SFB 944 Physiology & Dynamics of Cellular Microcompartments

3rd Symposium on

PHYSIOLOGY AND DYNAMICS OF CELLULAR MICROCOMPARTMENTS

November 22+23, 2013

Boothzaal, Utrecht University Library

The Netherlands

Organized by the Integrated Research Training Group
of the Collaborative Research Center 944

University of Osnabrück, Germany

Introduction

Dear all,

We would like to thank all of the participants for taking the time and energy to join our **3rd Symposium on "Physiology and Dynamics of Cellular Micro-compartments"**.

The Symposium is organized by the Integrated Research Training Group (IRTG) of our Collaborative Research Center (CRC) 944 and will take place in Utrecht at the University Library Uithof (Boothzaal, Universiteitsbibliotheek, Heidelberglaan 3). Aim of the Meeting is twofold: first, we want to give our PhD students the opportunity to present and discuss their research in the frame of an international meeting and, second, we want to invite local scientists who work on related topics in order to increase scientific exchange and foster potential collaborations between research groups in Osnabrück and the Netherlands. We are very happy that we could assemble a varied program emphasizing different facets of "Cellular Microcompartments" and that six guest speakers from Amsterdam and Utrecht followed our invitation. We thank all of you for sharing your expertise and knowledge and wish you an informative and productive time during the Symposium.

We would like to emphasize that the current meeting is the first one of the IRTG, which is taking place outside of Germany. As a result, the organization was more complex and demanding compared to a local meeting and we are grateful for the support from Joost Holthuis in identifying an appropriate venue, housing and catering. Part of the meeting will be a guided tour through the historical center of Utrecht, which features many buildings and structures from the Early Middle Ages. Coming from a young middle-sized University, it is also noteworthy that Utrecht houses the largest University of the Netherlands, which was established 1636.

Maps for the Meeting will be provided as an attachment to this booklet. In case you have questions, don't hesitate to contact our coordinator Katrin Klempahn or e-mail to "office.irtg@biologie.uni-osnabrueck.de".

We are looking forward to an exciting Symposium!

Roland Brandt (for the organizers)

Prof. Dr. Roland Brandt, Prof. Dr. Renate Scheibe, Prof. Dr. Heinz-Jürgen Steinhoff (Speakers of the IRTG)
Prof. Dr. Christian Ungermann (Speaker of the CRC 944)
Organizing committee:
Sigrid Bröcker-Smidt, Joost Holthuis and Katrin Klempahn

PROGRAM - FRIDAY, NOVEMBER 22

12:00 pm	***Lunch***
12:55 pm	OPENING REMARKS Roland Brandt (Speaker of the IRTG) / Joost Holthuis
	Session I Chair: Achim Paululat / Britta Barlag
01:00 pm	Johan de Rooij, PhD, The Hubrecht Institute, Utrecht **Mechanotransduction at the cadherin-actomyosin interface**
01:30 pm	Bárbara Rotstein Bajo **Analysis of cardiac ECM architecture**
01:50 pm	Wilrun Mittelstädt **SiiA and SiiB are novel subunits controlling the function of the SPI4-encoded type I secretion system of *Salmonella enterica***
02:10 pm	Christian Kock **Microcompartmentation of cell wall integrity sensors in *S. cerevisiae***
02:30 pm	Daniela Heine **In vitro analysis of the chitin synthase III complex in *S. cerevisiae***
02:50 pm	***Coffee*** + POSTER SESSION **(even numbers)**
	Session II Chair: Renate Scheibe / Jennifer Selinski
03:50 pm	Prof. Dr. J. C. M. (Sjef) Smeekens, Faculty of Science, Utrecht University **The ribosome as a micro-compartment involved in cellular signaling: the case of Arabidopsis S1 group bZIPs**
04:20 pm	Minhee Kang **Involvement of GapC in microcompartmentation and gene expression**
04:40 pm	Manuel Twachtmann **Dynamics of FNR recruitment into protein complexes**
05:00 pm	Nora Gutsche **Towards understanding the potential nuclear redox microcompartments and their influence on the transcription factor PERIANTHIA (PAN)?**
05:20 pm	Frans M. Klis, PhD, Swammerdam Institute for Life Sciences, University of Amsterdam **The role of the wall proteome of the medically important fungus *Candida albicans* in stress resistance and virulence**
06:00 pm	***„Warm-up“*** - ***Stadskasteel Oudaen***
07:15 pm	***Guided tour through the historical center of Utrecht***
08:15 pm	***Dinner – restaurant Djakarta***

PROGRAM - SATURDAY, NOVEMBER 23

	Session III Chair: Christian Ungermann / Meenakshi Rana
09:15 am	Prof. Dr. Anna Akhmanova, Cell Biology, Utrecht University **Control of cell architecture by microtubule end-binding proteins**
09:45 am	Lorène Penazzi **What can we learn about dendritic spine plasticity using a new mouse model of amyloidosis?**
10:05 am	Birol Cabukusta **Characterization of a Putative Ceramide Sensor: How is Mitochondrial Apoptosis Controlled in the ER**
10:25 am	***Coffee***
11:05 am	Prof. Dr. Judith Klumperman, University Medical Center Utrecht **The role of HOPS complex components in organizing the endo-lysosomal system**
11:35 am	Anna Lürick **The interaction of the multimeric HOPS complex with SNAREs**
12:00 pm	***Lunch***
01:15 pm	POSTER SESSION **(odd numbers)**
	Session IV Chair: Heinz-Jürgen Steinhoff/ Christian Rickert
02:30 pm	Prof. Dr. J. Antoinette Killian, Faculty of Science, Utrecht University **Detergent-free extraction of membrane proteins: the power of native nanodiscs**
03:00 pm	Daniel Klose **Tracing the transient conformational signal in bacterial phototaxis using molecular dynamics & SDSL-EPR spectroscopy**
03:20 pm	Christoph Drees **Towards *in situ* protein analytics: Upconversion nanoparticles as interaction sensors and photoactuators in living cells**
03:40 pm	CLOSING Christian Ungermann (Speaker of the CRC 944)

Mechanotransduction at the cadherin-actomyosin interface.

