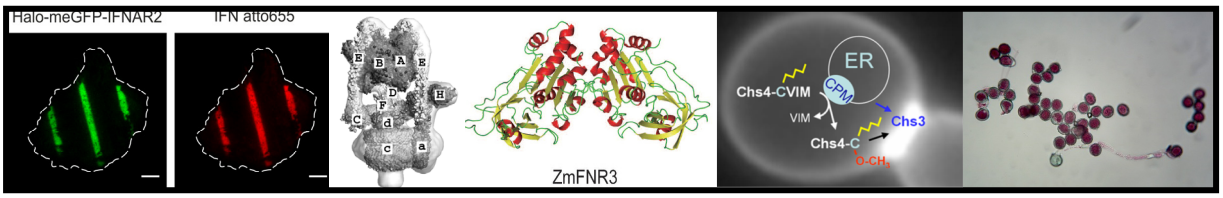
SFB 944 Physiology & Dynamics of Cellular Microcompartments

Lichtenberg PhD Program
MCC MEMBRANES AND CELLULAR COMMUNICATION

2. IRTG-Klausurtagung



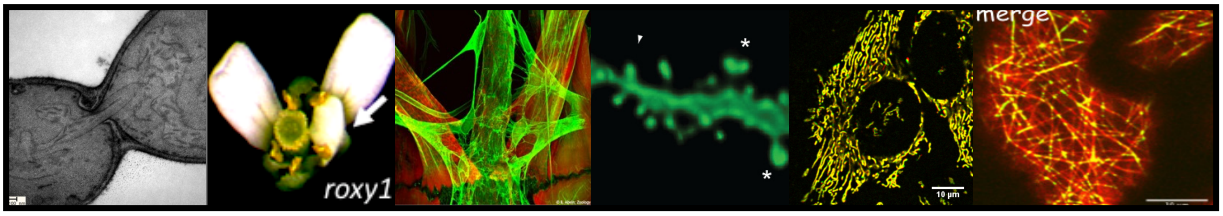
PHYSIOLOGY AND DYNAMICS OF CELLULAR MICROCOMPARTMENTS

November 29-30, 2012

Bohnenkamp-Haus of the Botanical Garden

Organized by the Integrated Research Training Group
of the Collaborative Research Center 944
and
The Lichtenbergkolleg PhD Program
“Membranes and Cellular Communication”

University of Osnabrück, Germany



Program

Thursday, November 29, 2012

14.15 h	Opening
	Session I: Cellular microcompartments, part I *Chair: Radhakrishnan Panatala*
14.20 h	Tracing the transient conformational signal in bacterial phototaxis using SDSL-EPR spectroscopy Daniel Klose (P10 Steinhoff/Klare)
14.40 h	Effector recruitment by the type I interferon receptor probed by single molecule imaging and cell micropatterning Sara Löchte (P8 Piehler)
15.00 h	Mind the gap: mechanism and impact of ceramide trafficking at the ER-mitochondria interface Amrita Jain (P14 Holthuis)
15.20 h	Function of the CORVET complex in tethering at the endosome Henning kleine Balderhaar (P11 Ungermann)
	Session II: Cellular microcompartments, part II *Chair: Daniela Heine*
15.40 h	Chs3 topology and the role of the 3TMS region in yeast chitin synthesis Simon Gohlke (P6 Merzendorfer)
16.00 h	The bud-neck microcompartment in the milk yeast *Kluyveromyces lactis* Dorthe Rippert (P3 Heinisch)
16.20 h	Coffee
16.50 h	Reversible disassembly of the yeast V-ATPase is regulated by intra- and extracellular pH Katharina Tabke (P12 Wieczorek)
17.10 h	Exploring FNR recruitment to the thylakoid membrane Manuel Twachtmann (P2 Hanke)
	Session III: Microcompartments between cells and in tissue, part I *Chair: Maik Drechsler*
17.30 h	The plastid-localized NAD-dependent malate dehydrogenase and its crucial role in energy supply during plant development Jennifer Selinski (P9 Scheibe)
17.50 h	The unique extracellular matrix protein Pericardin is important for heart integrity maintenance Ariane Schmidt (P7 Paululat)
18.10 h	Remodeling of the actin cytoskeleton during invasion of polarized cells by *Salmonella enterica* Alfonso Felipe-Lopez (P4 Hensel)
19.00 h	Spanferkel & Glühwein

Friday, November 30, 2012

	Session IV: Microcompartments between cells and in tissue, part II *Chair: Sarah Kopischke*
9.15 h	Nuclear PAN/ROXY activities Nora Gutsche (P13 Zachgo)
9.35 h	Sorting of synaptic vesicle proteins after exocytosis Rajit Rajappa (P5 Klingauf)
9.55 h	Spine changes in a mouse model of amyloidosis Lorene Penazzi (P1 Brandt)
10.15	Coffee
	Session V: New projects *Chair: Patrick Hörnschemeyer*
10.45 h	Dynamic mitochondrial supramolecular complex formation as functional microcompartmentation Bettina Rieger (AG Busch)
11.00 h	TOR1 dynamics and cell growth-induced microtubule reorientation in plants Henrik Buschmann
11.15 h	Analysis of Neprilysin activities in microcompartments of the sarcoplasmic reticulum Heiko Harten
11.30 h	Dynamic interaction between a sensor kinase and its periplasmic accessory protein mediates signal recognition Karoline Tschauner (AG Hunke)
11.45 h	Closing
12.00 h	Business Meeting for project leader

