, Karsten: *Analysis of the structure and function of protein phosphatase 2A.* (M. Hall, B. Hemmings & P. Matthias)

*Schomber, Tibor: *Transgenic models to study TGF-b function in hematopoiesis.* (R.Skoda, A. Rolink & U.A. Meyer)

Schreiber, Sylvia Nicole: *The transcriptional coactivator PGC-1 as a modulator of ERR and GR signaling: function in mitochondrial biogenesis.* (M. Hall, N. Kralli & U.A. Meyer)

*Schrenk-Siemens, Katrin: *The role of amyloid precursor proteins: Studies using neurons generated from wild-type and mutant embryonic stem cells.* (Y.A. Barde & M.A. Rüegg)

Scotton, Patrick: *New approaches to identify interactiors of MuSK, a receptor tyrosine kinase required for the formation of the nerve-muscle synapse.* (M.A. Rüegg & T. Meier)

*Stutvoet, Simone Tjitske Dorothea: *Jak-STAT Signaling in Liver Disease and Repair.* (M. Heim, U.A. Meyer & H.P. Hauri)

Tawfilis, Sherif: *Identification and analysis of Clp Protease substrates in C. crescentus.* (U. Jenal)

Troisfontaines, Paul: *The Ysa type IIi secretion system of Yersinia enterocolitica.* (G.R. Cornelis)

Urech, David: *Screening for extracellular protein – protein interactions in a novel yeast growth selection system.* (H. Riezman & M. Spiess)

Um Sung, He: *The role of S6K1 in development and maintenance of nutrient homeostasis.* (M. Hall, G. Thomas & J. Auwerx)

*Urbanowska, Teresa: *Development of a protein microarray platform for the multiplex analysis of biomarkers associated with Rheumatoid arthritis.* (Legay, Mangialaio & U.A. Meyer)

Vellore Palanivelu, Dinesh: *Structural investigation into recombinant eye lens aquaporin (AQPO) and the effector proteins (BEPS) from Bartonella henselae.* (C. Dehio & T. Schirmer)

Wullschleger, Stephan: *Characterzation of TOR complex 2 (TORC2) in Saccharomyces cerevisiae.* (M. Hall & M. Spiess)

Zamurovic, Natasa: *Transcriptional program of osteoblast differentiation; coordinated activation of Notch, Wnt, and TGF- signaling pathways in MC3T3 cell line.* (M. Hall, M. Spring & P. Matthias)

*Zimmermann, Bernhard: *Molecular diagnosis in neonatal medicine.* (W. Holzgreve, S. Hahn, R. Lindberg & U.A. Meyer)

* work performed outside of the Biozentrum

Advisory Board

Prof. Dr. Pascale Cossart
Unité des Interactions Bactéries-Cellules
INSERM U604
Institut Pasteur
28 Rue du Docteur Roux
F-75015 Paris Cedex 15
www.pasteur.fr

Prof. Dr. Gerald R. Fink
Whitehead Institute for Biomedical Research
Nine Cambridge Center
Cambridge, MA 02142-1479 / USA
www.wi.mit.edu

Prof. Dr. Beat Gähwiler
President of the Advisory Board
Institut für Hirnforschung
Universität Zürich
Winterthurerstrasse 190
CH-8057 Zürich
www.hifo.unizh.ch

Prof. Dr. Robert Huber
Max-Planck-Institut für Biochemie
Am Klopferspitz 18a
D-82152 Martinsried
www.biochem.mpg.de

Prof. Dr. Herbert Jäckle
Abteilung Molekulare Entwicklungsbiologie
Max-Planck-Institut für biophysikalische Chemie
Am Fassberg 11
D-3707 Göttingen
www.mpibpc.gwdg.de

Prof. Dr. Dieter Oesterhelt
Max-Planck-Institut für Biochemie
Am Klopferspitz 18a
D-82152 Martinsried
www.biochem.mpg.de

Sir George Radda, Prof. CBE FRS
University Laboratory of Physiology
Cardiac Science Centre
University of Oxford
Parks Road
UK-Oxford OX1 3PT
www.ox.ac.uk

Prof. Dr. Randy W. Schekman
Dept. of Mol. and Cell Biology
Howard Hughes Medical Institute
University of California
401 Barker Hall
Berkeley, CA 94720-3202 / USA
www.hhmi.org

Prof. Dr. Nigel Unwin
MRC Laboratory of Molecular Biology
Neurobiology Division
Hills Road
UK-Cambridge, CB2 2QH
www.mrc-lmb.cam.ac.uk

Focal Area
Cell Growth & Development

Most of the components that make up a cell are now known; a state of knowledge that was unimaginable a decade ago. This explosion in information, due to the recent sequencing of genomes from a wide variety of organisms, has created enormous opportunities for researchers studying the cell. The challenge now is to understand how all these components interact to constitute a functioning cell and ultimately a living organism. Research at the Biozentrum in the area of 'Cell Growth & Development' is dedicated to deepen the understanding of complex cellular systems. This focal area constitutes a large part of the overall research effort at the Biozentrum and is thus a hub for the smaller focal areas 'Neurobiology', 'Infection Biology', and the core program 'Genome Scale Biology & Bioinformatics'. 'Cell Growth & Development' also forms a synergistic alliance with the core program 'Structural Biology and Biophysics' working to understand the interactions within a cell at a quantitative and atomic level. The research programs of the individual groups in the focal area 'Cell Growth and Development' are described below.

Our focal area comprises ten research groups, headed by Profs. Markus Affolter, Thomas A. Bickle, Walter J. Gehring, Michael N. Hall, Hans-Peter Hauri, Urs Jenal, Paul Jenö, Walter Keller, Peter Philipsen and Martin Spiess. Prof. Thomas Bickle retired in 2005 after 33 years at the Biozentrum. We thank Tom for his many years of devotion to the Biozentrum. He has been an outstanding colleague who will be missed for his excellent science, teaching, and administration. Prof. Anne Spang was recruited to the Biozentrum in 2005, and we look forward to her joining us in early 2006. She studies intracellular protein and membrane transport in *S. cerevisiae* and *C. elegans*. To provide a platform for scientific exchange and discussions, a monthly seminar series of the focal area "Growth and Development" was initiated in 2005.

Synergies within the Biozentrum

Within the Biozentrum there are numerous fruitful collaborations between the research groups in the focal area 'Cell Growth and Development' and groups in the other focal areas and core programs. For example, Jenal has joined forces with Schirmer and Grzesiek to obtain structural information on a novel protein domain involved in bacterial cell fate determination and cell signaling. Affolter and Grzesiek collaborate to better understand morphogen signaling at the molecular and structural level, elucidating atomic structures of the relevant molecular complexes. Hall and Jenö are collaborating since

Professors
Markus Affolter, Thomas A. Bickle, Walter Gehring, Michael N. Hall, Hans-Peter Hauri, Urs Jenal, Walter Keller, Peter Philippsen, Anne Spang (starting in 2006), Martin Spiess

Scientists and Postdoctoral Fellows
Yoshitsugu Adachi*, Christian Appenzeller*, Marina Beaufils-Hugot*, Houchaima Ben Tekaya*, Heinz-Georg Belting*, Jorge Blanco, Alexander Böhm, Aude Bourniquel, Lionel Breuza*, Christopher Brink*, Clemens Cabernard*, Hélène Chanut-Delalande*, Adiel Cohen, Pascal Crottet, Sabine Dettwiler*, Bernhard Dichtl*, Wanda Dischert*, Caroline Dossenbach*, Andreas Ebner*, Elin Ellertsdottir*, Michael Friberg, Amy Gladfelter, Sacha Glardon*, Nicole Grieder, Britta Hartmann*, Marie Higy*, Stephen Helliwell*, Dominic Hoepfner*, Estela Jacinto*, Anja Jazwinska-Müller*, Alain Jung, Philipp Knechtle*, Philippe Laissue*, Sophie Lemire Brachat*, Lu Liang*, Robbie Loewith*, Anja Lorberg*, Jacob Malone*, Dietmar Martin, Karsten Meier*, Daniel Meyer*, Samir Merabet, Lydia Michaut, Helena Morales Johansson*, José Mulet-Salort, Marc Neumann*, Oliver Nufer*, Adriana Pagano, Ralf Paul, Frédéric Prince*, Georgios Pyrowolakis, Grégory Resch, Myriam Schaub, Tobias Schmelzle*, Hans-Peter Schmitz*, Alexandre Soulard, Hiroshi Suga, Stepanka Vanacova, Cécile Vedrenne, Andreas Vogel*, Markus Wendeler*, Stefan Wennmalm*, Stephan Wullschleger*

Graduate Students
Sören Abel, Christian Appenzeller*, Houchaima Ben Tekaya*, Claudia Birrer*, Kamila Boudier*, Clemens Cabernard*, Ilias Charlafti, Véronique Charpignon, Beat Christen, Matthias Christen, Nadine Cybulski*, Polychronis Dimitrakis, Caroline Dossenbach*, Anna Dürig, Andreas Ebner*, Monika Garas, Britta Hartmann*, Hans-Peter Helfer, Katrin Hungerbühler, Raphael Fünfschilling*, Maria Jimenez Sanchez*, Andreas Kaufmann, Lukas Keller, Anne Knoth*, Szymon Kobialka, Eva Kögler*, Michael Köhli, Andrea Kyburz, Philippe Laissue*, Assaf Levi, Li Lin*, Sandra Mitrovic, Klaus Molle, Marc Neumann*, Beat Nyfeler, Bertrand Paguet, Tobias Pauli*, Pazit Polak, Riccarda Rischatsch, Salome Röck, Salvatore San Paolo, Sylvia Schreiber*, Ryo Shioda, Hansruedi Stettler, Gregor Suri, Nicoleta Sustreanu*, Robin Vuilleumier, Alexander Weiss*

Focal Area
Cell Growth & Development

a number of years to characterize molecular interactions involved in growth control. Hall also works in close collaboration with the groups of Rüegg and Barde to study the role of TOR signaling in the postmitotic growth of muscle and neuronal cells. Several groups in other focal areas or core programs also pursue research programs which as such contribute to a better understanding of important aspects in the field of 'Cell Growth and Development'.

Synergies outside the Biozentrum

All groups have strong collaborations with experts around the world, both in academia and in industry. The Affolter and Gehring groups are members of a 'Network of Excellence' called 'Cells into Organs', which includes 24 groups in 12 universities across Europe. A summer school organized by this network will be held at the Biozentrum in autumn 2006. The Keller group is part of a European Network of laboratories studying different aspects of RNA biochemistry.

Teaching

All members of the focal area are involved in teaching at the undergraduate and postgraduate level. At the undergraduate level, a main part consists in the organization of different introductory lecture-courses. At the postgraduate level, all scientific topics covered by the research groups are the subject of advanced lecture series. The graduate teaching program of the Biozentrum includes nine topic-oriented cycles with a total of almost 50 lectures and courses. In addition, seminars with invited speakers are organized on a regular basis. A monthly seminar series of the focal area "Growth and Development" has also been started.

Master/Diploma Students
Simon Baeriswyl*, Claudia Birrer*, Yannick Blum*, Daniele Cavicchioli*, Sven Falk*, Mark Finlayson*, Stefan Gander, George Gentsch*, Martha Gerber*, Daria Graziussi*, Regula Halbeisen*, Sina Henrichs*, Sibylle Huber*, Benjamin Hurschler, Roman Lutz, Céline Maeder*, Flora Mauch*, Ann Mertz*, Dominique Meyer*, Borna Müller*, Juliet Odathekal*, Danilo Ritz*, Ivan Schlatter*, Marie Schmidt*, Andreas Stalder*, Nicoleta Sustreanu*, Sherif Tawfilis*, Patrick Tschopp*, Tineke van den Hoorn*, Lorenz Waldmeier*, Stephanie Wagner*

Laboratory Technicians
Paul Baumgartner, Nicole Beuret, Diana Blank, Käthy Bucher, Marc Folcher, Virginie Galati*, Tina Junne Bieri, Fabienne Hamburger, Urs Kloter, Véronique Koerin, Andrea Löschmann, Georges Martin, Ute Nussbaumer, Wolfgang Oppliger, Cristina Prescianotto-Baschong, Makiko Seimiya , Alexandra Schauerte*, Sylvia Voegeli, Verena Widmer

Floor Managers
Markus Hämmerle, Roland Kirchhofer, Marina Kuhn*, Markus Meier, Werner Metzger, Roger Sauder*, Ingrid Thönen*

Trainees
Caroline Kolb*, Tanja Schäuble, Volker Morath, Tomio Takahashi, Wiebke Wlotzka

Laboratory Helpers
Brigitte Berglas, Bernadette Bruno, Gina Evora, Monika Furrer, Angele Klein, Isabelle Lanz-Kalambay, Karin Maria Mauro, Eva Tamburini

Admininistrative Assistants
Elvira Amstutz, Greta Backhaus, Loredana d'Anna*, Liliane Devaja, Claudia Erbel*, Sandra Götz*, Maja Heckel*, Bettina Hersberger*, Liselotte König*, Marianne Liechti, Erica Oesch, Beatrice Thierrin*, Verena Zellweger

*employed during part of the report period

Cell signaling and cell rearrangement during organ morphogenesis

The organization of body pattern in developing multicellular organism is controlled to a large extend by cell-cell signaling. Great efforts have been devoted, in the past decades, to identify and characterize the molecular components of a relatively small number of diverse signaling cascades conserved throughout evolution. We have been studying two important developmental signals (Dpp/BMP and Fgf) with our efforts concentrated first on characterizing the signaling pathways in detail and deciphering their molecular logic, and second on understanding how these recurring pathways control exquisite cellular behavior during development, both in Drosophila and in zebrafish.

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 TGFβsuperfamily and over the past ten years we have characterized the Dpp signaling pathway in detail, in collaboration with the group of Konrad Basler in Zürich. Surprisingly, two nuclear proteins, Schnurri (Shn) and Brinker (Brk), play key roles in Dpp signaling and are responsible for much of Dpp's function as a morphogen. Brk is a DNA-binding transcription factor that counteracts responses to Dpp; Loss of Brk function causes overproliferation and ectopic expression of Dpp target genes, while gain of Brk function causes a general loss of Dpp signaling. We find that throughout development, Dpp causes the graded transcriptional downregulation of the *brk* gene, and it turns out that the inverse gradient of *brk* generated by the Dpp gradient is instructive and essential for organ development.

Since the conversion of an extracellular Dpp gradient into an inverse transcriptional gradient of *brk* is a (the) primary important event in the morphogen readout, we concentrated our efforts in understanding the regulatory steps involved at the molecular level. The dissection of the *brk* regulatory region identified two elements with opposing properties involved in the morphogen readout; a constitutive enhancer and a Dpp morphogen-regulated silencer. Furthermore, we find that the *brk* silencer serves as a direct target for a protein complex consisting of the Smad homologs Mad/Medea (which transduce the Dpp signal to the nucleus) and the zinc finger protein Shn. Together, these results provide the molecular frame-

Markus Affolter

Phone +41 61 267 20 72
Fax +41 61 267 20 78

markus.affolter@unibas.ch
www.biozentrum.unibas.ch/affolter

Group Members

Heinz-Georg Belting	Sibylle Huber
Yannick Blum	Anja Jazwinska
Clemens Cabernard	Alan Jung
Hélène Chanut-Delalande	Li Lin
Caroline Dossenbach	Georgios Pyrowolakis
Andreas Ebner	Samir Merabet
Elìn Ellertsdòttir	Marc Neumann
Sven Falk	Ute Nussbaumer
Britta Hartmann	Andreas Vogel

In Collaboration with

Konrad Basler (University of Zurich, Switzerland), Maria Leptin (University of Köln, Germany), Stephan Grzesiek (University of Basel, Switzerland), Antonio Jacinto (Gulbenkian Institute, Lisbon, Portugal)

Administrative Assistant

Liliane Devaja

Markus Affolter

work for a mechanism by which the extracellular Dpp morphogen establishes a finely tuned, graded read-out of transcriptional repression. Other morphogens, which pattern the nervous system or the limb fields in higher vertebrates, might use similar mechanisms.

Cell rearrangement in organ formation

To gain insight into how signaling pathways control complex cellular decisions during 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 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 of cell migration and cell rearrangement, and the regulation of the latter by extracellular components (Figures 1 and 2).

Studies on the development of blood vessels in higher organisms suggest strong parallels between tracheal development in insects and tube formation in the growing vasculature. 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 indeed find that our approach to study how cells rearrange during tracheal development can provide novel insight into how cells behave during angiogenesis in the zebrafish. We will now reinforce our efforts to study angiogenesis.

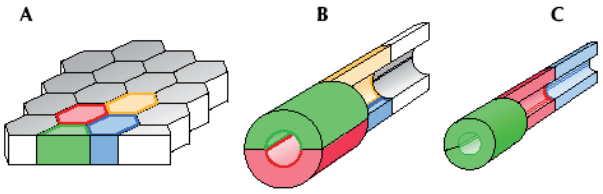

Figure 1. Cellular architecture of epithelial tubes. (A) All tubes of the tracheal system originate from a flat epithelial sheet via branching morphogenesis. (B) Large tubes are formed by several cells contributing to the luminal circumference. All adherence junctions (AJs) are formed between neighboring cells. (C) Fine tubes consist of single cells wrapped around the lumen. The lumen is sealed by autocellular AJs along the axis of the tube. These fine tubes arise from the larger, multicellular tubes via extensive AJ remodelling.

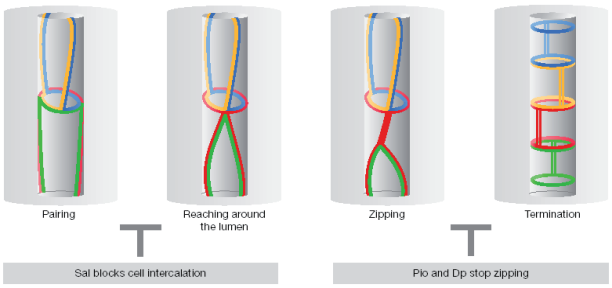

Figure 2: Steps of adherence junction remodelling during cell intercalation and tube elongation. For details, see Neumann and Affolter (2006) EMBO reports 7, 36-40.

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Protein-nucleic acid interactions

During much of the report period we have been winding down the group in preparation for shutting it down at the end of 2005, when T. Bickle retires. We have attempted to finish up as much as possible of the ongoing projects on complex DNA restriction and modification enzymes and the results are summarised below.

On the DNA cleavage mechanism of Type I restriction enzymes

Although the DNA-cleavage mechanism of Type I restriction-modification enzymes has been extensively studied, the mode of how these enzymes introduce DNA double-strand breaks still remains elusive. In this work, DNA ends produced by EcoKI, EcoAI and EcoR124I, members of the Type IA, IB and IC families, respectively, have been characterized by cloning and sequencing of restriction products from reactions with a plasmid DNA substrate containing a single recognition site for each enzyme. We show that all three enzymes cut this DNA randomly with no preference for a particular base composition surrounding the cleavage site, producing both 5'- and 3'-overhangs of varying lengths. EcoAI preferentially generated 3'-overhangs of 2-3 nucleotides, whereas EcoKI and EcoR124I displayed some preference for formation of 5'-overhangs in a length of about 6-7 and 3-5 nucleotides, respectively. A mutant EcoAI endonuclease assembled from wild-type and nuclease-deficient restriction subunits generated a high proportion of nicked circular DNA, whereas the wild-type enzyme catalyzed efficient cleavage of both DNA strands. We conclude that Type I restriction enzymes require two restriction subunits to introduce DNA double-strand breaks, each providing one catalytic center for phosphodiester bond hydrolysis and that cleavage of each strand of the DNA is independent of cleavage of the other.

Type I restriction-modification systems in the gram-positive *Lactobacillus delbrueckii* subsp. *lactis*: genetic organization and biochemical characterization.

We describe the first purification and biochemical characterization of type I restriction-modification (R-M) enzymes from a dairy bacterium and the second from a gram-positive bacterium. Three type I R-M systems were isolated from the two strains of *Lactobacillus delbrueckii* subsp. *lactis*, NCC88 and NCC82. The Type I restriction enzymes Lde88I, Lde88II and Lde82I were overexpressed heterologously in *E. coli* and purified to homogeneity using FPLC chromatography. All three enzymes were shown to be active for

Thomas A. Bickle

thomas-a.bickle@unibas.ch
www.biozentrum.unibas.ch/bickle

Group Members
A. Bourniquel
F. Hamburger
G. Resch

In Collaboration with
M. Szczelkun (University of Bristol, Great Britain), D. Krüger (Humboldt University, Berlin, Germany) and J. Meyer (Dental Institute, University of Basel, Switzerland)

Visiting Scientist
Prof. S. Hadi Aligarh Muslim (University, Faculty of Life Sciences, Aligarh, India)

Thomas A. Bickle

both methylase and endonuclease functions. Lde88I and Lde82I are encoded chromosomally in strains NCC88 and NCC82, respectively. The fully active enzyme, Lde88II, results from combining NCC88 chromosomally encoded HsdR and HsdM subunits with the HsdS subunit encoded on the NCC88 plasmid, pN42. NCC88 genomic DNA was found to be protected against restriction by both the chromosomally encoded enzyme Lde88I and the recombinational enzyme Lde88II, indicating that both enzymes coexist and methylate the host DNA in vivo.

The specific recognition sites of the NCC88 type I R-M systems were determined to be GAC-N5-GTT for Lde88I and GAA-N7-TTYG for Lde88II. The Lde88I site is the first type I enzyme recognition sequence to consist of two specific trimers. The recognition sites of all other naturally occurring type I restriction enzymes have the structure of Lde88II: a specific trimer and tetramer separated by a non-specific spacer of fixed length.

Reverse transcription and cDNA analysis showed that the NCC88 hsdR, hsdM and hsdS genes are expressed constitutively in vivo, as is the plasmid-encoded hsdS gene. Interestingly, all three genomic genes are expressed on a single, huge transcript "hsdR-hsdM-hsdS" of 5.9 kb, leading to the conclusion that regulation of the in vivo concentration of endonuclease with respect to the methylase must rely on the natural RNA instability.

Attempts at classification of theLde88I and Lde82I type I restriction enzymes give clear indications that lateral gene transfer played an essential role in the dissemination of type I restriction-modification systems.

The isolation and biochemical characterization of three type I restriction-modification (R-M) enzymes, Lde88I, Lde88II and Lde82I from strains NCC88 and NCC82 of *Lactobacillus delbrueckii* subsp. *lactis* led us to examine the evolutionary origin of type I R-M systems to determine whether they originate from the *Enterobacteriaceae*, as one might deduce from the literature available up to the mid-1990s. In the process of fitting the lactobacilli type I enzymes within the type I family sub-classification devised for *E.coli* and other enterobacterial type I R-M enzymes, we ascertained that far from being recent acquisitions, the lactobacilli R-M systems are very well adapted to their hosts at both the DNA level (G+C content 44%, typical for lactobacilli) and the enzyme level (high optimal activity temperatures of 60-70°C characteristic of enzymes from the thermophilic *L. delbrueckii*).
Phylogenetic trees were generated using the Phylip software from putative hsdM and hsdR gene sequences extracted from genome projects data collected in the REBASE database. Interestingly, the classical type IA, IB and IC families could be clearly distinguished as well as the more recently defined type ID family. This allowed us to identify six additional type I families that respect the evolutionary distance accepted for the families previously and extensively described. The phylogenetic trees generated in this work also show distinctly that type I R-M systems propagation within the bacterial kingdom has occurred essentially via lateral gene transfer.

A type III restriction enzyme in the oral pathogen, *Actinobacillus actinomycetemcomitans*

These enzymes were discovered by BLAST searches of the *A. actinomycetemcomitans* whole genome sequence with the subunit sequences of the enterobacterial EcoP15I type III enzyme. The genes were then amplified by PCR from the *A. actinomycetemcomitans* genome and cloned in *E. coli*. Unfortunately, the methylase subunit expressed in *E. coli* was insoluble and expression of the restriction subunit was lethal.

A by-product of this project was the discovery of a lytic enzyme coded by a prophage of *A. actinomycetemcomitans.* We have over-expressed and purified the enzyme and shown that it is active in vitro against several Gram negative pathogens. This enzyme may have therapeutic value and it will continue to be investigated in the laboratory of Jürg Meyer (Dental Institute, Basel University).

Thomas A. Bickle

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Bourniquel, A.A., Hamburger, F., Mollet B., & Bickle, T.A. (submitted). Type I restriction-modification systems in the gram-positive *Lactobacillus delbrueckii* subsp. *lactis*: genetic organization and biochemical characterization.

Bourniquel, A.A., Hamburger, F. & Bickle, T.A. (in preparation). Attempts at classification of the *Lactobacillus delbrueckii* subsp. *lactis* Lde88I and Lde82I restriction enzymes give clear indications that lateral gene transfer played an essential role in the dissemination of type I restriction-modification systems.

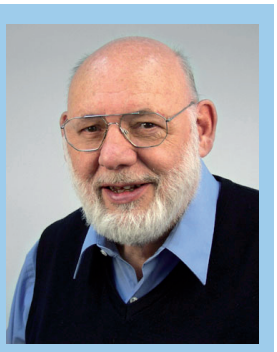
Bourniquel, A.A., Hamburger, F. & Bickle, T.A. (in preparation). Mobile genetic elements IS10 are able to move autonomously either as singletons or as IS10-IS10 tail-to-head duplexes within the genome of *Escherichia coli* strain JM109.

Hox and Pax Genes in Development and Evolution

E.B. Lewis has proposed a model of how homeotic (Hox) genes specify the body plan in insects, which can serve a paradigm for all metazoans since Hox genes are found in all bilaterian animals and homeobox-containing genes can be traced back all the way to coelenterates and sponges. The Lewis model assumes that there is an evolutionary developmental ground state represented in Drosophila by the second thoracic segment (T2) with a pair of legs (middle legs) and a pair of wings. Insects have evolved from crustacean ancestors, which had a pair of legs on each body segment, and dipteran insects arose from more primitive four-winged insects. Hox genes have evolved which remove the legs from the abdominal segments, leaving only three pairs of legs on the thorax, and the Hox gene *Ultrabithorax* converts the second pair of wings on T3 into small halteres (balancers). By deleting these genes, Lewis was able to reconstruct an 8-legged and a 4-winged fly. The Hox genes form a cluster of closely linked genes which are arranged in the same order along the chromosome as they are expressed along the anterior-posterior axis in both insects and vertebrates. Originally, Lewis concentrated on the genes specifying the thorax and the abdomen, and he proposed that there was one gene specifying one body segment. However, it turned out that there are only three Hox genes specifying the thorax and three Hox genes for the abdomen. Therefore, the Lewis model had to be modified to include segment-specific enhancers and most recently segment-specific microRNAs. T. Kaufman extended the Lewis model to the head region, which raised the question of the ground state which was thought to be formed in the absence of any Hox gene expression. However, our more recent studies favour the idea that the second thoracic segment indeed represents the ground state and that it is specified by the homeotic *Antennapedia* (Antp) gene.

Function of the homeotic Antennapedia gene

During the past year we have accomplished a little breakthrough concerning the function of the ***Antennapedia*** (*Antp*) gene, on which I have been working for the last 40 years. Carroll, S.B., Weatherbee, S.D. and Langeland, J.A. (Nature 375: 58-61, 1995) have postulated that the second thoracic segment (T2) represents the developmental ground state and develops without the input of homeotic genes. They also claim that there is no requirement for *Antp* function in Drosophila embryos or imaginal discs for the establishment of the wing primordia and morphogenesis of the wing. Indeed, they found wing disc primordia in $Antp^{-/-}$ embryos. However, they never



Walter J. Gehring

Phone +41 61 267 20 51
Fax +41 61 267 20 78

walter.gehring@unibas.ch
www.biozentrum.unibas.ch/gehring

In collaboration with
S. Artavanis-Tsakonas (Harvard); J. Bièrne and M. Tarpin (Reims); K. Ikeo and T. Gojobori (Mishima); S. Kurata (Sendai); P. Callaerts (Leuven); F. Munier (Lausanne); D. Schorderet (Sion); V. Schmid and H. Reichert (Basel); E. Saló (Barcelona); U. Walldorf (Homburg); K. Furukubo-Tokunaga (Tsukuba); H. Kondoh (Osaka); F. Girard (Montpellier); K. White (Yale)

Administrative Assistant
Greta Backhaus

showed that these wing disc precursors are in fact capable of developing into a wing imaginal disc and finally into a wing. Using genetic mosaics we now have shown that *Antp*$^{-/-}$ cells are **not** capable of developing into a wing disc, nor an adult wing, if surrounded by wildtype cells. Furthermore, ectopic expression of *Antp*$^{+}$ is capable of inducing eye into wing transformations. Therefore *Antp*$^{+}$ specifies the entire T2 segment including the ventral (leg) and the dorsal (wing) imaginal discs, and the notion of the ground state has to be revised.

One definition of the developmental ground state requires that loss-of-function mutations lead to transformations towards the ground state, whereas gain-of-function mutants show transformations in the opposite direction, away from the ground state. Using the nullo-promoter to drive Hox gene expression at the early embryonic preblastoderm and blastoderm stages, when the body plan is laid down, we have shown that ectopic expression (all over the blastoderm) of *Deformed*, a Hox gene specifying the maxillary and mandibulary segments of the head, is capable of inducing maxillary cirri on the thoracic segments (a transformation away from the T2 ground state). Similarly ectopic expression of *Abd B* all over the blastoderm leads to the transformation of all thoracic and abdominal segments into A8/9 abdominal segments, away from the ground state.

Whereas, our earlier studies had shown that the removal of the posterior Hox genes lead to a derepression of *Antp* at the transcriptional level, the ectopic expression of *Abd B* neither affects *Antp* transcription nor the accumulation of the ANTP protein suggesting the *Abd B* and *Antp* interact at the posttranslational level by protein-protein interactions. This interpretation is supported by our recent bimolecular fluorescence complementation experiments.

Our findings of protein-protein interactions between ANTP and BIP 2, a TATA-binding factor associated protein (homologous to TAF 155) have recently been confirmed by co-immunoprecipitation, showing that BIP 2 binds specifically to the YPWM motif of ANTP. This provides a first link between a Hox gene and the transcriptional machinery.

By using fluorescence cross-correlation spectroscopy on single molecules, we have determined the binding constant of the ANTP homeodomain to its DNA binding sites in solution. By some degree of automation we can now search the Drosophila genome for in vitro binding sites and compare the results with chromatin co-immunoprecipitation studies (ChIP on chip) carried out by the group of Kevin White at Yale in collaboration.

Pax 6 and Pax 2 in eye development and evolution

In our earlier studies we have identified Pax 6 as the master control for eye development. By targeted expression of Pax 6 we can induce ectopic eyes on the antennae, legs and wings of the fruit fly.
Using Affymetrix gene chips, we now have analyzed gene expression in eye morphogenesis during larval, pupal and adult stages to decipher the **eye morphogenetic programme**. During larval stages when an eye morphogenetic field is first induced, the majority of the genes which are induced are transcription factors involved in gene regulation. During pupal stages differentiation of the disc cells sets in and a number of differentiation genes, e.g. cuticular genes, are beginning to be expressed. Finally, in the adult eye all the genes involved in phototransduction are expressed. As the eye developmental programme unfolds progressively more “eye” genes are induced, 98 in the late larval eye disc, 409 in the pupae, and 528 in the adult eye. It is interesting to note that some 70% of the genes expressed in the Drosophila retina are also expressed in mouse, indicating that the eye developmental programme of insects and mammals have much in common, which is in line with my hypothesis of a monophyletic origin of the metazoan eye.

If eye development is controlled by *Pax 6* ranging from planarians to human, the genetic circuits, in particular the eye-specific enhancers, should also be conserved. We tested this hypothesis by using a lens specific gene, the δ1-crystallin gene of the chicken in collaboration with Hisato Kondoh’s group. This gene is one of the best characterized crystalline genes. Its lens-specific regulation is controlled by a 25 bp long DC 5 fragment localized in the third intron of the gene. DC 5 contains a Pax 6 and a Sox 2 binding site, and its activity depends on the cooperative binding of these two transcription factors. The Pax binding site is also “recognized” by Pax 2. To test the idea that Pax 6 and Sox 2, together with the DC 5 enhancer, could form a basic regulatory circuit shared by distantly related organisms, we introduced the DC 5 fragment of 25 bp into Drosophila to test its enhancer specificity. The results indicate that DC 5 enhancer is not only active in the Drosophila compound eye, but remarkably in those cells which secrete crystalline into the liquid lens, i.e. the cone cells. However, DC 5 is under the control of Pax 2 (rather than Pax 6) in combination with Sox 2. As

Pax 6 and Pax 2 derive from the same common ancestor, we propose that during evolution Pax 6 function in vertebrate lens development was retained by Pax 2 in Drosophila. It should be noted that also in chickens Pax 2 can bind to DC 5.

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TOR signaling and control of cell growth

Cell growth is highly regulated. Cells respond to nutrients or other appropriate growth stimuli by up regulating macromolecular synthesis, and thereby increasing in size. Conversely, cells respond to nutrient limitation or other types of stress by down regulating 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 same protein - the target of rapamycin TOR protein – and its namesake signaling network. TOR is a highly conserved protein kinase and the target of the immunosuppressive and anti-cancer drug rapamycin. The TOR signaling network controls cell growth by activating an array of anabolic processes including protein synthesis, transcription, and ribosome biogenesis, and by inhibiting catabolic processes such as bulk protein turnover (autophagy) and mRNA degradation, all in response to nutrients. Dysfunction of signaling pathways controlling cell growth results in cells of altered size and, in turn, causes developmental errors and a wide variety of pathological conditions. An understanding of the TOR signaling network may lead to novel drugs for the treatment of, for example, cancer, diabetes,

Michael N. Hall

Phone +41 61 267 21 50/51
Fax +41 61 267 21 48/07 59

m.hall@unibas.ch
www.biozentrum.unibas.ch/hall

Group Members

Adiel Cohen
Nadine Cybulski
Estela Jacinto
Maria Jimenez Sanchez
Veronique Koerin
Robbie Loewith
Anja Lorberg
Andrea Löschmann
Dietmar Martin
Karsten Meier
Klaus Molle
Helena Morales Johansson
José Mulet Salort
Wolfgang Oppliger
Pazit Polak
Tobias Schmelzle
Ryo Shioda
Alexandre Soulard
Stephan Wullschleger

Visiting Scientists

Takashi Ushimaru (University of Shizuoka, Japan)

Independent Subgroup

Stephen Helliwell, Céline Maeder, Ann Mertz, Danilo Ritz

In Collaboration with

Johan Auwerx (IGBMC, France), Yves-Alain Barde (Biozentrum, Basel), Michel Bellis (CRBM, France), Frank Cooke (University College London), Scott D. Emr (University of California, San Diego), Brian Fowler (University Children's Hospital, Basel), Stephan Grzesiek (Biozentrum, Basel), Alan Hall (University College London), Paul Jenö (Biozentrum, Basel), Trevor Lithgow (University of Melbourne, Australia), Yoshinori Ohsumi (National Institute for Basic Biology, Japan), Peter Parker (ICRF, London), Mike Primig (Biozentrum, Basel), Martin Raff (University College London), Markus Rüegg (Biozentrum, Basel), David Sabatini (Whitehead Institute, Cambridge), George Thomas (Friedrich Miescher Institute, Basel), Takashi Ushimaru (University of Shizuoka, Japan), Erik van Nimwegen (Biozentrum, Basel), Rudolf Wüthrich (University Hospital, Zürich).

Administrative Assistants

Maja Heckel, Verena Zellweger

Michael N. Hall

inflammation, muscle atrophy, learning disabilities, depression, obesity, and aging.

We are studying the TOR signaling network in the yeast *Saccharomyces cerevisiae* and in mammalian cells. We have found that the two TORs in yeast, TOR1 and TOR2, mediate numerous signaling pathways that fall into one of two major signaling branches. The two signaling branches integrate the temporal and spatial control of cell growth. The branch that determines when a cell grows (temporal control) utilizes TOR1 or TOR2 and is rapamycin sensitive. The branch that determines where a cell grows (spatial control) contains TOR2, but not TOR1, and is rapamycin insensitive. The rapamycin sensitive 'TOR-shared' branch controls several readouts that collectively determine the mass and thus the size of the cell. These readouts include protein synthesis and degradation, mRNA synthesis and degradation, ribosome biogenesis, nutrient transport, and autophagy. The known effector pathways that make up this branch involve either the type 2A-related phosphatase SIT4 or RAS and Protein Kinase A (PKA). The rapamycin insensitive 'TOR2-unique' branch controls the polarized organization of the actin cytoskeleton. This branch so far contains a single effector pathway consisting of the small GTPase RHO1, Protein Kinase C (PKC1) and a PKC1-regulated MAP Kinase pathway.

The elucidation of two major TOR signaling branches that integrate temporal and spatial control of cell growth was a major step in the understanding of TOR signaling and cell growth control. However, the two signaling branches also raised new and interesting questions. For example, what are the molecular determinants of the specificity and diversity of TOR signaling? Why can TOR2 signal in both branches whereas TOR1 is restricted to only one branch? Furthermore, how does TOR sense and discriminate between different nutrients? To answer these questions, we purified TOR1 and TOR2 in the hope of identifying co-purifying accessory proteins that mediate TOR function. Two functionally and structurally distinct, membrane-bound TOR complexes were identified. TOR complex 1 (TORC1) contains LST8, the uncharacterized protein KOG1 and either TOR1 or TOR2. TOR complex 2 (TORC2) contains TOR2, LST8 and the uncharacterized proteins AVO1, AVO2 and AVO3. As suggested by the fact that TORC1 contains either TOR1 or TOR2 whereas TORC2 contains only TOR2, TORC1 mediates the TOR-shared signaling branch and TORC2 mediates the TOR2-unique signaling branch. We also showed that both TORC1 and TORC2 are conserved in mammals (mTORC1 and mTORC2). mTORC1 consists of mTOR, raptor (also known as mKOG1) and mLST8, the mammalian orthologs of KOG1 and LST8, respectively. mTORC2 consists of rictor (also known as mAVO3) and mLST8. Like its yeast counterpart, mTORC2 is rapamycin insensitive and controls the actin cytoskeleton via a Rho-type GTPase. The two distinct TOR complexes constitute a primordial signaling network conserved in eukaryotic evolution to control the fundamental process of cell growth. Thus, the identification of TORC1 and TORC2 was a significant step in the ongoing characterization of TOR signaling.

The molecular functions of the individual TOR partner proteins are not known, but most of the partners are essential proteins (only AVO2 is not essential) and likely play an important role in determining the specificity and diversity of TOR signaling. In the case of TORC2, we showed that this complex is oligomeric, likely a TORC2-TORC2 dimer. AVO1 and AVO3 bind cooperatively to the N-terminal HEAT repeat region in TOR2, and are required for TORC2 integrity. AVO2 is a nonessential peripheral protein associated with AVO1 and AVO3. LST8 binds separately to the C-terminal kinase domain region in TOR2 and appears to modulate both the integrity and kinase activity of TORC2. TORC2 autophosphorylates sites in AVO1 and AVO3, but TORC2 kinase activity is not required for TORC2 integrity. We also demonstrated that mammalian TOR is oligomeric. The architecture of TORC2 has interesting implications with regard to TORC2 assembly and regulation. Our current and future work focuses largely on further characterizing the two TOR complexes and on elucidating the roles of the TOR partner proteins in yeast and mammals. We hope thereby to determine the molecular mechanisms by which TOR senses and signals nutrient availability in unicellular and multicellular organisms.

The regulation of ribosome biogenesis in response to environmental conditions is a key, but poorly understood, aspect of cell growth control. Ribosomal genes are regulated by the nutrient-sensitive TOR pathway. We described a mechanism by which yeast TOR regulates ribosomal protein (RP) genes via the Forkhead-like transcription factor FHL1. FHL1 has a dual role as an activator and a repressor of transcription that is determined by its direct interactions with the co-activator IFH1 and the co-repressor CRF1. TOR, via PKA, negatively regulates the kinase YAK1 and thereby maintains CRF1 in the cytoplasm. Upon TOR inactivation, activated YAK1 phosphorylates and activates CRF1. Phosphorylated CRF1 accumulates in the nucleus and displaces IFH1 from FHL1

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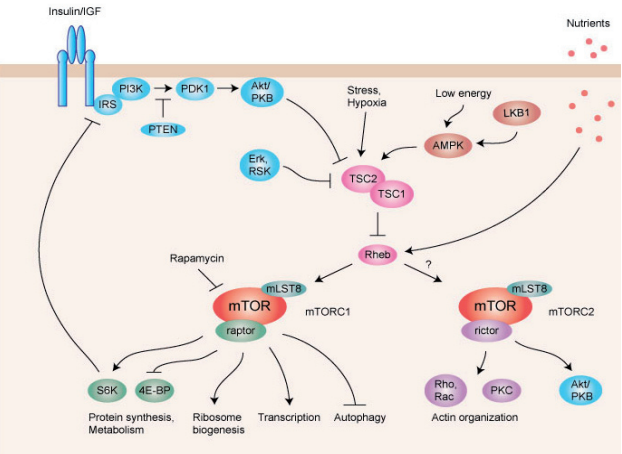
which is constitutively bound to RP gene promoters. Thus, we described a signaling mechanism linking an environmental sensor to ribosome biogenesis. This mechanism involves a novel mode of action for a Forkhead transcription factor.

The above describes only part of our recent findings and current research effort. Many other questions are being actively pursued concerning the role of TOR and the regulation of cell growth. For example, we have recently begun studying the role of TOR in the regulation of organismal metabolism. These ongoing studies have interesting implications when considering hormone- and nutrient-related processes such as appetite regulation and aging. Appetite and lifespan are regulated by hormones produced in response to nutrients. However, the nutrient sensor that signals the production of such hormones is not known and may involve TOR signaling.

TOR signaling pathways in mammalian cells integrate temporal and spatial control of cell growth.



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Protein traffic in the secretory pathway

Understanding the molecular mechanisms underlying the secretory pathway is a major goal of cell biology and biomedical research. In a eukaryotic cell, hundreds of different proteins are synthesized in the endoplasmic reticulum (ER) and subsequently sorted along the secretory pathway or secreted to the outside of the cell. The current and future 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 and how itinerant proteins are separated from resident proteins. We are studying major questions related to these issues in human and other mammalian cells using live cell imaging, biochemical, biophysical, and molecular approaches. Knowledge of these fundamental processes may ultimately lead to new strategies for the treatment of inherited and acquired diseases in which protein secretion is impaired.

Morphogenesis of the ER

The ER is an extended dynamic network of interconnected membrane cisternae and tubules in the cytoplasm. It is currently assumed that microtubules and kinesin motors determine the structure of the ER by active membrane expansion along microtubules. We discovered that the integral membrane protein CLIMP-63 acts as a morphogen by linking the ER to microtubules. Overexpression of wild-type CLIMP-63 changes ER and microtubule morphology in parallel (Fig.1). Overexpression of CLIMP-63 mutants impaired in microtubule binding results in a poorly extended ER without changes of the microtubular cytoskeleton. These results indicate that CLIMP-63-mediated stable anchoring of the ER to microtubules is required to maintain the spatial distribution of the ER. Thus, the concept emerges that ER shape is not only determined by motor driven processes but also by stable attachment of ER membranes to the microtubule cytoskeleton.

Identity and dynamics of the ERGIC

The ER-Golgi intermediate compartment (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 the ER to the Golgi. Are ERGIC clusters mobile transport containers or do they constitute a stationary compartment? To address this important question we established HeLa cell lines expressing GFP-ERGIC-53 and studied its dynamics using live cell imaging. GFP-ERGIC-53

Hans-Peter Hauri

Phone +41 61 267 22 22
Fax +41 61 267 22 08

hans-peter.hauri@unibas.ch
www.biozentrum.unibas.ch/hauri

Group Members

Christian Appenzeller-Herzog
Houchaima Ben Tekaya
Lionel Breuza
Käthy Bucher
Regula Halbeisen
Eva Kögler
Lu Liang
Sandra Mitrovic
Oliver Nufer
Beat Nyfeler
Cécile Vedrenne
Lorenz Waldmeier
Markus Wendeler

In Collaboration with

C. Barlowe and S. Otte (Dartmouth Medical School, Hanover, USA); P. Burkhard (Biozentrum, Basel, Switzerland); G. Cassata (IFOM-FIRC Institute of Molecular Oncology, Milano, Italy); D. Ginsburg and R. Kaufman (University of Michigan, Ann Arbor, USA); W. Hong (Institute of Molecular and Cell Biology, Singapore); P. Jenö (Biozentrum, Basel, Switzerland); K. Kato (Nagoya City University, Japan); D.R. Klopfenstein (Georg August University, Göttingen, Germany); C. Lopez-Otin (University of Oviedo, Spain); S. Michnick (University of Montreal, Canada); J.P. Paccaud (ATHELAS, Geneva, Switzerland); I. Pastan (NIH, Bethesda, USA); R. Pepperkok (EMBL Heidelberg, Germany); A.C. Roche (University of Orléans, France); W. Schamel (University of Freiburg, Germany); P. Seeberger (ETH Zurich, Switzerland); M. Tagaya (University of Pharmacy and Life Science, Tokyo, Japan); M. Zerial (Max-Planck-Institute, Dresden Germany)

Administrative Assistant

Marianne Liechti

Hans-Peter Hauri

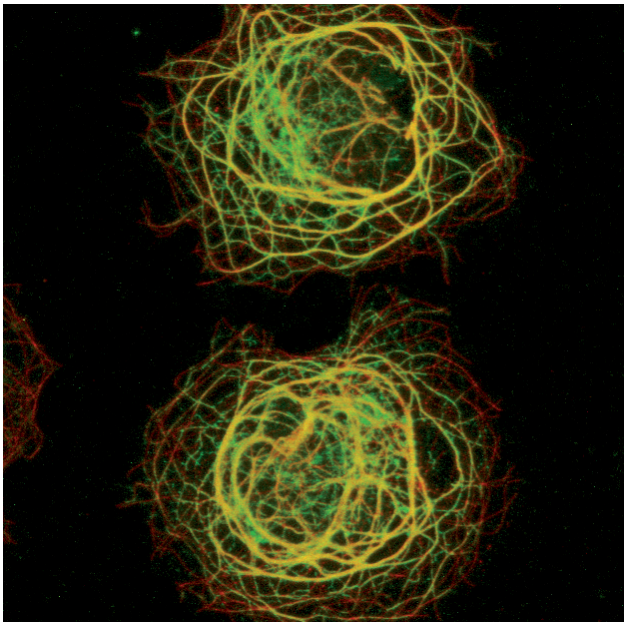

Fig.1: Overexpression of the morphogen CLIMP-63 induces bundling of ER membranes and microtubules to form rope-like structures (yellow).

was found to mainly localize at long-lived stationary and some short-lived highly mobile structures. Unlike the anterograde marker VSV-G-GFP, GFP-ERGIC-53 did not vectorially move to the Golgi upon exit from the ERGIC, as assessed by a novel quantitative vector field method. Dual colour imaging of GFP-ERGIC-53 and secretory dsRed revealed that the stationary structures are sites of repeated sorting of anterograde and retrograde cargo, and are laterally interconnected by highly mobile elements. These results show that ERGIC clusters are stationary membrane entities rather than mobile carriers. Tri-directional (i.e. anterograde, retrograde, and lateral) traffic from the ERGIC is dependent on an intact microtubular network. These results challenge the current most popular view that ERGIC clusters are mobile transport containers. Our working model on membrane traffic to and from the ERGIC is shown in Fig.2. Future efforts will focus on the molecular mechanisms controlling protein sorting in the ERGIC.

