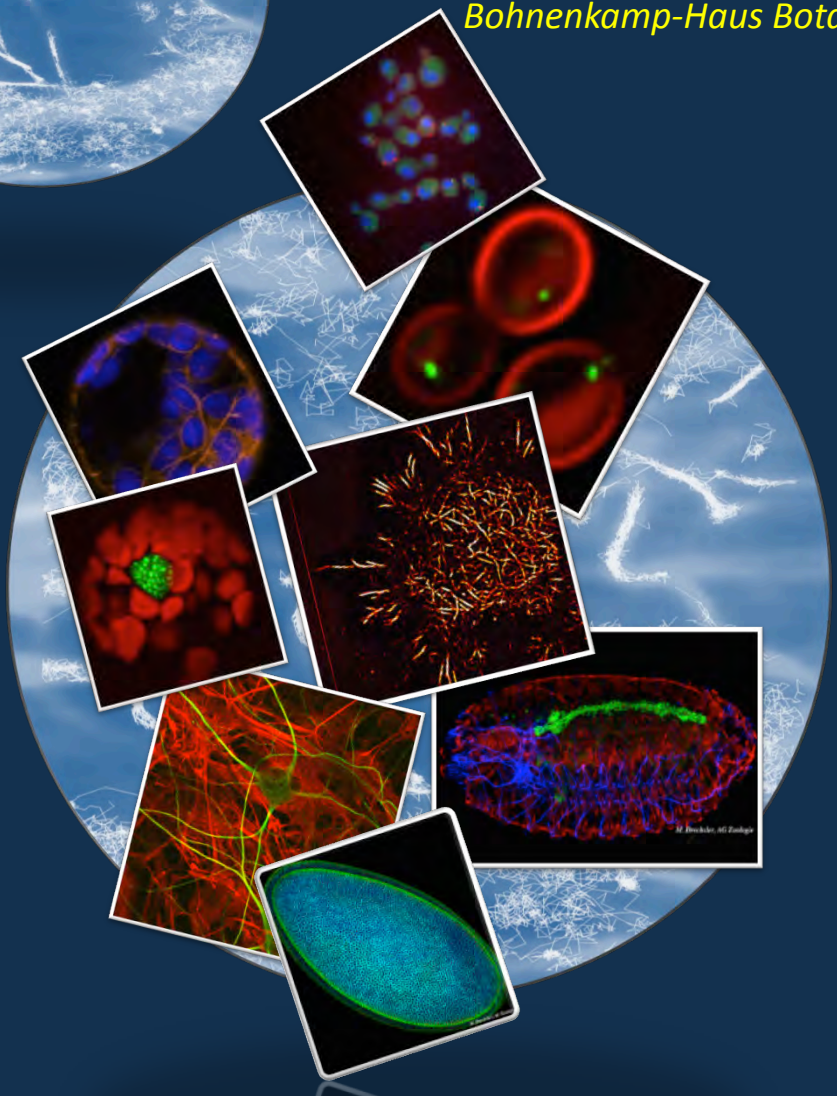
IRTG Summer School
&
6. Westerberger Herbsttagung

Physiology and dynamics of cellular microcompartments

September 21-22, 2012

University of Osnabrück
Bohnenkamp-Haus Botanical Garden



Contents

Welcome

Welcome to our **International Summer School 2012** on ”Physiology and Dynamics of Cellular Microcompartments”. The summer school is organized by the PhD students of the ”Integrated Research Training Group” (IRTG), which is part of the University’s Collaborative Research Center (CRC) ”SFB 944” and PhD students of the graduate training program on ”Membranes and Cellular Communication”. Within the CRC, several research groups of the Biology and Physics Departments from the Universities of Osnabrück and Münster work closely together with a common interest in studying microcompartments as basic functional units of a variety of cells.

The graduate training program on ”Membranes and Cellular Communication” was initiated in 2010 by research groups from the Physics, Chemistry and Biology Departments at the University of Osnabrück. The research and training activities are currently supported by eight PhD Lichtenberg-Fellowships provided by the government of Lower Saxonia. All Lichtenberg fellows participate also in the teaching program of the ”SFB944”.

The aim of the Summer School is to bring together distinguished scientists from different disciplines for intense scientific discussions on the topic of "Physiology and Dynamics of Cellular Microcompartments".

The summer school is organized as a joint meeting with the traditional biannual **”Westerberger Herbsttagung on the Perspectives of Molecular Neurobiology”**. This year, the ”6th Westerberger Herbsttagung” will be a compact session with a focus on ”Microcompartments in neurons and glia cells”.

The University of Osnabrück is a young University, founded in 1974, and is known for a large number of interdisciplinary degree programs, some of them rare or even unique among German Universities, including European Studies, Applied Systems Science and Cognitive Science. Currently, about 10,000 students are enrolled for studies. The Science Institutes of the University (Biology, Chemistry,

Physics, Cognitive Sciences) are located at the "Westerberg Campus", in close vicinity to the Botanical Garden of the University and the School for Applied Sciences ("Hochschule Osnabrück").

We are delighted to welcome all of you in Osnabrück. We thank you for your contribution and participation and look forward to an exciting meeting.

Organizing Team

The organizing team was headed by Katrin Klempahn, the coordinator of the IRTG. Members of the team are the following graduate students from the SFB944 projects and the Lichtenberg program:

Britta Barlag
Daniel Klose
Frederik Sündermann
Henning Arlt
Jörg Brühmann
Katharina Moschner
Katrin Klempahn
Lorene Penazzi
Manuel Twachtmann
Marco Kelkenberg
Minhee Kang
Mirko Hüsken
Nora Gutsche
Sara Löchte
Sascha Meyer

Schedule Summer School 2012

Friday 21st September:

12.45 a.m.	Opening: Michael Hensel, Sabine Zachgo & Roland Brandt
	Session 1: Chair - Roland Brandt **6th Westerberger Herbsttagung**
1.00 - 1.30 p.m.	John H. Carson (Farmington, USA): *Structure and function of RNA granules in neurons*
1.35 - 2.05 p.m.	Stefan Kindler (Hamburg, Germany): *Role of the postsynaptic density in the pathology of the fragile X syndrome*
2.10 - 2.30 p.m.	Eva-Maria Krämer-Albers (Mainz, Germany): *Myelin membrane traffic and the role of exosome secretion in axon-glia interaction*
2.35 - 2.45 p.m.	Progress Talk I - Dennis Janning (Osnabrück) *A novel interaction of Tau through its N-terminal projection domain*
2.45 - 3.00 p.m.	Progress Talk II - Frederik Sündermann (Osnabrück) *RNA-Binding protein (RBP) containing granules act as dynamic microcompartments in neural cells*
3.15 - 4.30 p.m.	Poster Session A & Coffee break
	Session 2: Chair - Jacob Piehler
4.30 - 5.15 p.m.	Kathryn Ayscough (Sheffield, UK): *Generating an actin network during endocytosis*
5.15 - 6.00 p.m.	Diane Lidke (Albuquerque, USA): *Diffusion, dimerization and domains: single quantum dot tracking to correlate membrane receptor dynamics with signaling*
6.00 p.m.	Dinner
	Key Note Lecture: Chair - Christian Ungermann
7.00 - 8.00 p.m.	Scott D. Emr (Ithaca, USA): *Kinase signaling cascade regulates endocytosis via an arrestin-related Ub ligase adaptor complex*
8.00 p.m.	Last night of summer with music and drinks

Saturday 22nd September:

	Session 3: Chair - Sabine Zachgo
9.00 - 9.45 a.m.	Andreas Meyer (Bonn, Germany): *Functional imaging of physiological parameters in subcellular compartments*
9.45 - 10.30 a.m.	Mark Aurel Schöttler (Potsdam, Germany): *Biogenesis and lifetimes of photosynthetic complexes in higher plants*
10.30 - 11.15 a.m.	Halina Gabryś (Kraków, Poland): *What´s going on at the surface of chloroplasts?*
11.15 - 11.35 a.m.	Coffee break
	Session 4: Chair - Achim Paululat
11.35 - 12.20 a.m.	Bruno Lemaitre (Lausanne, Switzerland): *The Drosophila gut: a new paradigm for epithelial immune response*
12.20 - 1.05 p.m.	Françoise Payre (Toulouse, France): *Die Haut in der sie wohnen: Development of the Drosophila epidermis*
1.05 - 2.05 p.m.	Lunch
2.05 - 3.15 p.m.	Poster Session B
	Session 5: Chair - Johann P. Klare
3.15 - 4.00 p.m.	Ariel Blocker (Bristol, UK): *Three-dimensional structure and mechanism of host cell-contact mediated activation of the Shigella T3SS needle tip complex*
4.00 - 4.45 p.m.	Enrica Bordignon (Zürich, Switzerland): *Properties and conformational transitions of ABC transporters in different enviroments: a site-directes spin labeling EPR approach*
4.45 - 5.30 p.m.	Gunnar von Heijne (Stockholm, Sweden): *Translocon-mediated assembly of membrane proteins*
5.30 p.m.	Closing: Christian Ungermann Award of poster prizes (sponsored by Nikon)

Location

The meeting will take place in the newly built Bohnenkamp-Haus at the "Botanischer Garten Osnabrück" (botanical garden). The garden is maintained by the University of Osnabrück and founded in 1984. It shows global plant biodiversity comprising the Mediterranean region, North America, Japan and China as well as protected regional plant populations. Plants are grown in two abandoned quarries of over eight hectares, giving the garden a special atmosphere with its setting surrounded by limestone, dating back over 200 million years. The collection consists of Alpine plants, Swabian Alpine plants, medicinal plants, ferns, and heather as well as several greenhouses including a rainforest house with a focus on Amazonian vegetation. The newly built Bohnenkamp-Haus serves as the garden's environmental education facility for activities for all ages. In an attractive ambience, it provides space for larger congresses and workshops as well as for small seminars and experimental practical courses.

There will be a chance to get a guided tour through the botanical garden by the director of the garden, Sabine Zachgo, on Friday.

We invite you to spend the last night of summer within the unique atmosphere of the botanical garden on Friday evening. You can relax with tropical feeling and cool drinks after a fascinating day of science. The evening will be opened with music by the guitar group "Absaits".

Directions

By bus:

Take bus line 21 going to "Atterfeld" from the train station or "Neumarkt", which is the central bus station in Osnabrück. The bus stop "Westerberger Hochschulen" is located just outside the main entrance of the botanical garden. Alternatively, you can use bus lines 11 and 13, direction "Eversburg" from "Neumarkt" at bay and exit at the bus stop "Sedanplatz" from where you have to go up the hill following Albrechtstraße.

On foot:

The botanical garden is also within walking distance (about 15 min uphill) from the Hotel Walhalla, if you prefer to walk. Leave the inner city through the "Heger-Tor" and cross the main road. Take the "Bergstraße" on your right hand and cross the "Bismarckplatz". Then take the "Edinghäuserweg" to your right and walk on straight until you reach the top of the Westerberg, where the botanical garden is located.

By car:

If you want to go by car and use a navigation system, direct it to "Albrechtstraße 29", zip code 49076, Osnabrück.

Speaker Portraits

John H. Carson

University of Connecticut Health Center, Farmington, USA

John H. Carson is Professor of Biochemistry at the University of Connecticut Health Center in Farmington (USA). He studied Biology at the Reed College in Portland, Oregon (USA) and obtained his PhD at the MIT in Cambridge, Massachusetts (USA). Afterwards he became a postdoctoral fellow first at the University of Bern (Switzerland) and then at McGill University in Montreal (Canada).

The research of the Carson lab is focused on RNA granules as trafficking and translation platforms containing multiple different RNA complexed with cognate RNA binding proteins and components of the translational machinery. John Carson discovered and named granules as RNA trafficking intermediates more than two decades ago. His lab uses biochemical methods to analyze combinatorial multivalent molecular interactions among granule components and single molecule imaging techniques to analyze translation in individual granules with molecular, spatial and temporal resolution. Recently the Carson lab has shown that CGC repeat RNA in the gene for fragile X mental retardation protein (FMRP) is incorporated into granules and inhibits translation. This may be the basis for a trinucleotide repeat neurodegenerative disorder called fragile X tremor ataxia syndrome.

Invited by the Department of Neurobiology - Westerberger Herbsttagung

Structure and function of RNA granules in neurons

John H. Carson

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RNA granules are large intracellular complexes held together through combinatorial multivalent interactions between RNA molecules, RNA binding proteins and scaffold proteins. RNA granules are localized and translated in dendritic spines producing proteins required for synaptic plasticity. Translation in individual granules can be either sporadic (monosomal) or bursty (polysomal) and is regulated by synaptic activity. A transgenic mouse lacking granule scaffold protein exhibits disrupted granule assembly, reduced bursty translation and impaired habituation. In fragile X tremor ataxia syndrome (FXTAS) premutation Fmr1 RNA containing expanded CGG repeats is incorporated into granules and inhibits translation, which may represent a molecular mechanism for FXTAS.

