BIENNIAL REPORT 2000-2001

BIOZENTRUM

DER UNIVERSITÄT BASEL

Biozentrum Basel
Klingelbergstr. 50 – 70
CH-4056 Basel
Switzerland

Tel.: +41 (0)61 267 2111
Fax: +41 (0)61 267 1712

E-mail: info@biozentrum.unibas.ch
www.biozentrum.unibas.ch

A printed copy of this report can be obtained via the Chairman's secretary at the above address or e-mail.

An online copy is available on our homepage.

Cover

The eyes shown on the cover belong to the people who have worked at the Biozentrum at the end of 2001. The list of people is not restricted to scientists only but also includes the members of the administration as well as the various internal services and craftsmen. This is to qualify the Biozentrum as a “lively house”, which for best function needs the input of all its “inhabitants”.

Creation, design and layout by Veit Goder

Impressum

Design and layout
Anka Stark

Edited by
Debbie Neyer, Anka Stark

Portraits and imaging support
Vreni Grieder, Annette Roulier, Ingrid Singh

Exposure and printing
Alban Druck Basel

CONTENTS

PREFACE

30 years Biozentrum! In December 2001 the Biozentrum celebrated its 30th anniversary with an evening for the general public, a 2-days scientific symposium and, last but not least, a party-night for the present and previous members of the Biozentrum. These events attracted a large number of participants, somewhere between 500-800 people each, including many colleagues from other universities and from the industrial neighborhood.

30 years ago, at a time when biology was still considered a “soft” science and chemistry and physics were the leading “hard” sciences it was a courageous and visionary decision to create a new building with some 20 professors and a yearly endowment of about 25 Mio CHF devoted exclusively to biology. A new name was created, the “Biozentrum” and for the next 15 years the “Biozentrum” would remain the first and only such center world-wide.

The success of the Biozentrum, gaining high international visibility in only a few years, attests primarily to the scientific quality of its research groups. This was helped, however, by implementing two new concepts. The first was to bring together under one roof apparently unrelated fields such as chemistry, physics, microbiology, cell biology, biochemistry, and pharmacology and to create an amalgam of these sciences with biology. The second was the development of a new curriculum organized around 2 month courses devoted to one topic only. Even though there was no established “market” for such an education and though it was initially unclear where the students would finally end up in their professional life, this curriculum became extremely popular. Some 550 students have by now received their diploma. In addition, more than 600 post docs have been trained. According to our last count more than 300 former students and post docs hold academic positions worldwide.

The 30 year anniversary has finally marked a step into the future. During the last 3 years a large part of the original faculty has retired, but 12 new faculty members could also be hired at an average age of 38. New blood is circulating in the arteries of the Biozentrum, fostering scientific research and education at the highest possible level.

Joachim Seelig
Chairman Biozentrum
April 2002

TEACHING

TEACHING ACTIVITIES

The Biozentrum educates scientists at the diploma, doctoral, and postdoctoral levels. The particular advantage of education at the Biozentrum is its direct integration into research. All lecturers are active, grant-holding scientists. The ratio of lectureres to students is also ideal and ensures an optimal support for every student during their studies.

The Diploma in Biology

Based on this education scheme, the two separate curricula called Biology I and Biology II taught by the Department of Integrative Biology and the Biozentrum were brought together in Fall 2000, to form the new curriculum in biology. This takes four years to complete and is taught by lecturers from both Departments.

During the first two years of basic studies, the students receive the essential grounding 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 as well as non-biological subjects.

After having completed the basic studies, the students must choose either molecular biology or organismic biology to be the main focus of their further studies, because the content of the third year, which consists of block courses lasting several weeks each, is determined by this choice. The block courses provide theoretical information which the students then turn into practice in the laboratory or in the field.

During the fourth and last year of the studies, the student works on his diploma thesis which is roughly equivalent to a Masters thesis in Anglo-Saxon universities. It is actually the student's real introduction into research. At the Biozentrum, the diploma students can specialize in biochemistry, bioinformatics, biophysics, developmental biology, genetics, immunology, microbiology, structural biology or cell biology. A complete list of the diploma theses completed during the report period can be found on page 7.

The interaction between the 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. These lecturers, the career tutors, are the students' contact person and personal advisors during the entire duration of their studies.

The PhD program

The Biozentrum also offers a doctoral degree studies. Besides the work on his/her dissertation, the student must attend lectures that take place in regular cycles within a time frame of 2 – 3 years. The current cycles cover molecular genetics, membranes, cell biology, biological systems, macromolecules, biotechnology, plant cell biology, molecular biology, virology/molecular medicine, neurosciences, and immunology. The work on a dissertation normally lasts about 3 years. After approval of the dissertation by the 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 page 9.

Seminars at the Biozentrum

The Biozentrum has a formal seminar series, the "Biozentrumsseminare", planned several months in advance and generally given by senior scientists. Many other seminars are also given that cannot be fitted into this formal series either because they are arranged at 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 post-doctoral position while sharing a post-seminar drink with the speaker.

Diploma Theses

*work performed outside the Biozentrum

*Agarwal Anni “Temporal and spatial Expression of Angiopoietic Genes during thymic development” (G. Holländer, M.A. Rüegg)

Antener Marcus “Production of mouse monoclonal antibody against recombinant human Fas and Fas ligand” (C. Erb, U. Jenal)

Barzaghi Patrizia “Muscle agrin rescues dystrophic symptoms in a mouse nodel for congenital muscular dystrophy: Characterisation of the molecular mechanisms involved in this process” (M.A. Rüegg)

*Bärtschi Markus “Does efflux contribute to bacterial resistance towards hydroxamic acids?” (M. Page, Ch. Thompson)

Baumli Sonja “Towards an Atomic Structure of Human Dopa Decarboxylase” (U. Aebi, P. Burkhard)

Bertapelle Marino “A screen for GLN3-Regulated Genes negatively controlling cell growth in *Saccharomyces cerevisiae”* (M. Hall, E. Jacinto)

*Bichsel Samuel “Regulation of NDR, a nuclear serine/threonine kinase” (B. Hemmings, T.A. Bickle)

Bittcher Godela “Characterization of a Transmembrane Form of Agrin” (M.A. Rüegg)

Blume Constanze “Topography of the Gap Junction investigated by Atomic Force Microscopy” (A. Engel)

*Bürgler Owen “Attempt to rescue the *labial* null mutant brain phenotype by misexpressing the *caudal* and empty *spiracles* gene in the *labial* domain of a *labial* null mutant background embryo using the Gal4/UAS system in *Drosophila melanogaster.”* (H. Reichert, M. Affolter)

Cabernard Clemens “Yeast two-hybrid screen with *Downstream* of *FGFR*, a gene required for cell migration in the mesoderm and trachea of *Drosophila melanogaster”* (M. Affolter)

*Caduff Judith “Generation and characterization of a fusion protein for the characterization of MAL during myelin formation and maintenance” (N. Schären-Wiemers, M.A. Rüegg)

Cavadini Gionata “Detection of T-cell receptor rearrangement circles in recent thyic emigrants as a parameter of thymic function during graft-versus-host disease” (M. Affolter, W. Krenger)

Degen Martin “Identification and characterization of ER-export signals in type I membrane proteins” (H.-P. Hauri)

*Dupasquier Marcel “Characterization of UBA2, a plant hnRNP-like protein” (W. Filipowics, T.A. Bickle)

Dossenbach Caroline “Function of different domains of the *Drosophila* FGF-receptor (DFGFR-1/DFR2) in branching morphogenesis of the developing tracheal system”. (M. Affolter)

Ernst Philipp “Interaction between the S. *Cerevisiae* Putative Cell Wall Sensors WSC1 and MID2 and Their Downstream Effectors” (M. Hall, P.-A. Delley)

Feddermann Bühler Nadja “Structure determination of inhibitor complexes of GABA-aminotransferase and investigation into a potential metal binding site.” (T. Schirmer, P. Storici)

Felix Daniel “Functional Dissection of the Antennapedia-Protein“ (W. Gehring, M. Affolter)

Fischer Beat “Genetic characterization of the *ftsH* gene in *Caulobacter crescentus*” (U. Jenal)

*Franzoni Stefania “Microgliosis in the central nervous system of Protease nexin-1 overexpressing mice” (D. Monard, M.A. Rüegg)

Friberg Michael “Cadherins The effect of dominant negative constructs on post-synaptic differentiation at the neuromuscular junction” (M.A. Rüegg)

Geering Barbara “Hunting the function of *C. crescentus CicA*: purification, phosphorylation analysis, site-directed mutagenesis and subcellular localization of *CicA*” (U. Jenal)

Gehrig Stefanie “Mutational analysis of the C-terminus of the flagellar motor protein FliF in *Caulobacter crescentus*” (U. Jenal)

*Grünig Anita “Analysis of different genetic factors influencing the heterogeneous clinical course inpatients with cystic fibrosis (CF)” (S. Liechti-Gallatti, U.A. Meyer)

*Heuss Adrian “Mutational effects of proviral insertions: investigating the ichthyosis mutation in mouse” (J. Stoye, T.A. Bickle)

Hippenmeyer Simon “Functional Analysis of Arc35p/End9p, the 35 kDa Subunit of the Arp2/3 Complex in *Saccharomyces cerevisiae*” (H. Riezman)

*Hoch Matthias “Preparation and evaluation of a novel drug-delivery system to melanoma by coupling a MSH-analog to sterically stabilized liposomes” (H.-P. Hauri)

*Isler Silvia “Characterization of the genomic locus of SPARC-like 1 (SPARCL1), a putative tumor suppressor gene of the lung” (U. Ludwig, M. Affolter)

Jauslin Matthias “Drug Discovery for Friedreich’s Ataxia: Metabolic Parameters in a Yeast Mutant deficient for Frataxin and in Human Fibroblasts from FRDA Patients” (Th. Meier)

Jeske Michael “Structural determinants of sorting of vasopressin and oxytocin into secretory granules” (M. Spiess)

Käli Roland “Characterization of a 70kDa glycoprotein interacting with ERGIC-53” (H.-P. Hauri)

*Kästli Mirjam “The expression of *Plasmodium falciparum var* gene family coding for the polymorphic variant surface antigen PfEMP1 in naturally infected blood samples” (H.P. Beck, T.A. Bickle)

Kaufmann Thomas “Expression, Purification and 2D-Crystallization of Lactose Permease from Escherichia coli (LacY) and Mechanosensitive Channel of Large Conductance from Mycobacterium tuberculosis (Tb-MscL)” (A. Engel)

*Kraft Claudine “A molecular analysis of the ER components associated with a membrane inserted fragment of the amyloid precursor protein” (H.-P. Hauri)

Krause Sarah “Folding and stability of foldon – the trimeric C-terminal domain from bacteriophage T_4 fibritin (T. Kiefhaber)

*Lepori Elisa “Cloning of the wild-type hepatitis A virus open reading frame and its expression in cell culture” (K. Bienz, T.A. Bickle)

Massner Jan “Investigation of signal functions of the GPI anchor in the ER Golgi transport of GPI anchored proteins in yeast (H. Riezman, M. Muñiz)

Meier Markus “Protein X-ray Crystallography Studies” (U. Aebi, P. Burkhard)

*Miglino Nicola “Characterization of new guidance cues for axonal pathfinding” (E. Stöckli, M.A. Rüegg)

Mitrovic Sandra “The lectin ERGIC-53 is required for efficient transport of sucrase-isomaltase from ER to Golgi” (H.-P. Hauri)

*Monigatti Flavio “Production of tyrosine sulfation sites within protein sequences using hidden Markov models”

*Moor Dominik “Evaluation of fluorescence-based enzyme-ligand binding assays for MurD (UDP-N-acetylmuramyl-L-alanine: D-glutamate ligase) from *Streptococcus pneumoniae*” (M. Gubler, T.A. Bickle)

*Müller Markus “Evaluation of HU1 snRNA-based antisense-RNA expression for induction of the Galα1,3 Gal epitope” (H.-P. Hauri)

Murphy Mark “Expression and regulation of the modification-dependent-restriction enzyme McrBC in *E. coli*” (T.A. Bickle)

Nervi Pierluigi “From soap to drug resistance. Role of detergents as specific modulators of ATPase activity of the multidrug transporter: A novel approach” (A. Seelig)

Röck Salome “Functional analysis of *Drosophila* FGF signaling in cell migration” (M. Affolter)

Rösch Karin “Topogenic contribution of signal cleavage and uncharged amino acids in the transmembrane segments of secretory and membrane proteins” (M. Spiess)

*Samandari Elika „Angiopoietin-3 expression during thymic organogenesis” (G. Holländer, T.A. Bickle)

Schäfer Katrin “Structure-function and topology study of *C. crescentus* ClpX with a new *in vivo* proteolysis system” (U. Jenal)

Schenk Andreas „AQP2: purification, crystallization and high resolution electron microscopy“ (A. Engel)

Schlatter Urs “An *in vitro* model to study protein expression under oxygen depletion in *Mycobacterium intracellulare*” (W.J. Philipp, Ch.J. Thompson)

*Schmidlin Heike “T-cell receptor rearrangement circles in murine bone marrow transplantation”. (W. Krenger, M. Affolter)

Schonegg Stephanie “Genetic Regulation of Rhodopsin Genes by Eye-Specific Genes in *Drosophila melanogaster*” (W. Gehring)

Schneider Michael “Cytosolic protein interaction with the H1 subunit of the asialoglycoprotein receptor (M. Spiess)

*Schweizer Roberto “Yeast bioassay for the screening of estrogen receptor β-isoforms in human breast cancer biopsis” (H. Müller, T.A. Bickle)

*Seiler Fabienne “Protease nexin-1 - where have you gone?” (D. Monard, M.A. Rüegg)

Studer Etienne „Investigations of the dynamics of biological networks by numerical simulations“ (Ch.J. Thompson)

*Valeggia Lucca “FtsA, a cell division protein from *Streptococcus pneumoniae*, interacts with UvrA in two-hybrid screens and oligonucleotide arrays” (R. Clerc, T.A. Bickle)

*Villalba Alberto “Engineering a fluorescence-based enzyme-ligand binding assay for SpnMurD: (UDP-N-acetyl-muramyl-L-alanine: D-glutamate ligase) from *Streptococcus pneumonias*” (M. Gubler, T.A. Bickle)

Weiser Stefan “Selection of high-affinity RNA binding ligands of Yhh1p/Cft1p and mapping of the Yhh1p/Cft1p protein binding domains to factors of CPF and CF IA” (W. Keller)

*Zürcher Alex “Search for novel extracellular matrix molecules and integrin ligands in the mammalian nervous system” (U. Müller, M.A. Rüegg)

Doctoral Dissertations

*work performed outside the Biozentrum

Aspöck Gudrun “Genes involved in development and differentiation of the nematode *Caenorhabditis elegans*” (W.J. Gehring, Th. Bürglin, M. Affolter)

Ayad-Durieux Yasmina „Genes controlling apical growth in the plant pathogenic fungus *Ashbya gossypii*” (P. Philippsen, H. Riezman)

Bachmann Annett “Intermediates in protein folding” (J. Seelig, T. Kiefhaber, J. Engel)

*Balciunaite Gina “About the role of WNT signaling in thymic development“ (U.A. Meyer, G.A. Holländer, E. Palmer)

*Bellahcene Mohamed “Endogenous protection against cardiac arrhythmias: Ischemic preconditioning against ventricular tachyarrhythmias during low-flow ischemia and reperfusion in rat hearts” (C.E. Zaugg, K.G. Hofbauer)

*Belluoccio Daniele “Characterization of clones from a subtracted, cartilage-specific cDNA library: MGP and matrilin-3” (J. Engel, B. Trueb, M. Chiquet)

Besson Sandrine “Translational control of thrombopoietin biosynthesis and pathogenesis of essential thrombocythemia” (U.A. Meyer, R. Skoda, P. Jenö)

Bieri Oliver “Elementary steps and the role of intermediates in protein folding” (T. Kiefhaber, J. Seelig)Chen Guo Jun “Investigation of the reaction mechanism of E. coli peptidyl-tRNA hydrolase” (Ch. Thompson, M. Page, T. Schirmer)

*Blecher Dinah Sarah «Validation studies on transgenic mouse models for analyzing somatic mutations *in vivo*” (U.A. Meyer, H.-J. Martus, C. Sengstag)

Bourniquel Aude Amande “Molecular insights into the metabolism and physiology of the lactic acid bacterium *Lactobacillus delbrueckii subsp. Lactis*” (T.A. Bickle, B. Mollet, W.M. de Vos)

Briguet Alexandre “Role of the ETS transcription factor GA-binding protein during postsynaptic differentiation at the neuromuscular junction” (M.A. Rüegg, Th. Meier)

Cassata Giuseppe “About ceh-14, a lim homeobox gene of *Caenorhabditis elegans*” (W.J. Gehring, F. Müller)

Delley Pierre-Alain “Control of cell growth upon environmental stress in *Saccharomyces cerevisiae*” (M.N. Hall, H. Riezman)

de Vries Henk “Characterization of cleavage factor II_m involved in 3’-end processing of mammalian messenger RNA precursors” (W. Keller, W. Filipowicz)

*Duckely Myriam “Agrobacterium T-DNA trafficking through the plant cell membrane and the plant Cytoplasm” (B. Hohn, A. Engel)

Fotiadis Dimitrios “Biochemical and structural analyses of membrane proteins in plants and animals” (A. Engel, P. Deen)

Goder Veit “Membrane protein topogenesis in the mammalian endoplasmic reticulum” (M. Spiess, H. Riezman)

Grünenfelder Björn “Proteolysis during the bacterial cell cycle” (Ch.J. Thompson, U. Jenal, H. Langen)

Handschin Christoph “A regulatory network of nuclear receptors for hepatic cytochrome P450 induction by drugs. Interactions between the xenobiotic-, the cholesterol- and the bile acid-sensors CXR, LXR and FXR in chicken liver” (U.A. Meyer, W. Wahli)

Hartmann Steffen “Protein C-mannosylation» (F. Meins, J. Hofsteenge, K. Kirschner)

Hasler Lorenz “Biochemical analyses and visualization of bacterial, plant and animal membrane proteins by electron and atomic force microscopy“ (A. Engel, J. Kistler)

He Xiaoyuan “Analysis of the rice tungro baciliform virus promoter” (Th. Hohn, M.N. Hall)

Hoepfner Dominic “Cytoskeletal components controlling dynamic behavior of nuclei and peroxisomes in yeast as revealed by life imaging” (P. Philippsen, M.N. Hall)

Horsch Kay “Control of mammary cell differentiation by lactogenic hormones and growth factors. Crosstalk between the epidermal growth factor receptor and the prolactin receptor” (T. Bickle, N. Hynes, P. Mathias)

Hübner Wolfgang “Characterization of new components involved in yeast and mammalian pre-mRNA 3’-end processing” (W. Keller, W. Filipowicz))

*Humar Rok “Response of vascular wall cells to hypoxia. Characterization of a novel Hypoxia-induced signaling pathway” (E. Battegay, D. Monard, M.A. Rüegg)

*Labuhn Martin “The frequency deleted INK4A/ARF locus on chromosome 9p21 is a prognostic factor in human glioblastomas” (A. Merlo, H. Reichert, M.A. Rüegg)

*Lambermon Mark Henricus Lucien “Functional characterization of the plant pre-mRNA maturation factor UBP1 and its interacting proteins” (W. Filipowicz, W. Keller)

Lewerenz Malte “Defending against viral infection: multiple interferon subtypes share receptor components but form different receptor complexes” (M.N. Hall, G. Uzé, M. Spiess)

Lombardi Ruben “Molecular dissection of the internalization step of endocytosis in Saccharomyces cerevisiae: Rvs167p and binding partners” (H. Riezman, M.N. Hall)

Manni Mara “Functional characterization of ϒ-secretase, a transmembrane domain-cleaving enzyme” (M. Spiess, P. Paganetti, H.-P. Hauri)

Marais Guy “Purification, characterisation, and molecular cloning of a phosphodiesterase from *Serratia marcescens* and use in the enzymatic synthesis of 2‘-modified nucleoside building blocks for pharmaceutical applications” (O. Ghisalba, Ch.J. Thompson)

Marty Thomas “Analysis of the nuclear events triggered by Decapentaplegic signaling in *Drosophila*” (M. Affolter, W.J. Gehring)

*Massa Steffen “How is the B cell-specific expression of the OBF-1 coactivator regulated and what genes are under its control?” (W. Keller, P. Matthias, R. Clerc)

*Minet Ariane Denis “Investigations on chicken Teneurin-1” (J. Engel, R. Chiquet-Ehrismann, D. Monard)

Moll Joachim “Role of muscle agrin in the treatment of muscular dystrophy in laminin α2-deficient mice and in the maturation of synaptic structures at the neuromuscular junction” (M.A. Rüegg, Th. Meier)

Morris Rowan Paul “Intrinsic multi-drug resistance in the antibiotic producing bacterium *Streptomyces*” (Ch.J. Thompson, U. Jenal)

Nickel Beatrice “Human T cell responses to a semi-conserved sequence of the malaria vaccine candidate antigen MSP-1” (N. Weiss, G. Pluschke, T. Bickle)

Niederhauser Olivier Mechanisms of neuronal cell death. Implications for therapeutic strategies in neurodegenerative disorders“ (H.-P. Hauri, M. Affolter)

Pappenberger Günter “Characterization of the rate-limiting steps in tendamistat folding” (T. Kiefhaber, R. Jaenicke)

Park Jongsun “Regulation and function of 3-phosphoinositide-dependent protein kinase-1 (PDK1), a key mediator in insulin signaling pathways” (T. Bickle, B.A. Hemmings, J. Hofsteenge)

Pende Mario “S6 kinases and the metabolic control of growth” (T. Bickle, G. Thomas, M. White)

Philippsen Ansgar “Development of a visualization tool for structural biology data & Crystallographic studies on transmembrane pores and a sorbitol dehydrogenase.” (T. Schirmer, A. Engel)

*Phinney Amie L. «Neuronal plasticity in aging and Alzheimer’s disease studies using mouse models” (U.A. Meyer, M. Jucker, G. Huber)

*Pogacic Vanda “Protein components and assembly of human H/ACA class small nuclear RNPs” (W. Filipowicz, W. Keller)

Punzo Claudio “Functional analysis of *Pax-6* genes during *Drosophila* eye development and evolution” (W.J. Gehring, M. Affolter)Rémigy Hervé-William “Methods for the reconstruction and characterization of membrane protein complexes” (A. Engel, A. Driessen)

Renold Anja “Analysis of post-Golgi sorting pathways of proteins in mammalian cells” (M. Spiess, H. Riezman)

Reymond Françoise “Characterization and functional analysis of human ubiquitin-conjugating enzyme CDC34 in cell cycle control” (M. Spiess, W. Krek, G. Thomas)

Richter Jan “Molecular cytogenetic model of bladder cancer development and progression” (U.A. Meyer, G. Sauter, T. Bickle)

*Ros Jacqueline “Secondary cerebral injury glutamate-induced lesion in vivo. Lesion characterization and effect of lactate and hypoxia” (U.A. Meyer, H. Landolt, L. Pellerin)

Schärer Florian “*Saccharomyces cerevisiae* Cnm67p acts as a spacer on the spindle pole body outer plaque and is phosphorylated in a cell cycle-dependent manner” (P. Philippsen, B. Winsor)

Schatt Stephan “An animal model for in utero HSC transplantation and the role of cytokine secretion by T- and NK cells in pregnancy” (T. Bickle, S. Hahn, P. Erb)

Sasse Bernd “On the assembly of the nucelar lamins” (U. Aebi, H. Herrmann)

Scheuring Simon “Atomic force and electron microscopic analysis of membrane channels and transporters” (A. Engel, J.-L. Rigaud)

Schmelzle Tobias “ Novel TOR effector pathways controlling cell growth in *Saccharomyces cerevisiae*” (M.N. Hall, G. Thomas)

*Schmutz Sanja „Molecular cloning of rat T-cadherin and regulation of expression in vascular smooth muscle cells” (J. Engel, T. Resink, G. Pluschke)

*Spengler Marianne “Isolierung von *GAL4* – Neuinserierungen auf 2.und 3. Chromosomen und Charakterisierung *GAL4*-induzierter Strukturvariationen in adulten zentralen Gehirnen von *Drosophila melanogaster*.“ (H. Reichert, M. Affolter, W.J. Gehring, K. Furukuba-Tokunaba)

Stöcklin Georg “A genetic approach towards identification of regulators involved in cytokine mRNA turnover” (Ch. Moroni, M. Spiess)

*Traechslin Jonas “Regulation of collagen XII expression by mechanical stress”(J. Engel, M. Chiquet, B. Trueb)

Vézina Julie “The modulation of anti-Fas induced apoptosis by endogenously processed fragments of wild-type Presenilin 1 in Jurkat T cells” (H. Riezman, M. Spiess)

Wagner Christian “Two-component signal transduction in *Streptococcus pneumoniae*” (Ch.J. Thompson, M. Page, U. Jenal)

*Wiederkehr-Adam Michèle “Specificity in the Jak/Stat signaling pathway” (U.A. Meyer, M. Heim, M.N. Hall)

Ziegler André “Noninvasive measurements of myocardial substrate preference and metabolism using *in vivo* ^{13}C NMR spectroscopy” (J. Seelig, C. E. Zaugg)

Ziegler Sandra “Specificity of cytokine signaling effects of thrombopoietin receptor mutants on megakaryopoiesis in vivo” (U.A. Meyer, R. Skoda, K. Bürki)

MEMBERS OF THE ADVISORY BOARD

Prof. Gerald A. Fink
Whitehead Institute for Biomedical Research
Cambridge, MA, USA

Prof. Beat Gähwiler (*Chair since 01.01.2002*)
Institut für Hirnforschung
Universität Zürich, Switzerland

Prof. Robert Huber
Max-Planck-Institut für Biochemie
Martinsried, Germany

Prof. Elizabeth F. Neufeld
University of California, Los Angeles
School of Medicine
Los Angeles, CA, USA

Prof. Dieter Oesterhelt
Max-Planck-Institut für Biochemie
Martinsried, Germany

Prof. Sir George K. Radda
Medical Research Council
London, UK

Prof. Harald Reuter (Chair until 31.12.2001)
Pharmakologisches Institut
Universität Bern, Switzerland

Prof. Randy W. Schekman
Howard Hughes Medical Institute
University of California
Berkeley, CA, USA

Prof. Lucy Shapiro
Stanford University
School of Medicine
Stanford, CA, USA

Prof. Nigel Unwin
Medical Research Council
Laboratory of Molecular Biology
Cambridge, UK

DIVISION OF CELL BIOLOGY

The research projects persued in the division of Cell Biology range from developmental genetics of *Drosophila* and neurobiology to the biochemistry of RNA processing and the molecular biology of evolution. The division consists of three senior groups headed by Markus Affolter, Walter Gehring, and Walter Keller, and the junior group of Silvia Arber. Thomas Bürglin accepted a position at the Karolinska Institute in Stockholm. The long term research efforts of the division of Cell Biology were recognized internationally by the award of the Kyoto Prize to Walter Gehring.

Professors

Markus Affolter, Silvia Arber*, Walter J. Gehring, Walter Keller

START fellow

Markus Affolter*

Independent scientist

Fritz Melchers*

Scientists and postdoctoral fellows

Silvia Barabino*, Meera Beery*, Jorge Blanco, Thomas Bürglin*, Bernhard Dichtl, Christine Dozier*, André Gerber*, Sacha Glardon*, Nicole Grieder*, Jay Groppe*, Wolfgang Hübner*, Anna Jazwinska-Müller*, Alain Jung*, Hiroshi Kagoshima*, Takekazu Kunieda*, Shiuyhang Kuo*, Lydia Michaut, Tomoko Nagao*, Martin Ohnacker*, Bertrand Paguet*, Valérie Petit*, Serge Plaza*, Kirsten Puls*, Claudio Punzo*, Myriam Schaub, Makiko Seimiya. Alessandra Vigano*, Henk de Vries*

Graduate students

Gudrun Aspöck*, Clemens Cabernard*, Giuseppe Cassata*, Sabine Dettwiler, Caroline Dossenbach*, Andreas Ebner, Jorge Espinoza*, Daniel Felix*, Britta Hartmann*, Simon Hippenmeyer*, Wolfgang Hübner*, Isabelle Kaufmann, Ina Kramer*, Thomas Marty*, Vera Niederkofler*, Tobias Pauli*, Frédéric Prince, Claudio Punzo*, Carlos Ribeiro, Martin Sadowski, Rishard Salie*, Henk de Vries*, Jeanette Wolf

Diploma students

Clemens Cabernard*, Caroline Dossenbach*, Daniel Felix*, Lukas Keller*, Andrea Kyburz*, Salome Röck*, Stephanie Schonegg*, Simon Stroebel*, Eline Vrieseling*, Stefan, Weiser*

Summer students

Beatriz Gafford (4 months) Dept. of Biology and Biochemistry, Univ. of Houston, Cristina Gonzalez (3 months.) Dept. Genética, Fac. Biologia, Univ. de Barcelona, Martina Schneider (2 1/2 months) Université de Besançon, Michel Tarpin (3 1/2 months) Depart. de Biologie-Biochimie, Univ. de Reims, Katja Tönsing (1/2 months) Dept. Biophysical and Physical Chemistry, Univ. of Bielefeld

Research associate

Salome Röck*

Technical personnel

Paul Baumgartner, Diana Blank, Susanne Flister, Jürgen Hench* (temporary), Urs Kloter, Georges Martin, Gisela Niklaus, Ute Nussbaumer, Markus Sigrist*, Verena Widmer*

Floor manager

Roland Kirchhofer

Secretariat

Greta Backhaus*, Liliane Devaja, Céline Knecht*, Erica Oesch-von Euw, Rose Marie Suter-Fritsche

Laboratory helpers

Bernadette Bruno, Pia Däppen*, Gina Evora*, Karin Mauro

*Employed during part of the report period

Induction and patterning in *Drosophila melanogaster*

M. Affolter, C. Cabernard, C. Dossenbach, A. Ebner, J. Groppe, B. Hartmann, A. Jazwinska, A. Jung, T. Marty, G. Niklaus, U. Nussbaumer, V. Petit, K. Puls, C. Ribeiro, S. Röck, A.M. Vigano

In collaboration with K. Basler (Uni Zürich), R. Schuh (MPI Göttingen)

Markus Affolter
Phone: +41 61 267 2077 Fax: +41 61 267 2078 E-mail: Markus.Affolter@unibas.ch
http://www.biozentrum.unibas.ch/affolter

Induction across germ layers is one of the most important mechanisms leading to the complex spatial organization of an animal as it emerges from a fertilized egg. In *Drosophila*, inductive cell-cell interactions have been described between the ectoderm, the mesoderm and the endoderm. As a result of these processes, positional information is transmitted between adjacent germ layers leading to their correct patterning along the body axis. Numerous signaling pathways have been linked to induction and we have been characterizing one of these in detail, the Dpp/ TGF β pathway.

Our and other groups' studies led to the identification of a number of gene products that constitute the core Dpp/ TGF β signaling pathway and are involved in signal reception, signal transduction and transcriptional interpretation. In addition to the ligand-activated heteromeric receptor complex and the signal-transducing intracellular Smad proteins, Dpp signaling requires two nuclear proteins, Schnurri (Shn) and Brinker (Brk), to prime cells for Dpp responsiveness. A complex interplay between the nuclear factors involved in Dpp signaling appears to control the transcriptional readout of the Dpp morphogen gradient, and we are trying to characterize this interplay at the molecular level. It remains to be seen whether similar molecular mechanisms operate in the nucleus of vertebrate cells.

During our analysis of a Dpp-responsive enhancer in the developing midgut, we made an interesting and important observation. We found that the Dpp-responsive enhancer of the *labial* (*lab*) gene requires for its signal-inducibility a direct interaction with the homeotic selector protein Lab and its cofactors Extradenticle and Homothorax. These results illustrated for the first time how a specific cellular response to Dpp can be generated through synergistic effects on an enhancer carrying both Dpp- and HOX-responsive (signaling- and selector-responsive) sequences. The cooperation of selector and signaling proteins in organ patterning has since received great attention and represents a key mechanism in organ development. We presently try to understand the molecular basis for the synergistic effects of these two classes of molecules.

Cell migration and extension during tracheal branching morphogenesis

Branching morphogenesis is a widely used strategy to increase the surface area of a given organ. A number of tissues undergo branching morphogenesis during vertebrate development, including the lung, the kidney, the mammary and numerous glands. Until recently, very little was known about the genetic principles underlying the branching process and about the molecules participating in organ specification and branch formation.

The tracheal system of insects represents one of the best-characterised branched organs. The tracheal network provides air to most tissues and we and others have intensively studied its development during embryogenesis at the morphological, cellular and genetic level. More than 30 genes have been identified and ordered into sequential steps controlling branching morphogenesis. These studies have revealed a number of important principles that might be conserved in other systems.

One of the most important factors for tracheal branching morphogenesis is Branchless (Bnl), a *Drosophila* FGF homologue. Bnl acts as a chemoattractant for tracheal cells and its developmentally regulated expression dictates the branching pattern. How this happens at the molecular level remains elusive, especially since each branch that forms requires, in addition to Bnl, different signalling input for proper development.

Using 4D confocal videomicroscopy in living embryos we have demonstrated that Bnl regulates filopodial activity in tracheal tip cells. While other signalling systems are also required for branch outgrowth, these other systems appear not to be required for motility per se, but for cells to be capable to undergo cell rearrangements allowing them to move towards Bnl sources. At present we try to decipher the molecular basis for Bnl-regulated chemotaxis and for signal-regulated branch formation and outgrowth.

Epithelial cells are fated to become tracheal cells during gastrulation upon the expression of the PAS-domain transcription factor Trachealess (Trh). It is the presence of this particular transcriptional regulator that instructs epithelial cells to undergo branching morphogenesis and to respond correctly to Bnl with directed motility. In order to understand this commitment at the molecular level, we are undertaking transcriptional profiling experiments using Affymetrix gene chips and different RNA populations. We hope to eventually isolate genes required for cells to undergo Trh-induced tubulogenesis and branching morphogenesis, with special emphasis on genes that allow the induction of filopodial activity in response to Bnl signalling. The identification of such genes would greatly contribute to understanding how branching processes can remodel pre-existing epithelial structures.

Publications

Affolter, M. (2000). Cell-cell interaction during Drosophila embryogenesis: novel mechanisms and molecules. *Ernst Schering Res Found Workshop* 29, 65-79.

Affolter, M. & Shilo, B. Z. (2000). Genetic control of branching morphogenesis during Drosophila tracheal development. *Curr Opin Cell Biol* 12, 731-5.

Amherd, R., Hintermann, E., Walz, D., Affolter, M. & Meyer, U. A. (2000). Purification, cloning, and characterization of a second arylalkylamine N-acetyltransferase from Drosophila melanogaster. *DNA Cell Biol* 19, 697-705.

Marty, T., Muller, B., Basler, K. & Affolter, M. (2000). Schnurri mediates Dpp-dependent repression of brinker transcription. *Nat Cell Biol* 2, 745-9.

Nussbaumer, U., Halder, G., Groppe, J., Affolter, M. & Montagne, J. (2000). Expression of the blistered/DSRF gene is controlled by different morphogens during Drosophila trachea and wing development. *Mech Dev* 96, 27-36.

Sivasankaran, R., Vigano, M. A., Muller, B., Affolter, M. & Basler, K. (2000). Direct transcriptional control of the Dpp target omb by the DNA binding protein Brinker. *EMBO J* 19, 6162-72.

Udagawa, Y., Hanai, J., Tada, K., Grieder, N. C., Momoeda, M., Taketani, Y., Affolter, M., Kawabata, M. & Miyazono, K. (2000). Schnurri interacts with Mad in a Dpp-dependent manner. *Genes Cells* 5, 359-69.

Affolter, M. & Mann, R. (2001). Development. Legs, eyes, or wings--selectors and signals make the difference. *Science* 292, 1080-1.

Affolter, M., Marty, T., Vigano, M. A. & Jazwinska, A. (2001). Nuclear interpretation of Dpp signaling in Drosophila. *EMBO J* 20, 3298-305.

Dossenbach, C., Rock, S. & Affolter, M. (2001). Specificity of FGF signaling in cell migration in Drosophila. *Development* 128, 4563-72.

Marty, T., Vigano, M. A., Ribeiro, C., Nussbaumer, U., Grieder, N. C. & Affolter, M. (2001). A HOX complex, a repressor element and a 50 bp sequence confer regional specificity to a DPP-responsive enhancer. *Development* 128, 2833-45.

Neuronal circuit formation in the developing spinal cord

S. Hippenmeyer, I. Kramer, V. Niederkofler, G. Niklaus, R. Salie, M. Sigrist, S. Stroebel, E. Vrieseling.

In collaboration with S.J. Burden (Skirball Institute, New York, USA), P. Caroni (Friedrich Miescher Institute, Basel), E. Frank (University of Pittsburgh, Pittsburgh, USA), C.E. Henderson (Marseille, France), T.M. Jessell (Columbia University, New York, USA).

Silvia Arber
Phone: 41 61 267 2057 Fax: +41 61 267 2078 E-mail: Silvia.Arber@unibas.ch
http://www.biozentrum.unibas.ch/arber

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 proprioceptive afferents in dorsal root ganglia (DRG) which establish monosynaptic connections with motor neurons (Ia afferents; Figure 1).

Transcription factors of the ETS class controlling the establishment of connections in the developing spinal reflex circuit

Different classes of transcription factors have been shown to control sequential steps in the hierarchy of differentiation of motor neurons in the developing spinal cord. For example, the homeobox transcription factor HB9 seems to play a crucial role in the initial specification of motor neurons, distinguishing motor neurons from surrounding interneurons in the spinal cord (Arber et al., 1999). Many of these early differentiation steps of motor neurons are thought to be controlled by cell-intrinsic cues and thus to occur independent of an influence from the target region. In contrast, the expression of members of one transcription factor family which has been the focus of our recent studies, the ETS transcription factor family, requires limb-derived signals for induction in motor neurons and DRG sensory neurons (Lin et al., 1998).

Within the ETS family of genes, we focus our attention on a small subfamily of genes (*Er81*, *Pea3* and *Erm*). In the developing avian embryo, ER81 and PEA3 are expressed in specific motor neuron pools, selectively innervating individual target muscles (Lin et al., 1998). In addition, ER81 and PEA3 are expressed by subpopulations of DRG muscle afferents. At the time that monosynaptic connectivity between proprioceptive afferents and motor neurons is being established in the spinal cord, motor pools expressing ER81 and PEA3 are innervated preferentially by sensory afferents expressing the same ETS gene (Lin et al., 1998). Taken together, these findings have suggested that ETS genes play a role in the establishment of neuronal connectivity at late developmental stages and that interactions between cell-intrinsic and cell-extrinsic cues are required for this process.

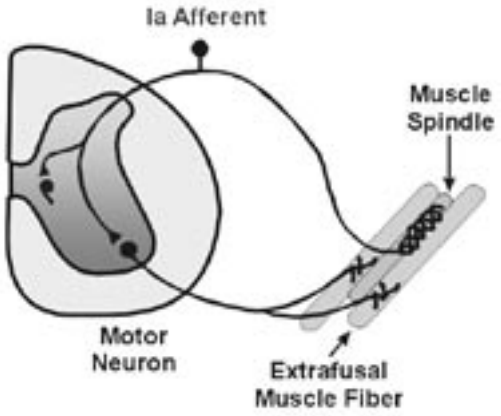


Figure 1:
Schematic representation of the spinal monosynaptic reflex circuit.

To begin to analyze the *in vivo* function of ETS proteins in the development of the spinal reflex circuit we generated *Er81* mutant mice (Arber et al., 2000). We found that soon after birth these mice develop a severe motor coordination phenotype. This behavioral phenotype is, however, not associated with a disruption

in the generation of the corresponding motor neurons or proprioceptive afferent neurons. Also, the initial axonal outgrowth pattern of these neurons is not affected by mutation of *Er81*. In contrast, a late developmental step in the differentiation of Ia afferents, the invasion of the correct termination zone within the spinal cord, is affected in *Er81* mutant mice (Arber et al., 2000). Electrophysiological analysis revealed that *Er81* mutant Ia afferents fail to make monosynaptic connections with motor neurons and an anatomical analysis showed that muscle afferents terminate aberrantly in the intermediate region of the spinal cord (Arber et al., 2000). In addition, we found that ETS genes are not only expressed by developing motor- and sensory neurons, but are also expressed in a third element of the spinal monosynaptic reflex circuit, in sensory organs innervated by Ia afferents in the periphery, the muscles spindles (Figure 2).

The role of motor neurons in the establishment of pattern in skeletal muscle

One key question in understanding the development of

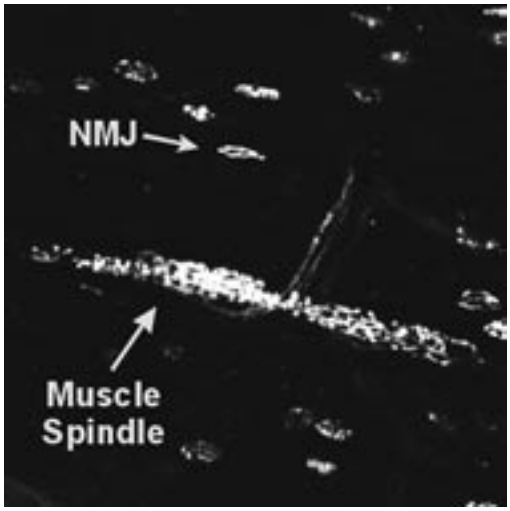


Figure 2:
Expression a Synaptophysin-eGFP fusion protein under the control of Thy1 regulatory elements to visualize peripheral endings of Ia afferents innervating muscle spindles and motor axons terminating at neuromuscular endplates (NMJ; in collaboration with P. Caroni).

neuronal circuits is to determine to which extent individual elements of a neuronal circuit depend on the presence of pre- and/or postsynaptic partners for their differentiation and for the establishment of correct connectivity.

The innervation of extrafusal muscle fibers (Figure 1) by motor axons and the simultaneous release of agrin by motor axons has been proposed to be a major determinant for the initiation of specific postsynaptic differentiation in developing muscles (Arber et al., 2002). Soon after motor axons invade a nascent muscle, pre- and postsynaptic elements have been shown to be aligned and a synaptic band within the muscle is established. We wanted to determine whether in the presence of motor axons invading the embryonic muscles is an absolute requirement for induction of postsynaptic differentiation or whether initiation of postsynaptic differentiation in the correct pattern within the muscle can be established even in the absence of motor axons.

In order to address this question we have applied a CRE-recombinase/Diphtheria toxin mediated binary genetic system in developing mouse embryos to eliminate motor neurons soon after their generation. To this end, we have generated mice where CRE-recombinase is expressed under the control of *Hb9* regulatory elements (Arber et al., 1999; *Hb9-CRE*) in combination with a strain of mice where silent Diphtheria toxin is activated via CRE-mediated excision of a STOP cassette under the control of *Isl2* regulatory elements. In these embryos, we found that despite the absence of motor axons, acetylcholine receptor clusters were present in developing muscles at the same sites where clusters are normally formed in the presence of motor axons (Yang et al., 2001). These experiments demonstrate that even in the complete absence of motor axons and thus of agrin, the initiation of postsynaptic differentiation within the muscle occurs normally. They also support the hypothesis that individual muscles are intrinsically programmed or prepatterned with respect to the site where postsynaptic differentiation is initiated and do not depend on the ingrowth of the presynaptic nerve for this event (Arber et al., 2002).

Publications

Lin, J. H., Saito, T., Anderson, D. J., Lance-Jones, C., Jessell, T. M. & Arber, S. (1998). Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. *Cell* 95, 393-407.

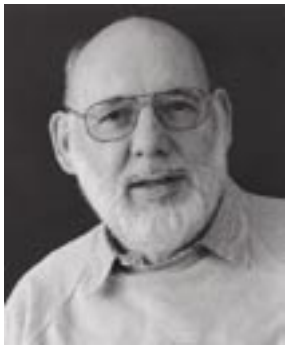
Arber, S., Han, B., Mendelsohn, M., Smith, M., Jessell, T. M. & Sockanathan, S. (1999). Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* 23, 659-74.

Arber, S., Ladle, D. R., Lin, J. H., Frank, E. & Jessell, T. M. (2000). ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. *Cell* 101, 485-98.

Yang, X., Arber, S., William, C., Li, L., Tanabe, Y., Jessell, T. M., Birchmeier, C. & Burden, S. J. (2001). Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. *Neuron* 30, 399-410.

Arber, S., Burden, S. J. & Harris, A. J. (2002). Patterning of skeletal muscle. *Curr Opin Neurobiol* 12, 100-3.

Pun, S., Sigrist, M., Santos, A.F., Ruegg, M.A., Sanes, J.R., Jessell, T.M., Arber, S. & Caroni, P. (2002). An intrinsic distinction in neuromuscular junction assembly and maintenance in different skeletal muscles. *Neuron* 34, 357-70.



Master control genes in development and evolution

P. Baumgartner, M. Berry, J. Blanco, J. Espinoza, D. Felix, S. Flister, S. Glardon, N. Grieder, L. Keller, U. Kloter, S. Kuo, T. Kunieda, F. Melchers, L. Michaut, T. Nagao, T. Pauli, S. Plaza, F. Prince, C. Punzo, S. Schonegg, M. Seimiya

Visiting scientists: M. Tarpin (University of Reims, France), K. Tönsing (University of Bielefeld, Germany)
In collaboration with S. Artavanis-Tsakonas (Harvard), J. Bièrne and M. Tarpin (Reims), K. Ikeo (Mishima), F. Munier and D. Schorderet (Lausanne), C. Remé and C. Grimm (Zürich), H. Reichert and V. Schmid (Basel), E. Salo (Barcelona), U. Walldorf (Homburg)

Walter J. Gehring
Phone: +41 61 267 2051 Fax: +41 61 267 2078 E-mail: Walter.Gehring@unibas.ch
http://www.biozentrum.unibas.ch/gehring

Our work in the context of developmental biology in Switzerland has been summarized in a special issue of the International Journal of Developmental Biology in an interview by Nik Walter and in review of our studies on eye development and evolution (Walter, 2002; Gehring, 2002).

Homeotic genes still keep revealing fascinating and unexpected facets. For example the essential differences between the homeotic *Antennapedia* and *Sex combs reduced* genes can be narrowed down to two single residues in the N-terminal arm of their homeodomains, which in *Sex combs reduced* can be phosphorylated. The phosphorylated form is inactive, and protein phosphatase PP2a is capable of specific dephosphorylation of the N-terminal arm and activation of the homeodomain (Berry and Gehring, 2000).

A novel concept emerged from our analysis of the role of *Antennapedia* in the inhibition of eye development which is under the control of *eyeless* (*ey*) a homolog of Pax 6. *Antennapedia* (*Antp*) antagonizes the *ey* gene cascade without interfering with transcription nor translation of *ey*. Using both *in vitro* and *in vivo* experiments, we demonstrated that this inhibitory mechanism involves direct protein-protein interactions between the homeodomain of *Antp* on the one hand, and the paired domain and the homeodomain of *ey* on the other (Plaza et al., 2001). These findings indicate that the paired- and homeodomains are not only DNA-binding domains, but also specific protein-protein interaction domains and lead to a new concept of gene regulatory networks, in which superimposed on the transcriptional network, a network of protein-protein interactions plays a crucial role.

Homeotic genes can induce cell fate changes only under certain preconditions. In the paper by Kurata et al. (2000) we show that *Notch* plays an important role in these cell fate changes. The *Notch* signaling pathway defines an evolutionarily conserved cell-cell interaction mechanism which throughout development controls the ability of precursor cells to respond to developmental signals. We were able to show that *Notch* signaling regulates the expression of the master control genes *eyeless*, *vestigial*, and *distal-less*, which in combination with homeotic genes induce the formation of either eyes, wings, antennae or legs on the head of the fly. Therefore, *Notch* is involved in a common regulatory pathway for the determination of the various appendages in *Drosophila*.

The genetic control of eye development and its evolutionary implications

Optix is a more recently discovered gene of the *sine oculis* family and presumably the homolog of *Six 3* in mice. Gain-of-function experiments indicate that *optix* plays an important role in eye development, which is clearly distinct from *sine oculis*, which is a direct target gene of *ey*. *Optix* is expressed independently of *ey*, and in contrast to *sine oculis*, *optix* alone is capable of inducing ectopic eyes in the antennal disc. These results suggest that *optix* is involved in eye-morphogenesis by an *eyeless*-independent mechanism (Seimiya & Gehring, 2000). The further analysis of *optix* has to await the isolation of loss-of-function mutants. The evolutionary studies have been extended to the base of the metazoan pedigree: Whereas a clear cut ortholog of Pax 6 has been found in planarians and nemerteans, the situation in cnidarians is more difficult to interpret (Miller et al. 2000; Gröger et al. 2000). However, medusae with well developed eyes remain to be studied.

The development of methods for RNA interference offers evolution biologists new possibilities to perform a functional genetic analysis. One might call this method also “poor man’s genetics”. In any case, in

collaboration with Emili Salo we have succeeded in knocking out *sine oculis* RNA in Dugesia, a flatworm, which renders these worms incapable of regenerating their eyes. This indicates that the gene cascade for eye morphogenesis in flatworms is similar to the one of insects and vertebrates.

Eye Organogenesis Programme

After many years of basic science in *Drosophila* and other model systems, I decided to try some possible medical applications. Seed money provided by the University was successfully used to launch a programme on age-related macula degeneration, a degenerative disease affecting a very large fraction of the aging population leading to blindness. We joined forces with the ophthalmologists in Lausanne (Munier and Schorderet) and in Zurich (Remé and Grimm), and try to transfer our knowledge gained on flies and mice to possible application in humans.

Publications

Berry, M. & Gehring, W. (2000). Phosphorylation status of the SCR homeodomain determines its functional activity: essential role for protein phosphatase 2A,B'. *Embo J* 19, 2946-57.

Engels, A., Reichert, H., Gehring, W. J. & Gartner, W. (2000). Functional expression of a locust visual pigment in transgenic Drosophila melanogaster. *Eur J Biochem* 267, 1917-22.

Groger, H., Callaerts, P., Gehring, W. J. & Schmid, V. (2000). Characterization and expression analysis of an ancestor-type Pax gene in the hydrozoan jellyfish Podocoryne carnea. *Mech Dev* 94, 157-69.

Kurata, S., Go, M.-J., Artavanis-Tsakonas, S. & Gehring, W.-J. (2000). *Notch* signalling and the determination of appendage identity. *Proceedings of the National Academy of Sciences of the United States of America* 97, 2117-2122.

Kurusu, M., Nagao, T., Walldorf, U., Flister, S., Gehring, W. J. & Furukubo-Tokunaga, K. (2000). Genetic control of development of the mushroom bodies, the associative learning centers in the Drosophila brain, by the eyeless, twin of eyeless, and Dachshund genes. *Proc Natl Acad Sci U S A* 97, 2140-4.

Miller, D. J., Hayward, D. C., Reece-Hoyes, J. S., Scholten, I., Catmull, J., Gehring, W. J., Callaerts, P., Larsen, J. E. & Ball, E. E. (2000). Pax gene diversity in the basal cnidarian Acropora millepora (Cnidaria, Anthozoa): implications for the evolution of the Pax gene family. *Proc Natl Acad Sci U S A* 97, 4475-80.

Pineda, D., Gonzalez, J., Callaerts, P., Ikeo, K., Gehring, W. J. & Salo, E. (2000). Searching for the prototypic eye genetic network: Sine oculis is essential for eye regeneration in planarians. *Proc Natl Acad Sci U S A* 97, 4525-9.

Seimiya, M. & Gehring, W. J. (2000). The Drosophila homeobox gene optix is capable of inducing ectopic eyes by an eyeless-independent mechanism. *Development* 127, 1879-86.

Gehring, W. J. (2001). The genetic control of eye development and its implications for the evolution of the various eye-types. *Zoology* 104, 171-183.

Kammermeier, L., Leemans, R., Hirth, F., Flister, S., Wenger, U., Walldorf, U., Gehring, W. J. & Reichert, H. (2001). Differential expression and function of the Drosophila Pax6 genes eyeless and twin of eyeless in embryonic central nervous system development. *Mech Dev* 103, 71-8.

Plaza, S., Prince, F., Jaeger, J., Kloter, U., Flister, S., Benassayag, C., Cribbs, D. & Gehring, W. J. (2001). Molecular basis for the inhibition of Drosophila eye development by Antennapedia. *Embo J* 20, 802-11.

Punzo, C., Kurata, S. & Gehring, W. J. (2001). The eyeless homeodomain is dispensable for eye development in Drosophila. *Genes Dev* 15, 1716-23.