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

Daniel Klose[1], Julia Holterhues[1], Meike Müller-Trimbusch[1], Enrica Bordignon[2], Ines Heinrich[3], Lin Li[3], Martin Engeldard[3], Johann P. Klare[1], Heinz-Jürgen Steinhoff[1]

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In microbial photo- and chemotaxis a two-component signaling cascade mediatesa 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 lightinduced 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 equilibriumwhich 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.

Effector recruitment by the type I interferon receptor probed by single molecule imaging and cell micropatterning

Sara Löchte, Sharon Waichman, Changjiang You, Philipp Selenschik, Christian P. Richter, Stephan Wilmes and Jacob Piehler
University of Osnabrück, Department Biophysics, Osnabrück, Germany

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 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 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 of individual molecules beyond the diffraction limit. Constitutive binding of STAT2 to IFNAR2 mediated via the coiled-coil domain of STAT2 was detected. In contrast, STAT1 binding was only observed in the presence of STAT2. Co-localization and co-tracking experiments could further demonstrate STAT2 – IFNAR2 interaction on a single molecule level. For quantitatively probing specific protein interactions, we established functional micropatterning of the signalling complex in living cells. To this end, biocompatible, microstructured surface architectures were implemented, which enabled covalent capturing of IFNAR subunits fused to the HaloTag into predefined areas. Thus, a contrast within individual cells was generated, allowing for qualitative and quantitiative protein interaction analysis with the micropatterned receptor subunit. Receptor integrity was confirmed by probing ligand binding and ternary complex formation as well as immunostaining of the phosphorylated Janus kinase. The dynamic interaction between effector proteins and immobilized IFNAR2 in patterned cells could be demonstrated by Fluorescence Recovery After Photobleaching (FRAP). Thus, we established a generic method for dissecting interactions of various effector proteins with the receptor subunits in a qualitative and quantitative manner.

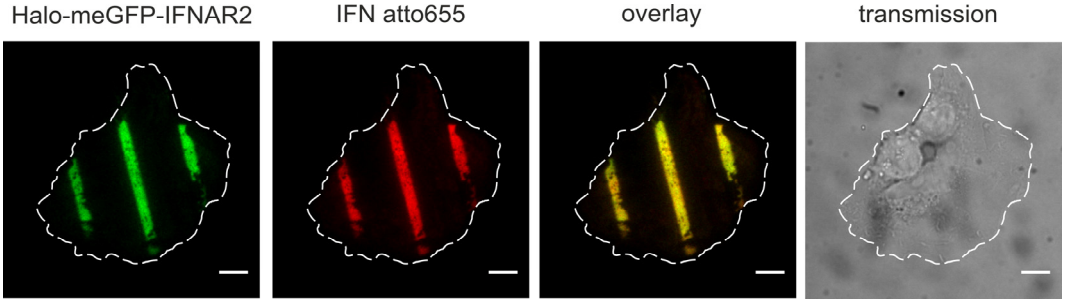


FIG: Immobilization of IFNAR2 fused to a Halo Tag and meGFP in living cells on patterned HTL-surface.

Mind the gap: Mechanism and impact of ceramide trafficking at the ER-mitochondria interface

Amrita Jain, P14 Holthuis

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 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 photo-activatable and clickable ceramide analogues to trace ceramide-binding proteins (CBPs) at ER-mitochondrial junctions and mitochondrial membranes. As a distinctive approach, we will redirect the biosynthetic ceramide flow from Golgi to Mitochondria by reengineered Ceramide Transfer Protein to verify the concept that mistargeting of ER ceramide to mitochondria commits cell to death. We will also manipulate the gap size and width at ER and mitochondria contact sites by drug inducible synthetic linkers to analyze the role of contact sites on ceramide trafficking. In view of the tumor suppressor activity of ceramides, we will evaluate newly identified components of the ceramide trafficking machinery, their effectors as well manipulation of ceramide flows to mitochondria as targets for modulating drug-induced apoptosis in cancer cells.

Function of the CORVET complex in tethering at the endosome

Henning kleine Balderhaar, P11 Ungermann

In the endomembrane system of eukaryotic cells, fusion events of vesicles and organelles with their target membranes are governed by highly specific and tightly regulated mechanisms. Beside distinct lipid compositions, SNAREs, small Rab GTPases and tethering complexes regulate fusion events in the endomembrane system. In the model organism *S. cerevisiae*, initial steps for fusion of endosomes and vacuoles require the action of the two relate tethering complex CORVET and HOPS, together with their corresponding Rab GTPases Vps21 and Ypt7. Both complexes are heterohexameric and share the same class C core subunits Vps11, Vps16, Vps18 & Vps33. In addition, both tethers contain two specific subunits; these are Vps3 & Vps8 for the CORVET and Vps39 & Vps41 for the HOPS complex.