References:
1. Gao Y, Tatavarty V, Korza G, Levin MK, Carson JH. (2008) Multiplexed Dendritic Targeting of alpha Calcium Calmodulin-dependent Protein Kinase II, Neurogranin, and Activity-regulated Cytoskeleton-associated Protein RNAs by the A2 Pathway. *Mol Biol Cell.* **19**:2311-27
2. Han SP, Friend LR, Carson JH, Korza G, Barbarese E, Maggipinto M, Hatfield JT, Rothnagel JA, Smith R. (2010) Differential subcellular distributions and trafficking functions of hnRNP A2/B1 spliceoforms.*Traffic.* **11**: 886–898
3. Tatavarty V, Ifrim MF, Levin M, Korza G, Barbarese E, Yu J, Carson JH. (2012) Single molecule imaging of translational output from individual RNA granules in neurons. *Mol Biol Cell.* **23(5)**:918-29

Stefan Kindler

University Medical Center Hamburg-Eppendorf, Germany

Stefan Kindler is a Group Leader at the Institute for Human Genetics at the University Medical Center in Hamburg, Germany. He studied Biology in Hamburg, performed his Diploma work at the Heinrich Pette Institute for Experimental Virology and Immunology, and obtained his PhD at the Center for Molecular Neurobiology at the University of Hamburg with Prof. Dr. Craig Garner. 1993 he became a postdoctoral fellow with Prof. Dr. M.B. Kennedy at the California Institute of Technology, Pasadena (USA), supported by a long-term fellowship from the Human Frontier Science Program Organization. 1995 he returned to Hamburg to become a Research Group Leader at the Institute for Cell Biology and Clinical Neurobiology, University Medical Center Hamburg-Eppendorf.
His research focuses on cellular and molecular mechanisms mediating the formation and modification of synapses. In particular, he is analyzing the contribution of mRNA transport into dendrites and regulation of local translation at physiological conditions and during diseases such as the *Fragile X-Syndrom*, in which a particular RNA-binding protein is not longer synthesized due to a genetic defect. A further research area represents the study of *spinocerebellar ataxia (SCA)*, a progressive, degenerative and genetic disease, in which disturbances in RNA metabolism appear to have an important role.

Invited by the Department of Neurobiology - Westerberger Herbsttagung

Role of the postsynaptic density in the pathology of the fragile X syndrome

Janin Ölschläger-Schütt, Sabine Kramp, Hans-Jürgen Kreienkamp, Stefan Kindler

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Functional absence of fragile X mental retardation protein (FMRP) causes the fragile X syndrome (FXS), a hereditary form of mental retardation characterized by a change in dendritic spine morphology. The RNA-binding protein FMRP has been implicated in regulating postsynaptic protein synthesis. Here, we have analyzed whether the abundance of scaffold proteins and neurotransmitter receptor subunits in postsynaptic densities (PSDs) are altered in the neocortex and hippocampus of FMRP deficient mice. Whereas the levels of several PSD components are unchanged, concentrations of Shank1 and SAPAP scaffold proteins and various glutamate receptor subunits are altered in both adult and juvenile knockout mice. With only one exception altered postsynaptic protein concentrations do not correlate with similar changes in total and synaptic levels of corresponding mRNAs. Thus, loss of FMRP in neurons appears to mainly affect the translation and not the abundance of particular brain transcripts. Semi-quantitative analysis of RNA levels in FMRP immunoprecipitates showed that in the mouse brain mRNAs encoding PSD components, such as Shank1, SAPAP1-3, PSD-95 and the glutamate receptor subunits NR1 and NR2B are associated with FMRP. Luciferase reporter assays performed in primary cortical neurons from knockout and wild-type mice indicate that FMRP silences translation of Shank1 mRNAs via their 3' untranslated region. Activation of metabotropic glutamate receptors relieves translational suppression. As Shank1 controls dendritic spine morphology, our data suggest that dysregulation of Shank1 synthesis may significantly contribute to the abnormal spine development and function observed in brains of FXS patients. To assess this hypothesis we are currently investigating whether a reduced *Shank1* gene dose may rescue the spine and/or behavioral phenotype of FMRP deficient mice. Thus, Shank1 may emerge as a novel drug target for treatment of FXS patients.

Eva-Maria Krämer-Albers

Johannes Gutenberg University, Mainz, Germany

Dr. Eva-Maria Krämer-Albers is a Lecturer and Principle Investigator at the Department of Molecular Cell Biology at the Johannes Gutenberg University in Mainz (JGU). She received a PhD in neurosciences at the University of Heidelberg (Germany) in 1997 and was further trained in molecular cell biology and neurogenetics at the Centre for Molecular Biology Heidelberg (ZMBH) and the MPI for Experimental Medicine (Göttingen). Her research interest is focused on cellular neurosciences in particular the molecular mechanisms of neuron-glia interaction and myelin biogenesis with implications for CNS disease. She studies the principles of membrane traffic in myelinating cells and the role of extracellular secreted vesicles in neural cell communication. Her research group is integrated in the interdisciplinary Focus Program of Translational Neuroscience at the JGU. In 2012 she became nominated Associate Editor of the Journal of Extracellular Vesicles.

Invited by the Department of Neurobiology - Westerberger Herbsttagung

Myelin membrane traffic and the role of exosome secretion in axon-glia interaction

Eva-Maria Krämer-Albers

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Brain function depends on coordinated interactions between neurons and glial cells. Oligodendrocytes ensheath axons with the myelin membrane and furthermore maintain long-term axonal integrity by an unknown pathway. We are studying myelin membrane traffic and its control by axon-glia interaction. Endocytic sorting and recycling appears to be a common mode of myelin protein trafficking. The major myelin membrane protein PLP is recycling through late endosomal compartments and multivesicular bodies (MVB). MVB fusion with the oligodendroglial plasma membrane results in secretion of exosomes into the extracellular space. These exosomes carry myelin proteins in addition to genuine exosome proteins and distinct species of RNA. Intriguingly, oligodendrocyte exosome release is stimulated by electrically active neurons via neurotransmitter secretion and activation of glial neurotransmitter receptors. Moreover, neurons internalize oligodendrocyte-derived exosomes by endocytosis and recipient neurons functionally recover the exosome cargo. Functional studies indicate that the transfer of oligodendrocyte-derived exosomes protects neurons from stress and starvation. We propose that oligodendroglial exosomes participate in bidirectional neuron-glia communication and mediate the transfer of bioactive molecules from glia to neurons. The signal-mediated transfer of exosomes from glia to neurons may be implicated in neuroprotection and glial maintenance of axonal integrity.

Kathryn Ayscough

University of Sheffield, United Kingdom

Kathryn is Professor of Molecular Cell Biology and Deputy Director of the Centre for Membrane Interactions and Dynamics (CMIAD) at the University of Sheffield. Her research focuses on the regulation of the actin cytoskeleton, and its interface with the endocytic machinery.

Kathryn obtained her PhD in 1993 undertaking studies on the Golgi apparatus in fission yeast in the lab of Graham Warren. This was followed by postdoctoral studies in the lab of David Drubin (U.C Berkeley). Foremost, in her post-doctoral research was the characterization of Latrunculin-A as a tool to study the importance of the actin cytoskeleton in yeast *in vivo* (Ayscough et al, *J.Cell Biol.* 1997). Initiating her independent career at the University of Dundee, Kathryn continued to develop ideas around links between the actin regulating machinery and endocytic proteins. In 2002 the lab used dual colour live cell imaging of proteins at actin patches to demonstrate direct coupling of actin associated proteins and endocytic machinery. Following a move to the University of Sheffield (2003), the lab revealed new links between actin dynamics and initiation of a cell death pathway (Gourlay et al, *JCB* 2004; *J.Cell Sci* 2005; *Mol Cell Biol* 2006). Most recently the lab has worked on trying to understand the perceived differences between the endocytic pathways in yeast and mammalian cells. Significantly the lab demonstrated that actin is critical during the invagination stage of endocytosis due to the effects of turgor pressure in yeast (Aghamohammadzadeh & Ayscough, *Nat Cell Biol*, 2009). Since then we have shown that, contrary to widespread belief, the yeast dynamin Vps1 plays an important role in endocytosis, interacting with the amphiphysin Rvs167, to bring about vesicle scission (Smaczynska de Rooij et al, *J.Cell Sci.* 2010, *Traffic*, 2012). Current studies are investigating the earliest stages of actin polymerisation at sites of endocytosis, and also aiming to determine how key factors are regulated to ensure unidirectional progress through endocytic stages.

Invited by the Department of Genetics

Generating an actin network during endocytosis

Kathryn Ayscough

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Endocytosis is a highly regulated and essential process in most eukaryotic cells. It is required for recycling of lipids and trafficking proteins, and for uptake or down-regulation of cell-surface receptors. During endocytosis the plasma membrane invaginates into the cell resulting in the production of a vesicle that then fuses with endosomes and enters the endolysosomal membrane system. This process involves at least 50 proteins that assemble transiently at sites on the plasma membrane. Work in *S. cerevisiae* has led to significant advances in our understanding of the distinct stages that take place during endocytosis in vivo. It is now widely believed that the broad stages of coat assembly (early), invagination (mid) and scission/inward movement (late) are conserved across evolution, and that in many cases direct homologues of proteins are responsible for carrying out equivalent steps in the process. In yeast, actin is critical for the endocytic process, and a framework generated by cross-linked bundles of actin must form in order to support the invagination process. Recently we have demonstrated that this framework is required in yeast to support the inward movement of membrane against turgor pressure. Our current work is focused on the earliest stages of actin filament formation responsible for driving the endocytic process.

Diane Lidke

University of New Mexico, USA

Diane Lidke is an Assistant Professor in the Department of Pathology at the University of New Mexico, School of Medicine (2006- present). In 2002 she received her PhD in Biophysical Sciences and Medical Physics from the University of Minnesota. Afterwards she joined the lab of Dr. Thomas M. Jovin at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany (2002-2005), where she was working on fluorescence microscopy and cell signal transduction. In 2005 she became a Research Assistant Professor in the Department of Pathology at the University of New Mexico, School of Medicine.

Diane Lidke´s research focuses on dynamics of protein interactions and cell signaling mechanisms in live cells using high-resolution bioimaging methods. She is working on the development and application of new techniques in fluorescence microscopy such as real-time visualization, FRET (Fluorescence Resonance Energy Transfer) microscopy, FRAP (Fluorescence Recovery After Photobleaching), single molecule tracking and anisotropy imaging. As a biological system the EGF receptor, the FcεRI and cell-cell interactions are of her main interest.

Invited by the Department of Biophysics

Diffusion, dimerization and domains: single quantum dot tracking to correlate membrane receptor dynamics with signaling

Diane Lidke

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Imaging technologies and biological tools have developed to a point where many fundamental biological questions can now be addressed at the molecular level. In particular, single particle tracking (SPT) using bright and photostable quantum dots (QDs) provides information about protein dynamics with high spatial ($\sim$10 nm) and temporal (>30 Hz) resolution. Using these techniques, we have examined the spatiotemporal regulation of the high affinity IgE receptor (FcεRI) and the erbB family on living cells, revealing new insights into the roles of receptor dynamics and membrane partitioning in the regulation of receptor interactions. Simultaneous observation of QD-IgE-FcεRI motion and GFP-tagged actin dynamics provided direct evidence that membrane-proximal actin bundles form a dynamic labyrinth that confines mobile receptors. We also demonstrate that co-confinement in membrane microdomains is important in promoting protein-protein interactions, enhancing erbB1 homodimerization. These results highlight the influence of lateral organization on signaling and demonstrate local membrane reorganization associated with signaling competent receptors.

Scott D. Emr

Cornell University, Ithaca, NY USA

Scott D. Emr is the Frank H.T. Rhodes Professor of Molecular Biology and Genetics at Cornell University (2007-present). He also is Director of the Weill Institute for Cell and Molecular Biology. He received his Ph.D. degree in Molecular Genetics from Harvard University in 1981. Dr. Emr has held positions at the University of California, Berkeley (Miller Research Scholar; 1981-1983), the California Institute of Technology (Assistant and Associate Professor; 1983-1991) and the University of California, San Diego School of Medicine (Distinguished Professor and Investigator in the HHMI; 1991-2007).

Dr. Emr's research focuses on the regulation of cell signaling and membrane trafficking pathways by phosphoinositide lipids, ubiquitin-mediated sorting reactions and vesicle-mediated transport reactions.