Callaerts, P., Lee, P. N., Hartmann, B., Farfan, C., Choy, D. W., Ikeo, K., Fischbach, K. F., Gehring, W. J. & de Couet, H. G. (2002). HOX genes in the sepiolid squid Euprymna scolopes: implications for the evolution of complex body plans. *Proc Natl Acad Sci U S A* 99, 2088-93.

Gehring, W. J. (2002). From transdetermination to the homeodomain at atomic resolution. An interview with Walter J. Gehring. *Int J Dev Biol* 46, 29-37.

Gehring, W. J. (2002). The genetic control of eye development and its implications for the evolution of the various eye-types. *Int J Dev Biol* 46, 65-73.

Onuma, Y., Takahashi, S., Asashima, M., Kurata, S. & Gehring, W. J. (2002). Conservation of Pax 6 function and upstream activation by Notch signaling in eye development of frogs and flies. *Proc Natl Acad Sci U S A* 99, 2020-5.

Punzo, C., Seimiya, M., Flister, S., Gehring, W. J. & Plaza, S. (2002). Differential interactions of eyeless and twin of eyeless with the sine oculis enhancer. *Development* 129, 625-34.

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

S. Barabino, D. Blank, S. Dettwiler, H. de Vries, B. Dichtl, A. Gerber, W. Hübner, I. Kaufmann, A. Kyburz, G. Martin, M. Ohnacker, B. Paguet, S. Röck, M. Sadowski, M. Schaub, S. Weiser, V. Widmer, J. Wolf

In collaboration with S. Doublié (University of Vermont, Burlington); P. Jenö (Biozentrum); H. Langen (Hoffmann La-Roche, Basel); M.A. O'Connell (MRC Edinburgh); E. Wahle (Universität Halle)

Walter Keller
Phone: +41 61 267 2060 Fax: +41 61 267 2079 E-mail: Walter.Keller@unibas.ch
http://www.biozentrum.unibas.ch/keller

The posttranscriptional processing reactions capping, splicing, 3'-end cleavage/polyadenylation and RNA editing are important steps in the maturation of nuclear messenger RNA precursors (pre-mRNAs) in eukaryotic cells. Differential splicing and RNA editing give rise to alternate protein products that can have drastically different functional properties. Likewise, the poly(A) tails have several distinct functions, such as increasing the efficiency of translation, mRNA export and the control of mRNA turnover. Elucidation of the functional and structural properties of the factors involved in these reactions is the main purpose of our research.

3'-end processing of pre-mRNAs

3'-end processing in mammalian cells proceeds in two major steps. Endonucleolytic cleavage at a specific site on the precursor RNA (pre-mRNA) is followed by the slow addition of approximately ten adenylate residues to the upstream cleavage product. The oligo(A) tail is subsequently rapidly elongated to its final size of 200 to 300 nucleotides. The reaction takes place within a surprisingly large multi-component complex, the assembly of which precedes the first reaction step. The pre-mRNA is recognized by the cleavage and polyadenylation specificity factor (CPSF) and the cleavage stimulation factor (CstF). CPSF binds to the highly conserved sequence AAUAAA upstream of the cleavage site and CstF binds to GU-rich or U-rich sequence elements located downstream of the poly(A) addition site. The RNA sequence signals serve as nucleation sites for the assembly of the 3'-end processing complex.

CPSF consists of four core subunits with molecular weights of 160, 100, 73, and 30 kD. The 160 kDa subunit contacts the AAUAAA signal in the pre-mRNA substrate. The 100 kDa and the 73 kDa subunits are related in their protein sequence but their function is not known. The 30kDa subunit of CPSF contains five zinc finger motifs and binds RNA nonspecifically. Data base searches have revealed the existence of additional putative CPSF subunits that are human homologues of known 3'-end processing factors in yeast. We are investigating two of these human proteins, hFIP1 and symplekin. A polypeptide corresponding to hFIP1 copurifies with CPSF over several chromatographic steps. Immunodepletion and reconstitution experiments indicate that hFIP1 represents an additional subunit of CPSF. We have expressed individual CPSF subunits and subunit combinations in insect cells with the help of baculovirus vectors. Preliminary experiments showed that baculovirus-expressed CPSF containing the core subunits as well as hFIP1 is more active in reconstitution assays than a control lacking hFIP1. We have recently found that hFIP1 is a target for phosphorylation. We will study this phenomenon in more detail in the future and we suspect that phosphorylation could have a role in the regulation of pre-mRNA 3'-end processing.

Symplekin is a possible human homologue of the yeast Pta1p protein, a subunit of the 3'-end processing factor CPF. In collaboration with Drs. Ilse Hofmann and Werner W. Franke (German Cancer Research Center, Heidelberg) we have found that the protein copurifies with CPSF and that anti-symplekin antibodies deplete CPSF from HeLa nuclear extracts and also inhibit the 3'-end processing reaction *in vitro*.

Four polypeptides of 72, 68, 59, and 25 kD copurify with the activity of human cleavage factor I_m (CF I_m). We have previously obtained complete cDNA clones coding for the 25 and the 68 kD subunits. Whereas the predicted amino acid sequence of the 25 kD polypeptide has no sequence features resembling motifs found in other proteins, the three large subunit isoforms have a domain organization found in spliceosomal SR-proteins. The proteins contain a classical RRM-type RNA binding domain near their N-

terminus, a proline-rich middle region and a C-terminal domain rich in mixed charge amino acids, such as RS dipeptide clusters and many glutamate and aspartate residues intermingled with serines. Analysis of the kinetics of the *in vitro* cleavage reaction indicates that interaction of CF I_m with the pre-mRNA is one of the earliest steps in the assembly of the 3'-end processing complex and facilitates the recruitment of other processing factors.

We have isolated cDNA clones coding for the 72 kD and the 59 kD polypeptides and we are trying to reconstitute alternative forms of the CF I factor in recombinant form with baculovirus vectors expressed in insect cells. We have also made mutants in the 68 kD subunit to better understand the function of its different subdomains in RNA recognition and in protein-protein interactions with other subunits.

We have purified cleavage factor II (CF II) and have isolated cDNAs coding for two of its potential subunits. The two proteins (hCLP1 and hPCF11) are mammalian homologues of subunits of the yeast cleavage factor IA (CF IA). We have found that hCLP1 specifically interacts with CF I and with CPSF suggesting that it bridges these two 3'-end processing factors within the cleavage complex. We have obtained full-length clones of hPCF11 and have raised antibodies directed against portions of the recombinant protein. These antibodies inhibit the pre-mRNA cleavage reaction. We have expressed hCLP1 and hPCF11 in the baculovirus system and have affinity-purified the complex. We are presently testing whether the two polypeptides are sufficient for the reconstitution of CF II activity.

In collaboration with Dr. Sylvie Doublié (University of Vermont, Burlington) we have solved the crystal structure of bovine poly(A) polymerase bound to the ATP analog cordycepin triphosphate at 2.5 Å resolution. The structure revealed expected and unexpected similarities to other proteins. As expected, the catalytic domain of poly(A) polymerase shares substantial structural homology with other nucleotidyl transferases such as DNA polymerase- and kanamycin transferase. The C-terminal domain unexpectedly folds into a compact domain reminiscent of the RNA recognition motif fold. Such non-canonical RNA binding domains are also found in other proteins, such as ribosomal protein S6, *Drosophila* sex-lethal protein and the mRNA export factor TAP. The three invariant aspartates of the catalytic triad ligate two of the three active site metals. One of these metals also contacts the adenine ring. Furthermore, conserved, catalytically important residues contact the nucleotide. These contacts, taken together with metal coordination of the adenine base, provide a structural basis for ATP selection by poly(A) polymerase. We are now trying to establish conditions for the co-crystallization of poly(A) polymerase with RNA oligonucleotides and we are mapping regions in the enzyme that are involved in protein-protein interactions with other components of the 3'-end processing apparatus.

3'-end processing of yeast pre-mRNAs requires four multisubunit factors which can be separated biochemically. Cleavage only occurs in the presence of cleavage factors I and II (CF I and CF II); specific polyadenylation of "pre-cleaved" RNA substrates requires CF I, polyadenylation factor I (PF I) and poly(A) polymerase. Recent results have shown that CF II contains a subset of polypeptides that are also present in PF I. A large complex of PF I together with poly(A) polymerase can 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. Affinity-purified CPF has been reported to contain nine polypeptides, most of which appear to be homologues of mammalian CPSF. We have recently identified six additional polypeptides by microsequencing (in collaboration with Dr. H. Langen, Hoffmann-LaRoche Ltd.). The new CPF polypeptides are a WD-40 protein (Cpf10p), Pti1p, protein phoshatase 1 (Glc7p), Ssu72p and the non-essential Ref2p and Cpf11p proteins. The new components are now being characterized biochemically and genetically.

The functional interactions of yeast pre-mRNA 3'-end formation factors with cis-acting RNA sequences are poorly understood. In the long run, we hope to determine a topological map of all the CF I and CPF protein-protein contacts, as well as the interactions with different RNA substrates. Employing an *in vitro* cleavage system with CPF and CF I we have shown that the so-called efficiency and positioning elements are dispensable for poly(A) site recognition within of a short *CYC1* substrate. Instead, U-rich elements immediately upstream and 10 to 15 nt downstream of the poly(A) site are required for cleavage site recognition within *CYC1* and *ADH1* pre-mRNAs. These elements act in concert with the poly(A) site to produce multiple contact points for interaction with the processing machinery since combinations of mutations within these elements were most effective in cleavage inhibition. Introduction of a U-rich element downstream of the *GAL7* poly(A) site strongly enhanced cleavage, underscoring the importance of downstream sequences in yeast pre-mRNAs in general. RNA binding analyses demonstrate that cleavage depends on the recognition of the poly(A) site region by CPF. We have derived a model for yeast pre-mRNA cleavage site recognition, which implies a previously unanticipated conservation between the yeast and mammalian 3'-end processing mechanisms.

Transcription by RNA polymerase II and pre-mRNA processing are coupled events *in vivo.* The carboxy-terminal domain (CTD) of RNA polymerase II is thought to mediate the cotranscriptional coordination of these processes through protein-protein interactions. We investigated the interactions of recombinant subunits of the polyadenylation machinery and the CTD *in vitro*. Mutations in the CF I subunits RNA14, *PCF11* and RNA15 disrupt transcription termination. Rna14p and Pcf11p specifically interact with the phosphorylated form of the CTD. Mutational analysis revealed that Pcf11p has two functional domains for CTD-binding and pre-mRNA 3'-end processing that are both essential for mRNA biogenesis and cell viability. Loss of CTD-binding activity does not affect the functions of Pcf11p in cleavage and polyadenylation and *vice versa*. Transcription run-on analysis of mutant *PCF11* alleles demonstrated that the CTD-interaction domain of Pcf11p is required for correct Pol II termination, whereas another domain located more C-terminal is necessary for cleavage and polyadenylation but not for transcription termination. Moreover, *YHH1*, the largest subunit of CPF, has a dual function as well. The protein interacts with yeast pre-mRNAs at the poly(A) site region and we have defined the RNA binding domain which mediates RNA recognition. Intriguingly, this domain is composed of predicted β-propeller forming repeats. In addition, Yhh1p specifically interacts with the phosphorylated CTD. We found that certain *yhh1* mutant alleles cause a strong defect in transcription termination, which does not correlate with a defect in cleavage activity. Thus, the coupling of transcription and 3'-end processing is mediated by subunits of CF I and CPF via a mechanism that is independent of RNA cleavage.

RNA-specific adenosine deaminases and RNA editing

Adenosine deaminases acting on RNA can be divided into two families: ADARs act on double-stranded RNA and edit several mRNA precursors whereas ADATs convert adenosine (A) to inosine (I) in transfer RNAs. ADAT1/Tad1p forms I at position 37 (3' of the anticodon) in eukaryotic tRNA-Ala. Previously, we identified a yeast adenosine deaminase that generates I at the wobble position of tRNAs (position 34). Inosine 34 is thought to enlarge the codon recognition capacity during protein synthesis. The heterodimeric enzyme consists of two related subunits, ADAT2/Tad2p and ADAT3/Tad3p. Comparison of adenosine deaminase domains revealed that ADAT2/3 may represent the evolutionary precursor of the deaminase domain found in ADAT1 and in ADARs.

We have recently cloned and expressed *Escherichia coli* and human tRNA:A34 deaminases. The prokaryotic enzyme (tadA, encoded in *E. coli* by the essential yfhC gene) has sequence similarity to the yeast tRNA deaminase subunit Tad2p. Recombinant tadA protein forms homodimers and is sufficient for site-specific inosine formation at the wobble position (position 34) of tRNA-Arg2, the only tRNA containing this modification in prokaryotes. With the exception of yeast tRNA-Arg2 no other eukaryotic tRNA substrates were found to be modified by tadA. However, an artificial yeast tRNA-Asp, which carries the anticodon loop of yeast tRNA-Arg, is bound and modified by tadA. Thus, the anticodon loop is a major determinant for tadA substrate specificity. We propose a possible scenario for the evolution of tRNA and pre-mRNA editing enzymes. Human ADAT2 and ADAT3 homologues were identified and tRNA:A34 deamination could be reconstituted from recombinant subunits. Moreover, antibodies raised against hADAT2 can immunodeplete tRNA:A34 deaminase activity from HeLa cell nuclear extracts.

We are mapping the critical amino acids in the yeast Tad2 and Tad3 proteins required for deamination activity and for tRNA binding. We found that site-specific mutation of conserved amino acid residues in the deaminase domain abolish tRNA deamination activity. Mutations in the deaminase domain of Tad3p abolished binding of Tad2p/3p to the tRNA whereas the equivalent amino acid substitutions in Tad2p did not. Our results indicate that Tad3p may play a critical role in tRNA recognition.

In collaboration with the laboratory of Dr. Mary O'Connell (MRC Edinburgh), we are investigating the substrate specificity of dADAR, the only pre-mRNA editing enzyme present in *Drosophila*.

Publications

Birse, C. E., Minvielle-Sebastia, L., Lee, B. A., Keller, W. & Proudfoot, N. J. (1998). Coupling termination of transcription to messenger RNA maturation in yeast. *Science* 280, 298-301.

Gerber, A. P. & Keller, W. (1999). An adenosine deaminase that generates inosine at the wobble position of tRNAs. *Science* 286, 1146-1149.

Barabino, S. M., Ohnacker, M. & Keller, W. (2000). Distinct roles of two Yth1p domains in 3'-end cleavage and polyadenylation of yeast pre-mRNAs. *EMBO J* 19, 3778-3787.

de Vries, H., Rüegsegger, U., Hübner, W., Friedlein, A., Langen, H. & Keller, W. (2000). Human pre-mRNA cleavage factor II_m contains homologs of yeast proteins and bridges two other cleavage factors. *EMBO J* 19, 5895-5904.

Fahrenkrog, B., Hübner, W., Mandinova, A., Pante, N., Keller, W. & Aebi, U. (2000). The yeast nucleoporin Nup53p specifically interacts with Nic96p and is directly involved in nuclear protein import. *Mol Biol Cell* 11, 3885-3896.

Keegan, L. P., Gerber, A. P., Brindle, J., Leemans, R., Gallo, A., Keller, W. & O'Connell, M. A. (2000). The properties of a tRNA-specific adenosine deaminase from Drosophila melanogaster support an evolutionary link between pre-mRNA editing and tRNA modification. *Mol Cell Biol* 20, 825-833.

Martin, G., Keller, W. & Doublié, S. (2000). Crystal structure of mammalian poly(A) polymerase in complex with an analog of ATP. *EMBO J* 19, 4193-4203.

Ohnacker, M., Barabino, S. M., Preker, P. J. & Keller, W. (2000). The WD-repeat protein Pfs2p bridges two essential factors within the yeast pre-mRNA 3'-end-processing complex. *EMBO J* 19, 37-47.

Paupard, M. C., O'Connell, M. A., Gerber, A. P. & Zukin, R. S. (2000). Patterns of developmental expression of the RNA editing enzyme rADAR2. *Neuroscience* 95, 869-879.

Vagner, S., Rüegsegger, U., Gunderson, S. I., Keller, W. & Mattaj, I. W. (2000). Position-dependent inhibition of the cleavage step of pre-mRNA 3'-end processing by U1 snRNP. *RNA* 6, 178-188.

Dichtl, B. & Keller, W. (2001). Recognition of polyadenylation sites in yeast pre-mRNAs by cleavage and polyadenylation factor. *EMBO J* 20, 3197-3209.

Gerber, A. P. & Keller, W. (2001). RNA editing by base deamination: more enzymes, more targets, new mysteries. *Trends Biochem Sci* 26, 376-384.

DIVISION OF STRUCTURAL BIOLOGY

The techniques applied in the Division of Structural Biology range from light, electron and scanning microscopy to X-ray crystallography and NMR spectroscopy. Using rigorous combinations of these techniques, we are in a unique position to determine structure of biological matter at all levels, from entire cells to atomic detail.

The Division currently consists of four senior research groups headed by Ueli Aebi, Andreas Engel, Tilman Schirmer, and Stephan Grzesiek who joined the Biozentrum by the end of 1999. In order to increase the critical mass in X-ray crystallography after the retirement of J.N. Jansonius, a new assistant professor position was created and successfully filled by Olga Mayans who joined us in December 2001. Two of the group leaders are also project leaders in the newly founded National Centers of Competence in Research (NCCR) programs 'Nanoscale Science' (U. Aebi and A. Engel) and 'Structural Biology' (A. Engel). The Division receives generous support by the M.E. Müller Foundation via the fully integrated M.E. Müller Institute for Structural Biology. During the last two years, major acquisitions of equipment were made possible by a joint donation from F. Hoffmann-La Roche and Novartis (800 MHz and 600 MHz NMR-spectrometers) as well as by funds from the University of Basel and the Swiss National Science Foundation (200 kV field emission transmission electron microscope).

The projects of the individual research groups are described below.

Professors

Ueli Aebi, Andreas Engel, Stephan Grzesiek, Tilman Schirmer

Assistant professor

Olga Mayans

Independent scientists

Peter Burkhard*

Cora-Ann Schönenberger*

University lecturers

Peter Burkhard*

Cora-Ann Schönenberger*

Scientists and postdoctoral fellows

Werner Baschong, Florence Cordier, Myriam Duckely*, Fabrice Dumas*, Birthe Fahrenkrog*, Bernd Feja*, Dimitrios Fotiadis*, Patrick Frederix*, Claire Goldsbury, Lorenz Hasler*, Daniel Häussinger, Bernard J. Heymann*, Zora Housley-Markovic, Victor A. Jaravine, Jan Kahmann*, Joachim Köser, Bohumil Maco*, Anna Mandinova*, Daniel J. Müller*, Shirley Müller, Giovanna Musco*, George L. Orriss, Ansgar Philippsen*, Hans-Jürgen Sass, Simon Scheuring*, Henning Stahlberg, Daniel Stoffler*, Paola Storici*, Sergei V. Strelkov, Nicole Taschner, Patrick van Gelder*, Paul Werten*

Graduate students

Thomas Braun, Melanie Börries*, Carmen Chan*, Gabriel Fedrigo*, Dimitrios Fotiadis*, Frédéric Grandjean*, Janelle Green*, Georgios Halambalakis*, Sergei Ivaninskii*, Thomas Kaufmann*, Aris Krikelis*, Markus Meier*, Sebastian Meier*, Clemens Möller*, Dinesh Palanivelu*, Ansgar Philippsen*, Hervé Remigy, Andreas Schenk*, Simon Scheuring*, Edwin B. Shillington*, Martin Stolz, Andreas Wild*

Guest graduate students

Milena Mourdjeva, Dept. of Molecular Immunology, Inst. of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria (4 months)

Maria Karlsson, Dept. of Plant Biology, Lund University, Lund, Schweden (1 month)

Isabel Dos Santos Cardoso, Inst. of Biomedical Sciences Abel Salazar, University of Porto, Portugal (2 months)

Silke Hippler, Max-Planck-Institut für molekulare Physiologie in Dortmund, Deutschland (10 days)

Diploma students

Markus Meier*, Constanze Blume*, Sonja Baumli*, Andreas Duijts*, Nadja Feddermann Bühler*, Gabriela Güntherodt*, Marco Gregorini*, Andreas Schenk*, Thomas Kaufmann*, Wanda Kukulski*, Martin Allan*, Stefan Langheld*

Visiting scientists

Christopher Bazinet Department of Biological Sciences, St. John's University, Jamaica, NY, USA; (7 months), Mohamed Chami, Laboratoire de Cristallogénèse, Institut Curie, Physico-Chimie, Paris, Frankreich (6 months), Joerg Kistler, University of Auckland, New Zealand (1 month), Nathan Nelson, Tel-Aviv University, Israel (1 month), Anna Rita Taddei, Centro Interdipartimentale di Microscopie Elettronice, Università degli Studi della, Chikara Sato Tsukuba, Science City, Japan (2 months), Tuscia Viterbo, Italien (3 months)

Laboratory personnel

Andrea Bürer*, Margrit Jenny, Alexandra Meli*, Barbara Merz, Rita Müller*, Vesna Olivieri*, Yvonne Rasser*, Joachim Scherf*, Klara Rathgeb-Szabo, Kitaru Suda, Rosmarie Sütterlin-Willener, Christine Widmer-Oetz, Sabine Wirtz*

Secretariat

Liselotte Walti, Sabine Frauenknecht*, Ute Grütter

Technical personnel

Roland Bürki, Robert Häring, Christine Saladin*, Robert Wyss, Marco Rogowski

Floor manager

Urs Fürstenberger*, Beat Schumacher*

*Employed during part of the report period

Structure-based functional analysis of proteins and their supramolecular assemblies

V.R. Alumella, W. Baschong, A. Bürer, B. Fahrenkrog, C. Goldsbury, J. Green, G. Güntherodt; G. Halambalakis, J. Köser, A. Krikelis, B. Maco, A. Mandinova, D. Stoffler, M. Stolz, R. Sütterlin, N. Taschner, A. Wild

In collaboration with T. Arvinte, P. Frey and M. Steinmetz (Novartis Pharma AG, Basel); D.A. Atar and T.F. Lüscher (University Hospital, Zürich); W. Baumeister and D. Typke (MPI Martinsried, Germany); M. Beckerle and K. Ullman (University of Utah Medical Center, Salt Lake City, USA); W. Brett, P. Hunziker, I. Martin and H.-R. Zerkowski (University Hospital, Basel); P. Burkhard, M. Dürrenberger, J. Engel, A. Hefti, W. Hübner, W. Keller, A. Lustig, S. Müller, U. Sauder, C.-A. Schoenenberger, J. Seelig, S. Strelkov, G. Vergères and F. Wohnsland (Biozentrum, Basel); M. Buschmann (Ecole Polytechnique, Montreal, Canada); I. Cardoso, A.M. Damas and M.J. Saraiva (University of Porto, Portugal); G. Cooper and J. Kistler (University of Auckland, New Zealand); N. Geisler (MPI, Göttingen, Germany); L. Gerace and G. Tinglu (Scripps Research Institute, La Jolla, USA); R.D. Goldman (Northwestern University Medical School, Chicago, USA); H. Gross (ETH-Hönggerberg, Zürich); C.W. Heizmann, E. Hahn and P. Wild (University of Zürich); H. Herrmann (DKFZ, Heidelberg, Germany); E. Hurt (ZMBH, Heidelberg, Germany); B. Kachar and P.M. Steinert (NIH, Bethesda, USA); J.-P. Kong and T.-T. Sun (NYU Medical Center, New York, USA); H.G. Mannherz (Ruhr University, Bochum, Germany); D.A.D. Parry (Massey University, Palmerston North, New Zealand); K. Scherrer (Institute J. Monod, Paris, France)

Ueli Aebi
Phone: +41 61 267 2261 Fax: +41 61 267 2109 E-mail: Ueli.Aebi@unibas.ch
http://www.biozentrum.unibas.ch/aebi

Our group explores the structure and function of proteins and their supramolecular assemblies by an interdisciplinary experimental approach that involves light, electron and scanning probe microscopies combined with X-ray crystallography (in collaboration with the group of P. Burkhard), biophysical, biochemical, cell and molecular biological approaches. Our activities broadly cover three topical areas: (1) the actin and intermediate filament (IF) cytoskeletons ; (2) the nuclear pore complex (NPC) and nucleocytoplasmic transport; and (3) structural dissection of the fibrillation mechanism of amyloid forming peptides.

Structural dissection of the interaction of thymosin ß$_4$ with F-actin filaments

Thymosin ß4 (Tß$_4$) is an actin sequestering protein that in some cells is present in high enough concentration to stabilize about 50% of the intracellular actin in an unpolymerized state. We produced a chemically cross-linked actin: Tß$_4$ complex that could be assembled into filaments in the presence of phalloidin and catalytic amounts of gelsolin: 2-actin complex [Ballweber et al. (2002) *J. Mol. Biol.* **315**, 613-625]. For structural analysis negatively stained Tß$_4$-decorated F-actin filaments were imaged by scanning transmission electron microscopy (STEM); determination of their helical parameters revealed an increase of the crossover spacing of the two right-handed long-pitch helical strands of the filaments from 36.0 to 40.5 nm. Difference map analysis of 3D helical reconstruction of control and actin: Tß$_4$ filaments yielded an elongated extra mass. Qualitatively, the overall size and shape of the difference mass accommodated published NMR data of the atomic structure of Tß$_4$. The deduced binding sites of Tß$_4$ to F-actin were in good agreement with those previously identified. Parts of the difference map might also represent subtle conformational changes of both proteins occurring upon complex formation.

Toward the atomic details of intermediate filament structure, assembly and dynamics

All intermediate filament (IF) proteins share a common tripartite structural organization of the dimer that represents the elementary 'building block' of the filament. This implies that the crystallographic detail we have obtained for the IF protein vimentin [Strelkov et al. (2001) *J. Mol. Biol.* 306, 771-779; Strelkov et al. (2002) *EMBO J.* 21, 1255-1266; see also P. Burkhard] may serve as a paradigm for the molecular organization of IFs in general. Moreover, our crystallographic data provide a solid structural basis for more systematically understanding the architecture and functioning of *bona fide* IFs at atomic detail. In addition, we suggest that the application of X-ray crystallographic techniques is not limited to the IF dimer. Firstly, future crystallographic studies will include homo- and heterotypic complexes formed by various IF fragments. In particular, this should provide atomic

resolution detail of the four distinct dimer-dimer interactions occurring in IFs and thus drastically extend the knowledge obtained from the cross-linking experiments. Secondly, we have recently discovered several point mutations in the full-length vimentin that arrest IF assembly at the unit-length filament (ULF) stage. As demonstrated recently for the ribosome, crystal structure determination of macromolecular assemblies such as ULFs with a molecular mass of about 1.7 MDa is no longer beyond the scope of the current methodology. The crystallographic data will be complemented by cryo-EM tomography and time-lapse atomic force microscopy that are likely to provide further structural insights into the successive dimer association events driving IF assembly.

Structural dissection of the nuclear pore complex and nucleocytoplasmic transport

Trafficking of cargoes between the cytoplasm and the nucleus is mediated by the nuclear pore complexes (NPCs), supramolecular assemblies that perforate the nuclear envelope (NE). Small molecules and ions traverse the NPC by passive diffusion, whereas macromolecules such as proteins, RNAs and ribonucleoprotein (RNP) particles usually require specific signals and factors that mediate their passage through the NPC. The limiting size of cargoes for passive diffusion has been reported to be ~9 nm in diameter, whereas that for signal-dependent macromolecular transport has been determined to be ~26 nm. Moreover, our molecular understanding as to how signal-bearing cargoes interact with the NPC, transport factors and the small GTPase Ran to assure effective vectorial transport has increased significantly over the past several years. To complement these functional data, we have determined the 3D architecture of the NPC by energy-filtering transmission electron microscopy (EFTEM) combined with tomographic 3D reconstruction of fully native *Xenopus* oocyte NEs embedded in thick amorphous ice. To chart the spatial organization of individual nucleoporins within the NPC, we are mapping by immuno-gold EM the epitopes of antibodies raised against different peptides of these nucleoporins (e.g., Nup153). To more directly correlate NPC structure with nucleocytoplasmic transport (NCT) at the level of individual NPCs, we have performed time-lapse atomic force microscopy of native NPC preparations kept 'alive' in physiological buffer, and monitored distinct structural changes in response to various chemical and physical effectors or stimuli. Last but not least, to better understand the actual translocation step of macromolecules through the central channel of the NPC, we are loading the cytoplasmic or nuclear compartment of *Xenopus* oocytes or cultured cells with GFP- or colloidal gold-labeled cargoes and tracking their time-dependent location by light, electron and atomic force microscopy. Cargoes include mono- and multimeric GFP constructs, anti-nucleoporin antibodies and engineered virus capsids. By this structure-based experimental approach, we should eventually gain a more mechanistic functional understanding of NCT at the single NPC level.

A molecular model for *in vitro* assembled transthyretin amyloid-like fibrils

Extracellular accumulation of transthyretin (TTR) variants in the form of fibrillar amyloid deposits is the pathological hallmark of Familial Amyloidotic Polyneuropathy (FAP). The TTR Leu55Pro variant occurs in the most aggressive forms of this disease. Inhibition of TTR wild-type (WT) and particularly TTR Leu55Pro fibril formation is of interest as a potential therapeutic strategy and requires a thorough understanding of the fibril assembly mechanism. To this end, we report on the *in vitro* assembly properties as observed by transmission electron microscopy (TEM), atomic force microscopy (AFM) and quantitative scanning transmission electron microscopy (STEM) for both TTR WT fibrils produced by acidification, and TTR Leu55Pro fibrils assembled at physiological pH [Cardoso et al. (2002) *J. Mol. Biol.* 317, 683-695]. The morphological features and dimensions of TTR WT and TTR Leu55Pro fibrils were similar, with up to 300 nm long, 8 nm wide fibrils being the most prominent species in both cases. Other species were also evident; 4-5 nm wide fibrils, 9-10 nm wide fibrils, and oligomers of various sizes. STEM mass-per-length (MPL) measurements revealed discrete fibril types with masses of 9.5 and 14.0 kDa/nm for TTR WT fibrils and 13.7, 18.5 and 23.2 kDa/nm for TTR Leu55Pro fibrils. These MPL values are consistent with a model in which fibrillar TTR structures are composed of 2, 3, 4 or 5 elementary protofilaments, with each protofilament being a vertical stack of structurally modified TTR monomers assembled with the 2.9 nm axial monomer-monomer spacing as evidenced by X-ray fibre diffraction data. *Ex vivo* TTR amyloid fibrils were also examined. Judged from their morphological appearance, the *in vitro* assembled TTR WT and Leu55Pro fibrils examined may represent immature fibrillar species. The *in vitro* system operating at physiological pH for TTR Leu55Pro and the model presented for the molecular arrangement of TTR monomers within fibrils may, therefore, describe early fibril assembly events *in vivo*.

Publications

Arcangeletti, C., De Conto, F., Sutterlin, R., Pinardi, F., Missorini, S., Geraud, G., Aebi, U., Chezzi, C. & Scherrer, K. (2000). Specific types of prosomes distribute differentially between intermediate and actin filaments in epithelial, fibroblastic and muscle cells. *Eur J Cell Biol* 79, 423-37.

Burkhard, P., Kammerer, R. A., Steinmetz, M. O., Bourenkov, G. P. & Aebi, U. (2000). The coiled-coil trigger site of the rod domain of cortexillin I unveils a distinct network of interhelical and intrahelical salt bridges. *Structure Fold Des* 8, 223-30.

De La Cruz, E. M., Mandinova, A., Steinmetz, M. O., Stoffler, D., Aebi, U. & Pollard, T. D. (2000). Polymerization and structure of nucleotide-free actin filaments. *J Mol Biol* 295, 517-26.

Fahrenkrog, B., Aris, J. P., Hurt, E. C., Pante, N. & Aebi, U. (2000). Comparative spatial localization of protein-A-tagged and authentic yeast nuclear pore complex proteins by immunogold electron microscopy. *J Struct Biol* 129, 295-305.

Fahrenkrog, B., Hubner, W., Mandinova, A., Pante, N., Keller, W. & Aebi, U. (2000). The yeast nucleoporin Nup53p specifically interacts with Nic96p and is directly involved in nuclear protein import. *Mol Biol Cell* 11, 3885-96.

Gietz, U., Arvinte, T., Haner, M., Aebi, U. & Merkle, H. P. (2000). Formulation of sustained release aqueous Zn-hirudin suspensions. *Eur J Pharm Sci* 11, 33-41.

Goldsbury, C., Goldie, K., Pellaud, J., Seelig, J., Frey, P., Muller, S. A., Kistler, J., Cooper, G. J. & Aebi, U. (2000). Conformation and amyloid fibril formation from full-length and fragments of amylin. *J Struct Biol* 130, 352-362.

Goldsbury, C. S., Wirtz, S., Muller, S. A., Sunderji, S., Wicki, P., Aebi, U. & Frey, P. (2000). Studies on the in vitro assembly of a beta 1-40: implications for the search for a beta fibril formation inhibitors. *J Struct Biol* 130, 217-31.

Hahn, E., Wild, P., Schraner, E. M., Bertschinger, H. U., Haner, M., Muller, S. A. & Aebi, U. (2000). Structural analysis of F18 fimbriae expressed by porcine toxigenic Escherichia coli. *J Struct Biol* 132, 241-50.

Herrmann, H. & Aebi, U. (2000). Intermediate filaments and their associates: multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr Opin Cell Biol* 12, 79-90.

Herrmann, H., Strelkov, S. V., Feja, B., Rogers, K. R., Brettel, M., Lustig, A., Haner, M., Parry, D. A., Steinert, P. M., Burkhard, P. & Aebi, U. (2000). The intermediate filament protein consensus motif of helix 2B: its atomic structure and contribution to assembly. *J Mol Biol* 298, 817-32.

Klainguti, M., Aigner, S., Kilo, J., Eppenberger, H. M., Mandinova, A., Aebi, U., Schaub, M. C., Shaw, S. G., Luscher, T. F. & Atar, D. (2000). Lack of nuclear apoptosis in cardiomyocytes and increased endothelin-1 levels in a rat heart model of myocardial stunning. *Basic Res Cardiol* 95, 308-15.

Kosova, B., Pante, N., Rollenhagen, C., Podtelejnikov, A., Mann, M., Aebi, U. & Hurt, E. (2000). Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p. *J Biol Chem* 275, 343-50.

Langelier, E., Suetterlin, R., Hoemann, C. D., Aebi, U. & Buschmann, M. D. (2000). The chondrocyte cytoskeleton in mature articular cartilage: structure and distribution of actin, tubulin, and vimentin filaments. *J Histochem Cytochem* 48, 1307-20.

Panté, N., Thomas, F., Aebi, U., Burke, B. & Bastos, R. (2000). Recombinant Nup153 incorporates in vivo into Xenopus oocyte nuclear pore complexes. *J Struct Biol* 129, 306-12.

Raska, I., Aebi, U. & Earnshaw, W. C. (2000). EMBO workshop report. An eclipse over the cell nucleus functional organization of the cell nucleus Prague, August 9-12, 1999. *EMBO J* 19, 3843-8.

Siniossoglou, S., Lutzmann, M., Santos-Rosa, H., Leonard, K., Mueller, S., Aebi, U. & Hurt, E. (2000). Structure and assembly of the Nup84p complex. *J Cell Biol* 149, 41-54.

Steinmetz, M. O., Hoenger, A., Stoffler, D., Noegel, A. A., Aebi, U. & Schoenenberger, C. A. (2000). Polymerization, three-dimensional structure and mechanical properties of Ddictyostelium versus rabbit muscle actin filaments. *J Mol Biol* 303, 171-84.

Stolz, M., Stoffler, D., Aebi, U. & Goldsbury, C. (2000). Monitoring biomolecular interactions by time-lapse atomic force microscopy. *J Struct Biol* 131, 171-80.

Wohnsland, F., Schmitz, A. A., Steinmetz, M. O., Aebi, U. & Vergeres, G. (2000). Influence of the effector peptide of MARCKS-related protein on actin polymerization: a kinetic analysis. *Biophys Chem* 85, 169-77.

Wohnsland, F., Schmitz, A. A., Steinmetz, M. O., Aebi, U. & Vergeres, G. (2000). Interaction between actin and the effector peptide of MARCKS-related protein. Identification of functional amino acid segments. *J Biol Chem* 275, 20873-9.

Wohnsland, F., Steinmetz, M. O., Aebi, U. & Vergeres, G. (2000). MARCKS-related protein binds to actin without significantly affecting actin polymerization or network structure. Myristoylated alanine-rich C kinase substrate. *J Struct Biol* 131, 217-24.

Baschong, W., Suetterlin, R. and Laeng, H. (2001). Control of autoimmunofluorescence of archival formaldehyde-fixed, paraffin-embedded tissue in confocal laser scanning microscopy (CLSM). *J. Histochem. Cytochem.* 49, 1565-1571.

Baschong, W., Suetterlin, R., Hefti, A. & Schiel, H. (2001). Confocal laser scanning microscopy and scanning electron microscopy of tissue Ti-implant interfaces. *Micron* 32, 33-41.

Brett, W., Mandinova, A., Remppis, A., Sauder, U., Ruter, F., Heizmann, C. W., Aebi, U. & Zerkowski, H. R. (2001). Translocation of S100A1(1) calcium binding protein during heart surgery. *Biochem Biophys Res Commun* 284, 698-703.

Fahrenkrog, B., Stoffler, D. & Aebi, U. (2001). Nuclear pore complex architecture and functional dynamics. *Curr Top Microbiol Immunol* 259, 95-117.

Goldsbury, C., Aebi, U. & Frey, P. (2001). Visualizing the growth of Alzheimer's A beta amyloid-like fibrils. *Trends Mol Med* 7, 582.

Martin, I., Suetterlin, R., Baschong, W. Heberer, M., Vunjak-Novakovic, G. and Freed, L.E. (2001). Enhanced cartilage tissue engineering by sequential exposure of chondrocytes to FGF-2 during 2D expansion and BMP-2 during 3D cultivation. *J. Cell Biochem* 83, 121-128.

Müller, S. A., Haener, M., Ortiz, I., Aebi, U. & Epstein, H. F. (2001). STEM Analysis of Caenorhabditis elegans muscle thick filaments: evidence for microdifferentiated substructures. *J Mol Biol* 305, 1035-44.

Pashmforoush, M., Pomies, P., Peterson, K. L., Kubalak, S., Ross, J., Jr., Hefti, A., Aebi, U., Beckerle, M. C. & Chien, K. R. (2001). Adult mice deficient in actinin-associated LIM-domain protein reveal a developmental pathway for right ventricular cardiomyopathy. *Nat Med* 7, 591-7.

Strelkov, S. V., Herrmann, H., Geisler, N., Zimbelmann, R., Aebi, U. and Burkhard, P. (2001). Toward the atomic details of intermediate filament structure, assembly and dynamics. *Proc. 10th Int. Wool Textile Res. Conf* KNL-4, 1-11

Strelkov, S. V., Herrmann, H., Geisler, N., Lustig, A., Ivaninskii, S., Zimbelmann, R., Burkhard, P. & Aebi, U. (2001). Divide-and-conquer crystallographic approach towards an atomic structure of intermediate filaments. *J Mol Biol* 306, 773-81.

Taschner, N., Muller, S. A., Alumella, V. R., Goldie, K. N., Drake, A. F., Aebi, U. & Arvinte, T. (2001). Modulation of antigenicity related to changes in antibody flexibility upon lyophilization. *J Mol Biol* 310, 169-79.

Ballweber, E., Hannappel, E., Huff, T., Stephan, H., Haener, M., Taschner, N., Stoffler, D., Aebi, U. & Mannherz, H. G. (2002). Polymerisation of chemically cross-linked actin:thymosin beta(4) complex to filamentous actin: alteration in helical parameters and visualisation of thymosin beta(4) binding on F-actin. *J Mol Biol* 315, 613-25.

Cardoso, I., Goldsbury, C.S., Wirtz, S., Mueller, S.A., Damas, A.M., Aebi, U. and Saraiva, M.J. (2002). Dynamcs of transthyretin fibrillogenesis entails the assembly of monomers. *J. Mol. Biol.* 317,683-695.

Fahrenkrog, B. & Aebi, U. (2002). The vertebrate nuclear pore complex: from structure to function. *Results Probl Cell Differ* 35, 25-48.

Kochs, G., Haener, M., Aebi, U. & Haller, O. (2002). Self-assembly of human MxA GTPase into highly-ordered dynamin-like oligomers. *J Biol Chem* 277, 14172-14176.

Lutzmann, M., Kunze, R., Buerer, A., Aebi, U. & Hurt, E. (2002). Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. *EMBO J* 21, 387-97.

Min, G., Stolz, M., Zhou, G., Liang, F., Sebbel, P., Glockshuber, R., Sun, T.-T., Aebi, U. and Kong, X.-P. (2002). Localization of uroplakin Ia, the bacterial FimH receptor, on the six inner domains of the 16-nm mouse urothelial plaque particle. *J. Mol. Biol.* 317, 697-706.

Strelkov, S. V., Herrmann, H., Geisler, N., Wedig, T., Zimbelmann, R., Aebi, U. and Burkhard, P. (*2002*). Evolutionarily conserved ?-helical segments 1A and 2B of the intermediate filament dimer: Their atomic structures and role in filament assembly. *EMBO J.* 21, 1255-1266.

Yang, Z., Madinova, A., Kozai, T., Joch, H., Aebi, U. & Luscher, T. F. (2002). Felodipine inhibits nuclear translocation of p42/44 mitogen-activated protein kinase and human smooth muscle cell growth. *Cardiovasc Res* 53, 227-31.

Herrmann, H., Wedig, T., Porter, R.M., Lane, E.B. and Aebi, U. (*in press*). Characterization of early assembly intermediates of recombinant human keratins. *J. Struct. Biol.*

Herrmann, H., Reichenzeller, M., Aebi, U., Hesse, M. and Magin, T.M. (*in press*). The functional complexity of intermediate filament cytoskeletons: From structure to assembly and to gene ablation. *Int. Rev. Cytol.*

Meier, M., Lustig, A., Aebi, U. and Burkhard, P. (*in press*). Removing an interhelical salt bridge abolishes coiled-coil formation in a *de novo* designed peptide. *J. Struct. Biol.*

Smith, T. A., Strelkov, S.V., Burkhard, P., Aebi, U. and Parry, D.A.D. (*in press*). Sequence comparisons of intermediate filament chains: Evidence of a unique functional/structural role for coiled-coil segment 1A and linker L1. *J. Struct. Biol.*

Membrane protein structure and function

T. Braun, P. Frederix, D. Fotiadis, M. Gregorini, L. Hasler, B.J. Heymann, T. Kaufmann, W. Kukulski, C. Möller, D.J. Müller, S. A. Müller, V. Olivieri, H. Remigy, A. Schenk, S. Scheuring, H. Stahlberg, K. Suda, P. Werten, C. Widmer

Visiting scientists: Joerg Kistler, University of Auckland, New Zealand, Nathan Nelson, Tel-Aviv University, Israel, Chikara Sato, Tsukuba Science City, Japan
In collaboration with U. Aebi (this Division), P. Agre (Johns Hopkins University, Baltimore, USA), W. Baumeister (Max-Planck Institut für Biochemie, Martinsried, Germany), Peter Deen (University Medical Center, Nijmegen, NL), A. Driessen and W. Konings (University of Groningen, Netherlands), J. Engel (Division of Biophysical Chemistry), Y. Fujiyoshi (Kyoto University, Japan), H. Grubmüller (Theor. Molecular Biophysics Group MPI for Biophysical Chemistry Goettingen), R. Kaback (University of California, Los Angeles, USA)

Andreas Engel
Phone: +41 61 267 2262 Fax: +41 61 267 2109 E-mail: Andreas.Engel@unibas.ch
http://www.biozentrum.unibas.ch/aengel

Membrane proteins represent one third of all proteins in a eucaryotic cell. They convert energy, receive and transmit signals, or act as transporters or ion channels. Our focus is on the structures and functions of membrane proteins. They are purified and reconstituted in the presence of lipids into tightly packed arrays. These 2D crystals are either rapidly frozen and analyzed by electron microscopy or adsorbed to mica and studied by atomic force miscopy (AFM). Both techniques give access to a membrane protein in its native state. The former technique allows the atomic structure of a frozen state to be determined at atomic resolution, while the latter allows the surface conformation and dynamics of the membrane protein to be measured in solution at a lateral resolution of 4-7 Å and a vertical resolution of 1 Å.

The combination of 2D crystallization, cryo-electron microscopy and AFM has been exploited to study different aquaglyceroporins and the rotors of the ATP synthase. We have invested a major effort in the improvement of 2D crystallization, because the production of highly ordered crystals is the prerequisite for structure determination by electron crystallography. An essential step forward was the acquisition of a 200 kV field-emission (FEG) microscope through funds from the University and from the Swiss National Foundation. In parallel to these activities, mass determination using the STEM is a service we maintain to provide consistently novel information of biological relevance.

Aquaglyceroporins

Aquaglyceroporins represent a family of over 300 membrane channel proteins in animals, plants and bacteriae. These channels allow water (aquaporins, AQPs) or small hydrophilic solutes (glyceroporins, GLPs) to pass unhindered, but block the passage of ions, thus allowing cells to adapt to osmotic variations. A major breakthrough was the elucidation of the atomic structure of human AQP1, the archetypal human erythrocyte aquaporin discovered by Peter Agre. While we produced the 2D crystals, the group of Yoshi Fujiyoshi at Kyoto University collected images and diffraction patterns using a 300 kV FEG microscope keeping the sample at 1 K in a Helium-cooled stage. Image processing was done in Kyoto as well, whereas data interpretation was shared, yielding the structure of AQP1 at 3.8 Å resolution. This structure led to a hypothesis as to how proton conduction by the single file of water molecules in the channel is suppressed. In collaboration with Helmut Grubmüller at the MPI Göttingen, the atomic model was refined to 3.5 Å and used for assessing the water flow by molecular dynamics simulations. This work confirms our initial hypothesis and demonstrates the quality of structural information collected by electron crystallography.

We also focussed on the crystallization of AQP0, the major integral protein (MIP) of lens fiber cells, as well as that of GlpF, the *E. coli* glycerol facilitator. The majority of AQP0 crystals occurred as two-layered 2D crystals that were precisely stacked by specific interactions of their extracellular loops. This indicated a dual function of AQP0: besides acting as water channel, this protein is also responsible for cell-cell adhesion. AQP0-knock-out mice developing cataracts immediately after birth support this view. GlpF yielded 2D crystals that diffracted to 3.7 Å and prompted a data collection session on the 300 kV He-FEG microscope in Frankfurt. While this project progressed rapidly, leading to a 3.7 Å projection structure and subsequently to a 6.9 Å 3D map, X-ray crystallography produced the 2.2 Å structure in parallel.

ATP synthases

ATP synthases are large complexes comprising a membrane resident (Fo) and a hydrophilic part (F1). They convert the electrochemical potential across

membranes into the chemical energy ATP. While the catalytic process of ATP synthesis in F1 involving rotation of the central subunit is largely elucidated, the generation of rotation through the flow of ions remains enigmatic. The visualization of the rotor in Fo was an important step: in collaboration with Norbert Dencher, Darmstadt, the stoichiometry of the chloroplast rotor was determined to be 14 subunits using AFM. Subsequently, a bacterial rotor was analyzed by AFM as well as electron crystallography in collaboration with Peter Dimroth, ETHZ. In this case, 11 subunits are required to build a rotor. Interestingly, the yeast rotors comprised of 10 subunits, as shown by X-ray crystallography. Thus, the textbook notion of a rotor with dodecameric symmetry needs to be revised; it seems that the number of subunits in the rotor is adapted in each organism to the average electrochemical potential available.

Controlled unfolding and visualization of single proteins by AFM

Progress in the use of the AFM has led to reproducible imaging at submolecular resolution. In addition, we have combined single molecule force spectroscopy with high-resolution imaging. In this way we can now pull a single molecule out of a supramolecular assembly and subsequently assess the damage generated. This combined technology initially applied to the S-layer of *Deinococcus radiodurans* was subsequently used to unfold bacteriorhodopsin molecules and to verify the nature of the damage by imaging the respective purple membrane patch. The power of this approach is currently further exploited to study AQP1 and the S-layer of *Corynebacterium glutamicum.*

STEM

Scanning transmission electron microscopy (STEM) is primarily used to determine the mass of biomacromolecules and their assemblies. Exploiting this feature leads to collaborations and projects covering a large spectrum of biology. Fimbriae mediate the adhesion of the bacteria to enterocytes. A mass and structural analysis carried out for the F18 fimbriae expressed by porcine toxigenic Escherichia coli strains showed these to be formed from axially repeating FedA monomers that alternate in a “zigzag” manner around the helical axis with an axial rise of 2.2 nm. The human Rad52 proteins have been shown to promote single-strand DNA annealing and to stimulate Rad51-mediated homologous pairing. Electron microscopic examinations of the human Rad52 proteins have revealed their assembly into ring-like structures in vitro. Using both conventional transmission electron microscopy and STEM, we found that the human Rad52 protein forms heptameric rings. A 3D reconstruction revealed that the heptamer has a large central channel. Examination of the McrBC restriction endonuclease of Escherichia coli revealed the association of a $McrB_L$ tetradecamer with one or two molecules of McrC, the subunit essential for DNA cleavage. In contrast, the McrB component alone exists as single heptameric rings as well as tetradecamers. The 6:3:1 stoichiometry indicated for the N-Ethylmaleimide-sensitive factor (NSF): soluble NSF attachment proteins (SNAPs): SNAP receptor (neuronal SNARE) complex indicated by previous binding studies was supported by mass measurements made on the 20S complex.

Future direction

Although many questions still need to be answered in the field of aquaglyceroporins, we now need to enlarge the repertoire of 2D crystals of membrane proteins from different families of medical importance. These include transporters, receptors and channels of the central nervous system and the gastrointestinal tract.

Two model proteins studied are the lactose permease and the multi-drug transporter LmrA. They are used to systematically improve 2D crystallization, which after all is the only method to study structure and function of membrane proteins under native conditions. We will enlarge the repertoire of model proteins by studying the vasopressin receptor, V2R, a G-protein coupled receptor.

To improve the output of high-resolution structures, a better image processing software is required. This project is funded by the National Center of Competence in Research, NCCR in Structural Biology. Another development is embedded in the NCCR in Nanoscale Sciences: we will produce novel tricks to simultaneously acquire multiple signals and to control the environment of functioning biomolecules. The mechano-sensitive channel MscL and the ATP synthase will be the major systems explored with this new instrumentation.

Publications

Braun, T., Philippsen, A., Wirtz, S., Borgnia, M., Agre, P., Kühlbrandt, W., Engel, A. & Stahlberg, H. (2000). GlpF: A structural variant of the aquaporin tetramer. In *Molecular biology and physiology of water and solute transport* (Hohmann, S. & Nielsen, S., eds.), Vol. ISBN 0-306-46501-9, pp. 13-22. Kluwer Academic, New York.

Braun, T., Philippsen, A., Wirtz, S., Borgnia, M. J., Agre, P., Kuhlbrandt, W., Engel, A. & Stahlberg, H. (2000). The 3.7 A projection map of the glycerol facilitator GlpF: a variant of the aquaporin tetramer. *EMBO Rep* 1, 183-9.

de Groot, B. L., Heymann, J. B., Engel, A., Mitsuoka, K., Fujiyoshi, Y. & Grubmuller, H. (2000). The fold of human aquaporin 1. *J Mol Biol* 300, 987-94.

Engel, A. (2000). Structural analyses of the aquaporin super-family. *Nephrol Dial Transplant* 15, 23-5.

Engel, A., Fujiyoshi, Y. & Agre, P. (2000). The importance of aquaporin water channel protein structures. *EMBO J* 19, 800-6.

Engel, A. & Muller, D. J. (2000). Observing single biomolecules at work with the atomic force microscope. *Nat Struct Biol* 7, 715-8.

Fotiadis, D., Hasler, L., Muller, D. J., Stahlberg, H., Kistler, J. & Engel, A. (2000). Surface tongue-and-groove contours on lens MIP facilitate cell-to-cell adherence. *J Mol Biol* 300, 779-789.

Heymann, J. B. & Engel, A. (2000). Structural clues in the sequences of the aquaporins. *J Mol Biol* 295, 1039-53.

Heymann, J. B., Pfeiffer, M., Hildebrandt, V., Kaback, H. R., Fotiadis, D., Groot, B., Engel, A., Oesterhelt, D. & Muller, D. J. (2000). Conformations of the rhodopsin third cytoplasmic loop grafted onto bacteriorhodopsin. *Structure Fold Des* 8, 643-53.