Transport signal-mediated protein export from the ER

It has become increasingly clear that 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 interacting with proteins of the COPII coat that shapes transport vesicles forming at ER exit sites. Thus, direct interaction of coat proteins with ER-export signals can actively recruit membrane proteins into transport vesicles. We defined three types of hydrophobic motifs, two of which need to be presented in oligomeric form to operate in ER export, whereas the third one, a C-terminal valine, also operates in monomeric proteins. Current efforts aim at the identification of the COPII coat subunits responsible for ER-export mediated by the different signals. Some soluble secretory proteins also leave the ER in an active way by interacting with a transport receptor which in turn binds to COPII, but the signals required for this cargo-receptor interaction are largely unknown. We found a novel type of signal that is composed of a high-mannose glycan intimately associated with a surface-exposed peptide beta-hairpin loop. The motif accounts for the lectin ERGIC-53-assisted ER-export of the lysosomal enzyme cathepsin Z. A similar oligosaccharide/peptide loop motif is present in cathepsin C. This is the first documentation of an ER-exit signal in soluble cargo in conjunction with its decoding by a transport receptor.

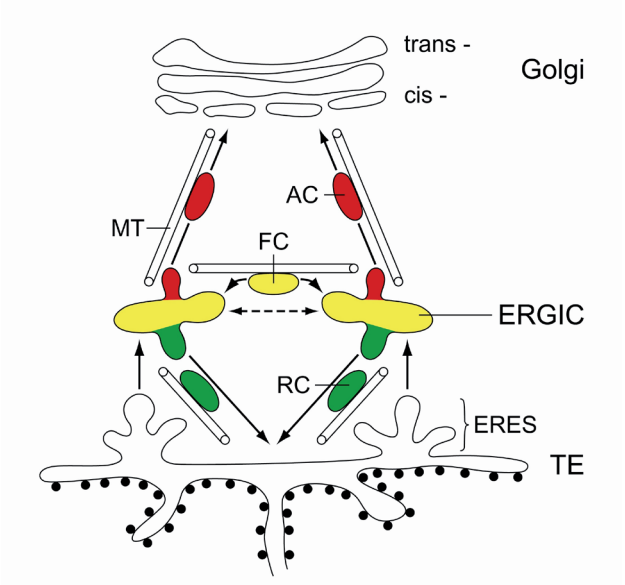

Fig. 2: Model of the organization of the early secretory pathway and sorting of anterograde and retrograde traffic in the ERGIC. AC, anterograde carrier; ERES, ER exit site; FC, fast-moving carrier; MT, microtubule; RC, retrograde carrier; TE, transitional elelment.

Cargo/receptor mechanism of ERGIC-53

ERGIC-53 is a hexameric membrane protein with lectin activity that operates as a cargo receptor mediating efficient transport of some glycoproteins including cathepsin Z and the blood coagulation factors V and XIII. Due to these features ERGIC-53 is an excellent model protein to study cargo receptor-

Hans-Peter Hauri

mediated glycoprotein transport early in the secretory pathway. Optimal binding of newly-synthesized glycoprotein cargo to ERGIC-53 in the ER requires glucose-trimming and high calcium. The precise carbohydrate specificity of ERGIC-53 is currently being analyzed by affinity chromatography and carbohydrate chip technology in collaboration with K. Kato and P. Seeberger. In vitro studies revealed efficient binding of ERGIC-53 to immobilized mannose at neutral but not slightly acidic pH. A conserved histidine in the centre of the carbohydrate recognition domain was identified as a molecular pH sensor. Cell acidification inhibited the association of the cargo protein cathepsin Z to ERGIC-53 and neutralization impaired cargo dissociation. The combined data suggest key roles of pH and calcium in the cargo/receptor mechanism of ERGIC-53. To test this hypothesis we need to establish the pH and the calcium concentration of the ERGIC, both of which are currently unknown.

Capturing protein interactions in the secretory pathway of living cells

Numerous transient 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. YFP fragments were fused to ERGIC-53, the ERGIC-53-interacting multi-coagulation factor deficiency protein MCFD2, and to ERGIC-53's cargo glycoprotein cathepsin Z. YFP PCA analysis revealed the oligomerization of ERGIC-53 and its interaction with MCFD2, as well as its lectin-mediated interaction with cathepsin Z. The YFP PCA analysis also discovered a carbohydrate-mediated interaction between ERGIC-53 and cathepsin C. We conclude that YFP PCA can detect weak and transient protein interactions in the secretory pathway and hence is a powerful approach to study luminal processes involved in protein secretion, including carbohydrate-mediated protein-protein interactions of low affinity.

In search of ERGIC's functions

Protein sorting is a major function of the ERGIC. An additional function of the ERGIC is to mediate initial concentration of abundant secretory cargo that leaves the ER by a non-selective process. By an organelle proteomic approach we found some ER-chaperones associated with the ERGIC suggesting that the ERGIC may contribute to protein quality control. The proteome approach also identified new proteins that are currently investigated and may shed new light on the functions of the ERGIC.

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Temporal and Spatial Control During the Bacterial Cell Cycle and Development

Our main objective is to understand temporal and spatial control mechanisms of the bacterial cell cycle and development on a molecular scale. We study cell dynamics in *Caulobacter crescentus*, an aquatic bacterium with a simple life cycle, which includes an asymmetric cell division and an obligate cell differentiation. During *Caulobacter* cell proliferation, a motile planktonic cell differentiates into a sessile, surface adherent cell type. This transition includes the continuous remodeling of the cell poles with different extra-cellular organelles being assembled and removed at the correct time and place. A single flagellum is assembled at the pole opposite the stalk preceding cell division. After a defined period the flagellum is replaced by the successive formation of adhesive organelles at the same cell pole, including pili, holdfast, and a stalk. Our work concentrates on the regulatory principles and pathways involved in *C. crescentus* pole development. In the past two years we have discovered that in this organism pole development is regulated by a novel bacterial signal transduction mechanism, which relies on the controlled synthesis and breakdown of the second messenger cyclic di-guanosine monophosphate (c-di-GMP). This and other studies have proposed that c-di-GMP is a general modulator of the transition from a motile, single cell state to a sessile, multicellular form of growth, which is often associated with biofilm formation and persistence in pathogenic bacteria. Our work has pioneered some of the emerging concepts of c-di-GMP signaling, including catalytic, structural, and regulatory aspects of the “make and break” of the second messenger (Fig. 1). This was made possible by a successful collaboration with the groups of B. Giese (Chemistry Department) and T. Schirmer (Structural Biology, Biozentrum). Ongoing work concentrates on the identification and characterization of additional components of the c-di-GMP signaling pathway in *C. crescentus*, on their temporal and spatial control, and on the elucidation of general regulatory principles of c-di-GMP signaling in bacteria.

Temporal and spatial control of pole development by coupled activation and polar sequestration of a diguanylate cyclase (Paul *et al*. 2004; Levi & Jenal 2006)

Pole development is coordinated with the *C. crescentus* cell cycle by two-component signaling proteins. The response regulator PleD is required for the correct timing of holdfast formation, stalk biogenesis, and flagellar ejection during cell differentiation. PleD is composed of two tandem CheY-like receiver domains and a GGDEF output domain of unknown

Urs Jenal

Phone +41 61 267 21 35
Fax +41 61 267 21 18

urs.jenal@unibas.ch
www.biozentrum.unibas.ch/jenal

Group Members

Sören Abel
Alexander Böhm
Beat Christen
Matthias Christen
Wanda Dischert
Anna Dürig
Mark Folcher
Martha Gerber
Assaf Levi
Jacob Malone
Flora Mauch
Dominique Meyer
Ralf Paul
Thierry Schaffner
Alexandra Schauerte
Sherif Tawfilis
Salvatore San Paolo

In Collaboration with

Tilman Schirmer (Biozentrum), Stephan Grzesiek (Biozentrum), J. Vohradsky (Prague, Czech Republic), H. Wennemers (University of Basel), B. Giese (University of Basel), M. Meuwly (University of Basel), B. Philipp (Universität Konstanz), M. Ackermann (ETH Zürich), D. Haas (Université Lausanne), B. Kazmierczak (Yale University, USA), K. Hellingwerf (University of Amsterdam, NL), S.I. Aizawa (Hiroshima University, Japan), W. Keck (Actelion, Basel)

Administrative Assistants

Loredana d’Anna, Claudia Erbel

Urs Jenal

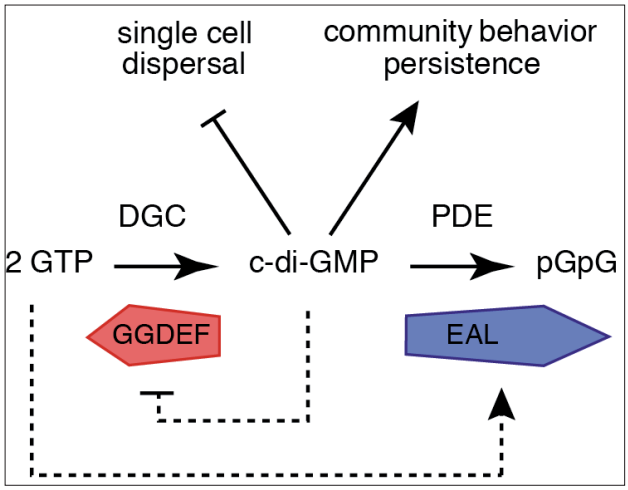

Fig. 1: Schematic of the "make and break" of cyclic di-guanosine monophosphate. DGC (diguanylate cyclase), PDE (phosphodiesterase); stippled lines indicate allosteric regulation of DGC and PDE activities.

function. Biochemical analysis of PleD revealed that the GGDEF domain is a diguanylate cyclase (DGC), which catalyzes the synthesis of c-di-GMP from two molecules of GTP (Fig. 1). This finding for the first time assigned a biochemical activity to this highly abundant family of bacterial signaling domains and proposed a global role for the second messenger c-di-GMP in bacterial signal transduction. In the meantime this has been substantiated by accumulating evidence in favor of a general role of c-di-GMP in bacterial persistence. PleD is activated through phosphorylation by DivJ and PleC, two polar sensor histidine kinases involved in *C. crescentus* cell differentiation. PleD activation is coupled to its sequestration to the differentiating cell pole (Fig. 2). Dynamic localization of PleD to the cell pole provides a mechanism to temporally and spatially control the signaling output of PleD during development. Preliminary evidence suggests that phosphorylation-mediated dimerization provides the signal for PleD sequestration. This has led to the proposal that localized activity of this novel-type signaling protein might constitute a general regulatory principle in bacterial growth and development. Ongoing studies are geared towards identifying c-di-GMP effector protein(s) of the PleD signal transduction pathway and to analyzing the role of PleD as a "localized pacemaker".

Structure function analysis of the PleD diguanylate cyclase (Chan *et al.* 2004)

In collaboration with the groups of T. Schirmer and B. Giese we have determined the crystal structure of PleD. While the receiver domains show the expected CheY-like fold, the novel C-terminal DGC domain of PleD is similar to the adenylate cyclase and palm domain of DNA polymerases. The structure allowed making specific predictions concerning activation of PleD by phosphorylation and dimerization, the catalytic mechanism, and allosteric control of the PleD diguanylate cyclase. Based on these predictions, we have shown that phosphorylation leads to PleD di-

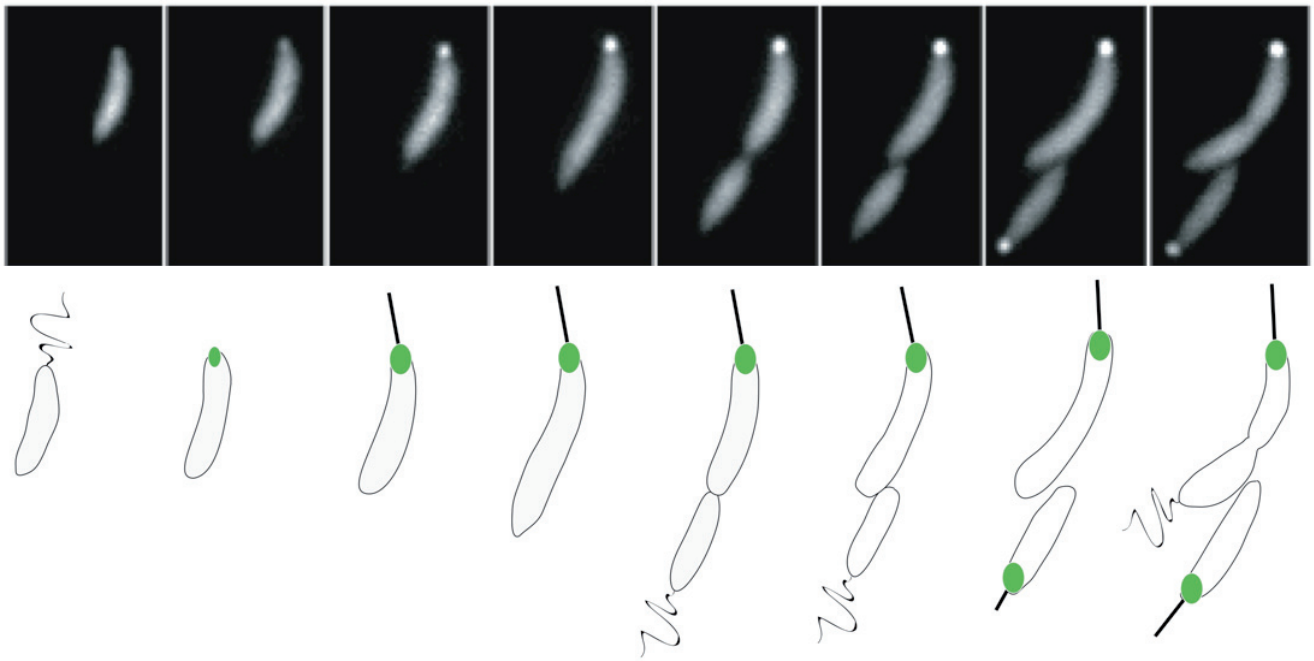

Fig. 2: Dynamic localization of the activated PleD response regulator during the C. crescentus life cycle (top: fluorescence time lapse; bottom: schematic)

merization and that the dimer constitutes the active enzymatic form. This is the first report demonstrating that bacterial DGCs function as dimers. Mutational analysis also confirmed the dimerization interphase predicted by the crystal structure. Similarly, mutational analysis was used to map key catalytic residues of the active site (A-site) of the PleD DGC domain.

The PleD structure revealed an unexpected second c-di-GMP binding site (I-site) located at the interphase between the DGC and second receiver domain. This and the observation that PleD activity is subject to strong non-competitive product inhibition (Ki~1µM) indicated a tight allosteric control mechanism (Fig. 1). Mutational analysis in combination with c-di-GMP binding studies and kinetic assays confirmed that the I-site constitutes a bona fide allosteric binding pocket. In the meantime we could demonstrate that negative allosteric control constitutes a general regulatory principle common to most DGC proteins in bacteria. We propose that this additional level of control allows bacteria to tightly control their cellular c-di-GMP levels and to couple the regulation of c-di-GMP synthesis to its controlled degradation by phosphodiesterases.

GTP controls a c-di-GMP specific phosphodiesterase (Christen *et al.* 2005)

It has been hypothesized that c-di-GMP specific phosphodiesterase activity resides in the highly abundant but functionally poorly characterized EAL domain (Fig. 1). We could show biochemically that CC3396, a *C. crescentus* GGDEF-EAL composite protein is a soluble PDE responsible for about 70% of the cytoplasmic PDE activity. The PDE activity, which rapidly converts c-di-GMP into the linear dinucleotide pGpG (Fig. 1), is confined to the C-terminal EAL domain of CC3396, depends on the presence of Mg^{2+} ions, and is strongly inhibited by Ca^{2+} ions. Remarkably, the associated GGDEF domain, which contains an altered active site motif (GEDEF), lacks detectable DGC activity. Instead, this domain is able to bind GTP and, in response, activates the PDE activity in the neighboring EAL domain. PDE activation is specific for GTP (K_D 4 µM) and operates by lowering the K_M for c-di-GMP of the EAL domain to a physiologically significant level (420 nM). Mutational analysis suggested that the substrate-binding site (A-site) of the GGDEF domain is involved in the GTP-dependent regulatory function, arguing that a catalytically inactive GGDEF domain has retained the ability to bind GTP, and in response, can activate the neighboring EAL domain. Based on these results we propose that the c-di-GMP-specific PDE activity is confined to EAL domains, that GGDEF domains can either catalyze the formation of c-di-GMP or can serve as regulatory domains, and that c-di-GMP-specific phosphodiesterase activity is coupled to the cellular GTP level in bacteria. To improve our understanding of the catalytic and regulatory mechanisms of bacterial PDEs, we are currently collaborating with the group of T. Schirmer to obtain a crystal structure of CC3396.

Cell cycle-dependent degradation of the FliF anchor protein (Grünenfelder et al. 2004; Kanbe *et al.* 2005)

Flagellar ejection is tightly coupled to the cell cycle in *C. crescentus* and coincides with the specific degradation of several structural motor proteins. In collaboration with the group of S.I. Aizawa *C. crescentus* hook basal body (HBB) structures were purified and characterized. Purified HBBs were less stable against acidic pH or protease treatment than HBBs of *Salmonella typhimurium*, supporting the view that flagellar ejection from *C. crescentus* is initiated by destruction of the fragile basal structures. The MS ring protein FliF, which anchors the flagellar structure in the inner membrane, is degraded coincident with flagellar release. We have identified ClpAP as the protease being responsible for cell cycle-dependent degradation of FliF. A C-terminal degradation tag mediates protease recognition of FliF. The analysis of the nature of the FliF degradation signal has indicated that both number and positioning of hydrophobic amino acids at the FliF C-terminus are critical for its degradation. These results contribute substantially to our understanding of how proteases specifically recognize and select proteins tagged for destruction. What remains to be elucidated are the regulatory mechanisms, which are responsible for the correct timing of proteolysis during the cell cycle.

Urs Jenal

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Processing and editing of eukaryotic messenger RNA precursors and of transfer RNAs

The processing reactions capping, splicing, 3' end cleavage/polyadenylation and RNA editing are important steps in the generation of nuclear messenger RNAs in eukaryotic cells. Differential splicing and RNA editing give rise to alternate protein products that can have drastically different functional properties. The cap at the 5' end and the poly (A) tails at the 3' end of mRNAs have multiple functions, such as increasing the efficiency of translation, mRNA export and the control of mRNA turnover. Moreover, the different processing steps are coupled to each other and to transcription by RNA polymerase II. Elucidation of the functional and structural properties of the factors involved in these reactions is the main focus of our research.

Mammalian pre-mRNA 3' end processing factors

3' end formation is an essential step in the maturation of almost all primary RNA polymerase II transcripts that encode proteins. The 3' end formation reaction includes endonucleolytic cleavage of the pre-mRNA, followed by polyadenylation of the upstream cleavage product. The addition of a poly(A) tail occurs cotranscriptionally and is dependent on functional interactions between the 3' end formation machinery and RNA polymerase II. Six trans-acting factors are sufficient to catalyze mammalian 3' end formation *in vitro*: cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I and II (CF I, CF II), poly(A) polymerase (PAP), and nuclear poly(A) binding protein (PABPN1; previously called PABII or PABP2). The functions of the large number of polypeptides constituting these factors include activities that are directly involved in the cleavage and polyadenylation steps and also in the coupling of this reaction to transcription and to other RNA processing reactions such as capping and splicing.

We have found previously that the cleavage and polyadenylation specificity factor CPSF interacts with the U2 snRNP. Furthermore, we showed that depletion of CPSF resulted in deficient 3' end cleavage as well as in reduced splicing. Both activities could be reconstituted with partially purified CPSF. Mutations in the U2 snRNP binding site resulted in reduction of the splicing and 3' end cleavage activities. To test whether the U2 snRNP itself is involved in 3' end processing, we digested the U2 snRNA of nuclear extract with RNase H and complementary oligonucleotides. Such treated extracts were not active in splicing and showed a clear reduction of 3' end cleavage activity.

Walter Keller

Phone +41 61 267 20 60
Fax +41 61 267 20 79

walter.keller@unibas.ch
www.biozentrum.unibas.ch/keller

Group Members

Diana Blank
Sabine Dettwiler
Bernhard Dichtl
Monika Garas
Isabelle Kaufmann
Anne Knoth
Andrea Kyburz
Georges Martin
Borna Müller
Bertrand Paguet
Salome Röck
Myriam Schaub
Marie-Joëlle Schmidt
Stepanka Vanacova
Stephanie Wagner
Verena Widmer
Wiebke Wlotzka

In Collaboration with

S. Barabino (University of Milano); S. Doublié (University of Vermont, Burlington); A. Gerber, (ETH Zürich); G. Keith (University of Strasbourg); H. Langen (Hoffmann La Roche AG, Basel); M. Mörl (Max-Planck-Institute for Evolutionary Anthropology, Leipzig); R. Stefl (ETH Zürich); C. Trotta (PTC Therapeutics, South Plainfield, New Jersey); E. Wahle (University of Halle-Wittenberg)

Administrative Assistant

Erica Oesch

Walter Keller

Cleavage factor I (CF I) consists of four polypeptides of 25, 59, 68_m and 72_m kDa. The 59 and 68 kDa proteins are similar in sequence and share some characteristic features. The 72 kDa polypeptide is a splice variant of CF I_m68 and also the 59 kDa protein consists of two alternative splice variants. All the large CF I_m subunits have a modular domain organization consisting of an N-terminal RNA-recognition motif (RRM) and a C-terminal alternating charge domain. The RRMs of CF I_m68 and CF I_m59 are not sufficient for RNA binding but are necessary for the association with the 25 kDa subunit. Efficient RNA binding requires a heterodimeric complex of either CF I_m68 or CF I_m59 with the 25 kDa protein and is mediated by the C-terminal alternating charged domain of the larger CF I_m subunits. Whereas multiple protein interactions with other 3' end processing factors are detected with CF I_m25, CF I_m68 in contrast to CF I_m59 interacts with members of the SR family of splicing factors via its C-terminal alternating charge domain. In collaboration with Dr. Silvia Barabino (University of Milano) we have found that this domain is also required for targeting CF I_m68 to the nucleus. However, CF I_m68 does not concentrate in splicing speckles but in foci that partially co localize with paraspeckles, a sub nuclear component in which other proteins involved in transcriptional control and RNA processing have been found.

In collaboration with Dr. Sylvie Doublié (University of Vermont, Burlington), we have examined a new crystal structure of bovine poly(A) polymerase (PAP). Although the structures of mammalian and yeast PAPs are known, their mechanism of ATP selection has remained elusive. In a recent bovine PAP structure complexed with an analog of ATP and $MnCl_2$, strictly conserved residues interact selectively with the adenine base, but the nucleotide was found in a "non-productive" conformation. In the new structure, obtained in the presence of $MgCl_2$, 3'-dATP adopts a "productive" conformation similar to that seen in yeast PAP or DNA polymerase ß. Mutational analysis and activity assays with ATP analogs suggest a role in catalysis for one of the two adenine-binding sites. The other site might function to prevent futile hydrolysis of ATP. Steady state kinetics experiments under distributive polymerization conditions suggest a sequential random mechanism *in vitro,* in the presence of ATP and RNA, without preference for a particular order of binding of the two substrates.

tRNA CCA transferases, tRNA maturing enzymes found in all organisms, and eubacterial poly(A) polymerases are so similar that until recently their biochemical functions could not be distinguished by their amino acid sequence. BLAST searches and analysis with the program "Sequence Space" for the prediction of functional residues revealed sequence motifs which define these two protein families. One of the poly(A) polymerase defining motifs specifies a structure that we propose to function in binding the 3' terminus of the RNA substrate. Similar motifs are found in other homopolyribonucleotidyl transferases. Phylogenetic classification of nucleotidyl transferases from sequenced genomes reveals that eubacterial poly(A) polymerases have evolved relatively recently and are found only in a small group of bacteria, and surprisingly also in plants, where they may function in organelles. In collaboration with Dr. Mario Mörl (Max-Planck-Institute for Evolutionary Anthropology, Leipzig) domains responsible for substrate specificity of bacterial poly(A) polymerases and tRNA CCA transferase were identified. We showed by domain substitution experiments that exchange of N- and C-terminal regions leads to chimeric enzymes with unexpected activities, indicating that tRNA nucleotidyltransferase carries an "anchor domain" in the C-terminal section that restricts polymerization to three nucleotides. A 27 amino acid region was identified that determines whether poly(A) or CCA is synthesized by the enzyme chimeras. Sequence alignments suggest that the catalytic cores of both enzymes carry identical components involved in nucleotide recognition and incorporation. This seems to be the prerequisite for the observed reprogramming of the catalytic center of PAP to incorporate a sequence of defined length and composition instead of long stretches of A residues.

3' end processing of mRNA precursors in yeast

Biochemical fractionation of yeast extract has shown that 3' end processing of yeast pre-mRNAs requires four multisubunit factors which can be separated by column chromatography. Cleavage only occurs in the presence of cleavage factors I and II (CF I and CF II), whereas specific polyadenylation of "precleaved" RNA substrates requires CF I, polyadenylation factor I (PF I) and poly(A) polymerase. We had previously shown that CF II contains a subset of polypeptides that are also present in PF I and that a large complex of PF I together with poly(A) polymerase could be isolated by affinity chromatography. This complex combines CF II/PF I and poly(A) polymerase activities and is now called cleavage and polyadenylation factor (CPF). Thus, the complete 3' end processing reaction can be reconstituted in vitro by combining CF I and CPF. Active CF IA and CPF can also be prepared by tagging one of their subunits and subsequent affinity purification.

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We have previously identified six new polypeptides in affinity-purified CPF fractions. The new proteins were a WD-40 protein (Swd2p), Pti1p, protein phosphatase 1 (Glc7p), Ssu72p and the non-essential Ref2p and Cpf11p proteins. We have characterized some of these new proteins genetically and biochemically in recent years. We will also continue with the analysis of the other subunits of the CPF complex.

The *Saccharomyces cerevisiae* WD-40 repeat protein Swd2p is found as a component of two functionally distinct multiprotein complexes: the cleavage and polyadenylation factor (CPF) that is involved in pre-mRNA and snoRNA 3' end formation and the SET1 complex (SET1C) that methylates histone 3 lysine 4. Based on bioinformatic analysis we predict a seven-bladed ß-propeller structure for Swd2p proteins. Northern, transcriptional run-on and in vitro 3' end cleavage analyses suggest that temperature sensitive *swd2* strains were defective in 3' end formation of specific mRNAs and snoRNAs. Protein–protein interaction studies support a role for Swd2p in the assembly of 3' end formation complexes. Furthermore, histone 3 lysine 4 di- and tri-methylation were adversely affected and telomeres were shortened in *swd2* mutants. Underaccumulation of the Set1p methyltransferase accounts for the observed loss of SET1C activity and suggests a requirement for Swd2p for the stability or assembly of this complex. We also provide evidence that the roles of Swd2p as component of CPF and SET1C are functionally independent. Taken together, our results establish a dual requirement for Swd2p in 3' end formation and histone tail modification.

A new poly(A) polymerase complex from S. cerevisiae

The most notable development in our recent research has been the characterization of a new type of protein complex with poly(A) polymerase activity from *Saccharomyces cerevisiae* that targets RNAs destined for degradation and also functions as an activator of the nuclear exosome. Eukaryotic cells contain several unconventional poly(A) polymerases in addition to the canonical enzymes responsible for the synthesis of poly(A) tails of nuclear messenger RNA precursors. The yeast protein Trf4p has been implicated in a quality control pathway that leads to the polyadenylation and subsequent exosome-mediated degradation of hypomethylated initiator $tRNA^{Met}$ ($tRNA_i^{Met}$; Kadaba et al., Genes Dev. 18: 1227-1240, 2004). We have found that Trf4p is the catalytic subunit of a new polyadenylation complex that contains Air1p or Air2p as potential RNA-binding subunits, as well as the putative RNA helicase Mtr4p. Comparison of native $tRNA_i^{Met}$ with its *in vitro* transcribed unmodified counterpart as substrates revealed that the unmodified RNA was preferentially polyadenylated by affinity-purified Trf4 complex from yeast, as well as by complexes reconstituted from recombinant components. These results and additional experiments with other tRNA substrates suggested that the Trf4 complex can discriminate between native tRNAs and molecules that are incorrectly folded. Moreover, the polyadenylation activity of the Trf4 complex stimulated the degradation of unmodified $tRNA_i^{Met}$ by nuclear exosome fractions *in vitro.* Degradation was most efficient when coupled to the polyadenylation activity of the Trf4 complex, indicating that the poly(A) tails serve as signals for the recruitment of the exosome. This polyadenylation-mediated RNA surveillance resembles the role of polyadenylation in bacterial RNA turnover.

RNA-specific adenosine deaminases and RNA editing

RNA-specific adenosine deaminases which convert adenosine to inosine residues are divided into two families: Adenosine deaminases acting on RNA (ADARs) act on double-stranded RNA and edit pre-mRNAs, whereas ADATs convert adenosine to inosine in tRNAs. Previously, we have characterized mammalian ADAR1 and ADAR2, as well as ADAT1/Tad1p from yeast, which forms inosine at position 37 (3' of the anticodon) in eukaryotic tRNA-Ala. We have also identified a yeast adenosine deaminase that generates inosine at the wobble position of tRNAs (position 34). This enzyme consists of two sequence-related subunits, ADAT2/Tad2p and ADAT3/Tad3p, which form a heterodimer. Comparison of the conserved active site domains of adenosine and cytidine deaminases revealed that ADAT2 may represent the evolutionary precursor of ADAT1 and ADARs. We are still in the process to identify, in the ADATs and in their RNA substrates, the features that are important for enzymatic activity, the capacity to bind specifically to different tRNAs and for the formation of the Tad2p/Tad3p heterodimer.

Walter Keller

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Networks controlling polar growth, nuclear division and peroxisome biogenesis in fungi

We investigate differences and similarities in cellular networks of two eukaryotic microorganisms, the unicellular fungus *Saccharomyces cerevisiae* and the filamentous fungus *Ashbya gossypii*. Despite their differences in life style and growth habitat, both organisms have 95 % of genes in common because they evolved from the same ancestor. Two key cellular functions, polar growth and timing of nuclear divisions, are controlled in both organisms by very similar sets of genes. The majority of these genes are present at syntenic positions confirming their evolution from common ancestral genes. During evolution, the individual role of many of these genes and the network interaction as a whole was modified to produce specific differences in growth and nuclear division observed today. Growth of *A. gossypii* is restricted to the tips thus forming long hyphae. The surface expansion rate is 20 to 40 times higher than in *S. cerevisiae* which displays alternating phases of polar and isotropic growth. We hypothesize that components controlling the actin cytoskeleton have evolved differently in both organisms. Mitotic divisions in multinucleated hyphae of *A. gossypii* are expected to occur synchronously based on the known controls of nuclear divisions in *S. cerevisiae*. However, nuclei in *A. gossypii* divide asynchronously despite the common cytoplasm. We hypothesize that novel modes of regulation of conserved cell cycle proteins direct this nuclear autonomous division cycle. Also cellular components other than nuclei are challenged by the different growth patterns of our two model organisms. Therefore, we additionally focus on the *de novo* synthesis and inheritance of peroxisomes; small single membrane-bound organelles involved in fatty acid metabolism. We have already identified components for slow cytoskeletal-based transport of peroxisomes in yeast and we are currently investigating how the same components could account for the rapid dynamics observed in *A. gossypii*.

Genomics as information basis

With the help of Syngenta, we sequenced the genome of the filamentous fungus *A. gossypii*. The annotated 4718 ORFs show extensive gene order conservation (synteny) with the *S. cerevisiae* genome. The complete synteny map allowed to reconstruct the ancient *S. cerevisiae* genome at the time of its duplication. The data are a rich source for novel approaches to analyze the components of fungal growth and nuclear division including evolution of promoters. The data also allowed important reannotations of the *S. cerevisiae* genome.

Peter Philippsen

Tel. +41 61 267 14 80
Fax +41 61 267 14 81

peter.philippsen@unibas.ch
www.biozentrum.unibas.ch/philippsen

Group Members

Claudia Birrer
Kamila Boudier
Daniele Cavicchioli
Mark Finlayson
Virginie Galati
Amy Gladfelter
Hans-Peter Helfer
Dominic Hoepfner
Katrin Hungerbühler
Andreas Kaufmann
Philipp Knechtle
Michael Köhli
Philippe Laissue
Sophie Lemire Brachat
Juliet Odathekal
Riccarda Rischatsch
Ivan Schlatter
Hans-Peter Schmitz
Nicoleta Sustreanu
Tineke van den Hoorn
Sylvia Voegeli

In Collaboration with

H. Tabak (University of Utrecht); F. Dietrich (Duke University); M. Primig (Biozentrum Basel); H.-P. Schmitz (University of Osnabrück); Y. Barral (ETH Zürich)

Administrative Assistants

Sandra Götz, Bettina Hersberger

Peter Philippsen

Nuclear division in multinucleated cells

Multinucleated cells are encountered in a variety of organisms and are integral to processes as diverse as the early development of the fruit fly, bone remodeling, and cancer metastasis. One molecular explanation for synchronous mitoses in multinucleated cells is that cyclin proteins and cyclin dependent kinases are shuttling between the nucleus and the cytoplasm. This enables the core cell cycle machinery to diffuse freely and influence multiple nuclei in the same cytoplasm. Thus, in multinucleated eukaryotic cells studied to date, continual communication between nuclei and the cytoplasm coordinates synchronous nuclear division. We have begun studying a novel asynchronous nuclear division system in the multinucleated fungus *A. gossypii*. Neighbouring nuclei in the hyphae divide at different times despite close physical proximity. Utilizing time-lapse video microscopy, we have generated nuclear pedigrees showing that most nuclei in *A. gossypii* cells have the capacity to divide but do so in nearly complete asynchrony. Analysis of spindle morphology suggests that neighboring nuclei exist in different stages of the cell cycle and that asynchrony is independent of cell shape. Additionally, asynchrony is a robust characteristic of the system and is regenerated rapidly upon release from an artificially generated synchronization. We showed that both G1 and mitotic cyclins co-exist in nuclei at all stages, and that Sic1p, the inhibitor of the cyclin-dependent protein kinase (CDK), may act as one oscillator because it is present in G1 to G2 nuclei but not during mitosis. Recently, we identified the *A. gossypii* S-phase cyclin as the only cyclin able to oscillate during the mitotic cycle. In addition, we also found that the *A. gossypii* homologue of Pds1 oscillates. These data represent the first molecular *in vivo* description of asynchronous mitoses in multinucleated cells and suggest that novel means of cell cycle regulation govern the nuclear division cycle in *A. gossypii*.

In search of a link between morphogenesis and nuclear division in *A. gossypii*

How mitosis is spatially regulated in a multinucleated filamentous fungus is largely unknown. We study this problem in the ascomycete *A. gossypii*, which is closely related to *S. cerevisiae* on a genome scale. Yet, *A. gossypii* displays an entirely different pattern of growth, leading to branched filaments whose nuclei multiply by asynchronous mitosis. This further raises the question whether, unlike in budding yeast, hyphal morphogenesis is independent of cell cycle stages in *A. gossypii*. In *S. cerevisiae*, the septins are thought to link bud formation to mitosis by recruiting Hsl1 and Hsl7, which are required for Swe1 inactivation and entry into mitosis. The proper assembly and organization of the septin collar at the neck is essential for the function of these cell cycle regulators. In *A. gossypii*, homologues to all key players of this morphogenesis checkpoint exist. To begin to investigate possible connections between the cell cycle and morphogenesis, we have focused on analyzing the structure and function of septin proteins in *A. gossypii* by using a Sep7-GFP strain. We detected some septin structures that were not described so far. Interestingly, most of the structures in *A. gossypii* seem to be composed of parallel bars, an instance which in yeast is only seen in certain mutants and is associated with higher Swe1 kinase activity. Heterologous expression of AgSep7-GFP in *S. cerevisiae* leads to the formation of normal yeast structures with continuous rings and double rings. This implies that the observed structures are not inherent to the Sep7 protein, but rather the result of different regulation or interaction in the two organisms.

Dynamics of the *A. gossypii* actin cytoskeleton

In *A. gossypii* the actin cytoskeleton is composed of long bundles of filaments (actin cables) and small punctate structures (actin patches) localized to growth sites and required for polar growth. We analyse different components of the actin cytoskeleton which have been labelled with Green Fluorescent Protein (GFP) by means of video microscopy, studying their structural and dynamic properties. Cap1 and Cap2 are the subunits making up capping protein which binds the barbed end of actin filaments and nucleates actin polymerisation *in vitro*. Cap1-GFP and Cap2-GFP co-localize with actin patches in rhodamine-phalloidin stainings. They are concentrated at the tip of hyphae, mostly cortical and in the subapical domain. Velocity measurements show that they are slower than actin patches in *S. cerevisiae* or *S. pombe*. Sequential recordings of the entire hypha show that the net movement of actin patches is backward. Co-stainings with the endocytosis marker FM4-64 show partial colocalization. These findings support the idea that actin patches are involved in endocytosis. Cap1-GFP and Cap2-GFP patches are immobilized by low doses of Latrunculin A, indicating that actin polymerization may be their mode of propulsion. Actin cables were made visible by using a GFP tagged variant of Abp140, an actin binding protein. Abp140-GFP co-localizes with actin cables in rhodamine phalloidin stainings. They are mostly cortical, often helical, and can be as long as 40 µm.

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They are very motile and show undulating movements. This is in contrast to the short, straight actin cables in *S. cerevisiae*, which have been shown to transport exocytic vesicles to the site where a new cell wall is formed. These results suggest that the structural basis and mechanisms for polarized growth are similar in *S. cerevisiae* and *A. gossypii*, yet differ markedly in dynamic behaviour.

Role of formins and RHO proteins for hyphal growth in *A. gossypii*

Formin homology proteins are known nucleators of actin cables in many different organisms. We study AgBni1 a formin homology protein from *A. gossypii*. AgBni1 is essential for hyphal formation and might be involved in tip branching, as shown by deletion mutants and activated alleles. Deletion mutants of AgBni1 have a defect actin cytoskeleton and do not form mature hyphae. In contrast, cells carrying the activated allele show tip-branching prior to emergence of lateral branches, which is never observed in the wildtype. In addition, we identified four different members of the family of small Rho-type GTPases that are capable of binding to the AgBni1 protein and therefore might be regulators of the latter. To further investigate the role of these members of the Rho family we integrated activated alleles into the genome of *A. gossypii*. However no single activated rho-allele was capable to mimic the phenotype of an activated AgBni1 indicating that either several different GTPases or other so far unknown factors are necessary for activation of AgBni1. We have begun to study the role of two additional *A. gossypii* formins, AgBnr1 and AgBnr2 which most likely function at sites of septation. Single deletions are viable but the double deletion is lethal.

***A. gossypii* homologues of *S. cerevisiae BUD* genes**

In *S. cerevisiae*, a series of so-called *BUD* genes has been identified which control establishment of new axis of polarity. The function of the *Ashbya BUD1/RSR1* and *BUD2* homologues were investigated by using time-lapse video microscopy. These movies revealed that *AgBUD2* is important for a stable growth axis of the elongating hyphae. *AgRSR1/BUD1* seems to play a role in the maintenance of hyphal tip growth and in the elongation of initiated hyphal branches. *Ashbya* hyphae lacking this gene frequently paused growth at the tip and showed many unsuccessful branching events. *A. gossypii* also carries homologues of *ScRAX1, ScRAX2, ScBUD7, ScBUD8, ScBUD9*. These genes encode putative transmembrane proteins. We are interested in determining the role of these homologues in the filamentous growth pattern of *A. gossypii*. In contrast to *A. gossypii* wild type, in *Agrax1*, *Agrax2* and *Agbud8* deletions up to four branches can simultaneously emerge from the main hypha, and additional new branches are formed between the already developed ones. Tip growth of the main hypha is markedly reduced when branches start growing. An altered branching pattern was not observed in the *Agbud7* deletion. AgRax2-GFP localizes at the tips of hypha and at the septum where it appears just after actomyosin ring formation, and before chitin deposition. AgBud7-GFP is found in dot-like structures which oscillate. The observed structures are able to divide keeping a similar density throughout the hyphae. We will complete the analysis by using video microscopy with different tip-located GFP marker proteins. *ScBUD8* and *ScBUD9* are twin genes (leftovers of the ancient whole genome duplication), and Ashbya carries a single homologue, *AgBUD8/9*. The protein encoded by *AgBUD8/9* is important for localization of AgRax2 to the growing tip.

Activity of syntenic *A. gossypii* promoters

Due to a high degree of synteny, our two model organisms represent an ideal system to study the conservation of entire promoters and promoter elements. A selection of promising *A. gossypii* promoters were chosen and fused to a promoterless *GFP* reporter gene. Evaluation of syntenic *A. gossypii* promoter strengths in *S. cerevisiae* revealed that 44% of the promoters tested exhibited significant green fluorescence. Two of those promoters, *ENO2P* and *PFK2P*, can be used as new heterologous promoters in *S. cerevisiae.* In order to identify conserved promoter elements we compared the *A. gossypii* promoters of *CLN1,2*, *ENO2*, *PFK2*, *TEF2*, *H3/H4*, *H2A/H2B*. and their *S. cerevisiae* homologues. Interestingly, we observed a high frequency of order conservation between *A. gossypii* and *S. cerevisiae* transcription factor binding sites. In contrast to that, the distances between the individual sites varied considerably between the two species.

Peroxisome formation via a novel ER pathway

Impairment of peroxisomal function is the cause of a number of diseases in humans ranging from single enzyme deficiencies to severe syndromes in which peroxisomes are completely absent. Peroxisome functions in fatty acid metabolism are quite well characterized but how peroxisomes are formed in eukaryotic cells is unknown. Both human and yeast

cells lacking peroxisomes due to mutations in *PEX3* or *PEX19* genes regenerate the organelles upon re-introduction of the corresponding wild-type version. To evaluate how and from where new peroxisomes are formed, we followed the trafficking route of newly made YFP-tagged Pex3 and Pex19 proteins by real-time fluorescence microscopy in *Saccharomyces cerevisiae*. Remarkably, Pex3 (an integral membrane protein) could first be observed in the endoplasmic reticulum (ER), where it concentrates in foci that then bud off in a Pex19-dependent manner and mature into fully functional peroxisomes. Pex19 (a farnesylated, mostly cytosolic protein) enriches first at the Pex3 foci on the ER and then on the maturing peroxisomes. This trafficking route of Pex3-YFP is the same in wild-type cells. These results demonstrate that peroxisomes are generated from domains in the ER.

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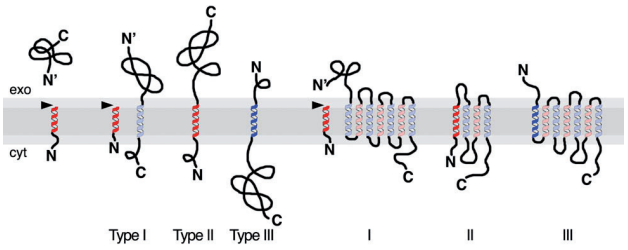
Membrane protein topogenesis and intracellular protein sorting

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 entirely *in vitro*.

Topogenesis of membrane proteins

Hydrophobic signal sequences target proteins to the ER membrane where the translocon mediates the passage of hydrophilic sequences across the membrane and the integration of apolar transmembrane segments into the lipid bilayer. In the process, the topology of the polypeptide a chain is determined, i.e. the orientation of the signal and of subsequent transmembrane segments with respect to the membrane.



Topologies of single-spanning membrane proteins of type I, II, and III, and examples of corresponding multi-span-

We are studying the determinants and the process of topogenesis in vivo by challenging mammalian cells with diagnostic mutant proteins. Signal orientation is determined by the flanking charges, hydrophobicity, and folding of N-terminal sequences. The results also suggest that the signal is exposed to the lipid membrane during topogenesis.
In parallel, we analyze the translocon in yeast by random and site-directed mutagenesis to generate mutants affected in topogenesis. The results indicate a role of the plug domain of Sec61p in topogenesis, probably by appropriately gating the channel. In addition, the plug is important for efficient assembly of the hetero-oligomeric translocon complex.

Martin Spiess

Tel. +41 61 267 21 64
Fax +41 61 267 21 49

martin.spiess@unibas.ch
www.biozentrum.unibas.ch/spiess

Group Members

Nicole Beuret	Szymon Kobialba
Pascal Crottet	Roman Lutz
Michael Friberg	Daniel Meyer
Stefan Gander	Adriana Pagano
Marie Higy	Christina Prescianotto-Baschong
Benjamin Hurschler	Hansruedi Stettler
Tina Junne Bieri	Gregor Suri

Visiting Scientist
J. Rutishauser (University Hospital, Basel)

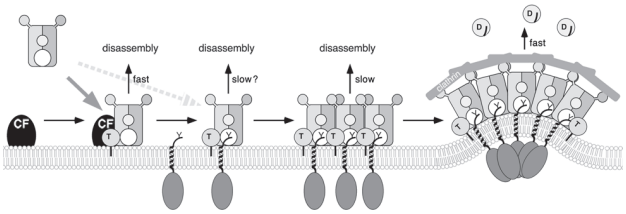
In Collaboration with
D. Cassel (Technion, Haifa); T. Schwede (Bioinformatics, Biozentrum)

Administrative Assistants
Maja Heckel, Verena Zellweger

Martin Spiess

Formation of transport vesicles

Transport between organelles is generally mediated by cytosolic coats which serve to create a scaffold to form coated buds and vesicles, and to selectively concentrate cargo by interacting with cytosolic sorting signals. Clathrin coats with different adaptor proteins (APs) are involved in transporting cargo proteins with characteristic tyrosine and dileucine motifs in various intracellular pathways. We have developed an assay using permeabilized cells to study the molecular requirements for the recycling of internalized receptors back to the plasma membrane. Manipulation of the cytosol used for reconstitution revealed that formation of recycling vesicles requires AP-1/clathrin coats and is regulated by rab4 and the connector rabaptin-5. The assay is being adapted to also analyze vesicle formation at the trans-Golgi network.