Dr. Emr counts among his early honors a Searle Scholars Award and an NSF Presidential Young Investigator Award. He has been elected a member of the National Academy of Sciences (2007), the American Academy of Arts and Sciences (2004), and the European Molecular Biology Organization (2008). In 2003, he was awarded the Hansen Foundation Gold Medal Prize for "elucidating intracellular sorting and transport pathways". In 2007, he was awarded the Avanti Prize for his "key contributions in understanding lipid signaling pathways".

Invited by the Department of Biochemistry

Protein quality control at the plasma membrane – Kinase signaling cascade regulates arrestin-related ubiquitin-ligase adaptors

Yingying Zhao, Bi Hsu, Chris Stefan, Jason MacGurn and Scott D. Emr

Department of Cellular and Molecular Biology & Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA

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Down-regulation of cell surface receptors and transporters is mediated by a series of membrane trafficking steps including ubiquitin-mediated endocytosis, ESCRT-mediated cargo(Ub) recognition, sorting and packaging into vesicles that bud into the lumen of the endosome (MVBs), and endosome-lysosome fusion that delivers sorted receptors into the lumen of the lysosome where degradation occurs. Failure to attenuate growth-factor receptor signaling at the plasma membrane (PM) by endocytic down-regulation can lead to cancer.
A key function of the ubiquitin-proteasome system is protein quality surveillance and the targeting of misfolded proteins for degradation. In the cytosol, specialized E3 ligases target soluble misfolded proteins for ubiquitination and subsequent proteasomal degradation. However, the case is more complicated for integral membrane proteins. Following co-translational insertion in the ER membrane, proteins that fail to fold properly in the ER are subject to ER-assisted degradation (ERAD), which involves retrotranslocation of proteins back into the cytosol followed by ubiquitin-dependent proteasomal degradation. Properly folded PM proteins, such as signaling receptors, ion channels, and nutrient transporters, exit the ER and traffic through the Golgi to the cell surface where they mediate their specific functions. Maintenance of proper PM proteostasis, particularly with respect to ion channels and nutrient transporters, is crucial to prevent loss of PM integrity and dissipation of essential ion and chemical gradients. As such, when PM resident proteins become damaged or misfolded, they must be recognized, removed by endocytosis and delivered to the lysosome for degradation. Thus, cells must maintain a "cradle to the grave" quality monitoring system for integral membrane proteins, yet the mechanisms of quality surveillance, particularly at the PM, remain poorly understood. We have evidence

that the E3 ubiquitin ligase Rsp5, the yeast homolog of Nedd4, is a key mediator of protein quality control at the PM. We show that proteotoxic stress triggers global activation of Rsp5-dependent ubiquitination, endocytosis, and vacuolar trafficking of PM proteins, and that yeast mutants attenuated for this response are highly sensitive to proteotoxic stress. These mutants exhibit toxic accumulation of integral membrane proteins at the cell surface and suffer catastrophic loss of PM integrity during misfolding stress. This stress-induced surface remodeling is mediated by a family of Rsp5 adaptors known as arrestin-related trafficking adaptors (or ARTs), which target Rsp5 ubiquitin ligase activity to specific PM proteins during proteotoxic stress. The Art1 protein undergoes a phosphoregulatory cycle. Dephosphorylation of Art1 triggers its activation and recruitment to PM cargo. Using genetics and biochemistry, we discovered that this phosphoregulatory cycle is mediated by a TORC1-Npr1 kinase signaling cascade.
We propose that the ubiquitin-mediated ART-Rsp5 PM surveillance system, together with other quality control pathways like ERAD, protects the cell from proteotoxic stress by limiting the toxic accumulation of misfolded integral membrane proteins in the PM.

Andreas Meyer

University of Bonn, Germany

Andreas Meyer is Professor and Head of the division "Chemical Signaling" of the Institute of Crop Science and Natural Resources at the University of Bonn. He received his PhD degree at the University of Karlsruhe in 1995. After that he was a postdoctoral fellow in the Plant Science department at the Oxford University, at the University of Freiburg and was one year a lecturer back at the University of Karlsruhe. In 2003 he became a Senior Researcher and group leader at the Heidelberg Institute for Plant Science at Heidelberg University (2003-2010). In 2008 he conducted his habilitation and got the *Venia Legendi* for Botany.

The research of Andreas Meyer focuses on the small tripeptide glutathione and its fundamental roles in the cell as a key component of the cellular defense system against reactive oxygen species and xenobiotics. He is trying to unravel the metabolic processes affecting the concentration and the oxidation of glutathione pool as well as downstream signaling processes. The model plant *Arabidopsis thaliana* is used, with its wide rage of available mutants affecting glutathione biosynthesis and other glutathione-related processes. To gain better insights into the cellular glutathione homeostasis and its role in redox signaling, novel live cell imaging techniques (e.g. roGFP) were developed and improved in his lab, allowing to quantify redox-potentials and changes in redox-levels. Combination of these data with the molecular analysis of genes of interest and comparison with the glutathione-related mutants will give further insights into the important role of glutathione within the cell.

Invited by the Department of Botany

Functional imaging of physiological parameters in subcellular compartments

Andreas Meyer

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Physiological processes are by definition dynamic with alterations in time and space. Deviations from steady state are particularly pronounced under conditions of environmental stress and might be exploited for signalling processes enabling organisms to adapt to adverse conditions. Eukaryotic cells are highly compartmentalized which implies that such signalling processes might originate or are even restricted to distinct compartments. While conventional destructive assays are capable of detecting alterations at single or only few time points it is rather difficult to analyse the full dynamics and the compartmentalization of such processes. During the last two decades fluorescent proteins have revolutionized cell biology by allowing researchers to peer into the cellular machinery. The ability to engineer fluorescent proteins for sensitivity to physiological parameters such as e.g. pH, Ca^{2+}, redox potential and H_2O_2 now enables minimal invasive visualisation of complex physiological and biochemical reactions in live cells. The ability to target genetically-encoded biosensors to distinct subcellular compartments provides opportunities for measurements at subcellular resolution. Another major advance over conventional chemical dyes is the reversible behaviour enabling dynamic measurements. The presentation will address engineering and application of different biosensors for functional imaging with emphasis on redox-related physiological parameters.

Mark Aurel Schöttler

Max-Planck-Institut of Molecular Plant Physiology, Potsdam, Germany

Mark Aurel Schöttler heads the "Photosynthesis Research Group" at the Max-Planck-Institute of Molecular Plant Physiology in Potsdam. He received his PhD in Biology at the University of Münster in 2005, and directly afterwards joined the newly established Department "Organelle Biology" at the Max-Planck-Institute. In 2006, he was appointed as junior research group leader, and in 2010, as regular group leader at the Max-Planck-Institute.

Dr. Schöttler´s research focusses on the regulation of photosynthesis in algae and higher plants. His group has developed spectroscopic techniques to simultaneously measure all redox-active components of the photosynthetic electron transport chain in intact leaves. Additionally, also the proton motif force across the thylakoid membrane and ATP synthase activity can be measured non-invasively. Using these spectroscopic techniques and transgenic approaches, the contribution of the different photosynthetic complexes to photosynthetic flux control was elucidated. Additionally, Dr. Schöttler´s group is interested in the biogenesis and lifetimes of the photosynthetic apparatus.

Invited by the Department of Plant Physiology

Biogenesis and lifetimes of photosynthetic complexes in higher plants

Mark Aurel Schöttler

Photosynthesis Research Group, Department III – Organelle Biology, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm

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With the exception of photosystem II, which has a rapid turn-over especially under light-stress conditions, not much is known about the lifetimes of the photosynthetic complexes in higher plants. Circumstantial evidence suggests that especially the cytochrome b_6f complex and photosystem I may be highly stable: In tobacco plants, their biogenesis seems to be restricted to young leaves, suggesting a high lifetime of the complex. To directly determine the lifetimes of all photosynthetic complexes, we employed an ethanol-inducible RNAi approach targeted against essential nuclear-encoded subunits of each complex. In case of the cytochrome b6f complex, we targeted the essential Rieske protein (PetC) and the small M subunit (PetM), whose function in higher plants is unknown. *PetM*- and *PetC*-RNAi transformants showed similar phenotypes after RNAi induction: Young expanding leaves bleached rapidly and developed necrosis, while mature leaves, whose photosynthetic apparatus was fully assembled prior to RNAi induction, stayed green. In line with these phenotypes, cytochrome b_6f complex accumulation, linear electron transport and leaf assimilation capacity were strongly repressed in young leaves. In mature leaves, all photosynthetic parameters were indistinguishable from the wild type even after 14 days of induction. As repression of the *PetM* and *PetC* mRNAs was equally efficient in young and mature leaves, these data strongly suggest a lifetime of the cytochrome b6f complex in the range of a few weeks. Based on the analysis of inducible *PsaD*-RNAi lines, also for photosystem I, a high lifetime could be confirmed. The biogenesis of both the cytochrome b_6f complex and photosystem I is indeed restricted to young leaves. To determine the molecular basis of the ontogenetic repression of their biogenesis in mature leaves, changes in transcription, transcript maturation, translation and complex assembly during leaf development were analyzed. The importance of these different levels in controlling photosynthetic complex biogenesis will be discussed.

Halina Gabryś

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Halina Gabrys is Head of the Department of Plant Biotechnology at the Jagiellonian University in Krakow. She is a plant physiologist and cell biologist interested in photobiology attempting to uncover the mechanism of action of plant photoreceptors, in particular phytochrome and the blue-UV photoreceptors in higher and lower plants. Among many other interests, she also focuses on light-induced changes in the dynamics of cytoskeletal proteins in protoplasts and cells in situ. She identified motor proteins involved in light-induced chloroplast movements and studies the ecological significance of chloroplast movements.

She has teaching experience of over 30 years, and coordinated various national and international projects contributing also as PI. Dr. Gabrys visited a series of labs as guest scientist, among others the University of Erlangen as Alexander von Humboldt Fellow, the University of Illinois at Urbana Champaign, USA, as Visiting Research Specialist and later on as Visiting Scholar, the Dartmouth College Hanover, New Hampshire, USA, as Visiting Scholar and as Fulbright Senior Grantee.

Invited by the Department of Plant Physiology

What's going on at the surface of chloroplasts?

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Chloroplasts are commonly identified as organelles performing photosynthesis, the primary energy supplying process on Earth. To optimize conditions for this process these organelles are in constant motion, directed by light in most plants. Their movements are powered by proteins present at their surface. The identity of these proteins is currently under debate. According to one view, the chloroplasts are moved by specific short actin filaments (cp-actin) attached to their surface. Light is proposed to influence polymerization/depolymerization processes of cp-actin, thereby changing its dynamics. A contrasting hypothesis is based on immunofluorescence studies. It proposes a more classical mechanism, with myosin as a partner of actin in the process of motive force generation, similar to other plant organelles. Another protein localized on the chloroplast surface is indispensable for the organelle movement. It is CHUP1, suggested as a possible factor linking chloroplast envelope to actin filaments. Several other novel proteins appear to play important regulatory roles in the movement mechanism. Among them are two kinesin-like proteins, KAC 1 and KAC2, which together with THRUMIN1 and CHUP1 are responsible for chloroplast interaction with actin filaments and its anchoring in the plasma membrane. JAC1 appears to act as a switch between responses of chloroplasts to weak and strong light. A complex of two other proteins WEB1/PMI2 blocks JAC1 in strong light, which causes an avoidance response of chloroplasts. All these interactions, critical for motive force generation and for determining the direction of movement take place in a very small volume of cytoplasm, between the plasma membrane and the chloroplast.

Bruno Lemaitre

École Polytechnique of Lausanne, Switzerland

Bruno Lemaitre obtained his PhD in 1992 with Dario Coen at the University Pierre and Marie Curie (Paris) on the P element transposition in Drosophila. Next, he joined the laboratory of Jules Hoffmann (CNRS, Strasbourg France) as a research associate where he began a genetic dissection of the Drosophila antimicrobial response. One of his initial findings demonstrated that the Toll receptor protein and its downstream signaling pathway are essential components of the fruit fly immune response (Lemaitre et al. (1996) Cell 86:973). This is a pioneer work in innate immunity which facilitated the identification of Toll-like receptors as crucial mediators of human innate immunity. In 1998, he started his own lab at the Centre Génétique Moléculaire a research institute of the Centre National de Recherche Scientifique at Gif-sur-Yvette (France). In 2007, he became professor at the École Polytechnique of Lausanne (EPFL). His laboratory uses the fruit fly as a model genetic system doing research in the field of innate immunity and host-pathogen interactions.