Holzl, H., Kapelari, B., Kellermann, J., Seemuller, E., Sumegi, M., Udvardy, A., Medalia, O., Sperling, J., Müller, S. A., Engel, A. & Baumeister, W. (2000). The regulatory complex of Drosophila melanogaster 26S proteasomes. Subunit composition and localization of a deubiquitylating enzyme. *J Cell Biol* 150, 119-30.

Lustig, A., Engel, A., Tsiotis, G., Landau, E. M. & Baschong, W. (2000). Molecular weight determination of membrane proteins by sedimentation equilibrium at the sucrose or nycodenz-adjusted density of the hydrated detergent micelle. *Biochim Biophys Acta* 1464, 199-206.

Manting, E. H., van Der Does, C., Remigy, H., Engel, A. & Driessen, A. J. (2000). SecYEG assembles into a tetramer to form the active protein translocation channel. *EMBO J* 19, 852-61.

Möller, C., Büldt, G., Dencher, N. A., Engel, A. & Müller, D. J. (2000). Reversible loss of crystallinity on photobleaching purple membrane. *J Mol Biol* 301, 869-879.

Müller, D. J., Fotiadis, D., Scheuring, S., Moller, C. & Engel, A. (2000). Conformational changes, flexibilities and intramolecular forces observed on individual proteins using AFM. *Single Mol.* 1, 115-118.

Müller, D. J., Heymann, J. B., Oesterhelt, F., Moller, C., Gaub, H., Buldt, G. & Engel, A. (2000). Atomic force microscopy of native purple membrane. *Biochim. Biophys. Acta* 1460, 27-38.

Müller, D. J., Stahlberg, H., Engel, A., Seelert, H. & Dencher, N. (2000). Direct imaging of native chloroplast F-0 atp synthase: Visualization of individual subunits. *Biophysical Journal* 78, 1655Plat.

Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J. B., Engel, A. & Fujiyoshi, Y. (2000). Structural determinants of water permeation through aquaporin-1. *Nature* 407, 599-605.

Nouwen, N., Stahlberg, H., Pugsley, A. P. & Engel, A. (2000). Domain structure of secretin PulD revealed by limited proteolysis and electron microscopy. *EMBO J* 19, 2229-36.

Oesterhelt, F., Oesterhelt, D., Pfeiffer, M., Engel, A., Gaub, H. E. & Muller, D. J. (2000). Unfolding pathways of individual bacteriorhodopsins. *Science* 288, 143-6.

Ringler, P., Heymann, B. & Engel, A. (2000). *Two-dimensional crystallization of membrane proteins*. Membr. Transp. (Baldwin, S. A., Ed.), Oxford University Press.

Scheuring, S., Tittmann, P., Stahlberg, H., Ringler, P., Borgnia, M., Agre, P., Gross, H. & Engel, A. (2000). The aquaporin sidedness revisited. *J Mol Biol* 299, 1271-8.

Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H. & Muller, D. J. (2000). Structural biology. Proton-powered turbine of a plant motor. *Nature* 405, 418-9.

Stahlberg, H., Braun, T., de Groot, B., Philippsen, A., Borgnia, M. J., Agre, P., Kuhlbrandt, W. & Engel, A. (2000). The 6.9-A structure of GlpF: a basis for homology modeling of the glycerol channel from Escherichia coli. *J Struct Biol* 132, 133-41.

Stasiak, A. Z., Larquet, E., Stasiak, A., Muller, S., Engel, A., Van Dyck, E., West, S. C. & Egelman, E. H. (2000). The human Rad52 protein exists as a heptameric ring. *Curr Biol* 10, 337-40.

de Groot, B. L., Engel, A. & Grubmuller, H. (2001). A refined structure of human aquaporin-1. *FEBS Lett* 504, 206-11.

Fotiadis, D., Jeno, P., Mini, T., Wirtz, S., Muller, S. A., Fraysse, L., Kjellbom, P. & Engel, A. (2001). Structural characterization of two aquaporins isolated from native spinach leaf plasma membranes. *J Biol Chem* 276, 1707-1714.

Müller, D. J., Dencher, N. A., Meier, T., Dimroth, P., Suda, K., Stahlberg, H., Engel, A., Seelert, H. & Matthey, U. (2001). ATP synthase: constrained stoichiometry of the transmembrane rotor. *FEBS Lett* 504, 219-22.

Müller, S. A. & Engel, A. (2001). Structure and mass analysis by scanning transmission electron microscopy. *Micron* 32, 21-31.

Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J. B., Engel, A. & Fujiyoshi, Y. (2001). Molecular basis of water selectivity on aquaporin-1. *Kidney Int* 60, 399.

Panne, D., Muller, S. A., Wirtz, S., Engel, A. & Bickle, T. A. (2001). The McrBC restriction endonuclease assembles into a ring structure in the presence of G nucleotides. *EMBO J* 20, 3210-7.

Sato, C., Ueno, Y., Asai, K., Takahashi, K., Sato, M., Engel, A. & Fujiyoshi, Y. (2001). The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. *Nature* 409, 1047-51.

Scheuring, S., Reiss-Husson, F., Engel, A., Rigaud, J. L. & Ranck, J. L. (2001). High-resolution AFM topographs of Rubrivivax gelatinosus light- harvesting complex LH2. *EMBO J* 20, 3029-35.

Stahlberg, H., Fotiadis, D., Scheuring, S., Remigy, H., Braun, T., Mitsuoka, K., Fujiyoshi, Y. & Engel, A. (2001). Two-dimensional crystals: a powerful approach to assess structure, function and dynamics of membrane proteins. *FEBS Lett* 504, 166-72.

Stahlberg, H., Heymann, B., Mitsuoka, K., Fushyihoshi, Y. & Engel, A., Eds. (2001). The aquaporin superfamily: structure and function. Vol. 51. Current Topics in Membranes. Edited by S. Hohmann, S. N., P. Agre. San Diego: Academic Press.

Stahlberg, H., Muller, D. J., Suda, K., Fotiadis, D., Engel, A., Meier, T., Matthey, U. & Dimroth, P. (2001). Bacterial Na(+)-ATP synthase has an undecameric rotor. *EMBO Rep* 2, 229-33.

Werten, P. J., Hasler, L., Koenderink, J. B., Klaassen, C. H., de Grip, W. J., Engel, A. & Deen, P. M. (2001). Large-scale purification of functional recombinant human aquaporin-2. *FEBS Lett* 504, 200-5.

Wimmer, C., Hohl, T. M., Hughes, C. A., Muller, S. A., Sollner, T. H., Engel, A. & Rothman, J. E. (2001). Molecular mass, stoichiometry, and assembly of 20 s particles. *J Biol Chem* 276, 29091-7.

Fotiadis, D., Scheuring, S., Müller, S. A., Engel, A. & Müller, D. J. (2002). Imaging and manipulation of biological structures with the AFM. *Micron* 33, 385-397.

Philippsen, A., Im, W., Engel, A., Schirmer, T., Roux, B. & Müller, D. (2002). Imaging the Electrostatic Potential of Transmembrane Channels: Atomic Probe Microscopy of OmpF Porin. *Biophysical Journal* 82, 1667–1676.

Scheuring, S., Müller, D., Stahlberg, H., Engel, H. & Engel, A. (*in press*). Sampling the conformational space of membrane protein surfaces with the AFM. *European Biophys J*.

Engel, A. & Stahlberg, H. (2002). Aquaglyceroporins: Channel proteins with a conserved core, multiple functions and variable surfaces. In *Molecular mechanisms of water transport across biological membranes. International Review of Cytology* (Zeuthen, T. & Stein, W. D., eds.). Academic Press, New York.

Fotiadis, D. & Engel, A. (*in press*). High resolution imaging of bacteriorhodopsin by atomic force microscopy. In *Atomic force microscopy: Methods and protocols in biomedical applications* (Braga, P. C. & Ricci, d., eds.). Humana Press Inc., Totowa, NJ.

Fotiadis, D., Suda, K., Tittmann, P., Jenö, P., Phillipsen, A., Müller, D. J., Gross, H. & Engel, A. (*in press*). Identification and structure of a putative Ca2+ binding domain at the C-terminus of AQP1. *J Mol Biol*.

Scheuring, S., Stahlberg, H., Chami, M., Houssin, C., Rigaud, J. L. & Engel, A. (*in press*). Charting and unzipping the surface-layer of Corynebacterium glutamicum with the atomic force microscope. *Mol Microbiol*.

High resolution NMR of biological macromolecules

M. Allan, F. Cordier, D. Häussinger, J. Kahmann, S. Langheld, G. Musco, S. Meier, H.-J. Sass,V. Jaravine

In collaboration with P. Wingfield, S. Stahl, A. Bax, D. Torchia (NIH, Bethesda, USA), J. Feigon, (University of California, Los Angeles, USA), M. Barfield (University of Arizona, Tucson, USA), L.K. Nicholson (Cornell University, USA), A. Dingley (University College, London, UK), B. König, G. Büldt, (Forschungszentrum Jülich, Germany), G. Barbato, R. Bazzo (IRBM, Rome, Italy), K. Saksela (University of Tampere, Finland), C. Hilbers, C. Kloks (University of Nijmegen, Holland), D. Riesner, G. Steger (Heinrich-Heine-Universität, Düsseldorf, Germany), G. Lippens (Institut Pasteur, Lille, France), M. Affolter, J. Engel, W. Gehring, M. Hall, T. Kiefhaber, C. Thompson (Biozentrum).

Stephan Grzesiek

Phone: +41 61 267 2100 Fax: +41 61 267 2109 E-mail: Stephan.Grzesiek@unibas.ch

http://www.biozentrum.unibas.ch/grzesiek

Scope of research

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 the following 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. 2. the study of macromolecular complexes where NMR yields information on interaction surfaces, the strength of the interaction and the dynamics. 3. the structure determination of membrane proteins and the gathering of structural information on biological molecules near the membrane surface. 4. the study of forces that are relevant for macromolecular folding.

Unsolved problems in NMR methodology that we consider the most important are: 1. the size limitation of current methods which is around 40-50 kDa for completely determined structures. 2. the accuracy by which these structures are determined. 3. the lack of automation in the data analysis and the structure calculations.

Scalar couplings across hydrogen bonds

Hydrogen 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 hydrogen bond related parameters such as reduced hydrogen exchange rates. In 1998, we and others discovered that surprisingly strong NMR-observable scalar couplings exist across hydrogen bonds. This effect can be used to “see” all three partners of a hydrogen bond directly in an NMR experiment, i.e. the donor, the acceptor and the proton itself. Thus, the hydrogen 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 hydrogen bond acceptor and hydrogen electronic orbitals and gives evidence for the correlation of electronic wave functions on both sides of the hydrogen bridge.

In the last two years we have summarized many of the results on scalar hydrogen bond coupling in several review articles. We have extended the original findings of scalar couplings between the ^{15}N-nuclei of donor and acceptor groups in Watson-Crick base-paired nucleic acids to other systems such as carbonylic hydrogen bonds in proteins and nucleic acids and have further elucidated the dependency of the size of the effect on the geometry of the hydrogen bonds. Based on this dependency, we could show that proteins undergo distinct changes in their hydrogen bond geometry during ligand binding and during temperature variations. The latter results give quantitative insights into the stability of individual protein components during temperature-induced denaturation. In a separate study, we could follow quantitatively the formation of individual hydrogen bonds during the folding process of a peptide. These measurements provide a direct proof for the Lifson-Roig theory of the coil-to-helix transition.

Structural projects

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. At present, we have solved the structure of the multidrug binding domain. The aim of further studies is the elucidation of the antibiotic regulation mechanism (in collaboration with C. Thompson).

Figure 1: the structure of the antibiotic binding part of TipA

Homoassociation of Cadherin. Cadherins are single pass transmembrane glycoproteins which mediate calcium dependent cell-cell adhesion by homophilic interactions. The structure of the first and the first two domains of E-cadherin have been solved by NMR spectroscopy and x-ray crystallography, respectively. However, an understanding of the calcium induced homoassociation of cadherin is missing. We have expressed and assigned the first two domains of E-cadherin in calcium-free and calcium-bound forms and have deduced the interaction interfaces for the homoassociation. In addition we could determine the relative orientations of the cadherin domains in the calcium-free, the calcium-bound monomeric, and calcium-bound complexed state. The results shed new light onto the mechanism of the calcium-regulated homoassociation (in collaboration with J. Engel).

Hepatitis C Protease. The protease of the hepatitis C virus is a promising target for drug development against the compelling medical problem of hepatitis C infections. In collaboration with the Instituto di Ricerche di Biologia Molecolare “P. Angeletti”, Rome, the structure of the hepatitis C protease was solved in complex with a covalently bound inhibitor.

Other structural projects. Further structural projects encompass the determination of the structure of the cold-shock domain of the human Y-box protein YB-1 (in collaboration with C. Hilbers, Nijmegen), of the Brinker transcription factor (in collaboration with M. Affolter), of several proteins of the TOR signalling pathway (in collaboration with M. Hall) and the eludication of interactions of master switch proteins of the eye development (in collaboration with W. Gehring).

Technique developments

We have shown that it is possible to observe high resolution NMR spectra of biomacromolecules within polyacrylamide gels. As one application, weak orientation of macromolecules can be induced by mechanical stress onto the gel matrix. This residual orientation can be used for the collection of information on bond orientations by residual dipolar couplings. Furthermore, we have developed a simple method for NMR structure calculations from residual dipolar couplings. This procedure obviates the necessity of a determination of the molecular alignment tensor prior to the calculations. In a separate development, it was shown that ‘transferred’ dipolar couplings can be observed on flexible ligands which are bound to oriented membrane receptors (in collaboration with A. Bax and B. König). These ‘transferred’ couplings give information about the conformation of the ligands and their orientation relative to the membrane receptors.

Publications

Barbato, G., Cicero, D. O., Cordier, F., Narjes, F., Gerlach, B., Sambucini, S., Grzesiek, S., Matassa, V. G., De Francesco, R. & Bazzo, R. (2000). Inhibitor binding induces active site stabilization of the HCV NS3 protein serine protease domain. *EMBO J* 19, 1195-206.

Cordier, F., Wang, C., Grzesiek, S. & Nicholson, L. K. (2000). Ligand-induced strain in hydrogen bonds of the c-Src SH3 domain detected by NMR. *J Mol Biol* 304, 497-505.

Dingley, A. J., Masse, J. E., Feigon, J. & Grzesiek, S. (2000). Characterization of the hydrogen bond network in guanosine quartets by internucleotide 3hJ(NC)’ and 2hJ(NN) scalar couplings. *J Biomol NMR* 16, 279-89.

Koenig, B. W., Mitchell, D. C., Konig, S., Grzesiek, S., Litman, B. J. & Bax, A. (2000). Measurement of dipolar couplings in a transducin peptide fragment weakly bound to oriented photo-activated rhodopsin. *J Biomol NMR* 16, 121-5.

Landrieu, I., Wieruszeski, J. M., Odaert, B., Inze, D., Grzesiek, S. & Lippens, G. (2000). Letter to the editor: sequence-specific 1H, 13C and 15N chemical shift backbone NMR assignment and secondary structure of the Arabidopsis thaliana PIN1At protein. *J Biomol NMR* 17, 271-2.

Sass, H.-J., Musco, G., Stahl, S. J., Wingfield, P. T. & Grzesiek, S. (2000). Solution NMR of proteins within polyacrylamide gels: diffusional properties and residual alignment by mechanical stress or embedding of oriented purple membranes. *J Biomol NMR* 18, 305-311.

Barfield, M., Dingley, A. J., Feigon, J. & Grzesiek, S. (2001). A DFT study of the interresidue dependencies of scalar J- coupling and magnetic shielding in the hydrogen-bonding regions of a DNA triplex. *Journal of the American Chemical Society* 123, 4014-4022.

Dingley, A. J., Cordier, F. & Grzesiek, S. (2001). An introduction to hydrogen bond scalar couplings. *Concepts in Magnetic Resonance* 13, 103-127.

Grzesiek, S., Cordier, F. & Dingley, A. J. (2001). Scalar couplings across hydrogen bonds. *Methods in Enzymology* 338, 111-133.

Jaravine, V. A., Alexandrescu, A. T. & Grzesiek, S. (2001). Observation of the closing of individual hydrogen bonds during TFE-induced helix formation in a peptide. *Protein Science* 10, 943-950.

Sass, H. J., Musco, G., Stahl, S. J., Wingfield, P. T. & Grzesiek, S. (2001). An easy way to include weak alignment constraints into NMR structure calculations. *J Biomol NMR* 21, 275-280.

Kloks, C. P., Spronk, C. A., Lasonder, E., Hoffmann, A., Vuister, G. W., Grzesiek, S. & Hilbers, C. W. (2002). The Solution Structure and DNA-binding Properties of the Cold-shock Domain of the Human Y-box Protein YB-1. *J Mol Biol* 316, 317-26.

Cordier, F. & Grzesiek, S. *(in press*). Temperature dependence of protein hydrogen bond properties as studied by high resolution NMR. *J Mol Biol*.

Protein X-ray crystallography

Pilar Garcia, Marco Marino

Olga Mayans
Phone: +41 61 267 2083 Fax: +41 61 267 2109 E-mail: Olga.Mayans@unibas.ch
http://www.biozentrum.unibas.ch/mayans

This group was newly established at Biozentrum in December 2001. Previously, I worked at EMBL-Hamburg Outstation (1997-2001) within the group of Matthias Wilmanns. During that time, I carried out crystallographic studies of two main systems:

i) Anthranilate phosphoribosyltransferase, a ribosylating enzyme from the metabolic pathway of tryptophan biosynthesis. This work completed the structural inventory of all seven enzymes from this pathway, the second major metabolic route under study after the glycolysis. Findings revealed cross-metabolic evolution between the tryptophan and nucleotide pathways. The project was in collaboration with Prof. K. Kirschner, formerly at Biozentrum.

ii) The gigantic muscle protein titin, its role in muscle development and elasticity. This work was in collaboration with PD Dr. Mathias Gautel at MPI for Molecular Physiology, Dortmund;

Although partial continuation of these topics will be pursued, the research focus of the group is on proteins of medical interest and, in particular, on alteration of signal transduction in disease. Projects are available in the context of neuromuscular disorders as well as on the physiology of the cell nucleus.

Crystallography of membrane proteins

C. Chan, V.P. Dinesh, N. Feddermann Bühler, P. van Gelder, M. Jenny, Z. Housley-Markovic, R. Müller, G.L. Orriss, A. Philippsen, Y. Rasser, J. Scherf, E. Shillington, P. Storici

In collaboration with A. Engel (Biozentrum/MIH), T. Kiefhaber, P. Phale, J.P. Rosenbusch (Biozentrum), P. Agre (Baltimore, USA), B. Erni (Univ. of Bern), S. Fischer (Univ. of Heidelberg, Germany), J. Kistler (Univ. of Auckland, New Zealand), M.A. Schmidt (Univ. Münster, Germany), R.B. Silverman (Northwestern Univ., Eaveston, USA)

Tilman Schirmer
Phone: +41 61 267 2089 Fax: +41 61 267 2109 E-mail: Tilman.Schirmer@unibas.ch
http://www.biozentrum.unibas.ch/schirmer

Structural knowledge of membrane proteins at atomic resolution is still scarce. We are employing X-ray crystallography for the structure determination of mainly bacterial membrane proteins. In particular, we are interested in the structural basis of solute translocation across the outer and the inner membrane.

Non-specific porins as models for ion channels

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. In collaboration with the group of J.P. Rosenbusch, Biozentrum, a large number of site-directed mutants have been produced, functionally characterized and their structures determined by X-ray crystallography. Recently we have focused on the role of the protein charges at the pore constriction, and how these charges influence ion selectivity and single channel conductance. We were able to relate quantitatively these electrophysiological properties to the respective crystal structures by simulating ion flow employing Brownian dynamics. Good agreement between simulation and experiment was obtained demonstrating that the essential factors governing ion flow through aqueous pores were captured faithfully by the simulations. It was found that anion/cation selectivity is crucially influenced by the charge constellation at the constriction, and that the presence of protein charges at the dipolar channel constriction increases channel conductance due to electrostatic steering.

Maltoporin, a specific passive channel

Maltoporin specifically facilitates the diffusion of maltodextrins across the outer membrane. The polypeptide chain is folded into an 18-stranded antiparallel β-barrel with three inwardly folded loops. Six contiguous aromatic residues line one side of the channel and form a hydrophobic path (‘greasy slide’) from the vestibule to the channel outlet.

Maltoporin is the prototype of a facilitated diffusion channel. Based on sugar-maltoporin structures, we propose that the greasy slide guides the long linear sugar substrates through the tight channel. At the start of translocation, the hydrophobic patches of the glucosyl moieties would adhere to the end of the greasy slide that is extending into the channel vestibule and which is easily accessible. This notion has recently been corroborated by results on side-directed mutants. Changing the ‘greasy slide’ residues to alanine reduced considerably the translocation efficiency *in vivo*. This is also true for the residues at the two ends of the slide that have been shown not to be involved in sugar binding at equilibrium.

After binding to the high-affinity site in the middle of the channel (as observed in the respective complex crystal structure), a maltodextrin molecule would have to perform a “register shift” for further translocation. The trajectory of this shift and the corresponding energetics have been calculated by the “conjugated peak refinement” method (in collaboration with S. Fischer and M. Karplus, Strasbourg). It was found that, during register shift, the maltoporin channel acts as a rigid complementary matrix. Due to the smooth and uninterrupted interaction with the greasy slide and the presence of a multitude of H-bonding partners no large energy barriers have to be overcome.

Active sugar transporters of the bacterial phosphoryl-transfer-system (PTS)

Active sugar transporters of the PTS couple sugar translocation across the bacterial inner membrane with its phosphorylation in the cytoplasm. The phosphoryl

group is delivered via several proteins, which become transiently phosphorylated at histidine or cysteine residues, to the sugar. We are aiming (in collaboration with B. Erni, Bern) to elucidate the structure of the multi-subunit complex of the mannose permease family which comprises the soluble subunits IIA and IIB and the membrane-spanning subunits IIC/IID.

During the few last years the X-ray structures of several soluble domains/subunits have been determined, i.e. the structure of the functional IIA dimer from *E. coli,* the structure of IIB from *B. subtilis* and of IIB from *K. pneumoniae*. 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 one helix from each protein.

Aquaporins and AIDA autotransporter

Aquaporins are found in various eukaryotic membranes and in the bacterial plasma membrane. They exhibit exquisite specificity for water or glycerol. We are working on the structure determination of

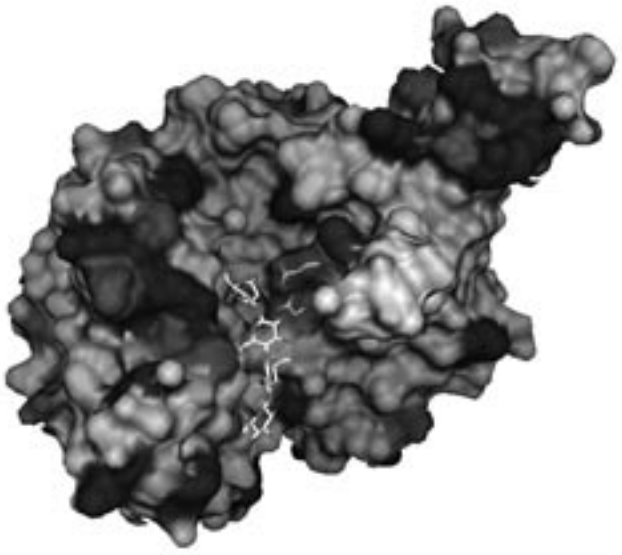


Figure 1:
Structure of hyaluronidase in complex with hyaluronic acid

MIP26 from mammalian eye lenses. Our collaborating partners are P. Agre, Baltimore, and A. Engel, Biozentrum, who have overexpressed the protein in yeast and developed a purification scheme.

Maintaining our interest in translocation across the outer membrane we are studying, in collaboration with M.A. Schmidt, Münster, the recombinant protein AIDA-I that forms adhesive structures at the surface of enteropathogenic *E. coli*. This protein is well suited to study protein export, since it harbors, at the C-terminus, its own translocation domain. Crystallization conditions are currently being screened for all these proteins.

Soluble proteins

GABA aminotransferase is an essential enzyme to maintain appropriate levels of the neurotransmitter GABA in the human brain. A few years ago, the structure of this vitamin B6 dependent enzyme was solved and we are now investigating, in collaboration with R.B. Silverman (USA), the binding mode of novel mechanism-based inhibitors of pharmaceutical importance.

The crystal structure of hyaluronidase, a major allergen of bee venom, has been determined. This sugar hydrolase exhibits the classical TIM barrel fold with an elongated deep groove for substrate (hyaluronic acid) binding. The structure, in complex with a tetrasaccharide product, strongly suggests an acid-base catalytic mechanism, in which Glu113 acts as the proton donor and the N-acetyl group of the substrate is the nucleophile (substrate assisted mechanism).

Publications

Markovic-Housley, Z., Miglierini, G., Soldatova, L., Rizkallah, P. J., Muller, U. & Schirmer, T. (2000). Crystal structure of hyaluronidase, a major allergen of bee venom. *Structure Fold Des* 8, 1025-1035.

Phale, P. S., Philippsen, A., Widmer, C., Phale, V. P., Rosenbusch, J. P. & Schirmer, T. (2001). Role of charged residues at the OmpF porin channel constriction probed by mutagenesis and simulation. *Biochemistry* 40, 6319-6325.

Schirmer, T. (2001). Membrane proteins with beta-barrel fold. *Chimia* 55, 493-496.

Van Gelder, P., Dutzler, R., Dumas, F., Koebnik, R. & Schirmer, T. (2001). Sucrose transport through maltoporin mutants of Escherichia coli. *Prot Eng* 14, 943-948.

Van Gelder, P., Dumas, F., Bartoldus, I., Saint, N., Prilipov, A., Winterhalter, M., Wang, Y., Philippsen, A., Rosenbusch, J.P. & Schirmer, T. (2002). Sugar transport through maltoporin of *Escherichia coli*: Role of the greasy slide. *J. Bacteriol.* 184, 2994-2999.

Macromolecular interactions in coiled coils, protein-ligand complexes and protein *de novo* design

S. V. Strelkov, M. Meier, S. Ivaninskii, S. Baumli, A. Meli, F. Grandjean

In collaboration with U. Aebi (Biozentrum), P. Cook (Univ. of Oklahoma, Norman, USA), H. Herrmann (DKFZ, Heidelberg, Germany), R. Hodges (UCHSC, Denver, USA), A. Hönger (EMBL, Heidelberg, Germany), J. P. Kraus (UCHSC, Denver, USA), A. Mozzarelli (Univ. of Parma, Parma, Italy), M. Spiess (Biozentrum), K. Weber (MPI, Göttingen, Germany)

Peter Burkhard
Phone: +41 61 267 2091 Fax: +41 61 267 2109 E-mail: Peter.Burkhard@unibas.ch
http://www.biozentrum.unibas.ch/burkhard

De novo design of α-helical proteins

The parallel two-stranded α-helical coiled coil is the most frequently encountered subunit oligomerization motif in proteins. Sequences of parallel left-handed coiled-coil proteins are characterized by a heptad repeat pattern of seven amino acids denoted **a** to **g** harboring mostly apolar residues in their **a** and **d** positions. Stability of coiled coils is achieved by the distinctive packing of the side chains of the amino acids in the **a** and **d** positions into a hydrophobic seam. We have *de novo* designed several highly charged two-heptad repeat long peptides which are stabilized by a complex network of different possible inter- and intrahelical salt bridge arrangements in addition to the well-known hydrophobic interactions occurring along the dimer interface. We are accessing the biophysical properties of these peptides by CD-spectroscopy, analytical ultracentrifugation and X-ray structure determination. The X-ray structure of these peptides confirmed our predicted intra- and interhelical salt bridge network. Such *de novo* designed peptides can be used as very short α-helical coiled coils for applications such as two-stage drug targeting and delivery systems, coiled coils as templates for combinatorial helical libraries for drug discovery, and as synthetic carrier molecules.

Structural investigations IF proteins

Our second related goal is to resolve the atomic structure of the intermediate filaments (IFs). A ~46 nm long, parallel α-helical coiled-coil dimer is the elementary building block of all IFs. We are designing a series of overlapping fragments of the 55-kDa type III IF protein vimentin for subsequent crystallization and X-ray structure determination. Knowledge of the atomic structure of an IF protein dimer is a prerequisite for a better understanding of how the many identified point mutations interfere with IF assembly, structure and dynamics.

So far we have designed, cloned, expressed and purified some 20 ‘miniconstructs’ of the intermediate filament protein vimentin. We have completed the 1.9 Å atomic structure of a fragment which involves the highly conserved TYRKLEGEE motif from the C-terminal part of the coiled-coil segment 2B. The structure of another fragment which includes most of the coiled-coil segment 2B, was established to 2.3 Å resolution. This structure reveals a coiled-coil ‘stutter’ caused by a discontinuity in the heptad periodicity. Our data show that the ‘stutter’ can be incorporated into a continuous coiled coil via a local partial untwisting of both individual α-helices and the supercoil. Finally, the structure of a 39-residue-long fragment could be determined to a resolution of 1.4 Å. This fragment corresponds to the highly conserved N-terminal region of the vimentin coiled coil and features a monomeric α-helix. Studies on additional fragments involving this interesting region are currently underway, and are expected to provide further information on its structure and oligomerization properties. (See also U. Aebi)

Structural investigations of vitamin B6 enzymes

DOPA decarboxylase (DDC): Parkinson’s disease is a chronic, progressive, neurological disorder characterized by tremor, bradykinesia, rigidity, and postural instability. Parkinson’s disease is thought to be the result of degeneration of dopamine-producing cells in the substantia nigra of the brain. Human cells synthesize endogenous dopamine from L-DOPA. The conversion of L-DOPA into dopamine is catalyzed by the vitamin B_6-dependent enzyme DDC. In collaboration with the group of C. Borri-Voltattorni we have determined the three-dimensional structure of DDC in its ligand-free form and in complex with the anti-Parkinson drug carbiDOPA. The crystal structure of DDC will now be used to assist in the design of more potent DDC inhibitors compared to the currently used drugs carbiDOPA or benserazide.

Cystathionine β-synthase (CBS): In collaboration with the group of J. Kraus we have recently determined the X-ray crystal structure of CBS. CBS is the first

enzyme of the transsulfuration pathway in which the toxic homocysteine is converted to cysteine. Deficiency of CBS activity is the most common cause of homocystinuria, an inherited metabolic disease characterized by dislocated eye lenses, skeletal problems, vascular disease and mental retardation. In our future work we want to assess the structures of the catalytic intermediates and we then want to address how CBS is it regulated in its catalytic activity and whether this regulation could possibly be modulated.

O-acetylserine sulfhydrylase (OASS): In collaboration with the group of P. Cook, we are investigating the structure-activity-relationship of OASS in greater detail. We have analyzed the structure of the external aldimine catalytic intermediate. Covalent binding of L-methionine as an external aldimine to OASS induces a large conformational change in the protein. When the inhibitor chloride binds to OASS this results in a new “inhibited” conformation, that differs from the “open” native or “closed” external aldimine conformations.

Publications

Burkhard, P., Kammerer, R. A., Steinmetz, M. O., Bourenkov, G. P. & Aebi, U. (2000). The coiled-coil trigger site of the rod domain of cortexillin I unveils a distinct network of interhelical and intrahelical salt bridges. *Structure Fold Des* 8, 223-30.

Burkhard, P., Meier, M. & Lustig, A. (2000). Design of a minimal protein oligomerization domain by a structural approach. *Protein Science* 9, 2294-2301.

Burkhard, P., Tai, C. H., Jansonius, J. N. & Cook, P. F. (2000). Identification of an allosteric anion-binding site on O-acetylserine sulfhydrylase: structure of the enzyme with chloride bound. *J Mol Biol* 303, 279-86.

Burkhard, P., Taylor, P. & Walkinshaw, M. D. (2000). X-ray structures of small ligand-FKBP complexes provide an estimate for hydrophobic interaction energies. *J Mol Biol* 295, 953-62.

Herrmann, H., Strelkov, S. V., Feja, B., Rogers, K. R., Brettel, M., Lustig, A., Haner, M., Parry, D. A., Steinert, P. M., Burkhard, P. & Aebi, U. (2000). The intermediate filament protein consensus motif of helix 2B: its atomic structure and contribution to assembly. *J Mol Biol* 298, 817-32.

Meier, M., Bider, M. D., Malashkevich, V. N., Spiess, M. & Burkhard, P. (2000). Crystal structure of the carbohydrate recognition domain of the H1 subunit of the asialoglycoprotein receptor. *J Mol Biol* 300, 857-65.

Tai, C. H., Burkhard, P., Jansonius, J. N. & Cook, P. F. (2000). Inhibition and structural changes of O-acetylserine sulfhydrylase-A from Salmonella typhimurium upon binding of sulfate and chloride anions. In *Biochemistry and molecular biology of vitamin B6 and PQQ dependent proteins*, pp. 271-276. Birkhäuser Verlag, Basel.

von Matt, A., Ehrhardt, C., Burkhard, P., Metternich, R., Walkinshaw, M. & Tapparelli, C. (2000). Selective boron-containing thrombin inhibitors--X-ray analysis reveals surprising binding mode. *Bioorg Med Chem* 8, 2291-303.

Bruno, S., Schiaretti, F., Burkhard, P., Kraus, J. P., Janosik, M. & Mozzarelli, A. (2001). Functional properties of the active core of human cystathionine beta- synthase crystals. *J Biol Chem* 276, 16-9.

Burkhard, P., Dominici, P., Borri-Voltattorni, C., Jansonius, J. N. & Malashkevich, V. N. (2001). Structural insight into Parkinson’s disease treatment from drug- inhibited DOPA decarboxylase. *Nat Struct Biol* 8, 963-7.

Burkhard, P., Stetefeld, J. & Strelkov, S. V. (2001). Coiled coils: a highly versatile protein folding motif. *Trends Cell Biol* 11, 82-8.

De Marco, V., Burkhard, P., Le Bot, N., Vernos, I. & Hoenger, A. (2001). Analysis of heterodimer formation by Xklp3A/B, a newly cloned kinesin-II from Xenopus laevis. *EMBO J* 20, 3370-9.

Janosik, M., Meier, M., Kery, V., Oliveriusova, J., Burkhard, P. & Kraus, J. P. (2001). Crystallization and preliminary X-ray diffraction analysis of the active core of human recombinant cystathionine beta-synthase: an enzyme involved in vascular disease. *Acta Crystallogr D Biol Crystallogr* 57, 289-91.

Meier, M., Janosik, M., Kery, V., Kraus, J. P. & Burkhard, P. (2001). Structure of human cystathionine beta-synthase: a unique pyridoxal 5‘-phosphate-dependent heme protein. *EMBO J* 20, 3910-6.

Strelkov, S. V., Herrmann, H., Geisler, N., Lustig, A., Ivaninskii, S., Zimbelmann, R., Burkhard, P. & Aebi, U. (2001). Divide-and-conquer crystallographic approach towards an atomic structure of intermediate filaments. *J Mol Biol* 306, 773-81.

Tai, C. H., Burkhard, P., Gani, D., Jenn, T., Johnson, C. & Cook, P. F. (2001). Characterization of the allosteric anion-binding site of O-acetylserine sulfhydrylase. *Biochemistry* 40, 7446-52.

Burkhard, P., Ivaninskii, S. & Lustig, A. (*in press*). Improving coiled-coil stability by optimizing ionic interactions. *J. Mol. Biol.*

Meier, M., Lustig, A. & Burkhard, P. (*in press*). Removing an interhelical salt bridge abolishes coiled-coil formation in a de novo designed peptide. *J. Struct. Biol.*

Smith, T. A., Strelkov, S. V., Burkhard, P., Aebi, U. & Parry, D. A. D. (*in press*). Sequence comparisons of intermediate filament chains: evidence of a unique functional/structural role for coiled-coil segment 1A and linker L1. *J. Struct. Biol.*

Strelkov, S. V. & Burkhard, P. (*in press*). Analysis of α-helical coiled coils with the program TWISTER reveals a structural mechanism for stutter compensation. *J. Struct. Biol.*

Strelkov, S. V., Herrmann, H., Geisler, N., Zimbelmann, R., Aebi, U. & Burkhard, P. (*in press*). Conserved segments 1A and 2B of the intermediate filament dimer: their atomic structures and role in filament assembly. *EMBOJ.*

Odds and ends of actin structure and function

M. Boerries (MD-PhD student), M. Mourdjeva (visiting PhD student; University of Sofia)

In collaboration with B. Fahrenkrog (M.E. Müller Institute, Biozentrum), A. Hoenger (EMBL, Heidelberg), A.A. Noegel (Institute for Biochemistry I, University of Cologne) C. Ringli (Institute of Plant Biology, University of Zürich), M.O.Steinmetz (Paul Scherrer Institute, Villigen), D. Stoffler (Scripps Research Institute, La Jolla), A. Remppis (Laboratory for Biochemical Cardiology, Medical University of Luebeck)

Cora-Ann Schoenenberger
Phone: +41 61 267 2096 Fax: +41 61 267 2109 E-mail: Cora-Ann.Schoenenberger@unibas.ch
http://www.biozentrum.unibas.ch/schoenenberger

Actin exhibits a myriad of diverse functions, most of which ultimately depend on its intrinsic ability to rapidly assemble and disassemble filamentous structures in a temporally and spatially well controlled and coordinated pattern. The crawling of a cell, for example, depends on the rapid polymerization of a dendritic actin network at the leading edge. In recent years the heptameric Arp2/3 complex has emerged as major organizer of this branched actin structure in cells. However, observations from our laboratory indicate that actin itself induces the formation of branched structures via a transiently formed 'lower dimer' (LD).

Actin lower dimer: an odd structure or the key to actin patterning ?

In previous work we have shown that the first oligomer, which is formed at the onset of actin polymerization *in vitro* is the 'lower dimer' (LD). We observed that LD formation and turnover depend on ionic strength, pH, as well as the concentration and species of actin. Although the contacts between two actin subunits in LD are not present in the mature filament, we have found that LD-like contacts are involved in the formation of experimentally induced actin assemblies such as crystalline tubes and paracrystalline filament bundles. In addition, under experimental conditions where LDs are stably incorporated into growing filaments, they give rise to branching and bundling. Our current working model predicts that LD initiates the branching of F-actin filaments and that the new structure is stabilized by the Arp2/3 complex or other actin-binding protein(s). Our experimental work is designed to elucidate the structure of the LD in detail and to define its involvement in the formation and patterning of supramolecular assemblies.

Actin in conformations other than those that occur within F-actin filaments appear to be present in specific cellular compartments. Consistent with this notion, actin's presence in the nucleus is nowadays undisputed, however, its structure and function remain enigmatic. A recent project in our lab addresses the role of nuclear actin and the possible involvement of LD in the organization of actin in the nucleus.

S100A1 and its interaction partners in neonatal rat cardiomyocytes

S100A1 is a soluble, EF-hand type Ca^{2+}-binding protein that is predominantly expressed in myocardial tissue and skeletal muscle. Its expression pattern and involvement in Ca^{2+} regulation predestinate S100A1 to play a role in myocardial contractility. In addition, a decrease in S100A1 levels has been observed in human cardiomyopathies. We have been analyzing the localization of S100A1 and potential interaction partners such as SERCA2, ryanodine-receptor, α-actinin, desmin, and the actin cytoskeleton in primary neonatal cardiomyocytes by confocal laser scanning microscopy. We have found that exogenously added human recombinant S100A1 is taken up preferentially by cardiac fibroblasts but to a lesser extent also by cardiomyocytes. We are currently analyzing the effects of increased S100A1 levels on the distribution of sarcomeric proteins by exogenous S100A1 stimulation as well as in transfection experiments using GFP fusion constructs. The same cellular system is used to test the structural effects of different actin isoforms and actin mutations in neonatal rat cardiomyoctes.

Atrial natriuretic peptide (ANP)

ANP is a cardiac hormone that elicits a profound diuresis, natriuresis, and hypotension. A high molecular weight precursor (preproANP) is constitutively synthesized in atrial cardiocytes, but its processing is catalyzed by yet ill-defined enzymes. Because ANP plays a role in the homeostasis of the heart, we have intitiated experiments to study its processing in cardiac fibroblasts and cardiomyocytes. To follow ANP on its way through the cell, we have fused GFP to the C-terminus of murine preproANP. Preliminary transfection experiments indicate that the fusion peptide enters the secretory pathway.

Publication

Steinmetz MO, H. A., Stoffler D, Noegel AA, Aebi U, Schoenenberger CA. (2000). Polymerization, three-dimensional structure and mechanical properties of Dictyostelium versus rabbit muscle actin filaments. *J Mol Biol.* 303.

DIVISION OF MOLECULAR MICROBIOLOGY

The projects of the Division of Molecular Microbiology range from the functioning of pathogens and restriction enzymes to cell aging and gene mapping. The Division consists of five groups headed by Tom Bickle, Guy Cornélis, Christoph Dehio, Charles Thompson, and Urs Jenal. It also includes the Institute of Applied Microbiology, led by Peter Philippsen.

This report period has been marked by the completion of the restructuring of the Division following the retirement of several prominent members. Guy Cornélis and Christoph Dehio have joined the Division and lead research groups working with the pathogens *Yersinia* and *Bartonella*, respectively. Infectious microbiology has thus become one of the research strengths of the Biozentrum.

The research projects undertaken in the division are described below.

Professors

Thomas Bickle, Guy Cornelis, Christoph Dehio*, Peter Philippsen, Charles Thompson

Independent scientist

Urs Jenal

Scientists and postdoctoral fellows

Christine Alberti-Segui, Phillip Aldridge*, Yasmina Bauer*, Aude Bourniquel*, Ingo Christiansen*, Michaela Dehio*, Geetrui Denecker*, Fred Dietrich*, Wanda Dischert*, Tibor Farkas, Mario Feldmann*, Amy Gladfelter*, Björn Grünenfelder*, Dominic Hoepfner*, Pavel Janscak*, Laure Journet*, Rowan Morris*, Jaime Mota*, Kien Nguyen, Liem Nguyen*, Magne Osteras*, Daniel Panne*, Ralf Paul*, Florian Schärer, Stephanie Schmid-Nuoffer, Hans-Peter Schmitz*, Ralf Schülein*, Dirk Schwartz*, Patrick Viollier*

Graduate students

Martin Ackermann, Christine Alberti-Segui, Yasmina Bauer*, Sophie Brachat-Lemire, Thomas Fuchs, Björn Grünenfelder*, Dominic Hoepfner*, Philipp Knechtle, Anne-Kathrin Köhler*, Philippe Laissue*, Christoph Meyer*, Henri Saenz*, Florian Schaerer, Michael Schmid*, Ralf Schülein*, Anja Seubert*, Christopher Snyder*, Sherif Tawfilis*, Jennifer Tenor, Paul Troisfontaines*, Stephan Wessely, Kamila Wojnowska*

Diploma students

Nijas Alijoski*, Simone Binggeli*, Michael Bloch*, Petr Broz*, Beat Christen*, Daniel Eglin*, Beat Fischer*, Patrick Garcia*, Barbara Geering*, Stefanie Gehrig*, Patrick Guye*, Hanspeter Helfer*, Katrin Hungerbühler*, Andreas Kaufmann*, Van-Duc Luu*, Mark Murphy*, Marcel Paris*, Riccarda Rischatsch*, Hansruedi Stettler*, Thomas Rhomberg*, Esther Wüest*

Visiting scientists

Ilana Camargo (1 month), Fundaçao Hemocentro de Ribeirao Preto, Sao Paulo, Brazil; Cecilia Hansen (1 month), Dept. of Molecular Evolution, Uppsala University, Sweden; Janos Kiss (6 months), Agricultural Biotechnology Center, Gödöllö, Hungary; Jana Novotna (10 months), Academy of Sciences of the Czech Republic, Prague, Czech Republik; Ferenc Olasz (10 months), Agricultural Biotechnology Center, Gödöllö, Hungary; Anna Maria Puglia (2 months), University of Palermo, Palermo, Italy; Monica Szabo (2 months), Agricultural Biotechnology Center, Gödöllö, Hungary; Jiry Vohradsky (4 months), Academy of Sciences of the Czech Republic, Prague, Czech Republic

Technical personnel

Marc Folcher, Hedi Frefel*, Fabienne Hamburger*, Ariane Hardmeyer*, Andreas Hefti*, Rosmarie Hiestand, Peter Jaeggi*, Irene Jakob, Marina Kuhn*, Anita Lerch, Lieu Nguyen, Maxime Québatte*, Gabi Rummel*, Ursula Sandmeier*, Alexandra Schauerte*, Margrit Steiner, Agathe Stotz, Ingrid Thoenen, Sylvia Vögeli, Marlies Zoller*

Floor manager

Werner Metzger*, Roger Sauder*, Fritz Zoller*

Secretariat

Elvira Amstutz, Yvonne Bächli*, Gisela Burkhalter*, Maja Frei*, Ursula Frei*, Sylvie Germano*, Esther Graf*, Regula Niederhauser*, Béatrice Thierrin

Laboratory helpers

Isabel Alameda, Brigitte Berglas, Fatma Duyar, Beatrix Lang, Sakine Uzundal

* Employed during part of the report period

DNA restriction and modification

A. Bourniquel, T. Farkas, P. Garcia, F. Hamburger, P. Janscak, A.-K. Köhler, C. Meyer, M. Murphy, D. Panne, M. Paris, U. Sandmeier, S. Schmid-Nuoffer, S. Wessely

In collaboration with E. A. Raleigh and F. Steward (New England Biolabs, Beverley MA, USA, A. Engel, S. Müller and S. Wirtz (Maurice Müller Institute, Biozentrum), B. Giese (Institute of Organic Chemistry, University of Basel), M. D. Szczelkun (University of Bristol, UK)

Thomas A. Bickle
Phone: +41 61 267 2120 Fax: +41 61 267 2118 E-mail Thomas-A.Bickle@unibas.ch
http://www.biozentrum.unibas.ch/bickle

DNA cleavage by NTP-dependent restriction enzymes

We are working with complex, NTP- hydrolysing DNA restriction enzymes. These include the ATP-dependent Type I and Type III systems and the GTP and methylated DNA-dependent McrBC. Over the past few years, we have come to understand a puzzling feature of the way in which these enzymes cut DNA. All of them need to find two recognition sites in a linear DNA molecule before they will cut it but the two recognition sites can be at great and variable distances from each other. It turns out that for the type I enzymes and for McrBC, an encounter with a recognition site stimulates translocation of DNA past the enzyme, which remains bound to its recognition site. The DNA is only cut when this translocation is blocked, generally by encountering another enzyme complex translocating from another site but also when translocation is blocked non-specifically, by a bound repressor or by a recombination intermediate, for example. In this case, a DNA molecule with a single recognition site and a bound repressor or a Holiday junction will be cleaved. The mechanism for type III enzymes is similar, except that a simple translocation block is not sufficient. DNA cleavage requires a specific protein-protein contact between two translocating enzyme complexes and a non-specific block; a repressor, for example, bound between the recognition sites, inhibits cleavage.

McrBC: structure and mechanism

McrBC, a GTP-requiring, modification-dependent endonuclease of *E. coli* K-12, specifically recognizes DNA sites of the form 5' $R^{m}C$ 3'. DNA cleavage normally requires two recognition elements separated by 40 bp to several Kb. We have investigated DNA binding and sequence recognition with gel-shift and DNAse I footprint analysis. In the gel-shift system, $McrB_L$ binding resulted in a fast-migrating specific gel-shift, in a manner requiring both GTP and Mg^{++}. The binding was specific for methylated DNA and responded to local sequence changes in the same way that cleavage does. Unusually, single-stranded DNA competed in a modification and sequence-specific fashion. A supershifted species was formed in the presence of McrC. In contrast, the number of recognition elements present did affect DNAse I footprints: a two-element substrate displayed protection of non-specific spacer DNA in addition to the recognition elements, suggesting a distinct conformation for the two-site complex in solution. Addition of McrC did not affect the footprint obtained. We infer an unusual conformation of the fast-migrating complex in gel-shift experiments or loss of the bound protein during electrophoresis. We propose that McrC effects a conformational change in the complex rather than a reorganization of the DNA : protein interface.

The nature of the communication between two recognition sites was analyzed on DNA substrates containing one or two recognition sites. DNA cleavage of circular DNA required only one methylated recognition site, whereas the linearized form of this substrate was not cleaved. However, the linearized substrate was cleaved if a lac repressor was bound adjacent to the recognition site. These results suggest a model in which communication between two remote sites is accomplished by DNA translocation rather than looping. A mutant protein with defective GTPase activity cleaved substrates with closely spaced recognition sites, but not substrates where the sites were further apart. This indicates that McrBC translocates DNA in a reaction dependent on GTP hydrolysis. We suggest that DNA cleavage occurs by the encounter of two DNA translocating McrBC complexes or can be triggered by non-specific physical obstacles like the Lac repressor bound on the enzyme's path along DNA. Our results indicate that McrBC belongs to the general class of DNA 'motor proteins', which use the free energy associated with nucleoside 5'-triphosphate hydrolysis to translocate along DNA.

Two proteins are expressed from the *mcrB* gene: a full-length version, $McrB_L$ and a short version, $McrB_s$. $McrB_L$ binds specifically to the methylated recognition site and is, therefore, the DNA binding moiety of the McrBC endonuclease. $McrB_s$ is devoid of DNA binding activity. *In vitro*, $McrB_L$, McrC and GTP are required for cleavage of cytosine methylated DNA. The optimal ratio of 3-5 $McrB_L$ per molecule of McrC suggests that DNA cleavage is accomplished by a multisubunit complex.

We observed that the quaternary structure of the endonuclease depends on binding of the co-factors. In gel filtration experiments, $McrB_L$ and $McrB_s$ form high molecular weight oligomers in the presence of Mg^{2+} and zzGTP, GDP or GTP-γ-S. Oligomerization did not require the presence of DNA and was independent of GTP hydrolysis. Electron micrographs of negatively stained $McrB_L$ and $McrB_s$ revealed ring-shaped particles with a central channel. Mass analysis by STEM indicates that $McrB_L$ and $McrB_s$ form single heptameric rings as well as tetradecamers. In the presence of McrC, a subunit that is essential for DNA cleavage, the tetradecameric species was the major form of the endonuclease. This leads to a model for the assembly of the McrBC endonuclease.

Subunit Assembly and DNA cleavage by the ytpe III restriction endonucleases

DNA cleavage by type III restriction endonucleases requires two inversely oriented asymmetric recognition sequences and results from ATP-dependent DNA translocation and collision of two enzyme molecules. In this study, we characterized the structure and mode of action of the related *Eco*P1I and *Eco*P15I enzymes. Analytical ultracentrifugation and gel quantification revealed a common Res_2Mod_2 subunit stoichiometry. Single alanine substitutions in the putative nuclease active site of ResP1 and ResP15 abolished DNA but not ATP hydrolysis, whilst a substitution in helicase motif VI abolished both activities. Positively supercoiled DNA substrates containing a pair of inversely oriented recognition sites were cleaved inefficiently whereas the corresponding relaxed and negatively supercoiled substrates were cleaved efficiently, suggesting that DNA overtwisting impedes the convergence of the translocating enzymes. *Eco*P1I and *Eco*P15I could co-operate in DNA cleavage on circular substrate containing several *Eco*P1I sites inversely oriented to a single *Eco*P15I site; cleavage occurred predominantly at the *Eco*P15I site. *Eco*P15I alone showed nicking activity on these molecules, cutting exclusively the top DNA strand at its recognition site. This activity was dependent on enzyme concentration and local DNA sequence. The *Eco*P1I nuclease mutant greatly stimulated the *Eco*P15I nicking activity while the *Eco*P1I motif VI mutant did not. Moreover, combining an *Eco*P15I nuclease mutant with wild-type *Eco*P1I resulted in cutting the bottom DNA strand at the *Eco*P15I site. These data suggest that double strand breaks result from top strand cleavage by a Res subunit proximal to the site of cleavage whilst bottom strand cleavage is catalysed by a Res subunit supplied *in trans* by the distal endonuclease in the collision complex.