To study the mechanism of coat formation, we reconstitute the recruitment of purified coat components to chemically defined liposomes presenting corresponding sorting peptides. AP-1 adaptors are recruited in the presence of specific lipids, Arf1·GTP, and sorting signals. They were found to oligomerize even in the absence of clathrin and to dissociate upon GTP hydrolysis induced by the GTPase activating protein ArfGAP1. AP-1 stimulates ArfGAP1 activity, suggesting a role of AP-1 in the regulation of the Arf1 “GTPase timer”. Interaction with cargo reduces the rate of GTP hydrolysis, thus contributing to efficient cargo sorting.

Secretory granules

The peptide hormone vasopressin is synthesized as a precursor protein, sorted into secretory granules, and released from the neurohypophysis in a regulated manner to control water reabsorption in the kidney. In collaboration with Jonas Rutishauser (University Hospital, Basel), we study how mutations cause familial central Diabetes insipidus, a dominant degenerative disease. Mutant proteins are retrotranslocated to the cytosol and degraded by the proteasome. A cytotoxicity assay is being developed to identify the mechanism of dominance.
In the course of these experiments, we discovered that expression of several regulated secretory proteins in nonendocrine cells generates granule-like structures which resemble secretory granules morphologically and biochemically. Co-expression studies show that granins enhance the efficiency of their formation and modulate their morphology. The results suggest that initial granule formation requires no additional machinery specific to regulated secretory cells besides the regulated cargo itself, and that granins have a supportive role in the process.

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Martin Spiess

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Focal Area
Infection Biology

Objective

The objective is the understanding of infectious diseases at the cell and molecular levels with the focus on bacterial infections. Three groups (G. Cornelis, C. Dehio and J. Pieters) decipher the pathogenesis of four different bacterial pathogens: *Bartonella* spp., *Capnocytophaga canimorsus*, *Mycobacterium* (M. tuberculosis, M. bovis BCG and *M. smegmatis*) and *Yersinia enterocolitica*. Each of these pathogens represents an archetype for a virulence mechanism (intracellular replication, immune evasion, type III secretion, type IV secretion etc.) and provides information which could lead to the design of treatment or vaccines against a whole range of pathogenic bacteria.

In studying these four pathogens, the research field includes Gram positive and Gram negative bacteria, extracellular and intracellular pathogens, well-recognized and less-known pathogens, microbial genetics and nanomachines, cellular trafficking and signal transduction. Thus, around a very focused central theme, the groups develop competence and collaborations covering many different and complementary aspects of the biology of infection. The expertise includes microbial genetics, cell biology, immunology, biochemistry, fluorescence and electron microscopy, and microarrays.

Extensive collaborations are taking place with our colleagues from the Core Programs “Structural Biology and Biophysics” as well as “Genome Scale Biology and Bioinformatics”.

The impact of this Research goes beyond the specific field of microbial pathogenesis, addressing basic questions in Biology such as the regulatory principles in nanomachines assembly (length control for instance), DNA transfer between microorganisms and the role of protein kinases in intracellular transport processes in macrophages.

Highlights

In 2003 the Cornelis group discovered that the length of the needle of the *Yersinia* injectisome (Type III secretion apparatus) is determined by a protein acting as a molecular ruler (see Report 2002-2003). Now, they showed that the needle must have a certain length to match the length of other macromolecules at the surface of the bacterium and the target animal cell (*Science*, 2005, 307:1278). They also demonstrated that the tip of the needle has a distinct structure made of protein LcrV and that this structure acts

Professors
Cécile Arrieumerlou (starting in 2006), Guy Cornelis, Christoph Dehio, Jean Pieters

Scientists and Postdoctoral Fellows
Imke Albrecht, Michaela Dehio*, Gottfried Eisner*, Edith Houben*, Rajesh Jayachandran*, Laure Journet*, Jaime Mota*, Liem Nguyen*, Nadine Schracke*, Gunnar Schröder, Ralf Schülein*, Hwain Shin, Sabrina Sonda*, Isabel Sorg, Varadharajan Sundaramurthy*, Anne Walburger*

Graduate Students
Céline Agrain, Petr Broz, Benoit Combaluzier*, Patrick Guye, Michel Letzelter, Manuela Mally, Jan Massner, Salome Meyer*, Catherine Müller, Thomas Rhomberg, Henri Saenz, Florine Scheidegger*, Hermine Schein*, Nicole Scherr*, Michael Schmid*, Stefanie Wagner*

Diploma/Master Students
Andrea Basler*, Philipp Engel*, Yvonne Lussi*, Kerstin Maylandt*, Anna Melone*, Philipp Müller*, Michèle Stöckli*, Isabel Toller*

Laboratory Technicians
Yvonne Ellner*, Claudia Erbel*, Giorgio Ferrari*, Lotte Kuhn, Marina Kuhn, Gabriele Kunz*, Nadege Devaux*, Cécile Pfaff, Cristina Presianotto-Baschong, Maxime Québatte, Doris Scheidegger, Nadine Silbereisen*, Jürg Widmer, Bettina Zanolari

Floor Managers
Marina Kuhn*, Werner Metzger, Roger Sauder, Ingrid Thönen*

IT-Coordinator
Andreas Hefti

Trainee
Emilie Clement*

Laboratory Helpers
Isabelle Lanz-Kalambay, Dominik Sutter*, Eva Tamburini, Michael Vanek*

Administrative Assistants
Elvira Amstutz, Loredana d’Anna*, Claudia Erbel*, Monika Föhner, Maja Heckel*, Liselotte König*, Beatrice Thierrin*

* employed during part of the report period

Focal Area
Infection Biology

as a platform for the assembly of a translocation pore into the membrane of the host cell (*Science*, 2005, 310:674-6).

The Dehio group discovered the *Bartonella* effector proteins (Beps), which are translocated into target host cells and subvert host cell functions. (*Proc Natl Acad Sci U S A*., 2005, 102 :856-61).

The Pieters group showed that a mycobacterial kinase is essential for escaping intracellular degradation within lysosomes and that a drug inhibiting this kinase allows the macrophage to kill mycobacteria (*Science*, 2004, 304(5678):1800-4).

Education

The members of the Focal Area “Infection Biology” together with U Jenal (Focal Area “Growth and Development”) organize a complete graduate teaching program on “Infection Biology” (Cycle A). The cycle was initiated during the 04-05 winter semester by a course on “General Mechanisms of Microbial Pathogenesis”. A course on “Major Microbial Diseases and Vaccine Development” was taught during the summer semester 05. A course on “Microbial Cell Structures and Drug Targets” takes place during the 05-06 winter semester.

The Focal Area also contributed to the „Joint Degree in Infectious Diseases, Vaccinology and Drug Discovery“ organized by the Novartis Institute for Tropical Diseases in Singapore (NITD), The University of Singapore, The Swiss Tropical Institute (Basel) and the Biozentrum.

Finally, the Focal Area organized a weekly seminar and a monthly Journal Club.

Molecular mechanisms of the pathogenesis of *Yersinia* sp and *Capnocytophaga canimorsus*

Type III secretion in *Yersinia*

We are studying type III secretion (T3S), a complex weapon, encountered in more than twenty animal, plant and insect Gram-negative pathogens. It consists of a nanosyringe derived from the flagellum (the injectisome) and an array of effector proteins. Bacteria docked at the surface of a eukaryotic host cell or included within a vacuole inject the effectors across the cellular membrane, into the cytosol of the host cell. The effectors disarm the cell, sabotage its communications or reprogram the cell to the benefit of the pathogen.

The Ysc-Yop system from *Yersinia* (*Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*) represents an archetype of T3S systems. The Ysc injectisome allows extra cellular bacteria to inject six different effectors called Yops into the cytosol of macrophages. Yops interrupt the signaling cascades triggering phagocytosis and the inflammatory response. The injectisome is made of 35 different proteins. It consists of a basal body embedded in the two bacterial membranes and the petidoglycan, extended by a 60-nm needle protruding outside the bacterial surface. The basal body contains the recognition and export apparatus, energized by an ATPase of the AAA+ family. Interestingly, this export apparatus serves to export the needle subunits during the assembly phase. It also serves to export LcrV, YopB and YopD, three proteins which are required for the formation of a translocation pore in the membrane of the target cell. The relation between the needle and the pore is still unclear (Figure 1). During the 2004-2005 period, our work focused mainly on the structure and assembly of the needle, the assembly of the translocation pore and the role of specific intrabacterial chaperones called the Syc (Specific Yop chaperones) proteins.

The needle length is controlled by a protein (YscP), which acts as a molecular ruler (see report 2002-2003). In 2004-2005, we demonstrated that the C-terminal domain of YscP switches the substrate-specificity of the export machine from early substrates (like needle subunits) to late substrates (Yops) when the assembly of the needle is completed. YscP is thus a ruler triggering a switch. The ruler protein (YscP) is exported by the growing injectisome during the assembly process. We showed that the ruler needs to be exported to exert an accurate length control. This observation strengthens the model for length control that we proposed in 2003. According to this model, the ruler resides within the growing needle, anchored by its N-terminus and gradually

Guy R. Cornelis

Phone +41 61 267 21 10
Fax +41 61 267 21 18

guy.cornelis@unibas.ch
www.biozentrum.unibas.ch/cornelis

Group Members

Céline Agrain
Petr Broz
Gottfried Eisner
Claudia Erbel
Laure Journet
Marina Kuhn
Michel Letzelter
Yvonne Lussy
Manuela Mally
Kerstin Mayland
Salome Meyer
Jaime Mota
Catherine Ann Müller
Cécile Pfaff
Nadine Schracke
Doris Scheidegger
Hwain Shin
Isabel Sorg
Stefanie Wagner

Visiting Scientists

K. Hughes (University of Utah)

In Collaboration with

M. Dürrenberger and U.Aebi (Microscopy Center, Uni Basel); A. Engel, S. Müller, P. Ringler, F. Erne (Biozentrum Basel and Maurice Müller Institute); S. Schuster (Pennsylvania State University, Philadelphia, US); U. Zähringer (Research Center Borstel, Germany); I. Attree (CEA Grenoble, France); D. Heinz and H. Niemann (GBF Braunschweig, Germany); I. Callebaut (CNRS/UMR 7590, Paris); E. Caron, (Imperial College, London); J. Tommassen and M. Koster (Utrecht, The Netherlands).

Administrative Assistants

Claudia Erbel, Monika Föhner, Michaela Hanisch (starting in 2006)

Guy R. Cornelis

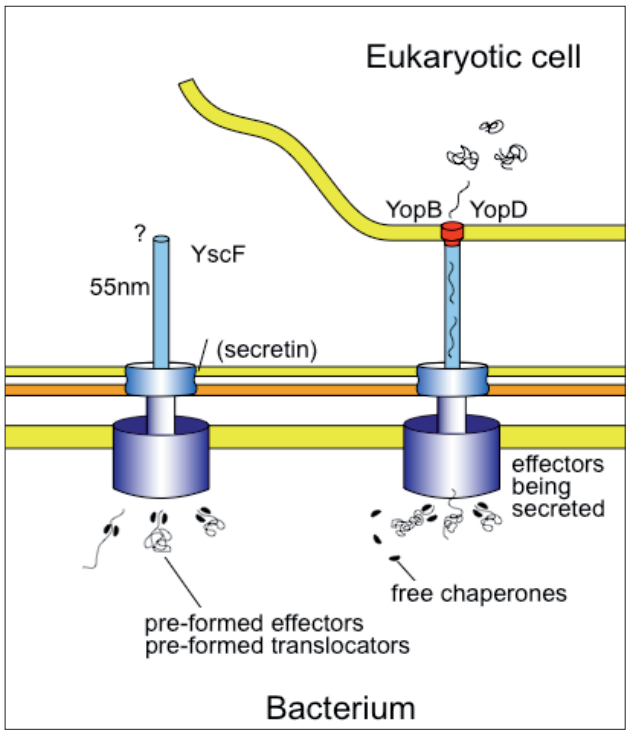

Figure 1: Left: The Yersinia *injectisome, prior to contact with a target cell. The basal body is made of two rings linked with a rod. The needle protrudes from the bacterial surface. Prior to their export, the effectors are bound to specific chaperones. Right: the injectisome in action. A pore made of YopB and YopD allows translocation of the effectors across the target cell plasma membrane. During the 2004-2005 period, we studied the relation between the needle and the pore.*

extends while the needle grows. When the needle has reached its final length, the C-terminal domain switches the substrate specificity and the ruler is released. We localized the export signal of YscP and discoverd that YscP is endowed with two different N-terminal export signals. Both are different from the export signals of the Yops, showing that the injectisome recognizes two different kinds of export signals; “early” and “late” export signals.

Finally, we asked the question why the needle length needs to be defined. By modulating the length of YscP, we engineered needles of different lengths and tested their efficacy. We observed that needles longer than normal are functional while needles that are shorter than normal are not. We then combined these needle constructs with three variants of the outer membrane protein YadA exhibiting also different sizes. We observed that short needles are functional in association with short molecules of YadA and that normal needles are less functional in association with longer molecules of YadA. We concluded that the needle length is adjusted in order to match different structures present at the surface of the bacterium (adhesin, lipopolysaccharide,...) and probably also the host cell.

For the assembly of the translocation pore, we first demonstrated that LcrV is required for the formation of the pore. Then, in collaboration with I. Attree (Grenoble), we demonstrated that YopB and YopD are inserted into the target cell membrane but that LcrV is not, suggesting that it could act as a platform or scaffold for the assembly of the translocation pore made of YopB and YopD. In excellent agreement with this hypothesis, in collaboration with the

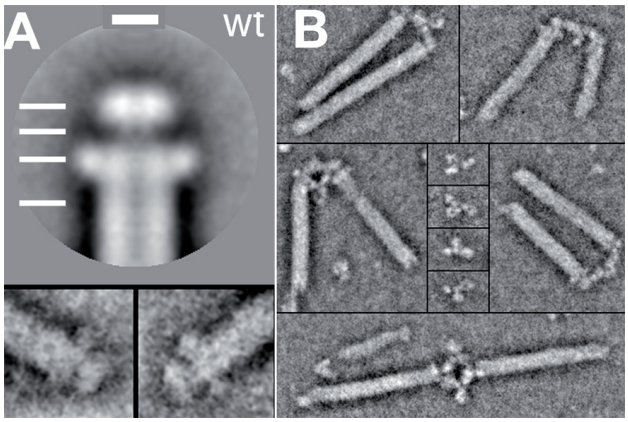

Figure 2: LcrV forms a structure at the tip of the needle of the Yersinia *injectisome. (A) Projection averages (top) and typical single STEM images (bottom) of the tip complexes formed by LcrV (left; resolution 1.5 nm), A central channel seems to permeate both the needle and the tip complex. (B) STEM images of pairs of needles connected by a divalent anti-LcrV antibody (negative staining). The antibodies generally attached to the head domain of the tip complex. The small central panels show individual antibodies. Scale bar: in A (top bar) 5 nm. From Mueller, Broz et al., Science 310, 674-676 (2005).*

group of A. Engel (Biozentrum), we discovered that LcrV forms a distinct structure, made of a head, a neck and a ring, at the tip of the needle (Figure 2). These observations explain the role of LcrV in pore formation and explain also why LcrV acts as a major protective antigen against plague.

Many effectors are associated with a specific Syc chaperone while they are stored inside the bacterium, prior to contact with a target. When the Yop is exported by the injectisome, the chaperone is removed by the ATPase and recycled inside the bacterium. The structure of such chaperones and the way they interact with their substrate is well character-

Guy R. Cornelis

ized but their main function remains elusive. During the 2004-2005 period, we discovered and characterized SycO, a new chaperone for the effector kinase YopO. The chaperone binding domain (CBD) within YopO coincides with the membrane localization domain (MLD) targeting YopO to the host cell membrane. In the absence of SycO, the CBD/MLD causes intrabacterial YopO insolubility. The binding of SycO prevents the aggregation of YopO but not folding and activity of the kinase. The CBD/MLD is not essential for YopO kinase activity. Thus, SycO masks, inside the bacterium, an aggregation-prone domain needed for proper YopO localization in the host cell. We propose that covering a MLD might be the essential function not only of SycO but also of other effector-chaperones.

Capnocytophaga canimorsus

In 2004, we initiated the study of the pathogenesis of *Capnocytophaga canimorsus*, a Gram-negative bacterium from the Order of Flavobacteria, commonly found in dog's mouths. It is responsible for fatal septicemia or meningitis after dog bites. In 2004-2005, we generated and sequenced two genomic libraries. The coverage is about 6.5 fold but the assembly of the contigs is still in progress. An overview of case reports suggests that the bacterium induces little inflammation during the early stages of the infection, until overwhelming bacterial growth and spread finally causes shock or meningitis. We characterized the inflammatory response of human and mouse macrophages upon *C. canimorsus* infection. Analysis of ten different strains of *C. canimorsus* showed absence of TNF-alpha and IL-1alpha release by infected mouse macrophages. Live and heat-killed Cc5, a strain isolated from fatal septicemia, did not induce IL-6, IL-8, IFN-gamma, MIP-1beta and nitric oxide in mouse and human macrophages. Failure of macrophages to promote inflammatory response coincided with the lack of NF-kappaB and mitogen-activated protein kinases (MAPK) activation as well as the inability of Toll-like receptor 4 (TLR4) to recognize and respond to Cc5. Moreover, Cc5 actively blocked release of TNF-alpha and nitric oxide, resulting in down regulation of TLR4 expression and dephosphorylation of p38 MAPK. Our findings highlight "passive" and "active" mechanisms of immune evasion by *C. canimorsus*, which may explain its capacity to subvert and escape from the host immune system.
During the 2004-2005 period, we have also developed an array of genetic tools for the study of *C. canimorsus*. This allowed us to generate a library of insertion mutants that will be screened for mutants unable to block the inflammatory response.

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Molecular analysis of type IV secretion systems in bacterial pathogenesis

The aim of our studies is to understand the molecular function of type IV secretion (T4S) systems in bacterial pathogenesis. The T4S systems of pathogenic bacteria have evolved from bacterial conjugation systems, which mediate the efficient spread of genetic traits, such as antibiotic resistance genes, among bacteria. Bacterial pathogens targeting eukaryotic host cells have adapted these DNA transfer systems for the delivery of virulence factors. A supramolecular assembly of 12 proteins is thought to span both Gram-negative bacterial membranes and the host cell membrane, allowing translocation of virulence factors from the bacterial cytoplasm directly into the host cell cytoplasm. An increasing number of human pathogenic bacteria uses T4S systems for the intracellular delivery of effector proteins that modify host cellular functions in favor of the pathogen. However, important questions regarding the molecular mechanism of T4S and the specific role of the translocated bacterial effector proteins within host cells have remained unanswered. Our recent work on the pathogenesis of the tumor-inducing bacteria of the genus *Bartonella* revealed central roles of two distinct T4S systems, VirB/VirD4 and Trw, in the ability of bacteria to colonize, invade and persist within vascular endothelial cells and erythrocytes, respectively. The identification of effector proteins transported by the VirB/VirD4 T4S system and the delineation of their secretion signal allowed us to better understand *Bartonella*-host cell interaction and modulation, as well as the molecular mechanistic aspects of T4S. Furthermore, the use of the delineated secretion signal for efficient intracellular delivery of engineered substrates into human cells may lead to an exploitation of T4S systems for novel therapeutic approaches.

Two distinct T4S systems are required for establishing *Bartonella* infection *in vivo*

The genus *Bartonella* comprises a large number of human and animal pathogens. Common features of bartonellae include transmission by blood-sucking arthropods and the specific interaction with endothelial cells and erythrocytes of their mammalian hosts. For each *Bartonella* species, the invasion and persistent intracellular colonization of erythrocytes is limited to a specific human or animal reservoir host. In contrast, endothelial cells serve as target cells in probably all mammals, including incidentally infected humans in the case of the animal-specific species (zoonotic infections). Laboratory rats experimentally infected with *Bartonella tribocorum* serve as a model for studying the characteristic intraerythrocytic bacteremia of bartonellae in their specific reservoir host. In

Christoph Dehio

Phone +41 61 267 21 40
Fax +41 61 267 21 18

christoph.dehio@unibas.ch
www.biozentrum.unibas.ch/dehio

Group Members

Andrea Basler
Michaela Dehio
Nadège Devaux
Yvonne Ellner
Philipp Engel
Patrick Guye
Maxime Québatte
Thomas Rhomberg
Henri Saenz
Florine Scheidegger
Hermine Schein
Michael Schmid
Gunnar Schröder
Ralf Schülein
Michèle Stöckli
Isabella Toller

Visiting Scientists

M. Vayssier-Taussat (UMR BIPAR, Paris)

In Collaboration with

S. Andersson (University of Uppsala, Sweden); P. Jenö (Biozentrum Basel); M. Llosa (University of Cantabria, Santander, Spain); M. Mann (Max Planck Institute, Munich, Germany); S. Suerbaum (Medical University, Hannover, Germany); T. Schirmer (Biozentrum Basel); S. Schuster (Penn State University, Pennsylvania, USA); A.C. Vergunst (Leiden University, Leiden); J. Waltenberger (University of Maastricht, Maastricht, The Netherlands); U. Zähringer (Research Center Borstel, Germany)

Administrative Assistants

Loredana d'Anna, Claudia Erbel

Christoph Dehio

this model, bacteria initially colonize a primary infection niche, which is composed of endothelial cells and potentially other target cell types. Within this primary niche, bacteria become competent for the subsequent invasion of erythrocytes, which occurs in characteristic intervals of ~5 days. A large-scale screen of 3084 signature-tagged transposon mutants of *B. tribocorum* allowed us to identify 99 bacterial genes essential for establishing intraerythrocytic bacteremia in rats (Saenz and Dehio, 2005). Fifteen of these essential pathogenicity genes map to the loci encoding the T4S systems VirB/VirD4 and Trw, highlighting earlier findings of our lab on the importance of these T4S systems for *Bartonella* pathogenesis. Importantly, the VirB/VirD4 system is required for infection of the primary niche, i.e. endothelial cells, while the Trw system appears to be essential for the subsequent infection of erythrocytes (Schröder and Dehio, 2005).

The VirB/VirD4 T4S system mediates subversion of endothelial cell functions

The zoonotic pathogen *Bartonella henselae* causes an asymptomatic intraerythrocytic bacteremia in the feline reservoir host, while it represents an important pathogen in incidentally infected humans. Remarkably, the broad range of disease manifestations includes proliferative disorders of the vascular endothelium resulting in the formation of vascular tumors (Dehio, 2005). We use primary human umbilical vein endothelial cells (HUVEC) as an *in vitro* model for studying this subversion of human endothelial cell functions by *B. henselae*. Interestingly, *virB4* mutants lacking a functional VirB/VirD4 T4S system were incapable of mediating most of the known physiological changes associated with *B. henselae* infection of HUVEC. These include (i) massive rearrangements of the actin cytoskeleton, resulting in the formation and internalization of large bacterial aggregates, (ii) nuclear factor kappa B-dependent pro-inflammatory activation, leading to cell adhesion molecule expression and chemokine secretion, and (iii) inhibition of apoptotic cell death, resulting in enhanced endothelial cell survival. Moreover, the VirB/VirD4 T4S system mediated cytostatic and cytotoxic effects at high bacterial titers, which interfered with the potent VirB/VirD4-independent mitogenic activity of *B. henselae* (Schmid *et al*., 2004). Taken together, the VirB/VirD4 T4S system was shown to be a major virulence determinant of *B. henselae*, required for targeting multiple endothelial cell functions exploited by this vasculotropic pathogen in the course of tumor formation (Fig. 1).

Intracellular protein delivery by the VirB/VirD4 T4S system mediates subversion of endothelial cell functions

In the course of characterizing the *virB/virD4* locus, we identified 7 adjacent genes that encode effector proteins translocated by the VirB/VirD4 T4S system into human endothelial cells. These *Bartonella*-translocated effector proteins (BepA-BepG) share a partly conserved C-terminal translocation signal, while their heterologous N-terminus is considered to serve effector functions after translocation into endothelial cells. Three of the seven Bep proteins carry conserved tyrosine phosphorylation motifs in this effector domain and become phosphorylated upon translocation into endothelial cells. Deletion of the locus encoding the Bep proteins resulted in a mutant phenotype indistinguishable from mutants lacking a functional VirB/VirD4 T4S system, demonstrating that the Bep proteins mediate all T4S system-dependent changes of endothelial cell function (Fig. 1)

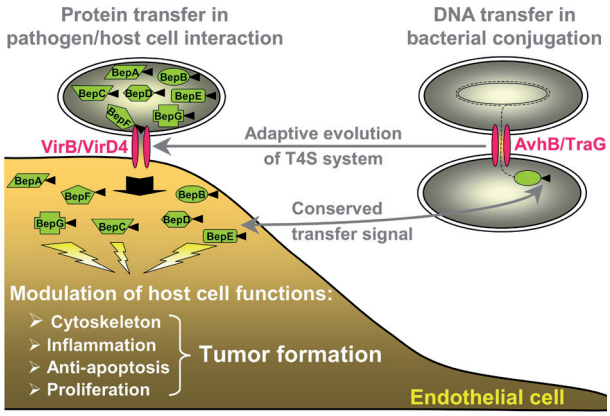

Figure 1: Effector proteins translocated by the Bartonella T4S system VirB/VirD4 subvert human endothelial cell functions.

The C-terminal translocation signal of the Bep proteins is bipartite, including a novel domain of approximately 140 amino acids termed BID (Bep intracellular delivery) followed at the very C-terminus by an unconserved tail sequence, which carries a net positive charge. Hidden-Markov models allowed us to identify BID domains also in several DNA-transfer proteins (relaxases) of plasmid-borne conjugation systems (e.g. of the *Agrobacterium tumfaciens* AvhB/TraG system), suggesting a recent history of adaptive evolution of the *Bartonella* VirB/VirD4 protein transfer system from these related conjugative DNA-transfer systems (Fig. 1).

Christoph Dehio

Fusion proteins composed of the C-terminal translocation signal of BepD and a heterologous reporter protein (e.g. the Cre recombinase of phage P1 or the Cya adenylate cyclase of *Bordetella pertussis*) were efficiently delivered by the VirB/VirD4 T4S system into human endothelial cells, demonstrating that the C-terminal translocation signal of the Bep proteins can be used for intracellular delivery of engineered protein substrates. Attenuated *B. henselae* strains may thus be used in humans for the intracellular targeting of proteins of therapeutic value, e.g. for the treatment of vascular disorders or for vaccine delivery (Schülein *et al.*, 2005).

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Analyzing the Interaction of Pathogens with the Host Immune System

When an organism is invaded by microbes, an immune response has to be established to clear the host from the invading microorganisms. Elimination of infectious agents is first of all ensured by the innate immune system. For extracellular pathogens, the defense consists largely of phagocytes that non-specifically engulf the microorganisms and destroy these within lysosomes. Degradation of the infectious agents also leads to the generation of fragments that can be displayed to T lymphocytes by Major Histocompatibility Complex (MHC) molecules, resulting in T cell activation and generation of specific immune responses.

My laboratory is interested in i) the intracellular trafficking events involved in the internalization and degradation of antigens and ii) the mechanisms that result in a proper T cell response. Many pathogenic microorganisms have gained the capacity to circumvent the effectiveness of the immune response at several levels, and a major focus of the laboratory is to decipher the mechanisms that are used by pathogens to escape immune recognition.

Antigen Internalization in Professional Antigen Presenting Cells

Presentation of antigens to T lymphocytes involves the entry and degradation of proteins and lipid molecules for presentation to T cells. One particular efficient internalization route occurs via macropinocytosis, which in addition can result in delivery of the antigens to the cytosol for presentation of antigenic fragments within the context of MHC class I molecules. This latter process, termed cross presentation, is crucial for the generation of immunity against virus-infected and tumor cells. We have analyzed the entry route for antigens internalized via macropinocytosis and found an essential role of the plasma membrane component cholesterol in macropinocytosis.

Interaction of Pathogenic Mycobacteria with Mammalian Cells

Several microorganisms have gained the capacity to invade and survive within mammalian host cells. Mycobacteria have the ability to enter and survive within eukaryotic cells, by preventing phagosome-lysosome fusion, making this an excellent model system to study both the pathogen-host interaction as well as the biochemical events involved in regulating transport events inside the host cell. We are interested in both the host as well as the mycobacterial factors contributing to mycobacterial survival.

Jean Pieters

Phone +41 61 267 14 94
Fax +41 61 267 21 49

jean.pieters@unibas.ch
www.biozentrum.unibas.ch/pieters

Group Members

Imke Albrecht	Philipp Müller
Benoit Combaluzier	Liem Nguyen
Giorgio Ferrari	Cristina Prescianotto-Baschong
Edith Houben	Nicole Scherr
Rajesh Jayachandran	Sabrina Sonda
Lotte Kuhn	Varadharajan Sundaramurthy
Gabriele Kunz	Anne Walburger
Jan Massner	Jürg Widmer
Anna Melone	Bettina Zanolari

In Collaboration with

Michel Steinmetz (PSI, Villigen, Switzerland); Hanno Langen & Bernd Mueller (F. Hoffman-La Roche, Basel, Switzerland); Ber Klebl, Gerald Bacher, Anil Koul (Axxima, Munich, Germany); Thomas Klimkait (Medical Microbiology, University of Basel); Claude Leclecrq (Institut Pasteur, Paris, France); Kris Huygen (Pasteur Institute Brussels, Belgium); Ton Rolink (DKBW, University of Basel); Regine Landmann (Department Forschung, Kantonspital Basel, Switzerland), Guilio C. Spagnoli (Departement Forschung, Kantonsspital Basel, Switzerland); Charles Thompson & Liem Nguyen (Department of Microbiology and Immunology, University of British Columbia, Canada), Paul Jenö, Daniela Nebenius-Oosthuizen & Markus Dürrenberger (Biozentrum Basel)

Administrative Assistants

Maja Heckel, Verena Zellweger

Jean Pieters

Macrophage Factors involved in the Modulation of Intracellular Trafficking

Analysis of the constituents of mycobacterial phagosomes revealed the accumulation of a host protein, termed coronin 1 or TACO (for *t*ryptophane *a*spartate containing *co*at protein) that is a crucial factor for mycobacterial survival inside macrophages. In the absence of coronin 1, mycobacteria are readily transferred to lysosomes.

We have biochemically characterized the organization of coronin 1 molecules. We showed that coronin 1 are coiled-coil-mediated homotrimeric complexes which associate with the plasma membrane and with the actin cytoskeleton via two distinct binding domains.

Association of coronin 1 with the actin cytoskeleton is mediated by C-terminal coiled-coil domain. In collaboration with Michel Steinmetz (Paul Scherrer Institute, Villigen CH) we have defined the crystal structure of the coiled coil domain of coronin 1, that we found to be a three stranded α-helical coiled coil. Within this coiled coil, a motif was found containing a critical arginine residue that is conserved in intracellular, extracellular, transmembrane, viral, and synthetic proteins harboring short trimeric coiled-coil domains; These findings contribute towards establishing sequence-to-structure rules for predicting coiled coils encoded in entire genomes and will also be of interest for viral drug development strategies.

In the absence of the coiled-coil domain, plasma membrane binding of coronin 1 still occurs suggesting that this is mediated by the N-terminal, WD repeat-containing domain. The capacity of coronin-1 to link F-actin filaments to the leukocyte plasma membrane may serve to integrate outside-inside signalling with modulation of the actin cytoskeleton.

Mycobacterial Factors involved in the Modulation of Intracellular trafficking

Besides the host factors involved in mycobacterial survival, we are analyzing the bacterial factors that allow pathogenic mycobacteria to survive within macrophages. We found that treatment of *Mycobacterium bovis* BCG (Bacillus Calmette-Guerin, which is the vaccine strain used as a model organism) with a eukaryotic protein kinase Cα inhibitor prior to infection of macrophages lead to an immediate transport of the mycobacteria to lysosomes suggesting the involvement of a PKCα-like kinase activity in blocking lysosomal delivery. Interestingly, eleven different eukaryotic-like protein kinases are encoded by pathogenic mycobacteria. Whereas most of the mycobacterial kinases are predicted to be transmembrane proteins with an intracellular kinase domain, two of these, protein kinase G and protein kinase K lack such a transmembrane segment. Interestingly, protein kinase G is retained in the downsized genome of the obligate intracellular pathogen *M. leprae* that carries the minimum set of mycobacterial genes necessary for intracellular survival. To analyze a role for protein kinase G in mycobacterial survival inside host cells, we disrupted protein kinase G in *M. bovis* BCG using phage-mediated homologous recombination. The resulting *M. bovis* BCG *pknG*-deletion mutant was immediately transferred to lysosomes and the mutant mycobacteria were unable to survive inside macrophages, defining protein kinase G as a crucial mycobacterial virulence factor (Figure 1).

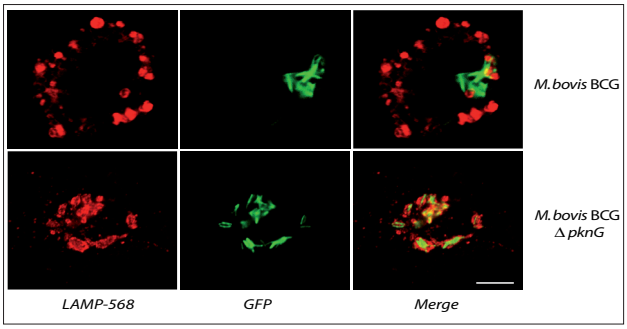

Figure 1: Pathogenic mycobacteria (in green), phagocytosed by macrophages remain outside lysosomal organelles (in red) (upper panels). However, when the eukaryotic-like serine/threonin kinase Protein Kinase G is deleted in these bacteria, they are readily transported to lysosomes (lower panels). Taken from Walburger et al., Science (2004)304:1800

Kinases are attractive drug targets, and in collaboration with Axxima (Munich, Germany) a protein kinase G inhibitor was characterized. This compound was able to induce transfer of mycobacteria to lysosomes and to mediate killing of mycobacteria inside macrophages. One possible advantage of targeting protein kinase G from pathogenic mycobacteria is that this will allow the macrophage to carry out its natural antibacterial activity of redirecting intracellularly surviving mycobacteria to lysosomes. Thus, pathogenic mycobacteria have evolved eukaryotic-like signal transduction mechanisms capable of modulating host cell trafficking pathways. Together, this work will help to understand how pathogenic mycobacteria evade the normal host immune responses. In addition, this work may reveal previ-

ously unknown mechanisms involved in the normal trafficking pathways as they are employed by these intracellular pathogens.

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Focal Area Neurobiology

Mechanisms controlling the development and function of the mammalian nervous system

A major challenge in biology is to understand how the nervous system is assembled in such a way that it can appropriately respond to a large range of stimuli and control complex functions such as body weight regulation, emotions and behavior. The nervous system must be capable of storing, integrating and retrieving information using mechanisms that are still poorly understood, but surely depend on the establishment of precise connections. How neurons 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 and injury of the nervous system. For example, 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 organize and teach a graduate and post-graduate program in Neurobiology. This includes a series of formal introductory lectures in Neurobiology, a 2-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 organised on Current Topics in Neurobiology, as well as 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

Professors
Silvia Arber, Yves-Alain Barde, Karl G. Hofbauer, Anita Lüthi, Heinrich Reichert (starting in 2006), Markus Rüegg, Kaspar Vogt (starting 2006)

Scientific Collaborators
Caroline Kopp-Chtarque*

Scientists and Postdoctoral Fellows
Gabriela Bezakova, Constanze Burkhardt*, Celine Costa*, Zhizhong Dong*, Patrick Dunant, Simon Hippenmeyer*, Ina Kramer*, David Ladle, Shuo Lin, Joseph Mascarenhas*, Tomoya Matsumoto*, Stan Nakanishi*, Janet Nicholson, Vera Niederkofler*, Ulrich Nordheim*, Jean-Christophe Peter*, Rishard Salie*, Claudia Senn*, Nicolas Wanaverbecq, Ulrike Wenzler-Hofsäss, Lixin Zhang*

Graduate Students
Rita Andraos, Christine Annaheim, Godela Bittcher*, Dimitri Clöetta*, Lucius Cueni*, Simon Dalla Torre*, Samuel Frère*, Andreas Friese, Milos Galic, Christoph Hangartner*, Simon Hippenmeyer*, Xian Chu Kong*, Ina Kramer*, Iwona Ksiazek*, Mira Kuisle, Jun Lee*, Marcin Maj, Vera Niederkofler*, Vassiliki Nikoletopoulou*, Nicolas Plachta, Stefanie Rauskolb, Rishard Salie*, Bernadette Saunier*, Patrick Scotton*, Anna Stepien*, Eline Vrieseling

Master/Diploma Students
C. Florian Bentzinger*, Deborah Heydet*, Roland Huber*, Cellia Längle*, Thomas Portmann*, Sarah Rüdiger*, Caterina Safina*, Michael Stebler*

Laboratory Technicians
Patrizia Barzaghi*, Valérie Crotet, Monika Mielich, Suzanne Moes*, Filippo Oliveri, Melanie Rittirsch, Markus Sigrist, Lazar Sumanovski, Monika Tetlak*, Annick Werner*, Fabian von Arx

Floor Managers
Markus Hämmerle, Roland Kirchhofer, Markus Meier

Laboratory Helpers
Bernadette Bruno, Gina Evora, Monika Furrer, Angele Klein, Karin Mauro

Administrative Assistants
Rose Marie Suter-Fritsche, Jny Wittker

Coordination "Basel Neuroscience Program"
Catherine Alioth, Simone Grumbacher-Reinert

*employed during part of the report period

Neuronal circuit assembly in the developing spinal cord

The aim of our studies is to understand the molecular and mechanistic basis involved in the establishment of specific neuronal connections within a circuit of interconnected neurons. A deep knowledge about the logic of how neuronal circuits are being assembled during development and which molecules are involved in the establishment of neuronal circuits may contribute to our understanding of the functioning of the mature nervous system. The main focus of our studies is to determine the principles of neuronal circuit formation in the developing vertebrate spinal cord. In the spinal monosynaptic reflex circuit many details of early neuronal specification as well as mature connectivity are already well understood. This neuronal circuit thus represents an ideal system to study how different neuronal subpopulations are being interconnected during development to establish mature neuronal circuits. In our studies, we mainly focus on the development of motor neurons in the ventral horn of the spinal cord and Ia proprioceptive afferents in dorsal root ganglia (DRG), which establish monosynaptic connections with motor neurons (Ia afferents; Figure 1).

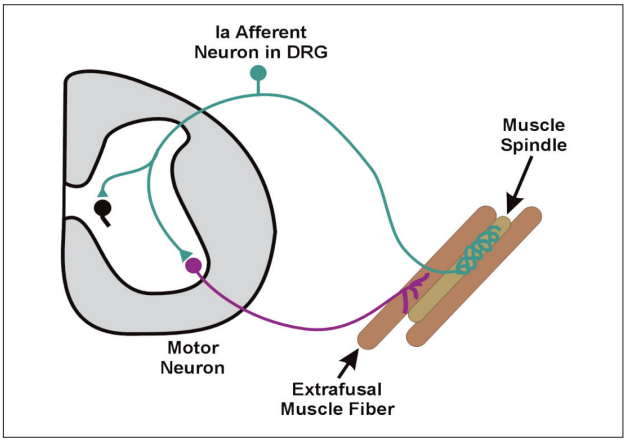

Fig. 1: Schematic representation of the mature spinal monosynaptic reflex circuit. Motor neurons project to extrafusal muscle fibers and receive direct synaptic input from Ia afferent sensory neurons.

Function of ETS transcription factors in the assembly of the vertebrate monosynaptic reflex circuit

In previous studies, we have identified two molecular pathways that link specific peripherally derived signals to the induction of the ETS transcription factors Pea3 and Er81 in defined subsets of motor neurons (Pea3) and dorsal root ganglia (DRG) sensory neurons (Er81). Furthermore, we found that these retrograde signals from an intermediate target region act selectively to control the progressive specification

Silvia Arber

Phone +41 61 267 20 57
Fax +41 61 267 20 78

silvia.arber@unibas.ch
www.biozentrum.unibas.ch/arber

Group Members

Simon Dalla Torre
Andreas Friese
Simon Hippenmeyer
Roland Huber
Ina Kramer
David Ladle
Celia Längle
Jun Lee
Monika Mielich
Stan Nakanishi
Vera Niederkofler
Thomas Portmann
Rishard Salie
Markus Sigrist
Anna Stepien
Eline Vrieseling

Visiting Scientists

J. Henaghan (The University in Albany, NY, USA)
Michael Röthlisberger (NCCR Lab rotation, University of Zurich)

In Collaboration with

P. Caroni (Friedrich Miescher Institute, Basel); J.A. Hassell (Mc Master University, Ontario, Canada); C.E. Henderson (Columbia University, New York, USA); T.M. Jessell (Columbia University, New York, USA); K.M. Murphy (Washington University, St. Louis, USA); A. Pierani (Ecole Normale Supérieure, Paris, France); W.D. Snider (University of North Carolina, Chapel Hill, USA)

Administrative Assistant

Rose Marie Suter-Fritsche

Silvia Arber

and differentiation of distinct neuronal subpopulations through the activation of the ETS transcription factors Pea3 and Er81 (Hippenmeyer *et al*., 2004).

In a recent study, we have addressed the question of how the temporal precision in the expression and activity of an ETS transcription factor controls the assembly of a neuronal circuit. We were able to show that upregulation of ETS transcription factor signaling specifically at a late stage of proprioceptive DRG sensory neuron differentiation is important to fulfill the appropriate developmental function (Hippenmeyer *et al*., 2005). We found that premature ETS signaling interferes with establishment of neuronal projections, acquisition of terminal neuronal traits and dependence on neurotrophic support (Figure 2). In contrast, late expression of the identical ETS transcriptional regulator in the same neuronal lineage can substitute for ETS gene function and promote neuronal differentiation. Together, these findings suggest that DRG sensory neurons undergo a temporal developmental switch, revealed by distinct responses to ETS transcription factor signaling at sequential steps of neuronal maturation (Hippenmeyer *et al*., 2005).

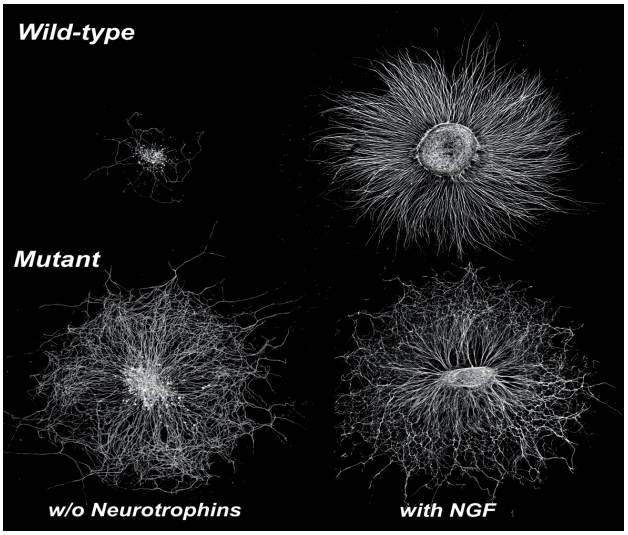

Fig. 2: Culture of DRG isolated from wild-type embryos (top; wild-type) or embryos with premature ETS signaling (bottom; mutant) cultured without neurotrophic support (left) or in presence of NGF (right). Note that mutant DRG neurons survive and grow axons in the absence of neurotrophic support. For details see Hippenmeyer et al., 2005.

Runx Transcription Factors and DRG Neuron Specification

Neuronal differentiation involves the acquisition of many specialized molecular properties that are essential for later neuronal function. The emergence of distinct subpopulations of DRG neurons is controlled by the selective activation of distinct transcriptional programs. Yet the transcriptional mechanisms that control the fragmentation of neuronal classes into distinct subtypes of primary sensory neurons remain obscure.

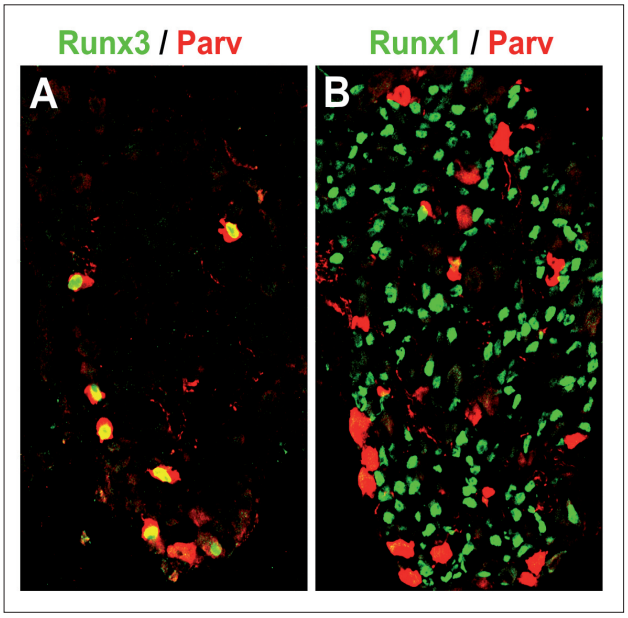

Fig. 3: Runx transcription factor expression in defined subpopulations of DRG neurons. In embryonic DRG neurons, Runx3 expression coincides with Parvalbumin and marks proprioceptive afferents (A). In contrast, Runx1 expression does not overlap with Parvalbumin and marks cutaneous afferents (B). For details see Kramer et al., 2006.

In a screen to identify genes selectively expressed by functionally distinct subpopulations of DRG neurons, we identified two members of the Runx family of transcription factors to mark TrkC$^+$ proprioceptive afferents (Runx3) and TrkA$^+$ cutaneous afferents (Runx1) respectively (Kramer *et al*., 2006; Figure 3).

Using genetic approaches in the mouse we have examined whether Runx transcription factor activity contributes to the acquisition of selective profiles of neurotrophic factor receptor and neuropeptide expression in different subpopulations of DRG sensory neurons, traits associated with functionally distinct neuronal subpopulations that ensure the maturation of these distinct sensory neuron subtypes. We found that runt domain transcription factor signaling plays

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an essential role in the emergence of key aspects of subpopulation character in sensory neurons, apparently by repression of alternate traits. Specifically, we found that Runx3 controls the establishment of a proprioceptor phenotype by promoting a selective TrkC phenotype through erosion of TrkB expression, whereas Runx1 expression within $TrkA^+$ cutaneous afferents represses the expression of the neuropeptide CGRP (Kramer *et al*., 2006).