Johan de Rooij

The Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, The Netherlands.
*Correspondence to j.derooij@hubrecht.eu

Classical Cadherins form connections between neighboring cells in all soft tissues. Cadherin adhesion complexes are not only structural components of cell-cell junctions, but they also serve as signaling centers to orchestrate processes including cell polarity, stem cell maintenance, differentiation and tissue remodeling. Regulation of actin dynamics plays a key role in these processes. Conversely, Cadherin complexes also transduce mechanical information from the associated actin cytoskeleton. This mechanotransduction is mediated by force-dependent unfolding of α-catenin and subsequent recruitment of Vinculin. It takes place at a specific type of cell-cell junction: radial actin-contacted, punctuate Cadherin junction that we named Focal Adherens Junction. Focal Adherens Junctions specifically exist during formation and remodeling of cell-cell adhesion where α -catenin-dependent Vinculin recruitment serves to strengthen Cadherin adhesion and accelerate formation or protect against disruption. Besides Vinculin, additional force-dependent proteins are present at Focal Adherens Junctions, including VASP and Zyxin. Their regulation by force is independent of Vinculin and this indicates that multiple mechanosensitive systems exist at the Cadherin-actin interface. The different machineries involved in Cadherin mechanotransduction and there possible functions in tissue remodeling will be discussed.

Analysis of cardiac ECM architecture

Rotstein Bajo, B[1]., Drechsler, M[1]., Schmidt, A[1]. and Paululat, A.[1]*

[1] University of Osnabrück, Department of Zoology and Developmental Biology, Osnabrück, Germany.

*Correspondence to paululat@biologie.uni-osnabrueck.de

The Extracellular matrix (ECM) is one of the most important structural and cellular regulators. The importance of the ECM´s correct deposition, composition and assembly is evidenced in many types of disorders, such as cardiovascular diseases (Tayebjee et al., 2003; Cox, TR. and Erler, JT., 2011), but we are still lacking a complete understanding of the general number of proteins participating in the formation of the cardiac ECM and how they influence the maintenance of cardiac ECM stability and functionality.

Recently our lab has identified Lonely heart as a new type of a secreted ECM structural protein adapter. Lonely heart selectively recruits one of the four collagens present in *Drosophila*, Pericardin, into the cardiac matrix (Drechsler at al., 2013). How Lonely heart and Pericardin assemble into the microcompartmentally organized cardiac ECM network need to be elucidated. Moreover, we would like to understand the precise molecular functions of both proteins and yet unknown interaction partners.

Today I would like to discuss with you how I will analyze Loh´s and Pericardin´s mode of action in the cardiac ECM.

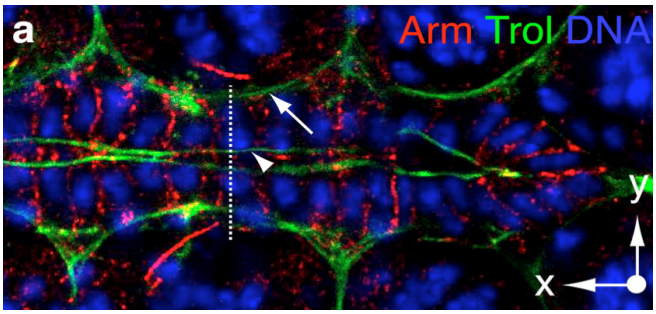

Figure1a: **The embryonic cardiac ECM**. Fluorescence micrograph showing the heart of a stage-17 *Drosophila* embryo stained for Draq5 (nuclei, blue), Armadillo/β-catenin (adherens junctions, red) and Trol/perlecan (basal and luminal compartment, green) (Adapted from Brandt, R. and Paululat, A., 2013)

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SiiA and SiiB are novel subunits controlling the function of the SPI4-encoded type I secretion system of *Salmonella enterica*

Thorsten Wille [1*], Carolin Wagner [2,3*], Wilrun Mittelstädt [3], Kathrin Blank [1], Daniela Döhler [2], Anna Lange [1], Michael Hensel [3+], Roman G. Gerlach [1]

[1]JRG3 RKI, Wernigerode, Germany
[2]Mikrobiologisches Institut, Universitätsklinikum Erlangen, Erlangen, Germany
[3]Abt. Mikrobiologie, Universität Osnabrück, Osnabrück, Germany
*Contributed equally
[+]Correspondence to michael.hensel@biologie.uni-osnabrück.de

Adhesion to polarized cells is an important hallmark of *Salmonella*-induced diseases and requires the function of a type 1 secretion system (T1SS) encoded by '*Salmonella* Pathogenicity Island 4' (SPI4) [1]. This T1SS has only one known substrate protein, namely SiiE, which is a highly repetitive non-fimbrial adhesin of 595 kDa mediating the binding of *Salmonella* specifically to the apical side of polarized cells [2]. SiiE-mediated adhesion is prerequisite for subsequent SPI1-mediated invasion.

Besides the canonical subunits SiiF (transport ATPase), SiiD (periplasmic adapter protein), and SiiC (outer membrane pore) of the T1SS, two additional subunits, SiiA and SiiB, are encoded by SPI4. Both proteins are not required for the secretion of SiiE by the T1SS [3], however previous experiments showed that mutant strains deficient in SiiA or SiiB are unable to adhere to and invade polarized cells, thus phenocopy *siiE* mutant strains. SiiA and SiiB are inner membrane proteins with one and three transmembrane (TM) helices, respectively. These show sequence similarity to proteins building proton channels, in detail TM2 and TM3 of SiiB are similar to members of the ExbB/TolQ family, and TM of SiiA to the MotB family. Mutation of a conserved aspartate residue in the TM of SiiA resulted in highly reduced SPI4-dependent invasion, showing its essential role for SPI4-encoded T1SS function. Using co-immunoprecipitation and bacterial two hybrid assays, homotypic and heterotypic interactions between SiiA and SiiB could be demonstrated. Furthermore, we were able to show heterotypic interactions between SiiB and SiiF whereas the Walker A box of SiiF is essential for this interaction. Taken these data together, we propose that SiiA and SiiB mediate a novel mechanism of control of T1SS function, most likely by formation of a proton-conducting channel and thereby linking surface expression of SiiE to the proton motive force (PMF).

In future, we will further test our hypothesis of a proton channel e.g. by depletion of the PMF, followed by quantification of adhesion to, and invasion of polarized epithelial cells and detection of SiiE surface expression. By this we will be able to unravel the underlying molecular mechanisms of the function of, and interplay between SPI4-T1SS components.