DNA cleavage by type I restriction enzymes

Type I restriction endonucleases are composed of multiple subunits, HsdS, HsdM and HsdR. A fully active endonuclease complex, composed of one HsdS, two HsdM and two HsdR subunits, requires the addition of Mg^{2+}, S-adenosylmethionine and ATP as cofactors. Nonmethylated DNA containing the appropriate recognition sequence is cleaved at a random location far away from the recognition sequence. Despite all the work that has been done with type I enzymes over the last 30 years, the DNA ends produced by these enzymes have never been characterised. We have now cloned and sequenced DNA molecules that have been restricted with the type I *Eco*R124II endonuclease. We show that *Eco*R124II cleaves a circular DNA substrate at random and has no preference for a particular base composition or base surrounding or at the cleavage site. The fragment ends created during the restriction reaction can be either blunt-ended, 3'- or 5'-recessed. From this data we conclude a mode of action in which two independent strand breaks take place, each caused by one of the HsdR subunits involved in the active endonuclease complex.

Publications

Janscak, P. & Bickle, T. A. (2000). DNA supercoiling during ATP-dependent DNA translocation by the type I restriction enzyme EcoAI. *J Mol Biol* 295, 1089-1099.

Stewart, F. J., Panne, D., Bickle, T. A. & Raleigh, E. A. (2000). Methyl-specific DNA binding by McrBC, a modification-dependent restriction enzyme. *J Mol Biol* 298, 611-622.

Janscak, P., Sandmeier, U., Szczelkun, M. D. & Bickle, T. A. (2001). Subunit assembly and mode of DNA cleavage of the type III restriction endonucleases EcoP1I and EcoP15I. *J Mol Biol* 306, 417-431.

Panne, D., Muller, S. A., Wirtz, S., Engel, A. & Bickle, T. A. (2001). The McrBC restriction endonuclease assembles into a ring structure in the presence of G nucleotides. *EMBO J* 20, 3210-3217.

Dussy, A., Meyer, C., Quennet, E., Bickle, T. A., Giese, B. a. & Marx, A. (2002). New light sensitive nucleosides for caged DNA strand breaks. *Chem Bio Chem* 3, 54-60.

Murphy, M., Schmid Nuoffer, S. & Bickle, T. A. (2002). Lack of regulation of the modification-dependent restriction enzyme McrBC in *Escherichia coli*. *J Bacteriol* 184, 1794-1795.

Molecular mechanisms of microbial pathogenesis: the *Yersinia* paradigm

(this report includes the work done by the group at the ICP, Brussels, during the 2000-2001 period)

P. Broz, G. Denecker, M. Feldman, L. Journet, L.J. Mota, M. Kuhn, P. Troisfontaines, E. Wüest

In collaboration with R. Benabdillah, S. Bleves, B. Foultier, N. Grosdent, C. Josenhans, I. Lambermont, M.N. Marenne, S. Müller, C. Neyt, N. Sauvonnet, I. Stainier, S. Tötemeyer, C. Youta, (Guy Cornelis' laboratory at ICP, Brussels, Belgium); L. Klüsener and J. Lee (Halle, Germany); W. Declercq and P. Vandenabeele (Ghent, Belgium); I. Maridonneau-Parini (Toulouse, France); J. Wehland and A. Sechi (GBF, Braunschweig, Germany); I. Attree (Grenoble, France); D. Vertommen and M. Ryder (ICP, Brussels); B. Pradet-Balade and J. A. Garcia-Sanz (CSIC, Madrid, Spain); T. Bossert and M. Dürrenberger (Biozentrum, Basel); F. Opperdoes (ICP, Brussels); P. van der Bruggen, J. Louahed, JC Renauld (LICR, Brussels); C. Parsot (I Pasteur, Paris)

Phone: +41 61 267 2110 Fax: +41 61 267 2118

Guy R. Cornélis
E-mail: Guy.Cornelis@unibas.ch
http://www.biozentrum.unibas.ch/cornelis

Introduction

We are studying the Yop virulon, a sophisticated weapon that allows bacteria from the genus *Yersinia* (*Y. enterocolitica* , *Y. pseudotuberculosis* and *Y. pestis*) to defeat the immune system of their host. This system allows extracellular bacteria adhering at the surface of eukaryotic cells to inject bacterial effector proteins called Yops into the cytosol of these cells, which disarms them or sabotages their communications. This system, encoded by a 70-kb plasmid, is an archetype for the so-called type III-secretion systems (TTSS), encountered in more than twenty animal, plant and insect pathogens.

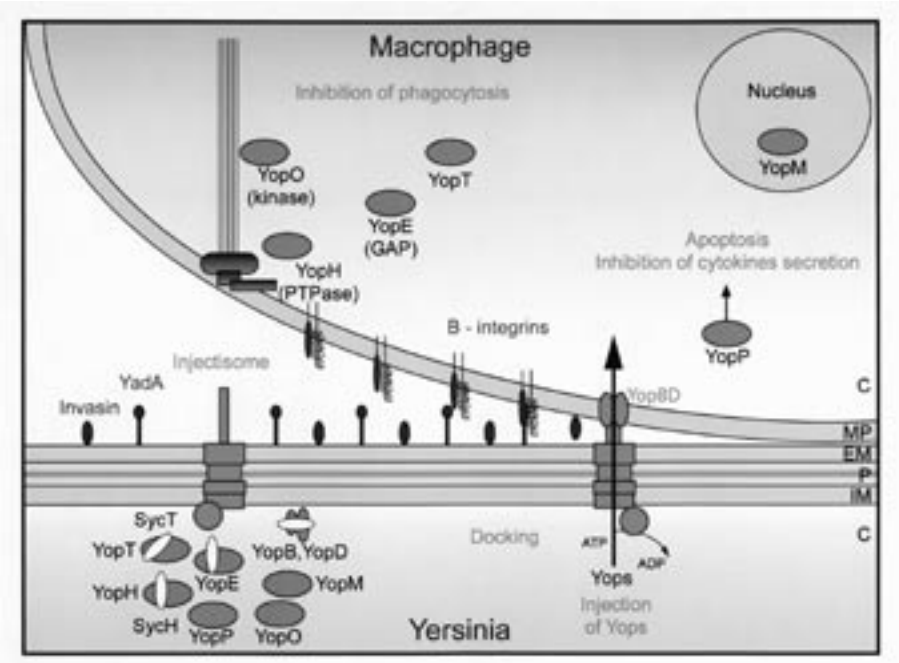


Figure 1: the basic model. When Yersinia are placed at 37 °C in a rich environment, the Ysc injectisome is installed and a stock of Yop proteins is synthesized. During their intrabacterial stage, Yops are capped with their specific Syc chaperone. When the bacterium enters into contact with a target cell, it docks tighly by the interaction between its adhesins Inv and YadA and cellular β-integrins. Then, the secretion channel opens and the Yops are exported. YopB and YopD form a pore in the target cell plasma membrane and the Yop effectors are translocated across this membrane into the cell's cytosol.

In *Yersinia*, the known effectors are YopE, YopH, YopO, YopM and YopT (Fig.1). YopH dephosphorylates proteins from the focal adhesion, YopT modifies RhoA, YopE activates the GTPase activity of Rac and YopO (YpkA) phosphorylates Rac and RhoA. YopP prevents the release of the proinflammatory cytokine TNFα by macrophages by interfering with the mitogen-activated proteins (MAP) kinases (MAPK) and NF-κB pathways. It also induces apoptosis of macrophages. Little is known about YopM, except that, unlike the other Yops, it is targeted to the nucleus of the cell.

Delivery of the Yop effectors requires the 25 YscA-Y proteins, YopB, YopD, LcrV and LcrG, which are encoded by four contiguous operons. The Ysc proteins constitute the Ysc "injectisome" (Fig. 2) which spans the two bacterial membranes and ends up with a needle protruding from the bacterial surface. YopB, YopD and LcrV are secreted by the injectisome and they form a pore in the animal cell membrane. Before contact with a target cell, the Ysc injectisome is supposed to be plugged with a protein called YopN. Yop

secretion can be artificially triggered in the absence of target cells by chelating Ca^{2+} ions. Synthesis of the Yops is under the dual control of temperature and the secretion apparatus (feedback inhibition). The proper operation of the system also requires the presence in the bacterial cytosol, of small individual chaperones, called the Syc proteins.

The Ysc injectisome

In our effort to characterize the injectisome, we studied a new component, called YscP. An *yscP*$_{\Delta 97\text{-}465}$ mutant was unable to secrete any Yop. Mechanical shearing, immunolabelling and electron microscopy experiments showed that YscP is exposed at the bacterial surface, when bacteria are incubated at 37°C but do not secrete Yops. At 37°C, when Ca^{2+} ions are chelated, YscP is released like a Yop protein. We concluded that YscP is an essential part of the injectisome, localized at the bacterial surface and destabilized by Ca^{2+}-chelation.

Several Ysc proteins, display homology with proteins of the flagellar basal body. To determine whether this relation extends to the regulatory pathways, we analyzed the role of *flhDC*, the master regulatory operon of the flagellum, on the *yop* regulon. In a *flhDC* mutant, the *yop* regulon was upregulated to the extend that Yops secretion occurred even at low temperature, a phenotype that was never observed before. The Ysc secretion machinery was thus functional at room temperature in the absence of flagella, this implying that in wild-type bacteria FlhD and/or FlhC, or the product of a FlhDC-downstream gene, represses the *yop* regulon.

Role of the SycE chaperone

Residues 1-77 of YopE contain all the information sufficient for the complete delivery of YopE into the target cell. Residues 15-50 represent the binding domain for SycE, the YopE-specific chaperone. To understand the role of this chaperone, we studied the delivery of various YopE-Cya reporters and deletion mutants of YopE by a polymutant *Yersinia* devoid of most of the effectors (ΔHOPEM). The results showed that residues 1-15 are sufficient for delivery into eukaryotic cells. However, concomitant production of another effector, had a strong inhibitory effect on the delivery of the proteins missing the chaperone binding domain. These results suggests that the SycE chaperone either facilitates secretion or introduces hierarchy among the effectors. To pursue our analysis, we engineered hybrid proteins made of part of YopE and three forms of the mouse dihydrofolate reductase (DHFR). We observed that secretion of hybrids made with wild type DHFR absolutely requires SycE. However, hybrids made with a DHFR mutant that cannot fold properly can be secreted without this chaperone. These results suggest that the Ysc secretion system of *Y. enterocolitica* can secrete only unfolded proteins and that Syc chaperones keep their cognate proteins in a secretion-competent state.

Study of the YopP effector

The delivery of YopP into macrophages prevents the release of the pro-inflammatory cytokines and triggers apoptosis. We analyzed the YopP-induced apoptosis. Generation of truncated Bid (tBid) was the first event that we observed. The subsequent translocation of tBid to the mitochondria induced the release of cytochrome c, leading to the activation of caspase-9 and the executioner caspases-3 and-7. The apoptotic cascade is thus triggered at an early stage but it is not clear yet whether YopP leads macrophages to apoptosis by a direct action or whether it acts indirectly by blocking the NF-κB-dependent rescue mechanisms. Recently it was reported that inhibition of the NF-κB pathway is dependent on a cysteine which would be the catalytic core of a protease activity (Orth et al., Science 2000,290:1594-1597). We found that disruption of this putative catalytic domain abolished the generation of tBid, reinforcing the link between apoptosis and NF-κB downregulation . We also analyzed the inflammatory response of human umbilical vein endothelial cells (HUVECs). We demonstrated that both the expression of the intercellular adhesion molecule ICAM-1 as well as the release of IL-6 and IL-8 are downregulated in a YopP-dependent way, thereby demonstrating that YopP plays a role in the inflammatory response of endothelial cells. Infection of HUVECs with several *Y. enterocolitica* strains showed that high-virulence biotype 1B strains are more efficient in inhibiting the inflammatory response than the low-virulence strains.

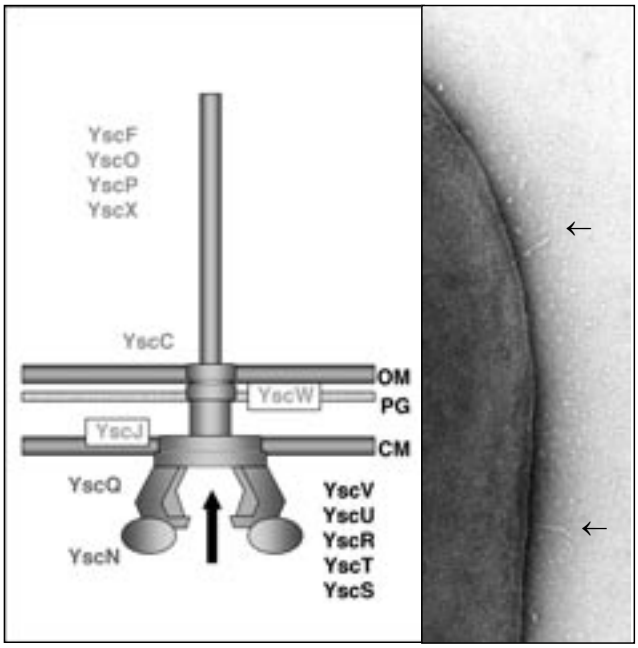
Role of the Yops and adhesins in resistance of *Y. enterocolitica* to phagocytosis

In addition to the Ysc type III secretion system, *Y. enterocolitica* is endowed with two adhesins, Inv and YadA. We tested the influence of all these virulence determinants on phagocytosis by PU5-1.8 and J774 cells as well as by human polymorphonuclear leucocytes. In the absence of opsonization, contact between bacteria and phagocytes was dependent upon Inv or YadA while in the presence of IgG and complement, contact was independent of bacterial adhesins. In both instances, the Ysc-Yop system conferred a significant level of resistance to phagocytosis. Single mutants lacking either YopE, -H, -T, -O were significantly more phagocytosed showing that each of these factors is necessary. Overexpression of any of YopE, -H, -T, -O, alone, did not confer resistance although these all affected the cytoskeleton. Thus, YopE, -H, -T, -O act synergistically to confer *Y. enterocolitica* resistance to phagocytosis by macrophages and polymorphonuclear leukocytes.

Characterization of a new pathogenicity locus in the chromosome of *Y. enterocolitica* and phylogeny analysis of type III secretion systems

In 2000, a TTSS, called Ysa was described in *Y. enterocolitica* 8081, a strain of serotype O:8 (Haller et al., Mol. Microbiol. 2000 : 36 : 1436-46.) In 2001, we characterized the *ysa* locus from A127/90, another strain of serotype O:8, and we extended the sequence to several new genes encoding Ysp proteins which are the substrates of this secretion system, and a putative chaperone SycB. This Ysa

Figure 2: the needle of the Ysc injectisome
left: *a schematic representation of the whole injectisome and localization of some components.*
right: *electron micrographs of needles protruding from the bacterial surface*



system is closely related to the Mxi-Spa TTSS of *Shigella* and to the SPI-1 encoded TTSS of *Salmonella enterica*. The *ysa* locus is only present in biotype 1B strains of *Y. enterocolitica*. Including this new Ysa system, a phylogenetic analysis of the 26 known TTSSs was carried out, based on the sequence analysis of three conserved proteins. All the TTSSs fall into five different clusters. The phylogenetic tree of these TTSSs is completely different from the evolutionary tree based on 16S RNA, indicating that TTSSs have been distributed by horizontal transfer.

Publications

Bleves, S. & Cornelis, G. R. (2000). How to survive in the host: the Yersinia lesson. *Microbes Infect* 2, 1451-1460.

Blocker, A., Holden, D. & Cornelis, G. R. (2000). Type III secretion systems: what is the translocator and what is translocated? *Cell Microbiol* 2, 387-390.

Boland, A. & Cornelis, G. R. (2000). Interaction of Yersinia with host cells. In *Bacterial invasion into eukaryotic cells* (Oelschlager, J. H. a. T., ed.), pp. 343-382. Plenum Press.

Boyd, A. P., Grosdent, N., Totemeyer, S., Geuijen, C., Bleves, S., Iriarte, M., Lambermont, I., Octave, J. N. & Cornelis, G. R. (2000). *Yersinia enterocolitica* can deliver Yop proteins into a wide range of cell types: development of a delivery system for heterologous proteins. *Eur J Cell Biol* 79, 659-71.

Boyd, A. P., Lambermont, I. & Cornelis, G. R. (2000). Competition between the Yops of *Yersinia enterocolitica* for delivery into eukaryotic cells: role of the SycE chaperone binding domain of YopE. *J Bacteriol* 182, 4811-21.

Cornelis, G. R. (2000). Molecular and cell biology aspects of plague. *Proc Natl Acad Sci USA* 97, 8778-8783.

Cornelis, G. R. (2000). Type III secretion: a bacterial device for close combat with cells of their eukaryotic host. *Philos Trans R Soc Lond B Biol Sci* 355, 681-693.

Cornelis, G. R. & Van Gijsegem, F. (2000). Assembly and function of type III secretory systems. *Annu Rev Microbiol* 54, 735-774.

Sory, M. P. & Cornelis, G. R. (2000). Virulence determinants of the bacterial pathogen *Yersinia enterocolitica*: mode of action and global regulation. In *Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis* (J. Linz, D. B., M. Stein and J.W. Cary, ed.), pp. 131-155. Technomic Publishing Company (USA).

Stainier, I., Bleves, S., Josenhans, C., Karmani, L., Kerbourch, C., Lambermont, I., Totemeyer, S., Boyd, A. & Cornelis, G. R. (2000). YscP, a *Yersinia* protein required for Yop secretion that is surface exposed, and released in low Ca2+. *Mol Microbiol* 37, 1005-18.

Boyd, A. & Cornelis, G. R. (2001). Yersinia. In *Principles of bacterial pathogenesis* (Groisman, E., ed.), pp. 227-264. Academic Press.

Cornelis, G. R. (2001). The Yersinia Ysc-Yop virulence apparatus. *Int. J. Med. Microbiol.*

Cornelis, G. R. & Denecker, G. (2001). Yersinia lead SUMO attack. *Nature Medicine* 7, 21-3.

Cornelis, G. R. & Rappuoli, R. (2001). Host-microbe interactions: bacteria. Microbiology will never be the same againellipsis. *Curr Opin Microbiol* 4, 13-15.

Denecker, G., Declercq, W., Geuijen, C. A., Boland, A., Benabdillah, R., van Gurp, M., Sory, M. P., Vandenabeele, P. & Cornelis, G. R. (2001). Yersinia enterocolitica YopP-induced apoptosis of macrophages involves the apoptotic signaling cascade upstream of bid. *J Biol Chem* 276, 19706-14.

Lee, J., Klusener, B., Tsiamis, G., Stevens, C., Neyt, C., Tampakaki, A. P., Panopoulos, N. J., Noller, J., Weiler, E. W., Cornelis, G. R., Mansfield, J. W. & Nurnberger, T. (2001). HrpZ(Psph) from the plant pathogen *Pseudomonas syringae* pv. phaseolicola binds to lipid bilayers and forms an ion-conducting pore in vitro. *Proc Natl Acad Sci U S A* 98, 289-94.

Müller, S., Feldman, M. F. & Cornelis, G. R. (2001). The type III secretion system of Gram-negative bacteria: a potential therapeutic target? *Expert Opin Ther Targets* 5, 327-339.

Tötemeyer, S. & Cornelis, G. R. (*in press*). Transport and intracellular Movement - Protein translocation via dedicated secretion systems. In *Methods and Microbiology* (Zychlinski, A. & Sansonetti, P.J. eds.). Academic Press Publisher.

Boyd, A. & Cornelis, G. R. (*in press*). In *Unraveling bacterial pathogenesis: a molecular mechanistic approach* (Taylor, R., ed.), pp. 227-264. Blackwell Science Publisher.

Foultier, B., Troisfontaines, P., Müller, S., Opperdoes, F. & Cornelis, G. R. *(in press*). Characterization of the ysa pathogenicity locus in the chromosome of Yersinia enterocolitica and phylogeny analysis of type III secretion systems. *J. Molecular Evolution*.

Molecular and cellular basis of bacterial persistence in the infected host

S. Binggeli, M. Dehio, P. Guye, R. Hiestand, M. Quebatte, T. Rhomberg, H. Saenz, M. Schmid, R. Schülein, A. Seubert, C. Snyder

Visiting scientist: C. Hansen (Uppsala University, Sweden)
In collaboration with S.G. Andersson (Uppsala University, Sweden), P. Jenö (Biozentrum, Basel), J. Koesling (Max Planck Institute for Infection Biolology, Berlin, Germany), Y. Piemont (University Louis-Pasteur, Strasbourg, France), G. Raddatz (Max Planck Institute for Developmental Biology, Tübingen, Germany), A. Sander (University of Freiburg, Germany), N. Suttorp (Humbolt-University, Berlin, Germany)

Christoph Dehio
Phone: +41 61 267 2140 Fax: +41 61 267 2118 E-mail: Christoph.Dehio@unibas.ch
http://www.biozentrum.unibas.ch/dehio

Scope of research

Bacteria capable of resisting the innate immune response of the host typically cause an acute infection. Progression to a chronic infection critically depends on the pathogen‘s ability to evade the triggered (B- and T-cell dependent) specific immune response. Several pathogens evade this second line of defence by persistently colonising an intracellular niche. This lifestyle requires adaptation to both the initial colonisation process (cell tropism, adherence and invasion) often associated with rapid bacterial growth, and the subsequent establishment of a chronic intracellular infection state with little or no growth. We have chosen the emerging pathogen *Bartonella* as a model to study the molecular and cellular basis of bacterial persistence in the infected host. *Bartonella* spp. are arthropod-born Gram-negative bacteria displaying a marked tropism towards endothelial cells and erythrocytes, which become infected providing an intracellular niche for long-term bacterial persistence. Depending on the level of adaptation between the *Bartonella* species/strain and the infected host, the clinical outcome ranges from asymptomatic infection to vasoproliferative tumour formation and life-threatening haemolytic anemia. During the past two years we have developed both rat and mouse models, which allowed us to dissect the *in vivo* course of persistent *Bartonella* infection in erythrocytes. We further established bacterial genetics resulting in the identification of bacterial pathogenicity factors involved in persistence. In addition, a genome-wide DNA-microarray has been set up with the aim of identifying sets of co-regulated genes required for distinct phases of establishing a persistent bacterial infection.

Animal models of bacterial persistence within erythrocytesH

We have analysed the long-lasting course of intraerythrocytic *Bartonella* infection by tracking green fluorescent protein-expressing *B. tribocorum* in the blood of experimentally infected rats. Following intravenous infection, bacteria are cleared from blood and colonise a primary niche, which may involve endothelial cells as a major target cell type. This primary niche seeds bacteria into the blood stream on day five of infection and subsequently in intervals of ~five days. In the blood stream, bacteria invade mature erythrocytes, replicate temporarily and persist intracellularly in a non-dividing state for the remaining life span of the infected erythrocytes. The non-haemolytic course of erythrocyte parasitism and the inaccessibility of the intraerythrocytic compartment to any type of host immune response permits *Bartonella* to persist for several weeks within the circulating blood, greatly facilitating transmission by blooding-sucking arthropods. A murine model of *B. grahamii* infection revealed that the abrogation of intraerythrocytic bacteremia typically observed within three months of infection critically depends on a triggered antibody response. While these antibodies do not effect intraerythrocytic bacteria, they appear to prevent the invasion of erythrocytes by extracellular bacteria released from the primary niche.

Bacterial pathogenesis factors required for establishing persistent erythrocyte infection

The recent establishment of genetic tools for *Bartonella* spp. facilitated the identification of pathogenicity factors involved in establishing persistent erythrocyte infection. *Bartonella* spp. encode a type IV secretion system homologous to the VirB system of *Brucella* spp. known to be involved in intracellular survival. *B. tribocorum virB* mutants do not establish an erythrocyte infection in the rat model, providing the first example of a *bona fide* pathogenicity locus in *Bartonella*. Application of the differential fluorescence induction technology resulted in the isolation of bacterial genes differentially

expressed during endothelial cell infection *in vitro* as well as erythrocyte infection *in vivo*. Among those identified is the complex *trw* locus comprised of 30 genes, which encodes a second type IV secretion system in *Bartonella*. Interestingly, Trw has no homologue in other pathogenic bacteria but is closely related to the conjugative pilus of the broad-host-range plasmid R388. *B. tribocorum trw* mutants do not infect erythrocytes in the rat model, demonstrating that *Bartonella* requires two distinct type IV secretion systems for establishing a persistent infection. While type IV secretion systems are important pathogenicity determinants, which are widely distributed among Gram-negative bacteria, no other pathogen is known to require two distinct type IV secretons for pathogenesis. It remains to be demonstrated at which step of establishing persistence VirB and Trw are implicated and which bacterial effector molecules are targeted by these transporters to the interacting host cells.

Genome-wide transcriptional profiling

The complete genome sequence of *B. henselae* was recently determined in the group of S.G. Andersson at Uppsala University. In collaboration with this group, we have established a spotted DNA-microarray composed of PCR products of essentially all encoded genes. The utility of the spotted DNA-microarray technology for genome-wide transcriptional profiling in *Bartonella* was demonstrated by studying the heat-shock response as a well-studied physiological process. Moreover, protocols for transcriptional profiling of the infection process in primary cell culture models were successfully established. A current focus of our research is to raise the sensitivity of transcript detection to permit transcriptional profiling on blood and tissue samples retrieved from infected animals. We assume that transcriptional profiling of the infection process will ultimately reveal distinct sets of co-regulated pathogenicity genes and metabolic pathways evolved by *Bartonella* to adopt its highly successful mode of persistent infection.

Publications

Andersson, S. G. & Dehio, C. (2000). *Rickettsia prowazekii* and *Bartonella henselae:* differences in the intracellular life styles revisited. *Int J Med Microbiol* 290, 135-141.

Bermond, D., Heller, R., Barrat, F., Delacour, G., Dehio, C., Alliot, A., Monteil, H., Chomel, B., Boulouis, H. J. & Piemont, Y. (2000). *Bartonella birtlesii* sp. nov., isolated from small mammals (*Apodemus* spp.). *Int J Syst Evol Microbiol* 50, 1973-1979.

Dehio, C., Gray-Owen, S. D. & Meyer, T. F. (2000). Host cell invasion by pathogenic *Neisseriae*. *Subcell. Biochem.* 33, 61-96.

Freissler, E., Meyer auf der Heyde, A., David, G., Meyer, T. F. & Dehio, C. (2000). Syndecan-1 and syndecan-4 can mediate the invasion of Opa_{HSPG}- expressing *Neisseria gonorrhoeae* into epithelial cells. *Cell Microbiol* 2, 69-82.

Muenzner, P., Dehio, C., Fujiwara, T., Achtman, M., Meyer, T. F. & Gray-Owen, S. D. (2000). Carcinoembryonic antigen family receptor specificity of *Neisseria meningitidis* Opa variants influences adherence to and invasion of proinflammatory cytokine-activated endothelial cells. *Infect Immun* 68, 3601-3607.

Rziha, H., Henkel, M., Cottone, R., Bauer, B., Auge, U., Gotz, F., Pfaff, E., Rottgen, M., Dehio, C. & Buttner, M. (2000). Generation of recombinant parapoxviruses: non-essential genes suitable for insertion and expression of foreign genes. *J Biotechnol* 83, 137-45.

Sander, A., Zagrosek, A., Bredt, W., Schiltz, E., Piemont, Y., Lanz, C. & Dehio, C. (2000). Characterization of *Bartonella clarridgeiae* flagellin (FlaA) and detection of antiflagellin antibodies in patients with lymphadenopathy. *J Clin Microbiol* 38, 2943-2948.

Dehio, C. (2001). *Bartonella* interactions with endothelial cells and erythrocytes. *Trends Microbiol.* 9, 279-285.

Dehio, C. (2001). The interactions of *Bartonella* with endothelial cells and erythrocytes. Response from Dehio. *Trends Microbiol.* 9, 531-532.

Dehio, C., Lanz, C., Pohl, R., Behrens, P., Bermond, D., Piemont, Y., Pelz, K. & Sander, A. (2001). *Bartonella schoenbuchii* sp. nov., isolated from the blood of wild roe deer. *Int J Syst Evol Microbiol* 51, 1557-1565.

Fuhrmann, O., Arvand, M., Gohler, A., Schmid, M., Krull, M., Hippenstiel, S., Seybold, J., Dehio, C. & Suttorp, N. (2001). *Bartonella henselae* induces NF-kappaB-dependent upregulation of adhesion molecules in cultured human endothelial cells: possible role of outer membrane proteins as pathogenic factors. *Infect Immun* 69, 5088-5097.

Koesling, J., Aebischer, T., Falch, C., Schulein, R. & Dehio, C. (2001). Cutting edge: antibody-mediated cessation of hemotropic infection by the intraerythrocytic mouse pathogen *Bartonella grahamii*. *J Immunol* 167, 11-14.

Raddatz, G., Dehio, M., Meyer, T. F. & Dehio, C. (2001). PrimeArray: genome-scale primer design for DNA-microarray construction. *Bioinformatics* 17, 98-99.

Schulein, R. & Dehio, C. (2001). A homologue of the *Agrobacterium* VirB type IV secretion system is essential for establishing intraerythrocytic infection by *Bartonella*. *Mol Microbiol*, submitted.

Schulein, R., Seubert, A., Gille, C., Lanz, C., Hansmann, Y., Piemont, Y. & Dehio, C. (2001). Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen *Bartonella*. *J Exp Med* 193, 1077-1086.

Differentiation in *Streptomyces* – multicellular development in a procaryote

I.Christiansen, M. Folcher, X.-M. Li, V.-D. Luu, R. Morris, K. Nguyen, L. Nguyen, D. Schwartz, H.R. Stettler, S. Tawfilis, J. Tenor, P. Viollier

Visiting scientists: J. Novotna and J. Vohradsky (Academy of Sciences, Prague, Czech Republic); A.M. Puglia (University of Palermo, Italy)
In collaboration with D. Aubel (Inst. Universitaire de Technologie, Villeurbanne, France); N. Bamas-Jacques and P.l Lacroix (Aventis Pharma SA, Vitry-sur-Seine, France); H. Langen and D. Röder (Hoffmann-LaRoche, Basel); G.E. Dale (Morphochem, Basel); H. Gaillard (Institute of Cell Biology, ETH-Hönggerberg, Zürich); M.J. Buttner, G.H. Kelemen and L. Marri (Dept. of Molecular Microbiology, John Innes Centre, Colney, Norwich, UK); M. Rinkel (Bio Media, Boussens, France); J.E. Bailey, A.D. Frey, C. Fux, M. Fussenegger, P.T. Kallio, H. Kaufmann, W. Minas, M. Rimann and B. von Stockar (Inst. of Biotechnology, ETH, Zürich)

Charles J. Thompson
Phone: +41 61 267 2116 Fax: +41 61 267 2118 E-mail: Charles-J.Thompson@unibas.ch
http://www.biozentrum.unibas.ch/thompson

Scope of research

Streptomyces are filamentous soil bacteria that undergo morphological changes coordinated with the synthesis of an enormous variety of antibiotics and other pharmaceutically important metabolites. A better understanding of the regulatory mechanisms which control this developmental program is of great interest, not only from the point of view of fundamental biology, but also for the discovery and engineering of new chemotherapeutic drugs.

Unlike better known unicellular bacteria that often have solitary lifestyles, *Streptomyces* are multicellular organisms that have evolved as coordinated communities of specialized, interactive cells. Whereas bacterial chromosome replication is typically followed by segregation into two cells that separate, *Streptomyces* genomes remain associated in a chain of cells forming a filament that elongates and branches. The dense interconnected network thus formed within the substrate enlarges into a colony. Developmental changes occur in localized regions of the colony generating groups of cells specialized for continued growth, programmed cell death, manufacture and circulation of nutrients, erection of aerial mycelium, spore formation, and biosynthesis of various secondary metabolites. This “tissue-specific” gene expression, coordinated by diffusible butyrolactone and peptide signaling compounds, is reminiscent of metazoans.

The erection of aerial mycelium, the most obvious visual manifestation of the developmental program, is linked to the biosynthesis of antibiotics. In general, these processes take place in substrate mycelium at about the same time; most mutants that cannot erect aerial mycelium (referred to as “bald”) are also unable to produce antibiotics. While several bald loci have been identified genetically as transcriptional and translational regulatory elements, the genetic functions they control are largely unknown.

Imbalances in physiology lead to accumulation of certain primary metabolites which are converted to novel compounds by the activation of alternative, “secondary metabolic” pathways. The secondary metabolites produced by *Streptomyces* have been the primary source of antibiotics and also have applications as herbicides, anticancer drugs, immunoregulators, and antiparasitic compounds. In order to avoid suicide, the biosynthesis of antibiotics must be associated with the expression of antibiotic resistance genes.

Antibiotic resistance and developmental genes have been the focus of this reporting period. We have identified systems that provide multidrug resistance in *Streptomyces*. The work has led to important pharmaceutical applications in drug discovery and gene therapy. Secondly, we obtained initial glimpses of links between metabolic, stress response, and developmental programs in *Streptomyces*.

Multidrug resistance (MDR) in organisms that produce multiple antibiotic compounds

Multidrug resistance controlled by a peptide antibiotic (Chiu, M. et al., 2001)

Sublethal exposure to the antibiotic thiostrepton induces multidrug resistance. One of these MDR systems is controlled by TipAL, a *Streptomyces* protein that activates its own transcription and provides resistance to diverse peptide antibiotics. Transcriptional activation is mediated by conformational changes induced by antibiotic binding to its C-terminal domain. Thiostrepton slightly enhanced the rate of specific association of TipAL with its promoter target (PtipA), but drastically lowered the rate of dissociation. TipAL-thiostrepton increased the affinity of RNA polymerase for PtipA more than tenfold. The molecular mechanism for the broad antibiotic recognition specificity displayed by TipA and the conformational changes leading to its activation is now being elucidated by analysis of NMR spectra in collaboration with Prof. Stephan Grzesiek's laboratory in the Division of Structural Biology.

Multidrug resistance regulated by a streptogramin antibiotic (Folcher, M., Morris, R.P. et al., 2001)

These studies were undertaken to gain a better understanding of how the transcription of bacterial multidrug resistance genes can be induced by diverse antibiotics. Our model system was a promoter (Pptr) activated by antibiotics having many different structures. A protein that bound to sequence motifs regulating the Pptr was purified and the corresponding locus identified as *pip*, encoding a protein similar to a procaryotic transcriptional repressor inactivated by tetracycline (TetR). Pip binding motifs were located upstream of the adjacent gene, encoding a major facilitator antiporter. Purified recombinant Pip was a dimer and displayed a high affinity for palindromic binding motifs. Surprisingly, the Pip/*ptr* promoter complex was dissociated only by one of the antibiotic classes able to induce transcription. While Pip was apparently not the regulatory element determining the multidrug response, its specificity for streptogramin antibiotics was exploited to design regulatory systems for repression or induction of cloned genes in mammalian and plant cells.

Streptogramin-based gene regulation systems for mammalian and plant cells *(Frey, A.D., 2001. Fussenegger, M., Morris, R.P. et al., 2000. Fussenegger, M., Thompson, C.J. et al., 2000)*

Systems to manipulate expression of foreign genes in mammalian cells will be essential tools for future human therapies, drug testing in animal models and biopharmaceutical manufacturing. We designed novel repressible (PipOFF) as well as inducible (PipON) systems for regulated gene expression in mammalian cells based on the repressor Pip (described above) of a streptogramin resistance operon of Streptomyces coelicolor. Expression of genes placed under control of these systems was responsive to clinically approved antibiotics belonging to the streptogramin group (pristinamycin, virginiamycin and Synercid®). The streptogramin-based expression technology was functionally compatible with the tetracycline responsive regulatory system, thus enabling the selective use of different antibiotics to independently control two different gene activities in the same cell. Systems based on the PIPpOFF and PIPpON concepts has also been adapted for use in plant cells to allow for pristinamycin-repressible or activated expression of a synthetic plant promoter. These systems provide an attractive extension of existing plant gene regulation technology for basic plant research or biopharmaceutical manufacturing.

Streptogramin responsive transcriptional reporter systems used in the design of a novel mammalian screening system for the detection of new antibiotics (Aubel, D., et al, 2001)

Screening and development of new antibiotic activities to counteract the increasing prevalence of multidrug-resistant human pathogenic bacteria has once again become a priority in human chemotherapy. Synthetically modified streptogramins are presently the sole effective agent in the treatment of some MDR nosocomial infections. We have designed a novel mammalian cell culture-based screening platform for the detection of streptogramin B antibiotics. Pip-based regulatory systems have been adapted to modulate reporter gene expression in Chinese hamster ovary cells (CHO) in response to streptogramin antibiotics. This screen is much more sensitive, rapid, and convenient than the traditional antibiotic sensitivity assay and enables detection of bioavailable and non-cytotoxic antibiotics.

Developmental control of stress stimulons in Streptomyces coelicolor revealed by statistical analyses of global gene expression patterns (Vohradsky, J. et al, 2000)

Stress-induced regulatory networks coordinated with a procaryotic developmental program were revealed by two dimensional gel analyses of global gene expression. Four developmental stages were identified by their distinctive protein synthesis patterns using principal component analysis. Statistical analyses focussed on expression of five stress stimulons (induced by heat, cold, salt, ethanol, or antibiotic shock) during development. Unlike other bacteria where various stresses induce expression of similar sets of protein spots, in *S. coelicolor* heat, salt and ethanol stimulons were composed of independent sets of proteins. Cluster analysis of stress protein synthesis profiles identified ten different developmental patterns or "synexpression groups". Proteins induced by cold-, heat-, or salt-shock were enriched in three

developmental synexpression groups. Thus, stress regulatory systems controlling these stimulons were implicated as integral parts of the developmental program.

Future analysis of developmental patterns of gene expression will rely on identification of spots in the proteome. An automated system has now identified 204 individual proteins. This proteome database is now available on a publicly available SWICZ server (http://proteom.biomed.cas.cz) where protein spots are linked to a set of search engines which allow record retrieval according to its name, spot ID, function, Mw and pI range, and corresponding genetic locus.

A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* *(Kelemen, G.H. et al, 2001)*

Morphological changes leading to aerial mycelium formation and sporulation in *S. coelicolor* rely on establishing distinct patterns of gene expression in separate regions of the colony. *SigH* was identified as an RNA polymerase subunit (sigma factor) associated with environmental stress responses in *S. coelicolor*. We showed that *sigH* is transcribed from two developmentally regulated promoters, *sigH*p1 and *sigH*p2. A protein possessing *sigH*p2 promoter-binding activity was purified to homogeneity from crude mycelial extracts of *S. coelicolor* and shown to encode the developmental regulatory protein BldD. *SigH*p2 transcription and *SigH* protein levels were no longer under developmental control in a *bldD* mutant. These data show that expression of *sigH* is subject to temporal and spatial regulation during colony development, that this tissue-specific regulation is mediated directly by the developmental transcription factor BldD, and that stress and developmental programs may be intimately connected in *Streptomyces* morphogenesis.

A connection between metabolism and development in *Streptomyces coelicolor* *(Viollier, P.H., Minas, W. et al., 2001. Viollier, P.H., Nguyen, K.T. et al., 2001)*

We have found that most developmental mutants that do not produce aerial hyphae or antibiotics are metabolically defective. They produce large amounts of organic acids, presumed to reflect reduced TCA cycle activity, perhaps as a consequence of limited oxygen availability as the densely packed substrate mycelium approached a critical mass. Projection of hyphae into the air may increase oxygen availability and thereby allow renewed TCA metabolic activity that consumes organic acids. These associations between organic acid metabolism and differentiation suggested that regulation of TCA cycle enzymes was linked to development and led to studies of aconitase and citrate synthase. Purification of aconitase (SacA) and citrate synthase (CitA) enzymes from *S. coelicolor* allowed cloning, sequencing, and disruption of their corresponding genes. Both *sacA* and *citA* mutants were acidogenic and unable to differentiate. They accumulated various organic acids as fermentation products and were impaired in the synthesis of antibiotics as well as aerial hyphae.

Pleiotropic functions of a *Streptomyces pristinaespiralis* autoregulator receptor in development, antibiotic biosynthesis, and expression of a superoxide dismutase *(Folcher, M., Gaillard, H. et al., 2001)*

In *Streptomyces*, a family of related butyrolactones and their corresponding receptor proteins serve as diffusible quorum sensing systems that can activate morphological development and antibiotic biosynthesis. *Streptomyces pristinaespiralis* contains a gene cluster encoding enzymes and regulatory proteins for the biosynthesis of pristinamycin, a clinically important streptogramin antibiotic. One of these proteins, PapR1, belongs to a well known family of *Streptomyces* antibiotic regulatory proteins (SARP). Gel shift assays using crude cytoplasmic extracts detected SpbR, a developmentally regulated protein that bound to the *papR1* promoter. SpbR was purified and its gene was cloned using reverse genetics. *spbR* encoded a protein similar to *Streptomyces* autoregulatory proteins of the butyrolactone receptor family including *scbR* from *S. coelicolor*. Purified SpbR and ScbR produced in *E. coli* bound a sequence motif immediately upstream of *pap*R1, *spbR*, and *scbR*. SpbR DNA binding activity was inhibited by an extracellular metabolite similar to the well known gamma butyrolactone signaling compounds. SpbR binding sites in the *papR1* promoter were homologous to other known butyrolactone autoregulatory elements. A nucleotide database search showed that these binding motifs were primarily located upstream of genes encoding SARPs and butyrolactone receptors in various *Streptomyces* species. Disruption of the *spbR* gene in *S. pristinaespiralis* resulted in severe defects in growth, morphological differentiation, pristinamycin biosynthesis, and expression of a secreted superoxide dismutase.

Publications

Fussenegger, M., Morris, R. P., von Stockar, B., Fux, C., Rimann, M., Thompson, C. J. & Bailey, J. E. (2000). Streptogramin-based gene regulation systems for mammalian cells. *Nature Biotechnol* 18, 1203-1206.

Fussenegger, M., Thompson, C. J. & Bailey, J. E. (2000). Patent deposited: “A novel streptogramin-based gene regulation system for mammalian cells”.

Vohradsky, J., Li, X.-M., Dale, G., Folcher, M., Nguyen, L., Viollier, P. H. & Thompson, C. J. (2000). Developmental control of stress stimulons in *Streptomyces coelicolor* revealed by statistical analysis of global gene expression patterns. *J Bacteriol* 182, 4979-4986.

Aubel, D., Morris, R. P., Rimann, M., Lennon, B., Kaufmann, H., Folcher, M., Bailey, J. E., Thompson, C. J. & Fussenegger, M. (2001). Design of a novel mammalian screening system for the detection of bioavailable, non-cytotoxic streptogramin antibiotics. *J Antibiotics* 55, 44-55.

Chiu, M. L., Viollier, P. H., Katoh, T., Ramsden, J. J. & Thompson, C. J. (2001). Ligand-Induced changes in the *Streptomyces lividans* TipAL protein imply an alternative mechanism of transcriptional activation for MerR-like proteins. *Biochemistry* 40, 12950-12958.

Folcher, M., Gaillard, H., Nguyen, L. T., Nguyen, K. T., LaCroix, P., Bamas-Jacques, N., Rinkel, M. & Thompson, C. J. (2001). Pleiotropic functions of a *Streptomyces pristinaespiralis* autoregulator receptor in development, antibiotic biosynthesis, and expression of a superoxide dismutase. *J Biol Chem* 276, 44297-44306.

Folcher, M., Morris, R. P., Dale, G., Salah-Bey-Hocini, K., Viollier, P. H. & Thompson, C. J. (2001). A transcriptional regulator of a pristinamycin resistance gene in *Streptomyces coelicolor*. *J Biol Chem* 276, 1479-1485.

Frey, A. D., Rimann, M., Bailey, J. E., Kallio, P. T., Thompson, C. J. & Fussenegger, M. (2001). Novel pristinamycin-responsive expression systems for plant cells. *Biotechnol Bioeng* 74, 154-163.

Kelemen, G. H., Viollier, P. H., Tenor, J., Marri, L., Buttner, M. & Thompson, C. J. (2001). A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* A3(2). *Mol Microbiol* 40, 804-814.

Viollier, P. H., Minas, W., Dale, G. E., Folcher, M. & Thompson, C. J. (2001). Role of acid metabolism in *Streptomyces coelicolor* morphological development and antibiotic biosynthesis. *J Bacteriol* 183, 3184-3192.

Viollier, P. H., Nguyen, K. T., Minas, W., Folcher, M., Dale, G. & Thompson, C. J. (2001). Role of aconitase in growth, metabolism, and morphological differentiation of *Streptomyces coelicolor*. *J Bacteriol* 183, 3193-3203.

Temporal and spatial control during the bacterial cell cycle

M. Ackermann, P. Aldridge, B. Christen, W. Dischert, D. Eglin, T. Fuchs, B. Geering, S. Gehrig, B. Grünenfelder, M. Osteras, R. Paul, G. Rummel, K. Schäfer, A. Schauerte

In collaboration with M.R.K. Alley (Dept. of Biochemistry, Imperial College, London), M. Dürrenberger (Division of Structural Biology, Biozentrum), H. Langen (Genetics Dept., Hoffmann-La Roche, Basel), P. Rainey (Dept. of Plant Sciences, Oxford University), J. Vohradsky (Academy of Sciences of the Czech Republic, Prague)

Urs Jenal
Phone: +41 61 267 2135 Fax: +41 61 267 2118 E-mail: Urs.Jenal@unibas.ch
http://www.biozentrum.unibas.ch/jenal

Scope of research

Our main objective is to understand temporal and spatial control mechanisms of the bacterial cell cycle and development with a particular emphasis on the role of controlled protein degradation. Specific degradation of key regulators and structural proteins is used by the cell to provide directionality and ordered progression to the cell cycle and developmental pathways. For our studies we have chosen *Caulobacter crescentus*, an organism in which cell cycle and cell differentiation are intimately connected. The *C. crescentus* life cycle includes an asymmetric cell division, which generates two different progeny, a sessile and replication-competent stalked cell and a motile but replication-inert swarmer cell. The latter has to differentiate into a stalked cell before it can grow, start a new round of chromosome replication and ultimately undergo another asymmetric cell division. Our research is geared towards understanding how asymmetry is generated in this system and how cell differentiation and cell cycle progression are intertwined.

Global analysis of cell cycle-dependent protein expression and degradation

Specific proteolysis in combination with timed synthesis of proteins leads to periodic fluctuations of cellular protein levels during bacterial development or cell proliferation. To provide a complete picture of how proteolysis contributes to the periodic events of cell proliferation in bacteria we have established a comprehensive catalog of proteins that are subject to timed synthesis and destruction during the *C. crescentus* cell cycle. By combining 2-D gel electrophoresis, sophisticated statistical analysis, and protein identification by mass spectrometry we found that over 15% of the *C. crescentus* proteins were differentially expressed during the cell cycle and about 5% of all proteins were rapidly degraded. Surprisingly, direct comparison of these two data sets revealed that more than half of the unstable proteins are also expressed in a cell cycle-dependent manner, implying that protein degradation plays a critical role in cell cycle control and that one of the main purposes of specific protein degradation is to ensure periodicity of a cell's replicative processes. The analysis of protease deficient mutants by 2-D gels and peptide mass fingerprinting is now used to unravel the identity of these proteins.

Role of the Clp protease in *C. crescentus* development and cell cycle control

The Clp protease, a structural homolog of the eucaryotic proteasome, consists of a barrel-like peptidase subunit, ClpP, and interchangeable ATPase subunits, which act in concert with ClpP and confer substrate specificity to the peptidase. The two known ATPases, ClpA and ClpX, have distinct substrate specificities and can either act independently as chaperones or, through unfolding of the protease substrates, can function as entrance guards of the ClpP peptidase cavity. Each *Caulobacter* cell contains about 1600 ClpP, 800 ClpX, and 300 ClpA oligomers. Since both chaperones compete for the available peptidase complexes, an exact control of the component stoichiometry is critical for the specific functioning of the ClpAP and ClpXP proteases. This is illustrated by the observation that overexpression of *clpX* reduces ClpAP, and overexpression of *clpA* reduces ClpXP activity, respectively. Cellular ClpX levels are subject to a sophisticated multi-level control. When *clpX* is overexpressed, ClpX levels are negatively controlled by a decrease of ClpX protein stability and by a ClpA- or ClpP-dependent repression of ClpX synthesis. While the exact nature of both control mechanisms remains to be elucidated this

suggested that the components of the Clp protease itself contribute to the maintenance of the correct complex stoichiometry.

We have found that in *C. crescentus* ClpX is engaged in cell cycle control while ClpA is recruited for cell differentiation. Both the *clpP* and *clpX* genes are required for growth and viability of *C. crescentus*. Studies with conditional *clpP* and *clpX* mutants revealed that the ClpXP protease is required for cell cycle progression and for the cell cycle-dependent degradation of CtrA, a master cell cycle regulator, which negatively controls replication initiation in *C. crescentus.* Consistent with the idea that ClpXP is required for replication control in *C. crescentus*, we found that depletion of ClpX blocked replication initiation while *clpX* overexpression lead to the accumulation of multiple chromosomes per cell. We are currently addressing the following questions: How is ClpXP activity temporally controlled during the *C. crescentus* cell cycle? And what are the ClpXP substrates that could explain the essential nature of the protease? To identify additional ClpXP substrates, protein stability was compared between wild-type and ClpX-depleted cells by pulse/chase and separation of labeled proteins by 2-D gel electrophoresis. In addition to CtrA, eight new protein spots were stabilized in the absence of ClpX, indicating that they represent substrates of the ClpXP protease. Experiments to verify this and to analyze the role of these proteins are under way.

Specific protein degradation is also involved in *Caulobacter* cell differentiation. Flagellar ejection during the swarmer cell differentiation is triggered by the specific degradation of the FliF flagellar anchor protein. To understand how FliF degradation is controlled during development we have identified and analyzed several components required for this event, including the protease responsible for FliF turnover, a degradation signal at the FliF C-terminus, and components of a signal transduction pathway involved in FliF degradation. *clpA* mutant strains are viable, but unable to degrade FliF during the cell cycle. This indicated that the membrane-integral FliF protein is degraded by the soluble cytoplasmic ClpAP protease. ClpA-dependent turnover required a defined number of hydrophobic amino acids at the very FliF C-terminus, which is exposed to the cytoplasm. We found that the number of hydrophobic amino acids determines the stability and protease specificity of FliF: While short stretches (2 - 5) of hydrophobic residues were sufficient to target FliF for cell cycle-dependent degradation by the ClpAP protease, the addition of longer hydrophobic tails tagged FliF for constitutive degradation by a yet unknown protease. Finally, we have identified a component of a signal transduction pathway involved in the control of FliF degradation: PleD is a novel type response regulator, the activity of which is modulated by two polar sensor kinases, DivJ and PleC. Genetic evidence suggested that PleD~P blocks flagellar rotation and is required for FliF degradation, flagellar loss, and stalk biogenesis. No function has so far been assigned to the PleD signaling domain, which is widely conserved in the bacterial kingdom. Recent evidence indicated that members of this domain family could be involved in the synthesis or breakdown of a secondary signal, cyclic-diGMP and predicted that they are functionally interchangeable. We have replaced the output domain of PleD with the corresponding domain of a PleD ortholog from *Pseudomonas fluorescence*, WspR, and generated a fully functional hybrid protein. Future work will concentrate on exploring a possible link between PleD function and cyclic-diGMP, and on how such a secondary signal might interfere with cell differentiation and Clp-dependent proteolysis.

CicA, a pioneer protein involved in *Caulobacter* morphogenesis

The careful analysis of the *clpP-clpX* intergenic region revealed that it contains a single gene, *cicA*, which codes for a novel bacterial morphogen with homology to a large family of phosphohydrolases. The *cicA* gene is essential for *C. crescentus* growth and, when overexpressed, leads to rapid morphological changes and eventually to cell lysis. Conserved aspartic acid residues, which had been proposed to participate in phosphotransfer reactions, are essential for both the function of CicA and for the high copy number toxicity effect. Cellular fractionation and immunolocalization experiments have provided strong indication that the CicA protein is located outside of the cell and is associated with the outer membrane. To genetically define the role of CicA we have isolated suppressor mutants that tolerate unphysiologically high CicA levels. Two spontaneous mutations that confer resistance to increased CicA levels have been mapped by transposon linkage and generalized transduction and are now being characterized in more detail.

Evolution of development and bacterial senescence

Two of the most fundamental questions of the *C. crescentus* life cycle and of developmental biology in general are: How is cellular asymmetry generated and what is the nature of the biological clock that determines the timing of cell differentiation? To address these questions and to unravel the genetic basis of *C. crescentus* cell differentiation, developmental mutants were isolated under conditions that released the selection pressure for asymmetry. Independent cultures were evolved in logarithmic growth for over 7000 generations. During this time mutants with faster growth could invade, resulting in a more than twofold fitness increase in all cultures. The steepest fitness

increase occurred during the first 2000 generations and was shown to be due to a dramatic shortening of the motile swarmer phase of the cell cycle indicating that the mutants had a defect in the proper timing of cell differentiation. Between generation 2000 and 7000 several mutants with pleiotropic developmental defects appeared, all of which had lost different aspects of cellular asymmetry, e.g. stalk biogenesis, polar pili formation, holdfast production or motility. The corresponding mutations are now being mapped and the analysis of the mutated genes will then provide insight into the temporal and spatial regulatory mechanisms employed by *C. crescentus* development.