Invited by the Department of Animal Physiology

The *Drosophila* gut: a new paradigm for epithelial immune response

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The gut combines and integrates very different physiological functions required for maintaining the equilibrium of the whole organism. In addition to its role in digestion, it is the main entry route for pathogens, and a reservoir for resident bacteria that must be tolerated. Finally, the intestinal epithelium undergoes a constant renewal required to maintain the integrity of this barrier. However, little is known about how these functions are regulated and coordinated, or what mechanisms are required to ensure gut homeostasis upon exposure to external challenges such as bacterial infection. Using an integrated approach, we are studying the mechanisms that make the gut an efficient and interactive barrier despite its constant interactions with microbes. We also focus our attention on the regulatory mechanisms that restore gut normal function upon challenge with bacteria. Our projects utilize integrated approaches to dissect not only the gut immune response, but also gut homeostasis and physiology in the presence of microbiota, as well as strategies used by entomopathogens to circumvent these defenses. We believe that the fundamental knowledge generated on *Drosophila* gut immunity will serve as a paradigm of epithelial immune reactivity and have broader impacts on our comprehension of animal defense mechanisms and gut homeostasis.

François Payre

Centre de Biologie du Développement, Toulouse, France

François Payre is currently Research Director from the Centre National de la Recherche Scientifique and faculty on Health and Biological Sciences at the University Paul Sabatier where he heads the Genes, Cells and Development program. He has received a BSc in Molecular Biology and a BSc in Biological Chemistry from Université Claude Bernard (Lyon, 1986), and a PhD in Molecular Genetics from the University of Toulouse in 1991. After post-doctoral training in the lab of A. Vincent where he studied evolution of gene regulation in flies, he set up his own lab in 1999 and was promoted Director in 2004. He has a long-standing interest in the mechanisms by which insect embryos build their external morphology during development (Payre et al., Nature 1999). In recent years, this work has led to insights into how gene regulatory networks determine morphological features during development and have evolved across species (Kondo et al., Science 2010; Frankel et al., Nature 2010, 2011). His research group has also identified the role of several regulators of localized cell shape changes during morphogenesis, cell migration and cell division. In 2010-2011, he was on a sabbatical stay at the Department of Biology of the New York University.

Invited by the Department of Zoology & Developmental Biology

Die Haut in der sie wohnen: Development of the *Drosophila* epidermis

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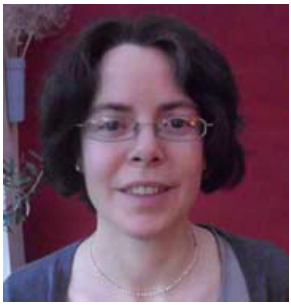
A challenging problem in biology is how the linear information encoded in the genome controls the formation of highly ordered 3-dimensional structures? The external morphology of insects is characterized a stereotyped array of cuticle extensions, called denticles or trichomes, resulting from the apical remodelling of underlying epidermal cells. Numerous studies using the trichome pattern as a readout have uncovered the gene regulatory networks that progressively pattern embryonic tissues during development. However, how these early regulatory cascades are translated, within individual cells, to program and realize the remodelling of their shape during terminal differentiation has remained elusive.
Combined to evolutionary studies, our genetic screenings identified a key transcription factor that determines which epidermal cells form trichomes. We have developed genome-wide molecular approaches to identify its downstream target genes in epidermal cells and, thereby, the repertoire of effectors directly responsible for trichome morphogenesis. Further investigation of their functions *in vivo* brought novel insights into the mechanisms underpinning a localized control of cell shape during normal development, as well as their corruption in certain human pathologies. Moreover, it has been recently discovered that a novel class of small peptides, encoded by apparently long noncoding RNAs, is required for epidermal development. We provide evidence that these peptides switch the activity of transcriptional pathways to provide a strict temporal control of cell differentiation. We will present recent advances towards understanding how these small peptides allow orchestrating changes in the shape of epidermal cells.

Ariel Blocker

University of Bristol, United Kingdom



Ariel Blocker obtained her PhD in 1995 from the University of Paris XI (Orsay). She worked at the EMBL in Heidelberg with Jean Gurenberg and Gareth Griffiths. Afterwards she became a postdoctoral fellow in Gareth Griffiths´ laboratory until she moved to Paris in 1997. There she worked at the "Institute Pasteur" as a postdoctoral fellow in Philippe Sansonetti´s laboratory. Since 2008 Ariel Blocker is part of the Schools of Cellular and Molecular Medicine & Biochemistry at the University of Bristol and since 2011 she is the reader in Microbiology.

In her research she focuses on the structural and functional analysis of the type III secreton of *Shigella flexneri*. She is interested in the mechanisms of protein targeting and secretion in prokaryotes and also in the mechanism of cell surface appendage assembly in Gram-negative bacteria. To reveal the macromolecular structures of the secreton, she uses e.g. electron microscopy.

Invited by the Department of Microbiology

Three-dimensional structure and mechanism of host cell-contact mediated activation of the Shigella T3SS needle tip complex

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Shigella flexneri is the causative agent of bacillary dysentery and utilises a Type Three Secretion System (T3SS) for cellular invasion. The T3SS resembles a molecular 'syringe' and is used by *S. flexneri* and many other Gram negative pathogens to secrete virulence effector proteins directly into the host cell, thereby instigating infection. At the distal end of the syringe's 50 nm-long and hollow needle sits the tip complex (TC). Its physical contact with the host cell is thought to result in activation of secretion. We previously provided evidence that the signal for secretion activation goes from the TC via the needle to a cytoplasmic regulator. Initial secretion leads to formation of a translocation pore (translocon) in host-cell membranes. The translocon is formed by conserved "translocator" proteins, IpaB-D. IpaD is hydrophilic and required for membrane insertion of the hydrophobic IpaB and IpaC. Prior to host cell contact, IpaD and IpaB localise to the distal needle tip, forming the TC. Upon host cell sensing, IpaB and newly secreted IpaC then insert into host plasma membranes, contiguously with needle secretion channel and the remainder of the effectors are translocated into the host cell. In order to understand how the TC senses host cells and leads to secretion activation, we used electron micrographs of negatively stained distal needle tips to reconstruct the structure of the TC atop needles in three dimensions (3D). This revealed the stoichiometry and unique organisation of IpaD and IpaB in the resting TC. Comparison of 3D reconstructions of TCs atop wild-type and mutants needles displaying premature secretion activation further reveal major conformational changes

within the TC during this process and suggest how these may be transmitted to the needle. We have validated this structural work using IpaD-IpaD cysteine crosslinking and conventional genetics. Our findings also reveal why antibodies to specific epitopes of LcrV and PcrV (*Yersinia* and *Pseudomonas* homologs of IpaD, respectively) are protective against disease.

Enrica Bordignon

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Enrica Bordignon is a Senior Researcher in the EPR spectroscopy group at the Department of Physical Chemistry of the ETH Zurich. She obtained her PhD at the University of Padova, Italy, with Prof. Carbonera investigating the photophysical properties of photosynthetic reaction centers and antenna complexes. Already during her PhD studies Dr. Bordignon was at the Max Volmer Laboratory for Biophysical Chemistry in Berlin.
As a postdoc, she joined the group of Prof. Steinhoff at the University of Osnabrück from 2003 to 2008 and was also a member of the Sonderforschungsbereich working on the bacterial phototaxis membrane protein complex *Np*SRII/*Np*HtrII by using site-directed spin labeling and EPR spectroscopy. Here Dr. Bordignon also started to work on ABC transporters, namely on the human TAP involved in antigen presentation in the immune system as well as on the maltose ABC transporters from *E.coli* and *S.tyiphimurium*. In 2008, she continued applying EPR spectroscopy to ABC transporters at the ETH Zurich in the group of Prof. Jeschke now investigating also the type I and type II ABC importers like ButCD-F, the ABC exporter MsbA for lipid transport as well as the vitamin B_{12} transporter.
Dr. Bordignon also was a Guest Professor at the Department of Physics at the Free University, Berlin, in the frame of the "Berliner Chancengleichheitsprogramm" and held a Humbolt Fellowship. In 2011, she won the Young Investigator Award of the International EPR Society.

Invited by the Biophysics Group, Department of Physics

Properties and structural transitions of ABC transporters in different environments investigated by site-directed spin labeling EPR

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Electron paramagnetic resonance (EPR) combined with site-specific labeling can provide information on structural transitions of large proteins and protein complexes in conditions close to the physiological ones[1], which can be difficult to assess with other established techniques such as X-ray crystallography and NMR spectroscopy. Here I will elucidate the application of a variety of EPR methods on the ABC transporters family, responsible for translocation of substrates across the membranes powered by ATP hydrolysis. Different states of the transporter proteins are populated at ambient temperature by addition of substrates and/or nucleotides both in micelles and liposomes and singly spin-labeled transporters can be used to gain information on changes in dynamics of different protein regions during the translocation cycle by CW EPR methods. Site-specific changes in water accessibility can be also obtained using established methods (e.g. determination of the g and A tensor components and power saturation techniques) or a dynamic nuclear polarization (DNP) method which was recently introduced to study water dynamics at ambient temperature on membrane protein complexes[2]. The same trapped states in the protein conformational cycle are studied at cryogenic temperature and doubly-labeled transporters can be used to perform the most informative EPR experiment, namely the measurement of distance distributions in the range between 1.8 and 8 nm by the pulsed EPR technique DEER (also known as PELDOR). I address important aspects of such structural characterization via interspin distance constraints in terms of measurement sensitivity[3], freezing conditions, choice of proper sites, reliability of the distance distribution, importance of broadband excitation. Examples of the type of agreement obtained between experimental DEER constraints and interspin distances simulated on available structural models[4] will be given. EPR spectroscopy made a significant contribution to the understanding of ABC transporters as dynamic molecules which can

communicate across the membrane through different subunits. Examples will be given on the maltose bacterial importer[5], the vitamin B_{12} bacterial importer[6], the lipid-A bacterial exporter MsbA[7] and the human exporter TAP[8].

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Nils Gunnar Hansson von Heijne

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Gunnar von Heijne has worked mainly on problems related to protein sorting and membrane protein biogenesis and structure. His work includes both bioinformatics methods development (e.g. methods for prediction of signal peptides and other sorting signals as well as prediction of membrane protein topology) and experimental studies in *E. coli* and eukaryotic systems.

The most important achievements include the discovery and experimental validation of the so-called "(-1,-3)-rule" (describes signal peptide cleavage sites; *EJB* 133:17, *JMB* 184:99) and the "positive inside" rule (describes membrane protein topology; *EMBO J* 5:3021, *Nature* 341:456, *Cell* 62:1135, *Cell* 77:401), the development of widely used prediction methods (e.g., TopPred, SignalP, TargetP, TMHMM; *JMB* 225:487, *Prot.Engineer.* 10:1, *JMB* 300:1005, *JMB* 305:567, *JMB* 327:735, *JMB* 340:783, *PNAS* 105:7177), the first proteome-wide theoretical and experimental studies of membrane protein topology in *E. coli* and *S. cerevisiae* (*Protein Sci.* 7:1029, *Science* 308:1321, *JMB* 35:489, *PNAS* 103:11142), the first quantitative analysis of the energetics of membrane protein assembly *in vivo* (*Nature* 433:377, *Science* 307:1427, *Nature* 450:1026, *PNAS* 106:11588), and recent theoretical and experimental studies of so-called dual-topology membrane proteins and their role in the evolution of membrane protein structure (*Nature Struct.Mol.Biol.* 13:112, *Science* 315:1282, *Science* 328:1698).

Invited by the Department of Microbiology

Translocon-mediated assembly of membrane proteins

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The process of translocon–mediated insertion of membrane proteins into target membranes has mostly been studied from a cell biological perspective, while biophysicists have focused on chemically well-defined model systems such as synthetic peptides interacting with pure lipid membranes or membrane-mimetics. We have tried to bring the quantitative thinking of the biophysicist to in vivo studies of membrane protein insertion in an attempt to bridge the gap between the mechanistic knowledge provided by cell biology and the physical chemistry perspective of biophysics. The results obtained so far support a simple partitioning model as the underlying principle of translocon-mediated membrane protein insertion.

Poster Abstracts

Endocytosis in the filamentous fungus *Ashbya gossypii*

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In a fast spreading mycelium of *Ashbya gossypii* the surface expansion rate of a hyphae is up to 40 times higher than that of a growing bud of its close relative *Saccharomyces cerevisiae*. To maintain polarity it is important to restrict the sites of surface growth to the hyphal tips and emerging branches. Therefore, polarity factors and excessive membrane material have to be internalized subapically. This is achieved by endocytosis. In *S. cerevisiae* the major pathway for endocytosis is clathrin and actin dependent and has been well characterized. Because of the highly conserved gene content compared to yeast, *A. gossypii* is especially applicable to study endocytosis during hyphal growth. First results showed that some steps in the process are up to 5 fold faster than in budding yeast. This might be caused by the requirement of a more efficient endocytic system during polar growth. Another major difference compared to *S. cerevisiae* is the role of clathrin in endocytosis. Live-cell imaging together with Total Internal Reflection Microscopy revealed that clathrin does not belong to the early endocytic proteins. Furthermore we could not detect colocalization of clathrin proteins with any of the other tested endocytic proteins. We assume that clathrin plays only a minor role in the endocytic processes of *A. gossypii*.