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Microcompartmentation of cell wall integrity sensors in *S. cerevisiae*

Christian Kock[1*], Janina Wittland[1], Henning Arlt[2], Rainer Kurre[3], Christian Ungermann[2] , Stefan Walter[1] and Jürgen Heinisch[1]

[1] University of Osnabrück, FB Biology/Chemistry, Genetics department, Osnabrück, Germany
[2] University of Osnabrück, FB Biology/Chemistry, Biochemistry department, Osnabrück, Germany
[3] University of Osnabrück, FB Biology/Chemistry, Biophysics department, Osnabrück, Germany
*Correspondence to christian.kock@biologie.uni-osnabrueck.de

The yeast cell wall ensures cell shape and integrity. Five plasma membrane sensors detect surface stress and activate a conserved MAPK cascade through Rom2, Rho1 and Pkc1. Downstream of the cascade Mpk1 activates the transcription factors Rlm1 and SBF. These regulate the expression of genes whose products are involved in cell wall synthesis and cell cycle control.

Two small protein families with Wsc1, Wsc2 & Wsc3 on the one hand and Mid2 & Mtl1 on the other hand constitute the surface sensors. They contain a highly mannosylated extracellular serine/threonine-rich region, a single transmembrane domain and a cytoplasmic tail. The Wsc-family sensors carry an additional cysteine-rich domain (CRD) and the Mid-type sensors a glycosylated asparagine residue as headgroup [1].

Since a quintuple deletion of all five sensors is not viable, we have constructed a strain which expresses two of the sensors under the conditional *GAL10* promotor to investigate the specific functions of single CWI sensors. In the wild-type, colocalization studies of the sensors with plasma membrane protein markers of the MCC (membrane compartment occupied by Can1), MCP (membrane compartment occupied by Pma1) and MCT (membrane compartment occupied by Tor2 complex) revealed that Wsc1 occupies a distinct plasma membrane domain, which is excluded from any of the three. Within the Wsc1 microcompartment (MCW) we are trying to identify Wsc1 interaction partners by establishing a large scale analysis using a "promiscuous" BirA* biotin ligase from *E. coli* [2].

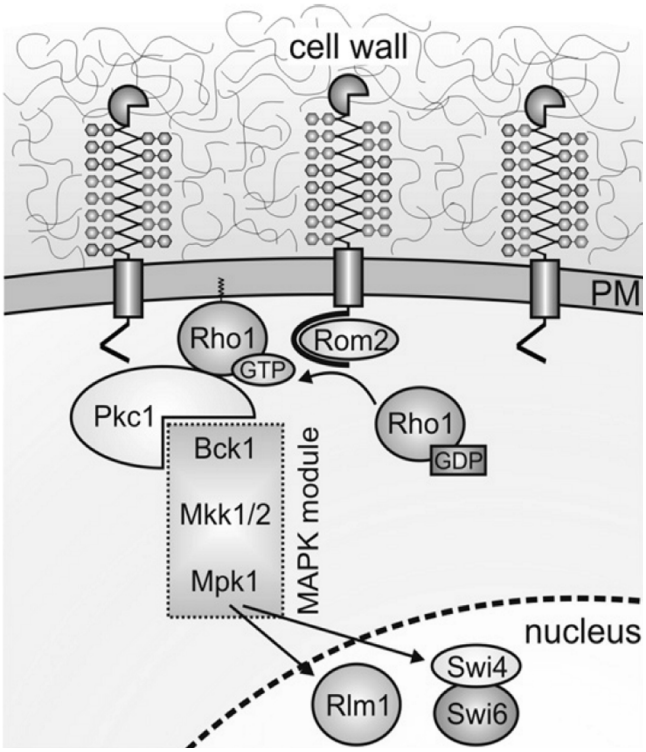

Figure 1. Simplified scheme of the cell wall integrity sensors, signaling cascade and target proteins in *S. cerevisiae* [1]

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In vitro analysis of the chitin synthase III complex in *S. cerevisiae*

Daniela Heine, Hans Merzendorfer

University of Osnabrück, Department of Biology/Chemistry, Osnabrück, Germany
*Correspondence to name Daniela.Heine@Biologie.Uni-Osnabrueck.de

The cell wall of fungi is essential for survival in hostile environments. Though the fungal cell wall varies in composition and structure, one common component is chitin, a β-(1-4)-linked linear polymer of N-acetyl-glucosamine. The general pathway of chitin synthesis is highly conserved. The last polymerization step is catalyzed by a membrane-integral β-glycosyltransferase known as chitin synthase (Chs).

In S. cerevisiae, there are three chitin synthases, of which one is part of a complex that produces more than 90 % of the cell wall chitin in vivo. This complex is named chitin synthase III (CSIII), and consists of the catalytic subunit Chs3 and the regulatory subunit Chs4.

To obtain a better understanding of Chs4 and its role in chitin synthesis, we focussed on protein-protein-interactions between Chs4 and different N-terminal domains of Chs3, which had been suggested previously to be involved in binding of Chs4. For this purpose, his-tagged versions of Chs4 and two Chs3 domains were expressed as recombinant fusion proteins with the maltose binding protein in E. coli, and purified via Ni-NTA and amylose columns. Binding studies and far-western blot analysis confirmed a physical interaction between the Chs3 domains and Chs4 so far.

Targeting of Chs3 to the bud neck is controlled by Pkc1-dependent phosphorylation. So, we further addressed the role of Chs3 phosphorylation in regulating protein interaction. Using Phos-tag gel electrophoresis and mass spectrometry, we could map four phosphorylation sites in the Chs3-C1 domain, while Chs3-C3 does not seem to be phosphorylated.

Finally, we aimed to identify unknown proteins interacting with Chs4. For this purpose, we coupled purified MBP-Chs4 (and MBP as a control) to amylose columns and incubated the columns with a yeast extract. Bound proteins were eluted by maltose and separated on SDS-PAGE. Bands were cut out and analyzed by mass spectrometry. In total, 321 proteins were identified, with 137 of them only interacting with Chs4. After all, 4 candidates seem to be promising to be real interacting partners of the CSIII complex.