We have also used *Caulobacter* to ask if bacteria are senescent and to test the hypothesis that replicative senescence could have evolved solely based on cellular asymmetry. We have established an experimental system that allows us to follow single *Caulobacter* stalked cells through the cell cycle and to monitor their reproductive performance and mortality over a large number of cell divisions. We found that *Caulobacter* stalked cells show a significant decline in growth rate and reproduction late in life. This is the first evidence for replicative aging in bacterial cells and provides the opportunity to investigate the molecular basis of aging in a simple unicellular organism. When mutants were analyzed for their senescence behavior which had been selected for increased fitness, we found that the reproductive output of one mutant line declined much earlier than in wild-type cells, indicating that, in this case, improved performance early in live has a negative effect on performance late in life.

Publications

Jenal, U. (2000). Signal transduction mechanisms in *Caulobacter crescentus* development and cell cycle control. *FEMS Microbiol Rev* 24, 177-191.

Osteras, M. & Jenal, U. (2000). Regulatory circuits in *Caulobacter*. *Curr Opin Microbiol* 3, 171-176.

Fuchs, T., Wiget, P., Osteras, M. & Jenal, U. (2001). Precise amounts of a novel member of a phosphotransferase superfamily are essential for growth and normal morphology in *Caulobacter crescentus*. *Mol Microbiol* 39, 679-692.

Grünenfelder, B., Rummel, G., Vohradsky, J., Röder, D., Langen, H. & Jenal, U. (2001). Proteomic analysis of the bacterial cell cycle. *Proc Natl Acad Sci U S A* 98, 4681-4686.

Fischer, B., Rummel, G., Aldridge, P. & Jenal, U. (*in press*). The FtsH protease is involved in development, stress response, and heat shock control in *Caulobacter crescentus*. *Mol Microbiol* 43.

Potocka, I., Thein, M., Edwards, L. C., Osteras, M., Jenal, U. & Alley, M. R. K. (*in press*). The degradation of a *Caulobacter* soluble cytoplasmic chemoreceptor is ClpX-dependent. *J Bacteriol*.

Genomics as information basis for investigating dynamics of growth and nuclear migration in fungi

Ch. Alberti-Segui, N.Alijoski, Y. Bauer, M. Bloch, F. Dietrich, H. Helfer, D. Hoepfner, K. Hungerbühler, A. Kaufmann, Ph. Knechtle, Ph. Laissue, A. Lerch, S. Lemire-Brachat, Ph. Lüdi, R. Rischatsch, F. Schärer, H.P. Schmitz, S. Voegeli, K. Wojnowska

In collaboration with B. André (Univ. Libre de Bruxelles, Belgium), J. Boeke (Johns Hopkins Univ., Baltimore, USA), H. Bussey (McGill Univ., Montreal, Canada), R. Davis (Stanford Univ., USA), R. Fischer (University of Marburg, Germany), F. Foury (Univ. de Louvain, Belgium), S. Friend (Rosetta Inpharmatics, Seattle, USA), T. Gaffney (Syngenta, Research Triangle Park, USA), J. Hegemann (Univ. Düsseldorf, Germany), E. Hurt (University of Heidelberg, Germany), M. Johnston (Washington Univ., St. Louis, USA), S. Kelly (Univ. of Wales, Aberystwyth, UK), P. Koetter (Univ. Frankfurt, Germany), B. Scherens (CERIA, Inst. de Recherches, Bruxelles, Belgium), J. Revuelta (Univ. de Salamanca, Spain), M. Snyder (Yale Univ., New Haven, USA), H. Tabak (University of Amsterdam, NL), G. Valle (Univ. di Padova, Italy), G. Volckaert (Katholieke Univ., Leuven, Belgium), R. Wing (Clemson Univ., USA)

Peter Philippsen
Phone: +41 61 267 1480 Fax: +41 61 267 1481 E-mail: Peter.Philippsen@unibas.ch
http://www.biozentrum.unibas.ch/philippsen

Introduction

Over the previous years our research was significantly influenced by the genomic sequencing projects of two eukaryotic microorganisms, the unicellular fungus *Saccharomyces cerevisiae* and the filamentous fungus *Ashbya gossypii*. We use this novel knowledge for diverse types of gene targeting and follow-up experiments with the aim to study two cellular processes: Dynamics of nuclei in *S. cerevisia*e and in *A. gossypii* and, as a new project, the control of hyphal growth in *A. gossypii*. One final aim is to understand coordination between growth and nuclear dynamics in this fungus.

Our interest in *Ashbya gossypii* originates from several interesting features of this organism. It belongs to the important group of filamentous Ascomycetes and grows as multinucleated mycelium. It has been described as a plant pathogen and it is able to overproduce riboflavin. Our analysis of the *A. gossypii* genome reveals that it consists of only $9x10^6$ base pairs, the smallest genome of a free-living eukaryote, coding for 4700 proteins (25% less than *S. cerevisiae*). Gene manipulations in this fungus by homologous recombination are straight forward allowing for example efficient PCR-based gene targeting including fusions to fluorescence markers like GFP.

Genomics as information basis for investigating growth and proliferation in fungi

With the completion of the DNA sequence of the *S. cerevisia*e genome in 1996 it became apparent that 40% of the 6‘200 predicted genes code for proteins of unknown function. In 1996 several European labs which had participated in the yeast genome sequence project initiated a functional analysis network (EUROFAN) with the aim to find the cellular roles of novel proteins. We participated in both phases of this EU program in order to find novel components involved in nuclear migration and division (see also previous reports). In addition, we coordinated during the second phase of EUROFAN the European activities of a transatlantic consortium which generated bar-coded deletions for all annotated *S. cerevisia*e open reading frames. This unique deletion collection is now used in academia and industry for functional profiling of the *S. cerevisia*e genome. It allows to apply an almost unlimited number of growth, stress, or selection conditions to liquid cultures each carrying the mixture of all gene deletions. The best growing and the non-growing deletions are detected with the help of the bar codes (two unique 20-mer sequences associated with each deletion). Fluorescently labeled copies of all bar codes present in the culture at the end of the experiment are prepared by PCR and hybridized to commercially available DNA chips carrying

complementary sequences to all bar codes.

Our group initiated in 1995 a genome project with the filamentous fungus *A. gossypii*. In 1997 we gained support from Novartis Agro (now Syngenta) which allowed us to complete over 90% of the genome analysis by the end of 2001. Key results are the almost complete map of close to 4700 ORFs which surprisingly shows extensive synteny to the gene order in *S. cerevisia*e and the apparent lack of gene duplications (with very few exceptions). The data are a rich source for novel approaches to analyze the dynamics of fungal growth and its molecular control.

Genes controlling nuclear migration in *S. cerevisiae*

S. cerevisiae is a budding yeast and nuclear migration comprises two major steps during the mitotic cycle. During the first step the nucleus moves from an apparently random position in the mother cell to a site close to the bud neck. The insertion of the dividing nucleus into the daughter cell during anaphase marks the second step. Nuclear migration and positioning of the mitotic spindle relative to the mother-daughter axis are dependent on the dynamic action of cytoplasmic microtubules. Other proteins required for correct migration and segregation of nuclei include microtubule-based motor proteins as well as putative components of the dynactin complex and the actin cytoskeleton.

The spindle pole body (SPB) of *S. cerevisia*e is the functional homolog of the mammalian centrosome, responsible for the organization of the tubulin cytoskeleton. Cytoplasmic (astral) microtubules essential for the proper segregation of the nucleus into the daughter cell are attached at the outer plaque on the SPB cytoplasmic face. Previously, we had shown that Cnm67p is an integral component of this structure; cells deleted for *CNM67* are lacking the SPB outer plaque and thus experience severe nuclear migration defects. With the use of partial deletion mutants of *CNM67*, we show that the N-and C-terminal domains of the protein are important for nuclear migration. The C terminus, not the N terminus, is essential for Cnm67p localization to the SPB. On the other hand, only the N terminus is subject to protein phosphorylation of yet unknown function. Electron microscopy of SPB serial thin sections reveals that deletion of the N- or C-terminal domains disturbs outer plaque formation, whereas mutations in the central coiled-coil domain of Cnm67p change the distance between the SPB core and the outer plaque. We conclude that Cnm67p is the protein that connects the outer plaque to the central plaque embedded in the nuclear envelope, adjusting the space between them by the length of its coiled-coil.

We also investigated the role of Spc72, the receptor of the cytoplasmic γ-tubulin complex. By using in-vivo fluorescence microscopy, we showed that cells lacking Spc72 can only generate very short (<1 μm) and unstable astral microtubules. Consequently, nuclear migration to the bud neck and orientation of the anaphase spindle along the mother-bud axis are absent in these cells. However, *SPC72* deletion is not lethal because elongated but misaligned spindles can frequently reorient in mother cells permitting delayed but otherwise correct nuclear segregation. High-resolution time-lapse sequences revealed that this spindle reorientation was most likely accomplished by cortex interactions of the very short astral microtubules. In addition, a set of double mutants suggested that reorientation was dependent on the SPB outer plaque and the astral microtubule motor function of Kar3 but not Kip2/Kip3/Dhc1, or the cortex components Kar9/Num1. Our observations suggest that Spc72 is required for astral microtubule formation at the SPB half-bridge and for stabilization of astral microtubules at the SPB outer plaque. In addition, our data excludes involvement of Spc72 in spindle formation and elongation functions.

Motor proteins involved in nuclear dynamics of *A. gossypii*

In order to follow nuclear movement within the hyphae during fungal growths an in-frame GFP fusion to the histone H4 has been carried out which resulted in a strong fluorescent labeling of nuclei. Video fluorescence microscopy and time-lapse studies revealed an active traffic of nuclei within the hyphae, including continuous oscillations, frequent mitotic events, bypassing of nuclei and movement through septa. This dynamic behavior results in a uniform distribution of nuclei along the hyphae. To better characterize nuclear movement, we also measured some dynamic parameters. Under time-lapse conditions (minimal medium, 24°C), we estimated the nuclear velocity to be 0.3 mm per minute which was similar to the hyphal tip extension, whereas oscillation velocity could reach 4 mm per minute.

We identified seven homologs to *S. cerevisia*e genes coding for molecular motors which interact with microtubules: Six kinesin-related proteins, *KRP1-6*, and one dynein heavy chain, *DHC1*. One-step gene targeting has been used to delete the corresponding genes in *A. gossypii*. Deletion of three of the kinesin-related proteins (*KRP1*, *KRP2* and *KRP4*) did not affect to an observable degree nuclear distribution or migration. Deletion of *KRP3* lead to a lower density and more random distribution of nuclei in the hyphae and to a prolonged time for mitosis. Analysis of deletions of *KRP5* revealed a role in nuclear oscillation. Analysis of *KRP6* is still in progress. We observed the most severe defect in nuclear migration when the complete dynein

heavy chain gene had been removed. In contrast to dynein mutants of *Aspergillus nidulans* where nuclei fail to move into the germ tube, we observed in the *DHC1* deletion of *A. gossypii* clumping of nuclei at the tip of hyphae. For the future we plan to study the deletion mutants of kinesin motors in hyphae with GFP-labeled spindle pole bodies and GFP-labeled organelles.

Functional similarities and differences between homologous growth control genes of *A. gossypii* and *S. cerevisiae*

In the filamentous ascomycete *Ashbya gossypii*, like in other filamentous fungi, onset of growth in dormant spores occurs as an isotropic growth phase generating spherical germ cells. Thereafter, a switch to polarized growth results in the formation of the first hyphal tube (monopolar germling). The initial steps of hyphal tube formation in filamentous fungi, therefore, resemble processes taking place prior to and during bud emergence of unicellular yeast-like fungi. In contrast to yeast species, where growth of the tip of an emerging bud is temporally limited, filamentous fungi exhibit sustained polarized growth of the hyphal tip. This, together with frequent lateral branching, generates the typical network of hyphae (mycelium).

The genome sequence of *A. gossypii* revealed homologs to all *S. cerevisiae* genes involved in polar growth despite substantial differences in the growth modes of these two organisms. Therefore it was very intriguing to analyze the function of the *Ashbya* homologs with respect to filamentous growth control.

Polarized cell growth requires a polarized organization of the actin cytoskeleton. Small GTP-binding proteins of the Rho-family have been shown to be involved in the regulation of actin polarization. Since a potential role of Rho-proteins has not been studied so far in filamentous fungi we isolated and characterized the *Ashbya gossypii* homologs of the *Saccharomyces cerevisiae CDC42*, *CDC24*, *RHO1*, and *RHO3* genes. The *AgCDC42* and *AgCDC24* genes can both complement conditional mutations in the *S. cerevisiae CDC42* and *CDC24* genes and both proteins are required for the establishment of actin polarization in *A. gossypii* germ cells. *Agrho1* mutants show a cell lysis phenotype. Null mutant strains of *Agrho3* show periodic swelling of hyphal tips that is overcome by repolarization and polar hyphal growth in a manner resembling the germination pattern of spores. Thus different Rho-protein modules are required for distinct steps during polarized hyphal growth of *A. gossypii*.

Next we characterized the *A. gossypii* homolog of the *S. cerevisiae BEM2* gene which is part of a network of Rho-GTPases and their regulators. ScBem2 is required for bud emergence and bud growth in yeast. We showed that the AgBem2 protein contains a GAP-(GTPase activating protein) domain for Rho-like GTPases at its carboxy terminus, and that this part of AgBem2p is required for complementation of an *Agbem2* null strain. Germination of spores resulted in enlarged *Agbem2* germ cells that were unable to generate the bipolar branching pattern found in wild-type germ cells. In addition, mutant hyphae were swollen due to defects in polarized cell growth indicated by the delocalized distribution of chitin and cortical actin patches in swollen hyphal tips. Surprisingly, the complete loss of cell polarity which leads to spherical hyphal tips was often overcome by the establishment of new cell polarities and the formation of multiple new hyphal tips. These results demonstrate that establishment of cell polarity, maintenance of cell polarity, and polarized hyphal growth in filamentous fungi require similar proteins as in *S. cerevisiae*.

We were also able to identify for the first time a fungal gene important for hyphal maturation. This novel *A. gossypii* gene encodes a presumptive PAK (p21-activated kinase)-like kinase. Its closest homolog is the *S. cerevisiae* Cla4 protein kinase; the *A. gossypii* protein is therefore called AgCla4p. *Agcla4* deletion strains are no longer able to perform the developmental switch from young to mature hyphae, and GFP (green fluorescent protein)-tagged AgCla4p localizes with much higher frequency in mature hyphal tips than in young hyphal tips. Both results support the importance of AgCla4p in hyphal maturation. AgCla4p is also required for septation, indicated by the inability of *Agcla4* deletion strains to properly form actin rings and chitin rings. Despite the requirement of AgCla4p for the development of fast-growing hyphae, AgCla4p is not necessary for actin polarization per se, because tips enriched in cortical patches and hyphae with a fully developed network of actin cables can be seen in *Agcla4* deletion strains.

The function of the *Ashbya BUD1/RSR1* and *BUD2* homolog were investigated by using time-lapse video microscopy. These movies revealed that *AgBUD2* is important for a stable growth axis of the elongating hyphae; *Ashbya* hyphae lacking this gene frequently change direction of growth. *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. Pausing of tip growth and frequent failures in lateral branch growth coincided with the loss of green fluorescence at tips and at the basis of branches in strains carrying a polarisome-GFP marker in addition to the *rsr1/bud1* deletion.

These first data on hyphal growth control allow the conclusion that homologous proteins of *S. cerevisia*e and *A. gossypii* like Cla4, Rsr1/Bud1 and Bud2 serve different functions in the cellular environments of both organisms.

Effects of myosin gene deletions on growth and development of *A. gossypii*

Three myosin genes, *AgMYO1*, *AgMYO2* and *AgMYO3* were identified in the genome of the filamentous fungus *Ashbya gossypii*. These encode type II, type V, type I myosins, respectively. Thus, *Ashbya* is the eukaryotic organism with the smallest number of myosins found so far. In order to learn whether these myosins fulfill similar functions in *A. gossypii* as their homologs in *S. cerevisia*e we performed a phenotypic analysis of deletion strains.

Agmyo1 null mutants grew at rates close to those of wild type. Hyphal morphology was unaltered. However, actin rings were absent, but septa still formed. Sporulation in this mutant was severely decreased. *Agmyo2* deletion mutants were non-viable. Although the knock out is lethal, spores still geminated and often grew to a stage of deformed germlings before growth arrested or lysis occurred. Enlarged forms of actin rings, thick actin ribbons as well as single cables, could be observed. *Agmyo3* deletion mutants showed severely reduced growth rates. This is most likely due to an impaired organization of the actin cytoskeleton, manifested in faint or entirely missing actin caps and heterogeneous patch size. In addition, this deletion did not sporulate. These data show that homologous myosins perform similar roles in *A. gossypii* and *S. cerevisiae*.

Publications

Ayad-Durieux, Y., Knechtle, P., Goff, S., Dietrich, F. & Philippsen, P. (2000). A PAK-like protein kinase is required for maturation of young hyphae and septation in the filamentous ascomycete Ashbya gossypii. *J Cell Sci* 113 Pt 24, 4563-75.

Brachat, A., Liebundguth, N., Rebischung, C., Lemire, S., Schärer, F., Hoepfner, D., Demchyshyn, V., Howald, I., Düsterhöft, A., Möstl, D., Pöhlmann, R., Kotter, P., Hall, M. N., Wach, A. & Philippsen, P. (2000). Analysis of deletion phenotypes and GFP fusions of 21 novel Saccharomyces cerevisiae open reading frames. *Yeast* 16, 241-53.

Hoepfner, D., Brachat, A. & Philippsen, P. (2000). Time-lapse video microscopy analysis reveals astral microtubule detachment in the yeast spindle pole mutant cnm67. *Mol Biol Cell* 11, 1197-211.

Wendland, J., Ayad-Durieux, Y., Knechtle, P., Rebischung, C. & Philippsen, P. (2000). PCR-based gene targeting in the filamentous fungus Ashbya gossypii. *Gene* 242, 381-91.

Wendland, J. & Philippsen, P. (2000). Determination of cell polarity in germinated spores and hyphal tips of the filamentous ascomycete Ashbya gossypii requires a rhoGAP homolog. *J Cell Sci* 113 (Pt 9), 1611-21.

Alberti-Segui, C., Dietrich, F., Altmann-Jöhl, R., Hoepfner, D. & Philippsen, P. (2001). Cytoplasmic dynein is required to oppose the force that moves nuclei towards the hyphal tip in the filamentous ascomycete Ashbya gossypii. *J Cell Sci* 114, 975-86.

Gadal, O., Strauss, D., Braspenning, J., Hoepfner, D., Petfalski, E., Philippsen, P., Tollervey, D. & Hurt, E. (2001). A nuclear AAA-type ATPase (Rix7p) is required for biogenesis and nuclear export of 60S ribosomal subunits. *EMBO J* 20, 3695-704.

Hoepfner, D., Schaerer, F., Brachat, A., Wach, A., and Philippsen, P. (2001). Reorientation of mispositioned spindles in the short astral microtubule mutant spc72Δ is dependent on the spindle pole body outer plaque and the Kar3 motor protein. *Mol Biol Cell* 13, 1366-1380.

Hoepfner, D., van den Berg, M., Philippsen, P., Tabak, H. F. & Hettema, E. H. (2001). A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in Saccharomyces cerevisiae. *J Cell Biol* 155, 979-90.

Requena, N., Alberti-Segui, C., Winzenburg, E., Horn, C., Schliwa, M., Philippsen, P., Liese, R. & Fischer, R. (2001). Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in Aspergillus nidulans. *Mol Microbiol* 42, 121-32.

Schaerer, F., Morgan, G., Winey, M. & Philippsen, P. (2001). Cnm67p is a spacer protein of the Saccharomyces cerevisiae spindle pole body outer plaque. *Mol Biol Cell* 12, 2519-33.

Wendland, J. & Philippsen, P. (2001). Cell polarity and hyphal morphogenesis are controlled by multiple rho-protein modules in the filamentous ascomycete Ashbya gossypii. *Genetics* 157, 601-10.

Wendland, J. & Philippsen, P. (*in press*). An IQGAP-related protein, encoded by AgCYK1, is required for septation in the filamentous fungus Ashbya gossypii. *Fungal Genetics and Biology*.

Giaever, G., Chu, A., Ni, L., Connelly, C., Riles, L., Bussey, H., Boeke, J., Snyder, M., Philippsen, P., Davis, R.W & Johnston, M. (*in press*). Functional Profiling of the *S. cerevisiae* Genome.

DIVISION OF BIOCHEMISTRY

The major research interests of the Division of Biochemistry are protein transport and signal transduction. The Department comprises three senior groups headed by Michael Hall, Howard Riezman, and Martin Spiess, one junior group headed by Anastasia (Natasha) Kralli, and a Protein Chemistry Service Group headed by Paul Jenö.

The Hall group studies signal transduction and growth control in yeast. The Riezman group studies membrane traffic in yeast. The Spiess group studies the asialoglycoprotein receptor and intracellular sorting of membrane proteins in mammalian cells. The Kralli group studies steroid hormone receptor activity in yeast and mammalian cells. The Protein Chemistry Service Group contributes to these studies and to the research efforts of others in the Biozentrum, and is developing microanalytical strategies for mass spectrometric determination of protein structure.

The research activities of each group are described in more detail below.

Professors

Michael N. Hall, Howard Riezman, Martin Spiess

University lecturer

Martin Horst*

Independent group leader

Anastasia Kralli

Scientists and postdoctoral fellows

Petek Cakar*, Christian Chatenay-Rivauday*, José Luis Crespo Gonzalez, Pascal Crottet, Pierre-Alain Delley*, Sylvane Desrivières*, Alex Garvin*, Kathleen D'Hondt*, Eric Dumermuth*,Klaus Fiedler*, Steffen Fredersdorf*, Sylvie Friant*, Koichi Funato, Veit Goder, Antje Heese-Peck*, Estela Jacinto, Elena Kouzmenko*, Dieter Kressler, Vivienne Laird*, Robbie Loewith*, Ruben Lombardi, Anja Lorberg*, Dietmar Martin*, Pierre Morsomme, Manuel Muñiz*, Adriana Pagano*, Harald Pichler*, Anja Renold*, Jonas Rutishauser*, Claudia Schärer-Brodbeck*, Tobias Schmelzle, Sabrina Sonda*, Beatrice Vallée, Reika Watanabe*, Andreas Wiederkehr*

Graduate students

Deborah Bonenfant, Cleiton De Souza*, Roger Emter, Michael Friberg*, Mary Higy*, Shashka Hristoskova*, Darko Knutti, Karsten Meier, Daniel Meyer, Helena Morales-Johansson, Debdyuti Mukhopadhyay*, Sylvia Schreiber, Stephan Wullschleger*

Diploma students

Marino Bertapelle*, Roky Bamert*, Kathrin Brogli*, Philipp Ernst*, Michael Jeske*, Szimon Kobialka*, Jan Massner*, Anja Meury*, Karin Rösch*, Michael Schneider*, Thomas Uhlmann*,

Visiting scientists

Asami Makino (1 week), Takashi Ushimaru (1 year/ 1 month/ 1 month), Michael Veit (2 weeks/ 2 weeks), Freie Universität Berlin, Berlin (Sokrates Programm)

Technical personnel

Thomas Aust*, Cristina Baschong, Nicole Beuret, Helena Bühler-Nurmi*, Luisita Dolfini*, Jeannette Holenstein*, Tina Junne, Véronique Koerin*, Andrea Löschmann, Thierri Mini, Wolfgang Oppliger, Joachim Scherf*, Martine Schwager*, Bettina Zanolari

Floor manager

Werner Metzger*

David Schütz*

Secretariat

Liselotte König*, Monika Lulik*, Verena Zellweger

Laboratory helpers

Elisabeth Hänggi, Isabelle Lanz*

* Employed during part of the report period

Signal transduction and control of cell growth

M. Bertapelle, J.-L. Crespo Gonzalez, P.-A. Delley, S. Desrivières, P. Ernst, S. Fredersdorf, E. Jacinto, R. Loewith, A. Lorberg, A. Löschmann, D. Martin, H. Morales, W. Oppliger, T. Schmelzle, S. Wullschleger

Visiting scientist: T. Ushimaru (University of Shizuoka, Japan)
In collaboration with K. Arndt (Wyeth-Ayerst Research, New York), B. Fowler (University Children's Hospital, Basel), S. Grzesiek (Biozentrum, Basel), P. Jenö (Biozentrum, Basel), P. Parker (ICRF, London), T. Powers (University of California, Davis), H. Riezman (Biozentrum, Basel), M. Rüegg (Biozentrum, Basel), D. Sabatini (Whitehead Institute, Cambridge), T. Ushimaru (University of Shizuoka, Japan)

Michael N. Hall
Tel: +41 61 267 2150/2151 Fax: +41 61 267 2149 E-mail: M.Hall@unibas.ch
http://www.biozentrum.unibas.ch/hall

Cell growth is a highly regulated process subject to temporal and spatial constraints. Cell growth (increase in cell mass or size) is usually coupled with cell division (increase in cell number) to give rise to an organ or organism of a characteristic size. In other cases, such as muscle hypertrophy in response to an increased workload or synapse strengthening during memory storage, cell growth and cell division are unlinked. Defects in cell growth cause a broad spectrum of diseases, ranging from cancer to depression. We are studying the signalling pathways that control cell growth, employing biochemical and genetic approaches with the yeast *Saccharomyces cerevisiae*. Within the last years, we have discovered novel signalling pathways involving TOR1 and TOR2. The TORs are the targets of the immunosuppressive and anti-cancer drug rapamycin, and the founding members of a family of phosphatidylinositol kinase-related protein kinases, first discovered by us in yeast and now found by others in humans, rats, flies, worms, and plants. The TOR signalling pathways, in response to nutrients, control an unusually large and diverse set of readouts (e.g., protein synthesis and degradation, transcription, ribosome and tRNA biogenesis, membrane traffic, autophagy, and organization of the actin cytoskeleton) all of which are important for cell growth, suggesting that the TORs play a central role in controlling and integrating cell growth. Our current work continues to focus on identifying and characterizing the upstream regulators and downstream effectors of TOR. Our findings may lead to novel therapeutic approaches in the treatment of, for example, cancer, muscle atrophy, and nerve damage.

The TOR signalling pathways

To study signal transduction pathways that control cell growth in yeast, we exploited the anti-fungal properties of rapamycin. Rapamycin-resistant yeast mutants were selected, and mutants altered in the two novel genes *TOR1* and *TOR2* (target of rapamycin) were obtained. The extremely large and highly homologous TOR1 and TOR2 proteins are structurally related to phosphatidylinositol kinases, and have become the founding members of a new class of signalling molecules that includes a mammalian TOR, the mammalian ataxia telangiectasia gene product (ATM), the DNA-dependent protein kinase (DNA-PK) and the yeast cell cycle checkpoint functions MEC1, TEL1, and RAD3.

TOR2 has two essential signalling functions. One function is shared with TOR1 and is required for signalling activation of translation initiation and thereby early G1 progression in response to nutrients. TOR may control eIF4E-dependent translation by activating a type 2A phosphatase inhibitor termed TAP42. The mammalian counterpart of this TOR signal transduction pathway appears to be a general mitogenic signalling pathway involving, in addition to mTOR, a tyrosine kinase (receptor or nonreceptor), the p110-p85 PI 3-kinase, PDK1, Akt (PKB) kinase, p70 S6 kinase ($p70^{S6k}$), and 4E-BP1 (PHAS-I). The mTOR signalling pathway activates eIF4E-dependent translation by causing the phosphorylation and inactivation of the eIF4E repressor 4E-BP1. The second TOR2 signalling function, which TOR1 is unable to perform, mediates the cell cycle-dependent organization of the actin cytoskeleton. The actin cytoskeleton is polarized in late G1 toward a future growth site, thereby orienting the secretory machinery toward this site. TOR2 signals to the actin cytoskeleton by activating a Rho-type GTPase switch consisting of the GTPases RHO1 and RHO2, the GDP/GTP exchange factor ROM2, and the GTPase activating protein SAC7. TOR2 activates this switch through ROM2. Activated RHO in turn signals

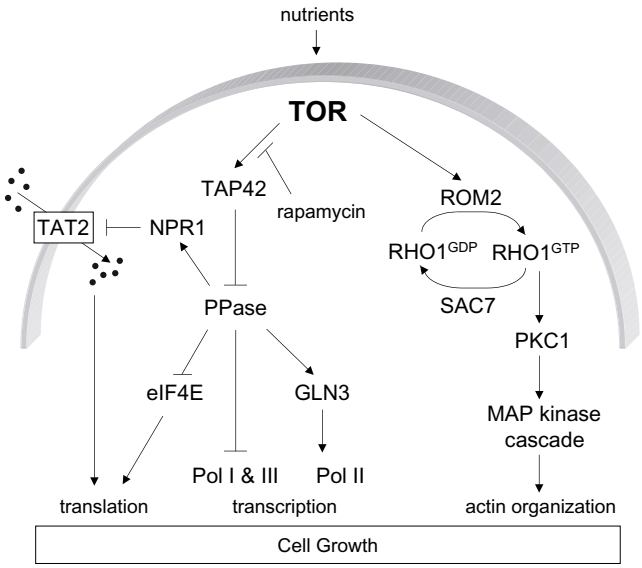


Figure 1: TOR signalling pathways integrate temporal and spatial control of cell growth.

to the actin cytoskeleton through the direct effector PKC1 and a downstream MAP kinase cascade. Thus, via two signalling pathways, TOR2 integrates temporal and spatial control of cell growth.

In addition to activating a cell growth program, as described above, the TOR nutrient signalling pathway also controls growth by inhibiting a stationary phase (G0) program. Loss of TOR or rapamycin treatment causes yeast cells to arrest growth in early G1 (G0) and to express several other physiological properties of starved cells. As part of this starvation response, high affinity amino acid permeases such as the tryptophan permease TAT2 are ubiquitinated, targeted to the vacuole, and degraded. The TOR pathway prevents the turnover of TAT2 in the presence of nutrients by phosphorylating and inactivating the Ser/Thr kinase NPR1. Thus, TOR negatively regulates the stationary phase program by turning off NPR1.

TOR also negatively regulates the stationary phase program by globally repressing starvation-specific transcription. TOR prevents transcription of genes expressed upon nitrogen limitation by promoting the association of the GATA transcription factor GLN3 with the cytoplasmic protein URE2. The binding of GLN3 to URE2 requires TOR-dependent phosphorylation of GLN3. Phosphorylation and cytoplasmic retention of GLN3 are also dependent on the TOR effector TAP42, and are antagonized by the type 2A-related phosphatase SIT4. TOR inhibits expression of carbon source-regulated genes by stimulating the binding of the transcriptional activators MSN2 and MSN4 to the cytoplasmic 14-3-3 protein BMH2. Thus, the TOR signalling pathway broadly controls nutrient metabolism by sequestering several transcription factors in the cytoplasm.

The inhibition of phosphatase activity appears to be a major mechanism of TOR signalling in both yeast and mammalian cells. In *Saccharomyces cerevisiae*, the rapamycin-sensitive TOR kinases negatively regulate the type 2A-related phosphatase SIT4 by promoting the association of this phosphatase with the inhibitor TAP42. TOR controls the interactions between SIT4 and TAP42 via TIP41, a conserved TAP42-interacting protein involved in the regulation of SIT4. Deletion of the *TIP41* gene confers rapamycin resistance, suppresses a *tap42* mutation, and prevents dissociation of SIT4 from TAP42. Furthermore, a *TIP41* deletion prevents SIT4-dependent events such as dephosphorylation of the kinase NPR1 and nuclear translocation of the transcription factor GLN3. Thus, TIP41 negatively regulates the TOR pathway by binding and inhibiting TAP42. The binding of TIP41 to TAP42 is stimulated upon rapamycin treatment via SIT4-dependent dephosphorylation of TIP41, suggesting that TIP41 is part of a feedback loop that rapidly amplifies SIT4 phosphatase activity under TOR-inactivating conditions.

The TOR kinases regulate a diverse set of cell growth-related readouts in response to nutrients. Thus, the yeast TOR proteins function as nutrient sensors, in particular as sensors of nitrogen and possibly carbon. However, the specific nitrogenous or carbon metabolites that act upstream of TOR are unknown. Glutamine, a preferred nitrogen source and a key intermediate in yeast nitrogen metabolism, is a possible regulator of TOR. The glutamine synthetase inhibitor L-methionine sulfoximine (MSX) specifically provokes glutamine depletion in yeast cells. MSX-induced glutamine starvation causes nuclear localization and activation of the TOR-inhibited transcription factors GLN3, RTG1, and RTG3, all of which mediate glutamine synthesis. The MSX-induced nuclear localization of GLN3 requires the TOR-controlled phosphatase SIT4. Other TOR-controlled transcription factors, GAT1/NIL1, MSN2, MSN4 and an unknown factor involved in the expression of ribosomal protein genes, are not affected by glutamine starvation. These findings suggest that TOR senses glutamine. Furthermore, as glutamine starvation affects only a subset of TOR-controlled transcription factors, TOR appears to discriminate between different nutrient conditions to elicit a response appropriate to a given condition.

TOR is peripherally associated with membranes. Subcellular fractionation and immunofluorescence studies indicate that TOR1 and TOR2 associate with the plasma membrane and a second compartment that is distinct from golgi, vacuoles, mitochondria and nucleus and may represent vesicular structures. Pulse-chase experiments show that association of TOR protein with plasma membrane and the second compartment is fast, does not involve components

of endocytic, secretory or golgi to vacuole transport pathways, and is not affected by rapamycin. Two domains within TOR2 independently mediate localization to both compartments. These domains are composed of HEAT repeats that are thought to act as protein-protein interaction surfaces. Thus, TOR proteins reside, possibly as part of a multi-protein complex, at the site of action of their known downstream effectors.

Many questions still remain concerning the TOR pathways. For example, how do the TOR kinases sense and respond to the availability of nutrients such as nitrogen and carbon? What are the direct regulators and substrates of TOR? How does TOR2 control the exchange factor ROM2? How is TOR2 different in performing its different signalling functions. What is the role of bona fide lipid kinases such as the phosphatidylinositol-4-phosphate 5-kinase MSS4 in TOR signalling? We are currently focusing on these and other questions related to the control of cell growth in yeast.

Publications

Beck, T. & Hall, M. N. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402, 689-692.

Beck, T., P.-A. Delley & Hall, M. N. (2000). Control of the actin cytoskeleton by extracellular signals. *Molecular Interactions of Actin*, eds. C. dos Remedios and D. Thomas. Heidelberg: Springer-Verlag Co. pp. 231-262.

Brachat, A., Liebundguth, N., Rebischung, C., Lemire, S., Scharer, F., Hoepfner, D., Demchyshyn, V., Howald, I., Dusterhoft, A., Mostl, D., Pohlmann, R., Kotter, P., Hall, M. N., Wach, A. & Philippsen, P. (2000). Analysis of deletion phenotypes and GFP fusions of 21 novel Saccharomyces cerevisiae open reading frames. *Yeast* 16, 241-53.

Cosentino, G. P., Schmelzle, T., Haghighat, A., Helliwell, S. B., Hall, M. N. & Sonenberg, N. (2000). Eap1p, a novel eukaryotic translation initiation factor 4E-associated protein in Saccharomyces cerevisiae. *Mol Cell Biol* 20, 4604-13.

Kunz, J., Loeschmann, A., Deuter-Reinhard, M. & Hall, M. N. (2000). FAP1, a homologue of human transcription factor NF-X1, competes with rapamycin for binding to FKBP12 in yeast. *Mol Microbiol* 37, 1480-93.

Kunz, J., Schneider, U., Howald, I., Schmidt, A. & Hall, M. N. (2000). HEAT repeats mediate plasma membrane localization of Tor2p in yeast. *J Biol Chem* 275, 37011-20.

Schmelzle, T. & Hall, M. N. (2000). TOR, a central controller of cell growth. *Cell* 103, 253-62.

Crespo, J. L., Daicho, K., Ushimaru, T. & Hall, M. N. (2001). The GATA transcription factors GLN3 and GAT1 link TOR to salt stress in Saccharomyces cerevisiae. *J Biol Chem* 276, 34441-4

Crespo, J.-L., T. Powers, B. Fowler & M. N. Hall. (*in press*). The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. *Proc. Natl. Acad. Sci. USA*.

Friant, S., Lombardi, R., Schmelzle, T., Hall, M. N. & Riezman, H. (2001). Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast. *EMBO J* 20, 6783-92.

Jacinto, E., Guo, B., Arndt, K. T., Schmelzle, T. & Hall, M. N. (2001). TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. *Mol Cell* 8, 1017-26.

Hall, M. N. & D. M. Sabatini. (*in press*). TOR and Control of Cell Growth. *Cahiers de la Fondation des Treilles*

Schmelzle, T., J.-L. Crespo & M. N. Hall. (*in press*). Translational control by amino acids and energy. *Handbook of Cellular Signaling, eds. R. Bradshaw and E. Dennis. San Diego: Academic Press.*

Schmelzle, T., S. B. Helliwell & M. N. Hall. (*in press*). YPK kinases and the RHO1 exchange factor TUS1 are novel components of the cell integrity pathway in yeast. *Mol. Cell. Biol.*

Membrane traffic in yeast

T. Aust, C. Prescianotto-Baschong, C. De Souza, K. D'Hondt, S. Friant, K. Funato, A. Heese-Peck, J. Holenstein, R. Lombardi, V. Koerin, J. Massner, K. Meier, A. Meury, P. Morsomme, D. Mukhopadhyay, M. Muñiz, H. Pichler, S. Sonda, C. Schärer-Brodbeck, B.Vallée, R.Watanabe, A.Wiederkehr B. Zanolari

In collaboration with T. Kinoshita (Osaka University, Japan), R. Schwarz (University of Marburg, Germany), H. Pelham (MRC, Cambridge, UK), S. Lemmon (Case Western Reserve University, Cleveland OH, USA), R. Haguenauer-Tsapis (Institut Jacques Monod, Paris, France), M. Peter (Institut Suisse de Recherches Expérimentales sur le Cancer, Epalinges), M. Geli (University of Heidelberg, Germany), A. Munn (Institute for Molecular Agrobiology, Singapore), D. Oesterhelt (Max Planck Institute, Martinsried, Germany)

Howard Riezman
Phone: +41 61 267 2160 Fax: +41 61 267 2149 E-mail : Howard.Riezman@unibas.ch
http://www.biozentrum.unibas.ch/riezman

We would like to understand the mechanisms of membrane lipid and protein synthesis, sorting, and transport through eucaryotic cells. For these studies we have chosen the yeast, *Saccharomyces cerevisiae*, where biochemical and genetic approaches to these problems are well established. Our work concentrates on three basic topics, synthesis and sorting of GPI-anchored proteins upon ER exit, endocytosis, ceramide synthesis and transport.

GPI anchored protein synthesis and sorting upon ER exit

GPI (glycosylphosphatidylinositol) is a glycolipid that is used to anchor proteins to membranes. GPI-anchored proteins are synthesized and segregated into the ER with an extra hydrophobic GPI attachment signal at their C-terminus which is recognized and cleaved by an enzyme that attaches the preformed GPI by a transamidation reaction. In a collaborative effort we have shown that enzymes involved in GPI synthesis, as well as the transamidase, are potential targets for chemotherapy against protozoal infections because this pathway is essential for viability of the blood stream form of *Trypansoma brucei*.

Previously we identified specific requirements for GPI-anchored protein transport to the Golgi apparatus in yeast. In order to test at which stage of transport they act, we have set up an *in vitro* assay that reconstitutes the transport of proteins from the ER to the Golgi apparatus. One of the requirements for the efficient transport of a particular GPI-anchored protein, Gas1p, to the Golgi is the protein Emp24p. This protein is a member of a family of proteins (8 members in yeast) that have been proposed to function in membrane trafficking. Several of the 8 yeast proteins can be found associated with each other in a protein complex and are functionally related. We could show that Emp24p is required for efficient packaging of Gas1p and another GPI-anchored proteins into vesicles that bud from the ER, without affecting other proteins. Emp24p and another subunit of the complex, Erv25p, could be specifically and directly cross-linked to Gas1p in ER-derived vesicles. From these studies we conclude that one function of the Emp24p complex is as a specific cargo concentrator in ER to Golgi transport.

Using an *in vitro* assay that reconstitutes a single round of budding from the endoplasmic reticulum, we found that GPI-anchored proteins and other secretory proteins exit the endoplasmic reticulum in distinct vesicles. Therefore, GPI-anchored proteins are sorted from other proteins, in particular other plasma membrane proteins, at an early stage of the secretory pathway. We have characterized cytosolic requirements for the sorting event. Surprisingly, proteins that have previously been identified as playing a role in the tethering of ER-derived vesicles to the Golgi apparatus are also involved in cargo protein sorting into vesicles. We also provided data that the same cargo sorting requirements are found *in vivo*. These studies open up great possibilities to study the mechanism of sorting of cargo proteins into distinct vesicle carriers from a single donor compartment.

Endocytosis

Endocytosis is the pathway whereby cells internalize portions of their cell surface to remove plasma membrane proteins in a regulated manner and to continuously sample the external environment.

Endocytosis is used by haploid yeast cells to down regulate mating pheromone receptors to ensure the continual capacity to detect a mating partner. We have visualized the pathway by electron microscopy following uptake of positively charged Nanogold™ with colocalization of specific protein markers of the endocytic pathway. These studies have identified and ordered distinct compartments in the pathway, first the early endosome, then the prevacuolar compartment and finally the late endosome, before arrival to the vacuole.

Our studies on the internalization step of endocytosis have revealed three interesting new principles concerning this process; the roles of ubiquitination, the actin cytoskeleton, and specific lipid requirements. We have shown that the α-factor receptor is modified by ubiquitin on specific lysines in its cytoplasmic tail upon addition of pheromone. Ubiquitination takes place at the cell surface prior to receptor endocytosis and depends upon prior receptor phosphorylation.

We have identified a series of proteins whose function is related to actin structure and which are required for endocytosis. We are currently exploring their interactions and their functions. End3p, contains an EH-domain and a putative phospatidylinositol bisphosphate binding site. We have shown that PIP_2-binding is regulated by occupancy of the EH domain. We have shown that the SH3 domain of the unconventional type I myosin stimulates actin polymerization *in vitro*. We have provided evidence that two other proteins, End6p and Rvs167p, which are essential for endocytosis and have homology to amphiphysin, function together *in vivo*. We have also identified a novel and functional interaction between the Arp2/3 complex and calmodulin.

Studies of two other *end* mutants led to the discovery that there are specific lipid requirements for the internalization step. End8p encodes a protein required for serine palmitoyltransferase activity, the first step of ceramide biosynthesis. Sphingoid bases are the relevant lipid and activate the *PKH1/2* kinases for their actin and endocytic functions. Our studies point to a role for sterols in receptor-mediated and fluid-phase endocytosis.

As part of a European Functional Analysis Network (EUROFAN) we have developed methods to systematically screen uncharacterized open reading frames for their functions in the endocytic pathway. This systematic approach has identified new genes involved in endocytosis as well as a protein required for the pathway used to recycle endocytic content.

Ceramide synthesis and transport

Ceramide is an important signaling molecule controlling stress responses and other crucial events. Many of the effects of ceramide can be correlated to new ceramide synthesis. Therefore, it is essential to understand how ceramide is made and how it is transported in the cell. We have identified two subunits of the ceramide synthase and are working on purification of the ceramide synthase complex. We have also developed assays to follow transport of ceramide from its site of synthesis in the ER to the Golgi apparatus *in vivo* and *in vitro*. Ceramide can be transported by both vesicular and non-vesicular mechanisms. The non-vesicular mechanism is cytosolic protein-dependent, but ATP-independent. Interestingly, transport is greatly stimulated by prior establishment of ER-Golgi membrane contacts. We are currently purifying the cytosolic factors required for the transport reaction.

Publications

D'Hondt, K., Heese-Peck, A. & Riezman, H. (2000). Protein and lipid requirements for endocytosis. *Annu Rev Genet* 34, 255-295.

Friant, S., Zanolari, B. & Riezman, H. (2000). Increased protein kinase or decreased PP2A activity bypasses sphingoid base requirement in endocytosis. *EMBO J* 19, 2834-44.

Gagny, B., Wiederkehr, A., Dumoulin, P., Winsor, B., Riezman, H. & Haguenauer-Tsapis, R. (2000). A novel EH domain protein of Saccharomyces cerevisiae, Ede1p, involved in endocytosis. *J Cell Sci* 113, 3309-19.

Geli, M. I., Lombardi, R., Schmelzl, B. & Riezman, H. (2000). An intact SH3 domain is required for myosin I-induced actin polymerization. *EMBO J* 19, 4281-91.

Lewis, M. J., Nichols, B. J., Prescianotto-Baschong, C., Riezman, H. & Pelham, H. R. (2000). Specific retrieval of the exocytic SNARE Snc1p from early yeast endosomes. *Mol Biol Cell* 11, 23-38.

Muniz, M., Nuoffer, C., Hauri, H. P. & Riezman, H. (2000). The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J Cell Biol* 148, 925-30.

Muniz, M. & Riezman, H. (2000). Intracellular transport of GPI-anchored proteins. *EMBO J* 19, 10-5.

Nagamune, K., Nozaki, T., Maeda, Y., Ohishi, K., Fukuma, T., Hara, T., Schwarz, R. T., Sutterlin, C., Brun, R., Riezman, H. & Kinoshita, T. (2000). Critical roles of glycosyl phosphatidylinositol for Trypanosoma brucei. *Proc Natl Acad Sci U S A* 97, 10336-41.

Ohishi, K., Inoue, N., Maeda, Y., Takeda, J., Riezman, H. & Kinoshita, T. (2000). Gaa1p and gpi8p are components of a glycosylphosphatidy linositol (GPI) transamidase that mediates attachment of GPI to proteins. *Mol Biol Cell* 11, 1523-33.

Schaerer-Brodbeck, C. & Riezman, H. (2000). Functional interactions between the p35 subunit of the Arp2/3 complex and calmodulin in yeast. *Mol Biol Cell* 11, 1113-27.

Schaerer-Brodbeck, C. & Riezman, H. (2000). Interdependence of filamentous actin and microtubules for asymmetric cell division. *Biol Chem* 381, 815-25.

Schaerer-Brodbeck, C. & Riezman, H. (2000). Saccharomyces cerevisiae Arc35p works through two genetically separable calmodulin functions to regulate the actin and tubulin cytoskeletons. *J Cell Sci* 113, 521-32.

Wiederkehr, A., Avaro, S., Prescianotto-Baschong, C., Haguenauer-Tsapis, R. & Riezman, H. (2000). The F-box protein Rcy1p is involved in endocytic memb*rane traffic and recycling out of an early endosome in* Saccharomyces cerevisiae*. J Cell Biol 149, 397-410.*

*Zanolari, B., Friant, S., Funato, K., Sutterlin, C., Stevenson, B. J. & Riezman, H. (2000). Sphingoid base synthesis requirement for endocytosis in Sacch*aromyces cerevisiae. *EMBO J* 19, 2824-33.

Friant, S., Lombardi, R., Schmelzle, T., Hall, M. N. & Riezman, H. (2001). Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast. *EMBO J* 20, 6783-92.

Funato, K. & Riezman, H. (2001). Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. *J Cell Biol* 155, 949-59.

Galan, J. M., Wiederkehr, A., Seol, J. H., Haguenauer-Tsapis, R., Deshaies, R. J., Riezman, H. & Peter, M. (2001). Skp1p and the F-box protein Rcy1p form a non-SCF complex involved in recycling of the SNARE Snc1p in yeast. *Mol Cell Biol* 21, 3105-17.

Lombardi, R., Friant, S. & Riezman, H., Eds. (2001). Endocytosis in Saccharomyces cerevisiae: involvement of actin, actin-associated protein complexes, lipids in the internalization step. *Frontiers in Molecular Biology. Edited by Marsh, M. Oxford, UK: Oxford University Press*.

Lombardi, R. & Riezman, H. (2001). Rvs161p and Rvs167p, the two yeast amphiphysin homologs, function together in vivo. *J Biol Chem* 276, 6016-22.

Muniz, M., Morsomme, P. & Riezman, H. (2001). Protein sorting upon exit from the endoplasmic reticulum. *Cell* 104, 313-20.

Schorling, S., Vallee, B., Barz, W. P., Riezman, H. & Oesterhelt, D. (2001). Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in Saccharomyces cerevisae. *Mol Biol Cell* 12, 3417-27.

Wiederkehr, A., Meier, K. D. & Riezman, H. (2001). Identification and characterization of Saccharomyces cerevisiae mutants defective in fluid-phase endocytosis. *Yeast* 18, 759-73.

Zahn, R., Stevenson, B. J., Schroder-Kohne, S., Zanolari, B., Riezman, H. & Munn, A. L. (2001). End13p/Vps4p is required for efficient transport from early to late endosomes in Saccharomyces cerevisiae. *J Cell Sci* 114, 1935-47.

Morsomme, P. & Riezman, H. (2002). The Rab GTPase Ypt1p and Tethering Factors Couple Protein Sorting at the ER to Vesicle Targeting to the Golgi Apparatus. *Dev Cell* 2, 307-17.

Prescianotto-Baschong, C. & Riezman, H. (2002). Ordering of compartments in the yeast endocytic pathway. *Traffic* 3, 37-49.

Membrane protein topogenesis and intracellular protein sorting

N. Beuret, P. Crottet, M. Friberg, V. Goder, M. Higy, M. Jeske, T. Junne, S. Kobialka, V. Laird, A. Pagano, A. Renold, M. Schneider

Visiting scientist: J. Rutishauser (University Hospital, Basel)
In collaboration with L. Vogel (Panum Institute, Copenhagen, DK)

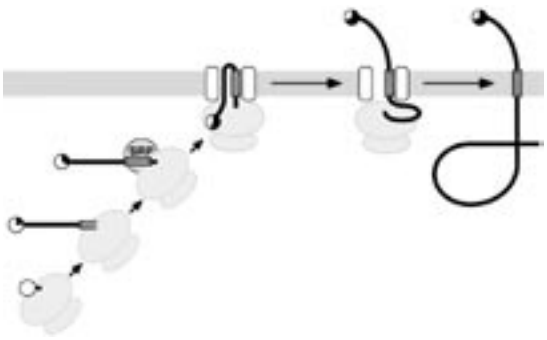
Martin Spiess
Phone: +41 61 267 2164 Fax: +41 61 267 2149 E-mail: Martin.Spiess@unibas.ch
http://www.biozentrum.unibas.ch/spiess

Proteins initially synthesized on cytosolic ribosomes must be sorted to the specific compartment(s) in which they perform their function. Proteins destined to 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 centers on (1) how membrane proteins are sorted to the ER and acquire a defined topology in the lipid bilayer, and (2) how they are sorted at the *trans*-Golgi or in endosomes.

Determinants of membrane protein topology

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. What determines the orientation of a signal in the membrane is not obvious, and the topogenesis of multi-spanning proteins is even less understood. We are analyzing the insertion process *in vivo* by challenging the insertion machinery with diagnostic mutant proteins. In addition to the well established effect of flanking charges, also the length and hydrophobicity of the apolar core of the signal as well as protein folding and glycosylation contribute to orienting the signal in the translocon. In multi-spanning membrane proteins, topogenic determinants are distributed throughout the sequence and may even compete with each other. During topogenesis, segments of up to 60 residues may move back and forth through the translocon, revealing unexpected dynamic aspects of topogenesis.

Figure 1:
A phosporylation site as a timer for ER targeting



To measure the kinetics of targeting and translocation, we have developed a novel assay using a short consensus sequence for phosphorylation by cytosolic protein kinase A. Incorporation of radioactive phosphate was shown to reflect the time of exposure to the cytosol, which depends on translation, targeting and translocation. By manipulating the translation rate, it was possible to determine that protein targeting is rapid and requires only a few seconds, and that the rate of N-terminal translocation proceeds at approximately 8 amino acids/second. This method may be applicable to other intracellular targeting processes.