Reconstruction of cell organelles via tracking and localization microcopy (TALM) of mitochondrial proteins in living cells

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Mitochondria can be found in almost all eukaryotic cells and underlie a constantly fusion and fission process. Despite, they are heterogeneous organelles with zones of different activity in single organelles. To dissect this heterogeneity, dynamics of mitochondrial membrane proteins was determined by TALM. We monitored the movement of single mitochondrial proteins fused to a HaloTag labeled specifically with a functionalized bright and photostable dye, Tetramethylrhodamin (TMRHTL), via fluorescence microscopy. Membrane proteins could be localized with a precision between 10-20 nm in life cells. Single microcompartments in individual organelles could be distinguished by comparing the superresolution image of proteins with their respective trajectories. By measuring this spatiotemporal dynamics of single respiratory chain complexes and diverse OMM proteins the dynamic structure of mitochondria could be monitored. Low mobile RCC were recorded in cristae and their trajectories were retraced to reveal nanometric structures of the inner mitochondrial membrane. However, mitochondria are tubular cell organelles with a radius of 500 nm to 800 nm. The projecton of trajectories in 2D reduced the spatial information, because the axial position of the fluorophores was neglected. By use of a cylindrical lens, also the information about the axial position of a single fluorophore can be obtained. The cylindrical lens induces a astigmatic point spread function (PSF) and blurs it along the y-axis or the x-axis. By calculating the different distortions, the z-positions of a single membrane protein in 3D can be localized. The goal is now to reconstruct 3D trajectories in combination with structural data obtained from superresolution fluorescence imaging and electron tomography of respective mitochondria.

Coordination of sorting complexes during endosomal maturation

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During endocytosis in eukaryotic cells, cargo proteins are incorporated into vesicles, which fuse with the early endosome. These endosomes then mature to late endosomes and fuse with the lysosome. The maturation of the organelle is facilitated by a number different proteins and protein complexes. The ESCRT machinery is required for luminal sorting of transmembrane proteins, retromer to recycle sorting receptors, and the tethering machinery, including Rab GTPases and the HOPS complex, that mediates fusion with the lysosome. It was shown recently that retromer and the fusion machinery are both regulated by the Rab GTPase Rab7/Ypt7, raising the possibility that there is crosstalk between different sorting events. To understand such a crosstalk it is crucial to know when these complexes are recruited to the endosome and when they are recycled back to the cytoplasm. In order to better understand endosomal maturation and possible crosstalk mechanisms, I analyze the ordering of sorting events in the endocytic pathway using fluorescently labeled peptides. In addition, I take advantage of the vacuole fusion system as a read-out of endosomal maturation defects. My data suggest that the alterations in fusion capacity of mutant organelles are caused by a defective crosstalk of the membrane remodeling machineries during endosomal maturation.

The AMP-activated kinase homologous SNF1 complex affects cell wall integrity in *Saccharomyces cerevisiae*

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The mammalian AMP-activated kinase (AMPK) serves in nutrient and energy sensing and is activated upon depletion of these sources. It forms an essential part in cell physiology and provides a target for drug-treatment of type-2 diabetes in humans.
The Snf1-kinase complex is the yeast ortholog of AMPK. Yeast cells deleted for *snf1* are defective in release from carbon catabolite repression and do not grow on non-fermentative carbon sources. Also, they do not accumulate glycogen. Furthermore, the Snf1-kinase has been found to be involved in the control of meiosis, sporulation and aging. We found that Snf1 affects the yeast cell wall structure, which consists of a polysaccharide network with attached protein and is remodeled for adjustment to needs during vegetative growth, cell fusion, and in response to environmental stress. This remodeling of the cell wall is governed by the cell wall integrity pathway (CWI pathway). As do mutants in this pathway, *snf1* cells display hypersensitivity to cell wall stress inducing agents and a reduction in cell wall thickness. Epistasis analyses and Mpk1 phosphorylation suggest a function in parallel to the CWI pathway.
A possible explanation for the cell wall phenotype of *snf1* cells is their reduced level of intracellular glucose-6-phosphate. Glucose-6-phosphate is necessary for synthesis of UDP-glucose, which in turn serves as a precursor of cell wall glucans. The deletion of the genes encoding the phosphofructokinase subunits á and â lead to an increased level of glucose-6-phosphate. Accordingly, *snf1 pfk1* mutants show a less pronounced sensitivity to cell wall stress like zymolyase which supports the assumption that the effect of Snf1 on the cell wall is exerted by its influence on glycolytic intermediary metabolites.

Cooperation of two protein secretion systems during the interaction of *Salmonella enterica* with host cells

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Salmonella enterica is an important gastrointestinal pathogen of humans and animals with the ability to invade non-phagocytic cells and to persist and proliferate within mammalian cells. For invasion in polarized epithelial cells, *Salmonella* uses two protein secretion systems1. The type 1 secretion system (SPI4-T1SS) is needed to get into closer contact with the epithelial cell via the giant, non-fimbrial adhesin SiiE3, which binds to an apical glycan structure[2]. The type 3 secretion system (SPI1-T3SS) translocates effector proteins leading to actin remodeling and uptake of Salmonella. For invasion of non-polarized cells, the giant adhesin SiiE is not necessary[1]. There is a cooperation of both secretion system during invasion of polarized cells[1], so we investigate the formation of a microcompartment of these two protein secretion systems at the zone of contact between *Salmonella* and its target cells. To reveal colocalization of both secretion systems, we perform variants of d STORM (direct Stochastic Optical Reconstruction Microscopy)[4] in non-invading bacteria and cells with invading bacteria. This enables us to gain a better understanding of *Salmonella* invasion of polarized cells. Furthermore EM (electron microscopy) and AFM (atomic force microscopy) help us to characterize in detail the structure, function and host cell binding of the giant adhesin SiiE under different conditions.

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Mammalian sucrose transporters

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According to the classic point of view, transport of sugars across animal plasma membranes is performed by two families of transporters. Secondary active transport occurs via Na^+ symporters of the SLC5 family, while passive transport occurs via facilitative transporters of the SLC2 family. Recently, a new family appeared in the scenery which was called the SLC45 family of putative sugar transporters because one of its members had been shown to transport sugars, and because all members exhibit an apparent amino acid sequence identity to plant sucrose transporters of slightly above 20%. Although the human SLC45 family members (A1-A4) show only 20-30% identity among each other, based on derived amino acid sequences, they all feature twelve transmembrane domains with a large intracellular loop between transmembrane domains VI and VII, and have a signature sequence R-X-G-R-[K/R] between transmembrane domains II and III, which is typical for plant sucrose transporters. Here we provide novel insights into the molecular features of SLC45 family members with a focus on their role as sugar transporters.

Walking on a Thin Line: Mechanism and Biological Impact of Ceramide Trafficking at the ER-Mitochondrial Interface

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Cells routinely synthesize ceramides in the endoplasmic reticulum (ER) as precursors for sphingolipids to form an impermeable plasma membrane. In addition to their role as central intermediates of sphingolipid biosynthesis, ceramides have been implicated as signaling molecules in cellular stress responses and apoptosis. Consequently, cells must regulate ceramide levels closely to meet metabolic demands without compromising their viability. We recently identified a protein sensor for ceramides and found that cells lacking a functional sensor commit suicide by mistargeting ER ceramides to mitochondria. How ER ceramides can reach mitochondria to trigger apoptosis is not known. The aim of this SFB project is to unravel the molecular principles that govern ceramide trafficking at the ER-mitochondrial interface and identify ceramide effectors responsible for mediating mitochondrial apoptosis. We will use photoactivatable and clickable ceramide analogues to trace ceramide-binding proteins at ER-mitochondrial junctions and mitochondrial membranes. As complementary approach, we will conduct a functional screen for proteins required for committing sensor-deficient cells to death. In view of the tumor suppressor activity of ceramides, we will evaluate newly-identified components of the ceramide trafficking machinery and their effectors as targets for modulating drug-induced apoptosis in cancer cells.

Structure and function analysis of the multisubunit HOPS complex

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Multisubunit tethering complexes are conserved among species and involved at different stages in intracellular transport pathways. The hexameric HOPS complex acts at the lysosome/ vacuole where it is responsable for several fusion events with different kinds of vesicles. In general, fusion recations require in addition to tethering complexes, SNAREs and small Rab GTPases. Mutations in the HOPS proteins lead to disruption of trafficking to the lysosome and therefore to several diseases. A missense mutation in the HOPS subunit Vps33A in mice causes progessive neurodegeneration due to a delivery defect to the lysosome. A mutation in the human Vps33B subunit leads to arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome, which might be cause by impaired endosomal biogenesis. It is therefore essential to understand the functionality and subunit arrangement of this tethering complex.

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Quantitative live cell imaging of microtubule dynamics in living cells

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Microtubules play a vital role in several aspects of cell physiology such as intracellular transport, mitosis or morphogenesis. In neuronal cells, a coordinated and dynamic reorganization of microtubules is required for the formation and maintenance of neurites. Disruption of microtubule dynamics can have major influences on the structure and function of neurons, as it becomes evident in several neurodegenerative diseases. We established a method to determine microtubule dynamics in living neural cells by expressing photoactivatable GFP (PAGFP)-tagged α-tubulin followed by live cell imaging after focal fluorescence activation. Using a modeling approach, microtubule dynamics can be quantitatively determined from fluorescence decay curves and the ratio of microtubules to free tubulin calculated. We demonstrate the effect of microtubule modulating agents and microtubule-associated proteins in living cells and show that DMSO, which is generally used as a carrier for hydrophobic substances, has a major impact on neuronal microtubule dynamics. The approach could be useful to determine the effect of novel microtubule modulating agents in a living system.

Arabidopsis TORTIFOLIA1 works downstream of auxin and controls cell elongation through its function in the microtubule-plus tip microcompartment

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Plant development requires the orchestration of cell division and cell elongation. Defects in cell elongation can lead to right- or left-handed organ twisting. Plant mutants with twisting phenotypes are either defective in microtubules, in auxin transport (a plant growth regulator) or in cell wall structure. This research aims to better understand how microtubules control cell elongation and how cell elongation is controlled by upstream growth regulators. To this end leaf growth of *tortifolia1* mutant leaves was studied in detail. Video analyses revealed that the mutant is defective in natural plant movements, so-called tropisms. It was found that these plant movements involve the plant-hormone auxin, however, *tortifolia1* was found to be partially auxin-insensitive. *TORTIFOLIA1* encodes a plant-specific microtubule-associated protein. Double labeling of TORTIFOLIA1 and microtubules showed that the protein labels microtubule plus-ends during growth and catastrophe. This unusual behavior suggests a novel function in the microtubule-plus tip microcompartment and provides insight for the proteins' function in cell wall synthesis and plant cell growth.

The cardiac extracellular matrix of *Drosophila melanogaster* constitutes a specialized structural microenvironment that facilitates organ integrity

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Extracellular matrices (ECM) constitute complex multifunctional protein scaffolds that promote tissue integrity, cellular adhesion and serve as signaling platforms. Mutations in genes encoding ECM proteins severely affect embryonic development and cause a broad range of birth defects. Molecular details of how specific ECMs are formed in complex tissues are rather elusive. In our studies we concentrate on the identification of genetic mechanisms leading to the formation and maintenance of functional matrices in the model *Drosophila melanogaster*.

Using classical genetic analysis we identified the *Drosophila* ADAMTS-like protein Lonely heart (Loh) as an essential factor for the organization of a cardiac matrix composed of the collagen Pericardin (Prc). Mutation of *loh* causes a progressive disruption of heart integrity, including the detachment of heart associated cells and a loss of hemolymph flow. We can show that Loh serves as a secreted mediator of matrix formation and is able and essential to recruit Prc to the ECM.

Our data, in combination with previous findings in mammals, demonstrates that the function of ADAMTS-like proteins in facilitating matrix formation and stability is evolutionary conserved.

The C-terminus of the Arabidopsis thaliana GRX ROXY1 is crucial for interaction

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Glutaredoxins (GRXs) are small glutathione-dependent oxidoreductases that participate in redox-regulated processes via thiol-modulation of proteins. In plants, GRXs are associated with stress response and recently also an involvement in flower development could be shown by us. The *Arabidopsis thaliana* GRX ROXY1 participates in petal primordia initiation and also later on in petal morphogenesis. ROXY1 belongs to a land-plant specific CC-type GRX group with 21 members (31 GRXs in total). It was previously shown that ROXY1 interact in the nucleus with floral TGA transcriptions factors and this interaction is a prerequisite for the function in Arabidopsis petal development. Deletion analysis further revealed the importance of the ROXY1 C-terminus for the interaction with TGAs and the function in petal development. By dissecting the ROXY1 C-terminus, crucial conserved motifs could be identified. Their requirement for the ROXY/TGA protein interaction, as well as for the biological ROXY1 function in petal initiation was investigated.