The ribosome as a micro-compartment involved in cellular signaling: the case of Arabidopsis S1 group bZIPs

Sjef Smeekens, Jingkun Ma, Maureen Hummel, Jeroen Lastdrager, Jebasing Selvanayagam, Johannes Hansonor

Department of Molecular Plant Physiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
Correspondence to j.c.m.smeekens@uu.nl

Plant growth depends on signaling systems that provide information on the status of cellular metabolites such as sugars and amino acids. Sugar availability is a powerful mediator of growth since sugars act both as immediate substrates for intermediary metabolism and as effective signaling molecules. Significant progress has been made in identifying plant growth controlling regulatory systems that receive input from sugars and sugar-derived metabolic signals. The C/S1 bZIP transcription factor network that in Arabidopsis encompasses several bZIP transcription factors of the S1 (bZIP1, 2, 11, 44, 53) and C (bZIP9, 10, 25, 63) group bZIP families. Heterodimers of these S1 and C class bZIPs are potent in planta transcriptional activators and provide the plant with extensive regulatory potential. Sucrose represses S1 class bZIP activity in a concentration-dependent way by arresting translation of S1 bZIP mRNAs via a ribosome stalling mechanism. Half maximum translational repression occurs at 10–20 mm sucrose. In the presence of sucrose the 'Sucrose Control' (SC) peptide encoded in the 5′-leader of the S1 mRNAs inhibits translation of the main bZIP ORFs. This SC peptide is evolutionary conserved in the plant kingdom and found only in homologous plant bZIP genes. Thus, bZIP-mediated reprogramming of metabolism and growth depends on the cellular sucrose level and increasing sucrose levels reduce S1 group mRNA translation.

Involvement of GapC in microcompartmentation and gene expression

Minhee Kang[1], Markus Schneider[1], Johannes Knüsting[1], and Renate Scheibe[1*]

[1]University of Osnabrück, FB Plant Physiology, Osnabrück, Germany
*Correspondence to Renate.Scheibe@Biologie.Uni-Osnabrueck.DE

Apart from glycolysis for energy production, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) participates in many other functions by its diverse activities accompanied by changes in its subcellular localization. The various subcellular localizations are usually associated with post-translational redox-modification of GAPDH, and these changes subsequently lead to the transient microcompartmentation and transcriptional changes in the nucleus which trigger cell death [1].

In plants, upon oxidative stress, GAPDH was found to be localized and accumulated in the nucleus [2] and its high redox-sensitivity may play a role in oxidative stress signalling, metabolic reprogramming or protection [3]. However, the function in the nucleus and the translocation mechanism of GAPDH are still unknown.

In this study, the nuclear localization of two cytosolic isoforms of phosphorylating GAPDH from *A. thaliana* (GapC1, GapC2) in oxidatively stressed protoplasts was directly visualized by BiFC. The function of GapC under various redox-states, its regulation by redox-modification and microcompartmentation are currently being analysed [4].

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Dynamics of FNR recruitment into protein complexes

Manuel Twachtmann[1*], Guy T. Hanke[1]

[1]Division of Plant Physiology, School of Biology/Chemistry, Osnabrück University, Germany
*Correspondence to guy.hanke@biologie.uni-osnabrueck.de

Photosynthesis demands dynamic changes in supercomplex composition in response to environmental and metabolic changes.

The enzyme ferredoxin:$NADP^+$ reductase (FNR) catalyses the final reaction step in photosynthetic electron flow by receiving electrons from the electron carrier protein Ferredoxin to synthesize NADPH for metabolic processes. FNR is present as a soluble protein and bound to the interacting proteins Tic62 and TROL at the thylakoid membrane [1].

Some short term adaptations involve changes in micro-compartments at the thylakoid membrane to maximize photosynthetic efficiency while maintaining low stress levels. FNR can be found recruited into several protein complexes of these micro-compartments [1]. The direct function of FNR in these micro-compartments remains unclear but we have identified specific structural features that determine FNR recruitment [2].

Elucidating the principle of dynamic release and recruitment of FNR we have investigated the interaction of three maize FNR isoforms with small peptide sequences, containing the FNR-binding-motif of Tic62 and TROL, by analytical size exclusion chromatography. In addition, we have analysed how these structural differences between maize FNRs affect the composition of supercomplexes in the thylakoid membrane of transgenic Arabidopsis plants with blue native PAGE.

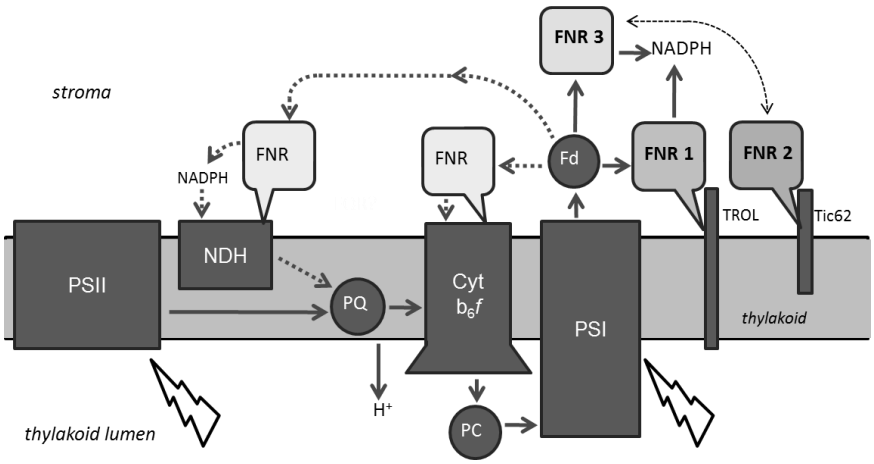

Figure 1: FNR is a soluble protein that synthesizes NADPH during photosynthesis. It can be bound to several interacting proteins on the thylakoid membrane. Recruitment into complexes with Tic62 and TROL seems to be isoform specific.

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Towards understanding the potential nuclear redox microcompartments and their influence on the transcription factor PERIANTHIA (PAN)?

Nora Gutsche*, Shutian Li, Sabine Zachgo

University of Osnabrueck, Department of Botany, Osnabrueck, Germany
*Correspondence to nora.gutsche@biologie.uni-osnabrueck.de

The development of a complex flower is a highly regulated process. Recently, an Arabidopsis mutant with a reduced petal number was found, which could be identified as a knockout mutant of the land-plant specific glutaredoxin *ROXY1*. Glutredoxins are oxidoreducatses known to post-translationally modify target proteins due to a redox-signal. The TGA-transcription factor PERIANTHIA (PAN) was isolated as an interaction partner of ROXY1. Both proteins act together in the same genetic pathway in petal initiation [1]. An interaction of both proteins in the nucleus is pivotal for governing normal petal initiation [2], but the function of the ROXY1 interaction with PAN remains enigmatic. The PAN protein comprises six cysteines, but for its *in planta* function in petal initiation only one cysteine, namely C340, is essential [1]. If post-translational modifications of the PAN cysteines are involved in the regulation of petal initiation it would be the first that time redox-regulation and flower development could be connected to a transcriptional regulator. This redox-regulation is mainly mediated by an altered 2GSH/GSSG ratio due to a higher level of reactive oxygen species (ROS) production. Plants have an excellent defence system against such molecules to protect the cell and to maintain a reduced cellular redox-status. Therefore the alteration of the redox-status must be a spatial and temporal highly regulated process within microcompartments.