In contrast to HOPS, only less is known about the CORVET complex, which was described five years ago in this laboratory. Towards obtaining a better structural and functional understanding, I constructed various CORVET-overproduction strains. By establishing the CORVET purification and the development of a new tethering assay, I was able to show that this complex can tether Vps21-positive structures *in vitro*. The same was true for complementary *in vivo* studies of CORVET-induced tethering events. In addition, my colleagues and I showed with several biochemical assays that the complex can bind lipids, the Rab GTPase Vps21-GTP and endosomal SNAREs. Moreover, the purified complex enabled a first structural insight in this tethering complex.
Recent studies as well as the presented data provide new insights into the function of the endolysosomal tethers.

Chs3 topology and the role of the 3TMS region in yeast chitin synthesis

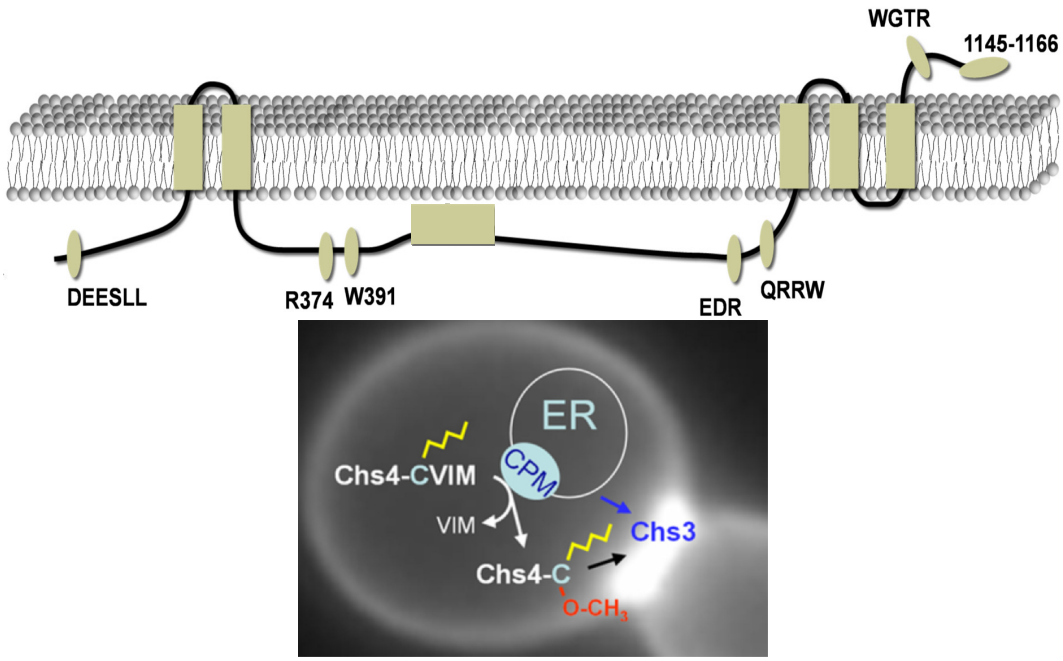
Simon Gohlke, P6 Merzendorfer

In *Saccharomyces cerevisiae*, the chitin synthase III complex produces about 90% of chitin for the lateral cell wall and is necessary for formation of the chitin ring at the bud neck [1].
The catalytic subunit of this complex, the integral membrane protein Chs3, is synthesized at the ER and routed via the trans-Golgi network to the plasma membrane (PM) where chitin synthesis occurs. The regulatory subunit of the chitin synthase III complex, Chs4, interacts with Chs3. It is required for Chs3 activity, but not for tethering Chs3 to the bud neck.
To understand complex formation and its regulation, we need to gain more insights into Chs3 topology, which is still uncertain. Therefore, site-directed mutagenesis and protease protection assays are used to determine the topology of transmembrane helices and soluble domains.
Moreover, we are interested in the function of the carboxy-terminal 3 transmembrane segment (3TMS) of Chs3. This region follows the catalytic domain and conserved WGTR motif and precedes a coiled-coiled region, and it has been suggested to be involved in pore formation and chitin extrusion [2]. Site directed mutagenesis reveals first insight into the roles of individual amino acids.

References

[1] Shaw JA, J Cell Biol. Jul;114(1):111-23 (1991)

[2] Van Leeuwen T, Proc Natl Acad Sci U S A. 20;109(12):4407-12 (2012)