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Understanding structural interactions within two component systems

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Bacteria have to deal with a large variety of environments and are urged to adapt most quickly to changing living conditions. The largest groups of regulatory signal transduction systems enabling this adaptation are two component systems (TCS). TCSs are typically composed of a transmembrane sensor Histidine Kinase (HK) and a cytoplasmic Response Regulator (RR) which facilitates the genetic response. Significant structural sequence identities have been found among TCSs. Nevertheless, TCSs comprise high specificity between the respective HK and RR pairs. Structural insights are fundamental for understanding protein-protein interactions. Hence, a structural homology model of the cytosolic part of the HK CpxA and its RR CpxR was developed, based on the HK-RR co-crystal structure solved by the group of Marina et al (2009). Putative interaction sites were derived from this model and investigated by MSPINE (2011). The identified interaction sites are currently under further *in vivo* analysis by BACTH, especially by creating mutants with substitutions at the positions of the respective interacting amino acids. All in all, our investigations contribute to a better understanding of the signal transduction mechanisms within TCSs.

A novel interaction of tau through its N-terminal projection domain

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The microtubule-associated protein tau plays an important role in several neurodegenerative diseases, including Alzheimer´s disease. However, its exact physiological functions are still unclear. The investigation of tau´s cellular interactions mediated by its functional domains could be crucial for a better understanding of the processes that lead to diseases. To investigate tau´s interactions and spatio-temporal dynamics by live cell imaging, a posttranslational labeling system was established. This allows the specific and stable *in situ* labeling of tau with membrane permeable and photostable dyes like tetramethylrhodamine (TMR). Using a tau deletion mutant lacking its microtubule-binding region, we observed cluster formation in living PC12 cells. Bleaching experiments indicated that tau molecules in the clusters did not undergo rapid exchange. Strikingly, co-localization experiments revealed an association of tau clusters with lipid droplets. Movement of tau clusters was studied by single particle tracking. Random motion as well as directed movement similar to lipid droplets was observed, which appears to be on microtubule tracks. By the use of several deletion mutants, the amino acid sequence required for this phenotype was narrowed down to the six amino acids Ser-Ala-Lys-Ser-Arg-Leu in tau´s N-terminal projection domain (amino acid position 238-243 in wild type tau). Taken together, the study reveals a prior unknown interaction of tau with lipid droplets. Since lipid droplets facilitate lipid storage including cholesteryl esters, tau might be linked to regulation of membrane homeostasis.

Functional analysis of the peritrophic matrix in Tribolium castaneum

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The peritrophic matrix (PM) is a highly specialized extracellular matrix, which is situated in the midgut of insects. It consists mainly of glycoproteins and to a lower degree of chitin. Only little is known about the composition and function of the PM in insects.
In the red flour beetle Tribolium castaneum, the PM is continuously produced at all feeding stages. It protects the midgut from mechanical damage and supports digestion by sequestering hydrolytic enzymes. The PM constitutes a poriferous matrix, which retains macromolecules with an exclusion size that varies between different insects. The exclusion size is believed to be determined by peritrophic matrix proteins (PMPs). They bind chitin microfibrils to form a network that allows other proteins to interact with the matrix. PMPs possess special chitin binding domains (CBDs), which can vary in number between different proteins.
To analyze the functions of PMPs, RNA interference experiments in combination with functional assays to determine PM permeability were applied. The latter assays are based on the distribution of fluorescent dextranes with defined molecular masses. After feeding the dextranes, they were found to be restricted to the gut lumen in wild type larvae, but entered the gut epithelium upon specific knockdown of PMP3 and PMP5-B. The knockdown of the latter genes resulted in a considerable reduction of the fat body and the body weight. Additionally insects failed to molt and died during molting. Knocking down PMP9 did not result in any phenotype. Our findings show that PMP3 and PMP5-B have pivotal functions in maintaining the integrity of the PM.

Dual targeting of the major myelin protein 36K of fish CNS

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Myelin is a multilamellar, lipid-rich membrane system that wraps jellyroll-like around axons. It serves as an insulator for saltatory impulse conduction in the central and peripheral nervous system (CNS; PNS) of vertebrates. Myelin contains a relatively simple array of highly specialized proteins, myelin basic protein (MBP) and the proteolipid proteins (PLP/DM20) being the two major CNS myelin proteins. In the CNS of phylogenetically older species such as bony fish a divergent protein pattern is observed. A quite remarkable major protein is encountered in the CNS myelin of fish, termed 36K after its apparent molecular weight (Jeserich, 1983). The hydrophobic 36K does not show a substantial sequence similarity with any of the known myelin proteins, but a moderate similarity with members of the short chain dehydrogenase family (SDR).

To study the functional properties, intracellular sorting and trafficking of this enigmatic myelin protein, a heterologous expression approach was accomplished using a rat oligodendroglial cell line (OLN-93, Richter-Landsberg and Heinrich 1996). With this integrative approach we were able to demonstrate, that a mitochondrial targeting signal (MTS) at the N-terminus of 36K is responsible for translocation into mitochondria. Deletion of the N-terminal region of 36K blocked the mitochondrial 36K import, while addition of this region to GFP leads to mitochondrial import. Furthermore, analysis of point mutation, introduced in the in the N-terminal phosphorylation sites, indicate that phosphorylation of the MTS inhibits 36K translocation to mitochondria. Masking of the MTS results in a distinct association to the plasma membrane. Our results point to a dual targeting of 36K and possibly to a dual biological role for it in the mitochondria that is likely to be different from its specific adhesive role in the cytoplasmatic leaflets. Summarized, our results reveal a striking similarity between the localization of 36K and the CNPase of higher vertebrates.

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Tracing the transient conformational signal in bacterial phototaxis using SDSL-EPR spectroscopy

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In microbial photo- and chemotaxis a two-component signaling cascade mediates a regulated response of the flagellar motor to environmental conditions. Upon activation, photo- and chemoreceptors transfer a signal across the plasma membrane to activate the histidine kinase CheA. Successive regulation of the CheY-phosphorylation level controls the flagellar motor.

In Natronomonas pharaonis a sensory rhodopsin II-transducer complex (SRII/HtrII) mediates negative phototaxis[1]. As the initial signal, a light-induced outward movement of receptor helix F leads to a conformational change of transducer helix TM2. The mechanism underlying the signal propagation to the adjacent HAMP domain[1,2] and subsequently to the kinase activation domain still remains unclear.

For the HAMP domain, a widely abundant signaling module, several mechanisms were suggested[3], all comprising two distinct conformational states of the HAMP domain. The two states can be observed by two-component cw-EPR spectra at ambient temperatures existing in a thermodynamic equilibrium which can be driven by salt-, temperature- and pH-changes[4].

To trace the conformational signal and its propagation throughout the elongated transducer, we applied cw- and pulse-EPR spectroscopy in conjunction with nitroxide spin labeling. We follow transient changes by time-resolved cw-EPR spectroscopy and compare the resulting spectral changes to difference spectra corresponding to the above shifts in the thermodynamic equilibrium.

The light-driven conformational changes are in agreement with a shift towards a more compact state of the HAMP domain. Following this signal beyond the HAMP domain requires a mechanism compatible with the formation of trimers of SRII/HtrII dimers which activate CheA. An activation scheme within the framework of hexagonal arrays formed by the trimers of SRII/HtrII will be the key step to understanding the enormous cooperativity leading to signal amplification via these receptor clusters.

Dynamics of sensors triggering cell wall integrity signaling in *S. cerevisiae & K. lactis*

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The yeast cell wall is essential to ensure cell shape and integrity. A set of plasma membrane spanning sensors detects cell surface stress and activates the small GTPase Rho1 via the GTP/GDP exchange factor (GEF) Rom2. The signal is transduced via Pkc1 to a conserved MAP (mitogen activated protein) kinase cascade. Ultimately, the CWI (cell wall integrity) signaling pathway governs appropriate gene transcription and cell cycle regulation. Two small protein families with Wsc1, Wsc2 & Wsc3 on the one hand and Mid2 & Mtl1 on the other hand constitute the sensors. They all contain a long, highly mannosylated extracellular serine/threonine-rich region, a single transmembrane domain and a short cytoplasmic tail, whereas the Wsc-family sensors in addition contain a cysteine-rich domain (CRD) as headgroup. The Wsc-family sensors accumulate in an endocytosis-dependent process at sites of polarized growth and are dynamically distributed between the cytoplasm, the plasma membrane and the vacuole. With GFP fusion proteins we could show that this accumulation in Arrestin-mediated endocytosis is reduced in *art5*Δ mutants. It was previously shown that CWI sensors show distinct clustering patterns under stress conditions. We want to show preliminary results on dynamic distribution and clustering of CWI sensors and present first hints on accumulation of sensors in the yeast microcompartments of Pma1 (MCP) and Can1 (MCC)/eisosomes in the baker´s yeast *Saccharomyces cerevisiae* and the milk yeast *Kluyveromyces lactis*.

The Msb3/Gyp3 GAP controls the activity of the Rab GTPases Vps21 and Ypt7 at endosomes and vacuoles

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Fusion of organelles in the endomembrane system depends on Rab GTPases that interact with tethering factors before lipid bilayer mixing. In yeast, the Rab5 GTPase Vps21 controls fusion and membrane dynamics between early and late endosomes. We identified Msb3/Gyp3 as a specific Vps21 GTPase-activating protein (GAP). Loss of Msb3 results in an accumulation of Vps21 and one of its effectors Vps8, a subunit of the CORVET complex, at the vacuole membrane in vivo. In agreement, Msb3 forms a specific transition complex with Vps21, has the highest activity of all recombinant GAPs for Vps21 in vitro, and is found at vacuoles despite its predominant localization to bud tips and bud necks at the plasma membrane. Surprisingly, Msb3 also inhibits vacuole fusion, which can be rescued by the Ypt7 GDP–GTP exchange factor (GEF), the Mon1–Ccz1 complex. Consistently, msb3Δ vacuoles fuse more efficiently than wild-type vacuoles in vitro, suggesting that GAP can also act on Ypt7. Our data indicate that GAPs such as Msb3 can act on multiple substrates in vivo at both ends of a trafficking pathway. This ensures specificity of the subsequent GEF-mediated activation of the Rab that initiates the next transport event.

Genetic analyses of the abundant wine yeast *Kloeckera apiculata* (= *Hanseniaspora uvarum*)

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The yeast *Kloeckera apiculata* (sexual form = *Hanseniaspora uvarum*) accounts for 50-90% of the total yeast population on wine grapes (Gafner, 2007). It is able to ferment sugars and produce ethanol, albeit to lower final concentrations than *Saccharomyces cerevisiae*. *K. apiculata* dominates the fermentation process during the first day of must fermentation, even with addition of *S. cerevisiae* starter cultures. It is known for its high capacity to produce flavour compounds, especially fruity esters, and its presence in wine production frequently leads to better products in so-called "wild fermentations". However, a high-level acetate production has also been reported for some strains of *K. apiculata*.

The long-term aim of this project is to obtain *K. apiculata* strains suitable for wine fermentation, e.g. by blocking the pathway to acetate production and by promoting the production of esters and glycerol, which improve wine quality. A first step to achieve this, is to establish a molecular genetics. As a basis, the genome annotation is in progress. About 93% of the genomic sequence, represented in 217 contigs, has been obtained. We are currently in the process to allocate these contigs to their respective chromosomes, which will further facilitate the genome annotation. Several thousand protein coding sequences have already been identified and some of them have been used for functional complementation in the respective *S. cerevisiae* mutants. This is a first step in order to provide the tools for the investigation of the metabolic pathways governing flavour production. In this context, the first selection markers have been isolated (*URA3*, *HIS3*).

In a more applied aspect of this project for the wine industry, we have employed the genome sequence information to generate a method for the detection of restriction fragment length polymorphisms (RFLP), in order to allow for the rapid identification of special strains of *K. apiculata*.