Here, *in vitro* EMSA binding studies are presented, which analyze whether the DNA binding of PAN to a known DNA-binding motif in a target gene depends on the redox-status of the PAN protein. Further *in vitro* characterization of the post-translational PAN modifications will address the regulatory mechanism of the petal initiation and the ROXY1 function.

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The role of the wall proteome of the medically important fungus Candida albicans in stress resistance and virulence

Frans M. Klis

Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands.
Correspondence to F.M.Klis@uva.nl

C. albicans is a polymorphic fungus that is a member of the human mycobiome and is frequently found in mucosal layers. When the immune system is compromised, it can penetrate deeper tissue layers, invade the bloodstream and cause systemic infections, which are often fatal. It has a sturdy cell wall consisting of an inner polysaccharide layer that is covered by a dense external layer of radially extending mannoproteins with an estimated surface density of 3×10^4 μm^{-2}. The outer protein layer consists of >20 different proteins with multiple functions. For example, they are involved in cell wall assembly and biofilm formation (transglycosylases) , recognition and binding of mammalian matrix proteins (adhesion proteins), detoxification of reactive oxygen species (superoxide dismutases), and acquisition of iron (heme-binding proteins) (1). We study the relative changes in the proteome of newly formed walls that occur in response to various stress conditions. For this we use mass spectrometric analysis of query walls mixed with ^{15}N-labeled reference walls as internal standard. To exemplify this approach, I will discuss the adaptations observed in the wall proteome of iron-deficient cells (2). Iron deficiency, which is the natural condition in human tissues and fluids, leads to a switch from growth in the yeast form to (pseudo)hyphal growth and can be induced by hypoxic conditions (the reductive uptake pathway of iron requires molecular oxygen), alkaline pH of the culture medium (ferric iron becomes insoluble), or by adding an iron chelator to the medium. Iron-deficient cells respond by strongly increased incorporation of all members of the heme-binding protein family (Csa1, Pga7, Pga10, and Rbt5), two adhesion proteins (Als3 and Als4), a superoxide dismutase (Sod4), and Hyr1, a protein involved in immune evasion. In contrast, the incorporation of Tos1, a putative β-1,3-glucanase, is strongly decreased. Our results show that the wall proteome of *C. albicans* is highly adaptable and plays a crucial role in virulence.

References

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Control of cell architecture by microtubule end-binding proteins

Anna Akhmanova

Cell Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht
The Netherlands
*Correspondence to a.akhmanova@uu.nl

Microtubule cytoskeleton plays an essential role in cell polarization, migration and division. Microtubules are polymeric tubes that are built and broken down from their ends. Proteins binding to microtubule ends can attach microtubules to various cellular structures and control different aspects of microtubule dynamics, such as microtubule polymerization rate and the frequency of transitions between growth and shortening. Recent studies showed that a sequence motif SxIP embedded in a basic, serine and proline-rich region can target a variety of proteins to growing microtubule ends by interacting with the members of End Binding (EB) family. By combining proteomics and bioinformatics approaches we have identified a broad set of regulators that control microtubule interactions with the plasma membrane, actin and different organelles. We have also characterized a protein family, which specifically associates with the growing microtubule minus ends. We have used cell biological and in vitro reconstitution experiments to obtain insight into how microtubule end-binding proteins control microtubule networks in interphase cells.

What can we learn about dendritic spine plasticity using a new mouse model of amyloidosis?

Lorène Penazzi*, Karolin Selle, Nataliya Golovyashkina, Christian Tackenberg, Roland Brandt and Lidia Bakota

Department of Neurobiology, University of Osnabrück, Osnabrück, Germany
*Correspondence to Lorene.Penazzi@Biologie.Uni-Osnabrueck.DE

Dendritic spines are small bulbous microcompartments, which receive the majority of excitatory synaptic input in the brain and are fundamental for memory formation and maintenance. Loss of spines occurring in several regions like the hippocampus is an early feature during neurodegeneration, which is for example strongly correlated with the progression of memory deficits in Alzheimer's disease (AD). Therefore it is fundamental to understand how dendritic spines are modulated by the presence of pathological level of disease-relevant factors such as beta amyloid peptides.
Here, we analyzed the effect of moderate level of Aβ on spine plasticity of pyramidal cells in the CA1 region of the hippocampus utilizing heterozygous APP_{SDL} mice that express human APP with a combination of three mutations found in familiar AD; Swedish, London and Dutch. Potential changes of spine density and shape were assessed by high-resolution cLSM and algorithm-based image analysis (Fig. 1).

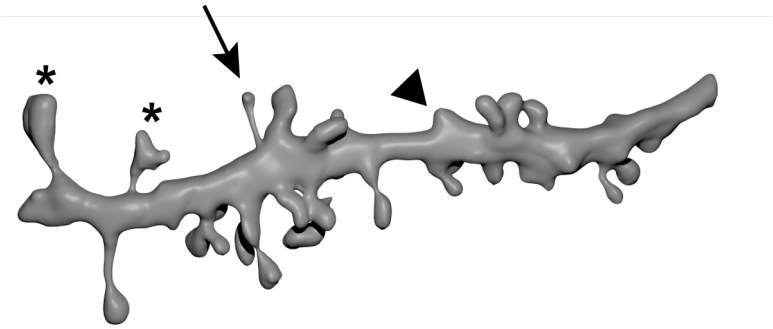

Figure 1. Example of a rasterized dendritic segment. High-resolution 3D cLSM image was taken of a dendritic segment with its spine protrusions. Arrow: thin spine; arrowhead: stubby spine; asterisk: mushroom spine.