The bud-neck microcompartment in the milk yeast *Kluyveromyces lactis*

Dorthe Rippert, P3 Heinisch

Within the last two decades, *K. lactis* has been established as an alternative and powerful model organism besides the budding yeast *S. cerevisiae*. A key feature related to its name is the ability to utilize lactose as a carbon source. In dairy industries, it is frequently employed to remove the disaccharide, e.g. for the sake of lactose-intolerant patients.
In fact, *K. lactis* also prefers glucose as a carbon source, has a life cycle supported by two mating types, a stable haploid and a semi-stable diploid phase, and can be induced to produce tetrads by nutrient starvation. Regarding the energy metabolism, *S. cerevisiae* displays the Crabtree effect and mainly produces alcohol from high-sugar contents even in the presence of oxygen. In contrast, *K. lactis* shows the Pasteur effect and relies on a predominantly respiratory metabolism.
In addition, in contrast to *S. cerevisiae*, *K. lactis* did not undergo a whole genome duplication (WGD) event. This largely explains why *K. lactis* displays a much lower redundancy of genes whose products are involved in central metabolism or signal transduction pathways, as compared to *S. cerevisiae*.
The formation of new buds, as well as cytokinesis, requires the formation of a septum built up of cell wall material, and depends on the remodeling of the cell wall during septum formation and prior to cell separation. In *S. cerevisiae*, the molecular mechanisms and regulation of cytokinesis are well described. *K. lactis* disposes of homologues of many of these components (e.g. *Kl*Cyk3, *Kl*Hof1, *Kl*Inn1, *Kl*Myo1). However, unlike *S. cerevisiae*, strains lacking either *Kl*Cyk3 or *Kl*Hof1 are not viable. A *Klinn1* deletion is lethal in both species. The deletion of *KlMYO1* gene produces perfectly viable progeny and shows no obvious phenotype on 30 °C, whereas in *S. cerevisiae* the *myo1*Δ deletion is lethal in most strain backgrounds and shows severe growth defects in the others. The deduced amino acid sequences of *Sc*Myo1 and *Kl*Myo1 are 44 % identical, with some highly conserved regions of more than 90 % identity near the N-terminus. The lack of an obvious phenotype of the *Klmyo1* deletion and the difference to *S. cerevisiae*, lead us to further investigate the role of *Kl*Myo1 in *K. lactis* cytokinesis, since it is supposed to be a central component of the contractile actomyosin ring (CAR). TEM images (Fig. 1) and *in vivo* localization studies of *Kl*Myo1-GFP fusions during cytokinesis reveal a higher degree of functional conservation than expected from the growth phenotypes.

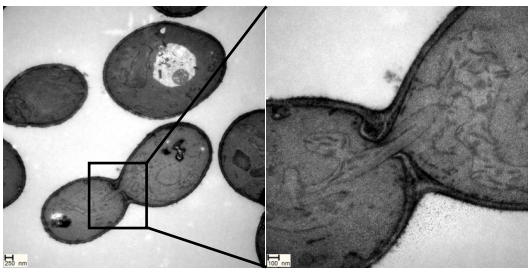


Figure 1: Electron micrographs of a cell lacking *KlMYO1* gene.

Reversible dissociation of the yeast V-ATPase is regulated by intra- and extracellular pH

Katharina Tabke, P12 Wieczorek

Vacuolar 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 the localisation of GFP-marked V-ATPase subunits in living yeast cells we found that only the V_1 subunit C but not the whole V_1 complex dissociates from the membrane under starvation conditions. Interaction studies done by FRET experiments support these observations.

Charge and structure of macromolecules depend on the protonation of amino acid residues. Organelles establish and maintain a distinct pH, and in the vacuole the V-ATPase is the main contributor of acidic pH. Here we report on the regulation of the yeast V-ATPase by the extra- and intracellular pH. Manipulation of the intracellular pH to either more acidic or more alkaline values leads to the abolishment of glucose dependent regulation of the V-ATPase. Our results contribute to the upcoming idea that the V-ATPase not only regulates the vacuolar pH but also acts as a pH sensor.

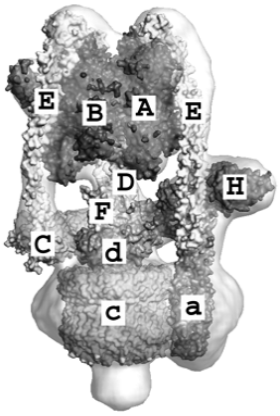


Figure 1: Structure of the V-ATPase.
Structure of the V-ATPase from *Manduca sexta* with cytosolic V_1 complex and membrane bound V_O complex [1].

[1] Muench *et al.*: Cryo-electron Microscopy of the Vacuolar ATPase Motor Reveals its Mechanical and Regulatory Complexity, *J. Mol. Biol.* **386**, 989–999 (2009).

Exploring FNR recruitment to the thylakoid membrane

Manuel Twachtmann, P2 Guy T. Hanke

In order to maximize the efficiency of photosynthesis a complex network of regulatory processes is required to balance out metabolic demand and available light intensity. One interesting and remaining question is the regulation of linear and cyclic electron flow during photosynthesis.
Ferredoxin:$NADP^+$ oxidoreductase (FNR) catalyses the final electron transfer step of the light reactions. We showed that its interaction pattern with different thylakoid membrane proteins might be altering the electron flux between cyclic and linear electron routes (Twachtmann et al., 2012). This indicates that the location of FNR could act as a molecular switch between the two electron pathways around the photosystems.
The basis for the dynamic interaction pattern of FNR is the structure of its N-terminal part, which we have shown by investigating the unique maize FNR (ZmFNR) isoproteins (Twachtmann et al., 2012). We now concentrate on these structural differences among the isoforms and investigate the flexibility of the N-terminal region. Crystal structures and native PAGE analysis indicate a possible formation of FNR dimers, which implies an even more complicated and dynamical recruitment pattern of this essential protein to the thylakoid membrane.