Our findings therefore provide evidence that Runx transcription factor signaling controls three critical elements of DRG neuronal phenotype: neurotrophin sensitivity, peptidergic neurotransmitter profile and axonal targeting. Thus Runx proteins serve as key transcriptional intermediaries in the assignment of the functional features of DRG sensory neuron subclasses that underlie the early steps of somatosensory processing (Kramer *et al*., 2006).

Functional Analysis of Repulsive Guidance Molecule (RGM) Family Members in the Mouse

Repulsive Guidance Molecule (*RGM*) had been implicated in the control of topography of retinal ganglion cell (RGC) axon termination zones along the anterior-posterior axis of the chick tectum. The molecular identification of RGM has recently opened the way for functional studies in the mouse. We found that there are three mouse proteins homologous to chick RGM (Niederkofler *et al*., 2004). Two members of this gene family (*mRGMa* and *mRGMb*) are expressed in complementary patterns in the nervous system, including the spinal cord and superior colliculus (Niederkofler *et al*., 2004). Functional studies in the mouse revealed a role for *mRGMa* in controlling cephalic neural tube closure but not anterior-posterior targeting of RGC axons to their stereotypic termination zones in the superior colliculus (Niederkofler *et al*., 2004).

The third member of the family (*mRGMc*) is expressed in skeletal muscles. Moreover, within the liver mouse *mRGMc* is selectively expressed by periportal hepatocytes (Niederkofler *et al*., 2005). Our recent analysis of *mRGMc* mutant mice revealed that *mRGMc* is involved in the control of iron metabolism and its mutation leads to juvenile hemochromatosis, a frequently fatal disease with early onset iron accumulation and absence of hepcidin expression (Niederkofler *et al*., 2005). Our findings therefore define a key role for *mRGMc* in dietary iron-sensing and also reveal that cytokine-induced inflammation regulates *hepcidin* expression through an *mRGMc*-independent pathway.

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Neuronal Survival and Axonal Elongation in Development and Diseases: Studies Using Embryonic Stem Cells and Animal Models

Scope of research

We are interested in the molecular pathways controlling neuronal survival, axonal elongation and activity-dependent plasticity in higher vertebrates. To understand mechanisms relevant to development and diseases of the nervous system, we use neurotrophins and their receptors as dissecting tools, as well as cellular assays based on mouse embryonic stem cells.

Neurotrophins

In mammals, the 4 members of the neurotrophin gene family are involved in virtually all aspects of the biology of neurons. These include the control of cell survival and of axonal elongation, as well as neurotransmission and activity-dependent modification of synaptic function. While this field of research has its conceptual and experimental origin in development biology, there is increasing interest in the role of neurotrophins in the adult nervous system, both intact and after lesion. For example, the neurotrophin receptor $p75^{NTR}$ plays a significant role in inhibiting axonal elongation and in causing cell death during development. But in the adult, this receptor is re-expressed by many neurons following lesions such as axotomy, brain ischemia and epilepsy. $P75^{NTR}$ is also known to bind a number of ligands others than neurotrophins, including a viral envelope glycoprotein and the plaque-forming peptide Aβ, thus further suggesting a link between this receptor and pathologies of the nervous system. With regard to the neurotrophin brain-derived neurotrophic factor (BDNF), a range of intriguing observations links the levels of expression of this neurotrophin with complex behavioural patterns in rodents, including the regulation of food intake and with experimental models of depression, and in human, with episodic memory. We recently generated mice that can reach the adult state while lacking BDNF following the selective deletion of the gene in neurons. These mice should be useful to better understand the role of BDNF in the adult nervous system, including the regulation of food intake (studies in collaboration with the group of Karl Hofbauer at the Biozentrum).

Embryonic Stem Cells

We use extensively mouse embryonic stem (ES) cells to complement with cellular assays our in vivo research. While these cells have an unlimited potential to divide they can also be selectively guided towards specific differentiation pathways. We found that ES cells can generate essentially uniform popu-

Yves-Alain Barde

Phone +41 61 267 22 30
Fax +41 61 267 22 08

yves.barde@unibas.ch
www.biozentrum.unibas.ch/barde

Group Members

Rita Andraos
Christine Annaheim
Valérie Crotet
Zhizhong Dong
Tomoya Matsumoto
Vassiliki Nikoletopoulou
Nicolas Daniel Plachta
Stefanie Rauskolb
Melanie Rittirsch
Ulrike Wenzler-Hofsäss
Annick Werner
Lixin Zhang

Visiting Scientists

P. Brawand (Université de Genève); R. Deogracias (Instituto de Investigaciones Biomedicas “Alberto Sols”, Madrid); J.M. Frade (Instituto Cajal (CSIC) Madrid); S. Gentz (GSF - National Research Center for Environmental and Health, Neuherberg); J. Hjerling-Leffler (Karolinska Institutet, Stockholm)

In Collaboration with

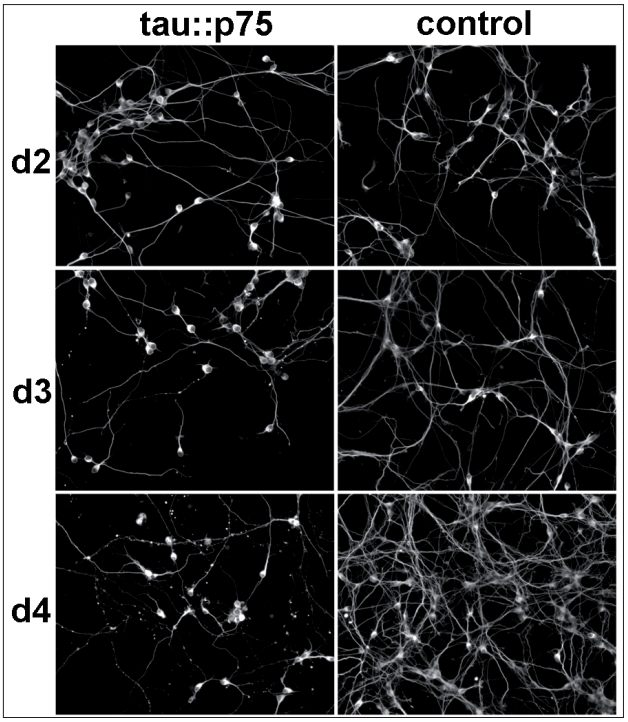
M. Götz and M. Korte (Max-Planck Institute of Neurobiology, Martinsried; GSF Neuherberg and Technical University Braunschweig); S. Hoving, J. Voshol, M. Bibel and P. Frey (Novartis)

Administrative Assistant

Jny Wittker

Yves-Alain Barde

lations of neurons that use glutamate as neurotransmitter. This was made possible following the accidental observation that ES cells can be directed to form an essentially pure population of a specific sub-type of neuronal precursors, namely Pax6-positive radial glial cells. Our ES cell-derived neurons respond to neurotrophins and we now use them to gain insight into the growth mediating effects of BDNF (work in collaboration with the groups of Markus Ruegg and Mike Hall at the Biozentrum). In addition, we engineered our ES cells to over-express $p75^{NTR}$ (see also illustration).



Legend: Neurons differentiated from ES cells rapidly develop long and branched processes. However, when they are engineered to over-express the neurotrophin receptor p75 as they differentiate into neurons, process elongation and branching stall and degeneration begins in a distal to proximal fashion, with cell bodies affected last.

This work has led to the establishment of a new model of axonal and dendrite degeneration and in collaboration with the proteomic group at Novartis, we are in the process of identifying proteins that may be causally involved in the death of neuronal processes. Finally, we also use mutant ES cells lacking genes necessary for the development of the central nervous system. In particular, we found in collaboration with the group of Magdalena Götz that ES cells lacking both functional copies of Pax6 develop differently from wild-type cells and that they generate inhibitory, as opposed to excitatory neurons. Also, these neurons were found to over-express $p75^{NTR}$ and to rapidly die in vitro. Upon re-examination of the Pax6 mutant cortex, we found that our in vitro findings could be verified in the developing cortex, thus suggesting that the ES cells differentiation system has a useful predictive value.

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New pharmacological concepts for the treatment of obesity and cachexia

The main research topic of the group “Applied Pharmacology” remained the validation of new concepts for the pharmacotherapy of metabolic risk factors. During the past two years the focus was on the treatment of cachexia by blockade of hypothalamic melanocortin-4 receptors (MC-4R). Cachexia is defined as a loss of fat and lean body mass in association with chronic diseases such as cancer or infections and is usually accompanied by inflammatory symptoms. As an independent risk factor for morbidity and mortality cachexia deserves medical attention and treatment. Dietary measures alone are not sufficient and pharmacotherapy is required. However, the currently available drugs are far from ideal and new pharmacological concepts are needed.

Inflammatory mediators appear to play an important role in the pathogenesis of cachexia. Circulating or brain cytokines can influence appetite and body composition via activation of specific hypothalamic receptors. These central effects might constitute a final common pathway in the development of cachexia which could explain why the body responds to various different underlying diseases with the same syndrome. Pharmacological blockade of this pathway should therefore be a useful therapeutic approach in treating cachexia regardless of its origin (for further details see Figure 1).

In collaboration with Santhera Pharmaceuticals AG in Liestal, various *in vivo* models were developed to validate the concept that blockade of MC-4R has beneficial effects in cachexia. These models include acute anorexia in lipopolysaccharide-treated rats and chronic cancer-induced cachexia in mice and can also be used to identify low molecular weight MC4-R antagonists and to characterize their acute effects on food intake. The chronic effects of MC-4R blockade on lean body mass were assessed with magnetic resonance relaxometry (MRR) in collaboration with Prof. Joachim Seelig’s group. This project was supported by a grant from the Swiss Commission for Technology and Innovation.

Another approach to block the melanocortin pathway consisted in the active immunization of rats with peptide sequences derived from the extracellular domains of the MC-4R. The functional role of the peptide sequences used as antigens was evaluated by using surface plasmon resonance. In these experiments an interaction between the third extracellular loop and the N-terminus of the MC-4R was demonstrated. Both domains were also found to interact with the endogenous inverse agonist Agouti-related protein (AgRP). These results suggested that the N-

Karl G. Hofbauer

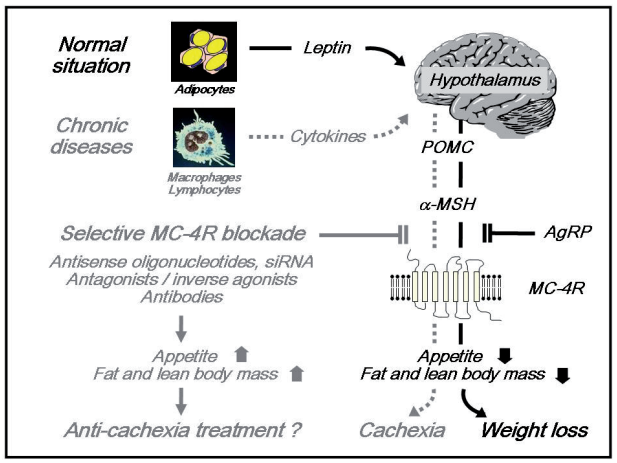
Phone +41 61 267 16 45
Fax +41 61 267 22 08

karl.hofbauer@unibas.ch
www.biozentrum.unibas.ch/hofbauer

Group Members
Patrick Dunant
Christoph Hangartner
Déborah Heydet
Suzanne Moes
Janet Nicholson
Ulrich Nordheim
Jean-Christophe Peter
Claudia Senn
Fabian von Arx

Administrative Assistant
Jny Wittker

Karl G. Hofbauer



The hypothalamic melanocortin system plays an important role in the regulation of energy balance and is part of the leptin signalling pathway (black letters, solid lines). Leptin is a small protein produced in adipose tissue in relation to the number and size of fat cells. It is secreted into the blood and reaches the hypothalamus where it activates specific receptors in the arcuate nucleus. Activation of these receptors results in an increased production of pro-opiomelanocortin (POMC), a precursor for several peptides including α-melanocyte stimulating hormone (α-MSH). This peptide acts on melanocortin-4 receptors (MC-4R) in the paraventricular nucleus which eventually leads to a reduction in appetite (anorexia) and an increase in energy expenditure. Another endogenous peptide, Agouti-related protein (AgRP), acts as an inverse agonist by reducing the constitutive activity of the MC-4R. Long-term stimulation of MC-4R induces a negative energy balance and a reduction in body fat mass. As a consequence circulating levels of leptin decrease which limits the amount of weight loss. In chronic diseases associated with cachexia cytokines are increased as part of the host defence reaction and stimulate the melanocortin pathway via specific cytokine receptors in the hypothalamus (grey letters, broken lines). In contrast to the normal situation where plasma levels of leptin decrease after weight loss thereby providing a negative feedback signal, cytokine levels remain elevated as long as the underlying chronic disease persists. Thus anorexia is maintained and the loss of fat and lean body mass continues. Experimental and clinical observations support the concept that blockade of the melanocortin pathway could improve this situation. Overexpression of AgRP or elimination of MC-4R in transgenic or ko mice results in obesity. Spontaneous mutations of the MC-4 receptor have been described in humans and cause severe forms of obesity. Such patients show increased height and elevated lean body mass. Similar changes occur in MC-4R ko mice. These observations support the notion that pharmacological MC-4R blockade could be a promising therapeutic approach to restore fat and lean body mass in patients with cachexia.

terminus may play a role in the constitutive activity of the MC-4R via its interaction with the third extracellular loop. It could therefore be an interesting template for the production of pharmacologically active antibodies.

Immunization of rats with peptide sequences of the N-terminus indeed resulted in a phenotype which consisted of an increase in body weight and fat mass. The post mortem evaluation of these rats revealed hepatic steatosis, a finding consistent with the existence of insulin resistance. Additional *in vitro* studies demonstrated that antibodies purified from the sera of these rats reduced the constitutive activity of the MC-4R, i.e. they acted as inverse agonists. Taken together these results showed that blockade of MC-4R led to an obesity-like syndrome. While such an effect would be undesirable in normal subjects it could be of therapeutic benefit in patients with cachexia.

Our immunization studies showed for the first time that it is possible to generate functionally active rat antibodies against extracellular domains of the MC-4R which can be used as pharmacological tools. In order to prepare sufficient amounts of such antibodies for further *in vivo* studies, polyclonal antibodies against the N-terminal domain of the MC-4R have been raised in rabbits and experiments in mice have been initiated to prepare monoclonal antibodies. The immunization studies in mice will also provide information about changes in lean body mass as determined by MRR.

The suitability of small interfering RNA (siRNA) for *in vitro* and *in vivo* studies on the MC-4R has been investigated. In these experiments it was shown that siRNA were equally effective as antisense oligonucleotides in knocking down MC-4R *in vitro*. However, they did not penetrate brain tissue after intracerebroventricular administration *in vivo*. Such problems may be overcome by improved solvents or vectors. Prolonged administration via osmotic minipumps could be another alternative to improve the efficacy of siRNA *in vivo*.

A new project on the central melanocortin pathway was started a year ago, in collaboration with Prof. Yves Barde's group, to investigate the role of brain-derived neurotrophic factor (BDNF) as a downstream mediator of the MC-4R. Since activation of this receptor affects not only energy intake and expenditure but also the cardiovascular system and sexual function, it would be interesting to identify specific mediators of each individual effect. This would make it possible to interact with these functions in a more

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specific manner. Studies with monoclonal antibodies against BDNF and other neurotrophins are being done in isolated hypothalami *in vitro* and in chronically cannulated rats *in vivo* to evaluate the role of these factors in response to MC-4R activation. The results of a first series of *in vivo* experiments showed that injection of BDNF monoclonal antibodies into the third cerebral ventricle of rats prevented the anorectic effect of an MC-4R agonist, while it had no influence on that of a cannabinoid-1 receptor antagonist, an agent considered to act independently of the MC-4R pathway. Further experiments are planned to evaluate which of the effects of MC-4R stimulation are mediated via BDNF.

The results of our experiments were presented at national and international meetings and are the subject of several publications. A book on "Pharmacotherapy of Cachexia" co-edited by Karl G. Hofbauer and Janet R. Nicholson appeared at CRC Press, Taylor & Francis Group, Boca Raton, USA, in January 2006. It is the first comprehensive volume on various aspects of this syndrome with a focus on drug treatment. Two invited reviews on the pharmacotherapy of obesity are in preparation.

Karl G. Hofbauer contributed to pharmacology teaching in the Natural Sciences Faculty and the Medical Faculty and has been made responsible for developing a new pharmacology curriculum for medical students in their first and second years. He is still engaged in several continuing education initiatives such as the Cardiovascular Pharmacology Seminars in Lucerne (co-organized with Prof. Paul Erne) and the Obesity, Metabolism and Nutrition (OMeN) lecture series at the University Hospital Basel (co-organized with several clinical colleagues).

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Towards the roles of sleep in neuronal functions

The quest for the role of sleep has a cultural and scientific history that starts with the origin of human civilization. The discussion of its role has explored a wide spectrum, ranging from its comparison to death, to its recognition as a prerequisite for mental and physical health, up to its appreciation as a promoter of human creativity. With the introduction of electroencephalography and electrophysiology in the 20th century, sleep became a subject of scientific research and was described as a heterogeneous, strictly organized series of physiological and behavioral states. Since then, it has been also established that sleep does not simply reflect a passively diminished waking, but is actively stabilized by sleep-promoting and antagonized by wake-promoting centers in the brain. Insufficient sleep has important consequences on the architecture and stability of sleep and arousal states, and additionally results in a decline of attention and motivation, in reduced short- and long-term memory, and in disturbed mental health. In western societies, a significant fraction of the health costs are caused directly or indirectly by the consequences of insufficient sleep. However, the biological mechanisms by which sleep exerts beneficial effects on brain functions are largely unknown. Recent approaches in behavioral and molecular neuroscience suggest that some of the beneficial effects of sleep manifest in genetic, developmental and circuit aspects of the establishment and remodeling of neuronal connections.

Our long-term goal is to understand the roles of sleep in brain functions at the cellular level. Ideally, we would like to identify the targets of the beneficial effects of sleep on diverse aspects of cognitive performance. The principal strategies we have been applying is to sleep-deprive mice, and perform the electrophysiological analysis of neuronal properties in brain slices of control and of sleep-deprived animals. So far, we have concentrated on synaptic plasticity in the hippocampus, which is a well established cellular correlate of learning. We also investigate ionic and synaptic mechanisms underlying thalamic function, in particular in relation to the generation of sleep-related neuronal oscillations. The thalamus is located at the interface between the sensory periphery and the cortical processing centers and is important in distinct aspects of sensory information transfer during waking and in the generation of sleep states. We have established thalamic slice preparations that have allowed, in the past years, to investigate some of the cellular correlates of thalamic functions

Anita Lüthi

Phone +41 61 267 22 46
Fax +41 61 267 22 08

anita.luthi@unibas.ch
www.biozentrum.unibas.ch/anitaluethi

Group Members
Lucius Cueni
Samuel Frère
Caroline Kopp-Chtarque
Mira Kuisle
Lazar Sumanovski
Nicolas Wanaverbecq

In Collaboration with
Làszlò Acsàdy (Institute of Experimental Medicine, Budapest); Tallie Z. Baram (UCIrvine); Bernhard Bettler (Pharmazentrum, Basel); Andreas Engel (Biozentrum, Basel); Karl Hofbauer (Biozentrum); Andrew Matus (FMI, Basel); Didier Pinault (Inserm, Strasbourg)

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We have achieved three major steps for further research on the role of sleep in brain function.

1. We have established a behavioral framework within which we are able to dissociate the role of sleep in brain function from confounding factors, such as stress, and prolonged sensory stimuli.
2. We have gained a mechanistic understanding of how neurons are capable of generating sleep-related oscillations, to the level of functional signaling complexes between different ion channels and intracellular signaling systems.
3. We have developed an *in vitro* approach to study how higher-order thalamic nuclei, essential for higher aspects of sensory processing and attentive mechanisms, are synaptically interconnected.

1. A role for sleep in homeostatic synaptic plasticity and NMDA receptor function (C. Kopp)

We have investigated the effects of sleep deprivation on homeostatic synaptic plasticity in the hippocampal CA1 area. Homeostatic synaptic plasticity refers to the susceptibility of synapses to undergo plastic changes depending on their prior history of activity. Homeostasis of synaptic function ensures that synaptic strength is maintained within working ranges and prevented from reaching saturation. We found that a stress-free sleep deprivation alters homeostatic synaptic plasticity in adult mice within a few hours only (*Figure 1*).
After sleep deprivation, the induction of synaptic potentiation was more difficult, whereas the induction of synaptic depression was facilitated. Moreover, recovery sleep reversed the effects of sleep deprivation. We could show that the alteration of homeostatic synaptic plasticity by sleep deprivation was not due to stress, nor to prolonged sensory exposure. Instead, it was caused by the lack of sleep. When investigating potential mechanisms underlying the alteration in homeostatic synaptic plasticity, we found that sleep deprivation induced a rapid and reversible change in the apparent subunit composition of N-methyl-D-aspartate (NMDA) receptors targeted by synaptically released glutamate. Therefore, the consequences of a stress-free sleep deprivation appear to manifest, at least in part, in measurable alterations in excitatory synaptic transmission and plasticity in brain slices. Moreover, the data suggest that a molecular basis for decreased learning and memory capacities after insufficient sleep, and perhaps cognitive function more generally, may be found in the properties of NMDA receptors.

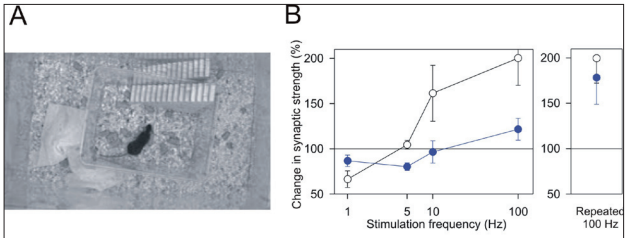

Figure 1.
A Experimental procedure to sleep-deprive a mouse by giving it access to an enlarged environment.
B Consequences of sleep deprivation on the frequency dependence of hippocampal synaptic plasticity. Open and filled circles are derived from brain slices prepared from control and sleep-deprived animals, respectively.

2. Ionic mechanisms underlying sleep-related neuronal oscillations in normal and epileptic animals (L. Cueni, S. Frère, M. Kuisle, N. Wanaverbecq)

We have been interested in the cellular and subcellular mechanisms underlying slow-wave sleep generation in thalamus and, in the long-term, how these contribute to the potentiation of slow-wave sleep after sleep deprivation. By using thalamic slice preparations that spontaneously generate sleep-related oscillations, we have studied the regulatory pathways of intrinsic thalamic pacemaker currents (Frère and Lüthi, 2004; Frère *et al*., 2004). A common theme arising from our recent work is that neuronal oscillations in thalamic neurons are based on a tight functional coupling between the low-threshold Ca^{2+} channels, the specific Ca^{2+}-dependent signaling elements, and the ionic channels acting as pacemakers for these oscillations. This is exemplified in both the thalamocortical cells and in the nucleus reticularis cells, the two key players in the generation of sleep-related oscillations. In thalamocortical cells, pacemaker currents are upregulated by cAMP that is produced via the Ca^{2+} entering during thalamic oscillatory burst discharges. This upregulation and its subsequent decay determine the periodic initiation and cessation of oscillatory burst discharges during sleep spindle waves and, in part, delta-rhythms. We also showed that in the Genetic Absence Epilepsy Rat from Strasbourg, a well-established rodent model of generalized epilepsy, the mechanisms underlying the termination of synchronized rhythms is disturbed. In thalamocortical cells of these animals, the regulation of pacemaker channels by cAMP is weakened, due to an enhanced expression of the mRNA for the cAMP-sensitive pacemaker channel subunits (manuscript submitted).
Recently, we identified a novel mechanism by which the timing of neuronal oscillations is controlled. Nucleus reticularis thalamic cells are key players in the initiation of spindle oscillations and spontaneously generate vigorous, but strongly dampened rhythmic discharges. These are generated by the interplay

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between low-threshold Ca^{2+} channels and Ca^{2+}-dependent SK potassium channels. We identified a crucial role for the sequestration of intracellular Ca^{2+} by the endoplasmatic reticulum (ER) in the timing of these neuronal oscillations (*Figure 2*). The ER seems to compete with the SK channels for the Ca^{2+} ions, thereby promoting the dampening of the oscillations. We also demonstrated that this competition is based on a selectivity of intracellular Ca^{2+} sequestration, perhaps due to the subcellular colocalization of ER compartments with the low-threshold Ca^{2+} and the SK channels.

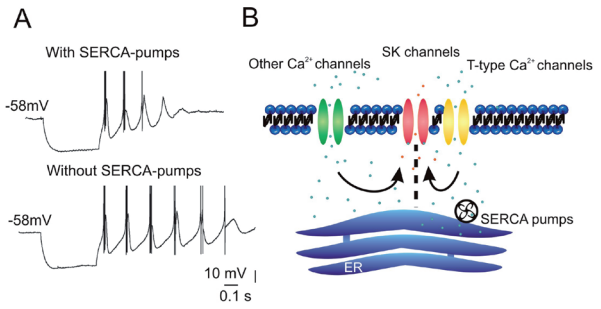

Figure 2.
A Sleep-related discharge pattern of a nucleus reticularis thalami cell is dependent on SERCA pumps. If these pumps are blocked, the discharge pattern is strengthened and looses the dampening.
B Hypothetical scheme illustrating the functional interaction between Ca^{2+} channels, SK channels and the SERCA pumps. Dashed lines delineate the potential compartmentalization.

3. Synaptic control of higher-order thalamic nuclei (S. Frère, M. Kuisle, N. Wanaverbecq)

The thalamus acts as a relay for sensory input to the cortex, but is also implicated in the coordination of multisensory and sensorimotor information. This latter function arises within the neuroanatomical and -physiological innervation pattern of higher-order, as opposed to first-order, thalamic nuclei. We have characterized a novel inhibitory afferent system into higher-order thalamic nuclei, which is part of a family of inhibitory projections arising in subcortical sensorimotor control nuclei, including basal ganglia (Bokor *et al*., 2005). These projections have an unusually large size of their synaptic terminals (up to 10 micrometer in diameter) and possess up to ~20 synaptic release sites. Therefore, they are expected to show a high efficacy of inhibitory transmission and to play a permissive role in the gating of sensorimotor information. We are currently studying their release properties and their impact on thalamic burst discharge by using brain slices labelled *in vitro* or mice with genetically encoded fluorescence in inhibitory neurons. Furthermore, to compare and contrast the properties of first-order and higher-order thalamic nuclei, we are currently studying the response properties of first- and higher-order thalamic neurons to arousing neurotransmitters, in particular to noradrenaline.

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Molecular mechanisms involved in synapse formation and neuromuscular disease

Among the most remarkable features of the brain is its capability to process information, which is, at least to a large part, determined by the complexity of the neural network. The functional units in these networks are synapses. To warrant efficient information transfer, synapses are highly specialized both on the pre- and on the postsynaptic side. It is evident that cellular differentiation, including the formation and remodeling of synaptic connections, depends on complex intracellular signaling networks that are activated by extracellular signaling molecules. Activation of such signaling pathways ultimately leads to stable changes in the gene expression program that determines the state of differentiation of a cell. The long-term goal of our research is to identify and characterize molecules that affect the formation and the modulation of synaptic connections both during development and the process of learning and memory.

Because of mainly technical limitations, it has so far been difficult to study synaptogenesis on a molecular level in the brain. In contrast, the neuromuscular junction (NMJ) is easily accessible for experimental manipulations and is thus the best studied synapse in the vertebrate nervous system. Both, the basic principle of chemical synaptic transmission (e.g. quantal release of neurotransmitter) and the molecular basis of synapse formation have been discovered using the NMJ. Improper function of the NMJ is the cause of many diseases that are often severe and eventually lead to premature death. They affect the pre- or the postsynaptic side and can have a genetic basis or might occur sporadically. This close relation between basic mechanisms involved in NMJ formation and disease has triggered also our interest in examining the molecular basis for the control of muscle function in normal and diseased state. This topic has become the second focus of our work.

Molecular mechanisms of NMJ development

During embryonic development, muscle fibers are formed by the fusion of precursor myoblasts. It was observed more than two decades ago and has more recently been studied in greater detail, that acetylcholine receptors (AChRs) form clusters before motor neurons innervate muscle. The formation of these pre-patterned AChR clusters requires the muscle-specific receptor tyrosine kinase MuSK. Upon innervation, nerve-released splice versions of the large heparansulfate proteoglycan agrin aggregate and maintain postsynaptic AChR clusters by activating MuSK underneath the nerve terminal. If agrin is not expressed, AChR aggregates disas-

Markus A. Rüegg

Phone +41 61 267 22 23
Fax +41 61 267 22 08

markus-a.ruegg@unibas.ch
www.biozentrum.unibas.ch/rueegg

Group Members

Patrizia Barzaghi	Shuo Lin
Gabriela Bezakova	Marcin Maj
Conrad Florian Bentzinger	Josef Mascarenhas
Godela Bittcher	Sarina Meinen
Constanze Burkhardt	Filippo Oliveri
Dimitri Cloëtta	Sarah Rüdiger
Celine Costa	Catherina Safina
Milos Galic	Patrick Scotton
Iwona Ksiazek	Michael Stebler
Xian Chu Kong	Monika Tetlak

In Collaboration with

J.L. Bixby (University of Miami, Miami, USA); H.R. Brenner (DKBW); J. Engel, J. Stetefeld and M. Hall (Biozentrum Basel); H. Lochmüller (Ludwig Maximillians-University Munich, Germany); T. Meier (Santhera Pharmaceuticals Ltd., Liestal); T. Oertner (FMI, Basel), J.R. Sanes (Harvard University, Cambridge, USA)

Administrative Assistant

Jny Wittker

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semble resulting in perinatal death due to respiratory failure. Agrin-MuSK signaling is also responsible to maintain gene transcription of postsynaptic genes in synaptic myonuclei.

To elucidate the signaling pathways activated during the formation of postsynaptic structures, we conducted a gene expression profiling screen using rat soleus muscle injected with recombinant neural agrin. Seven days after injection, when many postsynaptic structures are formed, we isolated mRNA from such muscles and compared their gene expression profile with muscles injected either with saline buffer or a splice version of agrin that cannot induce postsynaptic differentiation. Detailed analysis of the gene profiles revealed a set of immediate-early genes including Egr1 (Fig. 1A) that are known to be regulated by the mitogen-activated protein kinase (MAPK) pathway. Indeed, we find that phosphorylated forms of both the extracellular signal-regulated kinase (ERK) and c-Jun-N-terminal kinase (JNK) accumulate at the NMJ and at ectopic postsynaptic sites induced by agrin. In vivo perturbation experiments using RNA interference (RNAi) strongly indicate that MAPK signaling contributes to the formation and maintenance of postsynaptic structures in muscle. Interestingly, the MAPK pathway has also been implicated in changes of synaptic connections in response to learning and memory in the brain.

Copines are a new family of molecules involved in the formation and restructuring of synapses in the central nervous system

Another family of genes, that was discovered in the gene expression screen described above, are the copines. The copines are characterized by calcium-binding domains and their capability to form homo- and hetero-multimers. In mammals, eight copines have been annotated of which three are enriched in the central nervous system. Expression of some of the copines is also regulated during synapse formation in cultured hippocampal neurons. Functional studies using a combination of RNAi and overexpression show that a knock-down of copine 3 causes a reduction in the length and density of spines. Over time, the dendritic tree collapses and neurons die due to apoptosis. In contrast, a knock-down of copine 6 increases spine density and spine-head-diameter (Fig. 1B). Finally, neurons in which both copine 3 and copine 6 were down-regulated by RNAi do not die (as is the case in copine 3 knock-down experiments) but show the same effect as a knock-down of copine 6 (increase in spine density). Moreover, we find that copine 3 and copine 6 bind to each other and that copine 3 acts as a kinase.

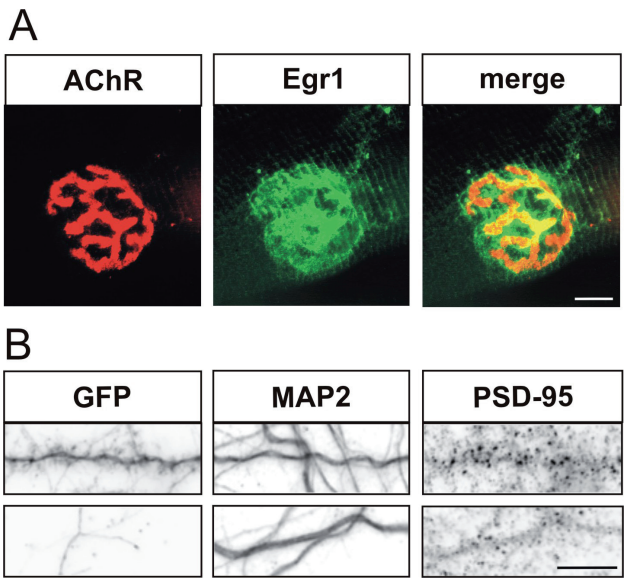

Figure 1: Proteins localized to the NMJ and to synapses in neurons
A: The NMJ is characterized by a high concentration of acetylcholine receptors (AChR, red). Similarly, one of the identified genes in the expression profiling screen, Egr1 (green), co-localizes to the NMJ thus confirming the expression data (see also merged picture). Scale bar = 10 µm.
B: Primary rat hippocampal neurons were transfected after 7 days (GFP-positive). Spines (PSD-95-positive) at the proximal segment of the dendrites (MAP2-positive) were analyzed five days later. Knockdown of copine 6 (top row) causes an increase of spine number by 46% and an increase of spine head diameter by 47% compared with non-transfected cells or neurons transfected with RNAi against CD4 (bottom row). Scale bar = 5 µm

Since the binding of copine 3 to copine 6 is calcium-dependent and since it is known that calcium influx is a primary signal in the strengthening and restructuring of synapses in long-term potentiation, translocation of copines into spines in response to depolarization was determined in collaboration with Thomas Oertner (Friedrich Miescher Institute, Basel). Copine 6 is enriched in spines and it becomes even more enriched in spines that are newly formed after depolarization of the neurons. All together, these experiments are strong evidence for copines representing a new family of calcium-sensitive proteins that play a role in the formation of spines during development and their restructuring in the adult triggered by electrical activity (e.g. learning and memory processes). This work thus provides strong experimental evidence for the idea that genes that are regulated dur-

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ing synapse formation at the NMJ may play a role in the formation of synapses in the brain.

Development of therapies for muscular dystrophies

We have recently shown in mice that a miniaturized form of the non-AChR-aggregating splice version of agrin, called “mini-agrin”, can greatly ameliorate a muscular dystrophy that is caused by mutations in the extracellular matrix molecule laminin-α2. The human disease, called MDC1A, is the most frequent congenital muscular dystrophy (incidence rate of approximately 0.5 x 10-5) and often leads to death in early childhood. Today, no treatment is available for this disease. By generating mice that allow temporal control of “mini-agrin” expression, we now tested whether even late onset of expression ameliorates the disease and whether full-length agrin or another artificially designed, extracellular matrix molecule would influence disease progression. In all these cases, we find a clear amelioration of behavioral, histological and biochemical parameters. For example, muscles of 4 week-old mice that express “mini-agrin” look as healthy as muscle from wild-type mice (Fig. 2).

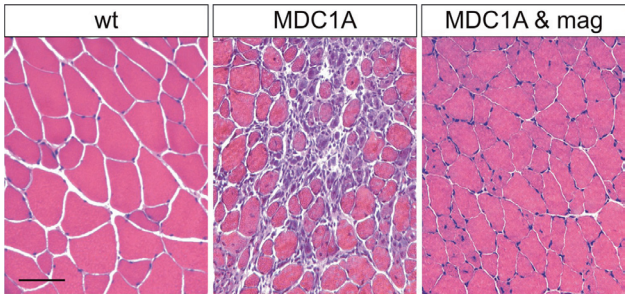

Figure 2: Histology of cross-sections from muscles isolated from mice with different genotypes. Muscles from wild-type (wt) mice are healthy, polygonally shaped. Muscle fibers from laminin-α2-deficient mice (MDC1A) are irregularly shaped and many non-muscle cells infiltrate the tissue. In contrast, muscle from laminin-α2-deficient mice that express “mini-agrin” (MDC1A & mag) looks similarly healthy as wild-type muscle. Scale bar = 50 µm

The role of interactors of mTOR in brain and muscle

Mammalian target of rapamycin (mTOR), originally identified in yeast by Michael Hall and collaborators (Biozentrum), is a central controller of cell growth. In collaboration with his laboratory, we have shown that mTOR, unlike previously assumed, assembles into two distinct protein complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), that can be distinguished based on their sensitivity to rapamycin mTORC1 (rapamycin-sensitive) consists of mTOR, mLST8 and raptor. mTORC2 (rapamycin-insensitive) contains mTOR, mLST8 and mAVO3, also called rictor. mTORC1 appears to be responsible for aspects involving cell growth by controlling protein translation, while mTORC2 affects the actin cytoskeleton.

Tissues where growth and not cell division is the main mechanism that accounts for the size of an organ, are the brain and muscle. In both of these organs, the main constituents (i.e. neurons in the brain and muscle fibers in muscle) are postmitotic, yet the cells must adapt to external stimuli by changing their size. In the developing brain, postmitotic neurons become highly polarized due to axonal and dendritic elongation. In the adult brain, growth and the formation of new synapses are adaptations to external stimuli underlying learning and memory. In adult muscle, exercise causes hypertrophy, while inactivity, due to disease or as a natural consequence of ageing, causes atrophy. Several lines of evidence strongly suggest that mTOR-signaling participates in the control of these processes. We are in the process of generating mice where raptor (mTORC1) or rictor (mTORC2) can selectively be inactivated in either muscle or the brain (conditional knock-out mice) and we will analyze the consequence of these knockouts in both tissues.

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Core Program Genome Scale Biology and Bioinformatics & SIB–Swiss Institute of Bioinformatics

Objective

Genome scale biology and Bioinformatics are at the crossroads of life sciences, mathematics and information technology. Their aim is to build a global perspective of biological systems, from which one can infer novel organizational and functional principles. Some of the more specific issues addressed are interpretation of gene and genome structure, identification of sequence elements and pathways, regulation of gene expression, prediction and visualization of 3D structures, and classification of functional domains in protein sequences. The projects in the interdisciplinary Core Program "Genome Scale Biology and Bioinformatics" are spanning this wide range of biological questions, often in collaboration with experimental groups. The Core Program consists of six groups headed by Mihaela Zavolan (RNA Regulatory Networks), Erik van Nimwegen (Genome Systems Biology), Torsten Schwede (Protein Structure Bioinformatics), Michael Primig (Meiotic transcriptional regulatory networks and Microarray Data Management/Analysis), Urs Meyer (Pharmacogenomics), and Peter Philippsen (Evolution of Cellular Networks) who is also associated with the focal area "Cell growth and development". The four Bioinformatics groups are members of the Swiss Institute of Bioinformatics (SIB). The research activities of the individual groups are described below.

Highlights

The group of Erik van Nimwegen has developed an algorithm for Reconstructing regulatory networks computationally by comparing genomes, which outperforms other existing methods on both synthetic data and real data from Saccharomyces genomes (PLoS Computational Biology, Volume 1, Issue7, December 2005).

The lab of Michael Primig has published an innovative tool for microarray data analysis and interpretation (Wrobel et al., Bioinformatics 2005) and participated in a study that identified a new surveillance mechanism of microtubule integrity during yeast meiosis (Hochwagen *et al*., Mol Cell Biol 2005).

The group of P. Philippsen sequenced and annotated, in collaboration with Syngenta, the genome of the filamentous fungus A. gossypii and showed that this fungus evolved from the same ancestor as the yeast S. cerevisiae (Dietrich et al. 2004, Science 304, 304-307). Combining the genome information and the available experimental tools now allow to analyze the divergent evolution of cellular networks

Professors
Urs A. Meyer, Michael Primig, Torsten Schwede, Erik van Nimwegen, Mihaela Zavolan

Associated Groups
Peter Philippsen

Scientists and Postdoctoral Fellows
Lorenza Bordoli, Nigel Brown, Frederic Chalmel, Ionas Erb, Carmela Gnerre*, Jürgen Kopp, Leandro Hermida, Diana Jung*, Maja Matis*, Christa Niederhauser-Wiederkehr, Mikael Oscarson*, Mikhail Pachkov, Anne-Kathrin Peyer, Michael Podvinec, Franck Rencurel*, Adrian Roth*, Christoph Rücker*, Marco Scarsi*, Alain Sewer, Viola Tamasi*, Mathias Vandenbogaert, Thomas Walther, Gunnar Wrobel

Graduate Students
James Battey, Sharon Blättler, Lukas Burger, Marie Cartron, Tzu-Ming Chern, Gudmundur Finnbogason*, Dimosthenis Gaidatzis, Taoufiq Harach*, Florian Kiefer Jose Ignacio Clemente Molina, Flavio Monigatti, Ulrich Schlecht, Holmfridur Thorsteinsdottir

Master/Diploma Students
Serej Ley*, Anca Moina, Christoph Wirth

Scientific Programming
Arnold Konstantin

Laboratory Technicians
Markus Beer, Michel Kaufmann, Renate Looser

Floor Managers
Markus Hämmerle, Jenni Roger, Markus Meier

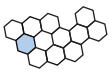
IT Coordination
Robert Gaisbauer, Reggie Hunziker, Roger Jenni, Rainer Pöhlmann

Technical Staff
Philippe Demougin, Faty Mahamadou, Nicodème Paul

Laboratory Helpers
Monika Furrer, Angele Klein

Admininstrative Assistants
Andrea Dornbierer Koch*, Marianne Liechti, Isabelle Steiger*, Yvonne Steger*

*employed during part of the report period



Core Program
Genome Scale Biology and Bioinformatics

controlling growth and nuclear division in these two simple eukaryotes.

Education

The members of the Core Program "Genome Scale Biology and Bioinformatics" organize the graduate teaching program on "Genomics & Bioinformatics " (Cycle F). The lecture series "Introduction to Bioinformatics I & II" accompanied by practical exercises is taught during the Winter and Summer Semester respectively, and is followed by students from the Biozentrum graduate program, as well as students in NanoSciences and Computer Science. In the winter semester 2005/2006 the first advanced lecture, "F10: Data Acquisition and Knowledge Representation", was introduced, and this will be followed by "F7: Protein Structure Bioinformatics" in summer semester 2006. Members of the Focal Area also contributed to the teaching of the Biozentrum curriculum lecture "Biologie 4", the Biochemistry block course, and several EMBnet training courses. In collaboration with the Swiss EMBnet node, Lorenza Bordoli provides user support and practical training courses in Bioinformatics.

Overview and Infrastructure

The Biozentrum's efforts to build a Bioinformatics division were supported by the Swiss Institute of Bioinformatics (SIB), Novartis and Roche. During the years 2004 and 2005, especially the younger groups in Bioinformatics have been able to increase in size by hiring PhD students, PostDocs and research programmers on endowment positions as well as through successful grant applications at the Swiss National Science Foundation SNF. We have established a fruitful collaboration between the Biozentrum, the Swiss Institute of Bioinformatics (SIB), and the Friedrich Miescher Institute (FMI) named Basel Computational Biology Center $[BC]^2$ to provide competitive IT infrastructure including application-, database-, and web-servers as well as large scale storage and backup facilities.

Conferences

The Basel Computational Biology Conference $[BC]^2$ is an annual interdisciplinary symposium organized at the Biozentrum to provide a platform for exchange of ideas in the field of computational biology. The symposium in 2004 "From Information to Simulation" has attracted more than 200 registered scientists from academic research institutes, the pharmaceutical industry and Biotech companies. The third symposium in 2005 "Biological Systems in silico", inspired by the exciting developments around Systems Biology in Basel, has continued the success of recent years. The theme of the fourth [BC]2 conference in March 2006 will be centered around comparative genomics.

Molecular mechanisms of diversity in response to drugs and chemicals in man

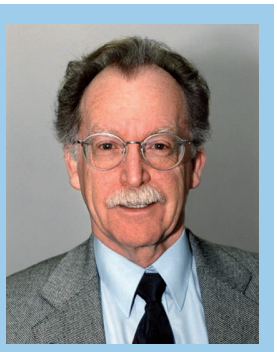
Genetic and environmental factors cause clinically important interindividual differences in the response to drugs and chemicals. Genetic polymorphisms of the genes coding for drug metabolizing enzymes, receptors and transporters are major contributors to this variability. A second major source of variability is the effect of drugs on gene expression, i.e. the transcriptional activation or repression of genes coding for drug-metabolizing enzymes, such as cytochromes P450, a phenomenon known as “induction”. The goal of our research is to understand the molecular mechanisms of variability in human drug response and to develop simple, non-invasive methods by which individuals at risk of developing adverse drug reactions, of suffering from drug inefficacy or drug-induced disease can be recognized.

Pharmacogenetics and Pharmacogenomics

Genetic polymorphisms of drug response are relatively common (>1%) monogenic traits caused by the presence of mutant alleles at the same gene locus and more than one phenotype in regard to drug interaction with the organism. Pharmacogenetic polymorphisms lead to subgroups in the population with altered responses to drugs and chemicals, e.g. a higher incidence of adverse drug reactions or a higher risk to develop a certain disease, for example cancer. In the past, we have elucidated the molecular mechanisms of several common genetic polymorphisms of drug metabolizing enzymes. For review, see Nature Rev./Genetics 5, 669-676, 2004. More recently, we have collaborated with other laboratories to study the variation of cytochrome P4502A6 (CYP2D6), the enzyme catalyzing the metabolism of nicotine. DNA tests derived from these projects are now being used in epidemiological studies throughout the world, particularly to evaluate hypotheses that implicate these polymorphisms in adverse drug reactions or in the pathogenesis of several diseases including cancer.