1) To dissect the mechanism how Aβ is acting on spines we used an *ex vivo* model of organotypic hippocampal slice cultures from APP_{SDL} transgenic and non-transgenic mice treated with the NMDAR antagonist CPP. EGFP expression was mediated via Sindbis virus. We observed that CPP abolished Aβ-mediated spine loss.
2) APP_{SDL} mice crossed with EGFP expressing mice, for the visualization of neuron population in different brain regions, were used for analysis to follow spine changes over time. Mice expressing EGFP alone served as control.
We observed in general reduced spine density at all ages, which on CA1 pyramidal neurons reached significance from 6 months on.
3) To analyze whether environmental enrichment has an effect on spines young APP_{SDL}/EGFP and EGFP mice were exposed during 4 months to housing conditions that facilitate enhanced sensory, cognitive, motor and social stimulations.
We observed that enriched environment abolished the difference in spine density between the two genotypes.
Taken together our data suggest that Aβ induces changes in spine density and morphology through NMDARs and that these changes can be modulated by enriched environment.

Characterization of a Putative Ceramide Sensor: How is Mitochondrial Apoptosis Controlled in the ER

Birol Cabukusta, Fikadu G. Tafesse, Ana M. Vacaru and Joost C.M. Holthuis

Molecular Cell Biology Division, University of Osnabrück, Osnabrück, Germany
bcabukusta@uos.de

The essential but potentially lethal nature of ceramides implies that cells must monitor their levels closely to avoid killing themselves during sphingolipid biosynthesis. We recently identified SMSr, an ER-resident ceramide phosphoethanolamine (CPE) synthase, as suppressor of ceramide-mediated cell death [1,2]. Disruption of SMSr catalytic activity causes a rise in ER ceramides and their flow in mitochondria, triggering a mitochondrial pathway of apoptosis. We find that SMSr-catalysed CPE production, although required, is not sufficient to suppress ceramide-induced cell death. Moreover, we show that SMSr-mediated ceramide homeostasis is critically dependent on the enzyme's *N*-terminal sterile-alpha motif or SAM domain and that SMSr forms oligomers through its SAM domain. Our results define ER ceramides as bona fide transducers of apoptosis and suggest a role of SMSr as ceramide sensor to protect cells against the inherent danger of sphingolipid biosynthesis. To further elucidate the mechanism by which SMSr controls ER ceramides, our ongoing work focuses on the functional analysis of oligomerization-defective SMSr mutants and on a proteome-wide search for SMSr binding partners.

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A The role of HOPS complex components in organizing the endo-lysosomal system

Judith Klumperman

Department of Cell Biology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
*Correspondence to j.klumperman@umcutrecht.nl

My lab has a long standing interest in the cellular pathways and mechanisms involved in lysosome biogenesis. Our recent studies focus on the role of components of the HOPS (Homotypic fusion and Protein Sorting) complex and the related CORVET (class C core vacuole/endosome tethering) complex in lysosomal biogenesis and functioning in mammalian cells. In yeast, HOPS (consisting of Vps39,41,18,11,33,16) and CORVET (consisting of Vps3,8,18,11,33,16) are well-established tethering complexes required for membrane fusion events at the endosomes and lysosome, respectively. In humans, the role of HOPS and CORVET is less well established, but their components are increasingly implicated in diseases. For example, Vps41 was recently linked to the development of gastric and colorectal cancers and has been identified as a protective factor in Parkinson disease. The ultimate goal of our studies is to understand how mutations in these HOPS and CORVET components cause human diseases.

Our previous studies have contributed to the understanding how mutations in Vps33B and Vps16B (VIPAR) can cause the multisystem disorder Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome1. Also, we have shown that LAMPs (lysosome associated membrane proteins) can travel directly from the trans-Golgi network (TGN) to late endosomes by a novel type of transport carrier1 that differs from clathrin coated vesicles involved in the delivery of lysosomal enzymes and requires Vps41 and the SNARE VAMP7 for fusion with late endosomes. In addition, we have found that Vps41 as well as Vps39 and Vps33B are required for homotypic fusion between late endosomes3. Currently, we are studying the knockdown phenotypes of various HOPS/CORVET genes, which is important to understand their precise role in endo-lysosomal functioning.

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The interaction of the multimeric HOPS complex with SNAREs

Anna Lürick[1*], Cornelia Bröcker[1], Anne Kuhlee[2], Stefan Raunser[2] and Christian Ungermann[1]

[1]University of Osnabrück, Department of Biology, Biochemistry Section, Germany
[2]Max Planck Institute of Molecular Physiology Dortmund, Department of Physical Biochemistry, Germany
*Correspondence to anna.luerick@biologie.uni-osnabrueck.de

The hexameric tethering HOPS complex plays an essential role for vacuolar/ lysosomal membrane fusion. The initial targeting of the complex to membranes is mediated via the Rab GTPase Ypt7, which allows subsequent interaction with SNARE proteins. Direct interaction between HOPS and both the monomeric Vam7 and an assembled SNARE complex has been reported previously (Krämer and Ungermann, 2011). In addition, disassembly of the trans-SNARE complex via Sec17 and Sec18 is inhibited by HOPS binding (Xu et al., 2010).
Sec1/Munc18 (SM) proteins play an essential role during membrane fusion reactions through the interaction with Syntaxins. The HOPS subunit Vps33 is homologous to such SM proteins.

Here, we investigate the interaction pattern between HOPS and vacuolar SNAREs. The Syntaxin Vam3 directly binds HOPS and a sub-complex composed of only Vps16 and Vps33. Pull down experiments with truncated Vam3 constructs identified the N-terminal H_{abc}-domain, which is characteristic for Syntaxins, as the binding domain. The interaction with the sub-complex was confirmed by electron microcopy analysis in which Vam3-His localization relative to HOPS was followed by Ni-NTA gold labeling.

Vacuoles covered with Vam3 lacking the N-terminal domain fuse less efficiently *in vitro*. However, vacuolar morphology and the localization of other vacuolar proteins is unaffected indicating that the H_{abc}-domain is not essentially required for fusion but might possess a regulatory function.

All together, these results indicate a participation of the Vam3-H_{abc}-domain in *priming* and *docking* steps of fusion.

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Detergent-free extraction of membrane proteins: the power of native nanodiscs.