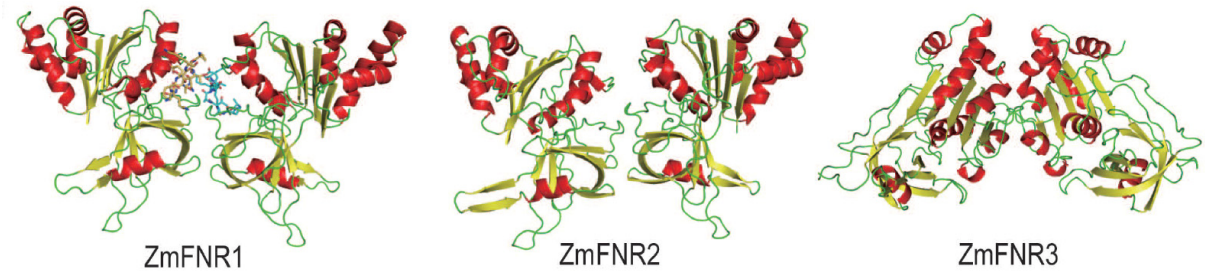


Figure 2: Crystal structures of recombinant maize FNR isoproteins which formed homodimers.

References:
Twachtmann, M., Altmann, B., Muraki, N., Voss, I., Okutani, S., Kurisu, G., Hase, T., and Hanke, G.T. (2012). N-terminal structure of maize ferredoxin:NADP+ reductase determines recruitment into different thylakoid membrane complexes. The Plant cell *24*, 2979-2991.

The plastid-localized NAD-dependent malate dehydrogenase and its crucial role in energy supply during plant development

Jennifer Selinski[a], Nicolas König[a], Guy Thomas Hanke[a], Vera Linke[a], Ekkehard H. Neuhaus[b] and Renate Scheibe[a]

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Malate valves are an essential feature for balancing metabolic fluxes, therefore, malate dehydrogenases play a key role in plant metabolism. Most cell compartments possess oxidoreductases that catalyze the interconversion of malate and oxaloacetate in a reversible reaction. These enzymes are either NAD^+- or $NADP^+$-specific [1].

During illumination, photosynthetic electron transport generates ATP and NADPH, and the removal of excessive NADPH through the malate valve enables continued ATP production. In addition to the redox-modulated NADP-MDH that is part of the malate valve in illuminated chloroplasts, plastids also contain NAD-MDH playing an important role in dark metabolism and in non-green tissues. For energy supply, NADPH is generated in the oxidative pentose-phosphate pathway. ATP is formed in substrate-chain phosphorylation during glycolysis, where NAD^+ needs to be continuously regenerated by plNAD-MDH [2], or can be imported via ATP/ADP transporters [3]. The expression of plNAD-MDH was found to be highest in heterotrophic and meristematic tissues. Heterozygous T-DNA insertion mutants of *A. thaliana* show an improved growth on ammonium as N-source and a higher expression of NADH-GOGAT. In-situ hybridization revealed that transcripts of plNAD-MDH were particularly abundant in the male and female gametophyte. While all ovules are fertilized and develop into viable seeds, the plNAD-MDH knockout has a strong effect on pollen tube growth. Therefore, no homozygous knockout plants can be obtained, but in vitro growth of pollen tubes lacking plNAD-MDH can be attained by feeding NADH-GOGAT substrates.

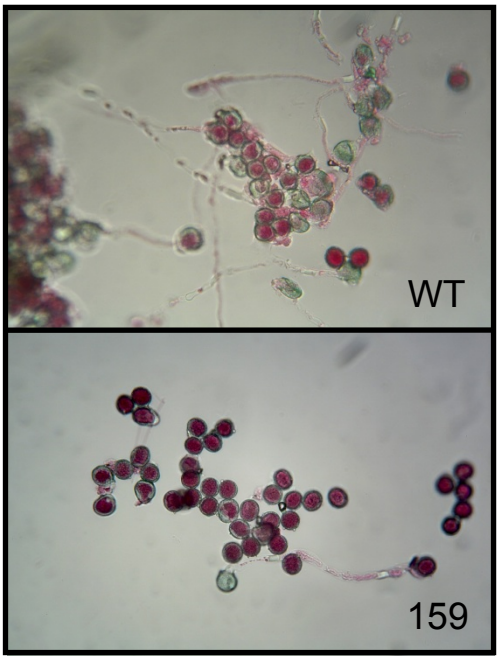

Figure 3: Pollen tube growth of *A. thaliana* wildtype (WT) and heterozygous plNAD-MDH knockout plants (159).