Mechanisms of Transcriptional Regulation of Cytochromes P450 (CYP) Genes by Drugs

A second major source of variability in drug response is the effect of drugs on gene expression, i.e. the transcriptional activation or repression of genes coding for drug-metabolizing enzymes and drug transporters. The present goal of our research team is to understand the molecular details of induction of drug-metabolizing enzymes and drug transporters. The following concept has emerged from studies in this and other laboratories. Inducers interact with the orphan nuclear receptors CAR (constitutive active or



Urs A. Meyer

Phone +41 61 267 22 20
Fax +41 61 267 22 08

urs-a.meyer@unibas.ch
www.biozentrum.unibas.ch/meyer

Group Members

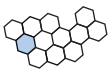
Markus Beer
Sharon Blättler
Gudmundur Finnbogason
Carmela Gnerre
Srinivas Gonukula
Taoufiq Harach
Diana Jung
Michel Kaufmann
Serej Ley
Renate Looser
Maja Matis
Stephan Nigg
Mikael Oscarson
Anne-Kathrin Peyer
Franck Rencurel
Adrian Roth
Christoph Rücker
Marco Scarsi
Viola Tamasi

In Collaboration with

U. Zanger, M. Eichelbaum (Stuttgart, Germany); David D. Moore (Houston, USA); Theo Wallimann, Dietbert Neumann (ETHZ); Benoit Viollet (Paris), Deborah Stroka, Daniel Candinas (Bern)

Admininstrative Assistant

Marianne Liechti



androstane receptor) or PXR (pregnane X receptor). These transcription factors form heterodimers with RXR (retinoid X receptor) and bind to enhancer motifs in the flanking regions of drug-regulated genes.

We have identified enhancer motifs in several drug-induced genes notably cytochromes P450 CYP2H1, CYP3A37 and 5-aminolevulinic acid synthase (ALAS1), the first and rate-limiting enzyme in heme biosynthesis. Moreover we have discovered a new xenobiotic-sensing nuclear receptor, named CXR (chicken xenobiotic-sensing receptor) and have demonstrated important links between cholesterol and bile acid homeostasis and drug metabolism. We have also developed a non-mammalian system, namely avian liver, avian hepatocytes and avian hepatoma cells (LMH), to study induction of cytochromes P450 and heme synthesis by drugs and chemicals. In LMH cells cytochromes P450 remain highly inducible by phenobarbital-like inducers in contrast to mammalian cell lines. Our experiments in LMH cells provide strong evidence for evolutionary conservation of the signaling pathways triggered by PXR, CAR and CXR. Thus, the phenobarbital-responsive enhancer units (PBRUs) of mouse Cyp2b10, rat CYP2B2 and human CYP2B6 were activated by the same compounds that activate the chicken CYP2H1 PBRU. Moreover, the mammalian receptors also bind to and activate the avian enhancer sequence. We could thus demonstrate that closely related nuclear receptors, transcription factors and signaling pathways are mediating the transcriptional activation of multiple genes by xenobiotics in chicken, rodents and man (Handschin *et al*., 2002, 2005). However, the reasons for the lack of drug induction in mammalian cell-lines remains obscure. One of our goals is to develop cell lines derived from human hepatocytes and hepatoma cells that maintain the response to phenobarbital.

How do drugs activate PXR and CAR?

There are major open questions regarding the mechanism by which drugs activate gene transcription. PXR and CAR both are mediators of the cellular response to xenobiotics, but their mechanisms of activation are different (Handschin & Meyer, 2003). PXR is apparently directly activated by xenobiotics that bind to its ligand-binding domain. CAR is unusual among the nuclear receptors in that it has high constitutive activity and is predominantly located in the cytoplasm. Treatment with phenobarbital, the classical inducer of drug metabolism, results in the translocation into the nucleus, where CAR (as heterodimer with RXR) transactivates the phenobarbital-response elements (PBRUs or PBREMs) of cytochromes P450s and other genes. The mechanisms by which phenobarbital-type inducers lead to the translocation of CAR are not known, nor are those by which CAR transactivates the PBRUs. Franck Rencurel in our team has discovered that the energy sensor AMPK (AMP activated kinase) is necessary for phenobarbital-type induction in a human hepatoma-derived cell line (Rencurel *et al*., 2005). He now has reproduced and extended these findings in human hepatocytes in primary culture. Sharon Blättler has reproduced these findings in LMH cells, providing evidence for an evolutionary conservation of this mechanism. In both human and chicken hepatocytes, phenobarbital increases the activity of AMPK, but the mechanism of this activation remains to be investigated. Apparently, it is not ATP depletion and we are presently testing a number of other hypotheses. Moreover, the target proteins of AMPK have been poorly studied in liver cells. The ultimate goal of all these studies, of course, is to understand the adaptation of the defense system against xenobiotics and to use this information in clinical therapy.

Crosstalk between xenobiotic-sensing nuclear receptors and receptors involved in cholesterol and bile acid homeostasis

We have extended our studies on the crosstalk between the nuclear receptors involved in cholesterol and bile acid homeostasis, namely LXR (the oxysterol receptor) and FXR (the bile acid receptor) and the xenosensing nuclear receptors PXR, CAR and CXR (Gnerre *at al*., 2005; Handschin *et al*., 2005) LXR, activated by oxysterols, inhibits the effects of inducer drugs by binding to the drug-response elements of several cytochromes P450. We have confirmed this mechanism by collaborative experiments in LXRα-/-, LXRβ-/- and LXRαβ double knockout mice. However, these *in vivo* experiments also have revealed unexpected and complex effects of LXR on other systems. More recently, by feeding mice a 1 % cholesterol diet, Adrian Roth has discovered that the target gene of LXR, Srebp1, directly interferes with coactivator recruitment by PXR and CAR and can explain the repression of xenobiotic metabolism by cholesterol (Roth *et al*., submitted). These data may explain clinical observations in rodents with disturbed lipid metabolism (e.g. the Fa/Fa Zucker rat) and in obesity in man.

At another level of regulation, Diana Jung has established that the bile acid receptor FXR regulates PXR expression by several FXR recognition sites in the promoter of PXR. This represents an additional

mechanism for the elimination of high levels of bile acids and drugs (Jung *et al*., submitted). In an additional study Carmela Gnerre has discovered that bile acids at physiological concentrations affect the expression of CYP3A4 via a previously unknown response element for FXR. This may be relevant for the clinically important, extremely variable expression of CYP3A4. Taken together, these recent experiments reveal an interesting coevolution of detoxication mechanism in the liver for drugs and bile acids (Gnerre *et al.*, 2004).

Role of heme synthesis during induction of cytochromes P450

Induction of cytochrome P450 (hemeproteins) requires a coordinated increase in heme synthesis. We have characterized several drug-responsive enhancer elements in the flanking region of the chicken, mouse and human 5-aminolevulinate synthase gene (ALAS1), the rate-limiting enzyme of heme synthesis. The chicken, mouse and human enhancers have been published (Fraser *et al*., 2002; 2003, Podvinec *et al.*, 2004). In an extension of these investigations, we have studied the effect of fasting on the regulation of ALAS1 in collaboration with Christoph Handschin, now in the laboratory of Bruce Spiegelmann (Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA). We have discovered that ALAS1 is regulated by the peroxisome proliferators-activated receptor γ–coactivator 1α (PGC-1α; Handschin *et al.*, 2005, editorial in Cell, commentary in Nature Medicine). These findings now allow us to restudy the mechanism of increase in heme synthesis during the induction of cytochromes P450 and to search for additional elements that respond to other inducer classes, a project carried out by Anne-Kathrin Peyer. By analyzing ultraconserved DNA sequences in the 5'flanking region of chicken, mouse and human ALAS1, a number of recognition sites for the transcription factor Hepatic Nuclear Factor 4α (HNF4α) and for FXR were discovered. These sites are presently studied by mutagenesis, chromatin immunoprecipitation and in vivo studies with mice with liver-specific knockouts of HNF4α (collaboration with Frank Gonzalez, NCI, NIH) and in FXR-/- mice.

In summary, our experiments all are designed to ultimately evaluate genotype-phenotype relationships in drug response and develop predictive tests for personalized medicine.

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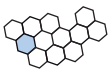

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Transcriptional regulatory networks governing meiotic cell cycle progression and gametogenesis in yeast and mammals

Meiosis leads to the formation of haploid gametes in nearly all sexually reproducing eukaryotes. This process promotes genetic diversity and is essential for the maintenance of the proper number of chromosomes during gametogenesis and subsequent fertilization. Progression through meiosis in yeast and mammals is regulated in part at the transcriptional level and often depends upon gene products specifically expressed during this important process. DNA microarray expression profiling studies identified hundreds of loci transcribed in sporulating yeast and in meiotic and post-meiotic germ cells of several higher organisms, including flies, worms, and rodents. While a few key regulators are known to mediate meiotic transcription in yeast, a full understanding of factors controlling this process is still lacking. Little is known about the regulation of meiotic transcription in other model systems or in humans.

While high-throughput approaches yielded major insight into transcriptional co-regulation of functions in cell cycle progression and gametogenesis, they produce a huge amount of information that is impossible for most life scientists and medical researchers to fully exploit without access to adequate bioinformatics tools. Development of user-friendly biological data storage and analysis solutions require standardized protocols and use of controlled vocabulary (ontology) that have begun to emerge only recently.

The yeast meiotic expression program

Dissecting the yeast meiotic transcriptional control network. In diploid yeast, nutritional and genetic cues trigger meiotic development yielding four haploid spores packaged in an ascus. The process depends upon two critical mitotic repressors of meiotic genes (Ume6 and Sum1), a general regulator (Abf1) and a meiosis-specific activator of middle and mid-late genes (Ndt80) all of which interact with specific target sites present in promoters that are induced during sporulation. Meiosis also depends on Ime1, a protein thought to control early meiotic gene activation via complex formation with Ume6. Significantly, the promoters of many meiotically up-regulated genes lack any of the known motifs for Ume6, Abf1 or Ndt80, implying that these loci are regulated by as yet unidentified factors (Schlecht and Primig, 2003). In a study with A. Amon (MIT, Boston), we investigated effects on meiotic transcription caused by spindle destabilization and meiotic arrest resulting from benomyl or low temperature (10°C) treatment. A novel spindle surveillance system arresting meiotic progression upon spindle destabilization was shown to cause induction of stress-response genes

Michael Primig

Phone +41 61 267 20 98
Fax +41 61 267 33 98

michael.primig@unibas.ch
www.biozentrum.unibas.ch/primig

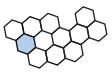
Group Members
Marie Cartron
Frederic Chalmel
Tzu-Ming Chern
Philippe Demougin
Faty Mahamadou
Leandro Hermida
Christa Niederhauser-Wiederkehr
Ulrich Schlecht
Thomas Walther
Gunnar Wrobel

Visiting Scientists
Rochelle Easton Esposito (The University of Chicago)

In Collaboration with
M. Hall (Biozentrum); P. Philippsen (Biozentrum);
A. Amon (Howard Hughes Medical Institute,
MIT Bosten); W. Wong (Harvard University);
R. Davis (Stanford University)

Admininstrative Assistants
Isabelle Steiger, Yvonne Steger

Member of the Swiss Institute of Bioinformatics
http://www.isb-sib.ch



and simultaneous down-regulation of nearly all meiotically induced genes independently of known spindle (Mad2) and recombination (Mek1) checkpoints (Hochwagen *et al*., 2005). Complementing this work on the regulation of meiotic transcription, we are also continuing our long-standing studies of the role of Abf1 in mitosis and meiosis (Prinz *et al.*, 1995). Abf1 (ARS binding factor 1) was originally isolated as a protein essential for DNA replication and later shown to participate in gene silencing, nucleotide excision repair, mRNA export and gene expression. The protein is thought to alter the chromatin structure of its target promoters rendering them accessible to other activators. However, the precise roles of Abf1 in transcriptional control of growth and development are still poorly understood. To learn more about Abf1 dependent gene regulation, we are carrying out an approach that includes expression profiling, a genome-wide protein-DNA interaction assay, binding site prediction and incorporation of protein-protein interaction data into the array data analysis procedure. We have identified many novel mitotic and meiotic Abf1 target loci involved in metabolic pathways, cytokinesis, mitotic and meiotic cell cycle progression, and spore development. Importantly, the upstream regions of genes down-regulated in the absence of functional Abf1 contain in most cases predicted Abf1 UAS target sites and the promoters are indeed often bound by the protein in vivo as assessed by genome-wide DNA binding assays (ChIP-Chip). Moreover, we find that Abf1 appears to regulate biological functions also via transcriptional control of proteins that physically interact with a large number of other factors (Schlecht *et al*., manuscript in preparation).

The core meiotic transcriptome in mammals

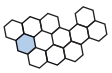
We recently reported (with B. Jégou, University of Rennes) the first large-scale expression profiling analysis of rat spermatogenesis (Schlecht *et al.*, Mol Biol Cell 2004). We next carried out a cross-species analysis of male germline transcription by analyzing GeneChip expression data from mouse and rat showing that data obtained in different labs using whole-organ and purified cell samples from rodents yield highly reproducible results (Wrobel and Primig, 2005). Based on this result we set out to identify the core protein-coding expression program of the mammalian male germline. We have now analysed purified germ cell samples and total testes controls from human, mouse and rat with whole-genome GeneChips in collaboration with B. Jégou (University of Rennes I) and D. Wolgemuth (Columbia University, New York). Hundreds of conserved genes showing highly reproducible transcriptional profiles across all three species were classified into somatic and germline expression clusters. The latter group contained known loci involved in meiosis, cell cycle progression, spermatogenesis or reproduction and many genes of unknown biological function. For these human loci, novel roles in male meiosis, gametogenesis and fertility are predicted by our data. These results will accelerate efforts to identify genes important for gametogenesis and they are likely to yield markers useful for the classification of reproductive pathologies related to meiotic failure (Rolland *et al.*, manuscript in preparation).

Microarray data management and analysis

Gene expression profiling is the most common application of microarrays and providing data via certified global repositories has now become mandatory for publishing in most scientific journals. To facilitate local array data management several solutions have been developed, but they cover different array platforms and often come with unintuitive graphical user interfaces. The key issue is that array data annotation (description) is a tedious process and no standardized ontology (controlled vocabulary) is yet available. Moreover, there is no integrated software suite that includes convenient functionalities specifically for GeneChip data storage and uploading to a certified repository as well as data analysis and interpretation. To address these important issues, we have developed the Microarray Information Management and Annotation System (MIMAS). The database is accessible at http://www.mimas.unibas.ch/ (Hermida *et al*., in press). Long-term maintenance of the project is ensured via a collaboration with V. Jongeneel (Swiss Institute of Bioinformatics; Vital-IT, http://www.vital-it.ch/, Lausanne).
MIMAS is being integrated with the certified public repository at the European Bioinformatics Institute (EBI) and with novel data mining tools such as GoCluster. The program was developed in our laboratory and reveals statistically significant enrichment of GeneOntology terms within groups of co-expressed genes obtained with a variety of clustering algorithms (Wrobel *et al.*, 2005).

A proto-type tool for community genome annotation

Classical approaches to knowledgebase development are based on the work of small groups of curators who process a rapidly increasing amount of information from the scientific literature. The large volume of data combined with the complexity of biological results means that some of the database



entries are incomplete, obsolete, and, on occasion, wrong. An important aim in the field of genome annotation is therefore to solicit contributions from life scientists who are asked to help keep entries complete and accurate. However, the established databases lack the appropriate submission/curation interface necessary for community annotation. With GermOnline we have developed the first such tool and we are carrying out a coordinated programme of large-scale cross-species community annotation organised according to research fields. In addition to providing a unique platform for biomedical research in germline development, GermOnline serves as a paradigm for knowledgebase development in other fields. A prototype server network is accessible via http://www.germonline.org/ (Primig *et al*., 2003; Wiederkehr *et al*., 2004a; Wiederkehr *et al*., 2004b).

In the future, we intend to integrate more array data and to expand the array platform types to include tiling arrays. Furthermore, once the necessary critical mass of core staff is obtained, we shall focus on gene annotation and information build-up with emphasis on cross-species description of conserved genes essential for the meiotic process. The data stored in GermOnline will be made available for downloading and computational analysis via the Internet.

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*these authors made an equal contribution.

Protein Structure Bioinformatics

The main goal of my group is to develop methods and algorithms to model, simulate and analyze three-dimensional protein structures and their molecular properties in order to apply these techniques to understand biological processes at a molecular level. Our current projects focus on three main areas:

Expert systems for comparative protein structure modelling

Three-dimensional protein structures are of great interest for the rational design of many different types of biological experiments, such as site-directed mutagenesis or structure-based discovery of specific inhibitors. However, the number of structurally characterized proteins is small compared to the number of known protein sequences; as of November 2005, more than 33'000 experimentally determined protein structures were deposited in the Protein Data Bank (PDB), while the UniProt protein knowledge database held more than 2 million sequences. Various computational methods for modelling three-dimensional structures of proteins have been developed to overcome this limitation. Since the number of possible folds in nature appears to be limited and the three-dimensional structure of proteins is better conserved than their sequences, it is often possible to identify a homologous protein with a known structure (template) for a given protein sequence (target). In these cases, homology modelling has proven to be the method of choice to generate a reliable three-dimensional model of a protein, as impressively shown in several meetings of the bi-annual CASP experiment. The huge amount of data originating from genome sequencing projects and structural genomics studies motivated the development of expert systems and fully automated methods which are sufficiently fast and reliable to generate accurate models on the scale of whole genomes.

The SWISS-MODEL server pioneered automated web-based comparative modelling. My group is developing and operating the SWISS-MODEL server as part of our activities within the Swiss Institute of Bioinformatics. Over the last 12 months, our server has treated more than 130'000 requests for individual 3-dimensional protein models. We have created the SWISS-MODEL Repository, a relational database of annotated protein models, which has been well accepted as a resource for model information. The aim of the SWISS-MODEL Repository is to provide access to an up-to-date collection of annotated three-dimensional protein models generated by automated homology modelling, narrowing the gap between sequence and structure databases. Currently,

Torsten Schwede

Phone +41 61 267 15 81
Fax +41 61 267 15 84

torsten.schwede@unibas.ch
www.biozentrum.unibas.ch/schwede

Group Members
Konstantin Arnold
James Battey
Nigel Brown
Lorenza Bordoli
Florian Kiefer
Jürgen Kopp
Flavio Monigatti
Michael Podvinec
Holmfridur Thorsteinsdottir

Visiting Scientists and Students
Tobias Kaillich

In Collaboration with
Nicolas Guex (GlaxoSmithKline R&D, Raleigh, USA); Manuel Peitsch (Novartis Pharma, Basel); András Aszódi (Novartis ISS, Vienna); Markus Meuwly (Department of Chemistry, University of Basel); Bernhard Erni (University Bern); Martin Spiess (Biozentrum Basel); Carlo Nicola (Fachhochschule Nordwest Schweiz); Mihaela Zavolan (SIB & Biozentrum University Basel); Vincent Zoete (SIB Lausanne); NITD Novartis Institute for Tropical Diseases, Singapore

Admininstrative Assistants
Isabelle Steiger, Yvonne Steger

Member of the Swiss Institute of Bioinformatics
http://www.isb-sib.ch

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Swiss-Model Repository holds 810‘000 three-dimensional protein models for 740‘000 different UniProt sequences. Since this year, SWISS-MODEL Repository is explicitly cross-referenced in the SwissProt and InterPro databases. In November 2005, after two years of development, we have released the next server generation, the SWISS-MODEL Workspace. It integrates programs and databases required for homology modelling in a web-based modelling environment, allowing the user to construct comparative protein models from a computer with web connection without the need for downloading and installing large program packages and databases.
Comparative modelling and structural genomics are complementing each other in the exploration of the protein structure space. In a comparative genomics analysis (collaboration with B. Erni, University of Bern), we are currently exploring the differences in the proteome between four bacterial species, *Escherichia Coli, Thermoanaerobacter tengcongensis, Thermotoga maritima* and *Aquifex aeolicus*, aiming to identify interesting target proteins for molecular characterization and experimental structure determination.

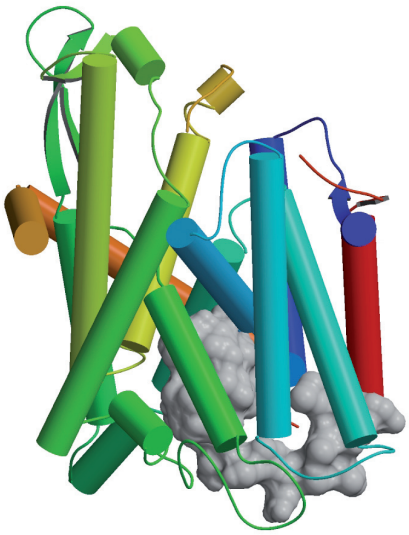

Figure 1: Comparative protein structure model of the yeast Sec61 hetero-trimer based on the archaeal SecY crystal structure from Methanococcus jannaschii. Residues forming the plug-domain are highlighted.

Comparative modelling applications in experiment design and drug discovery

Comparative protein modelling can support a variety of applications in life science research, such as the design of site directed mutagenesis experiments, the rationalization of the effects of sequence variations, or virtual screening and structure based drug design. We are collaborating with experimentalist and computational groups on specific practical applications.
Role of the plug domain of the Sec61p: Secretory and membrane proteins are sorted to the ER by signal sequences which upon insertion into the membrane are either cleaved or anchor the protein in the lipid bilayer. A conserved hetero-trimeric membrane protein complex, the Sec61 or SecY complex, forms a protein-conducting channel, allowing polypeptides to be transferred across or integrated into membranes. We have built a hetero-trimeric model for the yeast Sec61 translocon complex based on the experimental crystal structure of M. jannaschii SecY. In collaboration with Martin Spiess (Biozentrum Basel) this model was used to design site-directed mutations in Sec61p to study their influence on topogenesis. A designed deletion of the plug domain resulted in viable cells without growth defect, but showing effects on topogenesis.

Sequence variations and SNPs: Single nucleotide polymorphisms, or SNPs, account for the major part of inter-individual variation in the genome. The effects of genetic variations range from neutral mutations, through increased susceptibility for complex diseases and individual variation in drug response, to rare single-allele mendelian inherited diseases. Non-synonymous mutations located in protein coding regions, i.e. variations leading to an exchange of an amino acid, may possibly affect a protein’s stability or function. Since protein function strongly depends on the integrity of its three-dimensional structure, structural models of proteins can be used to analyze the functional role of the mutated residue and to provide insights into possible molecular consequences of mutations. We are currently performing a systematic analysis of pairs of protein structures with single point mutations to provide the basis for the development of suitable approaches for comparative modelling of SNPs.

Engineering of ATP-binding sites on protein kinases: Sequences of protein kinases are easily recognized by characteristic sequence motifs or homology with other kinases. Unfortunately, there is no comparable straight-forward approach to recognize the specific target of a protein kinase. The group of Kevan Shokat has introduced an elegant method to use protein design to engineer a functionally silent yet structurally significant mutation into the ATP binding site of the protein kinase. If this mutation is well designed, the protein functions similar to the wild-type enzyme with regard to the natural ATP substrate. However, due to the modified binding site, it may also accept

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chemically modified ATP radio-labelled derivatives, which act as non-specific inhibitors of the wild-type enzyme. We have used homology modelling to build a three-dimensional model for the protein kinase ATP binding pocket of human LKB1 based on the structure of murine cAMP dependent protein kinase and designed Shokat-type mutations (in collaboration with S. Antonarakis, University of Geneva). First analysis of the expressed protein show significant in vitro kinase activity for both ATP and the chemically N6-modified ATP analogue. Similar approaches in collaboration with other experimentalist groups (U. Meyer, Biozentrum, Basel; B. Hemmings, FMI, Basel) are currently in progress at different experimental stages.

Molecular modelling of protein-ligand complexes: Structure-based methods for drug discovery have made significant impact on the development of drugs in recent years. Several compounds discovered with the help of structural information have successfully passed clinical trials and have become approved drugs. One of the bottlenecks of structure-based methods is the availability of experimental structures of the target proteins. In these cases, protein structure homology models can provide a valuable alternative. The main concern of this approach is how errors and inaccuracies of the homology models influence the molecular modelling of the protein-ligand interaction. We have initiated a project (in collaboration with M. Meuwly, University Basel, and V. Zoete, SIB Lausanne) to explore the extent to which homology models can be used to study protein-ligand interactions. We have established a computational method for estimating the relative binding free energy of ligands in a model system (HIV-I protease) using restraint molecular dynamics methods (MD-GBSA) which correlates well with experimental data. By systematically introducing typical errors in the protein structure before simulation, we can explore the influence of inaccuracies of homology models on molecular modelling.

GRID computing as a platform for applied research in bioinformatics

Many approaches in computational biology are currently limited by the available computational resources. However, the combined theoretical computation power of Swiss scientific institutions is orders of magnitude larger than that available at each individual institution. We have developed a computational GRID infrastructure for bioinformatics applications which allows us to transparently compute ‚embarrassingly' parallel work units in a heterogeneous hardware and OS environment. We entered collaboration with several Swiss scientific centres (FMI, CSCS, SIB/VitalIT, FGCZ, Novartis) to form the „SwissBioGrid" initiative. The scientific application in our group which mainly benefits from this new infrastructure is virtual screening on Dengue viral target proteins. Dengue and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes. Dengue is primarily a disease of the tropics, and the viruses that cause it are maintained in a cycle that involves humans and Aedes aegypti, a domestic, day-biting mosquito that prefers to feed on humans. Infection with dengue viruses produces a spectrum of clinical illness ranging from a non-specific viral syndrome to severe and fatal hemorrhagic disease. We have entered a collaborative project which is aimed at the computational screening of small compounds available in public compound libraries to identify very early drug candidates, which could interact with molecular targets of the dengue viruses. We have selected the N-terminal domain of NS5 (RNA methyl transferase) as primary target, which is associated with the RNA capping reaction that puts a cap structure (7MeG5'-ppp5'-NMe) on the plus strand RNA genome and is crucial for the replicative cycle of the virus. Promising compounds arising from the virtual screening will be further analyzed *in vitro* by the Novartis Institute for Tropical Diseases (NITD, Singapore).

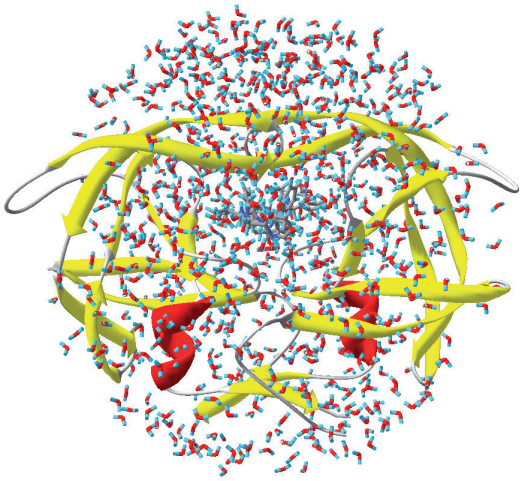
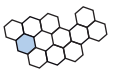


Figure 2: Structure model of a HIV-I protease dimer in complex with an inhibitor surrounded by water molecules. The relative binding free energy of different ligands can be estimated using restraint molecular dynamics MD-GBSA calculations.



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Principles of regulatory design

The proper functioning of a cell crucially depends on a network of complex regulatory systems that control when different proteins will be expressed, when and where they will be activated, what molecules are transported in and out of the cell, and so on. These regulatory systems have been designed by an evolutionary process that has taken billions of years and we currently understand very little about the way in which this evolutionary process has shaped the design of these regulatory systems. The main research interest of our group is the study of genome-wide regulatory systems, to reconstruct them from high-throughput molecular data, to understand and model how they have evolved, and to search for design principles in their construction.

The activities in our group revolve around the development and application of computational and analytical tools to study regulatory networks and their evolution. These studies can be roughly separated into three components. First, we are developing new algorithmic tools for the automated reconstruction of genome-wide regulatory networks from comparative genomic, ChIP-on-chip, and gene expression data. Second, we are developing methods for studying genome evolution and the evolution of regulatory networks in particular. Finally, we are studying the properties of the inferred regulatory networks and build evolutionary models that can account for the observed characteristics.

Genome-wide annotation of regulatory sites

An important research topic in my group in the last years has been the development and application of tools for the automated genome-wide annotation of regulatory sites based on comparative genomic, gene expression array, and ChIP-on-chip data. Existing motif finding methods either search for over-represented sequence motifs in a set of co-regulated sequences, or search for significantly conserved sequence segments in multiple alignments of orthologous intergenic regions. We have developed a novel algorithm, called PhyloGibbs, that combines these two approaches in a rigorous Bayesian frame work. The algorithm operates on collections of alignments of orthologous intergenic regions and searches through all possible ways in which an arbitrary number of binding sites for an arbitrary number of regulators can be assigned to the alignments. A probability is assigned to each such binding site configuration using an explicit mathematical model for the evolution of regulatory sites and nonfunctional DNA and taking into account the phylogenetic relationships of the species from which the sequences derive.

Erik van Nimwegen

Phone +41 61 267 15 76
Fax +41 61 267 15 84

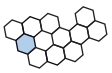
erik.vannimwegen@unibas.ch
www.biozentrum.unibas.ch/nimwegen

Group Members
Lukas Burger
Ionas Erb
Jose Ignacio Molina
Nicodeme Paul
Mikhail Pachkov

In Collaboration with
Eric D. Siggia (the Rockefeller University, New York); Michael Lenardo (NIH, Washington DC); Rahul Siddharthan (IMSc,Chennai); Piero Carninci (The RIKEN Institute, Tokyo)

Admininstrative Assistants
Isabelle Steiger, Yvonne Steger

Member of the Swiss Institute of Bioinformatics
http://www.isb-sib.ch



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We inferred the binding specificities for a large number of transcription factors in *Saccharomyces cerevisisia* by applying PhyloGibbs to a large ChIP-on-chip dataset in combination with the genomes of 5 recently sequenced *Saccharomyces* species. By comparing our results with known regulatory sites from the literature we can show that by combining information on evolutionary conservation with information on overrepresentation of sequence motifs, PhyloGibbs produces significantly more accurate results than existing methods. Since the ChIP-on-chip data have a high rate of false negatives, many functional sites are missed in this initial annotation. To remedy this, we have also developed a novel iterative procedure that takes the inferred motifs and scans the multiple alignments of all yeast intergenic regions for additional binding sites. Using this procedure we have produced a draft genome-wide annotation of regulatory sites in *S. cerevisiae* which contains over thirty thousand predicted binding sites for about 80 yeast transcription factors. In a collaboration with the group of Prof. Michael Primig we have been able to validate over 50% of the predicted binding sites for the factor ABF1 using independent ChIP-on-chip experiments. In addition, about 80% of the genes experimentally shown to be regulated by ABF1 have predicted binding sites in their promoter region.

We are now applying PhyloGibbs on a larger scale to produce genome-wide annotations of regulatory sites across many sequenced bacterial genomes. We have developed an automated procedure for identifying orthologous genes and producing syntenic alignments of the genomes of related bacteria. The automated procedure also reconstructs the phylogenetic tree relating the species and produces alignments of all orthologous intergenic regions between the species. We then run PhyloGibbs on these alignments to predict conserved binding sites genome-wide. We are validating our procedure using known sites in *E. coli* and *B. subtilis*. This procedure allows us to annotate regulatory sites in a large number of bacterial species for which virtually nothing is currently known about transcription regulation.

Finally, to disseminate our predictions to the scientific community worldwide we have developed a web-based database and genome browser (http://www.swissregulon.unibas.ch) that contains all our annotations and that allows users to browse the predicted regulatory sites on the genome.

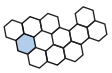
Computational reconstruction of bacterial signaling networks

Two-component systems are a large family of bacterial proteins that are responsible for a significant portion of signal transduction in bacteria. Each two-component system consists of a pair of proteins, with one containing a histidine kinase domain and the other containing a receiver domain. Although many bacteria have tens of such two-component systems with structurally highly similar kinase and receiver domains, the phospho-transfer is almost always highly specific, with each kinase showing a strong preference for phosphorylating its cognate partner receiver.

Our aim is to develop a computational model that is capable of predicting which kinase interacts with which receiver using only their amino acid sequences. Two-component systems are a very attractive model system for such a program because of several unique features they possess. First, all kinases and receivers exhibit a high degree of sequence similarity and are believed to fold into the same or very similar three dimensional structures. Second, thousands of two-component systems can be recognized in sequenced genomes, and their sequence similarity allows us to make accurate multiple alignments of all kinases and receivers. Finally, for a significant fraction of all two-component systems the cognate kinase/receiver pair is encoded by neighboring genes in a common operon on the genome. Using this large training set of cognate kinase/receiver pairs, we first identified pairs of positions in the proteins that show significant correlations in the amino acids that occur at these positions. We then created a probabilistic model for the joint distribution of amino acids at all correlated positions that allows us to distinguish interacting kinase/receiver pairs from non-interacting pairs. Preliminary results show that our model can accurately predict which pairs interact. In particular, our model allows us to predict interaction partners genome-wide for “orphan” kinases and receivers that occur in operons by themselves. Finally, by predicting all interaction pairs genome-wide we have started to reconstruct genome-wide signaling networks in bacteria, and this allows us to study the structural properties of these signaling networks.

Regulation and noise in alternative splicing

In collaboration with the group of Prof. Mihaela Zavolan and the RIKEN institute in Japan we have been studying alternative splicing in the mouse genome. The first aim of this project was to get an accurate



account of alternative splicing in mouse, to classify the types of alternative splice events, and to quantify the prevalence of different types of splice variation. After this we studied how and to what extent these splice variations are regulated versus just the result of noise in the splicing process, and we investigated to what extent the splice variations appear to be functional.

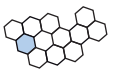
In order to identify splice variations we used genome alignments of the large FANTOM datasets of full-length cDNAs produced by the RIKEN institute. We found, however, that existing spliced alignment algorithms for mapping cDNAs to the genome did not reach the required level of accuracy for an accurate accounting of the splice variation evident in the data. In particular, existing algorithms often failed to map the starts and/or ends of the transcripts, and there were often inaccuracies in the placement of the splice boundaries. We therefore decided to develop a new alignment algorithm, called SPA for Spliced Alignment, which implements a Bayesian probabilistic approach. The main novel feature of our algorithm is that it uses explicit probabilistic models of gene structures and sequencing errors. This allows us to correctly trade-off the likelihood of different gene structures against the likelihoods of different sequencing errors. In addition, our algorithm uses a more sophisticated model of splice boundaries that allows us to correctly identify non-canonical splice boundaries, and even novel types of non-canonical splice boundaries. Application of SPA to the RIKEN FANTOM3 set of over 100,000 full-length cDNAs shows that SPA significantly improves on existing methods, in particular in the mapping of the 5' and 3' ends of the transcripts.

To study the contributions of noise and regulation in alternative splicing we have studied the two most common forms of splice variation; "cassette exons" that occur in some but not all transcripts, and small exon-length variations at acceptor and donor sites. Our studies show that the in- or exclusion of cassette exons is often dependent on the promoter that was used to initiate the transcript. This result suggests that exon inclusion might often be regulated at the transcriptional level. In addition, the data suggests that alternative exon inclusion events are highly biased to avoid frame-shifts from occurring, with different cassette exons of the same gene often showing correlated in- and exclusion so as to conserve the reading frame. This suggests that many of the cassette exon splice variations may be functional. In contrast, our analysis of the exon-length variations at acceptor and donor sites strongly suggests that these common splice variations are the result of noise in the splicing process. In particular, using a large number of exon boundaries that do not show splice variation, we built weight matrix models of the sequence-specificity of the spliceosomes at acceptor and donor boundaries. Using these weight matrices, we showed that the observed exon-length variations can be accurately explained by a simple physical model in which the spliceosome binds stochastically to neighboring splice sites with similar affinity.

Modeling the dynamics of cell-cultures

In modeling the dynamics of even very simple biological systems one is often faced with very incomplete information about the underlying components, reactions, and their kinetic parameters. The challenge in such situations is to still infer quantities of interest from this incomplete information. In collaboration with Michael Lenardo at the NIH in Washington DC, we developed a novel mathematical approach to such problems in the context of inferring key parameters of cell-culture dynamics from a set of measurements.

Lenardo's group has shown that the death of T-cells infected with HIV is not the result of apoptosis but rather necrosis, and that this cytopathicity is dependent on the expression of proteins from the HIV genome. The aim of our collaboration was to estimate the cytopathicity of different HIV deletion strains in order to identify the HIV genes involved in T-cell killing. We obtained time courses of infectivity (fraction of cells infected) and viability (fraction of life cells) in cultures of T-cells infected with different HIV strains. Our new approach was to fit these time courses to a set of coupled differential equations in which all complexities of the dynamics were subsumed into time-dependent 'effective rates'. Using these equations we were able to derive time-dependent upper and lower bounds on the cytopathicity of the different strains. This in turn allowed us to determine that the VPR gene makes an important contribution to the cytopathicity of HIV.



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Computational analyses of gene expression regulation

Recent probing of mammalian gene expression using high-throughput techniques revealed that much of the diversity of cell types in multi-cellular eukaryotes is due to the complex patterns of expression of gene structures. Most genes initiate transcription from multiple promoters, have complex patterns of splice variations and multiple poly-adenylation sites, and antisense transcription appears to be common. An entirely new form of gene expression regulation has been discovered and shown to involve small RNA molecules. The proportion of regulatory RNA genes in the human genome is now estimated to be at least a few percent of all genes in the genome, and their number is still growing. Computational methods, some developed in my group, have been instrumental in the identification and annotation of genes, splice variants and targets of regulatory RNAs.

Regulated and stochastic expression of splice forms

Since 60-70% of the human and mouse genes appear to have multiple splice forms, understanding the regulation of alternative splicing is key to understanding how mammalian cells differentiate and adapt to various environments. To take advantage of the vast amount of transcript data in the discovery of splice regulatory signals, we have developed an automated pipeline that maps EST and cDNA sequences to their genome with high accuracy and identifies alternative splice forms. The program for the cDNA-to-genome alignment was recently finalized through a collaboration with the group of Erik van Nimwegen. As a first application, we have analyzed the set of over 100,000 full-length mouse cDNAs that has been released this year by the Riken Institute in Japan.

We focused on the two most common forms of splice variation: cryptic exons (exons that are included in some transcripts and skipped in others) and small variations in exon length due to changes in the acceptor splice site. Our first goal was to determine whether the inclusion/exclusion of internal exons depends on the promoter from which the transcript was initiated. We focused specifically on transcripts that have been initiated from promoters that were independently validated using CAGE (cap analysis of gene expression) analysis, that was also performed by the Riken group. Our conclusion is that the inclusion of at least 5% of internal cryptic exons depends on the promoter from which the transcript was initiated. This is the first large scale computational study that provides statistical evidence for the coupling between transcription and splicing.

Mihaela Zavolan

Phone +41 61 267 15 77
Fax +41 61 267 15 84

mihaela.zavolan@unibas.ch
www.biozentrum.unibas.ch/zavolan

Group Members
Tzu-Ming Chern
Dimos Gaidatzis
Jean Hausser
Nicodeme Paul
Mirabela Rusu
Alain Sewer

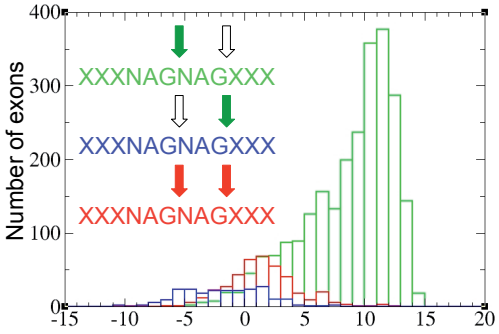
In Collaboration with
Jean Pieters (Biozentrum); Tom Tuschl Rockefeller University); Witek Filipowitz (FMI); Helge Grosshans (FMI); Nikolaus Rajewsky (New York University)

Admininstrative Assistants
Isabelle Steiger, Yvonne Steger

Member of the Swiss Institute of Bioinformatics
http://www.isb-sib.ch

Mihaela Zavolan

The second question that we addressed concerned what are now called NAGNAG acceptor sites. These are adjacent splice acceptor sites that can both be used in splicing, causing 3-nucleotide variations between the transcripts that harbor them. A few years ago, we described these variations as the most common exon length variations in mouse, and more recent studies argued that they are subject to selection pressure and play an important role in fine-tuning the proteome. We built a model of the sequence specificity of the factors that bind at the splice acceptor site, and we showed that NAGNAG sequences at which we observed splice variation can be distinguished from those that do not by the likelihood of the two neighboring candidate splice sites. Furthermore, analysis of the frequency of NAGNAG splice boundaries in protein coding and non-coding exons, as well as of the evolutionary conservation of the variant and invariant NAGNAG splice boundaries suggest that variant NAGNAG motifs are not under any specific selection pressure, but are most likely the results of neutral evolution.



Log-likelihood difference between the first and the second putative acceptor sites

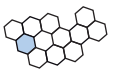
As shown in the figure above, most of the invariant exons that exhibit the NAGNAG sequence at the acceptor site use the first NAG site as acceptor, and this site has indeed a much higher likelihood than the second site (green). In contrast, the likelihood of the two sites is very similar for the exons that undergo alternative splicing (red). Thus, it appears that splice variation at NAGNAG sites occurs whenever evolution generates putative acceptors with comparable likelihoods, and at this point there is no evidence that a specific selection pressure operates to generate such sites. To continue our studies of the mechanism and implication of splice variation at NAGNAG sites, we have teamed up with the group of Stefan Stamm at the University of Erlangen, who will be testing our hypotheses using in vitro splicing assays and the group of Torsten Schwede at the Biozentrum, with whom we will be studying the impact of these single amino acid changes on protein function.

Identification of regulatory RNAs and of their mRNA targets

Small single-stranded RNAs exert a wide range or regulatory functions, from degradation and translational silencing of target mRNA, to transcriptional silencing of transposable elements and chromatin remodeling. We have collaborated for a number of years with the group of Thomas Tuschl at the Rockefeller University on projects aimed at discovering small regulatory RNAs. In the past year we have completed a study on virus-encoded miRNAs, we have characterized the miRNA profile of the zebrafish model organism, and we are in the process of releasing a large data set of over 400,000 small RNA sequences cloned from human, mouse and rat. This is the most comprehensive mammalian miRNA profiling study to date.

We are continuing to develop methods for predicting miRNA genes in single genomes. While we have initially used this methods to identify viral miRNAs, we have started to focus on mammalian genomes, and we were able to identify over 60 novel miRNA located within 10 kilobases of known miRNAs. This, as well as other prediction studies that appeared in the past year, indicate that the number of miRNA genes is much larger than initially anticipated.

The most important open question in the field of miRNA research is what mRNAs are being targeted by miRNAs. We have implemented and experimented with various methods for miRNA target prediction, and we just finalized a target prediction algorithm that uses the number and evolutionary conservation of putative target sites in a probabilistic framework to make predictions about specific miRNA-mRNA interactions. Based on the results that we have already obtained, we find an association between strongly conserved putative miRNA binding sites and various known A/U-rich elements that are known to bind various proteins involved in modulating mRNA stability. We are now initiating collaborations with experimental groups within the Biozentrum and the Friedrich Miescher Institute to test specific predictions of target genes and the motifs involved in miRNA-dependent translational regulation.



Mihaela Zavolan

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Core Program
Structural Biology & Biophysics

Goals

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 dynamic network of a living system.

It is our clear perspective that the understanding of biological function goes beyond a pure static, structural description and that additional information is needed from other biophysical techniques. In all of the research areas, 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. The structural techniques 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 and stopped-flow optical spectroscopies, 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 dynamic changes starting from picosecond time scales to very long periods, and to determine the energetics and thermodynamics of biomolecular interactions at very high precision.

Strong synergisms exist with all other research areas of the Biozentrum since many of the systems are shared projects with other biochemically or biologically oriented groups.

Teaching

Knowledge of structural principles of biomacromolecules and 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 at various levels by all members of the Core Program.

Professors
Ueli Aebi, Andreas Engel, Jürgen Engel*, Stephan Grzesiek, Thomas Kiefhaber, Dagmar Klostermeier (starting in 2006), Olga Mayans, Tilman Schirmer, Joachim Seelig

University Lecturers
Peter Burkhard*, Birthe Fahrenkrog*, Heiko Heerklotz, Cora-Ann Schoenenberger, Anna Seelig, Jörg Stetefeld, Sergei Strelkov*

Scientists and Postdoctoral Fellows
Thomas Ahrens*, Thomas Anderson*, Annett Bachmann, Werner Baschong, Arnaud Baslé*, Simon Bernèche*, Christophe Bodenreider, Sergei Boudko*, Mohamed Chami, Carmen Chan*, Florence Cordier*, Maria Dolores Crespo*, Sonja Dames, Myriam Duckely, Luminita Duma*, Birthe Fahrenkrog*, Patrick Frederix, Dimitros Fotiadis, Ewa Gatlik-Landwojtowicz, Elisabete Gonçalves*, Alexandra Graff*, Gernot Hänisch, Zora Housley-Markovic, Erik Hedboom*, Bart Hoogenboom, Ningping Huang*, Pernille Jensen, Larisa Kapinos Schneider*, Götz Kohler, Joachim Köser*, Laurent Kreplak, Frank Küster*, Roderick Lim*, Gia Machaidze*, Bohumil Maco, Marco Marino, Markus Meier*, Sebastian Meier*, Barbara Mouratou, Shirley Müller, Suat Özbek*, Dinesh Vellore Palanivelu*, Caroline Peneff-Verheyden, Ansgar Philippsen, Renate Reiter*, Hervé Remigy, Philippe Ringler, Hans-Jürgen Sass, Manuela Schätzle*, Bernhard Steinbauer*, Jörg Stetefeld*, Daniel Stoffler*, Martin Stolz, Mark Strohmeier*, Sergei V. Strelkov*, Anmin Tan*, Nicole Taschner*, David Tropel, Paul Werten, André Ziegler*

Graduate Students
Päivi Äänismaa, Teiba Al-Haboubi*, Martin Allan, Cedric Berger*, Melanie Börries*, Rainer Bucher, Fabio Casagrande*, Carmen Chan*, Arundhati Chattopadhyai*, Nora Eifler, Beat Fierz, Pilar Garcia Hermosa, Grégori Gerebtzoff, Marco Gregorini, Sarah Güthe*, Sergei Ivaninskii*, Karin Joder*, Thomas Kaufmann, Gabriela Klocek*, Florian Krieger*, Wanda Kukulski, Senthil R. Kumar, Stefan Langheld, Leticia Lemus*, Sonja Lorenz*, Christian Meesters*, Markus Meier*, Matthias Meier*, Sebastian Meier*, Andreas Möglich, Michael Mrosek, Stefan Nicolet*, Sivaraman Padavattan, Sara Paulillo, Marija Plodinec*, Elena Pokidysheva*, Manuela Schätzle*, Andreas Schenk, Ulrich Schröder*, Gian-Andrea Signorell, Kyrill Schwarz-Herion, Alekos Tsamaloukas, Navratna Vajpai*, Dinesh Vellore Palanivelu*, Paul Wassmann*, Andreas Wild, Christophe Wirth*

Core Program
Structural Biology & Biophysics

Undergraduate lectures are in part also attended by students of Pharmaceutical Sciences, Chemistry, Physics and Nano-Science.