Stefan Scheidelaar, Martijn Koorengevel, Jonas Doerr, Antoinette Killian

Membrane Biochemistry & Biophysics, Utrecht University
*Correspondence to j.a.killian@uu.nl

Membrane proteins are notoriously difficult to isolate and characterize due to their hydrophobicity and resulting problems in purification. Recently, a method has been discovered to solubilize membrane proteins directly from their native membrane by using an amphipathic copolymer, styrene-maleic acid (SMA). This polymer solubilizes membranes in the form of nanodiscs [1,2], thereby opening up the exciting possibility to purify and characterize membrane proteins while keeping their native environment intact.

Our first aim was to understand the physical properties of membranes that modulate this unique property of SMA. By using a systematic approach with vesicles of synthetic phospholipids, we found that SMA is an excellent membrane solubilizer and we developed a model for its mode of action.

Next we used the SMA technology to solubilize and purify a native membrane protein, the tetrameric potassium channel KcsA, from E.coli. This allowed us to characterize properties of the channel directly in its native membrane environment. In particular we investigated the thermal stability of the protein by using circular dichroism and fluorescence spectroscopy, and we investigated whether the protein has a preference for specific lipids by analyzing the lipid composition of the nanodiscs. Together, our findings highlight the potential of the use of these “native nanodiscs” as a general tool in the study of membrane proteins.

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

Daniel Klose[1*], Philipp S. Orekhov[2], Ioan Orban[1], Christian Rickert[1], Armen Mulkidjanian[1,2], Martin Engelhard[3], Johann P. Klare[1], Heinz-Jürgen Steinhoff[1]

[1]University of Osnabrück, Department of Physics, Osnabrück, Germany
[2]Moscow State University, School of Biology, Moscow, Russia
[3]Max-Planck-Institute for Molecular Physiology, Dortmund, Germany
*Correspondence to daniel.klose@uos.de

In *Natronomonas pharaonis* a sensory rhodopsin II – transducer complex (SRII/HtrII) mediates negative phototaxis.[1] 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 along the transducer still remains unclear.[1]

To trace the conformational signal and it's propagation throughout the elongated transducer, we follow transient changes upon illumination by time-resolved cw- and pulsed-Spin Labeling EPR spectroscopy. In the HAMP domain we find transient spectral changes that correspond to shifts in the previously found[2] thermodynamic equilibrium in the dark state.

Modeling the whole complex required for signaling and control of the subsequent kinase, we performed coarse grain molecular dynamic simulations in two adaptation states, comprising fully methylated or demethylated transducers. Since adaptation exhibits a well established relation to the extent of complex activation[3], this approach revealed the alteration of distinct dynamical states as a working model (Fig. 1) for the signaling mechanism.

Elucidating this mechanism at a higher level of detail is attempted by combining the experimental and simulation approaches, yet a complete activation mechanism requires a scheme of protein-protein couplings within the highly cooperative framework of hexagonal arrays formed by the trimers of SRII/HtrII dimers together with the kinases.

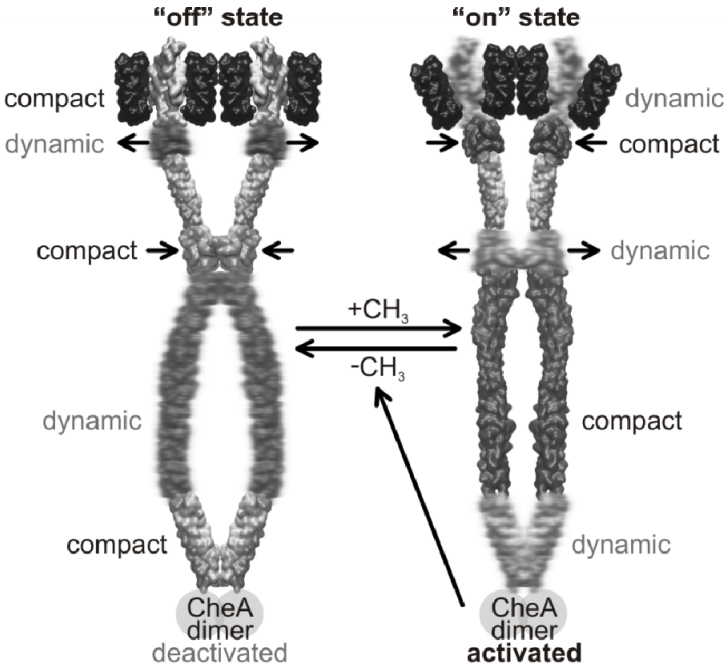

Figure 1. Model suggested for the signaling mechanism of SRII/HtrII trimer-of-dimer complexes.

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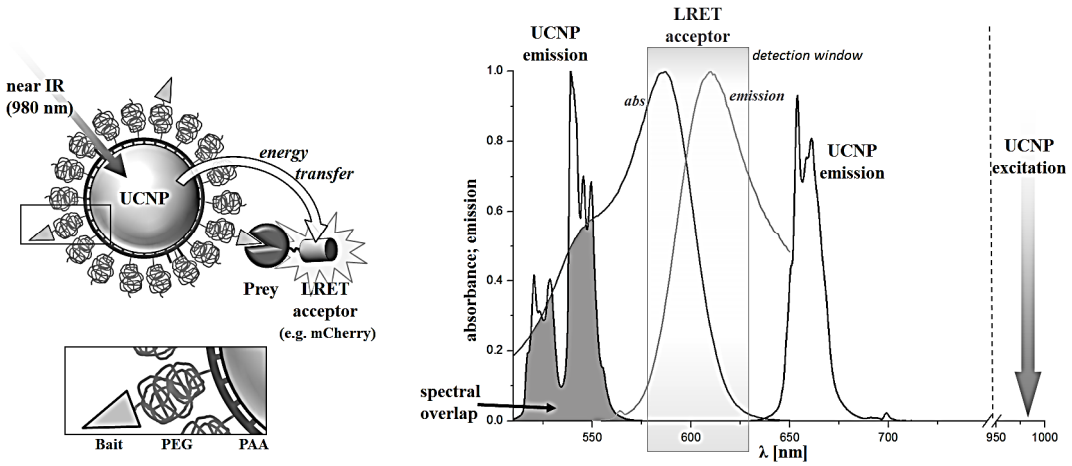
Towards *in situ* protein analytics: Upconversion nanoparticles as interaction sensors and photoactuators in living cells

Christoph Drees[1], Athira N. Raj[2], Jörg Nordmann[2], Markus Haase[2], Rainer Kurre[1] and Jacob Piehler[1*]