[1] Scheibe, R., (2004), Physiol. Plant., 120, 21; [2] Berkemeyer, M., *et al.* (1998), J. Biol. Chem., 273, 27927; [3] Reiser, J., *et al.* (2004), Plant Physiol., 136, 3524

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The unique extracellular matrix protein Pericardin is important for heart integrity maintenance

Ariane Schmidt, Maik Drechsler, Achim Paululat
University of Osnabrück, Developmental Biology, Osnabrück, Germany

The ECM of the *Drosophila* heart harbors the special Collagen IV like protein Pericardin. Together with other ECM components, the ECM forms a flexible but strong connection between the cardiomyocytes, pericardial nephrocytes and the alary muscles. Mutant analysis shows that the ECM of the *Drosophila* heart is important for organ integrity maintenance. We identified mutants for Pericardin that reveal such an essential function: If Pericardin fails to assemble properly or is totally missing the heart disintegrates and collapses. Here I will present my recent results of EMS induced *pericardin* mutants and the identification of potential components of the Pericardin biosynthesis pathway.

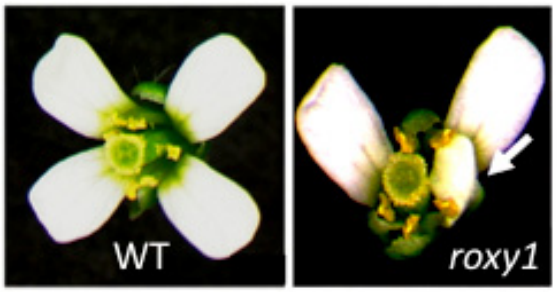
Remodeling of the actin cytoskeleton during invasion of polarized cells by *Salmonella enterica*

Alfonso Felipe-López, P4 Hensel

S. Typhimurium is able to adhere and invade epithelial cells of the intestine. *Salmonella* injects virulence proteins by the type 3 secretion system-1 (T3SS-1) into the host cell to re-organize the actin cytoskeleton allowing the bacterial internalization. Recently, the microvilli effacement of polarized epithelial cells caused by *Salmonella* was described as consequence of cooperation of the adhesin SiiE and the T3SS-1. To investigate the disruption of the brush border of polarized cells, SL1344 wild-type (WT), *sipA*-, *sopA*-, *sopB*-, *sopE*-, *sopE2*-mutant strains, M712 strain, which lacks the five mentioned effector proteins, and their complemented derivates were used for invasion experiments in MDCK Pf and C2*bbe1* cell lines. Disruption of the trans-epithelial electrical resistance (TEER) was evaluated in C2*bbe1* cells. Results showed that the single *sopE*-mutant strain was two third less invasive than the WT-strain. In contrast, one third of the invasiveness was recovered by the single introduction of *sopE* in M712. On the other hand, the M712 + *sopE* abrogated the TEER similarly to the WT strain. Moreover, actin-stained MDCK cells showed that *sopE*-single mutant strain did not disrupt the microvilli and only induced small ruffles. Some microvilli seemed to be fused to the *Salmonella* invasion point. Further results demonstrated that cells infected by WT strain presented reticular actin around the entry point at the apical side of the host cell. Additionally, M712 + *sipA* strain intimately anchored to the microvilli; M712 + *sopA* or + *sopB* enhanced the adherence compared to the background mutant strain; M712 + *sopE2* induced small accumulation of actin; finally, M712 + *sopE* effaced the microvilli and also induced reticular actin as observed with the WT strain. Interesting, chain-like structures at the former tight-junction were observed in ZO-1 stained C2*bbe1* cells and infected by M712 + *sopE*. To follow the invasion process, time lapse imaging using EGFP-LifeAct MDCK cells revealed an exponential decay of the F-actin signal during the ruffle formation caused by the infection of the WT- but not by *sopE*-deficient strain. Altogether, these results showed that microvilli effacement, reticular actin and disruption of tight junctions only depended on the presence of SopE. Deletion of other effector proteins also caused changes in the ruffle morphology and bacterial distribution. Further work is on progress to understand the molecular basis of the microvilli destruction caused by *Salmonella*.

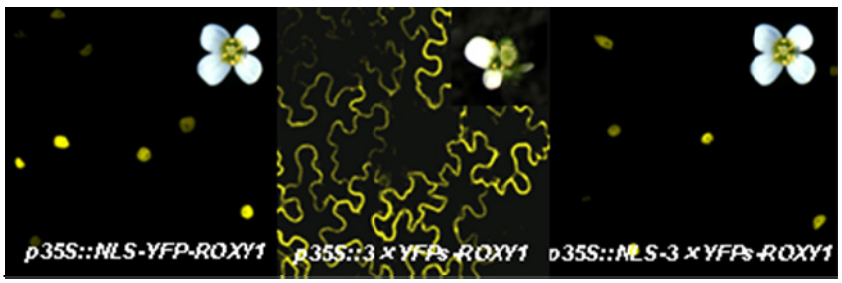
Nuclear ROXY1/PAN activities

Nora Gutsche, P13 Zachgo



In land-plants three groups of redox-dependent glutaredoxins (GRXs), small oxidoreductases, are present. Two are found from bacteria up to humans and a third group, comprising the ROXYs, is specific for land-plants. Out of this group, which comprises 21 members in *Arabidopsis thaliana*, ROXY1 is the best-studied. ROXY1 function is indispensable for petal initiation and further petal morphogenesis during flower development. Knock-out *roxy1* mutants form a reduced petal number and slightly abnormally shaped petals compared to the wildtype. TGA transcription factors were identified as ROXY1 interaction partners. One of them, PERIANTHIA (PAN), also participates in petal initiation and acts in the same genetic pathway as ROXY1.