New recruitment/Promotions/Awards/Highlights

At the beginning of 2006, the core program was further strengthened by Dagmar Klostermeier (formerly University of Bayreuth, Physics Department), who started as a newly appointed tenure track assistant professor (succession of Jürgen Engel). Her research focuses on single molecule and time-resolved fluorescence measurements of the dynamics of molecular motors.

In 2005 Drs. Birthe Fahrenkrog and Sergei Strelkov successfully passed their “Habilitation”.

At the end of 2005, Sergei Strelkov left the Biozentrum to accept an Associate Professorship at the Catholic University of Leuven, Belgium.

Heiko Heerklotz received the Stig Sunner Memorial Award for the year 2005 at the 59th Calorimetry Conference in Santa Fe, USA.

Joachim Seelig received the Avanti Lipid Award for the year 2005 at the Annual Meeting of the Biophysical Society, USA.

Stephan Grzesiek received the Laukien Prize for the year 2005 at the 46th Experimental Nuclear Magnetic Resonance Conference (ENC) in Providence, USA.

The EMBO Practical Course ‘Structure determination of biological macromolecules by solution NMR’ was organized by Stephan Grzesiek from September 7 to 14, 2005 at the Biozentrum.

Two group leaders (Ueli Aebi and Andreas Engel) are also project leaders in the National Centers of Competence in Research (NCCR) programs “Structural Biology” (AE) and “Nanoscale Science“ (UA and AE). As of June 2005, the latter has been renewed and funded for another four years.

External support by the M.E. Müller Foundation

The core program receives generous support from the M.E. Müller Foundation via the fully integrated M.E. Müller Institute for Structural Biology (research groups Ueli Aebi and Andreas Engel). The M.E. Mueller Foundation has agreed to extend this support until the end of 2011, amounting to a financial commitment of more than CHF 40 Mio. over a period of 25 years.

Master/Diploma Students
Regula Aregger*, Roger Benoit*, Fabio Casagrande*, Karin Joder*, Robert Kübler*, Martin Schaad*, Eleonore von Castelmur*, Daniel Vonwil*, David Walter*

Guest Master/Diploma and Graduate Students
Karin Doolewerdt*, Samuel Egli*, Rina Geissbühler*, Riccardo Gottardi , Stamatios Liokatis*, Johan Hebert*, Florian Rost*

Laboratory Technicians
Françoise Erne-Brand*, Grazyna-Maria Gantenbein*, Peter Ganz, Ruth Landwehr*, Xiaochun Li Blatter, Ariel Lustig*, Alexandra Meli*, Gianni Morson*, Rita Müller, Vesna Olivieri, Klara Rathgeb-Szabo, Marco Rogowski, Dietrich Samoray, Therese Schulthess, Kitaru Suda, Lazar Sumanovski, Rosmarie Sütterlin, Halina Szadkowski, Zöhre Ucurum Fotiadis, Joseph Wey

Floor Managers
Beat Schumacher, Hans Vogt

Technical Staff
Roland Bürki, Leonardo Falletti, Gernot Hänisch, Robert Häring*, Margrit Jenny, Simon Saner, Robert Wyss

Trainees
Vanessa Cardinali*, Math Cuajunco*

Laboratory Helpers
Beatrix Lang, Barbara Merz

Administrative Assistants
Ute Grütter, Sarah Güthe*, Brigitte Heiz Wyss*, Nicole Furler*, Debbie Neyer, Susanna Notz, Liselotte Walti

* employed during part of the report period

Structure-based functional analysis of cellular nanomachines by a hybrid methods approach

The long-term goal of the lab is to mechanistically understand the formation, turnover and functioning of cellular nanomachines, and more generally, supramolecular assemblies. This ambitious goal is achieved by a structure-based experimental approach which employs hybrid methods such as light, electron and scanning probe microscopies, X-ray crystallography, biophysics, biochemistry, and molecular cell biology. Being problem-driven, the lab focuses on (1) cytoskeleton structure, assembly, turnover and mechanics; (2) the nuclear pore complex and nucleocytoplasmic transport; (3) oligomerization and polymerization of amyloid forming peptides and how these spatially and temporally correlate with disease progression; and (4) developing and implementing novel optical and mechanical nano-sensors and nano-actuators for local diagnosis and therapy by minimally invasive interventions.
In addition to the research which is directed by U. Aebi, his lab hosts three groups headed by independent project leaders which have in part their own funding and collaborate on various projects with members of the Aebi group. PD Dr. Cora Schönenberger's main focus is on new forms and functions of actin in the nucleus, and on novel regulatory mechanisms controlling cardiac function; PD Dr. Birthe Fahrenkrog analyzes nuclear pore complex proteins in nucleocytoplasmic transport and other cellular processes, such as yeast apoptosis; and PD Dr. Sergei Strelkov employs X-ray crystallography and small-angle X-ray scattering (SAXS) to dissect the structural organization of intermediate filaments (IFs) at atomic detail to ultimately rationally understand IF-based human diseases. The progress of their projects is presented in independent reports. Following are a few selected results from the Aebi group.

Assessing the mechanical behavior of single intermediate filaments by scanning force microscopy

Despite their importance in determining cellular architecture and plasticity, few data are available on the mechanical properties of intermediate filaments (IFs) in vitro or within cells and tissues. In this context, Laurent Kreplak, a postdoc in the lab, has employed a scanning force microscopy (SFM) based protocol to directly probe the mechanical properties of single cytoplasmic IFs when adsorbed to a solid support in a physiological buffer environment. Depending on the experimental conditions chosen, the SFM tip was used to laterally displace or stretch single IFs on the support to which they had been adsorbed. Upon applying force, IFs were stretched on average 2.6 fold before breaking, with a maximum of 3.6 fold.

Ueli Aebi

Phone +41 61 267 22 61
Fax +41 61 267 21 09

ueli.aebi@unibas.ch
www.biozentrum.unibas.ch/aebi

Group Members

Werner Baschong
Riccardo Gottardi
Philip Haas
Ning-Ping Huang
Joachim Koeser
Laurent Kreplak
Roderick Lim
Ariel Lustig
Gia Machaidze
Bohumil Maco
Gianni Morson
Marija Plodinec
Tobias Reichlin
Daniel Stoffler
Martin Stolz
Rosmarie Sütterlin
Andreas Wild

Visiting Scientists

Dr. Nicole Foeger (DKFZ Heidelberg, Germany); Dr. Daphna Frenkiel-Krispin (Ben Gurion University, Ber-Sheva, Israel); Roberto Raiteri (University of Genova, Italy)

In Collaboration with

M. Dürrenberger, B. Fahrenkrog, O. Mayans, S.Müller, U. Sauder, C.-A. Schoenenberger, A. Seelig, J. Stetefeld and S.V. Strelkov (Biozentrum, Basel); T. Arvinte (Therapeomic AG, Basel); A. Aszodi and R. Fässler (Max-Planck Institute for Biochemistry, Martinsried, Germany); P. Frey (Novartis Pharma AG, Basel); P. Burkhard (University of Connecticut, Storrs, USA); G. Cooper and J. Kistler (University of Auckland, New Zealand); A.U. Daniels, P. Hunziker and I. Martin (University Hospital, Basel); J. Deng and K.H.A. Lau (Institute of Materials Research and Engineering (IMRE), Singapore); L. Forro (EPFL, Lausanne); N. Friederich (Bruderholzspital, BL); D. Goldman (Northwestern University Medical School, Chicago, IL, USA); C. Goldsbury and E. Mandelkow (DESY, Hamburg, Germany); Y. Gruenbaum (Hebrew University, Jerusalem, Israel); B. Hecht, M. Hegner and Y. Lill (Physics Department, University of Basel); H. Herrmann, J. Langowski and N. Mücke (DKFZ, Heidelberg, Germany); E. Hurt (ZBH, Heidelberg, Germany);

Ueli Aebi

Concomitant with this stretching, a large reduction of the apparent filament diameter. Exhibiting such mechanical properties which are quite distinct from those revealed by actin filaments or microtubules, suggests that in vivo IFs may serve as 'mechanical shock absorbers' or a 'security belt' in tissues exposed to extreme mechanical stress.

Tinkering with a cellular nanomachine - the nuclear pore complex

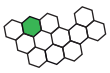
Natively unfolded phenylalanine-glycine (FG)-repeat domains which are grafted to about 30% of the nucleoporins, are alleged to form the physical constituents of the selective gate in nuclear pore complexes (NPCs) during nucleocytoplasmic transport (NCT). To provide a more mechanistic insight into the barrier mechanism controlling NCT, Roderick Lim, a postdoc in the lab, has designed an experimental platform to study surface-tethered FG-repeat domains at the nanoscale which mimics the general physical dimensions of NPCs. Scanning force spectroscopy measurements indicate that the collective behavior of such FG-repeat domains gives rise to a long-range exponentially decaying repulsive force in physiological buffer. The measured forces are appropriately described by polymer scaling theory which suggests that the molecules are thermally mobile and exist in an extended polymer brush-like conformation. This assertion is strengthened by observing that the brush-like conformation undergoes a reversible collapse transition in less polar solvent conditions. Taken together, these findings reveal how FG-repeat domains may simultaneously function as an entropic barrier and a selective trap in the near-field of native NPCs. Rod is now probing these ‚minimalist pores' with functionalized SFM tips, for example, with importin-beta, BSA, etc. As expected, importin-beta tips experience an attractive force, whereas BSA tips, i.e. representing a ‚passive' cargo, feel a repulsive force. Ultimately, by combining our ‚minimalist pores' with a two-compartment system harboring RanGTP in one compartment and RanGDP in the other, these should achieve receptor-mediated vectorial transport of signal-bearing (NLS or NES) cargos and, at the same time, act as a barrier to ‚passive' cargoes.

Multiple assembly pathways underlie amyloid-beta fibril polymorphism

The amyloid-beta protein transiently forms low and high molecular mass oligomers and protofibrils in vitro, and after longer incubation times assembles into polymorphic mature fibrils. The precursor-to-

A.V. Kajava (CNRS FRE-2593, Montpellier, France); J.-P. Kong and T.-T. Sun (NYU Medical Center, New York, NY, USA); R.H. Laeng (Kantonsspital Aarau); T.M. Magin (University of Bonn, Germany); L.N. Marekov, P.M. Steinert and A.C. Steven (NIH, Bethesda, MD, USA); O. Medalia (Ben Gurion University, Ber-Sheva, Israel); A.A. Noegel (University of Cologne, Germany); T. Pederson (University of Massachusetts Medical School, Worcester, MA, USA); J.-C. Perriard (ETHZ Zürich); U. Staufer (Institute of Microtechnology, University of Neuchâtel); M. Steinmetz (PSI Villigen); M.R. VanLandingham (Army Research Laboratory, Aberdeen Proving Ground, MD, USA)

Administrative Assistant
Liselotte Walti



product relationship of these species remains to be understood. Protofibrils are up to ~600 nm in length and have mass-per-lengths (MPLs) of 19±2 kDa/nm measured by scanning transmission electron microscopy. Two predominant mature fibril types, several microns in length and with MPLs of 18±3 and 27±3 kDa/nm, are identified after longer incubation times. The difference of ~9 kDa/nm between the two fibril types indicates a bona fide elementary protofilament subunit of this MPL. Fibrils in the 18±3 kDa/nm group often exhibited distinct coiling with axial cross-over spacings of ~25 nm. Although strikingly different in morphology, the MPL of these coiled fibrils is equivalent to that measured for protofibrils. They could therefore arise from a conformational change in the protofibril concurrent with coiling and rapid elongation. Alternatively, we cannot rule out an assembly pathway not directly related to protofibrils. In contrast, the 27±3 kDa/nm fibrils correspond to a MPL of 1.5x the protofibril and thus can neither arise from a simple conformational transition nor from lateral association of 19 kDa/nm protofibril precursors. Twisted ribbons with axial periodicities ranging from ~80 nm to 130 nm were prominent in the 27±3 kDa/nm group as well as more tightly coiled fibrils. Individual fibril ribbons had elongation rates of 20±12 nm/min when imaged by time-lapse scanning force microscopy. Protofibrils exhibited growth rates ~15x slower at 1.3±0.5 nm/min. These data support a model where concurrent multiple assembly pathways give rise to the various polymorphic fibril types.

Exploring mouse articular cartilage biomechanics by nano-indentation

In our attempts to more systematically probe the biomechanical properties of articular cartilage and to correlate these with the status of slowly progressing diseases such as osteoarthritis, Martin Stolz, a senior research associate in the lab, has employed indentation-type (IT) to follow the mechanical changes of the articular cartilage in the hips of strain-matched mice as a function of aging. As expected, while the articular cartilage layer gets thinner as the animals get older, the cartilage becomes stiffer when probed at the sub-micrometer scale. Concomitantly, by chemical analysis a systematic reduction of the glycosaminoglycan moiety is observed in the aging cartilage. While both changes are statistically significant, they are not as pronounced as they become in osteoarthritic cartilage observed in human biopsies. Very much like with porcine and human articular cartilage, the measured stiffness changes are strongly scale-dependent, i.e. they are only spotted when probed at the sub-micrometer but not at the micrometer to millimeter scale. Last but not least, we are in the process of developing IT SFM into a standardized procedure for rapid and reliable assessment of the mechanical quality of autologous and tissue-engineered cartilage aimed for transplantation into joint defects.

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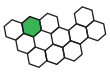

Ueli Aebi

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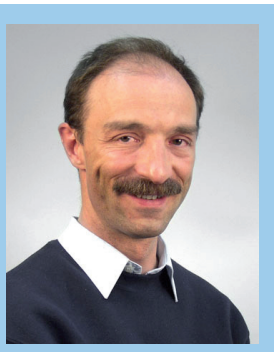
Nano-Biotechnology and Protein De Novo Design

Design of Peptide Nanoparticles

Artificial particulate systems such as polymeric beads and liposomes have many applications in drug delivery, drug targeting, antigen display, vaccination, and other technologies. We have recently designed a novel type of nanoparticle with regular icosahedral symmetry and a diameter of about 16 nm, which self-assembles from single polypeptide chains.
Remarkably, small viruses are composed of one single peptide chain folding into a capsid structure with icosahedral symmetry (Fig. 1a). By a superposition of different protein oligomerization domains onto the symmetry axes of the icosahedron, a nanoparticle with icosahedral symmetry can be designed (Fig. 1b,c). In agreement with the computer model, the electron micrographs of our designed nanoparticles show a diameter of about 18nm (Fig 1c,d). As shown in the schematic diagram (Fig 1e) such peptide nanoparticles can be functionalized in several ways:

(1) At either end the peptide chain can be extended with peptide sequences of a particular function. We have attached targeting peptides like somatostatin (Fig 1d) or bombesin to the nanoparticle. Such nanoparticles will interact with their respective receptors on cancer cells. As an advantage, the high density of ligands on the surface of the particle will increase the affinity for the specific receptor due to cooperativity of binding. Other functional peptides that can be attached include e.g. NLS sequences or cell penetrating peptides like TAT. By means of covalent linkage of specific dyes (fluoresceine), chelators (DOTA - for the binding of radionuclides for radiotherapy), or drugs can be attached at the ends of the peptide. The high density of dyes will significantly increase the signal to noise ratio in imaging applications.
(2) The central cavity of the nanoparticle with a diameter of about 6 nm is ideally suited for the encapsulation of quantum dots. For example, SeCd nanocrystals can be used as highly efficient fluorescence probes, gold nanoparticles as contrasting agents for electron microscopy and iron nanoparticles as probes for MRI imaging.
(3) The cavity of the pentameric coiled-coil domain allows to incorporate small hydrophobic molecules such as vitamin D3.
(4) Finally, the trimeric coiled-coil can be modified to allow binding of nucleic acids (like the trimeric coiled-coil of the macrophage scavenger receptor) and ultimately a gene delivery system can be engineered.



Peter Burkhard

Phone +41 61 267 20 91
Fax +41 61 267 21 09

peter.burkhard@unibas.ch
www.biozentrum.unibas.ch/burkhard

Group Members
Arundhati Chattopadhyay
Alexandra Graff
Sergei Ivaninskii
Senthilkumar Raman
Markus Meier
David Tropel

In Collaboration with
U. Aebi (Biozentrum); M. Bachmann (Cytos Biotechnology AG, Schlieren, CH); J. Cole (Univ. of Connecticut, Storrs, USA), P. Cook (Univ. of Oklahoma, Norman, USA); H. Herrmann (DKFZ, Heidelberg, Germany); R. Hodges (UCHSC, Denver, USA); D. Lanar (WRAIR, Silver Spring, USA); J. P. Kraus (UCHSC, Denver, USA); A. Mozzarelli (Univ. of Parma, Parma, Italy); F. Papadimitrakopoulos (Univ. of Connecticut, Storrs, USA); Cora-Ann Schönenberger (Biozentrum); J. Stetefeld (Biozentrum); T.A.P. Seery (Univ. of Connecticut, Storrs, USA)

Peter Burkhard

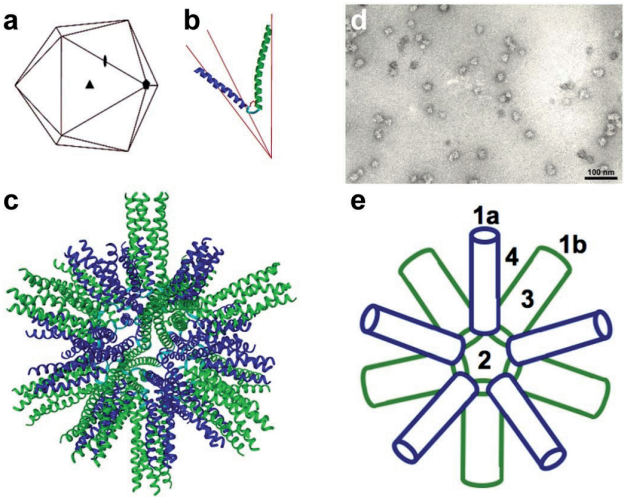


Figure 1. Design of peptide nanoparticles
a) Icosahedron with the symbols of the three different symmetry elements
b) Monomeric building block composed of a trimeric (blue) and pentameric coiled-coil-α-helix
c) Model of an assembled nanoparticle with icosahedral symmetry
d) Electron micrograph of the peptide nanoparticles functionalized with somatostatin
e) Schematic diagram of the particle with possible modification sites (see text)

Nanoparticles for Vaccine Design

The geometry of the nanoparticle and its resemblance to small viruses, will trigger a strong immune response based on repetitive antigen display; a concept that now is increasingly exploited for producing novel vaccines that yield high titers of specific antibodies by using virus-like-particles as repetitive antigen display systems. Such de novo designed nanoparticles eliminate the need for virus-based designs, and allow for high flexibility in vaccine design. Epitopes of any pathogen can readily be engineered onto the surface of the nanoparticle, thus allowing for the easy generation of a whole variety of different vaccines.
We are currently developing synthetic vaccine candidates against several major human pathogens. Immunogenic peptide sequences characteristic for these pathogens will be displayed on the surface of a peptide nanoparticle. In addition to its ability to elicit a high titer of serospecific neutralizing antibodies, the particulate structure will guide the nanoparticles to antigen presenting cells and induce CD4 proliferative responses and cytotoxic T lymphocytes, thus inducing long-term immunologic memory.
Using recombinant protein expression, we are now also designing nanoparticles presenting the epitopes of any of the major Plasmodium falciparum protein antigens causing malaria (e.g. from the MSP-1, the AMA-1, the CS proteins, etc.) and HIV (e.g. from the surface proteins gp41, or gp120) repetitively and rigidly on their surface. Using the same approach, synthetic subunit vaccines can also easily be designed against any other pathogens. Especially enveloped viruses (e.g. influenza, Ebola, SARS, etc.) represent an ideal target as their surface proteins are characterized by trimeric coiled-coil proteins, which are also building blocks of our nanoparticles.
Since the peptide nanoparticles will have the same immunological properties as VLPs and since this new technology allows for the design of novel types of vaccines, we expect that it will serve as a highly versatile platform for the design of synthetic subunit vaccines. The major advantages of this concept are i) its ease of handling in terms of protein expression (E. coli), protein purification and storage, hence leading to low production costs; ii) its flexibility allowing for a high throughput testing of different designs, which is especially important for HIV; iii) its suitability for the presentation of trimeric coiled-coil B-cell epitopes, which might prove to be an efficient vaccination strategy against enveloped viruses; iv) it does not need chemical coupling of the epitope to the particle as in some other approaches; and finally, v) it is not associated with a remaining risk factor as it is not infectious.

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Membrane protein structure and function

Membrane proteins are bilayer-embedded nanomachines that fulfill key functions such as energy conversion, solute transport, secretion, and signal transduction. The lack of structural information is related to the instability of membrane proteins in a detergent-solubilized state, making the growth of three-dimensional (3-D) crystals difficult. Two-dimensional (2-D) crystals of purified membrane proteins reconstituted in the presence of lipids provide a close to native environment and allow the structure and function of membrane proteins to be assessed. To this end, electron crystallography is used, and provides 3-D information at the atomic level. Membrane protein surfaces are studied by atomic force microscopy (AFM) at sub-nanometer resolution and in buffer solution, providing information about their conformational variability at the single molecule level that cannot be assessed by crystallographic methods. AFM also allows the molecular arrangements of proteins in native membranes to be assessed. Combining these methods we have studied different aquaglyceroporins, a bacterial photosynthetic system, and the packing arrangement of rhodopsin in murine disk membranes. In parallel, we use the STEM for mass determination and single particle imaging to acquire information of biological relevance.

Aquaporins (in collaboration with P. Kjellboom)

Maintaining water homeostasis is essential for all living organisms. Diffusion of water through pure lipid bilayers has a high activation energy (E_a >10 kcal/mol). The rapid flow of water through many specialized membranes in plants and higher organisms requires an E_a < 5 kcal/mol, explaining the existence of special channels for water, the aquaporins. After our work on AQP1, we have crystallized two further members of this important protein family, AQP2, the regulated water channel of the renal collecting duct, and SoPIP2;1, a major aquaporin of plant plasma membranes. Both types of crystals are well ordered, and have yielded the 3D structure at 4.5 Å (AQP2) and 5 Å (SoPIP2;1).

Rhodopsin (in collaboration with K. Palczewski)

G protein-coupled receptors (GPCRs) are involved in virtually all physiological processes. Crystal structures are available only for the detergent-solubilized light receptor rhodopsin. Based on our atomic model of rhodopsin's dimeric packing arrangement in native disk membranes, we have explored cross-linkers to provide a biochemical proof of this model. Moreover, different solubilization conditions using n-dodecyl-β-D-maltoside (DDM) showed the existence

Andreas Engel

Phone +41 61 267 22 62
Fax +41 61 267 21 09

andreas.engel@unibas.ch
www.biozentrum.unibas.ch/aengel

Group Members

Simon Bernèche	Wanda Kukulski
Fabio Casagrande	Shirley Ann Müller
Mohamed Chami	Ansgar Philippsen
Myriam Duckely	Hervé Remigy
Nora Eifler	Philippe Ringler
Françoise Erne-Brand	Andreas Schenk
Dimitros Fotiadis	Gian-Andrea Signorell
Patrick Frederix	Kitaru Suda
Marco Gregorini	Lazar Sumanovski
Thomas Kaufmann	Paul Werten

In Collaboration with

U. Aebi (this Division), P. Agre (Johns Hopkins University, Baltimore, USA); W. Baumeister (Max-Planck Institut für Biochemie, Martinsried, Germany); P. Bullough & N. Hunter (Krebs Institute, University of Sheffield, UK); G.R. Cornelis (Biozentrum, University of Basel); T. Economou (University of Crete, Greece); Y. Fujiyoshi (Kyoto University, Japan); H. Grubmüller (Theor. Molecular Biophysics Group MPI for Biophysical Chemistry Goettingen); M. Hegner & H. Hug (Physics Institute, University of Basel); P.Kjellbom (University of Lund, SE); A. Lüthi (Biozentrum, University of Basel); D.J. Müller (Univeristiy of Dresden, Germany); K. Palczewski (University of Washington, Seattle, USA); A. Pugsley (Institute Pasteur, Paris, France); U. Staufer (IMT, University of Neuchâtel)

Administrative Assistant

Liselotte Walti

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of rhodopsin dimers when assessed by blue native gel electrophoresis. Using milder detergents (n-tetradecyl-β-D-maltoside (TDM) and n-hexadecyl-β-D-maltoside (HDM)) yielded larger oligomers. Rhodopsin's capacity to activate transducin increased more than ten fold when HDM was used for solubilization than when using DDM.

PulD (in collaboration with A. Pugsley)

The outer membrane secretin PulD, an essential channel-forming component of the type II secretion system from *Klebsiella oxytoca*, was analyzed by limited proteolysis, mass spectrometry and mass measurements by STEM, and cryo-electron microscopy to generate a 3D model of the homomultimeric complex. The dodecameric channel-forming complex is composed of two rings that sandwich a closed disc. The two rings form chambers on either side of a central plug that is part of the middle disc. The PulD polypeptide comprises two major, structurally quite different domains, an N domain that forms the walls of one of the chambers and a trypsin-resistant C domain that contributes to the outer chamber, the central disc and the plug. The C domain contains a relatively low amount of β-structure typical of the transmembrane segments of classical outer membrane proteins. A thin cylindrical structure could be identified in top views and located in the 3D model. The chamber on the periplasmic side of the membrane is proposed to constitute a docking site for the secreted exoprotein pullulanase. The chamber on the outside of the membrane could allow displacement of the plug to open the channel and allow the exoprotein to escape.

VirE2 (in collaboration with M. Hegner)

Agrobacterium facilitates efficient inter-kingdom transfer of ssDNA from its cytoplasm to the host plant cell nucleus. This is executed by a type IV secretion system, which exports specific proteins, including the relaxase VirD2, which covalently binds to the 5'-end of the translocated ssDNA (T-DNA) and mediates ssDNA export. In *Agrobacterium tumefaciens*, another exported protein, VirE2, enhances ssDNA transfer efficiency 2000 fold. VirE2 binds cooperatively to the T-DNA and forms a compact helical structure, mediating T-DNA nuclear import. We discovered that by cooperatively binding to ssDNA, VirE2 proteins act as a powerful molecular machine, which is capable of pulling ssDNA against 50 pN loads. Because efficient ssDNA translocation relies on the presence of ssDNA binding proteins in the recipient in general, this could be a more general ssDNA import mechanism also occurring in bacterial conjugation and DNA uptake processes.
The native form of VirE2 in *Agrobacterium's* cytoplasm forms a complex with its specific chaperone VirE1. Unexpectedly, the VirE1VirE2 complex is able to bind to lipid vesicles, to form channels in black lipid membranes and to bind ssDNA. Electron microscopy showed that the VirE1VirE2-ssDNA complexes exhibited the helical structure previously observed for the VirE2-ssDNA complex. Upon ssDNA binding, VirE1 dissociates from the VirE2-ssDNA complex, implying that the stable VirE1VirE2 interaction is reversible and that the affinity of VirE2 for ssDNA is stronger than for VirE1. We conclude that VirE2 is a multifunctional protein, whose structure is urgently required to understand its function.

LcrV (in collaboration with G. Cornelis)

We have used scanning transmission electron microscopy (STEM) for mass determination of biomacromolecules and their assemblies. However, the outstanding imaging capability of the STEM has given exciting results on the fine structure of a molecular needle. Dark-field images of negatively stained needles of the *Yersinia* "Ysc" type-III secretion apparatus revealed a distinct tip structure containing LcrV, a protein necessary for pore formation and known since the mid-fifties as the protective antigen against plague.

Protein expression (in collaboration with A. Lüthi)

To produce sufficient material for structural analyses, we have invested a frustrating effort to obtain mammalian membrane proteins. Comparison of different systems for their capacity to express different membrane protein classes has not as yet given conclusive results. Yeast proved to be useful for the expression of aquaporins. In this case, the protein could be harvested and purified in large quantities and crystals could be grown. Baculovirus/Sf9 expressed a variety of channels and GPCRs, but at this time, we have not been able to purify enough protein for crystallization experiments. While HCN2 was functionally expressed in HEK cells infected with the Semliki Forest Virus as demonstrated with patch-clamp experiments by A. Lüthi (Biozentrum), we could not even solubilize sufficient amounts of protein to explore purification conditions. Two major, possibly connected hurdles prevented progress. Misfolded proteins are known to get stuck in the ER, as observed by immunofluorescence microscopy. However, this observation might also be explained

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by insufficient trafficking capacity; overexpressed proteins became stuck although they were properly inserted. These proteins could either not be solubilized, or if solubilized, they were susceptible to degradation.

Devolpments (in collaboration with H. Hug and U. Staufer)

We have pushed the development of a new atomic force microscope (AFM) that operates with small cantilevers and has a Fabry-Perot type deflection sensor. This AFM is more sensitive and faster than commercial AFMs and is expected to provide better images of soft, biological samples. First results indicate the power of this new instrument that now will be tested in multiple applications. A novel type of cantilever for measuring the topography simultaneously with the electronic properties of the sample has been developed. These cantilevers can be applied to a wide range of applications in biology, electrochemistry and materials processing.
A new tool has been introduced for 2D crystallization (2DX). It is based on previous experience with a system that allowed 2D crystals to be grown by dilution. The new 2DX robot operates on a 96 well plate and can control the crystallization process over weeks. A prototype has been built and is currently under test. With a new set of programs developed in the framework of the EU Network of Excellence, 3D-EM, progress has been achieved in the development of image processing tools for electron crystallography.
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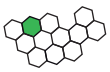

Andreas Engel

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Extracellular matrix, cell adhesion and cell communication

The group of Jürgen Engel terminated end of March 2004 because of his retirement. J. Engel continued some of the work as professor emeritus at the Biozentrum in collaboration with Thomas Kiefhaber and Annett Bachmann and members of other departments after his official retirement. He also worked during the summer periods of 2004 and 2005 as visiting scientist at the research department of Shriners Hospital for Children in collaboration with Hans Peter Bächinger. The present report describes a project on collagen folding, which has continued in Basel and in Portland. The project on the nematocyst wall is continued by PD Suat Özbeck in Heidelberg and Sebastian Meier in Stefan Grzesiek's research group. Daniel Häussinger and Stefan Grzesiek also continue to work on cadherins. The other projects described have been terminated.

Collagen folding

Different oligomerization domains were fused to both ends (N- and C-terminal) of the collagen model peptide $(GPP)_{10}$. The disulfide knot of collagen III and the foldon domain of T4 phage fibritin were used to induce trimerization of the chains. Trimerization is required for correct alignment and triple helix nucleation and is achieved in natural collagen by C-terminal domains called propeptides. With the help of our model system, we were able to show that triple helix formation can be nucleated at either end. This is of interest in view of recent findings that triple helix formation in a family of collagens with transmembrane domains at their N-termini may proceed from the N- to C-end.
In collaboration with Thomas Kiefhaber and Annett Bachmann we measured the rates of triple helix formation for the C-terminal crosslinked peptide of type III collagen in the absence of cis-trans isomerization of peptide bonds. This is normally the rate limiting step in collagen folding because of the abundance of proline and hydroxyprolines, which promote the cis-configuration. Refolding from an "all-trans" state was achieved by double jump experiments. By this experimental technique the triple helix is first unfolded by fast mixing with a denaturant. Following this step, the peptide bonds are maintained in trans configuration as they are present in the triple helix. Before the relatively slow trans to cis equilibration can occur the triple helix is refolded by fast dilution of the denaturant. It was found that triple helix formation is 3 to 4 orders of magnitude faster without the rate limiting step of cis-trans isomerization than with it. The rate is determined by triple helix nucleation in peptide bonds and it was also possible to determine the size of the nucleus. In collaboration with Hans

Jürgen Engel

Phone +41 61 267 22 50
Fax +41 61 267 21 89

juergen.engel@unibas.ch
www.biozentrum.unibas.ch/ret_jengel.html

Group Members
Thomas Ahrens
Sergei Boudko
Elena Pokedysheva
Ruth Landwehr
Suat Özbek
Therese Schulthess
Jörg Stetefeld

In Collaboration with
H.P. Bächinger (Shriners Hospital, Portland, Oregon. USA); R. Chiquet (Friedrich Miescher Institut, Basel); S. Grzesiek and D. Häussinger (Division of Structural Biology, Biozentrum); M. Rüegg (Division of Pharmacology/Neurobiology, Biozentrum); T. Sasaki (Max-Planck-Institut für Biochemie, München) and T. Holstein (University of Heidelberg)

Jürgen Engel

Peter Bächinger we also designed collagen-like systems without proline and hydroxyproline and will use them for comparative studies.

Nematocyst capsule wall

The project deals with the elucidation of assembly of the nematocyst wall from its two major constituents minicollagen and the glycoprotein NOWA. Both contain homologous Cys-rich domains of only about 20 residues with 3 disulfide bonds each. The structure of this domain in the C-terminal part of minicollagen-1 was explored by NMR spectroscopy. We were able to show that the enormous tensile strength of the capsule wall (it resists an osmotic pressure of more than 150 atmospheres) results from a cross-linking of the two proteins via disulfide bonds. Both proteins are produced as precursors with all disulfides internally linked within the same Cys-rich domain. We postulate a disulfide reshuffling mechanism, which is probably catalyzed by a disulfide isomerase. NOWA was shown to bind to the surface of lipid bilayers. It may mediate the contact of the protein layer of the wall with the inner part of the surrounding lipid membrane, which originates from a vesicle expelled by the Golgi apparatus of the nematocyst producing cells.

Mechanism of the homophilic interaction of cadherins

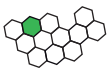
The interaction contacts between homophilically associating E-cadherins was studied by a combined NMR and crystallographic approach. The most salient feature of this work is the discovery of an activating conformational transition in domain CAD1, which is induced by proteolytic removal of the prodomain preceding CAD1. The prodomain and even a single amino acid residue attached to the N-terminus of CAD1 keep this domain in an inactive state. Only after its removal, a structural rearrangement takes place by which the essential tryptophane residue in position 2 of CAD1 can dock into the hydrophobic pocket of a CAD1 domain of an interacting cadherin molecule.

Interaction of agrin with laminin

We solved the crystal structure of the amino-terminal domain of agrin (NtA) and defined its binding site for laminin by site directed mutagenesis. It was shown before that this domain binds to the coiled-coil structure in the long arm of laminin. The binding site in laminin was mapped to a region of about 25 residues. Very interestingly this coiled-coil segment of three chains binds with its γ–1 chain to the C-terminal helix in NtA. A parallel alignment of the coiled-coil structure and a helix in a globular domain may be a rather general type of interaction for specific binding of partners to coiled-coil domains. The study adds structural and thermodynamic information to the biology of agrin in synapse formation.

Design of oligomeric proteins

We developed and tested varies oligomerization domains, and applied them for dimerization, trimerization and pentamerization of recombinantly expressed proteins: COMPcc (pentameric), matrilincc (trimeric), foldon (trimeric), disulfide knot of collagen III (trimeric), jun/foscc (dimeric). COMPcc is the five-stranded coiled-coil domain of cartilage oligomeric matrix protein, whose structure was solved by us several years ago and which we meanwhile used for oligomerization of cadherins. Matrilincc and jun/foscc are three- and two-stranded coiled-coil domains which we also used in cadherin research. The foldon domain of 29 residues is of a completely different type. It originated from T4-phage fibritin, combines to a very stable obligatory trimer and serves for nucleation of the alignment and folding of the fibritin coiled-coil structure. We learned about this domain from a collaboration with Vadim Mesyanzhinov, Moscow and used it extensively for trimerization of collagen model peptides. Fusion of polypeptide chains with the disulfide knot domain of collagen III was also found to be a very efficient way of cross-linking by recombinant technology.



Jürgen Engel

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

Our research focuses on the functional role of distinct nuclear pore complex proteins (nucleoporins) in nuclear transport as well as other cellular processes, such as (yeast) apoptosis. The nuclear pore complex (NPC) is the sole gateway between the nucleus and cytoplasm of interphase eukaryotic cells, and it mediates all trafficking between these two cellular compartments. NPCs are composed of about 30 different proteins, known as nucleoporins, and defects in nuclear transport as well as dysfunction of nucleoporins have been detected in distinct human diseases, such as cancer and autoimmune diseases. Based on the structure of the NPC, we are aiming to gain a more rational understanding of the functional role of individual nucleoporins for NPC structure and function.

Mapping the domain topology of individual nucleoporins within the 3D architecture of the NPC

The NPC is composed of about 30 nucleoporins, which are typically characterized by a distinct domain organization. A common feature of about one third of those nucleoporins is the presence of variable FG (phenylalanine-glycine)-repeat motifs, which are though to play critical roles in the interaction between nucleoporins and soluble transport receptors as they traverse the NPC. In order to better understand the role of FG-repeat and other nucleoporin domains for NPC structure, function and nuclear transport, we have mapped the domain topology of the vertebrate nucleoporins Nup153 and Nup214 for different nuclear transport states as well as in response to chemical effectors, such as Ca^{2+} and ATP by immuno-EM (in collaboration with K. Ullman, Huntsman Cancer Institute, University of Utah, Salt Lake City, and M. Powers, Emory University School of Medicine, Atlanta). For these studies, we are using antibodies specific to the distinct domains of Nup153 and Nup214 in combination with pre-embedding labelling of *Xenopus* oocyte nuclei. Moreover, we have expressed epitope-tagged versions of the two nucleoporins in *Xenopus* oocytes to confirm and strengthen the results we have obtained using the domain specific antibodies. By this approach, we could show that Nup153 and Nup214 are anchored to the NPC by their non-FG repeat domains, whereas their FG-repeat domains are highly flexible and mobile within the NPC (Fahrenkrog *et al.*, 2002; Fahrenkrog and Aebi, 2003; Paulillo *et al.*, 2005). Consistent with a role of FG-repeat domains in nuclear transport, we found that the location of the FG-repeat domains of Nup153 and Nup214 is correlated to the transport state of the NPC (Paulillo *et al.*, 2005), whereas chemical effectors have only minor

Birthe Fahrenkrog

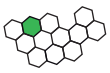
Phone +41 61 267 16 24
Fax +41 61 267 21 09

birthe.fahrenkrog@unibas.ch
www.biozentrum.unibas.ch/fahrenkrog

Group Members
Sara M. Paulillo
Kyrill Schwarz-Herion
David Walter

In Collaboration with
Ueli Aebi, Cora-Ann Schoenenberger, B, Maco, U. Sauder, and V. Olivieri (Biozentrum Basel); K.S. Ullman (Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA); M.A. Powers (Emory University School of Medicine, Atlanta, GA, USA); R.D. Goldman and D.K. Schumaker (Northwestern University, Chicago, IL, USA); K. Belanger (Colgate University, Hamilton, NY, USA); F. Madeo (Karl-Franzens University, Graz, Austria); D. Kraemer (University of Würzburg, Germany)

Administrative Assistant
Liselotte Walti



influence on their localization within the NPC (Paulillo *et al*., submitted). We have now switched our interest on the function of the non-FG-repeat domains of Nup153 and Nup214.

In search of the link between the nuclear pore complex and yeast apoptosis

In the past few years it became evident that mediating nuclear transport is not the only function of the NPC, but that the NPC is linked to, for example, chromosome segregation. kinetochore integrity, the secretory pathway and apoptosis (Fahrenkrog and Aebi, 2003; Fahrenkrog *et al*., 2004a). In this context, our studies on the yeast nucleoporin Nic96p have led us to the identification of the nuclear serine protease Nma111p, which we found to promote apoptosis in yeast (Fahrenkrog *et al*., 2004b). To elucidate the molecular function of Nma111p in yeast apoptosis, we were aiming to identify substrates of Nma111p that might be related to the yeast cell death program (in collaboration with F. Madeo, University of Graz). This led us to the identification of the inhibitor-of-apoptosis protein Bir1p as a direct interacting partner of Nma111p (Walter *et al*., 2006). Moreover, we could show that Bir1p is degraded by Nma111p *in vivo*, which enhances apoptosis, whereas overexpression of Bir1p inhibited the programmed cell death. We are now studying the interplay between Nma111p and Bir1p in more depth as well as the highly interesting link between the yeast NPC and yeast apoptosis.

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High resolution NMR of biological macromolecules

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.

Our work is directed towards problems in structural biology where NMR can give unique information: 1. The determination of biomolecular structures and interactions which are difficult to obtain by other methods. A special emphasis is put onto biomolecular pathogens, not completely folded structures, and membrane-associated proteins. 2. The study of macromolecular complexes where NMR yields information on interaction surfaces, the strength of the interaction and the dynamics, and thereby offers insights into biomacromolecular mechanisms 3. The study of forces that are relevant for macromolecular folding.

Structural Projects

Brinker

The Brinker (Brk) nuclear repressor is an essential component of the *Drosophila Decapentaplegic* morphogenic signaling pathway. Its N-terminal part binds to GC-rich DNA sequences and has weak homology to the Antennapedia homeodomain. We have determined the conformation and dynamics of the N-terminal 101 amino acids of Brk in the absence and presence of its cognate DNA by solution NMR spectroscopy (Figure 1). In the absence of DNA, Brk is unfolded at room temperature and highly flexible on the subnanosecond time scale throughout the entire backbone. Addition of cognate DNA induces the formation of a well-folded structure for residues R46 to R95. It consists of four helices forming a helix-turn-helix motif that differs from the three-helical homeodomains, but has fold similarity to the Tc3 transposase, the Pax-6 Paired domain, and the human centromere binding protein. The specificity of GC-rich DNA recognition can be rationalized by specific major groove hydrogen bond contacts from the N-terminal end of helix α2. The transition from a highly flexible, completely unfolded structure in the absence of DNA to a well-formed complex structure presents a very extreme case of the 'coupling of binding and folding' phenomenon (Cordier *et al*. manuscript in preparation; in collaboration with M. Affolter).

Stephan Grzesiek

Phone +41 61 267 21 00
Fax +41 61 267 21 09

stephan.grzesiek@unibas.ch
www.biozentrum.unibas.ch/grzesiek

Group Members

Martin Allan
Regula Aregger
Eunjung Bang
Florence Cordier
Sonja Dames
Luminita Duma
Daniel Häussinger
Pernille Jensen
Sebastian Meier
Klara Rathgeb-Szabo
Marco Rogowski
Hans-Jürgen Sass
Martin Schaad
Jörg Stetefeld
Mark Strohmeier
Navratna Vajpai

Visiting Scientists

Eunjung Bang (Korea Basic Sience Institute, Seoul); Luca Vangelista (Universität Milano); Uli Zähringer (Forschungszentrum Borstel)

In Collaboration with

M. Barfield (University of Arizona, Tucson, USA); M. Blackledge (IBS, Grenoble, Frankreich); R. Brüschweiler (NHMFL, Florida, USA); A.J. Dingley (University College, London, UK); W. Jahnke (Novartis, Basel); P. Lusso (DIBIT, Milano, Italy); Ch. Thompson (University of British Columbia, Vancouver, Canada; M. Affolter, J. Engel, M. Hall, T. Kiefhaber (Biozentrum)

Administrative Assistant

Debbie Neyer

Stephan Grzesiek

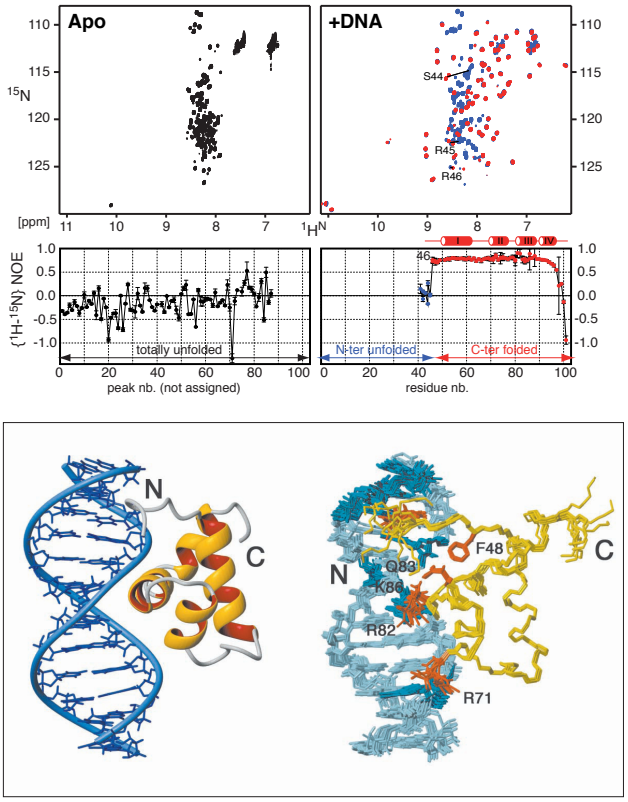


Figure 1: Interaction between Brinker and its cognate DNA. Top: NMR evidence that Brinker is unstructured in its apo form, but adopts a well-formed structure in the DNA complex. Bottom: solution structure of the Brinker/DNA complex.

T4 fibritin Foldon

We have obtained a very detailed NMR (Meier *et al.*, 2004a) and stopped-flow (Guthe *et al.*, 2004) characterization of the folding of foldon, which is the C-terminal domain of T4 fibritin (Figure 2).

This domain is obligatory for the formation of the fibritin trimer structure and can be used as an artificial trimerization domain. Its native structure consists of a trimeric β-hairpin propeller (PDB-code of solved NMR structure 1RFO). At low pH, the foldon trimer disintegrates into a monomeric (A-state) form that has similar properties as an early intermediate of the trimer folding pathway. The formation of this A-state monomer from the trimer, its structure, thermodynamic stability, equilibrium association and folding dynamics have been characterized to atomic detail by modern high-resolution NMR techniques. The foldon A-state monomer forms a β-hairpin with intact and stable H-bonds that is similar to the monomer in the foldon trimer, but lacks a defined structure in its N- and C-terminal parts. Its thermodynamic stability in pure water is comparable to designed hairpins stabilized in alcohol/water mixtures.

Details of the thermal unfolding of the foldon A-state have been characterized by chemical shifts and residual dipolar couplings (RDCs) detected in inert, mechanically stretched polyacrylamide gels. At the onset of the thermal transition, small uniform relative changes in RDC values indicate a uniform decrease of local N-H^{N} and C$^{\alpha}$-H$^{\alpha}$ order parameters for the hairpin strand residues. In contrast, near-turn residues show particular thermal stability in RDC values and hence in local order parameters. This coincides with increased transition temperatures of the β-turn residues observed by chemical shifts. At high temperatures, the RDCs converge to non-zero average values consistent with predictions from random chain polymer models. Residue-specific deviations above the unfolding transition reveal the persistence of residual order around prolines, large hydrophobic residues and at the β-turn.