AP-1/clathrin-coated vesicles

Sorting of membrane proteins 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 signals. The classical paradigm are clathrin coats and associated AP-2 adaptor proteins which cluster receptors with characteristic tyrosine and dileucine motifs during endocytosis. At the *trans*-Golgi, clathrin-coats containing AP-1 adaptors are formed in an ARF1-dependent manner and generate vesicles transporting cargo proteins to endosomes. The mechanism of site-specific targeting of AP-1 and

the role of cargo are poorly understood. We have developed an *in vitro* assay to study the recruitment of AP-1 adaptors to chemically defined liposomes presenting peptides corresponding to tyrosine-based sorting motifs. AP-1 recruitment was found to be dependent on myristoylated ARF1, GTP or nonhydrolyzable GTP-analogs, tyrosine signals and small amounts of phosphoinositides, most prominently phosphatidylinositol 4,5-bisphosphate, in the absence of any additional cytosolic or membrane bound proteins. The results suggest that cargo proteins are involved in coat recruitment and that the local lipid composition contributes to specifying the site of vesicle formation. The assay is being extended to include clathrin recruitment and vesicle formation, and to analyze the sequence preference of the ubiquitous isoform AP-1A vs. the epithelial-specific AP-1B, which is implicated in basolateral sorting.

Polarized sorting in epithelial cells

Whereas a number of cytosolic signals for basolateral sorting have been identified, much less is known about apical sorting determinants. In collaboration with Lotte Vogel (Panum Institute, Copenhagen), we compared closely related members of the serpin (serine protease inhibitor) family of proteins which are secreted from polarized Madin-Darby canine kidney (MDCK) cells either apically or in a non-polarized fashion. Chimeras composed of complementary portions of an apically targeted serpin and a non-sorted serpin identified an amino-terminal sequence of corticosteroid binding globulin (residues 1–19) that is sufficient to direct a chimera with antithrombin mainly to the apical side. The corresponding amino terminal sequences of α_1-antitrypsin and C1 inhibitor were also sufficient to confer apical sorting, although they do not share sequence homology. The apical sorting signal does not appear to be based on a conserved sequence motif, but is likely to involve a conformational contribution.

Secretory granules

In collaboration with Jonas Rutishauser (University Hospital, Basel), we further study the mechanism by which a signal sequence truncation in the vasopressin precursor protein causes familial central *Diabetes insipidus*, a dominant disturbance of water homeostasis. Normally, pro-vasopressin is synthesized in vasopressinergic neurons of the hypophysis and sorted at the *trans*-Golgi into secretory granules. At reduced serum osmolality, the granules are triggered to release the hormone into the bloodstream. The machinery required for the formation of and sorting into secretory granules is still poorly understood. In cells lacking a regulated secretory pathway, hormone precursors are expected to be secreted constitutively. However, we discovered by immunofluorescence that expression of pro-vasopressin and several other

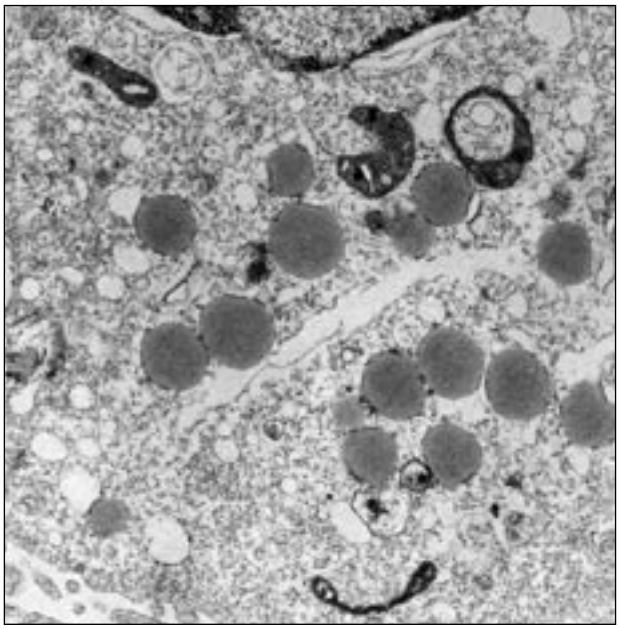


Figure 2
Electron microscopy of granule-like structures

regulated cargo proteins in constitutively secreting cells like COS and CHO cells generates granule-like structures. These structures represent a post-Golgi compartment, since they stain positive for sialic acid, and resemble granules that can be observed by electron microscopy. Coexpressed processing enzymes, but not constitutive secretory proteins, are corecruited into these structures. The results suggest that expression of regulated cargo proteins is sufficient to perform the initial stages of regulated secretion in the background of non-endocrine cells. Granule formation appears to be the result of self-organizing properties of regulated cargo, mainly via their ability to selectively aggregate under *trans*-Golgi conditions.

Publications

Cescato, R., Dumermuth, E., Spiess, M. & Paganetti, P. A. (2000). Increased generation of alternatively cleaved beta-amyloid peptides in cells expressing mutants of the amyloid precursor protein defective in endocytosis. *J Neurochem* 74, 1131-9.

Goder, V., Crottet, P. & Spiess, M. (2000). In vivo kinetics of protein targeting to the endoplasmic reticulum determined by site-specific phosphorylation. *EMBO J* 19, 6704-12.

Laird, V. (2000). Endocytosis meets exocytosis. In *Protein, Lipid and Membrane Traffic: Pathways and Targeting.* (Op den Kamp, J. A. F., ed.), pp. 211-219. IOS Press.

Laird, V. & Spiess, M. (2000). A novel assay to demonstrate an intersection of the exocytic and endocytic pathways at early endosomes. *Exp Cell Res* 260, 340-5.

Martín, M. E., Hidalgo, J., Rosa, J. L., Crottet, P. & Velasco, A. (2000). Effect of protein kinase A activity on the association of ADP-ribosylation factor 1 to Golgi membranes. *J Biol Chem* 275, 19050-9.

Meier, M., Bider, M. D., Malashkevich, V. N., Spiess, M. & Burkhard, P. (2000). Crystal structure of the carbohydrate recognition domain of the H1 subunit of the asialoglycoprotein receptor. *J Mol Biol* 300, 857-65.

Renold, A., Cescato, R., Beuret, N., Vogel, L. K., Wahlberg, J. M., Brown, J. L., Fiedler, K. & Spiess, M. (2000). Basolateral sorting signals differ in their ability to redirect apical proteins to the basolateral cell surface. *J Biol Chem* 275, 9290-5.

Rösch, K., Naeher, D., Laird, V., Goder, V. & Spiess, M. (2000). The topogenic contribution of uncharged amino acids on signal sequence orientation in the endoplasmic reticulum. *J Biol Chem* 275, 14916-22.

Goder, V. & Spiess, M. (2001). Topogenesis of membrane proteins: determinants and dynamics. *FEBS Lett* 504, 87-93.

Kuhn, A. & Spiess, M. (2002). Membrane protein insertion into bacterial membranes and the endoplasmic reticulum. In *Protein targeting, transport and translocation* (Dalbey, R. E. & von Heijne, G., eds.). Academic Press, London. p. 107-130.

Vogel, L. K., Sahkri, S., Sjöstrom, H., Norén, O. & Spiess, M. (2002). Secretion of antithrombin is converted from non-polarized to apical by exchanging its amino terminus for that of apically secreted family members. *J Biol Chem*. 277, 13883-13888.

Dumermuth, E., Beuret, N., Spiess, M. & Crottet, P. (2002). Ubiquitous 9-0-acetylation of sialoglycoproteins restricted to the Golgi complex. *J Biol Chem*. 277, 18687-18693.

Modulators of steroid hormone signaling

R. Bamert, K. Brogli, L. Dolfini, R. Emter, D. Knutti, D. Kressler, S. Schreiber, T. Uhlmann

In collaboration with R. Sitcheran, K.R. Yamamoto (University of California, San Francisco), A. Heese-Peck (Biozentrum, Basel)

Anastasia Kralli
Phone: +41 61 267 2162 Fax: +41 61 27 2148 E-mail: Anastasia.Kralli@unibas.ch
http://www.biozentrum.unibas.ch/kralli

Extracellular signals elicit cellular responses that are cell-type specific and physiological-state dependent. To elucidate the mechanisms that confer specificity and versatility to signal transduction pathways, we use steroid hormone signaling as a model system. Steroid hormones play important roles in mammalian development and physiology, and have widespread applications as drugs. They exert their effects by binding and activating intracellular receptors that are ligand-regulated transcription factors. Our studies focus on the receptor cofactors that modulate signaling and may confer specificity or regulation. To identify such modulators, we have exploited the ability of mammalian steroid receptors to function in yeast. Using yeast strains expressing the rat glucocorticoid receptor, we have performed genetic screens and identified yeast proteins that downregulate hormone response (e.g. Pdr5/Lem1p, Lem3p, and Erg6/Lem4p), and mammalian modulators that enhance signaling (such as the molecular chaperone p23, the chromatin assembly factor ASF1, and the transcriptional coactivator PGC-1). Understanding the mechanism of action of modulators may reveal novel intervention ways for activating, suppressing or altering the specificity of hormone action.

Yeast modulators

Studies of two yeast proteins that decrease the response to steroids have highlighted the regulatory capacity of plasma membrane proteins and lipids in steroid hormone action. The ABC transporter Pdr5/Lem1p exports selective steroids out of the cell, thereby restricting their availability to the intracellular receptor. An enzyme in sterol biosynthesis, Erg6/Lem4p, determines the type of sterols in the membrane and limits the rate of diffusion of hormone across the bilayer. Analogous functions in mammalian cells may regulate hormone availability in specific tissues. A third yeast modulator, Lem3p, highlights the ability of steroid receptors to integrate intracellular information, besides the hormonal signal. Lem3p belongs to a family of highly conserved eukaryotic proteins, all with no assigned function. It is an integral membrane protein of the endoplasmic reticulum, and it inhibits the activity of the nuclear, hormone-activated steroid receptors by an as yet unknown mechanism. Our findings suggest the existence of a Lem3p-dependent pathway by which the endoplasmic reticulum influences the activity of steroid receptors and other transcription factors in the nucleus. Future studies will elucidate the pathway by which yeast and human Lem3p affect the activity of nuclear transcription factors, and determine the signals to which this pathway responds.

The transcriptional coactivators PGC-1 and PERC

PGC-1, an inducible and tissue-specific coactivator of nuclear receptors, has been proposed to coordinate transcriptional programs important for energy homeostasis. To elucidate its role in steroid hormone signaling, we have dissected the domains and functions of PGC-1 that are required for enhancement of steroid receptor activity. Moreover, we have shown that PGC-1 confers novel properties to gluococorticoid signaling, by rendering it responsive to the stress-activated MAPK p38 pathway. Activation of the MAPK p38 counteracts the effect of a still unidentified repressor that associates with PGC-1 and restricts its function. Current efforts aim at identifying the PGC-1 repressor, which will likely contribute to the regulation of energy metabolism, and understanding the molecular mechanisms by which PGC-1 regulates gene expression.

PGC-1 is the founding member of a small family of sequence-related coactivators. In contrast to PGC-1, which enhances the activity of a wide range of nuclear receptors, we find that one of the close homologs, PERC, functions as a selective coactivator for the

estrogen receptor. Interestingly, PGC-1 and PERC enhance the activity of the estrogen receptor in distinct ways, showing specific preferences for the promoter and cellular context in which they do so. Thus, the two coactivators are likely to fulfill distinct physiological functions in estrogen signaling. Current studies aim in elucidating these functions.

Publications

Knutti, D., Kaul, A. & Kralli, A. (2000). A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. *Mol. Cell. Biol.* 20, 2411-22.

Sitcheran, R., Emter, R., Kralli, A. & Yamamoto, K. R. (2000). A genetic analysis of glucocorticoid receptor signaling. Identification and characterization of ligand-effect modulators in Saccharomyces cerevisiae. *Genetics* 156, 963-72.

Knutti, D. & Kralli, A. (2001). PGC-1, a versatile coactivator. *Trends Endocrinol. Metab.* 12, 360-5.

Knutti, D., Kressler, D. & Kralli, A. (2001). Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9713-8.

Kressler, D., Schreiber, S. N., Knutti, D. & Kralli, A. (2002). The PGC-1-related protein PERC is a selective coactivator of estrogen receptor α. *J. Biol. Chem.* 27, 13918-25.

Emter, R., Heese-Peck, A. & Kralli, A. (2002). *ERG6* and *PDR5* regulate small lipophilic drug accumulation in yeast cells via distinct mechanisms. *FEBS Lett. 521, p 57-61*

DIVISION OF BIOPHYSICAL CHEMISTRY

In the Division of Biophysical Chemistry, several methods are established including optical and NMR spectroscopy, high sensitivity titration, pressure perturbation and differential scanning calorimetry, stopped-flow and laserflash kinetics, measurements of membrane permeability and peptide synthesis.

The division of Biophysical Chemistry consists of three senior groups headed by Jürgen Engel, Thomas Kiefhaber and Joachim Seelig and one independent group headed by Anna Seelig. Gerhard Schwarz retired in February 2000 and was not replaced. His research focused on quantitative aspects of various biological and biochemical problems. The main topics were extracellular matrix proteins, protein folding, peptide-membrane interactions, membrane pores and *in vivo* magnetic resonance. In addition, Ariel Lustig provided service on analytical ultracentrifugation for the Biozentrum. In 2000 Joachim Seelig received the “Applied Physical Chemistry Award” from the “European Society of Applied Physical Chemistry”.

The projects of the individual research groups are described below.

Professors

Joachim Seelig, Jürgen Engel, Thomas Kiefhaber, Gerhard Schwarz*

University lecturers

Heiko Heerklotz, Anna Seelig, Mathias Winterhalter*

Scientists and postdoctoral fellows

Thomas Ahrens, Ognja Apostolov*, Annett Bachmann, Oliver Bieri*, Fabrice Dumas*, Sabine Frank, Gernot Hänisch, Heiko Heerklotz, Simon Hellstern, Richard Kammerer*, Larisa Kapinos Schneider*, Gia Machaidze, Suat Özbek*, Renate Reiter*, Roman Schwarz*, Bernhard Steinbauer*, Jörg Stetefeld, Anmin Tan*, Susanne Taylor*, Albert Tölle*, Patrick Van Gelder*, Ingrid Weis*, Torsten Wieprecht*, André Ziegler, Heidi Zinecker*

Graduate students

Naushaba Anjum*, Annett Bachmann, Oliver Bieri*, Sergei Boudko*, Gunnar Karelson, Sarah Krause*, Florian Krieger*, Ewa Landwojtowicz, Joseph Mascarenhas*, Pierluigi Nervi, Günter Pappenberger*, Daniel Poso*, Ignacio-Enrique Sanchez, Manuela Schätzle*

Guest graduate students

Sergey Driamov*, Claudia Broggini*, Alexandra Graff*, André Ziegler

Diploma students

Sarah Krause*, Beat Fierz*, Stefan Langheld*, Pierluigi Nervi

Technical personnel

Ingrid Bartoldus*, Franz Biry*, Leonardo Faletti, Charlotte Fauser*, Peter Ganz, Ruth Landwehr, Xiaochun Li Blatter, Ariel Lustig, Rita Müller*, Therese Schulthess, Martine Schwager*, Ralph Simon*, Halina Szadkowski*, Joseph Wey

Laboratory helper

Anna Varadi

Visiting scientists

Sarah Davies*, Jiayun Zhang*

Floor manager

Hans Vogt

Secretariat

Brigitte Heiz Wyss*, Erika Johner*, Monica Mayer*, Susanna Notz Ndombasi

* Employed during part of the report period

Extracellular matrix, cell adhesion and oligomerization

T. Ahrens, N. Anjum, S. Boudko, C. Fauser, S. Frank, S. Hellstern, R. Kammerer, R. Landwehr, J. Mascarenhas, S. Özbek, T. Schulthess, M. Schwager, J. Stetefeld

In collaboration with T.W. Holstein (Darmstadt University of Technology, Darmstadt, Germany), R. Timpl and L. Moroder (Max-Planck-Institut für Biochemie, Martinsried, Germany), D.D. Thomas (University of Minnesota, Minneapolis, USA), R.A. Kammerer (University of Manchester, GB), M. Koch (Harvard Medical School, Massachusetts, USA)

Jürgen Engel
Phone: +41 61 267 2250 Fax: +41 61 267 2189 E-mail: Juergen.Engel@unibas.ch
http://www.biozentrum.unibas.ch/jengel

Essentially all cells of an organism are surrounded by an extracellular matrix (ECM), which mediates cell adhesion, mechanical strength of tissues, cell communication, cellular differentiation and cell migration. ECM proteins are usually very large and composed of many domains with different functions. The ECM forms a tight and multifunctional network. Oligomerization domains such as collagen triple helices and α-helical coiled-coil domains are essential for network formation. Cellular receptors and specific adhesion proteins on plasma membranes are also involved in the network and connections exist to the cytoskeleton via membrane spanning receptors such as integrins.

Our research aims to elucidate the structure and function of adhesion- and ECM-proteins and in particular on their oligomerization and the mechanisms by which protein subunits with different functions are combined. Oligomerization leads to functional advantages of multivalency and high binding strength, increased structure stabilization and a combined function of different domains. Specifically we investigated the homophilic interaction between adhesion proteins of the E-cadherin family. This work was started by electron microscopy and crystallography but is currently continued by NMR- spectroscopy in close cooperation with the group of Stephan Grzesiek in the Department of Structural Biology. We also asked the question how the five-stranded coiled-coil domain of cartilage oligomeric matrix protein COMP is formed and how the channels formed in this way are used for binding of growth factors. We investigated how coiled-coil domains, in particular that of laminin, interact with binding partners, and how coiled-coil domains like those in matrilins interact with each other to form hetero-oligomers.

Oligomerization of matrices of high tensile strength frequently include covalent cross-links such as disulfide bridges between proteins. In cooperation with the group of Thomas Holstein in Darmstadt we selected the morphogenesis of the *Hydra* nematocyst wall as a model system and proposed a disulfide reshuffling mechanism leading to network formation of the wall-collagen in a distinct step of maturation. A non-collagenous glycoprotein NOWA is also involved in the wall assembly. It contains Cys-rich domains which are homologous to those found in minicollagen. We expressed both proteins in mammalian cells and are now studying their assembly.

Oligomerization properties seen in naturally occurring proteins can be designed by combining oligomerization domains with functional domains by protein engineering. A successful example studied in our laboratory is the homophilic interaction of adhesion proteins clustered by fusion with the pentameric coiled-coil domain of COMP. We also stabilized short segments of the collagen triple helix by cross-linking with oligomerization domains. Protein engineering is applied for the design of therapeutically important polyvalent inhibitors of multivalent receptors.

Publications

Engel, J. & Bächinger, H. P. (1999). Collagen-like sequences in phages and bacteria. *Proc. Indian Acad Sci.* 111, 81-86.

Pihlajamaa, T., Perala, M., Vuoristo, M. M., Nokelainen, M., Bodo, M., Schulthess, T., Vuorio, E., Timpl, R., Engel, J. & Ala-Kokko, L. (1999). Characterization of recombinant human type IX collagen. Association of alpha chains into homotrimeric and heterotrimeric molecules. *J Biol Chem* 274, 22464-8.

Engel, J. & Bächinger, H. P. (2000). Cooperative equilibrium transitions coupled with a slow annealing step explain the sharpness and nysteresis of collagen folding. *Matrix Biol* 19, 235-244.

Engel, J. & Kammerer, R. A. (2000). What are oligomerization domains good for? *Matrix Biol* 19, 283-8.

Erb, E. M. & Engel, J. (2000). Reconstitution of functional integrin into phospholipid vesicles and planar lipid bilayers. *Methods Mol Biol* 139, 71-82.

Frank, S., Kammerer, R. A., Hellstern, S., Pegoraro, S., Stetefeld, J., Lustig, A., Moroder, L. & Engel, J. (2000). Toward a high-resolution structure of phospholamban: design of soluble transmembrane domain mutants. *Biochemistry* 39, 6825-31.

Frank, S., Lustig, A., Schulthess, T., Engel, J. & Kammerer, R. A. (2000). A distinct seven-residue trigger sequence is indispensable for proper coiled-coil formation of the human macrophage scavenger receptor oligomerization domain. *J Biol Chem* 275, 11672-7.

Guo, Y., Kammerer, R. A. & Engel, J. (2000). The unusually stable coiled-coil domain of COMP exhibits cold and heat denaturation in 4-6 M guanidinium chloride. *Biophys Chem* 85, 179-86.

Hall, H., Bozic, D., Fauser, C. & Engel, J. (2000). Trimerization of cell adhesion molecule L1 mimics clustered L1 expression on the cell surface: influence on L1-ligand interactions and on promotion of neurite outgrowth. *J Neurochem* 75, 336-46.

Holler, N., Kataoka, T., Bodmer, J. L., Romero, P., Romero, J., Deperthes, D., Engel, J., Tschopp, J. & Schneider, P. (2000). Development of improved soluble inhibitors of FasL and CD40L based on oligomerized receptors. *J Immunol Methods* 237, 159-73.

Koch, A. W., Engel , J., Maurer, P. (2000). Calcium Binding to Extracellular Matrix Proteins, Functional and Pathological Effects. *The Molecular Basis of Calcium Action in Biology and Medicine; Kluwer Academic Publishers, Printed in the Netherlands*, 151-164.

Stetefeld, J., Jenny, M., Schulthess, T., Landwehr, R., Engel, J. & Kammerer, R. A. (2000). Crystal structure of a naturally occurring parallel right-handed coiled coil tetramer. *Nat Struct Biol* 7, 772-6.

Alexandrescu, A. T., Maciejewski, M. W., Rüegg, M. A., Engel, J., Kammerer, R. A. (2001). ^{1}H, ^{13}C and ^{15}N backbone assignments for the C-terminal globular domain of agrin. *J Biomolecular NMR* 20, 295-296.

Bächinger, H. P., Engel, J. (2001). Thermodynamic vs. kinetic stability of triple helices. *Matrix Biol* 20, 267-269.

Engel, U., Pertz, O., Fauser, C., Engel, J., David, C. N. & Holstein, T. W. (2001). A switch in disulfide linkage during minicollagen assembly in Hydra nematocysts. *Embo J* 20, 3063-73.

Frank, S., Kammerer, R. A., Mechling, D., Schulthess, T., Landwehr, R., Bann, J., Guo, Y., Lustig, A., Bachinger, H. P. & Engel, J. (2001). Stabilization of short collagen-like triple helices by protein engineering. *J Mol Biol* 308, 1081-9.

Hellstern, S., Pegoraro, S., Karim, C. B., Lustig, A., Thomas, D. D., Moroder, L. & Engel, J. (2001). Sarcolipin, the shorter homologue of phospholamban, forms oligomeric structures in detergent micelles and in liposomes. *J Biol Chem* 276, 30845-52.

Kammerer, R. A., Jaravine, V. A., Frank, S., Schulthess, T., Landwehr, R., Lustig, A., Garcia-Echeverria, C., Alexandrescu, A. T., Engel, J. & Steinmetz, M. O. (2001). An intrahelical salt bridge within the trigger site stabilizes the GCN4 leucine zipper. *J Biol Chem* 276, 13685-8.

Stetefeld, J., Jenny, M., Schulthess, T., Landwehr, R., Schumacher, B., Frank, S., Ruegg, M. A., Engel, J. & Kammerer, R. A. (2001). The laminin-binding domain of agrin is structurally related to N-TIMP-1. *Nat Struct Biol* 8, 705-9.

Boudko, S., Frank, S., Kammerer, R. A., Stetefeld, J., Schulthess, T., Landwehr, R., Lustig, A., Bächinger, H. P., Engel, J. (*in press*). Nucleation and Propagation of the Collagen Triple Helix in Single-chain and Trimerized Peptides: Transition from Third to First Order Kinetics. *J Mol Biol*.

The mechanism of protein folding

A. Bachmann, O. Bieri, B. Fierz, R. Müller, L. Kapinos, S. Krause, F. Krieger, G. Pappenberger, D. Poso, I.E. Sanchez, M. Schätzle, J. Wey, H. Zinecker

In collaboration with H. Aygün (Biospring AG, Frankfurt/Main), J.W. Engels (Universität Frankfurt/Main), G. Fischer (MPG, Halle/Saale), R. Givens (University of Kansas), K. Koller (Aventis AG, Frankfurt)/Main), A.E. Merbach (Université de Lausanne), J. Wirz (Physikalische Chemie, Universität Basel)

Thomas Kiefhaber
Phone: +41 61 267 2194 Fax: +41 61 267 2189 E-mail: T.Kiefhaber@unibas.ch
http://www.biozentrum.unibas.ch/kiefhaber

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 on the characterization of the rate-limiting steps in the folding process. Studies of 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 in the time range of nanoseconds to hours.

Elementary steps in protein folding

During protein folding, contact formation between specific parts of the polypeptide chain allow a search for energetically favorable conformations. Intrachain diffusion can thus be regarded as the elementary step in the folding process and it should determine the maximum rate at which a protein can fold. We developed a model system to directly measure contact formation between two defined points on a polypeptide chain using triplet-triplet energy transfer.

We used model peptides to study the effects of amino acid sequence, chain length and temperature on the process of contact formation. Single exponential kinetics of contact formation on the nanosecond time scale were observed for most peptides. The rates depend on the number of amino acids separating donor and acceptor and show a maximum of 5-10 ns in water 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 30-50 ns. The time constant for contact formation at the ends of very tight glycine-containing turns of 3-4 amino acids, which are often found at the end of ?-hairpins, is in the range of 5-10 ns.

Folding of Tendamistat

Several small proteins were shown to fold very fast (typically on the millisecond time scale) and without

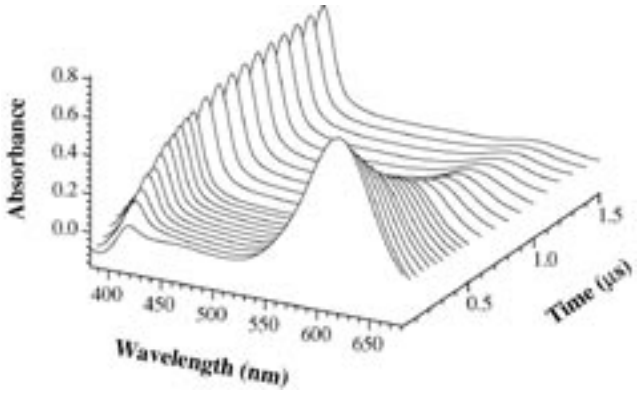


Figure 1: Kinetics of triplet-triplet energy transfer from a donor to an acceptor molecule measured by changes in triplet absorbance.

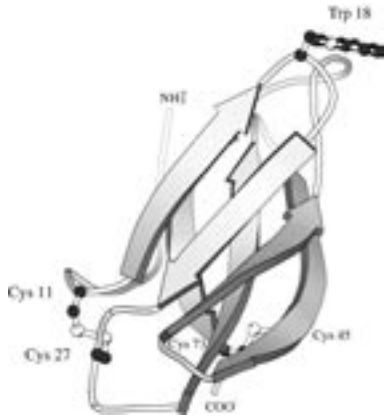


Figure 2

detectable intermediate states. Our group uses the small all--sheetprotein tendamistat as a model system to study folding and stability of a fast folding protein.

The transition state of tendamistat folding was characterized by measuring the denaturant dependence, the temperature dependence and the pressure dependence of the folding and unfolding reactions. The experiments showed that folding of tendamistat proceeds in at least two consecutive steps through an obligatory metastable intermediate. We further observed that changes in solvent conditions and amino acid replacements can lead to changes in the rate-limiting step for folding. These results showed that defined folding pathways can also exist for apparent two-state folders.

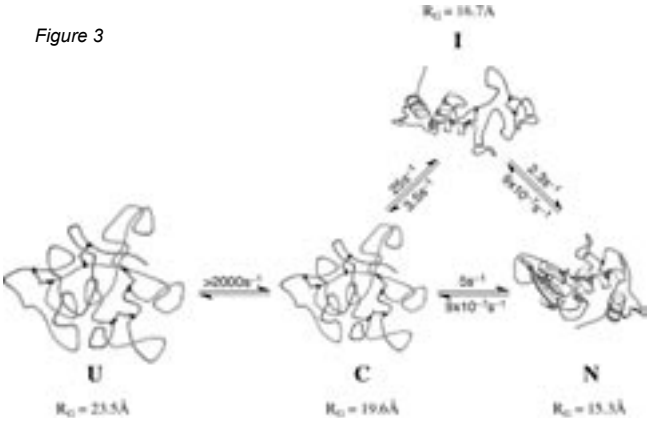
Studies on slow folding processes revealed that folding of about 5 % of unfolded tendamistat molecules is limited by the *cis->trans* isomerization of non-prolyl peptide bonds. The large number of peptide bonds in a protein leads to a significant fraction of unfolded molecules with at least one non-native *cis* peptide bond isomer, although the *cis*/*trans* equilibrium at peptide bonds strongly favors the *trans* form. *Cis->trans* isomerization of non-prolyl peptide bonds has long been speculated to cause slow steps in protein folding, since it is an intrinsically slow process with a high activation energy. However, it has previously not been demonstrated that it actually limits protein folding reactions for a part of the unfolded molecules. Our results imply that isomerization at non prolyl peptide bonds will dramatically effect folding of large proteins. For a protein with 500 amino acid residues, more than 50 % of the unfolded molecules have at least one non native peptide bond isomer.

Folding of hen lysozyme

Lysozyme from hen egg-white is one of the best studied model systems for protein folding. The protein consists of an -domain with helical structure and a -domain with predominately -sheets. During refolding of lysozyme the polypeptide chain rapidly collapses to a compact globular state, which is still largely solvent accessible. We could show that the transition from the unfolded protein to the collapsed state is a two state transition (first-order transition). Subsequently, a partially folded state with native-like secondary structure in the -domain is formed. We have previously shown that formation of the intermediate is not an obligatory step in lysozyme folding. An additional fast track of folding exists on which native lysozyme forms directly from the collapsed state without detectable intermediates.

Kinetic modeling of all events occurring during pulse-labeling hydrogen exchange experiments allowed us to resolve the puzzling observation that hydrogen bonds seemed to form much faster during refolding than changes in spectroscopic properties and in chain dimensions. The results showed that the apparent faster formation of hydrogen bonds is due to kinetic competition between folding and labeling

Figure 3



during the exchange pulse. The 4-state mechanism shown in the figure above is in accordance with all experimental data.

Folding and stability of the C-terminal domain from T4-fibritin

We recently started to work on the folding mechanism of the C-terminal domain of the trimeric T4-fibritin. Fibritin is located at the “neck” of the T4 phage and its N-terminal part forms a long coiled–coiled structure. The C-terminal small globular domain (25 amino acid residues per monomer) accelerates folding of the N-terminal domain dramatically so its functions seems to be the facilitation of the association/folding process. The isolated C-terminal fragment is stable in solution. The thermal and denaturant-induced unfolding transitions are reversible and can be described by a two state process from the folded trimer to the unfolded monomer. Kinetic refolding studies revealed very rapid structure formation in the monomer followed by concentration dependent association steps.

Publications

Bieri, O., Wirz, J., Hellrung, B., Schutkowski, M., Drewello, M. & Kiefhaber, T. (1999). The speed limit for protein folding measured by triplet-triplet energy transfer. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9597-9601.

Bieri, O. & Kiefhaber, T. (2000). Kinetic models in protein folding. In *Frontiers in molecular biology: Mechanisms of protein folding* (Pain, R., ed.), pp. 34-64. Oxford University Press, Oxford, UK.

Boxer, S. G., Benner, S. A., Brunori, M., Chothia, C., Kiefhaber, T., Luthey-Schulten, Z., Matthews, B. W., Onuchic, J. N., Rigler, R. & Schuster, P. (2000). What are the constraints on the evolution and diversity of biopolymers? In *Simplicity and complexity of proteins and nucleic acids* (Frauenfelder, H., Deisenhofer, J. & Wolynes, P., eds.), pp. 365-382. Dahlem University Press, Berlin.

Pappenberger, G., Saudan, C., Becker, M., Merbach, A. E. & Kiefhaber, T. (2000). Denaturant-induced movement of the transition state of tendamistat folding revealed by high pressure stopped-flow measurements. *Proc. Natl. Acad. Sci. U.S.A.* 97, 17-22.

Bachmann, A. & Kiefhaber, T. (2001). Apparent two-state tendamistat folding is a sequential process along a defined route. *J. Mol. Biol.* 306, 375-386.

Bieri, O. & Kiefhaber, T. (2001). Origin of apparent fast and non-exponential kinetics of lysozyme folding measured in pulsed hydrogen exchange experiments. *J. Mol. Biol.* 310, 919-935.

Pappenberger, G., Aygün, H., Engels, J. W., Reimer, U., Fischer, G. & Kiefhaber, T. (2001). Non-prolyl *cis* peptide bonds in unfolded proteins cause complex folding kinetics. *Nature Structural Biology* 8, 452-458.

Bachmann, A., Segel, D. & Kiefhaber, T. *(in press)* Test for cooperativity in the early lysozyme folding intermediate. *Bioph. Chem.*

Molecular functions and mechanisms of membrane active agents

Gerhard Schwarz (Group leader, retired in 2000)
F. Dumas, P. van Gelder, A. Graff, R. Reiter, S. Taylor, I. Weis, M. Winterhalter, J. Zhang

Owing to the retirement of G. Schwarz as a professor at the University of Basel his group was disbanded by the end of March 2000. Nevertheless, he continued his active work in relevant theoretical research and in collaboration with the laboratory of M. Winterhalter at the University of Toulouse (France).

We study the interaction of substances, particularly pep-tides and proteins with lipid membranes, based on physical chemical principles. This involves a quantitative analysis of pertinent molecular mechanisms, directed towards understanding the working of biological membrane functions.

The mass conservation plot approach

Conventional binding data when evaluated in an appropriate model-free way (using a so called mass conservation plot) lead to the underlying thermodynamic asso-ciation isotherm no matter how complex the case may be. In addition, different physical binding signals resulting from potentially different binding structures can so be established. This novel method opens up quite versatile applications.

Monolayers

The mass conservation plot method has been very usefully applied to the issue of lipids and peptides in monolayers at the air/water interface imitating membrane conditions. A number of biolipids have to form insoluble films at this interfaced. Their equation of state could be described in a comparably simple fashion. Lipids of somewhat reduced hydrocarbon chain length (C10, C8) revealed, however, a marked tendency of dissolution into the subphase accompanied by aggregation in either environment upon compression. In the case of a HIV fusion peptide analogue a high affinity to form a monolayer was observed and its structure elucidated by means of spectroscopic and microscopic techniques.

Peptide-liposome interactions

The basic mastoparan X peptide from wasp venom binds to cell membranes and induces various functions. Negatively charged lipids enhance the binding affinity and give rise to a pronounced biphasic kinetics. The mass conservation plot reveals the existence of two classes of bound peptide structures with substantially different fluorescence signals which have a tendency to aggregate. The slower forming structure exerts negative cooperativity so that the binding degree decreases in the long run.

Electric field effect on membrane transport

Porin channels of outer bacterial membranes mediate sugar transport. A basic model theory describing such a translocation mechanism has been developed and was successfully tested with experimental data.

Publications

Schwarz, G. (2000). A universal thermodynamic approach to analyze biomolecular binding experiments. *Biophys. Chem.* 86, 119-129.

Taylor, S. E., Debat, B., Blaudez, D., Jacobi, S., Chi, L. F., Fuchs, H. & Schwarz, G. (2000). Structure of a fusion peptide analogue at the air/water interface, determined from surface activity, infra-red spectroscopy and scanning force microscopy. *Biophys. Chem.* 87, 63-72.

Van Gelder, P., Dumas, F. & Winterhalter, M. (2000). Understanding the bacterial outer membrane channels by reconstitution into black lipid membranes. *Biophys. Chem.* 85, 153-167.

Weis, I., Welzel, P. B., Bähr, G. & Schwarz, G. (2000). Equations of state for POPX lipids at the air/-water interface. A comprehensive study. *Chem. Phys. Lipids* 105, 1-8.

Schwarz, G. & Reiter, R. (2001). Negative cooperativity and aggregation in biphasic binding of mastoparan X peptide to membranes with acidic lipids. *Biophys. Chem.* 90, 269-277.

Schwarz, G. & Zhang, J. (2001). Chain length dependence of lipid partitioning between the air/water interface and its subphase. Thermodynamic and structural implications. *Chem. Phys. Lipids* 110, 35-45.

Membrane interactions and *in vivo* magnetic resonance

O. Apostolov, S. Driamov, P. Ganz, H. Heerklotz, G. Karelson, S. Keller, G. Machaidze, B. Steinbauer, R. Schwarz, A. Tan, T. Wieprecht, A. Ziegler

In collaboration with M. Beyermann (Forschungsinstitut für Molekulare Pharmakologie, Berlin-Buch), E. Breukink (University of Utrecht, Utrecht), E. Seifritz (Psychiatrische Universitätskliniken, Basel)

Joachim Seelig
Phone: +41 61 267 2190 Fax: +41 61 267 2189 E-mail: Joachim.Seelig@unibas.ch
http://www.biozentrum.unibas.ch/jseelig

Lipid-peptide interactions are different from chemical reactions in that no covalent bonds are formed or broken. Instead, the association of a peptide with a pure lipid membrane corresponds to a physical adsorption process which can be divided into at least three molecular steps (i) electrostatic adsorption (if peptide and membrane are charged), (ii) partitioning into the membrane, be it between the lipid headgroups or into the hydrophobic core of the bilayer, and (iii) conformational changes of the peptide in the new environment (most often a transition to a partially α-helical structure).

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.

Hydrophobic partitioning

A good example for a purely hydrophobic partitionig is cyclosporin A, a peptide which has found wide-spread pharmaceutical applications as an immunosuppressant. It is a cyclic peptide of 11 amino acids, barely soluble in water but easily dissolved in oil. The molecule is difficult to measure with spectroscopic techniques since it carries no characteristic chromophor. However, the binding (the physical adsorption) of the peptide to the lipid bilayer is associated with a considerable release of heat and the partitioning into the membrane can thus be studied with high-sensitivity isothermal titration calorimetry (ITC). Temperature changes of 10^{-6} °C in volumes as small as 1 mL can be detected. Thus it became possible to perform a complete thermodynamic analysis of the cyclosporin A-membrane equilibrium. The characteristic parameters determined were the binding isotherm, the partition constant $K = 5 \times 10^3$ M^{-1} (at 25°C), and the partition enthalpy $\Delta H^0 = + 5.9$ kcal/mol (Schote *et al.* 2002, *Pharm. Sci. 91*, 856-72).

Electrostatic adsorption

Nisin Z, a 34-residue peptide, is an antimicrobial, cationic peptide of the lantibiotics family which has found applications as a food preservative. The peptide renders the bacterial cytoplasmic membrane permeable to ions, amino acids etc. and interacts with most membranes in a non-specific manner. The effective charge of nisin Z is z ~ +3.8 and it is strongly attracted to negatively charged membranes. Isothermal titration calorimetry was used to provide a quantitative thermodynamic description of nisin Z adsorption. For biologically relevant conditions with a membrane surface potential of –40 mV, the electrostatic attraction increases the peptide concentration near the membrane surface by 2-3 orders of magnitude compared to the bulk concentration. Electrostatic attraction is strong but hydrophobic insertion is weak. Based on the surface concentration of nisin Z the intrinsic partition constant is only $K = 1.8$ M^{-1}. The positive charges of the peptide interact specifically with the negative lipid headgroups and the peptide remains fixed to the membrane surface (Breukink *et al.* 2000, *Biochemistry 39*, 10247-54).

Key-lock interactions

Specific phsopholipid-peptide interactions are rare. One of the very few known exceptions is the tetracyclic peptide Ro 09-0198 (cinnamycin) which like nisin Z also belongs to the group of lantibiotics. Ro 09-0198 forms a tight equimolar complex with

phosphatidylethanolamine. We have established the thermodynamic parameters of this equilibrium with isothermal titration calorimetry and have studied the structural consequences with NMR. Complex formation is characterized by a large binding constant of $K_0 = 10^7 – 10^8$ M^{-1}. The reaction enthalpy is strongly temperature dependent and varies between zero at about 10°C and -10.5 kcal/mol at 50°C. Addition of Ro 09-0198 to PE containing bilayers eliminates the typical bilayer structure (Machaidze *et al*. 2002, *Biochemistry 41*, 1965-71).

Membrane-induced random coil-α-helix transition

Magainins are antimicrobial peptides of the innate immune response system of the African frog *Xenopus laevis*. The positively charged peptides act by enhancing the permeability of the microbial target membrane. Magainins adopt a random coil conformation in water, but fold into an amphipathic α-helix upon binding to the lipid membrane. We have measured the thermodynamics of this process for magainin and for a second peptide, the signal sequence of rat mitochondrial rhodanese. For both systems the same concept was applied: the parent peptide was compared with analogues in which two neighboring amino acids were replaced by their D-isomers. The double-D substitution results in a local disturbance of the α-helical conformation without modifying other important properties. By correlating the experimental results obtained with calorimetric and spectroscopic methods it is possible to extract the contribution of α-helix formation to the binding process. Helix-formation at the membrane surface is an enthalpy-driven reaction with ΔH_{helix} ~-0.7 kcal/mol per residue which contributes considerably to the free energy of binding (more than 50% of the total free energy). Peptides which adopt an α-helical conformation thus bind stronger to the membrane than peptides which are hindered from doing so. The binding constant increases several orders of magnitude due to helix formation (Wieprecht *et al.* 1999, *J. Mol. Biol. 294*, 785-94; Wieprecht *et al.* 2000, *Biochemistry 39*, 15297-305).

Detergent-membrane interactions

Membrane binding and micelle formation of detergents are related processes as both are driven essentially by the 'hydrophobic effect'. They can be measured conveniently with isothermal titration calorimetry (Heerklotz & Seelig 2000, *Biochim. Biophys. Acta 1508*, 69-85). We have measured the membrane partition coefficients for a large variety of non-ionic detergents and have found a linear correlation between the free energies of membrane partitioning and micelle formation (Heerklotz & Seelig 2000, *Biophys. J. 78*, 2435-40). The surfactant-to-lipid ratio, R_b^{sat}, at which the membrane is saturated with surfactant and starts to disintegrate can be predicted by the simple relationship $R_b^{sat} \approx K \cdot CMC$.

The lipopeptide surfactin consists of a cyclic heptapeptide moiety attached to a β-hydroxy fatty acid. Studies of the membrane partitioning and self-association of surfactin revealed a dramatic perturbation of the membrane structure (Heerklotz & Seelig 2001, *Biophys. J. 81*, 1547-54). This effect may also account for the antibiotic activity.

***In vivo* magnetic resonance**

Magnetic resonance of the human brain. Using fMRI we have studied the effect of ethanol on the response to acoustic stimulation. The intake of alcohol drastically reduced the sensitivity to acoustic stimulation and also led to a desensitation with respect to monoaural stimulation (Seifritz *et al.* 2000, *Psychiatry Res. 99*, 1-13). Using MRS we measured the N-acetylaspartate levels in the frontal lobe of IV heroin-dependent patients (Haselhorst *et al.* 2002, *Neurology 58*, 305-7).

Metabolic studies of animals. Hepatic glucose-6-phosphate (G6P) was monitored non-invasively in rat liver by *in vivo* ^{13}C NMR after infusion of [1-^{13}C] glucose. ^{13}C-NMR spectroscopy at 7 Tesla provides the sensitivity and spectral resolution to detect hepatic G6P at sub-millimolar concentration and allows the hepatic glucose $\rightarrow$ G6P reaction to be assessed in situ (Künnecke *et al.* 2000, *Mag. Reson. Med. 44*, 556-62). Myocardial substrate uptake, substrate preference and metabolism are difficult to assess non-invasively. We have extended cardiac ^{13}C-NMR spectroscopy to the *in vivo* situation ('closed-chest model') and have quantitated the myocardial metabolism *in vivo*. Overnight-fasted Sprague-Dawley rats received intravenous infusions of non-radioactive ^{13}C-labeled glucose, 3-hydroxybutyrate, and acetate as markers for glycolysis, metabolism of ketone bodies and direct incorporation into tricarboxylic acid (TCA) cycle, respectively. *In vivo* ^{13}C-NMR spectra were acquired from the myocardium with a time resolution of 6 min. 3-Hydroxybutyrate and acetate were rapidly extracted by the myocardium and supplied 42 ± 6 and 53 ± 9% of the acetyl-CoA for TCA cycle operation whereas glucose, although also well extracted, did not contribute to myocardial oxidative metabolism (Ziegler *et al.* 2002, *NMR Biomed. 15*, 1-13). We have measured the gastrointestinal transit times in mice and humans with ^{27}Al- and ^{19}F-nuclear magnetic resonance. Al^{3+} bound to ion-exchange resin and perfluorononane were administered orally as selective and specific markers for the stomach and the entire GI tract, respectively. ^{27}Al- and ^{19}F-NMR were employed to follow boli of the mixed markers. The MR-data revealed that gastric emptying in humans proceeded linearly whereas in mice an exponential decay was observed (Schwarz *et al.*, *in press*, *Mag. Reson. Med.*).

Publications

Wieprecht, T., Apostolov, O., Beyermann, M. & Seelig, J. (1999). Thermodynamics of the α-helix-coil transition of amphipathic peptides in a membrane environment: implications for the peptide-membrane binding equilibrium. *J Mol Biol* 294, 785-794.

Breukink, E., Ganz, P., de Kruijff, B. & Seelig, J. (2000). Binding of Nisin Z to bilayer vesicles as determined with isothermal titration calorimetry. *Biochemistry* 39, 10247-10254.

Haselhorst, R., Kappos, L., Bilecen, D., Scheffler, K., Mori, D., Radü, E. W. & Seelig, J. (2000). Dynamic susceptibility contrast MR imaging of plaque development in multiple sclerosis: application of an extended blood-brain barrier leakage correction. *J Magn Reson Imaging* 11, 495-505.

Heerklotz, H. & Seelig, J. (2000). Correlation of membrane/water partition coefficients of detergents with the critical micelle concentration. *Biophys J* 78, 2435-2440.

Heerklotz, H. & Seelig, J. (2000). Titration calorimetry of surfactant-membrane partitioning and membrane solubilization. *Biochim Biophys Acta* 1508, 69-85.

Seifritz, E., Bilecen, D., Hänggi, D., Haselhorst, R., Radü, E. W., Wetzel, S., Seelig, J. & Scheffler, K. (2000). Effect of ethanol on BOLD response to acoustic stimulation: implications for neuropharmacological fMRI. *Psychiatry Res* 99, 1-13.

Wieprecht, T., Apostolov, O., Beyermann, M. & Seelig, J. (2000). Membrane binding and pore formation of the antibacterial peptide PGLa: thermodynamic and mechanistic aspects. *Biochemistry* 39, 442-52.

Fahr, A. & Seelig, J. (2001). Liposomal formulations of Cyclosporin A: a biophysical approach to pharmacokinetics and pharmacodynamics. *Crit Rev Ther Drug Carrier Syst* 18, 141-72.

Heerklotz, H. & Seelig, J. (2001). Detergent-like action of the antibiotic peptide surfactin on lipid membranes. *Biophys J* 81, 1547-1554.

Haselhorst, R., Dürsteler-MacFarland, K. M., Scheffler, K., Ladewig, D., Müller-Spahn, F., Stohler, R., Seelig, J. & Seifritz, E. (2002). Long-term IV heroin is associated with reduced N-acetylaspartate levels in the frontal cortex. *Neurology* 58, 305-307.

Heerklotz, H. & Seelig, J. (2002). Application of pressure perturbation calorimetry to lipid bilayers. *Biophys J* 82, 1445-1452.

Machaidze, G., Ziegler, A. & Seelig, J. (2002). Specific binding of Ro 09-0198 (cinnamycin) to phosphatidylethanolamine. A thermodynamic analysis. *Biochemistry* 41, 1965-1971.

Schote, U., Ganz, P., Fahr, A. & Seelig, J. (2002). Interactions of cyclosporins with lipid membranes as studied by solid-state nuclear magnetic resonance spectroscopy and high sensitivity titration calorimetry. *Pharm Sci* 91, 856-872.

Seelig, A. & Seelig, J. (2002). Membrane structure. In *Encyclopedia of Physical Science and Technology* 3rd edition edit. (Meyers, R. A., ed.), Vol. 9, pp. 355-367. Academic Press, New York.

Ziegler, A., Zaugg, C. E., Buser, P. T., Seelig, J. & Künnecke, B. (2002). Noninvasive measurement of myocardial carbon metabolism using *in vivo* ^{13}C NMR spectroscopy. *NMR Biomed* 15, 1-13.

Goldsbury, C., Goldie, K., Pellaud, J., Seelig, J., Frey, P., Müller, S. A., Kistler, J., Cooper, G. J. & Aebi, U. (2000). Amyloid fibril formation from full-length and fragments of amylin [In Process Citation]. *J Struct Biol* 130, 352-362.

Künnecke, B., Küstermann, E. & Seelig, J. (2000). Simultaneous in vivo monitoring of hepatic glucose and glucose-6-phosphate by (13)C-NMR spectroscopy [In Process Citation]. *Magn Reson Med* 44, 556-562.

Mader, I., Roser, W., Kappos, L., Hagberg, G., Seelig, J., Radü, E. W. & Steinbrich, W. (2000). Serial proton MR spectroscopy of contrast-enhancing multiple sclerosis plaques: absolute metabolic values over 2 years during a clinical pharmacological study [In Process Citation]. *AJNR Am J Neuroradiol* 21, 1220-1227.

Wieprecht, T., Apostolov, O., Beyermann, M. & Seelig, J. (2000). Interaction of a mitochondrial presequence with lipid membranes: role of helix formation for membrane binding and perturbation [In Process Citation]. *Biochemistry* 39, 15297-15305.

Wieprecht, T., Apostolov, O. & Seelig, J. (2000). Binding of the antibacterial peptide magainin 2 amide to small and large unilamellar vesicles [In Process Citation]. *Biophys Chem* 85, 187-198.

Schwarz, R., Kaspar, A., Seelig, J. & Künnecke, B. (*in press*). Gastrointestinal transit times in mice and humans measured with ^{27}Al- and ^{19}F-nuclear magnetic resonance. *Magn Res Med*.

Wieprecht, T., Beyermann, M. & Seelig, J. (*in press*). Thermodynamics of the coil-α-helix transition of amphipathic peptides in a membrane environment: the role of vesicle curvature. *Biophys Chem*.

Wieprecht, T. & Seelig, J. (*in press*). Calorimetry to elucidate hydrophobic and electrostatic interactions between peptides and bilayers. In *Current Topics in Membranes Peptide Lipid Interactions* (Simon, S. A. & McIntosh, T. J., eds.).

Membrane barriers: passive diffusion and active transport

E. Landwojtowicz, X. Li Blatter, P. Nervi

In collaboration with R. Glück and R. Zurbriggen (Swiss Serum & Vaccine Institute, Bern), G. Isenberg (Tech. University, Munich), H.P. Merkle and C. Broggini (Institute for Pharmaceutical Sciences, ETH, Zurich)

Anna Seelig
Phone: +41 61 267 2206 Fax: +41 61 267 2189 E-mail: Anna.Seelig@unibas.ch
http://www.biozentrum.unibas.ch/aseelig

Biological membranes are selective diffusion barriers. The barrier permeability is regulated by the lipid composition which, in turn, determines the lateral packing density of the lipid bilayer (Seelig & Seelig 2002, in *Encyclopedia of Physical Science and Technology*). Partitioning of compounds into the cell membrane is modulated by transporters of the ATP binding cassette family such as P-glycoprotein (Pgp) and the multidrug resistance protein (MRP1). They extrude a wide variety of chemically unrelated exogeneous and endogeneous compounds out of the cell membrane at the expense of ATP hydrolysis. These transporters are expressed at high levels in membranes with protective functions such as the blood-brain (BBB) and the intestinal (IB) barrier. They are also expressed in the membranes of certain tumors and bacteria to which they confer *multi drug resistance*.

Passive diffusion through the BBB and the IB

A fast and simple technique has been developed to reliably predict passive diffusion of a drug through specific biological membranes (Fischer *et al.* 1998, *J. Membr. Biol. 165*, 201-11). It is based on surface activity measurements which provide four parameters: (i) the air-water partition coefficient, K_{aw}, (ii) the cross-sectional area, A_D, (iii) the critical micelle concentration, CMC, and (iv) the solubility limit. Parameters (i-iii) were calibrated with a large number of compounds with known ability to diffuse through the intestinal barrier (IB) and/or the blood-brain barrier (BBB). The limiting size of the parameters was shown to differ for the two barriers.

Knowledge of K_{aw} and A_D of drugs and of the packing density, π, of the lipid bilayer allows direct estimation of the lipid-water partition coefficient, K_{lw} (Fischer *et al.* 1998, *J. Membr. Biol. 165*, 201-11).