Within the C-terminal helix of ROXY1 two motifs were found to be crucial for normal petal development, the ALWL motif and the recently identified L**LL motif. Interestingly, only the L**LL motif is essential for interaction with TGAs in the nucleus. We could in addition show that a nuclear ROXY1 localization is pivotal for governing a wildtype flower development.
GRXs of the other two groups are known to exert post-translational protein modifications. Degluathionylation and/or cleavage of disulfide bridges in target proteins are confirmed functions. Until now, little is known about the biochemical role of ROXYs. The functional importance of cysteine 340 in the PAN protein hints towards a possible contribution of ROXY1 in redox-based post-translational modifications and indicates that ROXY1 might be able modify the PAN activity. Elucidating the function of ROXY1 in association with the transcription factor PAN in the nucleus is now the next step. Thereby, we aim to understand if and how redox-processes are involved in nuclear microcompartments in controlling transcriptional activities during flower development.



Sorting of synaptic vesicle proteins after exocytosis

Rajit Rajappa, P5 Klingauf

Fusion of synaptic vesicles (SVs) during synaptic transmission is mediated by SNARE complex assembly formed by the coil-coiling of three members of the SNARE protein family. In order to maintain neurotransmission exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. I identified dimerisation of the vesicular SNARE synaptobrevin2 (syb2) as a first important step in self-assembly of endocytosing SVs. In cultured hippocampal neurons, using a combination of two pH-sensitive dyes (pHluorin and cypHher) I could show that dimerization of syb2 is necessary for proper sorting into endocytosing SVs. This is corroborated by Fluorescence Photo-Activation Localization Microscopy (FPALM) of membranes of secretory (PC12) cells, where exocytosed syb2 re-assembles into clusters harbouring a few ten molecules, which is abolished when dimerization is stopped by mutation of a glycine residue.

Spine changes in a mouse model of amyloidosis

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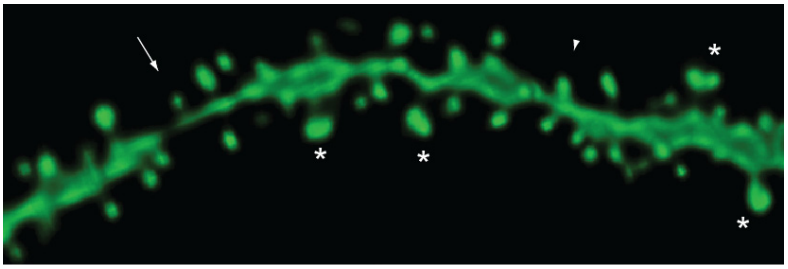
Alzheimer's disease (AD) is a highly prevalent neurodegenerative disorder in elderly, which is characterized by progressive cognitive decline. Morphological changes as well as dendritic spine loss in several regions of the hippocampus and then in the neocortex are early and invariant features of this dementia. Indeed, it exists a strong correlation between modifications in synaptic connections and progression of memory deficit in AD. Here, we investigated the effect of chronic presence of low amount of Aβ on dendritic spines of pyramidal cells in hippocampus and parietal 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 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 observed a decreased spine density in pyramidal cells of the hippocampus and an increased density in the cells of the parietal cortex. Whereas spines in the hippocampus CA3 region exhibited morphological changes both in stubby and thin spines (transient spines) in young and aged APP_{SDL} mice, spines in CA1 region showed a shift from mushroom (persistent spine) towards stubby with aging. No morphological changes were observed in the cells of the parietal cortex of aged transgenic mice.
The data indicate that chronic presence of Aβ induces morphological changes at synapses and spine loss, which are age and region-specific.



Dynamic mitochondrial supramolecular complex formation as functional microcompartimentation

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Mitochondria possess structurally and functionally distinct areas and are dynamically controlled by organelle fusion and fission, adapting the membrane potential ($\Delta\psi$), ΔpH (together constituting the proton motive force, PMF) and morphology of the mitochondrial compartment to the metabolic needs of the cell. For efficient harvesting of the PMF across the inner mitochondrial membrane, energy loss must be minimized, and this is achieved partly by restricting certain events to specific microcompartments on bioenergetic membranes [1]. Arrangement of respiratory chain complexes into supramolecular complexes or supercomplexes (SC) consisting of complex I, III and IV in various combinations has been demonstrated in mitochondria in vitro. The plasticity model [2] suggests a dynamic rearrangement for regulation (of e.g. electron transfer). But whether single mitochondria can be heterogeneous in $\Delta\psi$, ΔpH and also distribution and arrangement of respiratory chain complexes is not finally proven. Our attempt is the monitoring and analysis of functional supercomplex assembly in mitochondria of living cells.
We applied Förster resonance energy transfer in combination with fluorescence lifetime imaging microscopy (FLIM-FRET) in HeLa cells (Fig. 1). As proof of concept that FLIM-FRET allows for quantitative evaluation of SC $I_1III_2IV_1$ formation, we also show moderate FRET between the TOM core complex subunit Tom22 and the loosely associated subunit Tom20. Here, average FRET efficiency can be elevated by lactacystin, demonstrating dynamic SC formation. Moreover, we are able to determine matrix and intermembrane space (IMS) pH specifically by utilization of a pH-sensitive, ratiometric GFP-derivative (Fig. 1) in correlation to SC formation. Now, analysis of functional microcompartments can be done more accurately on the single mitochondrial level.