These results show that a very large number of physico-chemical parameters of the folding process can be obtained from a rigorous combination of modern high-resolution NMR techniques. In particular, $^{1}D_{NH}$ and $^{1}D_{C\alpha H\alpha}$ RDCs provide new information on structural preferences within the unfolded and partially unfolded monomer states. A further increase in the number of determined RDCs is possible, e.g. by the detection of sidechain or long-range RDCs or by using different alignment conditions. This may ultimately lead to a complete quantification of local order in folding/unfolding transitions (in collaboration with T. Kiefhaber).

Homoassociation of Cadherin

Cadherins are single pass transmembrane glycoproteins, which mediate calcium dependent cell-cell adhesion by homophilic interactions. A detailed understanding of the calcium-induced homoassociation of cadherin is missing. In the last years, we have extensively characterized this homoassociation by NMR spectroscopy in vitro. Cadherins are made as immature precursor proteins, which are activated by the proteolytic cleavage of an N-terminal prosequence. We have now been able to follow this proteolytic activation mechanism in atomic detail for a model precursor in the NMR tube (Haussinger *et al.*, 2004) (in collaboration with J. Engel).

Stephan Grzesiek

The multidrug resistance protein TipA

In recent years, research on multidrug resistance proteins and their regulation has gained increased interest as increasing numbers of microbial organisms develop resistance to antibiotics. The TipA protein is one such multidrug resistance protein in *Streptomyces lividans*, which regulates its own expression by binding to its own promotor upon antibiotic binding. The protein consists of a multidrug binding domain and a DNA binding part. After solving the structure of the drug binding TipAS domain in the free form, we have now solved one structure in complex with the antibiotic Nosiheptide. The results show that upon drug binding, the protein undergoes a dramatic structural change from a half-folded form to a fully folded form (Jensen *et al.*, in preparation; in collaboration with C. Thompson).

TOR FATC

The target of rapamycin, TOR, is a highly conserved ser/thr kinase that plays a central role in the control of cellular growth. TOR has a characteristic multi-domain structure. Only the kinase domain has catalytic function; the other domains are assumed to mediate interactions with TOR-substrates and -regulators. Except for the rapamycin-binding domain, there is no high-resolution structural data available for TOR. We have carried out a structural, biophysical, and mutagenesis study of the extremely conserved carboxy–terminal FATC domain of TOR (Dames *et al.*, 2005). The importance of this domain for TOR function has been demonstrated in several publications. Our data show that the FATC domain in its oxidized form exhibits a novel structural motif consisting of an α-helix and a C-terminal disulfide-bonded loop. Upon reduction, the flexibility of the loop region increases dramatically (in collaboration with M. Hall).

Minicollagen cysteine-rich domains

Minicollagens from *Hydra* are the smallest known collagens to date. They are found in the walls of nematocysts, which are explosive organelles formed from a post-Golgi vesicle in *Hydra*, jellyfish, corals and other *Cnidaria*. Nematocyst walls are stabilized by a collageneous matrix, which depends on disulfide crosslinking to withstand the extreme osmotic pressure of up to 150 bar. The *Hydra* capsule wall mainly consists of the proteins minicollagen and NOWA. Both proteins contain highly homologous cysteine- and proline-rich domains, called minicollagen cysteine rich domains (MCRDs). Minicollagens are expressed as soluble precursor forms with intramolecular disulfide bonds in their MCRDs. In nematocyst development a wall hardening occurs, during which the intramolecular MCRD disulfide bonds are most likely reshuffled to intermolecular bonds, thereby crosslinking molecules within the capsule wall.

We have determined a high-precision solution structure of the C-terminal minicollagen cysteine rich domain of *Hydra* using modern heteronuclear and weak alignment NMR techniques at natural isotope abundance (PDB-code 1SP7) (Meier *et al.*, 2004b; Pokidysheva *et al.*, 2004). The domain consists of only 24 amino acids, six of which are prolines and six are cysteines bonded in disulfide bridges that constrain the structure into a new fold. With six cysteines in a folded core of only 20 structured amino acids, this MCRD represents one of the sequences

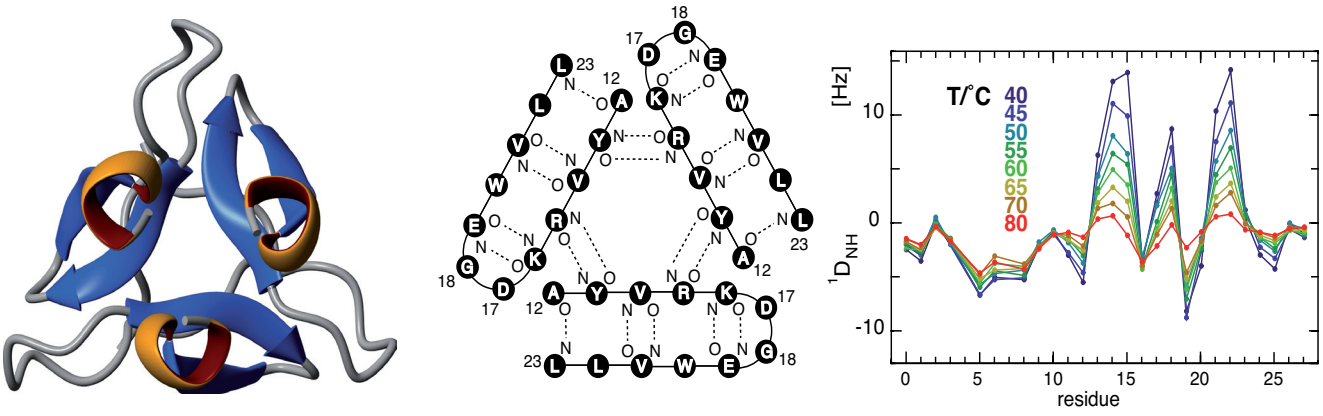
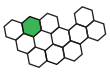


Figure 2: Structure and thermal unfolding of T4 fibritin foldon. Left: NMR structure. Middle: H-bond network. Right: Loss of structure during thermal unfolding as observed by $^1D_{NH}$ RDCs.



with the highest densities of disulfide bonds known so far. The redox equilibrium of the structure has been characterized from a titration with glutathione. No local native structures are detectable in the reduced form. Thus oxidation and folding are tightly coupled (in collaboration with J. Engel).

NMR technique development

Hydrogen bond detection

Hydrogen bonds (H-bonds) are essential for the stabilization of protein and nucleic acid secondary structure and often play a fundamental role in the regulation of enzymatic reactions. However, until recently, most of the evidence for hydrogen bonds in biological macromolecules was indirect either from the spatial proximity of donor and acceptor groups or from H-bond related parameters such as reduced hydrogen exchange rates. In 1998, we and others discovered that surprisingly strong NMR-observable scalar couplings exist across H-bonds. This effect can be used to "see" all three partners of a H-bond directly in an NMR experiment, i.e. the donor, the acceptor and the proton itself. Thus, the H-bond connectivity pattern of biomacromolecules (and therefore the secondary structure) can be established directly via a COSY experiment. The effect itself is caused by the overlap of H-bond acceptor and hydrogen electronic orbitals and thus can be related to the H-bond geometry. For recent review see (Grzesiek *et al*., 2004).

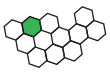
In the last two years, we have extended the research on this effect in several ways. (1) We have quantitatively characterized the backbone H-bond network of the partially folded A-state of ubiquitin (Cordier and Grzesiek, 2004). The results show intact, native-like H-bonds of the first β-hairpin β1/β2 and the α-helix, albeit at lower strength, whereas the H-bonds in the C-terminal part change from a pure β-structure in the native state to an all-α-helical $H^N(i) \rightarrow O(i-4)$ connectivity pattern in the A-state. (2) We have characterized strength of the internal H-bond network of a DNA guanine quadruplex for various cation-bound forms over a wide temperature range (Dingley *et al*., 2005). The variations in H-bond strength exhibit an overall trend $Na^+ > K^+ > NH_4^+$ and correlate with the different cation positions and N2–H2•••N7 H-bond lengths in the respective structures. The temperature dependence revealed a significantly higher thermal stability of H-bond geometries in the DNA quadruplexes as compared to nucleic acid duplexes, which can be rationalized by the cation coordination and extensive H-bond network of the quadruplexes. (3) We have quantified the effect of amide hydrogen/deuterium exchange on the strength of protein H-bonds, which leads to a small decrease in donor acceptor wavefunction overlap (Jaravine *et al*., 2004).

New structural and dynamical information from weak alignment techniques

In recent years, the weak alignment of biomolecules in anisotropic liquid phases has become a tool to directly monitor average net orientations and order parameters of individual bonds by residual dipolar couplings (RDCs). We are using and developing (Jensen *et al*., 2004) this technology with the aim to obtain new and highly precise information on the structure and dynamics of folded and unfolded proteins.
In particular, a collaboration with the groups of Martin Blackledge from the IBS in Grenoble, and Rafael Brüschweiler at the US National High-Field Magnet Lab has resulted in a novel approach to characterize the slow time scale dynamics of proteins in solution (Bouvignies *et al*., 2005). Analysis of a very large set of RDCs by a 3D axial fluctuation model revealed an intriguing pattern of standing waves across the β-sheet of protein G involved in binding to immunoglobulin G implying maximal conformational sampling at the interaction site. These findings suggest that dynamical information can be transmitted across a long-range network of hydrogen bonds in proteins.

Award

The work on H-bond scalar couplings was honored by the Laukien Prize for the year 2005 at the 46th Experimental Nuclear Magnetic Resonance Conference (ENC).



Stephan Grzesiek

Publications

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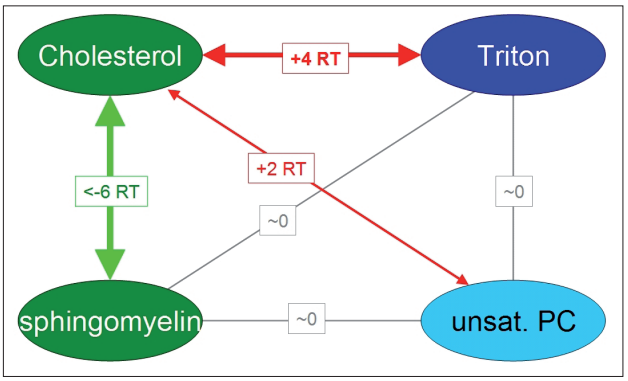
Detergent-resistant membranes and lipid rafts

Lipid rafts are considered functional membrane domains in vivo that sequester certain proteins and play a key role in signalling and other functions. It has been assumed that such rafts can be isolated from biological membranes as so-called detergent-resistant membrane (DRM) fragments and much of what we mean to know about rafts is based on this technique. However, as discussed in our paper in TiBS (Lichtenberg *et al*., 2005), there is no convincing proof for the idea that DRMs are identical with in vivo rafts. Instead, the detergent isolation technique could, in the worst case, even induce the formation of artificial domains that are then mistaken for rafts. Van Rheenen *et al.* (2005) have tested our prediction of detergent-induced domain formation and confirmed its relevance *in vivo*.

The phenomenon of detergent-induced domain formation in membranes is confirmed but it may be counterintuitive that a membrane-disordering agent like Triton should induce and/or promote the formation of ordered domains. To shed light on this issue, we established a simple model describing the equilibrium of two lipids and a detergent in four phases (ordered and disordered membrane phases, micelles, monomers) and studied the effect of varying detergent concentration on the existence, abundance and composition of the different phases (Keller *et al.*, 2005). Different scenarios are obtained, and detergent-induced domain formation is predicted specifically for the case of an unfavourable nonideal interaction between the detergent and an order-loving lipid such as cholesterol or sphingomyelin.



We have, indeed, observed such unfavourable interactions between Triton and cholesterol (Tsamaloukas *et al*. 2006a). In detergent-free systems, there is also a certain tendency towards de-mixing that is based on favourable SM-cholesterol and unfavourable POPC-cholesterol interactions, but this effect may be overcompensated by the entropy of ideal mixing. The addition of Triton shifts the balance be-

Heiko Heerklotz

Phone +41 61 267 21 80
Fax +41 61 267 21 89

heiko.heerklotz@unibas.ch
www.biozentrum.unibas.ch/heerklotz

Group Members
Alekos Tsamaloukas
Halina Szadkowska

In Collaboration with
K. Gawrisch (NIH); T. Gutberlet (Paul Scherrer Institute, Villigen); P. Slotte (Åbo Akademi, Turku)

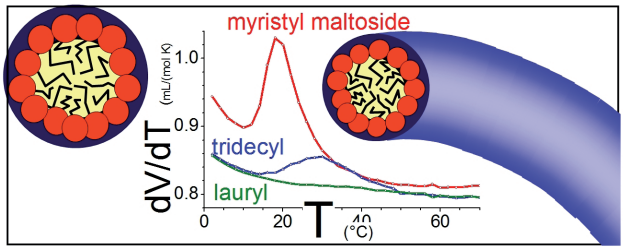
Heiko Heerklotz

tween mixing entropy and domain-promoting non-ideality in favour of a separation of POPC-Triton from SM-cholesterol.

In order to assess affinities and interaction enthalpies of cholesterol with PC vs. SM (Tsamaloukas *et al.*, 2006b), we have established three new assays for isothermal titration calorimetry that are based on the re-partitioning of cholesterol between membranes and complexes with cyclodextrin in solution (Tsamaloukas *et al.*, 2005).

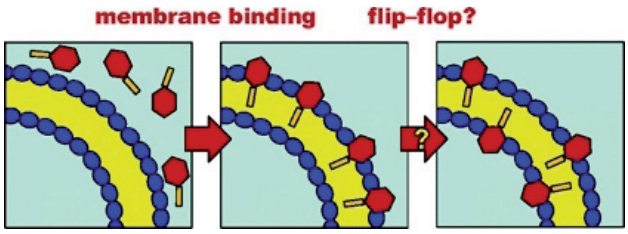
Relationship between volumetric, thermodynamic and structural properties in colloidal systems

The rather new method of pressure perturbation calorimetry provides information on the temperature-dependent thermal volume expansion of a solute or colloidal particle in aqueous dispersion. The goal of the project is to explore the potential of this parameter for a better understanding of colloidal systems, primarily their packing and hydration characteristics. To obtain a comprehensive picture, PPC experiments are combined with structural investigations (light scattering, SANS) and thermodynamic techniques. We have applied this approach to micelle formation (in preparation), a micellar sphere-to-rod transition (Heerklotz *et al.*, 2004) and the putative liquid disordered – liquid ordered phase equilibrium in lipid vesicles containing cholesterol (submitted).



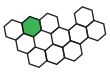
Interactions of drugs and antibiotic peptides with membranes

We have been continuing our studies on the mechanism of action of membrane-active compounds. Deuterium NMR studies provided information on the localization of surfactin, an antibiotic peptide from bacillus subtilis, in membranes and its effect on membrane structure (Heerklotz *et al.*, 2004). A key property of drugs and membrane-active compounds is their membrane permeation rate and mechanism. Our approach to this problem is based on a combination of solute uptake and release assays, the model for which was recently extended to ionic species (Keller *et al.*, 2006a). This method was also applied to characterize the membrane permeabilization of transdermal penetration enhancers (Barrow *et al.*, 2005).



Rationalization and optimization of the solubilization of G-protein coupled receptors (GPCRs)

GPCRs are the largest and most important class of drug targets, but relatively little is known about their structure and mode of action. One reason for that is the problem to solubilize them in a functional state, a question that usually requires many years of largely empirical search for suitable conditions. Our project aims at establishing a rational approach to the choice of detergents and conditions for solubilization and reconstitution of GPCRs. An empirically optimized protocol for the peripheral cannabinoid receptor CB2 is, for example, based on a solubilization buffer containing three different detergents in certain proportions, gylcerol and salts (Yeliseev *et al.*, 2005). Starting with a visit to the NIH in 2005, we have been investigating the effect of changes of these optimized conditions (e.g., addition of other detergents etc.) on the stability and activity of the receptor. Furthermore, we are developing quantitative models for describing membrane solubilization by such a complex mixture; existing models are largely limited to the practically unrealistic case of a single detergent component.



Heiko Heerklotz

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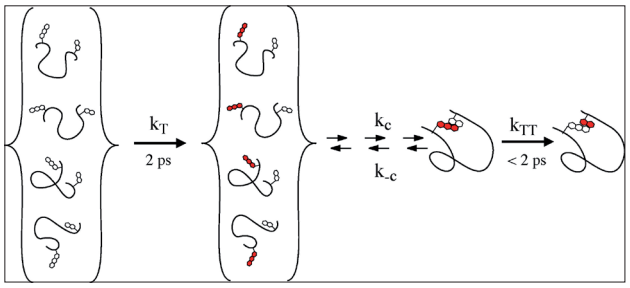
Mechanism and Dynamics of Protein Folding

Folding of a protein starting from the random coil is a spontaneous process in vitro. We use different approaches to gain insight into the mechanism of protein folding. Kinetic measurements of unfolding and refolding reactions of small model proteins are aimed at the detection and characterization of partially folded intermediates between the unfolded protein and the native state and at the characterization of the rate-limiting steps in the folding process. Studies on peptide models investigate the earliest steps in protein folding like intrachain diffusion and secondary structure formation. Different experimental techniques are applied that allow us to study conformational dynamics from the femtoseconds to the hours time range.

Elementary steps in protein folding.

During folding a protein has to form interactions between specific parts of the polypeptide chain. Intrachain diffusion can thus be regarded as the elementary step in the folding process, which determines the maximum rate at which a folding polypeptide chain can explore conformational space. We have developed a model system to directly measure contact formation between two defined points on a polypeptide chain using triplet-triplet energy transfer (TTET; for a review see Fierz & Kiefhaber, 2005).



Schematic representation of TTET experiments from a triplet donor to a triplet acceptor group to measure intrachain diffusion in polypeptide chains.

In collaboration with the group of W. Zinth (LMU München) we characterized all photochemical reactions involved in the TTET process. The results showed that the photochemistry of this system is fast and thus allows measurements of chain dynamics of processes slower than 5-10 ns (Satzger *et al.*, 2004). We measured TTET in various model peptides and natural loop sequences from proteins to study the effects of amino acid sequence, chain length, temperature, and solvent properties on the process of intrachain diffusion (Krieger *et al.*, 2004; Möglich *et*

Thomas Kiefhaber

Phone +41 61 267 21 94
Fax +41 61 267 21 89

t.kiefhaber@unibas.ch
www.biozentrum.unibas.ch/kiefhaber

Group Members

Annett Bachmann	Stefan Langheld
Christophe Bodenreider	Leticia Lemus
Maria Dolores Crespo	Sonja Lorenz
Beat Fierz	Andreas Möglich
Sarah Güthe	Rita Müller
Karin Joder	Andreas Reiner
Florian Krieger	Manuela Schätzle
Robert Kübler	Therese Schulthess
Frank Küster	Joseph Wey

In Collaboration with

Bächinger, H.P. (Portland Shriners Research Center); Engel, J. (Biozentrum, Basel); Fischer, G. (MPG Halle/Saale); Glockshuber, R. (ETH Zürich); Grzesiek, S. (Biozentrum, Basel); Henklein, P. (Humboldt Universität Berlin); Kammerer, R. (University of Manchester, UK); Tavan, P. (LMU München); Wennemers, H. (Department Chemie, Universität Basel); Zinth, W. (LMU München)

Administrative Assistants

Brigitte Heiz Wyss, Sarah Güthe

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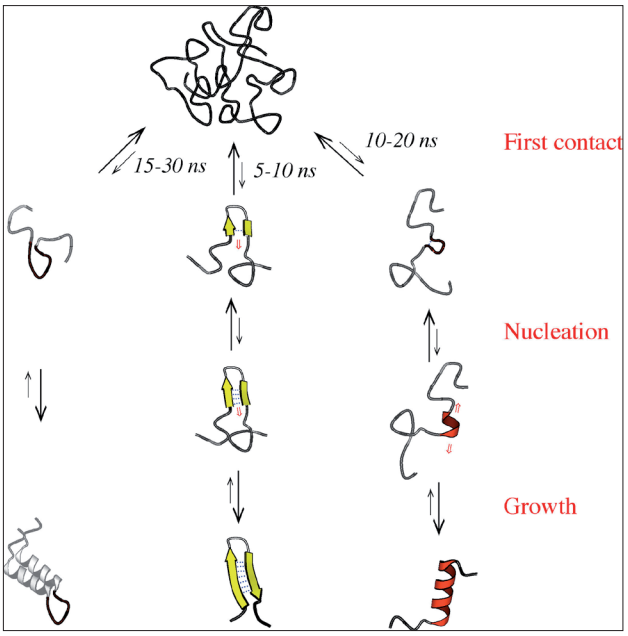
al., 2005; Krieger *et al.*, 2005). Single exponential kinetics of intrachain contact formation on the nanosecond time scale were observed for most peptides. The dynamics depend on the number of amino acids separating donor and acceptor and show a maximum time constant of 5-10 ns for flexible chains. These results set an upper limit for the speed of formation of the first side chain contacts during protein folding and suggest that typical protein loops can form on a timescale of 30 to 50 ns. The minimum time constant for contact formation at the ends of very tight turns and in elements of secondary structure is in the range of 5-10 ns.



Schematic representation of the time constants for the first steps in formation of loops, β-hairpins and α-helices during protein folding derived from TTET experiments.

Folding Mechanism of Small Single Domain Proteins

Several small proteins were shown to fold very fast (typically on the millisecond time scale) and without detectable intermediates. Our group uses several single domain proteins including the DNA-binding domain of brinker, Hsp15, parvalbumin and tendamistat as model systems to study folding and stability of fast folding proteins. To obtain insight into the barriers for protein folding we applied various kinetic concepts from physical organic chemistry to protein folding reactions (Bodenreider & Kiefhaber, 2005; for a review see Bachmann *et al.*, 2005). Analysis of folding data from our lab and of data reported in literature on other proteins allowed us to obtain a general picture of the properties of free energy landscapes for protein folding reactions. The results indicate that folding transition states are narrow regions on the free energy landscape which are robust against perturbations like mutations or changes in solvent conditions. Apparent transition state movements frequently observed in mutational studies were shown to be due to ground state effects, most commonly caused by structural changes in the unfolded state. We could show that the sensitivity in the detection of transition state movements can be improved by applying multiple perturbations. This allowed a more detailed characterization of the shape of the free energy barriers for protein folding reactions (Schätzle & Kiefhaber, 2006).

Folding of Multidomain and Oligomeric Proteins

Model proteins for studies on the mechanism of protein folding are usually not optimized for rapid folding but rather for function in the cell. We have investigated more complex folding reactions of two proteins, which have a strong evolutionary pressure for rapid and efficient folding. The first system is a protease from Semliki Forest Virus (SFVP), which is a two-domain protein and has to cleave itself out of a large polyprotein chain cotranslationally during protein synthesis. Rapid folding and cleavage of SFVP is essential for the biogenesis of the more C-terminal peptide segments in the viral polyprotein, since self-cleavage of the SFVP releases a signal peptide sequence at the newly generated N-terminus of the nascent chain. This directs the remaining polyprotein to the endoplasmic reticulum. To test whether fast co-translational folding of SFVP is an intrinsic property of the polypeptide chain or whether folding is accelerated by cellular components, we investigated spontaneous folding of recombinant SFVP in vitro, in collaboration with the group of R. Glockshuber (ETH Zürich). The results showed that SFVP folds more than two orders of magnitude faster than any previously studied two-domain protein (τ=50 ms), and that structure formation in the N-terminal domain precedes folding of the C-terminal domain. This demonstrates that co-translational folding of SFVP does not require additional cellular components and suggests that rapid folding is the result of molecular evolution towards efficient virus biogenesis (Sánchez *et al.*, 2004).
The second model protein to study a complex folding process is the C-terminal domain from the trimeric phage T4 protein fibritin. The only known function of this small domain (3x27 amino acids) is the promotion of rapid folding and association of fibritin. In collaboration with the group of S. Grzesiek (Biozentrum) we could show that a chemically synthesized

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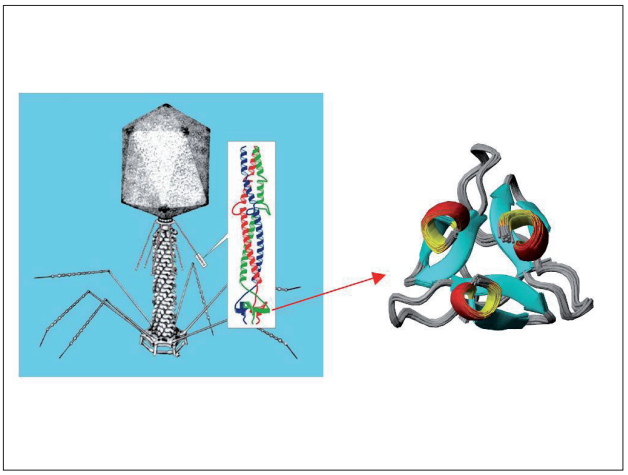
foldon-domain folds into the same trimeric β-propeller structure as in full-length fibritin (3x486 amino acids) and undergoes a two-state unfolding transition from folded trimer to unfolded monomers (Güthe *et al.*, 2004).



Structure of the T4 fibritin protein and of the trimeric foldon domain.

The folding kinetics of the foldon domain involve several consecutive reactions. Structure formation in the region of the single β-hairpin of each monomer occurs on the sub-milliseconds time scale. This reaction is followed by two consecutive association steps with rate constants around $6 \cdot 10^6$ $M^{-1}s^{-1}$, which is similar to the fastest reported bimolecular association reactions in protein folding. At low protein concentrations folding follows apparent third-order kinetics. At high protein concentrations, folding becomes independent of protein concentration with a half-time of about 3 ms, indicating that a first-order folding step from a partially folded trimer to the native protein becomes rate-limiting. Our results suggest that all steps on the folding/trimerization pathway of the foldon domain are evolutionary optimized for very rapid and specific initiation of trimer formation during fibritin assembly. The results further imply that optimized folding landscapes for oligomeric proteins have different properties than those for monomeric proteins.

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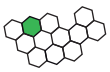

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Protein X-ray crystallography

Dystrophia Myotonica Kinase

Dystrophia Myotonic Kinase (DMPK) is the defining member of a family of complex, multidomain kinases of major biomedical relevance. These are characterized by a highly conserved catalytic domain and a coiled-coil fraction (CC) involved in the regulation of their activity. DMPK is thought to be involved in modulation of plasma membrane depolarization, reorganization of the actin cytoskeleton during tissue development and, possibly, synaptic plasticity. DMPK might be related to a progressive neuromuscular disorder known as Myotonic Dystrophy (DM), the most common form of muscular dystrophy in adults - although, its involvement in disease remains polemic.

We aim at understanding the complex mechanism of regulation of this kinase, in particular its activation by dimerization and its intrasteric modulation. Self-assembly is crucial for the regulation of DMPK and related kinases. Their CC domains are thought to form dimeric arrangements and, thus, to mediate dimerization in these enzymes. We have established the principles of DMPK assembly by a combination of X-ray crystallography, small angle X-ray scattering (SAXS), ultracentrifugation and size exclusion chromatography coupled to multi-angle static light scattering (SEC-MALS) applied to full-length DMPK and its component domains (Garcia *et al*., 2004; Garcia *et al*., 2006). Contrary to expectations, the self-assembly of DMPK is not dictated by the association properties of its CC domain, which per se does not form dimers. Instead, it appears driven by sequence segments flanking both N- and C-termini of the catalytic kinase fraction, leading to the formation of head-to-head dimers. Our findings support a shared pattern of assembly across DMPK, ROCKs and MRCK members of this family. Although it is yet to be proven whether these kinases follow the same regulatory principles.

Molecular basis of muscle elasticity

The giant muscle protein titin constitutes the third filament system in skeletal and cardiac muscle of vertebrates. It is composed of 27’000 to 33’000 residues (according to isoforms), which fold into ~300 Ig and FnIII modules assembled into linear arrays that span over 1 µm length - from the Z-disc, at the beginning of the sarcomere, to the central M-line.
Among other cellular roles, titin contributes to sarcomere assembly, stretch-stress response and muscle elasticity. The latter is achieved via passive restoring forces generated by its elastic central I-band region,

Olga Mayans

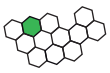
Phone +41 61 267 20 83
Fax +41 61 267 21 09

olga.mayans@unibas.ch
www.biozentrum.unibas.ch/mayans

Group Members
Marco Marino
Pilar Garcia
Rainer Bucher
Michael Mrosek
Erik Hedbom
Zöhre Ucurum
Eleonore von Castelmur

In Collaboration with
Reinhard Sterner (Department of Biochemistry, University of Regensburg); Claudia Muhle-Goll (MPI, Heidelberg); Laurent Kreplak (Biozentrum/MIH, Basel); Siegfried Labeit (Universitätsklinikum, Mannheim); Dmitri Svergun (EMBL, Hamburg Outstation)

Administrative Assistant
Ute Grütter



which primarily comprises tandem repeats of multiple Ig domains. We have recently performed a comprehensive analysis of the building-blocks of these tandems (Marino *et al*., 2005).

Filamentous and modular protein architectures, such as those of titin, are characterized by intrinsic flexibility and a poorly defined long-range order, which defies characterization by X-ray crystallography. Despite this challenge, we have recently suceeded in solving the crystal structures of several poly-Ig fragments from titin. For the smallest of those fragments, that of the N-terminus of titin comprising two-Ig (Z1Z2) connected by a flexible linker, we have performed a comprehensive characterization of its conformational dynamics using X-ray crystallography, SAXS, NMR relaxation studies and residual dipolar couplings (Marino *et al*., 2006). The existence of a possible preferential conformational pathway in this molecule is under analysis. Z1Z2 is the binding site of a small protein relevant to muscle disease, telethonin, which caps the titin filament, anchoring it to the Z-disc. The structure of Z1Z2 in complex with telethonin has been recently elucidated (Zou *et al*., 2006). Z1Z2 constitutes the first Ig-tandem of titin for which an architectural, dynamic and binding characterization becomes completed. This is expected to serve as a model system to understand further the molecular mechanisms of poly-Ig arrays of titin.

Metabolic Evolution

The tryptophan biosynthesis pathway is the major metabolic route under study after the glycolysis. Data on its genetics, biochemistry and the structure of its enzymes makes it now a unique model for the elucidation of multi-level relations in enzyme evolution.

We are engaged in the study of several members of the pathway. One of them, anthranilate phosphoribosyltransferase (AnPRT, also known as TrpD), is a key evolutionary link in metabolic nucleotide/nucleoside processing. The metabolic synthesis and degradation of essential nucleotide compounds is primarily carried out by phosphoribosyltransferases (PRT) and nucleoside phosphorylases (NP), respectively. Despite the resemblance of their reactions, five unrelated families of PRTs and NPs exist, where AnPRT constitutes the only evolutionary link between synthesis and salvage enzymes. We have characterized the active site of dimeric AnPRT by elucidating crystal structures of the wild-type enzyme complexed to its two natural substrates: anthranilate and PRPP/Mg2+. These bind into two different domains within each protomer and are brought together during catalysis by rotational domain motions as shown by small angle X-ray scattering data. Steady-state kinetics of mutated AnPRT variants address the role of active site residues in binding and catalysis. Results allow the comparative analysis of PRT and pyrimidine NP families and expose recurrent structural motifs, which having evolved independently, reveal the common basis of nucleotide/nucleoside recognition across the unrelated enzymes.

This work illustrates how metabolic enzymes have a high potential for interconversion of their activities and, thus, can be recruited by other cellular pathways according to organismic requirements.

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Crystallography of bacterial membrane and signaling proteins

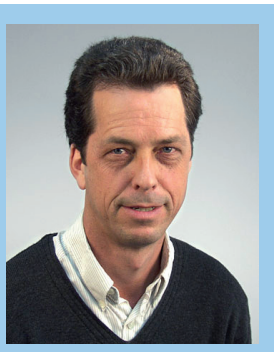
We are studying the structural basis for solute translocation across the outer and the inner bacterial membrane. Our second focus is on proteins that are involved in signal transduction utilizing the cyclic dinucleotide c-diGMP, a recently discovered second messenger in prokaryotes.

Sugar transporters of the bacterial phosphoryl-transfer-system

The PEP-dependent 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). Latter are comprised of several structural domains or subunits: IIA and IIB are hydrophilic cytoplasmic components and catalyze the phosphorelay, whereas IIC (IID) is integrated in the plasma membrane and catalyzes translocation.

We have been studying the mannose specific PTS and have solved the structures of the IIA and IIB domains. The transition state for phosphoryl transfer between IIA and IIB has been modeled based on the individual X-ray structures. The model of the complex shows surface and charge complementarity at the interface. The penta-coordinated phosphoryl group appears to be stabilized by the partial dipole charges of two helices, one from each protein.

Presently, the major effort of our research is the structure determination of sucrose permease IIBC from *Salmonella typhimurium*. IIBC is composed of two domains: membrane-spanning IIC domain that is covalently linked to a cytosolic IIB domain. This protein was chosen, because its IIB domain may 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. Extensive screening of crystallization conditions has been undertaken, but crystals of suitable quality for structure determination have not yet been obtained. In parallel, we are attempting to crystallize IIBC in complex with an antibody fragment. Respective monoclonal antibodies have been produced and selected for their ability to bind conformational epitopes. Crystallization assays of Fab-IIBCSuc complex are underway.



Tilman Schirmer

Phone +41 61 267 20 89
Fax +41 61 267 21 09

tilman.schirmer@unibas.ch
www.biozentrum.unibas.ch/schirmer

Group Members
Arnaud Baslé
Carmen Chan
Zora Markovic-Housley
Sivaraman Padavattan
Dinesh Vellore Palanivelu
Caroline Peneff
Dietrich Samoray
Paul Wassmann
Christophe Wirth

Visiting Scientists
J. Saito, Kyowa Hakko Kogyo Co. Ltd., Shizuoka, Japan

In Collaboration with
G. Cornelis, Ch. Dehio, A. Engel, U. Jenal (Biozentrum); P. Agre (Baltimore, USA); G. Condemine (CNRS, Villeurbanne, France); B. Erni (Univ. of Bern); S. Fischer (Univ. of Heidelberg, Germany); B. Giese (Univ. of Basel); R. John (Cardiff, UK); J.Kistler (Univ. of Auckland, New Zealand); R.B. Silverman (Northwestern Univ., Eaveston, USA)

Administrative Assistant
Ute Grütter

Tilman Schirmer

Porins

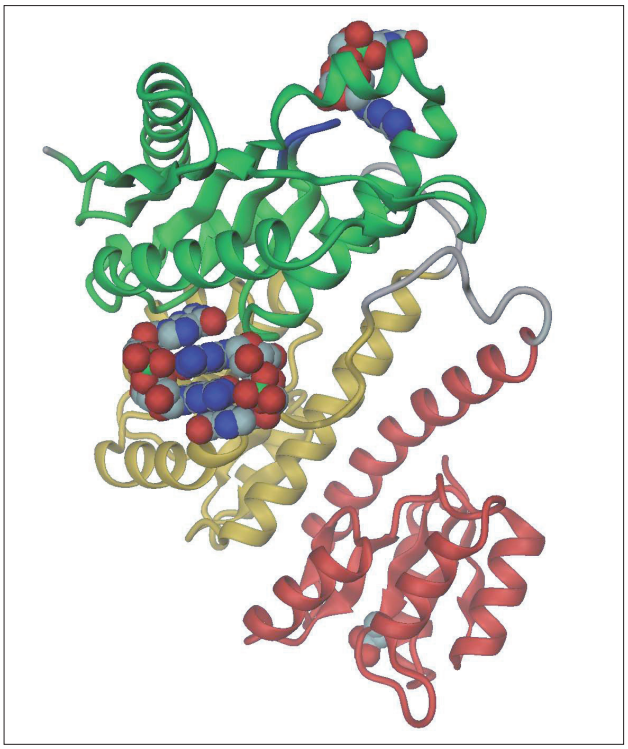
Porins are integral membrane proteins from the outer membrane of Gram-negative bacteria. They allow the uptake of nutrients by passive diffusion through an intrinsic pore that extends along the axis of the transmembrane β-barrel structure. Recently, several new porins displaying properties different to those of the classical and structurally characterized porin family have been reported. We are studying KdgM, a major outer membrane protein of the plant pathogen *Erwinia chrysanthemi*, that is thought to act as an oligogalacturonate specific porin used by the bacteria during the course of plant infection. In collaboration with G. Condemine (Villeurbanne, France), KdgM, as well as homologues from *Escherichia coli* (NanC) and *Yersinia pestis* (KdgN), have been overexpressed and purified. Reproducible protein crystals have been obtained, but are still not of sufficient quality. The X-ray structure of this porin together with further functional characterization should shed light onto the structure-function relationships of this new class of porins.

Aquaporin from the mammalian eye lens

Aquaporins are found in various eukaryotic membranes and in the bacterial plasma membrane. They exhibit exquisite specificity for water or glycerol. In collaboration with P. Agre (Baltimore, USA) and A. Engel (Biozentrum) we have obtained crystals of recombinant eye lens AQP0 that diffract to 7 Å resolution. Despite the poor resolution, we were able to determine the crystal packing by molecular replacement. Octamers that are formed by tight head-to-head association of tetramers are the building blocks of the lattice. Possibly, this interaction reflects the *in vivo* situation, where AQP0 is known to be essential for mediating the tight stacking of lens fiber cell membranes.

Two-component response regulator PleD

Recent discoveries suggest that a novel second messenger, c-diGMP, is extensively used by bacteria to control multicellular behavior. Condensation of two GTP to the dinucleotide is catalyzed by the widely distributed diguanylate cyclase domain that occurs in various combinations with sensory and/or regulatory modules. PleD, which is part of a signaling pathway regulating the developmental cycle of *Caulobacter crescentus*, is composed of two (CheY like) input domains and an output domain that bears no obvious structural homology to any existing fold. Recently, the group of U. Jenal (Biozentrum) showed that PleD is a tightly regulated diguanylate cyclase. In collaboration with the Jenal group and the group of B. Giese (Chemistry Department), we have determined the crystal structure of full-length PleD in complex with its product c-diGMP. The structure reveals the catalytic mechanism for the condensation of two GTP molecules to c-diGMP by the adenylate cyclase like output domain, but also reveals an unexpected second product binding site that is responsible for strong feed-back inhibition. A mechanistic model for the regulation of this response regulator („activation by dimerization, inhibition by domain immobilization“) has been proposed.

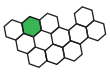


Crystal structure of the response regulator PleD. C-diGMP is seen bound to the active site (top) and, as a dimer, to the allosteric inhibition site (left).

To contribute further to our knowledge about the regulation of c-diGMP levels in bacterial cells, we have recently started to investigate c-diGMP specific phosphodiesterases.

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. In collaboration with the Dehio group (Biozentrum), we are working on the



structure elucidation of Bep proteins from *Bartonella hensellae* of the T4SS that are composed of FIC and BID domains responsible for pathogenic action in the host cell and translocation, respectively. Structural knowledge of the BID domain, in particular, will reveal the requirements for efficient targeting of passenger domains into the host cell.

Plant and insect allergens

Allergic reactions caused by insects and plants are the paradigm of IgE-mediated hypersensitivity reactions which affect more than 20% of the population. Specific immunotherapy (SIT) is the only effective treatment, but it is hampered by the danger of IgE-mediated anaphylactic side effects. Successful SIT operates at the level of T-helper cells leading to the modulation of the immune response. Therefore, for safe SIT, allergen variants should be created which possess intact T-cell recognition sites, whereas IgE binding sites are abolished. With this aim, we determined the structure of hyaluronidase, the main allergen of bee venom, in complex with a specific Fab antibody fragment. It was revealed that the Fab fragment recognizes a protruding helix-turn-helix substructure of the antigen. Truncation of this motif may lead to a hypoallergen for SIT. Also, we have determined the structure of celery allergen Api g 1, a homologue of the major birch pollen allergen Bet v 1. The analysis of its surface revealed three conserved epitopes which may account for cross-reactivity with Bet v 1 and provide a molecular basis for a better understanding of the pollen induced fruit and vegetable cross-reactivity.

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New forms of actin in the nucleus or how an old dog learns new tricks

Actin is one of the most abundant proteins in the cytoplasm of eukaryotic cells. In recent years, a role for actin in classic nuclear functions such as gene transcription, RNA processing and ribonucleoprotein transport has emerged and has settled the long-standing debate over the existence of actin in the nucleus. Although the dual localization of actin in cytoplasm and nuclei is meanwhile accepted, the form of actin in the nucleus remains enigmatic. Evidence for an uncommon polymeric actin in the nuclear periphery and the detection of numerous proteins that regulate actin patterning in the nucleus, suggest that both monomeric and oligomeric or polymeric forms of actin are at play and that the equilibrium between them, perhaps differentially regulated at various intranuclear sites, may be a major determinant of nuclear function. We are analyzing the structure and function of nuclear actin in different model systems.

Conformation-specific antibodies reveal distinct actin structures in the nucleus and the cytoplasm

One approach we have adopted to study different forms of actin is to generate specific actin monoclonal antibodies (mAbs). For example, we used chemically cross-linked actin 'lower dimer' (LD) to produce 1C7, a mAb that recognizes an epitope buried in the F-actin filament. In monomeric actin, as well as in the LD, the epitope is surface-exposed. In immunofluorescence studies with different cell lines, 1C7 selectively reacts with non-filamentous actin in the cytoplasm and detects a discrete form of actin in the nucleus. Upon latrunculin-induced disassembly of the filamentous cytoskeleton in fibroblasts, we observed a perinuclear accumulation of the 1C7-reactive actin conformation, as well as an assembly of phalloidin-staining actin structures in chromatin-free regions of the nucleus. Our results indicate that distinct actin conformations and/or structures are present in the nucleus and the cytoplasm of different cell types and that their distribution varies in response to external signals.

LD is a unconventional dimeric form of actin that we and others have observed at the onset of polymerization in vitro (Schoenenberger *et al*., 1999; 2002). In this transiently formed actin species, the subunits assume a conformation that does not occur in F-actin filaments at steady state. In contrast, LD accumulates in the presence of the actin depolymerizing drug latrunculin. Because in vitro experiments have suggested that LD might be implicated in supramolecular actin patterning [Reutzel *et al*., 2004; Schoenenberger *et al*., 2002], and because prelimi-

Cora-Ann Schoenenberger

Phone +41 61 267 20 96
Fax +41 61 267 21 09

cora-ann.schoenenberger@unibas.ch
www.biozentrum.unibas.ch/schoenenberger

Group Members
Teba Al Haboubi (partial)
Melanie Boerries (partial)
Ulrich Schroeder

In Collaboration with
Prof. U. Aebi (M.E. Müller Institute); Prof. B.M. Jockusch, TU Braunschweig; Prof. P. Burkhard, University of Connecticut; Dr. P. Most, Internal Medicine III, University of Heidelberg; Prof. J. Walker, MRC Cambridge UK

Administrative Assistant
Liselotte Walti

Cora-Ann Schoenenbergerr

nary data in latrunculin-treated cells reveal specific forms of actin in the nucleus, we are studying the significance of LD with regard to nuclear actin.

To generate Abs that react with LD exclusively, we have designed peptides that correspond to surfaces involved in the intersubunit contact of the LD and are engineering these peptides onto the surface of peptidic nanoparticles. Nanoparticles devised in P. Burkhard's laboratory mimic small, rigid viral capsids, which, because of the repetitive antigen, display have been shown to elicit a high titer of serospecific neutralizing antibodies. To test the antigenicity of nanoparticles we grafted actin sequences representing the hydrophobic plug, which is buried in the actin filament, onto their surface. Immunization with 'plug particles' indeed produced a high titer immune response in mice and a number of actin-specific mAb were established. Immunofluorescence studies on cells revealed that these mAbs labeled actin filaments only once the epitope had been exposed. We have also engineered nanoparticles that display an LD-related epitope on their surface and the resulting antibodies are under investigation.

Novel regulatory mechanisms for S100A1 in cardiac function

Heart Failure is a condition initiated by impairment of the heart's function as a pump. Factors that lead to abnormal contraction and relaxation in the failing heart include insufficient energy metabolism as well as abnormal Ca^{2+}-handling. We have shown that the Ca^{2+}-binding protein S100A1, which is the most abundant S100 protein in the heart, modulates the Ca^{2+} turnover in cardiomyocytes. A rise in intracelullar S100A1 leads to an improved Ca^{2+}-cycling which, depending on the intracellular location of the extra S100A1, involves an increased sarcolemmal Ca2+ extrusion through the sodium-calcium exchanger (NCX) or an enhanced SERCA2a activity. In collaboration with P. Most's group, we have found that cardiac-restricted overexpression of S100A1 in the failing heart leads to increased myocardial contractility *in vitro* and *in vivo*. Based on our recent discovery of an interaction of S100A1 with the F_1-ATPase, new functions for S100A1 in cardiac energy metabolism are emerging. In order to elucidate the role of S100A1 in cardiac energy metabolism, we have started to examine the effects of overexpression and knockdown of S100A1 protein levels on the ATPase activity in cardiomyocytes. Specifically, we have established that differences of intracellular S100A1 protein levels significantly modify both ATP production and Ca^{2+}-homeostasis. Future studies aim at unveiling the role of S100A1 in linking Ca^{2+}-cycling to the heart's energy metabolism.

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Membrane-Mediated Substrate Transporter Interactions

P-glycoprotein (Pgp, MDR1, ABCB1) and related ATP-binding cassette (ABC) transporters are present in most living organisms and contribute to the cellular defense system. Pgp prevents cytosolic accumulation of exogenous toxins or drugs and endogenous waste products by binding them in the cytosolic plasma membrane leaflet and flipping them to the outer leaflet, using the energy of ATP hydrolysis. The activity of Pgp is enhanced when the cells are weakened e.g. due to the lack of nutrients (Gatlik-Landwojtowicz *et al.* 2004). In humans Pgp is expressed e.g. in cells of the intestinal barrier, IB, and, to an even higher extent, in the endothelial cells of the blood vessels in the brain, constituting the blood-brain barrier, BBB. Various cancer cells also express high levels of Pgp. Cells, which exhibit the multidrug resistance phenotype can over-express Pgp and other efflux transporters after exposure to a single chemical agent (e.g. a drug or a food component). As a result, these cells become resistant to the selective agent and cross-resistant to a broad spectrum of structurally and functionally dissimilar drugs.

Most well characterized transporters, such as peptide or sugar transporters move either just one substrate or a single class of substrates across the membrane whereby, substrate binding and release occurs in the aqueous phase. Pgp significantly differs by taking care of a broad range of chemically diverse substrates whereby substrate binding and release takes place in the lipid phase. This has significant consequences for the thermodynamics and kinetics of substrate-transporter interactions (Seelig & Gatlik-Landwojtowicz, 2005). Direct measurement of substrate-transporter recognition in the lipid phase has not yet been possible. Visualization of substrate-transporter complexes by means of X-ray crystallography must be considered with great caution since the essential element for Pgp activity, the lipid membrane, is missing.