Structure - activity relationship for Pgp and MRP1

The analysis of the three-dimensional structures of a large number of Pgp and MRP1 substrates revealed recognition elements (Seelig *et al.* 2000, *Int. J. Clin. Pharm. Ther. 38*, 111-21; Seelig & Landwojtowicz 2000, *Eur. J. Pharm. Sci. 12*, 31-40; Seelig, *in press*, in *Drug Bioavailability/Estimation of Solubility, Permeability, and Absorption*) consisting of two or three electron donor groups with specific spatial separations. Both transporters seem to recognize the same three-dimensional arrangement of electron donor groups, most probably via hydrogen bond formation. They differ, however, in their charge specificity (Fischer *et al.* 1998, *J. Membr. Biol. 165*, 201-11).

Drug-induced Pgp activation in living cells

A new assay based on measurements of the extracellular pH by means of the Cytosensor® technique was developed to monitor the kinetics of drug-induced Pgp-ATPase activation in living cells overexpressing Pgp. The activation profiles were interpreted in terms of a model assuming activation with one substrate molecule bound and inhibition with two molecules bound to the transporter. The data reveal direct coupling between proton extrusion and intracellular phosphate release (Landwojtowicz *et al.* submitted, *Biochemistry*).

Publications

Fischer, H., Gottschlich, R. & Seelig, A. (1998). Blood-brain barrier permeation: molecular parameters governing passive diffusion. *J Membr Biol* 165, 201-211.

Seelig, A. & Landwojtowicz, E. (2000). Structure-activity relationship of P-glycoprotein substrates and modifiers. *Eur J Pharm Sci* 12, 31-40.

Seelig, A., Li Blatter, X., Frentzel, A. & Isenberg, G. (2000). Phospholipid binding of synthetic talin peptides provides evidence for an intrinsic membrane anchor of talin. *J Biol Chem* 275, 17954-17961.

Seelig, A., Li Blatter, X. & Wohnsland, F. (2000). Substrate recognition by P-glycoprotein and the multidrug resistance-associated protein MRP1: a comparison. *Int J Clin Pharmacol Ther* 38, 111-121.

Zurbriggen, R., Novak-Hofer, I., Seelig, A. & Glück, R. (2000). IRIV-adjuvanted hepatitis A vaccine: *in vivo* absorption and biophysical characterization. *Prog Lipid Res* 39, 3-18.

Seelig, A. & Seelig, J. (2002). Membrane structure. In *Encyclopedia of Physical Science and Technology* 3rd edition edit. (Meyers, R. A., ed.), Vol. 9, pp. 355-367. Academic Press, New York.

Landwojtowicz, E., Nervi, P. & Seelig, A. (*in press)*. Real-time monitoring of P-glycoprotein activation in living cells. *Biochemistry*.

Seelig, A. (*in press)*.Towards P-glycoprotein structure-activity relationships. In *Drug Bioavailability/ Estimation of Solubility, Permeability, and Absorption* (Waterbeemd, V. d., Lennernäs & Artursson, eds.). Wiley-VCH, Weinheim.

DIVISION OF PHARMACOLOGY/NEUROBIOLOGY

In contrast to other divisions of the Biozentrum, which are part of the Natural Science Faculty of the University (Phil. II), the Division of Pharmacology/Neurobiology is also an institute of the Medical Faculty of the University of Basel. In this context it is responsible for the teaching of pharmacology and pharmacotherapy to students of medicine and dentistry. In the biology curriculum, the members of the division have a major role in teaching of neurobiology, physiology, and cell biology for undergraduate students. They also teach neurobiology and molecular medicine in the graduate program of the Biozentrum. The division had a leading role in planning and implementing the new biology curriculum of the University of Basel that was introduced in 2000. The division also hosts the MyoContract GmbH, the first University spin-off biotech-company in the Biozentrum. The 2000 Robert Bing Preis was awarded to Markus Rüegg of our Division.

The Division of Pharmacology/Neurobiology at present houses 4 senior research groups headed by Hans-Peter Hauri, Karl Hofbauer, Urs A. Meyer and Markus Rüegg, and 2 junior groups headed by Andreas Lüthi and Anita Lüthi. Independent research is also carried out by Dieter Walz. Karl Hofbauer holds an endowed chair financed by the Novartis Research Foundation and the group of Andreas Lüthi is financed by the Borderline Personality Disorder Research Foundation.

The research projects undertaken in the division are described below.

Professors

Hans-Peter Hauri, Karl G. Hofbauer, Andreas Lüthi, Anita Lüthi, Urs A. Meyer, Markus A. Rüegg

University lecturer

Dieter Walz

Scientific collaborators

Andreas Crameri*, Esther Gerez*, Simone Grumbacher-Reinert

Scientists and postdoctoral fellows

Remo Amherd*, Albert Angele*, Gabriela Bezakova*, Lionel Breuza*, Alexandre Briguet*, Giuseppe Cassata*, Karol Dokladny*, David Fraser, Carmela Gnerre*, Christoph Handschin*, Franziska Kuhn*, Lu Liang*, Shuo Lin, Ralf Meyer*, Joachim Moll, Ulrich Nordheim*, Mikael Oscarson*, Nicole Probst*, Adrian Roth*, Claudia Senn*, Cecile Vedrenne*

Graduate students

Christian Appenzeller, Manuel Baader, Houchaima Ben Tekaya*, Alexandre Briguet*, Stephanie Bissiere*, Godela Bittcher*, Alexander Eusebio, Samuel Frère*, Christoph Handschin*, Christoph Hangartner*, Xian Chu Kong, Iwona Ksiazek*, Nicola McIntyre*, Sandra Mitrović*, Joachim Moll, Oliver Nufer, Michael Podvinec, Gayathri Sadasivam*, Patrick Scotton, Sandra Ziegler*

Diploma students

Christian Bacher*, Patrizia Barzaghi*, Godela Bittcher*, Sharon Blättler*, Martin Degen*, Michael Friberg*, Roland Kälin*, Sarina Meinen*, Sandra Mitrovic*, Beat Nyfeler*, Adrian Reisch*, Beat Ryff*, Alfred Schnüriger*, Christine Wong*, Adrian Zumsteg*

Technical personnel

Patrizia Barzaghi*, Markus Beer, Käthy Bucher, Francine Hoffmann*, Jeannette Holenstein, Michel Kaufmann, Ronald König*, Renate Looser*, Bettina Ludwig*, Suzanne Moes*, Filippo Olivieri*, Beat Schumacher*, Romana Tomka*, Wolfgang Ziemke*

Floor manager

Roger Jenni

Secretariat

Marianne Liechti, Jny Wittker

Laboratory helpers

Angele Klein, Monika Furrer

* Employed during part of the report period

Protein traffic in the secretory pathway

C. Appenzeller, H. Ben Tekaya, L. Breuza, K. Bucher, G. Cassata, M. Degen, R. Kälin, F. Kuhn, L. Liang, S. Mitrovic, O. Nufer, B. Nyfeler, A. Schnüriger, G. Sadasivam, C. Vedrenne

In collaboration with K. Bienz (Univ. Hospital, Basel), R. Baumeister (Univ. Munich), R. Kaufman (Univ. Michigan), J. Klumperman (Univ. Utrecht), A. Lustig and B. Kammerer (Biozentrum, Basel), K. Matter (Univ. College, London), J.-P. Paccaud (Univ. Geneva), I. Pastan (NIH, Bethesda), A.-C. Roche (Univ. Orléans), H. Riezman (Biozentrum, Basel), Riordan (Mayo Clinic, Rochester), P. St George-Hyslop (Univ. Toronto), E. Sztul (Univ. Alabama), U. Seligsohn (Sheba Medical Center, Israel), K. Tani and M. Tagaya (Univ. Tokyo)

Hans-Peter Hauri
Phone: +41 61 267 2222 Fax: +41 61 267 2208 E-mail: Hans-Peter.Hauri@unibas.ch
http://www.biozentrum.unibas.ch/hauri

The secretory pathway has moved to center stage in biomedical research in recent years. Many inherited human diseases are due to defects of protein maturation and secretion. 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 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 mammalian cells using morphological, biochemical, biophysical, molecular and genetic approaches. Knowledge of these fundamental processes of cell biology may ultimately lead to new strategies for the treatment of inherited and acquired diseases in which protein secretion is impaired.

Organization and dynamics of the early secretory pathway

The molecular basis of how the 3 major compartments of the early secretory pathway, the ER, the ER-Golgi intermediate compartment (ERGIC) and the Golgi apparatus, adopt and maintain characteristically different structures remains largely unknown. The ERGIC is now best defined by the cycling type I membrane marker protein ERGIC-53 that accumulates in this organelle. Our morphological studies show that the ERGIC is a dynamic membrane system composed of a constant average number of tubulovesicular clusters near the Golgi apparatus and in the cell periphery, always close to ER membranes. A major function of the ERGIC is the sorting of anterograde (to the Golgi) and retrograde (to the ER) protein traffic. We are currently studying this bidirectional traffic in living cells to obtain insight into the transport mechanisms and the dynamics of the ERGIC.

Biogenesis of ER subdomains

The structure and dynamics of the ER depends on membrane-cytoskeleton interactions. We have discovered that the integral ER membrane protein CLIMP-63, formerly termed p63, binds microtubules in vivo and in vitro. Further analysis of CLIMP-63 provided novel information on the mechanism of ER-subdomain-specific protein localization. CLIMP-63 is excluded from the nuclear envelope. Using mutagenesis in conjunction with GFP technology, and structural analysis, including CD spectroscopy, electron microscopy and analytical ultracentrifugation, we found that the luminal domain of CLIMP-63 is necessary and sufficient for oligomerization into α-helical complexes that prevent nuclear membrane localization. Concentration of CLIMP-63 into patches may enhance microtubule binding on the cytosolic side and contribute to ER morphology by the formation of a protein scaffold in the lumen of the ER.

Lectin-mediated glycoprotein transport

Intracellular animal lectins play important roles in quality control and glycoproteins sorting along the secretory pathway. The major cycling lectin is ERGIC-53. ERGIC-53 is a mannose lectin that operates as a cargo receptor mediating efficient transport of an increasing number of secretory glycoproteins including cathepsin C, cathepsin Z and blood coagulation factors V and VIII. Non-functional ERGIC-53 is the molecular basis of the human genetic disease combined factor V and VIII deficiency leading to hemophilia. ERGIC-53

is the most prominent member of a family of animal lectins that also includes VIP36. These lectins are related to leguminous plant lectins. We have now cloned other members of this family and are currently characterizing them.

Mechanism of protein export from the ER

Protein sorting in the ER is believed to occur by two opposing processes, selective ER retention and selective ER export. We use ERGIC-53 as a model protein to uncover the mechanism of selective ER-export. In this study we have discovered an unanticipated role of cytosolically exposed C-terminal amino acids of type I membrane proteins in ER export. Three different minimal motifs were defined that facilitate ER export. The motifs are common among type I membrane proteins. They mediate binding to COPII proteins that drive transport vesicle formation at the ER. Most strikingly a single C-terminal valine can act as a transport signal when attached to a reporter protein. These result support the notion that ER export is signal-mediated. The precise mechanism of signal/coat interaction remains to be determined.

Role of ERGIC-53 in *C. elegans*

ERGIC-53 has been conserved from *C. elegans* to man and there is a homologous protein in yeast. To explore the function of ERGIC-53 in a primitive multicellular organism we have included C. elegans into our analysis. ERGIC-53 was found to be expressed in all cells of C. elegans with high levels in actively secreting cells. Inactivation of ERGIC-53 by RNAi leads to an egg laying defect that may reflect a secretion problem. This project is currently continued as a collaboration with G. Cassata and R. Baumeister (University of Munich).

Search for novel proteins cycling in the early secretory pathway by proteomics of the ERGIC

Proteins cycling in the early secretory pathway play important roles in membrane trafficking. To identify novel cycling proteins we took advantage of the observation that upon treatment of cells by brefeldin A (BFA), such proteins accumulate in the ERGIC defined by ERGIC-53. This is in marked contrast to resident Golgi proteins that accumulate in the ER after BFA treatment. We developed a new method to highly purify ERGIC membranes from BFA-treated human cells. The purified fraction was resolved by SDS-PAGE and subjected to tandem mass spectrometry. Major identified proteins corresponded to known cargo receptors and proteins involved in vesicular transport or quality control. Novel and non-characterized proteins are being cloned and characterized.

Publications

Appenzeller, C., Andersson, H., Kappeler, F. & Hauri, H. P. (1999). The lectin ERGIC-53 is a cargo transport receptor for glycoproteins. *Nature Cell Biol* 1, 330-334.

Linstedt, A. D. & Hauri, H. P. (1999). *Giantin*. Guidebook to the Cytoskeletal and Motor Proteins (Vale, T. K. a. R., Ed.), Oxford University Press, New York.

Moussalli, M., Pipe, S. W., Hauri, H. P., Nichols, W. C., Ginsburg, D. & Kaufman, R. J. (1999). Mannose-dependent ERGIC-53-mediated ER to Golgi trafficking of coagulation factors V and VIII. *J Biol Chem* 274, 32539-32542.

Cassata, G., Röhrig, S., Kuhn, F., Hauri, H. P., Baumeister, R. & Bürglin, T. R. (2000). The Caenorhabditis elegans Ldb/NLI/Clim orthologue ldb-1 is required for neuronal function. *Develop Biol* 226, 45-56.

Hauri, H., Appenzeller, C., Kuhn, F. & Nufer, O. (2000). Lectins and traffic in the secretory pathway. *FEBS Lett* 476, 32-37.

Hauri, H. P., Kappeler, F., Andersson, H. & Appenzeller, C. (2000). ERGIC-53 and traffic in the secretory pathway. *J Cell Sci* 113, 587-596.

Mizoguchi, T., Nakajima, K., Hatsuzawa, K., Nagahama, M., Hauri, H. P., Tagaya, M. & Tani, K. (2000). Determination of functional regions of p125, a novel mammalian Sec23p-interacting protein. *Biochem Biophys Res Commun* 279, 144-149.

Muniz, M., Nuoffer, C., Hauri, H. P. & Riezman, H. (2000). The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J Cell Biol* 148, 925-930.

Alvarez, C., Garcia-Mata, R., Hauri, H. P. & Sztul, E. (2001). The p115-interactive proteins GM130 and giantin participate in endoplasmic reticulum-Golgi traffic. *J Biol Chem* 276, 2693-2700.

Klopfenstein, D. R., Klumperman, J., Lustig, A., Kammerer, R. A., Oorschot, V. & Hauri, H. P. (2001). Subdomain-specific localization of CLIMP-63 (p63) in the endoplasmic reticulum is mediated by its luminal α-helical segment. *J Cell Biol* 153, 1287-1300.

Rust, R. C., Landmann, L., Gosert, R., Tang, B. L., Hong, W., Hauri, H. P., Egger, D. & Bienz, K. (2001). Cellular COPII proteins are involved in production of the vesicles that form the poliovirus replication complex. *J Virol* 75, 9808-9818.

Nufer, O., Guldbrandsen, S., Degen, M., Kappeler, F., Paccaud, J. P., Tani, K. & Hauri, H. P. (2002). Role of cytoplasmic C-terminal amino acids of membrane proteins in ER export. *J Cell Sci* 115, 619-628.

Hauri, H. P., Nufer, O., Breuza, L., Ben Tekaya, H. & Liang, L. *(in press)*. Lectins and protein traffic early in the secretory pathway. *Biochem Soc Symp*.

Chair for Applied Pharmacology, Novartis Research Foundation

K. Dokladny, C. Hangartner, S. Moes, U. Nordheim, C. Senn

In collaboration with: D. Guerini, C. Huppertz, B. Nüsslein (all Novartis)

Karl G. Hofbauer
Phone: +41 61 267 1645 Fax: +41 61 267 2208 E-Mail: Karl.Hofbauer@unibas.ch
http://www.biozentrum.unibas.ch/hofbauer

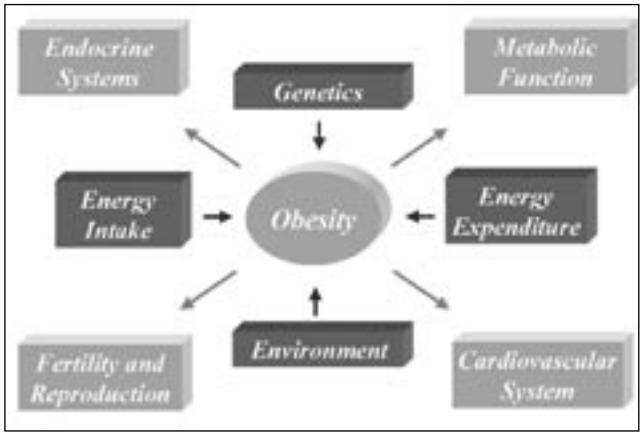
The group, which was established in October 2000 through a donation by the Novartis Research Foundation, received in 2001 additional support by Novartis Pharma to initiate a joint program with 3 postdoctoral fellows. The work in this program, which represents a new model for the collaboration between industry and academia, is performed in laboratories at the Biozentrum and at Novartis Pharma Research Basel. By July 2001 the postdoc positions were filled and the regular group meetings started.

Three research programs on different topics (central regulation of appetite, thermogenesis and energy expenditure, overweight and cardiovascular function) but under a common theme (pharmacological concepts for weight loss and reduction of risk factors in the treatment of obesity) were defined. A PhD student working on the design of antisense oligonucleotides as pharmacological tools also contributes to the program. The initial efforts were mainly devoted to methodological aspects and the establishment of the necessary techniques including animal models and biochemical assays. By the beginning of 2002, most of the required methodology had been established and first experimental results became available. Depending on the development in the programs, one additional PhD student will be recruited during the course of 2002.

A program on *in silico* simulation of the regulation of energy balance was started early in 2002. This entails computer modelling of selected pathways controlling energy intake and expenditure and complements experimental results with theoretical analysis.

The integration of the group into the Biozentrum proceeded well, although the installation of the *in vivo* research facilities was delayed due to the reconstruction of the animal house. When the experimental facilities become available, the equipment for chronic cardiovascular and metabolic studies in mice and rats will be installed at the Biozentrum. Although primarily intended for the use by our group, help and support in the phenotyping of genetically modified mouse strains will be offered to other groups of the Biozentrum.

Figure1:
The size of body fat mass is the result of the balance between energy intake and energy expenditure. If intake is too high for a given expenditure or if expenditure is too low for a given intake, obesity will develop over the long term. An imbalance between energy intake and expenditure can be caused by genetic or environmental factors. In most cases, both genetic and environmental factors act together in the pathogenesis of overweight and obesity. Adipose tissue is not only an important energy depot, but also an active endocrine organ, which has an influence on cardiovascular and metabolic function, fertility and endocrine systems. It is therefore understandable that obesity is associated with various cardiovascular, metabolic and endocrine diseases.



The group also became an official member of the Pharmazentrum and established close contacts to the university hospital by co-founding the OMeN (Obesity, Metabolism and Nutrition) seminar series. This initiative was started by several academic investigators with a common interest in this field and could be the nucleus for future joint research projects.

The scientific output during the build-up phase consisted in several invited lectures and in reviews on the pathophysiology and pharmacotherapy of obesity, published in or submitted to various international journals. In collaboration with the Swiss Society for Pharmacology and Toxicology, a one-day congress for cardiologists (blockade of the renin-angiotensin system in the treatment of congestive heart failure) was organized.

Publications

Chiesi, M., Huppertz, C. & Hofbauer, K. G. (2001). Pharmacotherapy of obesity - Targets and perspectives. *Trends Pharm Sci* 22, 247-254.

Hofbauer, K. G. (*in press)* Pathophysiologie der Adipositas: Moleküle, Mechanismen, Modelle. *Swiss Medical Forum.*

Hofbauer, K. G. & Huppertz, C. Pharmacotherapy and Evolution (*in press*). *Trends in Ecology and Evolution*.

Hofbauer, K. G. Molecular Pathways to Obesity. *Review submitted to Int J Obesity*.

Neural substrates of emotions

S. Bissière, N. Kemp, R. König

In collaboration with S. Arber (Biozentrum, Basel), B. Bettler (Institute of Physiology, Basel), W. Brake (UCSB, Santa Barbara), I. Mansuy (ETH, Zurich), B. McEwen (Rockefeller University, NewYork), H.-R. Olpe (Novartis, Basel)

Andreas Lüthi
Phone: +41 61 267 1644 Fax: +41 61 267 2208 E-mail: Andreas.Luthi@unibas.ch
http://www.biozentrum.unibas.ch/andreasluethi

How does the brain form memories of emotional events? My laboratory is interested in understanding the molecular and cellular mechanisms underlying the formation and retrieval of emotional memories. Using *in vitro* and *in vivo* electrophysiological methods, we are investigating activity- and experience-dependent changes in the neural circuitry of the amygdala of the mouse, a brain structure that plays a pivotal role in emotional behavior, particularly fear, in both humans and animals.

One of the most powerful models to study fear and anxiety is classical fear conditioning, a form of Pavlovian conditioning. In Pavlovian fear conditioning, the subject, often a rat or a mouse, is exposed to an unconditioned stimulus, a noxious stimulus such as a footshock, in conjunction with a conditioned stimulus, a neutral stimulus such as a tone or a light. As a result, the tone acquires aversive properties and will, on next occurrence, trigger a fear response. Behavioral and *in vivo* electrophysiological experiments suggest that associative synaptic plasticity in the amygdala underlies Pavlovian fear conditioning. However, the molecular and cellular mechanisms of associative synaptic plasticity in the amygdala are poorly understood.

Molecular and cellular mechanisms of synaptic plasticity in the amygdala

A hallmark of Pavlovian fear conditioning is the requirement for temporal coincidence of the conditioned and the unconditioned stimulus during training. To understand the mechanisms underlying the induction of associative synaptic plasticity during fear conditioning, we have determined the time windows during which coincident activity in the lateral amygdala (LA) induces long-term potentiation, a form of associative synaptic plasticity. Our results indicate that associative synaptic plasticity, considering the low average firing rate of LA neurons, is precise and input-specific. We are investigating the molecular and cellular mechanisms underlying the induction of associative synaptic plasticity, how the timing rules can be modulated, and how input-specificity is achieved.

The activity of LA neurons is tightly controlled by GABAergic inhibition. In a second project we could show that inhibitory synaptic transmission prevents the induction of associative synaptic plasticity, and that it is strongly depressed by dopamine. Given that dopaminergic input to the amygdala is required for the acquisition and retrieval of fear memories, we hypothesize that dopamine might be a key factor controlling the induction of associative synaptic plasticity in the LA. We are investigating how dopamine affects associative synaptic plasticity, and, by using transgenic mice expressing Green Fluorescent Protein in specific LA interneurons targeted by dopaminergic afferents, determining how dopamine depresses synaptic inhibition.

Impact of early life stress on amygdala function

There is converging evidence that a number of psychiatric disorders involving anxiety and emotional dysregulation, such as posttraumatic stress disorder or borderline personality disorder, are associated with a dysfunction of the amygdala. Based on the association of borderline personality disorder with early life stress, we are interested in the long-term consequences of early stress on the physiology of the amygdala and the medial prefrontal cortex, a brain region that strongly modulates the amygdala. To this end, we are using mice that have been repeatedly separated from their mother during the first two weeks of postnatal development as an animal model for early life stress. We are applying behavioral, biochemical, and electrophysiological techniques to correlate changes in behavior with physiological alterations.

Ultimately, we expect that a combined *in vitro* and *in vivo* approach, aimed at understanding the synaptic and cellular mechanisms of fear learning and their regulation by environmental and genetic factors in the mouse, will help us to reveal some of the mechanisms underlying anxiety disorders in humans.

Publications

Benke, T. A., Lüthi, A., Isaac, J. T. R., Anderson, W. W. & Collingridge, G. L. (2001). Non-stationary noise analysis in branched structures: limitations and usage for determination of changes in the unitary conductance of synaptic AMPA receptors. *J Physiol (Lond)* 537, 407-420.

Bissière, S. & Lüthi, A. (2001). Dopaminergic modulation of inhibitory synaptic transmission in the mouse lateral amygdala in vitro. *Soc Neurosci Abstr* 27, 143.

Lüthi, A., Schwyzer, L., Mateos, J. M., Gähwiler, B. H. & McKinney, R. A. (2001). NMDA receptor activation limits the number of synaptic connections during hippocampal development. *Nat Neurosci* 4, 1102-1107.

Subcellular, cellular and network pacemakers in the sleeping and epileptic brain

A. Angele, S. Frère, A. Reisch

In collaboration with Andreas Engel (Division of Structural Biology and Maurice E. Müller Institute, Biozentrum); Urs A. Wiedemann (CERN, Geneva)

Anita Lüthi
Phone: +41 61 267 2246 Fax: +41 61 267 2208 E-mail: Anita.Luthi@unibas.ch
http://www.biozentrum.unibas.ch/anitaluethi

Subcellular, cellular and network pacemakers in the sleeping and epileptic brain

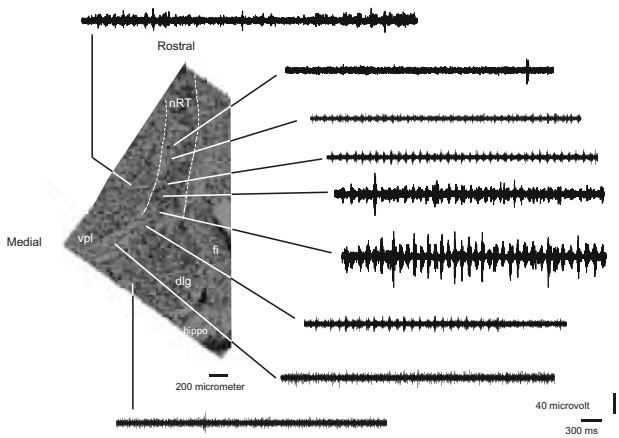
Rhythmic activities, such as those occurring during heart beat and respiration, are of fundamental importance in life. To enable rhythmicity, nature has crafted internal clocks or pacemakers, each of which ticks at its intrinsic frequency. The term “clock” appears even only as a weak metaphor, considering the power of those rhythms, which coordinate the state of arousal with variations in body temperature, locomotion, breathing rates and secretion of hormones – thus defining the entire state of our body.

Biological systems make much wider use of rhythms than is intuitively apparent: ever since the discovery of electrical activity in the nervous system we know that, during sleep, wave-like electrical impulses rhythmically inundate large areas of the brain. These rhythms contribute to disconnecting us from the sensory environment while we are asleep. Abnormal function of rhythm generators further illustrates the importance of proper pacemakers: some epileptic patients suffer from periodic, sudden attacks that are generated within an important part of the brain responsible for generating sleep-related rhythms, the thalamus. In our electrophysiological research, we are interested in the regulation of cell-intrinsic and circuit elements that determine normal and abnormal rhythm generation, as well as other, functions in the thalamus.

Generation of rhythmic synchronized discharges in neural networks *in vitro*

How does the brain autonomously generate rhythmicity during sleep and how could we study the networks involved? We have developed an *in vitro* slice from mouse thalamus that maintains the thalamic network in high intactness. Extracellular recordings reveal that the neural networks within this slice synchronize spontaneously to generate a rhythmic discharge that is typical for early periods of mammalian sleep, indicating that essential cellular and network pacemakers of sleep physiology can be preserved in a rodent brain slice. The “*in vitro* sleeping” thalamic slice shows synchronized network rhythms that appear as 3-10 cycles of 5-11 Hz discharges followed by a silent period of 5-30 seconds. Neurons from the caudal nucleus reticularis thalami (nRT) show repetitive burst discharges in synchrony with rhythm generation (see Figure 1). We currently assess the involvement of three characteristics of nRT neurons in neural synchronization *in vitro*: 1) excitation of nRT neurons via glutamatergic synaptic innervation by thalamocortical neurons 2) electrical coupling within the nRT via gap junctions 3) intrinsic repetitive burst discharges of nRT cells at 10 Hz, resulting from the cyclical activation of Ca^{2+} currents and Ca^{2+}-activated K^+ currents, and the modulation of these by neurotransmitters. Elucidation of the role of these

Figure 1



Topographic distribution of spontaneous oscillations illustrated in a Nissl-stained mouse slice. The region of strongest oscillations is restricted to ~0.5 mm in the most caudal area of the nucleus reticularis (nRT). Weak oscillations are also observed in the ventroposterolateral nucleus (vpl) from presumed thalamocortical (TC) neurons.

factors in rhythm generation provides mechanistic insight into physiologically relevant network function and may help delineate critical factors involved in epilepsy (e.g. potentiation of intrinsic bursting of nRT cells), generalized anesthesia and sleep disorders.

Regulation of cell-intrinsic pacemakers by cAMP

A central pacemaker for thalamic rhythmicity resides within an intrinsic electrical current that flows across cellular membranes and periodically dictates electrical discharge capabilities. This voltage-dependent ionic current is also found in the heart where it contributes to modulation of heart rate in response to signals from the autonomous nervous system. The pacemaker current is increased when intracellular cAMP levels are raised, and this modulation is involved in the transition between different modes of thalamic function. To gain insight into the dynamics of cAMP synthesis and degradation, and to assess the regulatory profile of thalamic adenylyl cyclases (ACs), we measure pacemaker currents via whole-cell patch-clamp recordings. Initial experiments revealed that at least three types of ACs with unique regulatory properties appear to be functionally coupled to the pacemaker current. Some of these ACs are tonically active, some others appear to be inhibited or stimulated exclusively under defined patterns of activation of G-protein-coupled neurotransmitter receptors. Interestingly, some of these patterns lie close to those found during epileptiform activity in the thalamus, suggesting that the regulation of thalamic ACs and the pacemaker current appears to represent a site of subcellular interpretation of the physiological context to which thalamic cells are exposed. Therefore, ACs may serve as a target to pharmacologically control transitions between different modes of thalamic activity.

Publications

Santoro, B., Chen, S., Luthi, A., Pavlidis, P., Shumyatsky, G. P., Tibbs, G. R. & Siegelbaum, S. A. (2000). Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J Neurosci* 20, 5264-75.

Luthi, A. (2001). Spontaneous rhythm generation in a thalamic slice preparation from mouse. *Soc Neurosci Abstr* 27, 605.

Luthi, A., Di Paolo, G., Cremona, O., Daniell, L., De Camilli, P. & McCormick, D. A. (2001). Synaptojanin 1 contributes to maintaining the stability of GABAergic transmission in primary cultures of cortical neurons. *J Neurosci* 21, 9101-11.

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

R. Amherd, M. Baader, Ch. Bacher, M. Beer, S. Blättler, A. Crameri, D. Fraser, Ch. Handschin, G. Gnerre, F. Hoffmann, M. Kaufmann, R. Looser, B. Ludwig, R. Meyer, M. Oscarson, M. Podvinec, N. Probst, A. Roth, B. Ryff, A. Zumsteg

In collaboration with M. Affolter (Biozentrum); U. Zanger, M. Eichelbaum (Stuttgart, Germany); Heme/Biotech A/S Hillerod, Denmark); Epidauros, Biotechnologie AG (Bernried, Germany); Clondiag Technologies (Jena, Germany).

Urs A. Meyer
Phone: +41 61 267 2220 Fax: +41 61 267 2208 E-mail: Urs-A.Meyer@unibas.ch
http://www.biozentrum.unibas.ch/meyer

Introduction

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 for 40 years 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 to develop adverse drug reactions, 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 more than one allele 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. Over the last few years, we have investigated the molecular mechanisms of several common genetic polymorphisms of drug metabolizing enzymes: 1) the debrisoquine/sparteine or cytochrome P4502D6 (CYP2D6) polymorphism; 2) the mephenytoin or cytochrome P4502C19 (CYP2C19) polymorphism, and 3) the polymorphism of N-acetyltransferases (NAT). These polymorphisms are transmitted as autosomal recessive traits and cause impaired inactivation of numerous clinically important drugs or chemical carcinogens in so-called “poor metabolizer” individuals. For each of these 3 polymorphisms the target enzymes have been purified in our laboratory from human liver, the normal and mutant genes cloned, sequenced and expressed and mutations causing these variations identified. 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

Numerous drugs and chemicals induce the expression of drug- and steroid-metabolizing enzymes in the liver. Induction of these enzymes has a major impact on drug effects including drug-drug interactions, drug-toxicity and xenobiotic carcinogenicity and contributes to interindividual variability in drug response. Induction most often occurs at the level of transcription and prototypical drug- and other xenobiotic inducers include polycyclic aromatic hydrocarbons (e.g. dioxin), phenobarbital (PB), peroxisome proliferators (e.g. clofibrate) and steroids (e.g. dexamethasone). Each of these classes of compounds induce a different but overlapping pattern of enzymes. Recent advances in our understanding of the induction response suggest the following general mechanisms of transcriptional activation: The xenobiotics interact with intracellular proteins either of the basic helix-loop-helix class (polycyclic aromatic hydrocarbons, AhR system) or with so-called orphan nuclear receptors such as PPAR (peroxisome proliferator activated receptor), CAR (constitutively activated receptor, affected by phenobarbital and phenobarbital-like-inducers) or PXR (pregnane x receptor, activated by dexamethasone, rifampicin, etc.). Each of these intracellular proteins forms heterodimers with other proteins (AhR with Arnt,

CAR and PXR with the retinoid x receptor RXR) and the heterodimer binds to DNA recognition motifs, so-called drug or xenobiotic response elements. Many of the details of the induction mechanism remain to be elucidated. In particular, the endogenous ligands of PXR and CAR and the detailed mechanisms of transcriptional activation are unknown. Inducers of cytochrome P450s of the CYP3A family apparently can directly bind to the ligand binding domain and activate PXR. It remains unclear, how phenobarbital-like inducers increase CAR-RXR binding to the DNA recognition sequences characterized as phenobarbital-response units (PBRU) or motifs (PBREM). Moreover, phosphorylation/dephosphorylation events, cytokines and hormones affect the induction response by as yet unknown mechanisms. This lack of knowledge is in part due to methodological difficulties. Most of the known phenobarbital- and rifampicin-inducible enzymes are not expressed or not inducible in presently known stable mammalian cell lines.

We are using 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 chicken embryo liver in ovo, in primary cultures of chicken embryo hepatocytes, and in LMH cells cytochromes, P450 remain highly inducible by phenobarbital-like inducers.

In reporter gene studies in LMH cells with the 5'-flanking region of chicken CYP2H1 we have discovered a phenobarbital-responsive 264 bp enhancer unit. Within this enhancer element, we have identified a conserved nuclear receptor consensus sequence (a DR-4 element) which mediates induction in reporter gene assays. In parallel, we have used homology concepts to clone the avian nuclear receptor CXR (chicken xenobiotic sensing receptor). This orphan nuclear receptor has properties of both PXR and CAR and may represent the evolutionary precursor of these transcription factors. CXR also affects the drug-induction in chicken liver of two additional cytochromes P450s recently cloned and characterized in our laboratory, CYP3A37 and CYP2C45. Our experiments in LMH cells also suggest 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 and 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.

As induction of CYPs by drugs is observed in all eucaryotes (a notable exception is yeast) we have speculated that drug-induction of CYPs involves an endogenous regulation common to all living organisms. Firstly, CYPs catalyze not only the metabolism of xenobiotics, but also steroids, fatty acids, cholesterol and bile acids, and secondly, CAR, PXR and CXR belong to the subfamily of orphan nuclear receptors comprising also receptors involved in cholesterol homeostasis, including the oxysterol receptor LXR (liver X receptor) and the bile acid receptor (BAR). In the absence of uptake of exogenous cholesterol, intracellular cholesterol content is controlled via de novo synthesis of cholesterol from acetylcoenzymeA and catabolism of cholesterol to bile acids. We therefore have tested the hypothesis that the mechanism of induction of CYPs can be explained by interactions between xenobiotic-sensing nuclear receptors and nuclear receptors involved in cholesterol and bile acid homeostasis. We compared the effect of squalestatin (SQ1, a potent inhibitor of cholesterol synthesis), oxysterols and bile acids on both the basal and inducible expression of CYP2H1 and CYP3A37, in LMH cells. SQ1 potently induced both CYPs and shared signaling pathways with the drug regulation of these genes. Moreover, we discovered that bile acids also induce CYP2H1 and confirm recent data in mammals that bile acids regulate CYPs. Interestingly, hydroxylated bile acids and hydroxycholesterols inhibit drug activation of CYP2H1 and this is due to activation of LXR. LXR apparently binds to the same response elements as CXR, CAR and PXR and inhibits the induction of drug-metabolizing CYPs. These interactions between cholesterol homeostasis mechanisms and induction of CYPs is now further investigated in mouse lines with deficiencies of LXR, CXR, CAR and PXR.

Role of heme synthesis during induction of cytochromes P450

An important aspect of CYP regulation is the role of heme synthesis in drug-mediated induction of CYP-hemeproteins. This has been a controversial issue for many years. The supply of heme for the assembly of hemeproteins in the liver is controlled by the activity of 5-aminolevulinic acid synthase (ALAS1). Drug-induction of CYPs in the liver and other tissues requires coordinated increases in ALAS1 and apocytochromes. How does the cell achieve this coordination?

The expression of the ALAS1 protein is tightly controlled by a regulatory heme pool, which affects different negative feedback mechanisms. Heme inhibits induction of ALAS1 in the liver and depresses ALAS1 activity by blocking mitochondrial import of ALAS1 preprotein and by destabilizing ALAS1 mRNA. Therefore, it was assumed that induction of apocytochromes P450 by drugs would deplete the

regulatory heme pool and derepress heme-mediated inhibition of ALAS1 synthesis. Our own studies have demonstrated that induction of CYPs by drugs involves parallel increases in the transcription of ALAS1 and CYP genes and is not dependent on apocytochrome synthesis. The molecular mechanism that determines the drug-induced, tissue-specific transcription of the ALAS1 gene has not been studied. It concerns one of the main projects of our research team. As a first step in this direction, we have cloned the flanking region of chicken, mouse and human ALAS1 and have discovered drug-responsive enhancer elements.

The mechanism by which ALAS1 is induced by drugs is of particular importance in diseases associated with deficiencies in hepatic heme synthesis, such as inducible hepatic porphyrias. In these disorders, drugs including phenobarbital, rifampicin, and other CYP inducers precipitate acute attacks of neuropsychiatric dysfunction and these attacks are associated with massively induced ALAS1. We have developed a transgenic mouse model of a partial heme deficiency which is unique as an experimental tool and has been successfully used to study the effects of heme limitation on the transcription of ALAS1 and CYP genes and to assess new treatment models to suppress the induction of ALAS1 in patients with porphyria. We intend to use these mice to understand how xenobiotics induce ALAS1 and precipitate acute attacks of porphyria.

Publications

Pharmacogenetics/Pharmacogenomics

Amherd, R., Hintermann, E., Walz, D., Affolter, M. & Meyer, U. A. (2000). Purification, cloning, and characterization of a second arylalkylamine N-acetyltransferase from *Drosophila* melanogaster. *DNA Cell Biol* 19, 697-705.

Eiselt, R., Domanski, T. L., Zibat, A., Mueller, R., Presecan-Siedel, E., Hustert, E., Zanger, U. M., Brockmoller, J., Klenk, H.-P., Meyer, U. A., Khan, K. K., He, Y.-A., halpert, J. R. & Wojnowski, L. (2001). Identification and functional characterization of eight CYP3A4 protein variants. *Pharmacogenetics* 11, 447-458.

Gellner, K., Eiselt, R., Hustert, E., Arnold, H., Koch, I., Haberl, M., Deglmann, C. J., Burk, O., Buntefuss, D., Escher, S., Bishop, C., Koebe, H.-G., Brinkmann, U., Klenk, H.-P., Kleine, K., Meyer, U. A. & Wojnowski, L. (2001). Genomic organization of the human *CYP3a* locus: identificationof a new, inducible *CYP3A* gene. *Pharmacogenetics* 11, 1-11.

Wong, J. M. Y., Harper, p. A., Meyer, U. A., Bock, K. W., Mörike, K., Lagueux, J., Ayotte, P., Tandale, R. F., Sellers, E. M., Manchester, D. K. & Okey, A. B. (2001). Ethnic variability in the allelic distributionof human aryl hydrocarbon receptor codon 554 and assessment of variant receptor function *in vitro*. *Pharmacogenetics* 11, 85-94.

Meyer, U. A. (2000). Drugs and toxins - drug handling. In *Liver Cirrhosis and its Development* (Boyer, J. L. e. a., ed.), pp. 236-241. Kluwer Academic Publishers.

Meyer, U. A. (2000). Pharmacogenetics. In *Melmon & Morelli's Clinical Pharmacology* (Carruthers, G. S., Hoffman, B. B., Melmon, K. L. & Nierenberg, D. W., eds.), Vol. 4th edition, pp. 1179-1205. McGraw-Hill, New York.

Meyer, U. A. (2000). Pharmacogenetics and adverse drug reactions. *Lancet* 356, 1667-71.

Meyer, U. A. (2001). Pharmacogenetics - Clinical Viewpoints. In *Pharmacogenomics* (Kalow, W., Meyer, U. A. & Tyndale, R. F., eds.), pp. 135-150. Marcel Dekker, Inc., New York, Basel.

Meyer, U. A. (2001). Pharmakogenomik. *Medgen* 13, 246-248.

Kalow, W., Meyer, U. A. & Tyndale, R. F. (eds.) (2001). *Pharmacogenomics*, Marcel Dekker, New York, Basel.

Meyer, U. A. & Gut, J. (*in press*). Genomics and the prediction of xenobiotic toxicity.

Meyer, U. A. (*2002*). Introduction to pharmacogenomics: promises, opportunities, and limitations. In *Pharmacogenomics* (Licinio, J. & Wong, M., eds.). Wiley-VCH.

Transcriptional regulation of cytochromes P450 by drugs

Handschin, C. & Meyer, U. A. (2000). A conserved nuclear receptor consensus sequence (DR-4) mediates transcriptional activation of the chicken CYP2H1 gene by phenobarbital in a hepatoma cell line. *J Biol Chem* 275, 13362-9.

Handschin, C., Podvinec, M. & Meyer, U. A. (2000). CXR, a chicken xenobiotic-sensing orphan nuclear receptor, is related to both mammalian pregnane X receptor (PXR) and constitutive androstane receptor (CAR). *Proc Natl Acad Sci U S A* 97, 10769-74.

Ourlin, J. C., Baader, M., Fraser, D., Halpert, J. R. & Meyer, U. A. (2000). Cloning and functional expression of a first inducible avian cytochrome P450 of the CYP3A subfamily (CYP3A37). *Arch Biochem Biophys* 373, 375-384.

Handschin, C., Podvinec, M., Stöckli, J., Hoffmann, K. & Meyer, U. A. (2001). Conservation of signaling pathways of xenobiotic-sensing orphan nuclear receptors, chicken xenobiotic receptor, constitutive androstane receptor, and pregnane X receptor, from birds tu humans. *Mol Endocrinol* 15, 1571-1585.

Handschin, C., Podvinec, M., Looser, R., Amherd, R. & Meyer, U. A. (2001). Multiple enhancer units mediate drug-induction of CYP2H1 by xenobiotic-sensing orphan nuclear receptor Chicken Xenobiotic Receptor. *Mol Pharm* 60, 681-689.

Baader, M., Gnerre, C., Stegeman, J. J. & Meyer, U. A. (2002) Transcriptional activation of cytochrome P-450 CYP2C45 by drugs in mediated by the chicken xenobiotic receptor CXR interacting with a phenobarbital-response enhancer unit. *J Biol Chem*. 277, 15647-15653.

Ourlin, J. C., Handschin, C., Kaufmann, M. & Meyer, U. A. (2002). A Link between Cholesterol Levels and Phenobarbital Induction of Cytochromes P450. *Biochem Biophys Res Commun* 291, 378-384.

Podvinec, M., Kaufmann, M. R., Handschin, C. & Meyer, U. A. (2002) NUBIScan, an in-silico approach for prediction of nuclear receptor response elements. *Mol Endocrin*. 16, 1269-1279.

Handschin, C., Podvinec, M., Amherd, R., Looser, R., Ourlin, J.C. & Meyer, U.A. (2002). Cholesterol and bile acids regulate xenosensor signaling in drug-mediated induction of cytochromes P450. *J. Biol. Chem.* 10, 1074.

Heme synthesis

Fried, R. & Meyer, U. A. (2000). Porphyrien. In *Klinische Gastroenterologie und Stoffwechsel* (Adler, G. & Meyer, U. A., eds.), pp. 837-845. Springer-Verlag, Berlin.

Schuurmans, M. M., Hoffmann, F., Lindberg, R. & Meyer, U. A. (2001). Zinc mesoporphyrin represses induced hepatic 5-aminolevulinic acid synthase and reduces heme oxygenase activity in a mouse model of acute hepatic porphyria. *Hepatology* 33, 1217-1222.

Synapse formation and neuromuscular diseases

P. Barzaghi, G. Bezakova, G. Bittcher, A. Briguet, A. Eusebio, M. Friberg, I. Ksiazek, X. C. Kong, S. Lin, S. Meinen, J. Moll, F. Oliveri, P. Scotton, B. Schumacher, Ch. Wong

In collaboration with J.L. Bixby (University of Miami, Miami, U.S.A.); H.R. Brenner (Institute of Physiology, Basel); U. Certa (Hoffmann-LaRoche, Basel); J. Engel & J. Stetefeld (Div. of Biophysical Chemistry, Biozentrum); H. Lochmüller (Genzentrum Munich, Germany); T. Meier (MyoContract Pharmaceutical Research Ltd., Biozentrum); U. Müller (Friedrich-Miescher Institute, Basel); J.R. Sanes (Washington University, St. Louis, U.S.A)

Markus A. Rüegg
Phone: +41 61 267 2223 Fax: +41 61 267 2208 E-Mail: Markus-A.Ruegg@unibas.ch
http://www.biozentrum.unibas.ch/rueegg

Among the most remarkable feats of the brain is its capability to process information, which is to a large part determined by the complexity of the neural network. The cellular units underlying information processing in the brain are synapses. To warrant efficient information transfer, synapses are highly specialized both on the pre- and on the postsynaptic site. Pathological alterations in synaptic functions are a hallmark in many psychotic and neurodegenerative diseases, such as Depression, Parkinson's and Alzheimer's disease. Despite the fact that synapses play a central role in brain function and dysfunction, very little is known about the molecules that are involved in the formation and the modulation of synapses in the brain.

The long-term goal of our research is to identify and characterize molecules that affect the formation and the modulation of synaptic connections and to apply this knowledge to disease. Technical limitations have made it difficult to study these events on a molecular level in the brain. In contrast, the cholinergic neuromuscular junction (NMJ) allows the detailed study of its structure and function. Hence, most of our knowledge on the molecular mechanisms involved in the formation of synapses, derives from studies on the NMJ.

Development of the neuromuscular junction

Formation of synaptic structures at the NMJ is independent of electrical activity but requires the exchange of trophic factors between the presynaptic motor neuron and the postsynaptic muscle fiber. One of the hallmarks of postsynaptic differentiation is the aggregation of acetylcholine receptors (AChRs) underneath the nerve terminal. Subsequently, gene transcription of "synaptic" genes is selectively ceased in non-synaptic myonuclei but is maintained in the myonuclei underneath the nerve terminal. All these events result in a highly specialized and complex structure at the site of contact between motor neuron and muscle fiber (Fig. 1).

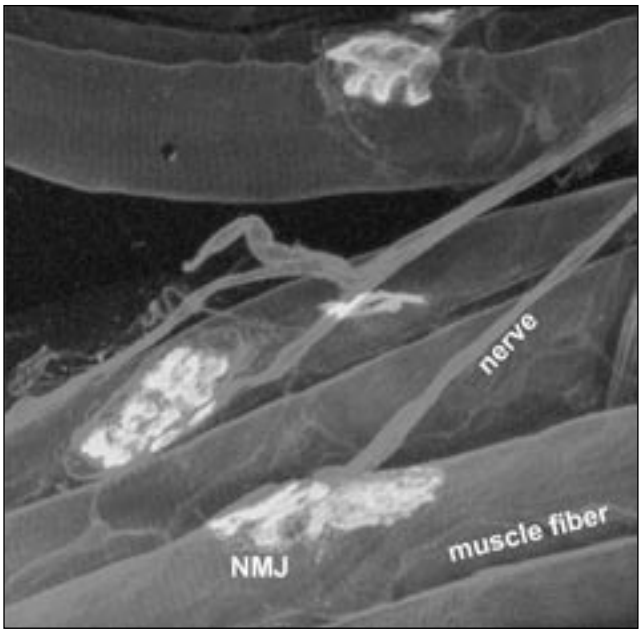


Figure 1:
Whole mount view of muscle fibers that are innervated by single motor neurons. At the neuromuscular junction (NMJ), hallmarks of postsynaptic specializations include aggregates of acetylcholine receptors (light grey) opposite to the innervating nerve.

Still little is known about the molecules involved in the induction of the presynaptic nerve terminal. In contrast, research over the last decade has provided ample evidence that the heparan sulfate proteoglycan agrin, which is released from developing motor neurons and tightly binds to synaptic basal lamina, is required and sufficient to induce the formation of the postsynaptic apparatus. This postsynapse-inducing activity of agrin is mediated by the activation of a receptor tyrosine kinase, called MuSK. Activation of

MuSK then triggers an intracellular signaling cascade, which results in changes of cytoskeletal organization and activation of a secondary pathway that causes changes of gene transcription in subsynaptic myonuclei. This secondary pathway involves the neuregulin/erbB system and the ETS transcription factor GABP.

One agrin gene, several activities

Agrin cDNAs encode a protein of more than 2'000 amino acids with modules that are characteristic for extracellular matrix molecules. In addition, the protein is heavily glycosylated both at N- and O-linked docking sites, assigning agrin to the family of heparan sulfate proteoglycans. Alternative mRNA splicing at the amino-terminal end generates two distinct isoforms of agrin. One represents a secreted form characterized by a cleaved signal sequence followed by a domain that is required and sufficient for the binding of agrin to extracellular matrix. The second amino-terminus encodes an internal, non-cleaved signal peptide that converts agrin to a type II transmembrane protein. The expression of these splice variants differs between tissues and is likely to be important for the proper localization of agrin in the respective tissues that do or do not contain basal lamina (e.g. the NMJ versus the brain). Besides the N-terminal differences, additional splicing events near the C-terminus have a decisive influence on agrin's function to induce postsynaptic structures *in vivo*. Only those isoforms that contain amino acid inserts at these splice sites are capable of inducing postsynaptic structures. Interestingly, the expression pattern of the carboxy-terminal splice variants also varies between different tissues. In particular, neurons express splice versions containing amino acid inserts (i.e. postsynapse-inducing forms) and non-neuronal cells those that lack amino acid inserts (i.e. inactive forms). These data show that agrin's activities are under a tight developmental and tissue-specific control.

Role of agrin isoforms in the brain and at the NMJ

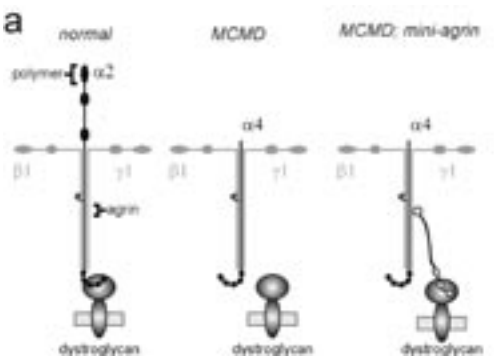
The organizing role of neural agrin in the development of the NMJ and the expression of agrin during synapse formation in the brain would argue for its role as a general molecular code that orchestrates synaptogenesis. Agrin-deficient mice die perinatally because of the failure to form NMJs, thus making it impossible to analyze synapse structure and function in these mice at postnatal stages. To determine the role of agrin in the CNS, we are currently investigating the brain phenotype of agrin-deficient mice whose NMJ phenotype was rescued by the transgenic expression of chick neural agrin in motor neurons. Such mice survive birth but are still grossly abnormal and die at the age of 3 weeks to approximately 3 months. The brains of these agrin-deficient mice show no gross abnormality in the overall structure but a highly significant decrease in the number of synaptic boutons. These results indicate that agrin deficiency influences neuron-to-neuron synapses and they provide first *in vivo* evidence for a role of agrin in the central nervous system. We are currently analyzing the molecular mechanisms underlying this loss of synapses and their consequences on brain function.