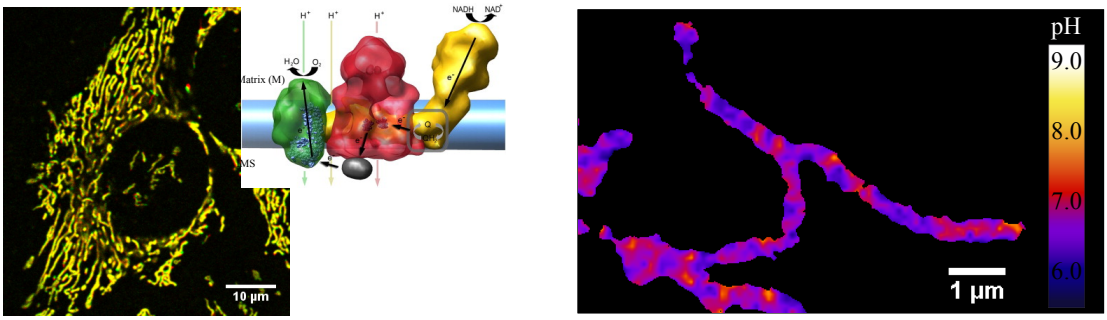

Fig. 1: Left Emission-overlay of mEGFP (CIII) and mDsRed-Halo-TMR (CIV) as used for FLIM-FRET and model of SC $I_1III_2IV_1$ [3] **Right** Monitoring pH inside living HeLa cell mitochondria at CIV on the IMS site.

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[2] Acin-Perez R, Fernandez-Silva P, Peleato ML, Perez-Martos A, Enriquez JA (2008) *Mol Cell* 32: 529-539.
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TOR1 dynamics and cell growth-induced microtubule reorientation in plants

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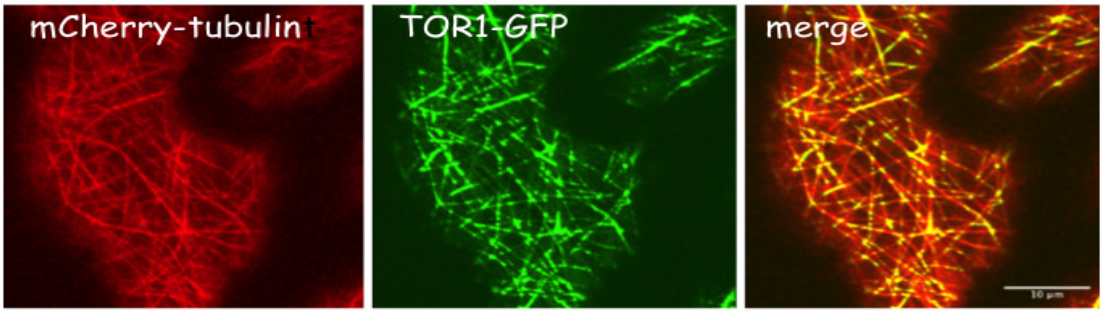
Plant development requires the orchestration of cell division and cell elongation. Defects in cell elongation can lead to right- or left-handed organ twisting. Plant mutants with twisting phenotypes are either defective in microtubules, in auxin transport (a plant growth regulator) or in cell wall structure. 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.



Analysis of Neprilysin activities in microcompartments of the sarcoplasmic reticulum

Heiko Harten

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 was apparently dispensable. A Yeast two-hybrid screen confirmed by pull-down assays identified a yet uncharacterized carbohydrate kinase as a first interaction partner of the intracellular domain of Nep4. Our data represent the first report of an intracellular neprilysin domain being involved in muscle integrity.

Dynamic interaction between a sensor kinase and its periplasmic accessory protein mediates signal recognition

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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 protein CpxP inhibits autophosphorylation of CpxA and supports the degradation of misfolded pilus subunits [3]. Previous functional and structural studies suggest that CpxP might be involved in sensing misfolded pilus subunits, pH, and salt [4]. By employing membrane-SPINE [5] and bacterial two-hybrid system, we were now able to demonstrate the direct physical interaction between CpxP and CpxA *in vivo* in *Escherichia coli*. Furthermore, our data show that salt and misfolded pilus subunits induce the release of CpxP from CpxA. Release of CpxP from CpxA is assumed to result in the autophosphorylation of CpxA [3]. Thus, our data assign CpxP as the sensor for theses specific Cpx-inducing stimuli. In sum, our combined results lead to a deeper insight into the signal recognition in TCS in general.

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