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Spatio-temporal dynamics of effector recruitment by a cytokine receptor probed by single molecule imaging and cell micropatterning

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The key signaling pathway activated by cytokine receptors involves tyrosine phosphorylation of signal transducers and activators of transcription (STAT) proteins by receptor-associated Janus kinases. We aim to unveil of the very early events of signal activation by the type I interferon receptor, which is comprised of the receptor subunits IFNAR1 and IFNAR2. To this end, we have established methods for probing the spatiotemporal dynamics of STAT recruitment to IFNAR1 and IFNAR2 at the plasma membrane by single molecule imaging and micropatterning of functional signalling complexes in live cells. The interaction of STATs to membrane-proximal sites was detected by total internal reflection fluorescence microscopy, allowing for localizing and tracking individual molecules beyond the diffraction limit. Constitutive binding of STAT2 but not of STAT1 to IFNAR2 mediated via the coiled-coil domain of STAT2 was detected. In contrast, STAT1 binding was observed to be increased after receptor stimulation. As lateral diffusion turned out to be the limiting factor for quantitatively probing STAT binding, we established functional micropatterning of the signalling complex in living cells. Receptor subunits fused to the HaloTag were covalently captured into predefined areas, thus generating a contrast within individual cells, allowing for qualitative and quantitative protein interaction analysis with the micropatterned receptor subunit. The dynamic interaction between effector proteins and immobilized IFNAR2 in patterned cells could be demonstrated by FRAP and single molecule tracking. Stable association of the Janus kinases to the micropatterned receptor was confirmed as well as constitutive binding of STAT2. Moreover, ligand-dependent association of STAT1 into micropatterns confirmed functional organization of the signaling complex. Thus, we established a generic method for probing effector interactions with the receptor subunits in a quantitative manner.

Extracellular multisite recording of synaptic potentials in human model neurons

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Extracellular recording of human model neurons (NT2N cells) revealed spontaneous spiking activity initially in the third week after replating. Furthermore, biphasic voltage pulses above 500 μV evoked fEPSPs up to 3.7 mV. By ombined application of the cholinergic antagonists tubocurarine (10 μM) and scopolamine (10 μM) a 15% reduction in fEPSP amplitudes was revealed while in the presence of the AMPA/kainate receptor blocker CNQX (10 μM) amplitudes were diminished by 33%. The high reproducibility of fEPSP amplitudes observed especially in astroycte-NT2N co-cultures renders this cell culture system a highly promising tool for future experimental studies on the effects of neuroactive substances in a human system.

A novel role for the non catalytic intracellular domain of Neprilysins in muscle physiology

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Neprilysins are membrane bound M13-endopeptidases responsible for the activation and/or inactivation of peptide signaling events on cell surfaces. By hydrolyzing their respective substrates, mammalian neprilysins are involved in the metabolism of numerous bioactive peptides especially in the nervous, immune, cardiovascular and inflammatory systems. Based on their involvement in essential physiological processes, proteins of the neprilysin family constitute putative therapeutic agents as well as targets in different diseases, including Alzheimer´s disease.

We here demonstrate that overexpression of Neprilysin 4 (Nep4) in *Drosophila melanogaster* leads to a severe muscle degeneration phenotype. This phenotype is observed for overexpression of full length Nep4 in somatic muscles and is accompanied by severely impaired movement of larvae and lethality in late larval development. On the other hand, downregulation of expression caused only the latter two effects. By expressing several mutated and truncated forms of Nep4 in transgenic animals, we show that the intracellular domain is responsible for the observed phenotypes while catalytic activity of the enzyme is apparently dispensable. A Yeast two-hybrid screen identified a yet uncharacterized carbohydrate kinase as a first interaction partner of the intracellular domain of Nep4. To our knowledge, this is the first report of an intracellular neprilysin domain being involved in muscle integrity.

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Human Aβ peptides induce changes in spine shape and reduce cortical thickness in a mouse model of Alzheimer´s disease

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Alzheimer´s disease (AD) is a highly prevalent neurodegenerative disorder in elderly. Among the pathological features changes in spine density and loss of neurons can be indicators for long-term cognitive impairments during AD. We investigated the effect of chronic presence of Aβ on loss of neurons in different cortical areas and synaptic connectivity of pyramidal cells in parietal association cortex using a transgenic mouse model. APP_{SDL} mice express human APP with combination of three mutations; Swedish, London and Dutch. The mice produce equimolar amounts of Aβ40 and Aβ42, which increase during aging and lead to deposits at 20 months. To evaluate the impact of elevated quantity of human Aβ peptides on neurodegeneration, we measured cortical thickness in aged mice. We found significant reduction of thickness of the cortex in both regions analyzed. To determine whether the thinning of the cortical mass is due to loss of neurons we applied a machine learning approach on micrographs of NeuN stained coronal sections for unbiased determination of neuron density. We observed a slightly higher neuron density in most of the cortical regions analyzed indicating loss of parenchymal tissue but no loss of neurons. To assess potential changes in synaptic connectivity, APP_{SDL} mice were crossed with the GFP M line to determine changes of spine density and shape by high-resolution cLSM. We found that spine density increased and the morphology exhibited a shift from mushroom towards stubby in aged Aβ producing mice.
The data indicate that chronic presence of Aβ induces changes in spine shape and reduces cortical thickness without neuron loss.

Mapping Functional Interactions in Heterodimeric Phospholipid Pumps

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P_4-ATPases catalyze phospholipid transport to generate phospholipid asymmetry across membranes of late secretory and endocytic compartments, but their kinship to cation-transporting P-type transporters raised doubts on whether P4-ATPases alone are sufficient to mediate flippase activity. P_4-ATPases form heteromeric complexes with Cdc50 proteins. Studies of the enzymatic properties of purified P4-ATPase/Cdc50 complexes showed that catalytic activity depends on direct and specific interactions between Cdc50 subunit and transporter, while *in vivo* interaction assays suggested that the binding affinity for each other fluctuates during the transport reaction cycle. The structural determinants that govern this dynamic association remain to be established. Using domain swapping, site-directed and random mutagenesis approaches, we here show that residues throughout the subunit contribute to forming the heterodimer. Moreover, we find that a precise conformation of the large ectodomain of Cdc50 proteins is crucial for the specificity and functionality to transporter/subunit interactions. We also identified two highly conserved disulfide bridges in the Cdc50 ectodomain. Functional analysis of cysteine mutants that disrupt these disulfide bridges revealed an inverse relationship between subunit binding and P_4-ATPase-catalysed phospholipid transport. Collectively, our data indicate that a dynamic association between subunit and transporter is crucial for the transport reaction cycle of the heterodimer. To address whether Cdc50 subunits play a direct role in P_4-ATPase-catalysed lipid transport, we have set up a wheat germ based cell-free expression system allowing detergent-free incorporation of P_4-ATPases and Cdc50 subunits into unilamelar liposomes. We use the same as an alternative strategy to circumvent some of the bottlenecks associated with traditional approaches for the structural and functional analysis of membrane proteins.

FRET reveals lactacystin-induced increase of Tom20/TOM assembly

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The Translocase of the Outer mitochondrial Membrane (TOM) is a protein complex that translocates nuclear encoded proteins into mitochondria. The corresponding receptor subunits Tom20 and Tom70 are only loosely associated as proved by western blot studies. Interestingly, the Tom20 protein level might depend on physiological demands and there is evidence that the specific proteasome inhibitor lactacystin considerably raises this level. Our attempt is to monitor Tom20/TOM assembly at the single cell level and find out whether lactacystin-induced Tom20 accumulation increases assembly of Tom20 into the complex. Furthermore, we want to check the effect of lactacystin on lysosomal turnover, delta-pH (via sEcGFP), reactive oxygen species (ROS) production and morphology of HeLa cell mitochondria. To investigate possible variations in Tom20/TOM assembly, we applied Förster resonance energy transfer in combination with fluorescence lifetime imaging microscopy (FRET-FLIM). As FRET donor, eGFP was fused to Tom22 and Tom20 to DsRed (single) or DsRed-Halo/TMR (double) as FRET acceptor. For calculation of the average FRET efficiency (E = (1-tDA/tD)x100), the average fluorescence lifetime of the donor was measured in presence (tDA) and absence (tD) of acceptor. Using the double acceptor construct, the mean FRET almost doubled to a value of 6.57 (SD = 0.82). Lactacystin-treatment (10 μM, 24 h) engendered a further significant increase of FRET. Although the delta-pH across the inner mitochondrial membrane was disrupted and mitochondrial ROS content was elevated, lysosomal turnover and mitochondrial morphology was not altered. We show that interaction between two candidate proteins of the TOM complex, Tom22 and Tom20, can be monitored in living cells using FRET-FLIM. Moreover, specific inhibition of the proteasome apparently enhances the assembly of Tom20 into the TOM complex. In addition, lactacystin also disrupts the delta-pH and elevates mitochondrial ROS in HeLa cells.

The AMP-activated kinase homolog *Kl*Snf1 affects cell wall integrity in the milk yeast *Kluyveromyces lactis*

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The milk yeast *Kluyveromyces lactis* has been established as an important model organism besides *Saccharomyces cerevisiae*. In contrast to the fermentative *S. cerevisiae*, *K. lactis* is a Crabtree-negative yeast with a predominantly respiratory metabolism. Its cell wall is considerably thinner than that of *S. cerevisiae* (Backhaus *et al.*, 2010), for which we found an influence of SNF1 signaling on cell wall structure. The SNF1-kinase complex belongs to the family of AMP-activated kinases (AMPK), which triggers different signaling cascades in mammals. Yeast Snf1 is required for a proper derepression upon glucose limitation, allowing for growth on alternative carbon sources, as well as for the response to environmental stresses. To follow-up our findings in *S. cerevisiae*, we started with the investigation of the SNF1-kinase complex in *K. lactis*, with its less redundant genome. We found that a *Klsnf1* deletion also affects the *K. lactis* cell wall structure, resulting in a reduced thickness when grown on media with 1M sorbitol, as judged from measurements of transmission electron microscopy images. In addition, mutants of the cell wall integrity (CWI) pathway, which regulate the remodeling of the cell wall, and *snf1* cells are hypersensitive to cell wall stress inducing agents such as Calcofluor white and Congo red. In addition, *snf1 rlm1* mutants (which lack the major transcription factor of the CWI pathway) are synthetically lethal. These epistasis analyses indicate, that SNF1 signaling acts in parallel to the CWI pathway to control cell wall synthesis. To identify the downstream targets, we currently investigate Msn2 as a possible effector of SNF1 signaling both in *S. cerevisiae* and in *K. lactis*. A metabolic explanation for the cell wall phenotype of *Klsnf1* cells may be provided by phenotypic analysis of glycolytic *pfk* mutants, which appear to be hyper-resistant to cell wall stress agents.

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Atomic Force Microscopy (AFM) as a tool to unravel nanomechanical properties of cell wall integrity sensors and to measure cell wall thickness in live yeast cells

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Thickness and structural properties of microbial cell walls are usually investigated by electron microscopy, which is very time consuming and may be prone to preparation artefacts. In addition, it requires vacuum conditions and due to sample fixation requirements live cell imaging is prohibited [1]. With Atomic Force Microscopy (AFM) it is not only possible to scan cell surfaces of live cells, but also to determine properties of single molecules [2]. The latter was used to establish a set of *in vivo* molecular rulers, using a modified version of the yeast cell wall integrity (CWI) sensor Wsc1 [3]. Thus, His-tagged Wsc1 sensors of different lengths are employed to determine the cell wall thickness in live cells, through their accessibility to force spectroscopy. We will provide some examples of mutants interconnected to the CWI pathway.
Furthermore, one can use indentation curves to calculate the Youngś modulus of microbial cell walls, which ultimately indicates the elasticity, and thereby the degree of cross-linking, of the yeast cell wall [4]. We will present our preliminary data on mutant analyses with this method.

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Engineering nanobody fusion proteins for specific type I IFN targeting

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Type I interferons (IFNs) play a key role in the early response to pathogen infections by activating innate and adaptive immune defenses. They bind to a cell surface receptor comprised of the subunits IFNAR1 and IFNAR2, which is expressed at the membrane of all nucleated cells. Owing to the antiviral, antiproliferative and immunomodulatory responses elicited by IFNs they have valuable therapeutic potential for the treatment of diseases such as hepatitis, multiple sclerosis and cancer. The successful application of IFNs, however, is thwarted by its strong side effects because all cells of the body are affected. Here, we aimed to increase the specificity of IFNs towards target cells by fusion to a nanobody binding to a marker protein expressed on the cell surface. To this end, we explored the affinity-activity relationship for IFNα2 mutants, which revealed a non-linear correlation upon increasing the binding affinity compared to wildtype IFNα2. As a possible mechanism responsible for this effect, we characterized ligand-induced endocytosis by single molecule fluorescence microscopy. Rapid clathrin-dependent uptake of the ligand and enrichment of signaling complexes in endosomes was confirmed. Taking this effect into consideration, we designed IFNα2 mutants with decreased affinity towards IFNAR2 fused to a nanobody for specific targeting via a flexible linker. By using a cellular model system based on a nanobody against the mouse leptin receptor, highly efficient IFN targeting was achieved. The functional properties of several nanobody-IFNα2 fusion proteins was explored in detail by cellular and biophysical assays as well as advanced fluorescence imaging techniques.