As outlined above, agrin isoforms expressed in non-neuronal tissue, which lack the postsynapse-inducing activity of neural agrin, do not play a primary role in the induction of postsynapses at the NMJ. In an attempt to unravel the function of non-neuronal agrin at the NMJ, we use mice in which this agrin isoform is overexpressed in muscle. We find a profound difference in synapse maturation as their NMJs continue to look "immature" for at least six months. This phenomenon is likely to be due to an increased stability of postsynaptic structures and the failure of reshaping the synapse. Consistent with this interpretation, electron microscopy shows that postsynaptic folds and AChRs are detected at sites that are devoid of presynaptic terminals. These experiments are direct evidence that non-neuronal agrin, via its binding to α-dystroglycan and to laminin (see also Figure 2a next page), plays a role in later stages of synapse development.

Gene expression profiles during synapse formation

One of the biggest challenges in our research field is to unravel the molecular mechanisms involved in the formation and the maintenance of synaptic connections between neurons. It is an old question whether the NMJ is a good model to understand synapse formation and function in the CNS. The past has provided ample evidence that the principal mechanisms of synaptic transmission are identical at the NMJ and at CNS synapses. Thus, it is highly probable that the molecular principles that govern the formation and maintenance of synaptic connections are also similar. Because it is still impossible to devise experimental paradigms that are selective enough to specifically identify candidate genes involved in synapse formation in the brain, we identify such genes at the NMJ. To this end, we employ a newly developed technique where postsynaptic structures are induced in non-synaptic regions of rat muscle by the application of recombinant neural agrin. This method allows impregnation of the entire muscle basal lamina and to control the time of muscle exposure to agrin. As a result of such treatment, many postsynapse-like structures are induced that have all the features characteristic of the postsynaptic apparatus at the NMJ. These include the activation/silencing of gene transcription in subsynaptic myonuclei. With this method we are currently analyzing gene expression

Figure 2a:
Schematic model of the connection between the extracellular matrix molecule laminin-2 (α2, β1, γ1) and the muscle cell receptor dystroglycan in wild-type mice (normal). Regions in the α2 chain necessary for laminin polymerization (polymer) and binding to agrin are indicated. In mice deficient of the laminin α2 chain (MCMD), the homologous laminin α4 chain is upregulated and combines with the β1 and the γ1 chain to laminin-8. The laminin α4 chain is truncated at the amino-terminus and binds only weakly to dystroglycan. The mini-agrin, which binds to laminin-8 and dystroglycan, was used to test whether it could restore linkage of muscle extracellular matrix and the muscle cell (MCMD; mini-agrin).



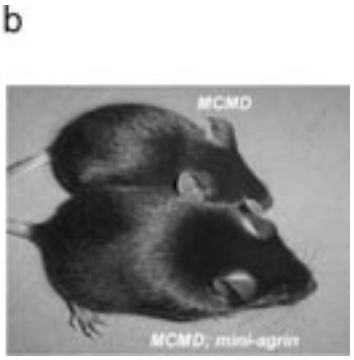


Figure 2b:
The mice overproducing mini-agrin (MCMD; mini-agrin) are bigger and healthier than their littermates that are deficient of the α2 chain of laminin (MCMD).

profiles induced by the formation of postsynaptic structures *in vivo*. This is done using state-of-the-art genomics in collaboration with Hoffmann-LaRoche and the Core Lab for Genomics at the Biozentrum.

We are also in the stage of devising experimental paradigms that allow us to study the function of candidate genes *in vivo*. In one approach, we use RNA interference (RNAi), which has successfully been used to silence gene transcription in *C. elegans* and *Drosophila* but not in mammals. Our preliminary results strongly indicate that we can specifically perturb the formation of postsynaptic structures by co-injection of dsRNA encoding genes known to be required for NMJ formation. This is the first time that RNAi has successfully been applied in adult mammals *in vivo* and we will use this technique to study the function of genes identified in the screen outlined above.

Neuromuscular diseases

Pathological changes of neuromuscular connection and of muscle fiber integrity result in muscle weakness, progressive muscle atrophy and muscle wasting. In the most severe cases, patients die of respiratory failure. The finding that some of the genes involved in NMJ development are also relevant in the pathology of muscular diseases has lead us to test whether "replacement therapies" can successfully be used to treat such diseases. We tested this hypothesis for "merosin-deficient congenital muscular dystrophy", a relatively rare and severe muscle wasting disease, caused by mutations in the α2 chain of the most abundant laminin isoform found in skeletal muscle and peripheral nerve (Fig. 2a). The laminin α2 chain is the main extracellular ligand of the muscle membrane receptor α-dystroglycan. Because the non-neuronal form of agrin binds with high affinity to extracellular matrix (i.e. laminin) and α-dystroglycan, we hypothesized that introducing this ligand may compensate for the loss of the laminin α 2 chain. To test this we generated genetically engineered mice that overproduce a miniaturized version of the agrin protein (called mini-agrin). Indeed, muscle function and structure is restored in mini-agrin overproducing mice, and they survive much longer than their non-transgenic littermates (Fig. 2b).

Our studies are the first example of functional compensation of a diseased phenotype by an engineered molecule of the extracellular matrix, whose design was based on functional and not on structural similarity between the mutated α2 chain of laminin and agrin. Our results are thus "the proof-of-principle" that rational design and the use of small mimetics of large proteins can be useful for gene therapy. Such mimetics can be incorporated into existing viral vectors and are less likely to cause immunological responses. In the case of agrin, such gene therapy may not require high transfection efficiency because mini-agrin is secreted from muscle fibers and can act on neighboring, non-transfected fibers. In addition, the use of pharmacologically active compounds that increase the level of expression of the endogenous agrin could be another promising strategy in the treatment of congenital muscular dystrophy. In the future, we will critically verify this concept by using a set of other rationally designed molecules that also share a common function. In addition, it will be important to show that these mimetics can ameliorate the phenotype in more advanced stages of the disease. If successful, our concept may become a starting point for devising new strategies in the treatment of not only muscular dystrophies but of several genetically-defined diseases.

Publications

Briguet, A. & Ruegg, M. A. (2000). The ets transcription factor GABP is required for postsynaptic differentiation in vivo. *J Neurosci* 20, 5989-5996.

Halfter, W., Dong, S., Schurer, B., Osanger, A., Schneider, W., Ruegg, M. & Cole, G. J. (2000). Composition, synthesis, and assembly of the embryonic chick retinal basal lamina. *Dev Biol* 220, 111-128.

Meier, T. & Ruegg, M. A. (2000). The role of dystroglycan and its ligands in physiology and disease. *News Physiol Sci* 15, 255-259.

Sommer, B., Barbieri, S., Hofele, K., Wiederhold, K., Probst, A., Mistl, C., Danner, S., Kauffmann, S., Spooren, W., Tolnay, M., Bilbe, G., van der Putten, H., Kafmann, S., Caroni, P. & Ruegg, M. A. (2000). Mouse models of α-synucleinopathy and Lewy pathology. *Exp Gerontol* 35, 1389-1403.

van der Putten, H., Wiederhold, K. H., Probst, A., Barbieri, S., Mistl, C., Danner, S., Kauffmann, S., Hofele, K., Spooren, W. P., Ruegg, M. A., Lin, S., Caroni, P., Sommer, B., Tolnay, M. & Bilbe, G. (2000). Neuropathology in mice expressing human α-synuclein. *J Neurosci* 20, 6021-9.

Alexandrescu, A. T., Maciejewski, M. W., Ruegg, M. A., Engel, J. & Kammerer, R. A. (2001). 1H, 13C and 15N backbone assignments for the C-terminal globular domain of agrin. *J Biomol NMR* 20, 295-6.

Moll, J., Barzaghi, P., Lin, S., Bezakova, G., Lochmuller, H., Engvall, E., Muller, U. & Ruegg, M. A. (2001). An agrin minigene rescues dystrophic symptoms in a mouse model for congenital muscular dystrophy. *Nature* 413, 302-7.

Neumann, F. R., Bittcher, G., Annies, M., Schumacher, B., Kroger, S. & Ruegg, M. A. (2001). An alternative amino-terminus expressed in the central nervous system converts agrin to a type II transmembrane protein. *Mol Cell Neurosci* 17, 208-25.

Ruegg, M. A. (2001). Molecules involved in the formation of synaptic connections in muscle and brain. *Matrix Biol* 20, 3-12.

Stetefeld, J., Jenny, M., Schulthess, T., Landwehr, R., Schumacher, B., Frank, S., Ruegg, M. A., Engel, J. & Kammerer, R. A. (2001). The laminin-binding domain of agrin is structurally related to N-TIMP-1. *Nat Struct Biol* 8, 705-9.

Thermodynamics and kinetics of energy conversion

Dieter Walz (GL, will retire in 2002)
W. Ziemke

In collaboration with S.R. Caplan (Weizmann Institute of Sciences, Rehovot, Israel), R. Naumann (Max-Planck-Institute for Polymer Research, Mainz, Germany), T. Walz (Harvard Medical School, Boston, USA)

Energy conversion is a key feature of biological systems. The free energy associated with a given process is not entirely dissipated as entropy while the process is running „downhill“ along its thermodynamic force, but is in part transferred to another process which then can run „uphill“ against its force.

The bacterial flagellar motor converts the energy of the electrochemical potential difference for protons across the cytoplasmic membrane into mechanical energy of the flagellar rotation. As mentioned in the previous report we have designed an electrostatic model for this motor which closely reproduces a large number of experimental data from different laboratories. This model was extended to include Brownian motion of the rotor and the elasticity of the stalks of the force generating units. Monte Carlo simulations with the extended model confirmed the results obtained with the deterministic description used previously. Moreover, the “locking” of the motor under de-energized conditions could be simulated with the assumption that the channels are closed under such conditions, for which some experimental evidence exists. The variance in rotation period was also calculated from the Monte Carlo simulations, and the experimentally observed dependence of this variance on rotation frequency could be reproduced with rigid as well as elastic stalks, providing appropriate values for the parameters describing the elasticity are chosen. This dependency has been interpreted as arising from a stepping of the motor, but such a feature is not inherent in our model. Monte Carlo simulations with several stepping motor models indeed showed that this interpretation is not correct.

The H^+/ATP synthase converts the energy of the electrochemical potential difference for protons across the cytoplasmic, the mitochondrial or the thylakoid membrane into chemical energy of the phosphorylation reaction. A rotary part of the enzyme that forms a stalk (γ and ε subunit of F_1) is twisted by the proton movement in F. The torsional energy thus stored is used for ATP synthesis in F_1 whereby the twist of the stalk is released. We work on a kinetic model for this mechanism by modifying and/or extending already existing models such that it will be suitable for simulations with SPICE (Walz et al., 1995). This model shall enable us to simulate the behavior of the enzyme if subjected to AC membrane potentials in a large frequency range. It should then be possible to interpret experimental data obtained by R. Naumann with H^+/ATP synthase reconstituted into membranes, which are attached to a gold electrode by means of an array of spacer molecules with a defined length.

A model for the binding of transferrin to the transferrin receptor in the presence and absence of the competing ligand HFE is currently being worked out. It shall serve to evaluate the experimental data obtained by T. Walz using electron microscopic images of single particles.

The experimental work on the estimation of the volume per chlorophyll of thylakoid membranes was concluded. The difference in values mentioned in the previous report, and found with different radio-labelled markers on the one hand and with ^3H-H_2O on the other hand could be confirmed, thus corroborating the concept of a hydration layer on the membrane into which markers other than ^3H-H_2O cannot penetrate.

Publications

Walz, D., Caplan, S. R., Scriven, D. R. L. & Mikulecky, D. C. (1995). *Methods of mathematical modelling*. *Bioelectrochemistry: Principles and Practice. Bioelectrochemistry: General Introduction.* (Caplan, S. R., Miller, I. R. & Milazzo, G., Eds.), 1, Birkhäuser Verlag, Basel.

Amherd, R., Hintermann, E., Walz, D., Affolter, M. & Meyer, U. A. (2000). Purification, cloning, and characterization of a second arylalkylamine N-acetyltransferase from *Drosophila melanogaster*. *DNA and Cell Biol* 9, 697-705.

Walz, D. & Caplan, S. R. (2000). An electrostatic mechanism closely reproducing observed behavior in the bacterial flagellar motor. *Biophys J* 78, 626-51.

Walz, D. & Caplan, S. (2002). Bacterial flagellar motor and H^+/ATP synthase: two proton-driven rotary molecular devices with different functions. *Bioelectrochemistry* 55, 89-92.

Teissié, J., Walz, D. & Milazzo, G. (*in press*). *Bioelectro-chemistry: Principles and Practice. Bioelectrochemistry of Membranes*, 6, Birkhäuser Verlag, Basel.

DIVISION OF BIOINFORMATICS

SWISS INSTITUTE OF BIOINFORMATICS BASEL

Bioinformatics is an exciting branch of modern information technology that applies massive computing power to biological data management and analysis. This rapidly evolving field was spawned by recent technological advances in molecular biology that produced an unprecedented amount of information about DNA, RNA and proteins. A particularly promising application of bioinformatics is comparative protein modeling that predicts protein structures on the basis of similarities to known 3D structures. This approach should facilitate drug design as well as fundamental research that aims at unraveling mechanisms of action of important proteins.

The Division of Bioinformatics was established in 2001. It will ultimately consist of four independent research groups. A 48 processor Beowulf Cluster financed by Novartis provides large-scale computing resources. Torsten Schwede's lab works on comparative protein structure modeling with emphasis on its large-scale genomic application including web accessible databases of predicted protein structures. Michael Primig's lab develops a novel approach to knowledgebase construction in the field of gamete formation and germline development. In addition to that, he is involved in a Swiss-wide effort focusing on microarray data management and analysis within the framework of the Swiss Array Consortium (SAC).

During 2002, the University of Basel and the Swiss Institute of Bioinformatics (SIB) launched a collaborative effort to promote Bioinformatics at the Biozentrum. This constitutes the first step towards the establishment of an internationally competitive research and teaching program in a key area of biological research.

Professors
Michael Primig*, Torsten Schwede*

Scientists and postdoctoral fellows
Reinhold Koch*, Jürgen Kopp*

Graduate students
Flavio Monigatti*, Franziska Röder*

Visiting scientist
Michel Bellis (CRBM, Montpellier)

Technical personnel
Thomas Aust*, Philippe Demougin*, Jeannette Holenstein

Unix system administration
Rainer Pöhlmann

Floor manager
Roger Jenni

Secretariat
Doris Kündig

* Employed during part of the report period

Meiotic development in yeast

T. Aust, R. Koch

Visiting scientist: M. Bellis (CRBM, Montpellier)
In collaboration with S. Esposito (University of Chicago), B. Braun (University of Washington, Seattle), N. Lamb (IGH, Montpellier) and P. Jenoe (Biozentrum, Basle).

Michael Primig
Phone: +41 61 267 2098 Fax: +41 61 267 3398 E-mail: Michael.Primig@unibas.ch
http:www/biozentrum.unibas.ch/primig

Introduction

Diploid yeast cells vegetatively grow or, in the absence of nitrogen and a fermentable carbon source, undergo meiotic development. This process involves two rounds of chromosome segregation without an intervening S-phase, resulting in the production of four haploid spores. During sporulation in yeast at least 900 genes display a measurable transcriptional response as determined by whole-genome expression profiling using GeneChips. Most of these genes are expressed also during vegetative growth including some that display cell cycle regulated expression patterns (e.g. CLBs). The transcriptional profiles of these genes fall into 7 broad categories (expression clusters); it is noteworthy that timing of induction and time of function correlate well. The promoters of some of these genes contain binding sites for the Ume6/ Ime1, Abf1 and Ndt80 transcription factors known to be involved in meiotic gene expression. However, the 5'-upstream regions of the vast majority of meiotically regulated genes lack known regulatory elements, these genes should therefore be dependent upon as yet unidentified factors required for meiotic gene expression.

Expression profiling Ume6

Ume6 is the DNA binding subunit of a conserved chromatin-remodelling factor involving Sin3/Rpd3 and Isw2, that governs the transition from mitotic growth to meiotic differentiation in yeast. A large number of target genes containing the Ume6 binding site (URS1) were previously identified, but for most of these genes no evidence for regulation *in vivo* by Ume6p was available. Ume6 is known to be required for mitotic repression of certain meiotic genes. We are therefore particularly interested in the subset of ORFs/genes that are de-repressed in a mitotically growing ume6 mutant and induced in a sporulating wild type strain. We used whole-genome expression profiling to determine the pattern of deregulation in wild type versus ume6 deletion strains in the SK1 and W303 strain backgrounds. We compared the data sets obtained cells cultured in the presence of fermentable (glucose) and non-fermentable (acetate) carbon sources. 114 genes were identified as affected by the deletion of *UME6*, 49 of these have the cognate URS1 core DNA target sequence. The 114 genes were corroborated using two genetically distinct yeast strains (SK1 and W303) during growth in both glucose and acetate media. A high proportion of these genes fall into the functional classes of cell growth, mitotic division and DNA synthesis. A comparison of the genome-wide expression patterns indicates that W303 expressed genes required for gluconeogenesis (the synthesis of glucose from acetate) at much higher levels than SK1. Since glucose inhibits sporulation these results provide an explanation for SK1's rapid and W303's slow entry into the meiotic differentiation program after incubation in pre-sporulation medium containing acetate. A subgroup of 59 de-repressed genes and ORFs were also found to display meiotic induction following broader than expected expression patterns. These findings indicate that Ume6 regulates many more meiotic genes than initially anticipated.

A proteomics approach to the analysis of meiotic gene expression

Meiotically expressed genes fall into three categories. There are those that are meiosis specific and others that are constitutively expressed during both mitosis and meiosis. Another group are those that display cell cycle regulated expression pattern during mitotic growth and complex patterns of up-and downregulation during meiotic development. How are these complex regulatory events coordinated? To date we know only three site-specifically DNA binding transcription factors involved in meiotic gene expression (Ume6, Abf1 and Ndt80), while

several hundred meiotically regulated loci have been identified whose promoters do not contain matches to their target sites. In collaboration with Paul Jenoe, we will use affinity purification protocols, SDS and 2D gel analysis followed by Mass Spectrometry to identify novel DNA binding proteins specifically present in sporulating wild type but not in meiosis-deficient control strains. It likely that such factors play transcriptional roles. Furthermore, we wish to analyse the state of modification of these factors in the absence of Ime2, Rim11 and Rim15 protein kinases known to be important for meiotic gene expression through their interaction with Ume6 and Ime1 (a factor that binds Ume6 to form an activating complex); the aim of this experiment being to identify novel substrates of these kinase. This should lead to a better understanding of important and conserved landmark events during gametogenesis in yeast and, ultimately, in higher eukaryotes as well.

The GermOnline knowledgebase

Background and rationale

GermOnline (http://germonline.igh.cnrs.fr) is a unique subject-oriented gateway for gametogenesis. This new database differs from most of the major biological databases that contain information on specific experimental systems (e.g. SGD, Flybase, Wormbase, MGD) or biomolecules (Swissprot). In these databases direct comparison of data from different organisms is difficult. The primary goal of GermOnline is to provide rapid access to comprehensive information about genes, expression data and protein functions implicated in germline development, meiosis and gamete formation across systems. The project currently covers 11 key model systems as well as humans. A second important feature of GermOnline is that it enables the scientific community studying these processes (rather than annotators surveying the literature), to directly contribute, curate and update such information. An international board of curators is being assembled to oversee the database, thereby ensuring the highest scientific quality of its contents. To provide a world-wide round-the-clock service we have installed a network of GermOnline servers in the US and Europe. The database is currently hosted by Michael Primig at the Biozentrum (Basel, Switzerland), Bob Braun at the University of Washington (Seattle, US) and Ned Lamb at the Institute of Human Genetics (Montpellier, France). The database currently contains ~140000 gene information pages from 12 species that organise access to internal and external sources of information about genes, genomic expression patterns, gene products and protein-protein interactions.

Publications

Primig, M., Sockanathan, S., Auer, H. & Nasmyth, K. (1992). Anatomy of a transcription factor important for the start of the cell cycle in Saccharomyces cerevisiae. *Nature* 358, 593-7.

Prinz, S., Klein, F., Auer, H., Schweizer, D. & Primig, M. (1995). A DNA binding factor (UBF) interacts with a positive regulatory element in the promoters of genes expressed during meiosis and vegetative growth in yeast. *Nucleic Acids Research* 23, 3449-56.

Primig, M., Williams, R., Winzeler, E., Tevzadze, G., Conway, A., Hwang, S., Davis, R. & Esposito, R. (2000). The core meiotic transcriptome in budding yeasts. *Nature Genet.* 26, 415-424.

Rabitsch, K., Toth, A., Galova, M., Schleiffer, A., Schaffner, G., Elisabeth Aigner, Rupp, C., Penkner, A., Moreno-Borchart, A., Primig, M., Esposito, R. K., Franz , Knop, M. & Nasmyth, K. (2001). A screen for genes required for meiosis and spore formation based on whole genome expression. *Current Biology* 11, 1001-1009.

Protein structure bioinformatics (Swiss Institute of Bioinformatics)

Jürgen Kopp (starting 1.1.02), Flavio Monigatti (starting 1.3.02), Franziska Röder (starting 1.3.02)

In collaboration with Nicolas Guex (Swiss Institute of Bioinformatics & GlaxoSmithKline, Geneva), Manuel Peitsch (Novartis Pharma, Basel), Rolf Apweiler (European Bioinformatics Institute, Hinxton, UK), Stylianos Antonarakis (University of Geneva Medical School, Geneva), Peter Berndt (Roche, Basel), and Michael Primig (Division of Bioinformatics).

Torsten Schwede
Tel: +41 61 267 1581 Fax: +41 61 267 1584 E-mail: Torsten.Schwede@unibas.ch
http://www.biozentrum.unibas.ch/schwede

Understanding the molecular function of a protein is greatly facilitated by analysis of its 3-dimensional structure. Experimental determination of protein structures by X-ray crystallography and NMR is often hampered by technical problems; as a consequence we currently know 100 times more peptide sequences than 3D structures. Therefore, computational methods of structure prediction have gained much interest in recent years. My group is focusing on the development and application of methods in molecular modeling.

Comparative protein structure modeling

Comparative protein structure modeling (or homology modeling) predicts the three-dimensional structure of a given protein sequence (target) based on an alignment of this protein to one or more homologues proteins of known structure (templates). Despite recent improvements in *ab initio* structure predictions, comparative modeling is the most detailed and accurate of all current structure prediction techniques. All homology-modeling methods consist of the four steps: fold assignment and template selection, target-template alignment, model building and evaluation of the model structure.

It has been estimated that at the moment about one third of all sequences has detectable similarity to one or more known protein structures, indicating that homology modeling can be applied to about 230'000 of the currently 700'000 protein sequences in the SWISS-PROT and TrEMBL database. With regard to ongoing structural genomics efforts, the usefulness of comparative modeling is increasing steadily. It is anticipated that within less than a decade most structural folds of soluble non-membrane proteins will be known, and homology modeling can be applied to most protein sequences.

Facing the huge amount of data originating from genome sequencing projects and structural genomics studies, it is necessary to develop expert systems and fully automated methods that are fast and reliable enough to generate accurate models on the scale of whole genomes. The SWISS-MODEL server has been pioneering the field of automated homology modeling since 1994. With more than 100'000 modeling requests in 2001, SWISS-MODEL is the most widely used web-based modeling server. Our group in Basel is contributing to the development of SWISS-MODEL within the Swiss Institute of Bioinformatics in collaboration with N. Guex (GlaxoSmithKline, Geneva). The refinement of homology modeling techniques will be a major focus during the next years.

Integration of Protein Structure into Biological Knowledge Databases

The "Swiss Model Repository" is a database of annotated comparative protein structure models. It contains the results of automated large-scale modeling for sequences from the SwissProt / TrEMBL database using the SWISS-MODEL server pipeline. The integration of structural models with biochemical knowledge databases is a crucial step in any computational analysis of sequence-structure-function relationships. The links established from the Swiss Model Repository to InterPro or GermOnline will allow visualizing 3-dimensional protein models, highlighting regions of functional relevance. This integration will have direct applications in the validation of sequence-based assignments in a 3-dimensional structural context.

Structural bases of inherited human disease

Effects of genetic variations in the human genome range from neutral mutations over increased susceptibility for complex diseases, individual variation in drug response, to rare single-allele

mendelian inherited diseases. A large body of knowledge about disease related genetic variations have been accumulated in specific databases (e.g. OMIM, HGMD), including non-synonymous mutations in coding regions that are likely to affect protein function. Their correct identification is of great interest for studies of protein function and their implication in disease.

Three dimensional protein structure models are valuable to provide insights in the molecular bases of these mutations. In recent studies on a rare form of inherited deafness, we used homology modeling of the enzyme TMPRSS3 to rationalize the impact of the observed mutations on the protein function. While visual inspection of the structural context of mutations provides valuable insight in single cases, the development of general objective scoring functions is still an unsolved problem.

Our preliminary studies on mutations of human phenylalanine hydroxylase causing phenylketonuria showed that computational approaches based on protein structure models could significantly contribute to an objective quantification of mutational effects. The large-scale numerical analysis provides a better and quantitative understanding about the molecular mechanisms underlying a pathological phenotype. We now plan to extend our preliminary studies on phenylalanine hydroxylase on a broader basis of proteins.

Publications

Guex, N., Schwede, T. & Peitsch, M. C. (2000). Protein Tertiary Structure Modeling. In *Current Protocols in Protein Science*, pp. 2.8.1.-2.8.17. Wiley and Sons, New York.

Peitsch, M. C., Schwede, T. & Guex, N. (2000). Automated protein modelling--the proteome in 3D. 1, 257-66.

Schwede, T., Diemand, A., Guex, N. & Peitsch, M. C. (2000). Protein structure computing in the genomic era. *Research in Microbiology* 151, 107-12.

Masmoudi, S., Antonarakis, S. E., Schwede, T., Ghorbel, A. M., Gratri, M., Pappasavas, M. P., Drira, M., Elgaied_Boulila, A., Wattenhofer, M., Rossier, C., Scott, H. S., Ayadi, H. & Guipponi, M. (2001). Novel missense mutations of TMPRSS3 in two consanguineous Tunisian families with non-syndromic autosomal recessive deafness. *Human Mutation* 18, 101-8.

Guex, N., Schwede, T. & Peitsch, M. C. (2002). Imaging Science in Biochemistry. In *Encyclopedia of Imaging Science and Technology* (Hornak, J. P., ed.), pp. 693-709. Wiley and Sons, New York.

Wattenhofer, M., Di Iorio, M. V., Rabionet, R., Dougherty, L., Pampanos, A., Schwede, T., Montserrat-Sentis, B., Arbones, M., Iliades, T., Pasquadibisceglie, A., D'Amelio, M., Alwan, S., Rossier, C., Dahl, H. H. M., Petersen, M. B., Estivill, X., Gasparini, P., Scott, H. S. & Antonarakis, S. E. (2002). Mutations in the TMPRSS3 gene are a rare cause of childhood non-syndromic deafness in Caucasian patients. *Journal of Molecular Medicine* 80, 124-131.

THE LIFE SCIENCES TRAINING FACILTY

P. Demougin

In collaboration with E. Domany (Weizman Institute, Rehovot), M. Bellis (CRBM, Montpellier), E. Oakeley (FMI, Basle) and the members of the Swiss Array Consortium (http://www.sacnet.ch

Michael Primig
Phone: +41 61 267 2098 Fax: +41 61 267 3398 E-mail: Michael.Primig@unibas.ch
http:www/bioz.unibas.ch/corelab

Introduction
DNA microarrays have become an increasingly powerful tool in biomedical research. This technology is currently applied in two different ways: firstly, the molecular classification and characterisation of gene expression profiles underlying diseases like e.g. cancer, muscular pathophysiologies and the response to host-pathogen interactions. This type of experiment includes also the identification of complex patterns of transcriptional regulation during cell cycle progression in humans and yeast as well as gametogenesis for example in *C. elegans* and yeast. Secondly, analysis of genomic DNA to identify polymorphisms and genetic rearrangement as well as gene deletion and duplications.

High density oligonucleotide microarrays (GeneChips) & Microarray production
The LSTF provides the infrastructure for carrying out research projects based upon the Affymetrix GeneChip system. This includes training in RNA preparation, target synthesis and quality control, microarray hybridisation and scanning as well as data management & analysis. The LSTF provides indirect support for the Microarray Labs located at the Biozentrum and the Swiss Tropical Institute. This ensures that the hardware, software and know-how required to produce and process microarrays are firmly established in Basle for the benefit of the local scientific community.

Bioinformatics
Information management has become a major issue in biological research and a global standard has now been proposed that sets the rules for publishing genomics research (Minimum Information About a Microarray Experiment - MIAME). A major project in this field is GeneX that offers an open source standard for information management. The Swiss Array Consortium (SAC) has agreed to evaluate and possibly implement this solution in Switzerland. We have recently initiated the joint development of a microarray information analysis and management system in cooperation with the lab of Edward Oakeley at the FMI within the framework of the SAC whose aim it is to promote genomics & bioinformatics in Switzerland. It is our goal to develop a database and information exchange platform that integrates various commercially available programs needed for data production (Microarray Analysis Suite 5.0) and data analysis (GeneSpring 4.2, GeneSight 2.0). We are also in the process of implementing solutions provided by Eytan Domany (C2WC cluster server) and Michael Bellis (Arrayon 1.0 analysis and database suite) within the framework of scientific collaborations.

Publications

Primig, M., Sockanathan, S., Auer, H. & Nasmyth, K. (1992). Anatomy of a transcription factor important for the start of the cell cycle in Saccharomyces cerevisiae. *Nature* 358, 593-7.

Prinz, S., Klein, F., Auer, H., Schweizer, D. & Primig, M. (1995). A DNA binding factor (UBF) interacts with a positive regulatory element in the promoters of genes expressed during meiosis and vegetative growth in yeast. *Nucleic Acids Research* 23, 3449-56.

Primig, M., Williams, R., Winzeler, E., Tevzadze, G., Conway, A., Hwang, S., Davis, R. & Esposito, R. (2000). The core meiotic transcriptome in budding yeasts. *Nature Genet.* 26, 415-424.

Rabitsch, K., Toth, A., Galova, M., Schleiffer, A., Schaffner, G., Elisabeth Aigner, Rupp, C., Penkner, A., Moreno-Borchart, A., Primig, M., Esposito, R. K., Franz , Knop, M. & Nasmyth, K. (2001). A screen for genes required for meiosis and spore formation based on whole genome expression. *Current Biology* 11, 1001-1009.

PROTEIN CHEMISTRY

The availability of burgeoning sequence data bases demand ever faster and more efficient methods for global screening of protein expression. Two-dimensional gel electrophoresis, which allows one to visualize several hundred proteins at a time, offers the possibility to collect information on translational regulation, post-translational modifications and turnover rate for each protein on a proteome wide level. Today, more than 300 proteins corresponding to the products of 279 genes on a reference map of *Saccharomyces cerevisiae* have been identified (see also http://www.ibgc.u-bordeaux2.fr/YPM).

The complexity of the cellular proteome expands enormously if protein post-translational modifications are taken into account. Since many cellular responses to external stimuli involve protein phosphorylation as a means of transducing signals, there is a need for efficient methods for analyzing in parallel a large number of proteins involved in signal transduction pathways at as close to physiological conditions as possible. In particular, for the study of signal transduction cross-talk, tracking the changes in phosphorylation of serine/threonine and tyrosine under various conditions is essential. In order to develop and assess the technology required for efficient phosphoprotein analysis, a thorough investigation of selected signalling components of the rapamycin-dependent TOR-signalling pathway in the yeast *Saccharomyces cerevisiae* was initiated (see report of M. Hall).

Independent scientist
Alex Garvin

Graduate student
Debora Bonenfant

Technical personnel
Thierry Mini

In collaboration with M. Hall (Biozentrum) and H. Boucherie (IBGC, Bordeaux).

Paul Jenö
Phone: +41 61 267 2168 Fax: +41 61 267 2148 E-mail: Paul.Jenoe@unibas.ch
http://www.biozentrum.unibas.ch/jenoe

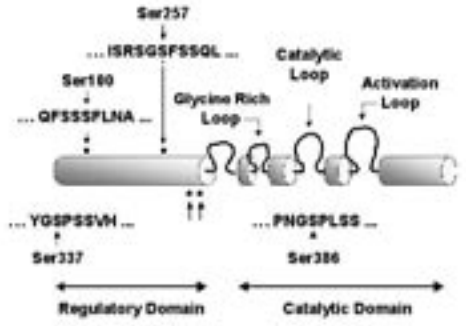
Mass spectrometric analysis of the rapamycin-dependent phosphorylation sites of the yeast protein kinase NPR1

Inactivation of TOR by the immunosuppressant drug rapamycin or by nitrogen starvation leads to a reprogramming of the cell's inventory of amino acid transporters: while high affinity transporters such as the tryptophan permease TAT2 are rapidly degraded by targeting to the vacuole, the broad range amino acid transporter GAP1 is upregulated and stabilized upon nitrogen starvation. The reprogramming of permeases in response to nutrient concentration is controlled via the Ser/Thr kinase NPR1, which becomes phosphorylated by the TOR signalling pathway. In cells growing in rich medium, NPR1 is highly phosphorylated; upon nitrogen starvation, or rapamycin treatment, NPR1 becomes rapidly dephosphorylated. To elucidate the TOR-specific phosphorylation sites on NPR1, a thorough mass spectrometric analysis of the phosphorylation pattern was carried out by comparing NPR1 from non-treated and rapamycin-treated yeast cells. The rapamycin sensitive sites were mapped to S/P or T/P sites and on serine residues followed by a hydrophobic phenylalanine (Fig. 1). The phosphorylation pattern strongly resembles the rapamycin-sensitive sites found in p70s6 kinase.

Quantification of changes in site-specific phosphorylation

Quantification of changes in the extent of phosphorylation presents an even greater analytical challenge than does the determination of the sites of phosphorylation. This is because the ionization efficiency of the corresponding pair of phosphorylated and nonphosphorylated peptide is not identical. We therefore devised a method which allows precise quantification of changes in the extent of

Figure 1



phosphorylation in proteins by mass spectrometry. In short, a phosphoprotein derived from a pool of cells (e.g., the wild type) is purified and digested with trypsin in normal (^{16}O) water, while the same protein (e.g. from a mutant strain) is digested in water enriched in ^{18}O (> (>96%). Equal amounts of the two pools are mixed, phosphopeptides specifically selected and the phosphate removed with alkaline phosphatase. In the spectra recorded on a MALDI-TOF instrument the signals from the wildtype and the mutant protein can easily be discerned by a 4 Da shift due to the incorporation of ^{18}O into the C-terminus of the peptide. The change in the extent of phosphorylation is easily calculated from the isotope ratio of the ^{16}O and ^{18}O

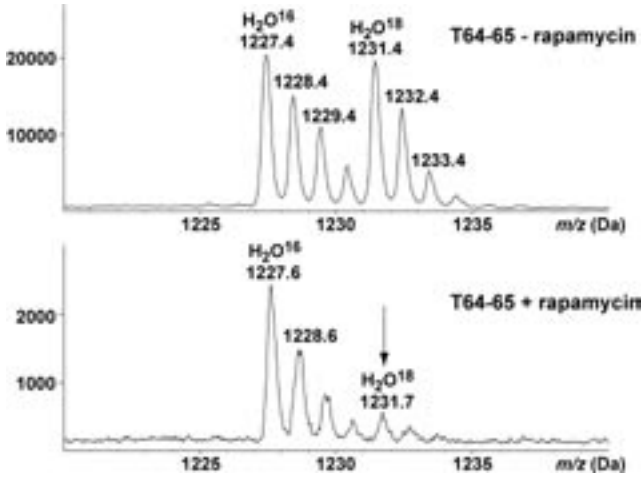


Figure 2

signal of the peptide. We applied the method to determine the differences in phosphorylation of NPR1 from untreated or rapamycin treated yeast cells. For example, decreased phosphorylation of the peptide encompassing residues 353-363 of NPR1 is evident in the spectrum recorded from non-treated (upper panel Fig. 2) or rapamycin-treated (lower panel, Fig. 2) yeast cells.

Publications

Radimerski, T., Mini, T., Schneider, U., Wettenhall, R. E., Thomas, G. & Jeno, P. (2000). Identification of insulin-induced sites of ribosomal protein S6 phosphorylation in Drosophila melanogaster. *Biochemistry* 39, 5766-74.

Fotiadis, D., Jeno, P., Mini, T., Wirtz, S., Muller, S. A., Fraysse, L., Kjellbom, P. & Engel, A. (2001). Structural characterization of two aquaporins isolated from native spinach leaf plasma membranes. *J Biol Chem* 276, 1707-14.

Haurie, V., Perrot, M., Mini, T., Jeno, P., Sagliocco, F. & Boucherie, H. (2001). The transcriptional activator Cat8p provides a major contribution to the reprogramming of carbon metabolism during the diauxic shift in Saccharomyces cerevisiae. *J Biol Chem* 276, 76-85.

Bonenfant, D., Mini, T. & Jeno, P. (2002). Mass spectrometric analysis of protein phosphorylation. In *The protein protocols handbook* (Walker, J. M., ed.), pp. 609-622. Humana Press.

Frank, S., Schulthess, T., Landwehr, R., Lustig, A., Mini, T., Jeno, P., Engel, J. & Kammerer, R. A. (2002). Characterization of the matrilin coiled-coil domains reveals seven novel isoforms. *J Biol Chem* 14, 14.

ZMB

Center of microscopy of the University of Basel

SERVICE, TEACHING, AND DEVELOPMENT OF INSTRUMENTS

The ZMB is a shared facility of the University of Basel, including the Biozentrum, which covers the field of electron microscopy and confocal laser scanning microscopy. The ZMB offers instrumentation, service, training, and advice. All members of the University of Basel are welcome to collaborate with the ZMB. In second priority we collaborate with other Universities and the private industry.

Laboratory personnel

Tamara Bossert*, Marcel Düggelin, Andi Hefti*, Daniel Mathys, Karin Missuk*, Ursula Sauder, Robert Wyss

Secretariat

Beatrice Schwyzer*

* Employed during part of the report period

Markus Dürrenberger
Phone: +41 61 267 2075 Fax: +41 61 267 1410 E-mail: Markus.Duerrenberger@unibas.ch
http://www.unibas.ch/zmb

Historical change 2000/2001

The Interdepartmental Electron Microscopy (IEM) of the Biozentrum and the REM-Labor of the University of Basel have been fused to the ZMB in September 2001. The ZMB is embedded in the administration of the Biozentrum.

Prof. Richard Guggenheim, the leader of the REM-Labor, has been retired after 30 successful years of work with scanning electron microscopes for the University of Basel. His tradition will be continued within the Zentrum für Mikroskopie.

Service

The ZMB houses a range of instruments such as TEM, REM and confocal laser scanning microscope. The following instruments are available: two transmission electron microscopes (TEM) including equipment for preparation, one cryo scanning electron microscope (SEM), one field emission SEM with EDX, one field emission environmental scanning electron microscope (ESEM) with EDX, including equipment for preparation. Also one confocal laser scanning microscope (CLSM) with image processing hard- and software is at your disposal.

About 350 service projects and collaborations were handled during the time period of this biennial report.

Teaching

The ZMB, in collaboration with the Maurice E. Müller Institute (Structural Biology), is responsible for the 'Grundkurs Mikroskopie' (basic course in microscopy) for students of the biology curriculum.

In addition, the ZMB has an active role in the transdisciplinary curriculum for microscopy that is offered by the head organization 'Interdisziplinäres Zentrum Mikroskopie Basel'. The courses offered in this curriculum are offered to those members of the university who need to make extensive use of light and electron microscopy in their research projects. The ZMB takes part in: 'Basiskurs Licht- und Elektronenmikroskopie' (course in basics of light- and electron microscopy. Duration: two weeks, all day;

registration necessary) and several courses of the three to five days long advanced courses hold every second year.

The IEM is also co-organizing the 'Kolloquium über Mikroskopie: Vom Mikro- zum Nanobereich' (one day meeting of all microscopists of the region, takes place every semester).

Development of instruments

Improving Environmental Scanning Electron Microscopy (ESEM)

The ZMB started collaboration with the Heinrich Pette Institute in Hamburg (Germany) (responsible person: Dr. H. Hohenberg) with the aim to improve the reliability of the environmental-SEM mode of our field-emission philips XL30 SEM. After initial success with living mites (*R. Guggenheim* et al.), the range of samples need to be extended to more sensitive ones from biology and medicine. This implies changes in the mechanical setup of the microscope.

Publications

Baur, B., Fröberg, L., Baur, A., Guggenheim, R. & Haase, M. (2000). Ultrastructure of snail grazing damage to calcicolous lichens. *Nordic Journal of Botany* 20 (1), 119 - 128.

Geng, J., Oelhafen, P., Mathys, D., Düggelin, M., Guggenheim, R., Brunold, S. & Gantenbein, P. (2000). *Copenhagen*.

Kaupp, A., Guggenheim, R. & Nagel, P. (2000). Die Eischale als Gegenstand der phylogenetischen Forschung bei Paussinae und anderen Carabidae (Coleoptera: Adephaga). *Entomologica Basiliensia* 22, 149 - 154.

Kaupp, A., Guggenheim, R. & Nagel, P. (2000). Egg-shell structure of Paussinae and other Carabidae, with notes on its phylogenetic relevance (Coleoptera). *Natural History and Applied Ecolog of Carabid Beetles*, 111-122.

Luginbuhl, P., Indermuhle, P.-F., Grétillat, M.-A., Willemin, F., Rooij de, N. F., Gerber, D., Gervasio, G., Vuilleumier, J.-L., Twerenbold, D., Düggelin, M., Mathys, D. & Guggenheim, R. (2000). Femtoliter injector for DNA mass spectrometry. *sensors and actuators* B 63, 167 - 177.

Malmström, J., Reusser, E., Guggenheim, R., Düggelin, M., Mathys, D., Gieré, R., Lumpkin, G. R. & Blackford, M. G. (2000). *12th European Congress on Electron Microscopy, Brno*.

Petrova, T., Rumbolz, J., Guggenheim, R., Stern, W. B. & Frey, M. (2000). *12th European Congress on electronic microscopy, Brno*.

Rumbolz, J., Büche, C., Kassemeyer, H. H. & Guggenheim, R. (2000). Differentiation of Plasmopara viticola preceeding sporulation. *Phytopathology* 90 (6), 67 - 68.

Rumbolz, J., Kassemeyer, H.-H., Steinmetz, V., Deising, H. B., Mendgen, K., Mathys, D., Wirtz, S. & Guggenheim, R. (2000). Differentiation of infection structures of the powdery mildew fungus Uncinula necator and adhesion to the host cuticle. *Can. J. Bot. (Canadian Journal of Bot.)* 78, 409 - 421.

Zhang, H., Vollmer, M., De Geyter, M., Litzistorf, Y., Ladewig, A., Dürrenberger, M., Guggenheim, R., Miny, P., Holzgreve, W. & De Geyter, C. (2000). Characterization of an immortalized human granulosa cell line (COV434). *European Society of Human Reproduction and Embryology* 6 (2), 146 - 153.

Bojat, N. C., Dürrenberger, M. & Haase, M. (2001). The spermatheca in the land snail, *Arianta arbustorum* (Pulmonata: Stylommatophora): muscle system and potential role in sexual selection. *Invertebrate Biology* 120 (3), 217-226.

Duffner, K., Schruft, G. & Guggenheim, R. (2001). Passive dispersal of the grape rust mite Calepitrimerus vitis Nalepa 1905 (Acari, Eriophyoidea) in vineyards. *Anz. Schädlingskunde/J. of Pest Science* 74, 1-6.

Rumbolz, J., Kassemeyer, H.-H. & Guggenheim, R. (2001). Infektionsprozess beim echten Mehltau. *Der deutsche Weinbau* Nr. 10, 48 - 51.

Acknowledgements with considerable service work

2000, S. P. Benjamin and R. Jocqué, Two new species of the genus Suffasia from Sri Lanka (Araneae: Zodariidae)

2000, M. Haase, E. Weigand and H. Haseke, Two new species of the family Hydrobiidae (Mollusca: Caenogastropoda)

2000, M. Haase, A Revision of the genus Belgrandia with the description of a new species from France (Caenogastropoda: Hydrobiidae)

2000, M. Haase and A. Karlsson, Mating and the inferred function of the genital system of the nudibranch, Aeolidiella glauca (Gastropoda: Opisthobranchia: Aeolidioidea)

2000, W. Halfter, S. Dong, B. Schurer, A. Osanger, W. Schneider, M. Ruegg and G. J. Cole, Composition, Synthesis, and Assembly of the Embryonic Chick Retinal Basal Lamina

2000, H. Hess, Saccocomen aus dem Malm Süddeutschlands und ihre Lebensweise

2000, Z. Kang, Morphology of plant pathogenic fungi under scanning electron microscope

2000, Z. Kang, Ultrastructure of Plant Pathogenic Fungi

2000, K. A. f. U. Luzern, Der Boden lebt - Broschüre zum Bodenlehrpfad Meggerwald

2000, K. Weber, Boden 2000

2001, D. Bernoulli and B. Gunzenhauser, A dolomitized diatomite in an Oligocene-Miocene deep-sea fan succession, Gonfolite Lombarda Group, Northern Italy

2001, G. Betz, R. Imboden and G. Imanidis, Interaction of liposome formulations with human skin in vitro

2001, L. Gasperini, D. Bernoulli, E. Bonatti, A. M. Borsetti, M. Ligi, A. Negri, R. Sartori and K. von Salis, Lower Cretaceous to

Eocene sedimentary transverse ridge at the Romanche Fracture Zone and the opening of the equatorial Atlantic

2001, W. J. Gehring, The genetic control of eye development and its implications for the evolution of the various eye-types

2001, D. J. Miller, A. F. Budd and Issue Eiditors, Morphology and

evolution of the late cenozoic marine biota of tropical america

2001, M. Stalder, T. Deller, M. Staufenbiel and M. Jucker, 3D-Reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid

2001, P. E. Tschudin, Shell Morphology, Shell Texture And Species Discrimination Of Caribbean Tucetona (Bivalvia, Glycymeridae)

MYOCONTRACT PHARMACEUTICAL RESEARCH LTD.

Founded in 2000 by scientists of the Pharmacology/Neurobiology Departement, MyoContract is the first start-up company of the Biozentrum.

For more information, please have a look at http://www.myocontract.com

R. Amherd, D. Bleckmann, A. Briguet, I. Courdier-Fruh, M. Jauslin, T. Meier, F. Schnüriger, F. Schoumacher

In collaboration with M.A. Rüegg (Division Pharmacology, Biozentrum Basel, CH), N. Mermod (EPFL, Lausanne, CH), H. Lochmüller (Genzentrum München, D), 5th Framework Program of the European Commission (Pierre Rustin, Hopital Necker, Paris, F; Michel Koenig, IGBMC, Illkirch, F; Joerg B. Schulz, Department of Neurology, Tübingen, D; Francoise Foury, Unité de Biochimie Physiologique, Louvain-la-Neuve, B)

Thomas Meier
Phone: +41 61 267 2108 Fax: +41 61 267 2108 E-mail:Thomas.Meier@unibas.ch
http://www.biozentrum.unibas.ch/meier

Friedreich Ataxia

Friedreich Ataxia (FA) is the most prevalent inherited ataxia, with an estimated frequency of 1 in 30000 individuals. This progressive neurodegenerative disorder is an autosomal recessive disease with onset in early childhood. It is characterized by gait and limb ataxia, lack of tendon reflexes, loss of position sense, dysarthria and pyramidal weakness of the legs.

Loss of large sensory neurons in the dorsal root ganglia is one of the early pathological changes and life-threatening hypertrophic cardiomyopathy is observed in most patients. The disease is caused by the lack of expression of Frataxin, a nuclear encoded mitochondrial protein whose expression levels correlate well with the main sites of pathological changes. Most patients are homozygous for large expansions of GAA repeats in the first intron of the gene. This particular DNA structure is thought to trap RNA polymerases in a triple helix structure, thereby preventing proper transcription. Long expansions correlate with early onset of the disease symptoms.

Early studies have shown mitochondrial iron accumulation in Friedreich ataxia. Subsequently, yeast models showed the importance of frataxin in mitochondrial iron homeostasis.

Using the YFH1 yeast frataxin homolog knockout model, we have completed a high throughput screening for compounds that rescue the impaired growth of this mutant in the presence of iron or copper. A number of substances have been successfully identified with this approach. The next step towards tests in animal models is the validation of these compounds in a mammalian cell culture system. We have initiated the development of such a model using primary cultures of fibroblasts from patients and from control donors. These FA cells do not display any specific phenotype under normal culture conditions. We have therefore established culture conditions that specifically kill FA cells by interfering with one of the cell's detoxification mechanisms. Active substances were expected to prevent FA cell death.

With this assay, we verified the activity of the compounds previously identified in the yeast screen. In addition, we are using now this cellular assay to identify small molecule enzyme mimetics that will be useful for the treatment of this disease.

General muscle atrophy

Beside relatively rare diseases such as Friedreich Ataxia, our research interests also focus on general

muscle wasting which affects a large number of patients. General muscle wasting occurs after severe skeletal surgeries and also after severe non-skeletal surgeries (disuse atrophy). Extensive physiotherapy is currently the only safe and effective therapeutic option for these patients to regain muscle mass and muscle strength.

Muscle atrophy also occurs in cancer disease and HIV-positive patients (cancer and AIDS related muscle wasting). Cancer related muscle wasting or cancer cachexia affects approximately 80 % of terminally ill cancer patients and often leads to death by itself. Approximately 25 % of HIV-positive people experience some degree of weight loss and/or wasting. So far, there are no drugs on the market showing a significant impact on quality of life, weight gain, or muscle strength.

Ageing is associated with a progressive loss of muscle mass and muscle strength (age related muscle wasting). In a considerable number of cases this has a significant impact on essential mobility functions such as for example walking or eating.

Supported by KTI (Kommission für Technologie und Innovation) and in collaboration with the group of Professor Markus A. Rüegg at the Biozentrum, we addressed the question how muscle atrophy can be prevented and how muscle regeneration can be improved. So far we concentrated on muscle-specific kinase (MuSK), a receptor tyrosine kinase. Several lines of evidence indicate that alterations of MuSK expression and activation is correlated with the atrophy status of the muscle. For example MuSK expression levels increase in denervated muscle.

Our group has developed two assays to screen for small-molecule MuSK activators: a first assay is based on a cell-free system where substances directly acting on the intracellular kinase domain of MuSK can be screened for. A second system comprises a cell-based assay, which allows for the screening of MuSK activating substances in a muscle cell line. Both assays form the basis for the screening of large libraries of small molecules.

Neuronal agrin (the endogenous activator of MuSK) does not directly interact with MuSK. Therefore, a MuSK co-receptor has been proposed by several groups as part of a agrin-receptor/MuSK complex. Current experiments are focussing on the identification of MuSK binding proteins. MuSK co-immunoprecipitations are performed in order to isolate and identify MuSK binding proteins. New MuSK interacting proteins would not only allow a better understanding of the mechanisms underlying agrin stimulation but would represent new candidate drug targets.

Publications

Meier, T. & Rüegg, M.A. (2000). The role of dystroglycan and its ligands in physiology and disease. *News Physiol. Sci*. 15, 255-259

Courdier-Fruh I., Barman L., Briguet A. & Meier T. *(in press).* Glucocorticoid-mediated regulation of utrophin levels in human muscle fibers. *Neuromuscul. Disord*.

CORE FACILITIES
PERSONNEL

Core Secretariat
Urs Fürstenberger
Ursula In-Albon, Ronja Cueni*, Cinar Kara*, Monica Keller*, Elisabeth Loiseleur, Marisa Müller*, Silvia Schad, Irène Schmucki, Caroline Schneider*, Christine Saladin, Vera Sinniger

Purchase and stores
Konrad Weber
Karl-Heinz Heuser*, Gioacchino Romagnoli, Hans-Rudolf Zeller

Mechanical workshop
Raymond Strittmatter
Alois Affeltranger, Rudolf Eberhard, Olivier Jenni*, Bernhard Leinweber, Michel Schaffhauser, Patrick Schlenker

Electronical workshop
Paul Henz, Jürg Wenger

Technical services
André Hassler
Christian Herzog, Beat Hostettler*, Anton Schürmann*

Safety and radiation protection
Christoph Stürzinger

Web-Design
Werner Indlekofer*
Margrit Jaeggi

Photolab
Verena Grieder, Annette Roulier*, Ingrid Singh*

Public relations
Anka Stark*

Animal station
Roland Geiser
Urs Berglas, René Weber, Renato Zedi*

Library
Karin Flügel

Building maintenance
Daniel Oeschger
Alois Brändle, Bruno Marioni*, Alfred Schütz*

Switchboard
Irene Dürr
Suzanne Stöckli

Information Technology
Franz Biry
Rainer Pöhlmann
Isabelle Altherr

***Employed only part of the report period**