In comparison to the aqueous milieu, the anisotropic lipid environment changes many physical-chemical parameters. Among others, it partially or totally strips off the hydration shell of the partitioning molecule, induces preferential molecular orientation, stabilizes secondary structural elements such as α-helix and β-sheet, in the case of peptides, and increases the rate of translational diffusion and thus the rate of approach to the protein target. It also enhances electrostatic interactions, including H-bonding interactions, due to its lower dielectric constant. Membrane-mediated processes have been discussed in the field of substrate-*receptor* interactions (Seelig *et al.*, 1996). However, membrane-mediated substrate-*transport-*

Anna Seelig

Phone +41 61 267 22 06
Fax +41 61 267 21 89

anna.seelig@unibas.ch
www.biozentrum.unibas.ch/aseelig

Group Members
Päivi Äänismaa
Samuel Egli
Ewa Gatlik-Landwojtowicz
Grégori Gerebtzoff
Xiaochun Li-Blatter
Renate Reiter

In Collaboration with
Gunda I. Georg (Department of Medicinal Chemistry, University of Kansas, 1251 Wescoe Hall Drive, Lawrence, KS 66045, USA); Lawrence P. Wennogle, Ph.D., Drug Discovery, Intra-Cellular Therapies, Inc. (ITI), New York ; Jörg Huwyler (F. Hoffmann-La Roche, Basel); Ueli Aebi (Biozentrum, Basel)

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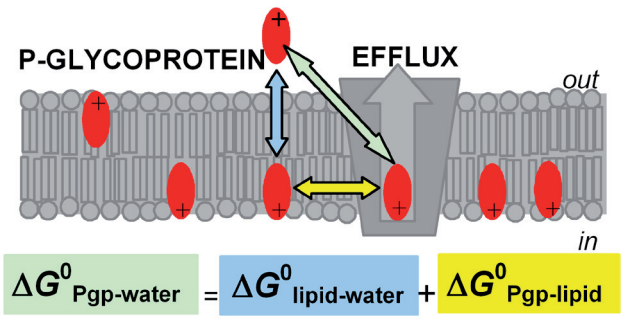
er interactions have only recently been investigated in depth (for review see (Seelig & Gatlik-Landwojtowicz, 2005)). To contribute to the understanding of membrane-mediated substrate-Pgp interactions we asked the following questions: (i) How large is the contribution of the lipid membrane to substrate-transporter binding? (ii) How can a single transporter achieve recognition of so many chemically diverse substrates? (iii) Why are the rates of Pgp ATPase activity and the rates of apparent substrate transport not identical?

How large is the contribution of the lipid membrane to substrate-transporter binding?

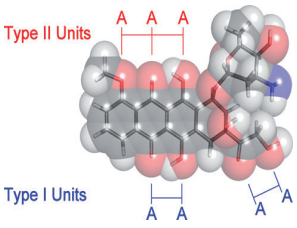
Binding of a substrate from water to the activating binding region of P-glycoprotein occurs in two steps, a partitioning step from water to the lipid membrane, characterized by a lipid-water partition coefficient, K_{lw}, and a binding step from the lipid membrane to the transporter, characterized by the transporter-lipid binding constant, K_{tl}. The transporter-water binding constant, K_{tw}, can thus be expressed as product of two individual binding constants K_{lw} and K_{tl}. We selected 15 structurally diverse drugs and measured the binding constants from water to the activating binding region of P-glycoprotein, $K_{tw,}$ as well as the lipid-water partition coefficients, K_{lw}. The former were obtained by measuring the concentrations of half-maximum activation in living NIH-MDR-G185 mouse embryo fibroblasts using a Cytosensor microphysiometer and the latter were derived from surface activity measurements. This allowed determination of the membrane concentration of drugs at half-maximum P-glycoprotein activity (C_b = (0.02 to 67) mmol/L lipid). The membrane concentration of drugs at half-maximum P-glycoprotein activity is thus greater by a factor of 10^2 to 10^4 than the corresponding aqueous concentration.On this basis the free energy of substrate-transporter binding in the lipid membrane could be determined (ΔG^0_{tl} = (-7 to -27) kJ/mol) (yellow arrow).



The direct interactions between drugs and P-glycoprotein are thus, on the average, quite weak, which is typical for transporters but contrasted with general expectations. Despite the relatively weak Pgp-substrate interactions, cells expressing high levels of Pgp are well protected against intruding drugs or toxins, since the lipid membrane acts as a drug scavenger (Seelig & Gatlik-Landwojtowicz, 2005, Gatlik-Landwojtowicz *et al*, 2006).

How can a single transporter achieve recognition of so many chemically diverse substrates?

To address the most intriguing question as to the nature of the substrate-transporter interactions in the lipid membrane an educated guess is still required. Based on the analysis of hundreds of substrates, the minimal common element of Pgp substrates, was found to consist of a specific geometric pattern of two hydrogen bond acceptor groups (called type I and type II units) (Seelig, 1998) .



We definied an intrinsic Pgp substrate as a compound that (i) is cationic or non-charged, (ii) is able to reach the inner cytosolic leaflet of the cytoplasmic membrane, and (iii) carries minimally one type I or type II unit. A single compound can carry up to seven or more such patterns. The hydrogen bond acceptor patterns (type I and type II units) are likely to react with hydrogen bond donor groups, which are abundant in the putative transmembrane sequences of P-glycoprotein (Seelig *et al*., 2000). Since the free energies of binding from the lipid membrane to the transporter have been shown to differ considerably for the different substrates investigated (see above), substrate-transporter binding cannot be explained by a classical one-pharmacophore model. However, substrate-transporter binding can be well interpreted with a modular binding concept, where the biding-modules consist of hydrogen bond acceptor patterns (type I and type II units). The average free energy per hydrogen bond formed was determined as ΔG^0_{Hi} ≈ -4 kJ/mol, which is in agreement with expectations (Gatlik-Landwojtowicz *et al,* 2006). The hydrogen bond acceptor hypothesis was further tested by Georg and her research group. They synthesized paclitaxel analogs with different numbers of hydrogen bond acceptor patterns and demonstrated that the affinity of the analogs to Pgp decreases with decreasing number of hydrogen bond acceptor patterns (Ge *et al*., 2006, Spletstoser *et al*., 2006).

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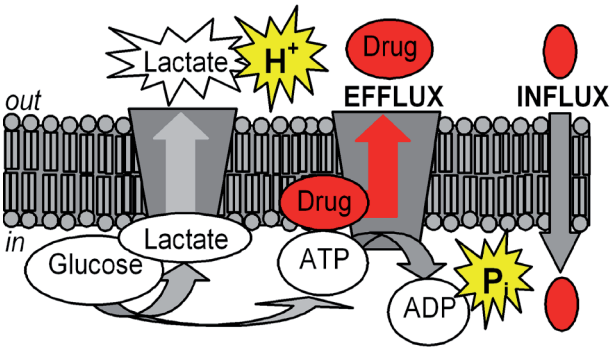
What is the difference between an intrinsic and an apparent substrate for Pgp?

Understanding and predicting transport of lipid-soluble substrates was a further challenge (Seelig & Gatlik-Landwojtowicz, 2005). The intrinsic drug transport rate is directly proportional to the rate of ATP hydrolysis or the turnover number of Pgp. However, intrinsic substrates are not necessarily identified in transport experiments across cell monolayers, nevertheless, they modulate drug absorption. The net influx, J, of a drug into the cell is the sum of the active efflux, $-V$, (Gatlik-Landwojtowicz *et al*, 2004) and the passive influx, Φ (Gerebtzoff *et al*., 2004).



Whether or not a compound is an apparent substrate thus depends on the relationship between passive influx and active efflux. The latter is proportional to the lipid-water partition coefficient, K_{lw}, which depends on the cross-sectional area, A_D, of the molecule and the lateral packing density, π_M, of the lipid bilayer (Gatlik-Landwojtowicz *et al*, 2006). Intrinsic substrates with large cross-sectional areas or high charge diffuse slowly and thus have a high chance to appear as apparent substrates. Small intrinsic substrates that diffuse more rapidly may appear as “non-substrates” since Pgp is unable to cope with their influx. Certain compounds (e.g. verapamil) can appear as apparent substrates in cell lines with a high packing density, π_M, and as non-substrate in cell-lines with a lower packing density. This explains the numerous conflicting results in literature. We have developed a program for the prediction of the cross-sectional area, A_D, of a drug (Gerebtzoff & Seelig, Submitted) and have determined the lateral packing density, π_M, of several model and biological membranes (Gatlik-Landwojtowicz *et al*, 2006) which allows calculation of whether or not a compound is an apparent substrate in a given system (cf. Seelig & Gatlik-Landwojtowicz, 2005).

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Membrane interactions and *in vivo* magnetic resonance

Thermodynamics of peptide-membrane interactions is a complex process. It depends on the chemical nature of the lipids, peptides, and carbohydrates involved and also on the mechanistic nature of the processes investigated. Different rules apply for trans-membrane insertions than for half-sided embedding. Electrostatic forces (both coulombic attraction / repulsion and dipolar interactions), hydrogen bond formation, and hydrophobic interactions play equally important roles. The enormous interest in "lipid rafts" has sensitized the scientific community to realize that the lipid part of the membrane is not simply a homogenous grease but comprises an enormous variety of lipid molecules of hitherto unknown functions. Depending on the membrane composition, groups of specific lipids may aggregate into patches with physical properties distinctly different from those of other membrane domains. The interaction of a peptide with the lipid membrane can be divided into three steps. (i) Binding is initiated by the electrostatic attraction of a usually cationic peptide to the anionic membrane. (ii) The next step is the transition of the peptide into the plane of binding which is followed by (iii) a conformational change of the peptide in the new environment (most often a transition to a partially α-helical structure).

The second topic of our research is *in vivo* magnetic resonance. *In vivo* Magnetic Resonance Imaging (MRI) provides anatomic images of living systems. Functional magnetic resonance imaging (fMRI) goes one step further and provides insight into brain activity. Magnetic Resonance Spectroscopy (MRS) detects the metabolic processes in well-defined spatial areas of the living animal without the use of invasive methods.

Thermodynamics of lipid–peptide interactions

This review is focused on peptide molecules which exhibit a limited solubility in the aqueous phase and bind to the lipid membrane from the aqueous medium. Surface adsorption, membrane insertion, and specific binding are usually accompanied by changes in the heat content of the system and can be measured conveniently with isothermal titration calorimetry, avoiding the necessity of peptide labeling. The driving forces for peptide adsorption and binding are hydrophobicity, electrostatics, and hydrogen bonding. An exclusively hydrophobic interaction is exemplified by the immunosuppressant drug cyclosporine A. Its insertion into the membrane can be described by a simple partition equilibrium $X_b=K_0C_{eq}$. If peptide and membrane are both charged, electrostatic interactions are dominant leading to nonlinear binding

Joachim Seelig

Phone +41 61 267 22 06
Fax +41 61 267 21 89

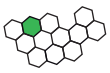
joachim.seelig@unibas.ch
www.biozentrum.unibas.ch/jseelig

Group Members
Thomas Anderson
Peter Ganz
Elisabete Gonçalves
Gabriela Klocek
Götz Kohler
Matthias Meier
Florian Rost
Bernhard Steinbauer
Anmim Tan
André Ziegler

Visiting Scientist
B. Meinlschmidt (Technische Universität München)

In Collaboration with
M. Beyermann (Forschungs-Institut für Molekulare Pharmakologie, Berlin-Buch); B. Künnecke (Roche Ltd. Basel); A. Seelig (Biozentrum Basel); M. Steinmetz (Paul Scherrer Institut, Villigen); J. Buchner (Technische Universität München)

Administrative Assistant
Susanna Notz



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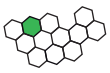
curves. The concentration of the peptide near the membrane interface can then be much larger than its bulk concentration. Electrostatic effects must be accounted for by means of the Gouy–Chapman theory before conventional binding models can be applied. A small number of peptides and proteins bind with very high affinity to a specific lipid species only. This is illustrated for the antibiotic cinnamycin (Ro 09-0198) which forms a 1:1 complex with phosphatidyethanolamine with a binding constant of 10^8 M^{-1}. Membrane adsorption and insertion can be accompanied by conformational transitions facilitated, in part, by hydrogen bonding mechanisms. The two membrane-induced conformational changes to be discussed are the random coil-to-α-helix transition of amphipathic peptides and the random coil-to-β-structure transition of Alzheimer peptides. (Seelig 2004)

Interaction of the Protein Transduction Domain of HIV-1 TAT with Heparan Sulfate: Binding Mechanism and Thermodynamic Parameters

The positively charged protein transduction domain of the HIV-1 TAT protein (TAT-PTD; residues 47-57 of TAT) rapidly translocates across the plasma membrane of living cells. This property is exploited for the delivery of proteins, drugs and genes into cells. The mechanism of this translocation is, however, not yet understood. Recent theories for translocation suggest binding of the PTD to extracellular glycosaminoglycans as a possible mechanism. We have studied the binding equilibrium between TAT-PTD and three different glycosaminoglycans with high sensitivity isothermal titration calorimetry (ITC) and provide the first quantitative thermodynamic description. The polysulfonated macromolecules were found to exhibit multiple identical binding sites for TAT-PTD with only small differences between the three species as far as the thermodynamic parameters are concerned. Heparan sulfate (HS, MW 14.2 $\pm$ 2 kDa) has 6.3 $\pm$ 1.0 independent binding sites for TAT-PTD which are characterized by a binding constant $K_0 = (6.0 \pm 0.6) \cdot 10^5$ M^{-1} and a reaction enthalpy $\Delta H^0_{pep} = -4.6 \pm 1.0$ kcal/mol at 28°C. The binding affinity, ΔG^0_{pep}, is determined to an equal extent by enthalpic and entropic contributions. The HS–TAT-PTD complex formation entails a positive heat capacity change of ΔC^0_p = + 135 cal/mole peptide which is characteristic of a charge neutralization reaction. This is in contrast to hydrophobic binding reactions which display a large negative heat capacity changes. The stoichiometry of 6-7 TAT-PTD molecules per HS corresponds to an electric charge neutralization. Light scattering data demonstrate a maximum scattering intensity at this stoichiometric ratio, the intensity of which depends on the order of mixing of the two components. The data suggest crosslinking and/or aggregation of HS-TAT-PTD complexes. Two other glycosaminoglycans, namely heparin and chondroitin sulfate B, were also studied with ITC. The thermodynamic parameters are $K_0 = (6.0 \pm 0.8) \cdot 10^5$ M^{-1} and ΔH^0_{pep} = -5.1 $\pm$ 0.7 kcal/mol for heparin and $K_0 = (2.5 \pm 0.5) \cdot 10^5$ M^{-1} and ΔH^0_{pep} = -3.2 $\pm$ 0.4 kcal/mol for chondroitin sulfate B at 28°C. The close thermodynamic similarity of the three binding molecules implies also a close structural relationship. The ubiquitous occurrence of glycosaminoglycans on the cell surface together with their tight and rapid interaction with the TAT protein transduction domain makes complex formation a strong candidate as the primary step of protein translocation. (Ziegler and Seelig 2004)

The Cationic Cell-Penetrating Peptide CPP^{TAT} Derived from the HIV-1 Protein TAT Is Rapidly Transported into Living Fibroblasts: Optical, Biophysical, and Metabolic Evidence

Cell-penetrating peptides (CPPs) are cationic peptides which, when linked to genes, proteins, or nanoparticles, facilitate the transport of these entities across the cell membrane. Despite their potential use for gene transfer and drug delivery, the mode of action of CPPs is still mysterious. It has even been argued that the observed transport across the cell membrane is an artifact caused by chemical fixation of the cells, a common preparation method for microscopic observation. Here we have synthesized a fluorescent derivative of the HIV-1 TAT protein transduction domain [Fg-CPPTAT(PTD)] and have observed its uptake into nonfixated living fibroblasts with time-lapse confocal microscopy, eliminating the need for fixation. We observe that Fg-$CPP^{TAT(PTD)}$ enters the cytoplasm and nucleus of nonfixated fibroblasts within seconds, arguing against the suggested artifact of cell fixation. Using differential interference contrast microscopy, dense aggregates are detected on the cell surface. Several observations suggest that these aggregates consist of Fg-$CPP^{TAT(PTD)}$ bound to membrane-associated heparan sulfate (HS). The aggregates grow in parallel with Fg-$CPP^{TAT(PTD)}$ uptake and are detected only on fibroblasts showing Fg-$CPP^{TAT(PTD)}$ uptake. These observations resemble earlier reports of “capping” of cell surface molecules combined with a polarized endocytotic flow. Enzymatic removal of extracellular HS reduced the rate of both Fg-$CPP^{TAT(PTD)}$ uptake and aggregate formation, demonstrating that HS is involved in the uptake mechanism. The functionality of the fibroblasts during the CPP uptake was investigated with a cytosen-



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sor microphysiometer measuring the extracellular acidification rate (ECAR). Short exposures (2.5 min) to the CPP reduced the ECAR which was, however, reversible upon reperfusion with buffer only. In contrast, no recovery to baseline values was observed after repeated exposures to the CPP, suggesting that the CPP is toxic in long-term applications. (Ziegler *et al.* 2005)

Binding of Oligoarginine to Membrane Lipids and Heparan Sulfate: Structural and Thermodynamic Characterization of a Cell-Penetrating Peptide

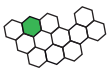
Cell-penetrating peptides (CPPs) comprise a group of arginine-rich oligopeptides that are able to deliver exogenous cargo into cells. A first step in the internalization of CPPs is their binding to the cell surface, a reaction likely to involve membrane phospholipids and/or heparan sulfate proteoglycans (HSPGs). The present work characterizes the interaction of R9, one of the most efficient CPPs, with either heparan sulfate (HS) or lipid vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol (POPG). Isothermal titration calorimetry shows that R_9 binds to HS with high affinity. Assuming that HS has *n* independent and equivalent binding sites for R_9, we find an association constant of 3.1×10^6 M^{-1} at 28°C. At this temperature, the reaction enthalpy is ΔH^0_{pep} = -5.5 kcal/mol and ~7 R_9 molecules bind per HS chain, which is equivalent to ~0.95 cationic/anionic charge ratio. ΔH^0_{pep} decreases in magnitude upon an increase in temperature, and the reaction becomes entropy-driven at higher temperatures (≥37°C). The positive heat-capacity change entailed by this reaction (ΔC^0_p = +167 cal mol^{-1} K^{-1}) indicates the loss of polar residues on R_9-HS binding, suggesting that hydrophobic forces play no major role on binding. Calorimetric analysis of the interaction of R_9 with POPC/POPG (75:25) vesicles reveals an association constant of 8.2×10^4 M^{-1} at 28°C. Using a surface partition equilibrium model to correct for electrostatic effects, we find an intrinsic partition constant of ~900 M^{-1}, a value that is also confirmed by electrophoretic mobility measurements. This corresponds to an electrostatic contribution of ~33% to the total free energy of binding. Deuterium nuclear magnetic resonance (NMR) shows no change in the headgroup conformation of POPC and POPG, suggesting that binding takes place at some distance from the plane of the polar groups. ^{31}P NMR indicates that the lipid bilayer remains intact upon R_9 binding. The fact that R_9 binds with greater affinity to HS than to anionic lipid vesicles makes the former molecule a more likely target in binding this CPP to the cell surface. (Gonçalves *et al.* 2005)

Structural and Thermodynamic Aspects of the Interaction between Heparan Sulfate and Analogues of Melittin

Melittin is an amphipathic cationic peptide derived from honeybee venom with well-known cytolytic and antimicrobial properties. When coupled to cationic polymers or lipid molecules, it forms conjugates with high transfection efficiency and low toxicity with promising applications in gene therapy. A first step in the internalization of melittin and its conjugates is their binding to the cell surface, a reaction likely to involve heparan sulfate proteoglycans (HSPG). In the present work, we characterize the binding equilibrium of heparan sulfate (HS) with two melittin analogs, [Cys^1]melittin (mel-SH) and retro-inverso [Cys^1]melittin (ri-mel-SH). The terminal cysteine found in these peptides replaces the N-terminal glycine present in native melittin, and allows covalent binding to other molecules. Isothermal titration calorimetry (ITC) reveals a high affinity of each melittin analog to HS. Association constants of 4.7×10^4 M^{-1} and 3.5×10^5 M^{-1} are found at physiological ionic strength and 15 °C for ri-mel-SH and mel-SH, respectively. The reaction enthalpy measured under these conditions is $\Delta H^o_{pep} = 4.2 kcal/mol$ for ri-mel-SH and $\Delta H^o_{pep} = 1.1 kcal/mol$ for mel-SH. The peptide-to-HS stoichiometry is ~20 for ri-mel-SH and ~14 for mel-SH under the same conditions. Temperature dependence studies using ri-mel-SH (mel-SH) show that ΔH^o_{pep} decreases in magnitude upon increase in temperature, which results in a molar heat capacity of $\Delta C^o_{pep} = -322$ cal $mol^{-1}K^{-1}$ (-45 cal $mol^{-1}K^{-1}$). Such a negative heat capacity change is not expected for a purely electrostatic interaction and indicates that hydrophobic and other interactions are also involved in the binding equilibrium. Salt dependence studies of the binding constants confirm that non-electrostatic forces are an important component of the HS-melittin interaction. Binding to HS induces conformational changes in both peptides, with ri-mel-SH showing a 6-fold increase of the α-helix content when incubated with HS under saturation conditions. (Gonçalves *et al.* 2006 (in press))

Calorimetric Measurement of Phospholipid Interaction with Methyl-β-Cyclodextrin

Cyclodextrins are able to bind hydrophobic molecules in their interior cavity and as such have received a great deal of attention as carriers of cholesterol, lipophilic drugs, and other sparingly soluble com-



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pounds. Despite the importance of these biochemical applications, relatively little is known about the interactions of cyclodextrins with phospholipid membranes. Here we characterize the binding of randomly methylated β-cyclodextrin (mβCD) to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) using right-angle light scattering and isothermal titration calorimetry. Existing models of lipophilemembrane interactions are inadequate to describe the observed binding; we introduce a modified chemical reaction model in which the chemical activity of the phospholipid is independent of its concentration. We find that an average of four mβCD molecules bind to each POPC molecule with an enthalpy of reaction of 46 kJ mol^{-1} and an equilibrium constant of 90 M^{-3}. These results are consistent with earlier qualitative observations and suggest that disruption of phospholipid membranes may be minimized if the concentration of mβCD is kept below about 15 mM. (Anderson *et al.* 2004)

Structural properties of perfluorinated linear alkanes: a 19F and 13C NMR study of perfluorononane

Liquid perfluorocarbons exhibit unique physical–chemical characteristics such as extraordinary stability, combined hydrophobia and lipophobia, low surface tension and a capacity to carry large quantities of gas. They have found widespread use in industry, medicine and biology even though the molecular origin of these properties is not fully understood. The objective of the present work was to elucidate the physical behavior of perfluorinated linear alkanes by investigating their intramolecular electronic environment using 13C and 19F NMR techniques in combination with theoretical calculations of molecular orbitals. Particular advantage was taken of 19F–19F through-space couplings, which led us to propose a molecular model in which delocalized p-electrons of the fluorines cover the entire surface of the molecule in two pairs of intertwined helices. Experimental data are presented for *n*-perfluorononane and supported by corresponding measurements with shorter and longer perfluorinated alkanes. (Schwarz *et al.* 2004).

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Structural and functional diversity generated by alternative mRNA

Alternative mRNA splicing is becoming increasingly recognized as an important mechanism for the generation of structural and functional diversity in proteins. Recent estimations predict that about 50% of all eukaryotic proteins can be alternatively spliced. Several lines of evidence suggest that alternative mRNA splicing results in small changes in protein structure and is likely to fine-tune the function and specificity of the affected protein. However, the knowledge of how alternative splicing regulates cellular processes on the molecular level is still limited.

Agrin is a multidomain heparansulfate proteoglycan best known for its function to induce and maintain postsynaptic specializations at the neuromuscular junction (NMJ). The agrin gene undergoes alternative mRNA splicing at several sites which results in several protein isoforms that differ in expression and function. Alternative usage of exons at the 5' end of the gene results in two different ways of attachment to cellular structures.

Two additional splice sites are located in the C-terminal domains G2 and G3, respectively. The four amino acid insert in G2, called A in chicken and y in rodents, encodes a heparin binding site. This insert is included in agrin transcripts expressed in neurons and glial cells while non-neuronal cells in the periphery, such as muscle, Schwann and kidney cells, lack this insert. The second splice site, called B in chicken and z in rodents, results in proteins with 8, 11 or 19 (8 + 11) amino acid inserts in the G3 domain, which is essential for AChR aggregation in vitro and the formation of functional NMJs in vivo. The recent determination of the structures encoding G3-B0, G3-B8 and G3-B11 has helped to elucidate the structural basis underlying these functional differences. The B splice insert is flanked by several loops, which form a surface corresponding to the molecular recognition interface in the family of LNS proteins. NMR relaxation data show that this surface in all G3 domains is subject to dynamic flexibility on the microsecond to millisecond time scale. Beside a remarkable structural similarity to the immunoglobulin fold, the plasticity of this interaction interface is similar to that of hypervariable loop regions in antibodies (CDR`s). This might enable agrin for selective discrimination between different binding partners through induced fit mechanisms.

Jörg Stetefeld

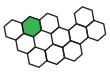
Phone +41 61 267 20 91
Fax +41 61 267 21 09

joerg.stetefeld@unibas.ch
www.biozentrum.unibas.ch/stetefeld

Group Member
Joseph Bosco Mascarenhas

Visiting Scientists and Students
Helena Fracha, Franziska Boegli

In Collaboration with
Prof. Manuel Koch (University Cologne); Markus A. Ruegg (Biozentrum); Peter Burkhard (University of Connecticut)



Jörg Stetefeld

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Intermediate filaments: from molecular structure to understanding human disease

The cytoskeleton of all metazoan cells contains three major filament systems: microfilaments, microtubules and intermediate filaments. The integrated network formed by these three filament types is responsible for the mechanical integrity of the cell and is critically involved in its division, motility and plasticity. The structural principles of IFs are still far from being fully understood [see e.g. Strelkov, Herrmann and Aebi (2003) Bioessays, 25, 243-251]. However, the knowledge of the IF molecular architecture is essential for the understanding of the structural mechanism of human diseases (cardiomyopathies, laminopathies, skin, neuronal diseases and many others) that have been linked to mutations in IF proteins. Few years ago, we have pioneered the use of X-ray crystallography towards studying the atomic structure of an IF protein vimentin [Strelkov *et al.* (2001) J. Mol. Biol. 306, 773-781; Strelkov *et al.* (2002) EMBO J. 21, 1255-1266]. In addition, we employ further structural biology techniques such as electron microscopy, atomic force microscopy and solution small-angle X-ray scattering [Strelkov *et al.* (2004) Meth. Cell Biol.].

Head-to-tail assembly of lamin dimers

The nuclear IF proteins lamins assemble together with several proteins on the inner nuclear membrane into a filamentous scaffold called the nuclear lamina. In man, three types of lamins with significant sequence identity are expressed. The first assembly step of lamins is a head-to-tail association of dimers (as opposed to vimentin where a A11-type lateral tetramer is formed first). Towards obtaining the molecular detail of this interaction, in collaboration with PD Dr. H. Herrmann (DKFZ Heidelberg), we have prepared N- and C-terminal lamin rod fragments as well as a further series of fragments of variable size, including those of human lamins A, B1 and B2. Recombinant expression in E.coli and a His-tag based purification is being used to obtain mg quantities necessary for extensive crystallisation screening. Recently, we have solved the crystal structure of the coiled-coil dimer from the second half of coil 2 from human lamin A at 2.2Å resolution [Strelkov *et al.* (2004) J. Mol. Biol.]. Comparison to the crystal structure of the homologous segment of human vimentin reveals a similar overall structure but a different distribution of charged residues and a different pattern of intra- and interhelical salt bridges. These features may explain, at least in part, the differences observed between the lamin and vimentin assembly pathways. Employing a modeled lamin A coil 1A dimer, we propose that the head-to-tail association of two lamin dimers involves strong electrostatic at-

Sergei V. Strelkov*

Group Members
Dr. Larisa Kapinos
Stefan Nicolet

Visiting Scientist
Dr. Anna V. Sokolova

In Collaboration with
Prof. Dr. Ueli Aebi, PD Dr. Jorg Stetefeld, Dr. Laurent Kreplak (Biozentrum Basel); PD Dr. Harald Herrmann and MD Harald Bar (DKFZ Heidelberg, Germany); Prof. Dr. Peter Burkhard (University of Connecticut, USA); Prof. Dr. D.A.D. Parry (Massey University, New Zealand); Dr. Dmitry Svergun (EMBL Hamburg, Germany); Prof. Dr. Vadim Mesyanzhinov (Institute of Bioorganic Chemistry, Moscow, Russia); MD Dr. Lev Goldfarb (NIH Bethesda, USA); Prof. Dr. Alfred Wittinghofer (MPI, Dortmund, Germany)

* **New address**
Prof. Sergei V. Strelkov
Laboratorium Analytische Chemie Faculteit
Farmaceutische Wetenschappen, O&N II
Herestraat 49 bus 822
B-3000 Leuven, Belgium

Phone +32 16 33 04 85
Fax +32 16 32 34 69

sergei.strelkov@pharm.kuleuven.be

Sergei V. Strelkov

tractions of distinct clusters of negative charge located on the opposite ends of the rod domain with arginine clusters in the head domain and the first segment of the tail domain. Moreover, lamin A mutations, including several ones in coil 2B, have been associated with human laminopathies. Based on our data most of these mutations are unlikely to alter the structure of the dimer but may affect essential molecular interactions occurring in later stages of filament assembly and lamina formation. In addition, we have explored the conditions under which pairs of N- and C-terminal fragments form stable complexes using analytical ultracentrifugation [Schumacher *et al.* (2006)]. The obtained complexes are being screened for crystallisation.

Molecular basis of desmin myopathy

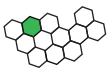
We are investigating the structural effect of several disease-related mutations in desmin, a muscle-specific IF protein *[Bar et al*. (2004) J. Struct. Biol.]. In particular, deletions of residues 359-361 and 366 within coiled-coil segment 2B were associated with severe cardio- and myopathy. Using molecular modeling we have shown that both deletions cause a local change in the coiled-coil twisting, which however has a drastic effect on the dimer structure [Kaminska *et al.* (2004) Human Genetics]. As the result, dimer-dimer interactions are altered, leading to abnormalities in the filament structure and ultimately causing the disease. To provide experimental proof for our findings, we work towards determining the crystal structures of desmin fragments carrying such mutations. A synthetic peptide corresponding to desmin segment containing deletion at position 366 as well as further recombinant desmin fragments carrying point mutations L345P, A360P, L370P, L385P and E401S were prepared. Extensive crystallisation screening with these desmin fragments is being done using nanolitre-scale robotic facilities at the University of Zurich and EMBL Hamburg. In addition, our collaborators Dr. Harald Bar (DKFZ Heidelberg) and Dr. Laurent Kreplak (Biozentrum) are using electron and atomic force microscopies to investigate the assembly of the full-length desmin with the corresponding mutations. These efforts are complementary to the crystallographic studies and computer modelling.

Structure and assembly of complete IFs

IF assembly can be initiated *in vitro* by increasing the ionic strength or lowering of pH. Recently, we were first to use small angle X-ray scattering (SAXS) to investigate the *in vitro* assembly of the wild-type human IF protein vimentin and its elongation-deficient mutant K139C by systematically varying the pH and ionic strength conditions, and complemented these experiments by electron microscopy and analytical ultracentrifugation [Sokolova *et al*. (2006)]. The SAXS data collection using synchrotron radiation was carried out at the EMBL Outstation in Hamburg. While vimentin solution in 5mM Tris-HCl (pH8.4) yields predominantly tetramers, we show that further lateral assembly induced by NaCl addition leads to formation of a distinct octameric intermediate, and octamers eventually associate into the unit-length filament (ULF). Based on the SAXS experiments supplemented by crystallographic data and additional structural constraints, first ever three-dimensional molecular models of vimentin tetramer, octamer and the ULF are constructed. This structural information is discussed in the context of IF assembly in vivo. As the next step, we plan to expand our small-angle scattering studies of the filament assembly process to further IF proteins such as desmin and its disease-related mutants.

The right-handed coiled coil of the VASP tetramerisation domain

Apart from IFs, our research interests include the structural principles of α-helical coiled coils. In particular, the vasodilator-stimulated phosphoprotein (VASP) is a key regulator of actin dynamics. In collaboration with the group of Prof. A. Wittinghofer (MPI Dortmund, Germany) we have determined the 1.3 Å resolution crystal structure of the 45-residue long tetramerization domain (TD) from human VASP [Kuhnel et al (2004) PNAS]. By using our computer program Twister [Strelkov and Burkhard (2002) J. Struct. Biol. 137, 54–64] we demonstrate that this domain forms a right-handed α-helical coiled-coil structure with a similar degree of supercoiling as found in the widespread left-handed coiled coils with heptad repeats. The basis for the right-handed geometry of VASP TD is a 15-residue repeat in its amino acid sequence, which reveals a characteristic pattern of hydrophobic residues. Hydrophobic interactions and a network of salt bridges render VASP TD highly thermostable with a melting point of 120°C. Comparison of the VASP TD with other structures containing heptad and 11-residue repeats sheds light on the general principles of the coiled-coil formation.



Sergei V. Strelkov

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LSTF
Life Sciences Training Facility

The Life Sciences Training Facility provides a GeneChip system, Q-PCR and bioinformatics tools for quantative DNA and RNA analysis

High Density Oligonucleotide Microarrays (Gene Chips) have become a major tool in basic and applied biomedical research (http://www.affymetrix.com/). GeneChips enable scientists to study DNA mutations (SNP-Chips), RNA concentrations (Expression profiling arrays) and genome-wide protein-DNA interactions (ChIP-Chip).

The Life Sciences Training Facility (LSTF) provides free access to the Affymetrix 7G GeneChip scanner 3000 system, capable of scanning the newest generation of GeneChips. This includes all-exon and tiling arrays that cover the complete genome sequences of human and mouse. The LSTF trains users in total RNA preparation, cDNA/cRNA target synthesis and quality control as well as microarray hybridisation and scanning. A Corbett RG 3000A system is operational and allows sophisticated quantitative PCR approaches to studying RNA concentrations and/or validating microarray data.

To facilitate and accelerate annotation of high-throughput expression profiling experiments, the Microarray Information Management and Annotation System (MIMAS) was developed. It provides life scientists with a highly flexible and focused GeneChip data storage and annotation platform, essential for subsequent analysis and interpretation of the experimental results with clustering and mining tools. The system is accessible to registered users, who work at a research institute that is a member of the Swiss Array Consortium (SAC) at http://www.mimas.unibas.ch/. Furthermore, a free array data storage solution is offered via the Basel Computational Biology Center [BC]2 (http://www.bc2.ch). To further support users of the LSTF, microarray data analysis training courses focussing on commercially available software solutions are offered. Finally, training courses covering open source packages (CTWC, http://ctwc.bioz.unibas.ch/; ExpressionProfiler, http://ep.ebi.ac.uk/EP/) are organised in cooperation with the laboratories of E. Domany (Weizmann Institute, Rehovot, Israel) and A. Brazma (EBI, Hinxton, UK).

Michael Primig

Phone +41 61 267 20 98
Fax +41 61 267 33 98

michael.primig@unibas.ch
www.biozentrum.unibas.ch/primig

Group Member
Philippe Demougin
In Collaboration with
H. Reichert (Biozentrum), C. Moroni (DKBW), R. Skoda (DF), M. Tanner (STI), M. Fey (University of Bern), E. Domany (Weizmann Institute) and A. Brazma (EBI)

Homepage Corelab
www.biozentrum.unibas.ch/corelab

Publications

Wrobel, G., Chalmel, F. & Primig, M. (2005). goCluster integrates statistical analysis and functional interpretation of microarray expression data. *Bioinformatics* 21, 3575-7.

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Proteomics Analysis at the Biozentrum with a New Look

After nearly a decade, the mass spectrometry lab of the Biozentrum of the University of Basel has been equipped with new instruments. In addition to an LCQ ion trap mass spectrometer used as a workhorse for daily analytical routine, Finnigan's newest mass spectrometry development, the LTQ-Orbitrap, has been taken into operation. In proteomics analysis, safe identification of proteins in ever more complex mixtures has become a necessity to help to understand fundamental biological phenomena, such as the construction of protein-protein networks and signaling pathways involved in pathological and developmental processes. Instrumentation that maximize confident protein identifications in combination with computational resources are therefore highly desirable.

The LTQ Orbitrap is a newly developed hybrid mass spectrometer with two analyzers, a linear ion trap and an Orbitrap. Both ion traps have high ion storage capacities as well as high scan rates and MS/MS sensitivities. The Orbitrap achieves excellent mass resolution and mass accuracy in full MS and MS/MS modes of operation.

The resolution in an Orbitrap analysis depends on the acquisition time of an ion. For a resolution of 7,500, the ion is detected for about 90 ms, whereas a resolution of 60,000 requires a detection time of 750 ms. There are various scanning modes that can be chosen depending on the needs of the experiment. For example, while the Orbitrap measures the mass of a precursor ion accurately, the LTQ ion trap acquires several MS/MS scans concurrent to the acquisition of a high resolution MS spectrum in a fully automated data dependent manner. On the other hand, for protein digests of average complexity, both, precursors and fragment ions can be measured in the Orbitrap analyzer, yielding accurate mass information of both, precursors and fragment ions.

In addition to using the instrument routinely for protein identifications, we have successfully identified potential protein substrates for the yeast Npr1p kinase. A protein library that had been generated from chemically digesting extracts of yeast cells was not only successfully identified, but also, the site of the in vitro phosphorylated candidate substrate was been identified, despite the low abundance of the substrate in a very complex mixture of proteins.

Paul Jenö

Phone +41 61 267 21 56
Fax +41 61 267 21 48

paul.jenoe@unibas.ch
www.biozentrum.unibas.ch/jenoe

Group Member
Patrick Altermatt, Stefan Gander, Suzette Moes
In Collaboration with
W. Keller, J. Pieters, M. Hall, C. Dehio, Y.–A. Barde, U. A. Meyer (Biozentrum Basel)

Homepage Mass Spectrometry
www.biozentrum.unibas.ch/mass_spec

The instrument is capable of mass resolution in excess of 40,000 and mass accuracies of less than 10 ppm are easily achievable during routine operation, resulting in high confidence peptide and protein identifications. Therefore, the instrument will be extremely well suited for future proteomics applications.

Publications

Otto, H., Conc, C., Maier, P., Wolfle, T., Suzuki, C., Jenö, P, Rücknagel, P., Stahl, J. & Rospert, S. (2005). The chaperones MPP11 and Hsp70L1 form the mammalian ribosome-associated complex. *Proc Natl Acad Sci U S A* 102, 10064-10069.

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TMCF
Transgenic Mouse Core Facility

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

Service

TMCF offers the injection of conventional DNA constructs and BAC's into the nuclei of fertilised oocytes, and carries out the injection of ES cells into blastocysts, and mouse line rederivation by embryo transfer. TMCF provides material and methods for ES cell work. The aim is to work closely with the researchers and give technical support throughout the duration of an experiment. TMCF also evaluates the requirements and extends the services offered accordingly.

Set up

The facility consists of an injection suite, lab space for general 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, and a mouse stem cell lab.

Daniela Nebenius-Oosthuizen

Phone +41 61 267 14 42
Fax +41 61 267 14 14

d.nebenius-oosthuizen@unibas.ch
www.biozentrum.unibas.ch/tmcf

Group Members
Roland Geiser
Annette Klewe-Nebenius
Laurent Morawiec

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.
For further details on time schedule and registration, please contact the Units’ secretary.

Research

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

Markus Dürrenberger

Phone +41 61 267 14 04
Fax +41 61 267 14 10

markus.duerrenberger@unibas.ch
www.biozentrum.unibas.ch/duerrenberger

Group Members
Marcel Düggelin
Daniel Mathys
Rolf Plank*
Ursula Sauder
Beatrice Schwyzer
Vesna Olivieri*
Robert Wyss (part time)

In Collaboration with
Biozentrum Basel, Maurice E.Müller Institute, Basel

Homepage Centre of Microscopy
http://www.unibas.ch/zmb/

*employed during part of the report period

BioPhIT
Biozentrum/Pharmazentrum Information Technology and Bioinformatics System Administration

BioPhIT and Bioinformatics System Administration act together to administer and maintain the central as well as local IT infrastructure provided to research groups throughout the Bio-/Pharmazentrum and, in particular, the Division of Bioinformatics.

User Support

More than 430 desktop workstations are supported by BioPhIT within the Bio-/Pharmazentrum. This is comprised of approx. 250 Windows workstations, 80 Macintosh systems and about 100 notebook systems. Additionally, about 20 highly specific workstations attached to data acquisition instruments (e.g. microscops, patch clamp setups, etc) are maintained. On average, five support tickets submitted via the central ticketing system and approx. ten support requests by phone or email are handled on a daily basis. Several central knowledge databases and management tools are used to effciently handle these requests. In order to facilitate and improve workstation setup and rollout, BioPhIT implemented a hardware independent cloning system for Intel CPU systems.

Windows ADS Environment

The central Windows Active Directory Server Environment for the Bio-/Pharmazentrum was upgraded from Windows 2000 to Windows 2003 server. A second failover NAS system, for the central user home and group area, was implemented in order to dramatically reduce recovery time in case of major failure events.

Web Services

The webserver infrastructure was consolidated and upgraded to reflect modern requirements in terms of performance, security, and disaster recovery. Web pages for serveral institutes (Biozentrum, Pharmazentrum, Inst. of Zoology), as well as project and event related pages (Basel Computational Biology Conference $[BC]^2$), $[BC]^2$ Discussion Forum in collaboration with the i-net Initiative of the Kanton Basel-Stadt, Bench to Bedside Symposium, Basel Neuroscience Program are hosted. In addition, several research project websites including scientific services are hosted: SwissModel Server, Workspace and Repository, NubiScan, Mirz, SPAED, SwissRegulon, Eyebase, Ashbya Genome Database, Gemonline.

Roger Jenni

Phone +41 61 267 22 35
Fax +41 61 267 22 09

roger.jenni@unibas.ch
www.biozentrum.unibas.ch/biophit

Coordinator
Roger Jenni

Administration: Bioinformatics Systems, Web Systems and EMBOSS
Robert Gaisbauer*, Rainer Pöhlmann

Windows 2000 Server Administration
Jsabelle Altherr

Windows Systems
Franz Biry, Dany Fröhlich, Keith Potter

Macintosh Systems
Dany Fröhlich

Hardware
Franz Biry

Administration
Reggie Hunziker

Apprentice
Ismerai Steiner

* employed during part of the report period

BioPhIT
Biozentrum/Pharmazentrum Information Technology and Bioinformatics System Administration

EiWAP2

In the past two years, BioPhIT has implemented and maintained the server infrastructure and disaster recovery architecture for the second generation of the Bio-/Pharmazentrum ERP system EiWAP2 (Einkauf, iShop, Waren, Apparate, Personal).

Bioinformatics System Administration

The High Performance Computing Environment of the Bioinformatics Division was further expanded. The infrastructure now consists of a 50 node Beowulf cluster connected to 4 login nodes, two Oracle database servers, one MySQL/Postgres database server as well as about 8 TB of storage. A dedicated management server including an automated monitoring tool facilitates administration of the whole environment. Automated weekly update and processing routines maintain local copies of major DNA and protein databases.

We provide graphical user and command line interfaces for the EMBOSS software package (The European Molecular Biology Open Software Suite) for users at the Biozentrum. A second EMBOSS server open to all users at the University of Basel, is jointly maintained with the URZ.

PC Grid

Windows ADS connected PC's in the Bio-/Pharmazentrum and the Friedrich-Miescher Institute (FMI), as well as PC's from public computer rooms maintained by the University Computing Centre (URZ), were connected to a United Devices (UD) MP Platform to form a grid. Idle CPU cycles from a total of 450 PCs could be harvested to run data parallel applications like e.g. protein-ligand docking on them.

Calendaring System

Several calendaring systems have been evaluated during the past year. The goal was to find a platform independent solution that will be able to synchronize with Palm, PocketPC and Symbian based PDA's. Deployment of the final system is scheduled for the first quarter of 2006.

In Collaboration with
Andreas Hefti (Division of Molecular Microbiology, Biozentrum Basel); Leandro Hermida (Division of Bioinformatics); Dean Flanders (Friedrich Miescher Institute, Basel); Reto Schaub (Department Forschung, Kantonsspital Basel)

Financing
Institute of Zoology (Altherr); Department of Clinical and Biological Sciences DKBW (Fröhlich); Division of Bioinformatics (Potter); Biozentrum (Pöhlmann); University of Basel (Biry, Fröhlich, Hunziker); Swiss Institute of Bioinformatics (Gaisbauer)

Services & Administration

Admininstrative Office
Urs Fürstenberger (Administrative Director)
Monica Gassmann*
Ursula In-Albon
Cinar Konuk
Denise Locher
Marisa Müller
Doris Rossi*
Christine Saladin
Caroline Schneider
Vera Sinniger

Animal House
René Zedi (Team Leader)
Urs Berglas
Roland Geiser
René Weber
Saskia Wüthrich
Stefanie Wüthrich

Building Administration and Maintenance
Daniel Oeschger (Team Leader)
Alois Brändle
Bruno Marioni
Christine Widmer

Electronical Workshop
Robert Häring* (Team Leader)
Jürgen Wenger* (Team Leader)
Paul Henz

Library
Karin Flügel

Mechanicals Workshop
Raymond Strittmatter (Team Leader)
Sandro Belbey
Antonio Catania
Peter Jaeggi
Oliver Jenni*
Bernhard Leinweber
Markus Plozza
Michel Schaffhauser
Patrick Schlenker
Tim Spiegel*
Marcel Wittlin*

Occupational Health and Safety Advisor
Karin Hinni*
Christoph Stürzinger*

Photo and Web Design
Werner Indlekofer (Team Leader)
Verena Grieder
Margrit Jaeggi
Annette Roulier
Ingrid Singh

Public Relations
Anka Stark*
Alexandra Weber*

Purchasing, Store and Supply
Alexander Träris (Team Leader)
Luciana Das Neves Pedro*
Karl-Heinz Heuser*
Gioacchino Romagnoli
Hans-Rudolf Zeller

Reception
Irene Dürr
Suzanne Stoeckli

Secretary to the Chair
Doris Kündig

Technical Services
André Hassler (Team Leader)
Christian Herzog
Beat Hostettler

*employed during part of the report period