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RNA-binding protein (RBP) containing granules act as dynamic microcompartments in neural cells

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Tau is a microtubule associated protein (MAP) that is enriched in the axon of neurons and is involved in several neurodegenerative diseases collectively called tauopathies. Mechanisms, which lead to an enrichment of tau in the axon are thought to involve transport and compartment-specific binding of the protein but also mechanisms, which operate on the level of the mRNA, may contribute to tau localization. It is known that regulation of mRNA translation, transport and decay occurs in different types of RNA granules. These RNA granules can be considered as dynamic cellular microcompartments, which contain a subset of different mRNA species and various mRNA-binding proteins (RBPs). To determine morphological and functional features of tau RBPs, fusion constructs coding for G3BP-1 and IMP-1, which have been previously shown to interact with tau mRNA (Atlas, 2004), and fluorescence proteins were prepared and expressed in differentiated PC12 cells and primary neurons. Using computer-assisted image analysis of laser scanning micrographs we report that G3BP-1 and IMP-1 containing granules have similar volumes and mobility, but show distinct exchange rates of their molecular components. Tracking experiments indicate that the granules do not undergo directed transport. Expression of both proteins induces morphological differentiation and increases the amount of big tau isoforms in PC12 cells. Our data indicate that G3BP-1 and IMP-1 containing granules act as dynamic microcompartments in neural cells and contribute to regulate the differentiation of neurons.

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The reversible dissociation of yeast V-ATPase *in vivo*

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Vacuolar H^+-ATPases (V-ATPases) are proton pumps found in every eukaryotic cell. During starvation conditions, they are regulated by reversible dissociation of the ATP-hydrolyzing V_1 complex from the membrane bound, proton translocating V_o complex. This type of regulation was first observed in the non-feeding, moulting tobacco hornworm (*Manduca sexta*). In the baker's yeast *Saccharomyces cerevisiae*, dissociation of the V-ATPase holoenzyme is induced by glucose deprivation. The shut-down of this energy consumptive pump appears to be an economic mode during diauxic shift. The re-addition of glucose induces the rapid and efficient reassembly of the holoenzyme without the need for biosynthesis of new subunits. By analysing localisation of GFP-marked V-ATPase subunits in living yeast cells, we found that only the V_1 subunit C and not the whole V_1 complex dissociates from the membrane under starvation conditions. Furthermore interaction studies done by FRET experiments support these observations. We could also show that a decreased intracellular cAMP concentration, the inhibition and/or deletion of Protein kinase A subunits and the luminal pH are involved in regulating the dissociation of subunit C. Furthermore, dissociation is dependent on microtubules and the glycolytic enzyme aldolase. The interaction of subunit C with the cytoskeleton, especially with microtubules, seems to be a universal property. Finally we found that the heterotrimeric complex RAVE may play a role not only in biosynthetic assembly of the V-ATPase but also in the reassociation of subunit C to the holoenzyme.

Protein-protein interaction within the Cpx-two component system

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Two-component signal transduction systems (TCS) are the main mechanisms by which bacteria sense and respond to environmental stimuli [1]. TCS typically consist of a sensor kinase (SK) and a response regulator (RR). The SK autophosphorylates upon detecting an inducing cue and transfers the phosphoryl group to its cognate RR which now promotes changes in cellular physiology or behavior [1]. To keep the TCS in balance, the RR gets dephosphorylated intrinsic or due to the phosphatase activity of the SK [1]. However, the mechanistic details about the precise signal integration and transfer remain still unknown [2].

The Cpx-envelope stress system is a well established TCS composed of the membrane-bound SK CpxA, the cytosolic RR CpxR and in addition of the accessory protein CpxP [3]. Factors that cause cell envelope stress as e.g. pH stress, salt stress and misfolded proteins induce the Cpx-TCS [3]. The accessory CpxP inhibits autophosphorylation of CpxA and supports the degradation of misfolded pilus subunits [3]. Previous functional and structural studies suggest not only that CpxP inhibits CpxA through a direct protein-protein interaction but also indicate how CpxP act as a sensor for misfolded pilus subunits, pH, salt [4] and zinc [6]. By employing membrane-SPINE [5] and bacterial two-hybrid system, we were now able to demonstrate the direct physical protein-protein interaction between CpxP and CpxA *in vivo* in *Escherichia coli*. Furthermore, our data show under several Cpx-inducing conditions that CpxP is released from CpxA assigning CpxP as the sensor for specific Cpx-inducing stimuli. Release of CpxP from CpxA is assumed to result dimerization and consequently in the autophosphorylation of CpxA [1, 3]. Thus, our combined results lead to a deeper insight into the signal recognition in TCS in general.

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Recruitment of maize FNR into membrane complexes depends on N-terminal structure

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Ferredoxin-$NADP^+$-Reductase (FNR) shows a dynamic behaviour within the thylakoids of plant cells in terms of its localization and function. There are different isoforms of FNR, which show altered localization dependent on the physiological state of the plant. In *Arabidopsis thaliana* there are two isoforms which are both soluble and membrane bound. However, in *Zea mays* there are three isoforms: ZmFNR1 (predominantly membrane bound), ZmFNR2 (soluble and membrane bound) and ZmFNR3 (predominantly soluble). These isoforms differ predominantly in the N-terminal part of the protein, which suggests that it determines specific protein-protein interaction. Recently, two FNR binding proteins, Tic62 and TROL, were discovered which tether FNR to different positions of the thylakoid membrane [1,2]. Maize is a better model to investigate the dynamics and mechanisms of FNR-thylakoid interactions because of its three diverse FNR isoforms. The dynamic behaviour of FNR affects the electron partitioning during photosynthesis, which in C_4-plants like maize may either promote linear electron flow or cyclic electron flow. The latter is dedicated to photophosphorylation instead of photoreduction of $NADP^+$ by FNR. In conclusion, the regulation of FNR is a way for plants to response to different physiological states concerning energy demand and redox state. Here we present that the N-terminal end of FNR is important for the interaction with the FNR binding proteins Tic62 and TROL and that differential recruitment of FNR to the thylakoid membrane may inflict changes in electron flow during photosynthesis[3].

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Proteomic Analysis of *Salmonella*-modified Membranes

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Salmonella enterica is a food-borne, facultative intracellular pathogen. After infection, *Salmonella* is able to massively remodel the host endosomal system, resulting in various, in part tubular membrane structures such as *Salmonella*-containing vacuoles (SCV), *Salmonella*-induced filaments (SIF), sorting nexin tubules, *Salmonella*-induced secretory carrier membrane protein 3 tubules and lysosome-associated membrane protein 1-negative tubules, summarized here as so-called *Salmonella*-modified membranes (SMM) [1-3]. A common feature of SMM is the presence of effector proteins of the *Salmonella* Pathogenicity Island 2 (SPI2)-encoded type III secretion system (T3SS). Apart from these SPI2-T3SS effector proteins and a few canonical organelle markers, the cellular origin and precise membrane composition of SMM is largely unknown.

To determine origin and composition of these SMM we developed a specific enrichment based on affinity purification of the membrane-integral SPI2-T3SS effector proteins. We modified effector proteins with epitope-tags and protease cleavage sites optimized for immunoaffinity precipitation and release of the recovered material. In combination with liquid chromatography-tandem mass spectrometry we probed the composition of SMM during infection of RAW264.7 macrophages. This enabled us to identify known and novel components and will lead us to unravel the origin of these unique cellular compartments.

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Synaptophysin1 targets and sorts synaptobrevin2 presynaptically

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Fusion of synaptic vesicles (SVs) during synaptic transmission is mediated by SNARE complex assembly formed by coiled-coiling of the vesicular SNARE protein synaptobrevin2 (syb2) and the presynaptic membrane SNAREs syntaxin-1A and SNAP-25. In order to maintain neurotransmission, exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. Previously, we have identified retrieval from a pre-assembled surface pool of SV constituents at the periactive zone, the so-called 'readily retrievable pool', as the major route of compensatory endocytosis. Here we identify dimerization of syb2 as a first important step in self-assembly of these surface nanodomains. Moreover, we show that efficient targeting and sorting of syb2 dimers is dependent on the tetraspan membrane protein synaptophysin 1 (syp1), a SV protein of still controversially discussed function. TIRF-PALM of PC12 cell membranes reveals clustering of exocytosed syb2 into nanodomains of a few ten molecules, which however is abolished when expressing a monomeric syb2 (G100Y) mutant. Hippocampal synapses expressing monomeric syb2 display reduced numbers of SVs and occurrence of multivesicular bodies and endosome-like structures. Moreover, in both, differentiated PC12 cells as well as neurons mutant monomeric syb2 is mislocated. In hippocampal synapses the syb2 surface pool is dramatically decreased for monomeric syb2. Besides dimerization, presynaptic targeting and sorting of syb2 depends on the SV protein syp1. Using PC12 cells, we find that co-expression of syp1 targets wt, but not mutant syb2 to the distal part of neurites. In contrast, shRNA-mediated knock-down of syp1 prohibits the enrichment of syb2 at the tips and reduces overall membrane expression. In neurons co-expression of syp1 and wt, but not mutant syb2, enhances targeting of syb2 into functional SVs. These data show that syp1 is responsible for targeting and sorting of syb2 dimers to the presynaptic compartment.

Dynamic mitochondria display a mosaic arrangement of cristae with respect to the composition of OXPHOS complexes

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Mitochondria are involved in many cellular processes including energy supply, signaling and apoptosis. Their ability to fuse and divide provides functional and morphological flexibility and is a key feature in mitochondrial quality maintenance by enabling the re-composition of the mitochondrial material. In contrast to outer membrane proteins and matrix proteins which mix fast and homogeneously in dynamic mitochondria, the mixing of inner membrane proteins is obviously inhomogeneous. In the context of mitochondrial dynamics as a putative rescue mechanism to avoid the accumulation of damaged compounds, this observation is of high significance. To comprehend this on the molecular level, we here analyzed the spatial distribution of OXPHOS complexes in dynamic mitochondria by immunoelectron microscopy. We found inner membrane segments (cristae) with mixed composition of OXPHOS complexes but also regions with cristae that apparently had not mixed their composites, though. Our data suggest a mosaic composition of the inner mitochondrial membrane. Two conjunctures might work together here: the maintenance of cristae during mitochondrial fusion and fission and limited diffusion of inner membrane proteins between cristae.

Heterodimerization of the type I IFN receptor on single molecule level

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Type I Interferons (IFN) are key cytokines in the regulation of the innate immune response. All IFNs bind to a shared cell surface receptor comprised of two subunits, IFNAR1 and IFNAR2. Detailed structure-function analysis of IFNs has established that the dynamics and affinities of IFN interactions with the receptor subunits play a critical role for regulating signalling specificities. Here we have explored the IFN-induced assembly of the ternary signalling complex at physiological expression level by dual color single imaging. To this end, we employed highly specific and efficient orthogonal posttranslational labelling approaches with photostable organic fluorophores combined with TIRF-microscopy. By single molecule co-localization and co-tracking analysis we have mapped lateral distribution and diffusion of the two receptor subunits with very high spatial and temporal resolution. Thus, we were able to monitor IFN induced assembly, co-diffusion and dissociation of individual ternary signaling complexes in the plasma membrane. These studies clearly establish that IFNAR1 and IFNAR2 are not stably preassociated prior to IFN binding. However, measurements on IFN induced ternary complex formation revealed a surprisingly rapid two dimensional association kinetics. Particle image cross-correlation spectroscopy (PICCS) revealed that IFNAR1/2, although not pre-associated, seem to be pre-organized on submicron scale, which could facilitate receptor assembly. Quantification of receptor dimerization allowed assessing the equilibrium between binary and ternary complexes in the plasma membrane. Systematic modulation of the binding affinities towards the receptor subunits revealed that binding properties of IFNα2 are optimized for ternary complex formation at physiological receptor surface concentrations.

Dynamic submicroscopic signaling zone revealed by TALM and image correlation analysis

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By using monofunctional quantum dots labeling type I interferon (IFN) receptor IFNAR1 and IFNAR2 expressed at endogenous level in living cells, we have probed the diffusion and ligand induced assembly of IFN signaling complex individually in living cell plasma membrane. We thus take this opportunity to investigate the critical initiating stage of IFN signaling at single molecule level. The spatiotemporal dynamics of individual receptor subunits and cytosolic effector proteins before and after stimulation by the ligand were probed by time-lapse dual color imaging over extended time periods. By localizing and tracking individual receptor subunits, super-resolution images were obtained with a resolution of $\sim$10 nm (tracking and localization microscopy, TALM). In order to quantitatively analyze the temporal and spatial dynamics of receptor and cytosolic effector proteins, we implemented image correlation analysis of such TALM images. Long-term tracking of individual ternary complexes was possible based on pair correlation and particle correlation TALM (pcTALM). Thus, the life-time of the ternary complex of > 15 s was revealed as well as their submicron confinement at plasma membrane where transient recruitment of effector proteins ($\sim$ 1 sec) upon IFN stimulation was observed.

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