[1] University of Osnabrück, Department of Biology, Barbarastr. 11, 49076 Osnabrück, Germany
[2] University of Osnabrück, Institute of Chemistry, Department of Inorganic Chemistry I-Materials Research, Barbarastr. 7, 49069 Osnabrück, Germany
* Correspondence to piehler@uos.de

Upconversion nanoparticles (UCNP) are excited by multiphoton absorption of NIR light and emit photons in the UV and visible range. In contrast to multiphoton absorption of organic dyes, upconversion is much more efficient and can be achieved by continuous wave excitation. Owing to these properties, UCNP can be excited with negligible background and thus are promising probes for labelling of cells as tissues (1). Moreover, luminescence resonance energy transfer (LRET) from the UCNP to molecules in the immediate proximity opens exciting possibilities as spectroscopic reporters or photoactuators with very high spatial resolution (2). The aim of this collaborative project is to exploit these features of UCNP for developing novel tools for spatially resolved quantitative protein interaction analysis in living cells. To this end, core-shell UCNPs with enhanced luminescence quantum yield were synthesized and rendered water soluble by means of polyelectrolyte coating. Using surface PEGylation and functionalization by the HaloTag ligand, specific targeting of these UCNP to HaloTag proteins in the cytosol of living cells was achieved. In parallel, surface architectures were developed for detecting and quantifying LRET to proteins specifically captured to the surface of UCNP. For this purposes, we established microscopic and spectroscopic set-ups for efficient UCNP excitation and LRET detection. These tools will be employed for systematically optimizing the properties of UCNP and surface functionalization for LRET, which will be required for successful applications as reporters and photoactuators in living cells.



UCNP-LRET reporter system. Left: Schematic view on a UCNP bound to a reporter (Prey-LRET acceptor). **Inset:** The bio-coating consist of poly(acrylic acid) (PAA: water solubility), poly(ethylene glycole) (PEG: bio-compatibility) and a bait. **Right:** Experimental setup (spectra). UCNPs are excited at ~980 nm and emit at much lower wavelengths (530-560 nm). If a suitable LRET acceptor (spectral overlap) is bound to the particle, energy will be transfered from the UCNP to the acceptor. This leads to acceptor emission which can be monitored separately within a defined detection window (emission filter).

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Super Resolution Localization Microscopy: Visualization of secretion systems cooperation in *Salmonella enterica*

Britta Barlag[1], Oliver Beutel[2], Dennis Janning[3], Jacob Piehler[2], Michael Hensel[1*]

University of Osnabrück, Faculty Biology, Osnabrück, Germany
Divisions of Microbiology[1] Biophysics[2] and Neurobiology[3]
*Correspondence to michael.hensel@biologie.uni-osnabrueck.de

Salmonella enterica uses the *Salmonella* Pathogenicity Island (SPI)1 type III secretion system (T3SS) and SPI4-T1SS to invade polarized cells. The SPI4-T1SS mediates the intimate contact with the epithelial cell via the giant, non-fimbrial adhesin SiiE [2], followed by the translocation of effector proteins by the SPI1T3SS [1]. Since both systems act cooperatively during invasion of polarized cells [1], we investigated the formation of a microcompartment of these two secretion systems at the zone of contact between *Salmonella* and its target cell. It is still unclear whether secretion systems are randomly distributed or are localized in preformed clusters.

Unraveling structures in bacteria is often limited by the diffraction limit of 200 nm of conventional light microscopy. Super Resolution Localization Microscopy is a versatile tool to overcome the diffraction limit, increasing the resolution by ~10-fold [3]. This technique is based on stochastical activation of photoactivatable or blinking fluorophores and allows high resolution analyses of secretion system localization in bacteria [3].

To study the localization and movement of the secretion systems in the cell envelope, we fused different subunits of SPI1-T3SS and SPI4-T1SS to the HaloTag. This engineered halogenase forms covalent products with chloralkane substrates conjugated to fluorescences dyes compatible with single molecule imaging techniques. Since the ligand permeates bacterial membranes without influence on cell viability, labeling of subunits of secretion systems is possible in living bacteria.

We observed that SPI1-T3SS and SPI4-T1SS are localized in clusters in the cell envelope in the growth phase of highest invasiveness. Dual-color, live-cell Stochastic Optical Reconstruction Microscopy (STORM) revealed clusters of both systems localized in the same region of the envelope. Single particle tracking (SPT) showed only inter-cluster movement of the tagged subunits of secretion systems. These findings suggest a preformed distribution of cooperating secretion systems.

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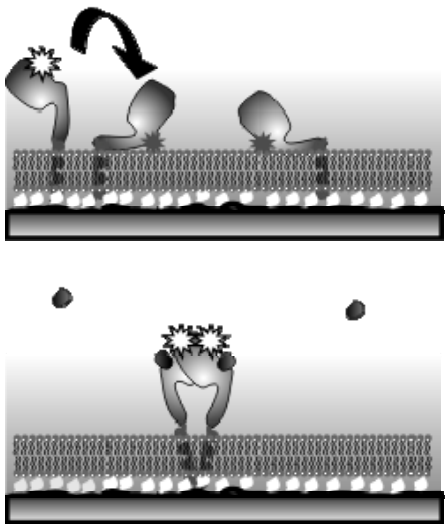
Surface architecture for probing receptor interactions and conformations in polymer-supported membranes

Oliver Birkholz[1*], Friedrich Roder[1], Suman Peel[2] and Jacob Piehler[1]

[1]University of Osnabrück, Dpt. of Biology/Chemistry, Div. of Biophysics, Osnabrück, Germany
[2]University of Bristol, School of Biochemistry, University Walk, BS8 1TD Bristol
*Correspondence to oliver.birkholz@biologie.uni-osnabrueck.de

Transport and communication across biological membranes is mediated by transmembrane protein complexes, which are structurally organized in the context of the lipid bilayer. The conformation of such proteins and protein complexes therefore depend on interactions with membrane lipids, which can be modulated by protein-protein interactions. In this project, we aimed to establish a generic approach for analysing the structural organization of transmembrane proteins and its regulation by lipids and proteins.



Surface-modulated fluorescence quenching of model receptors in a polymer-supported membrane.

To this end, we develop surface architectures comprising a polymer-supported membrane (PSM) in defined proximity to a spectroscopic optic modulator layer. Based on this architecture, the distance of a fluorescence probe within the membrane protein of interest from the surface can be monitored with high spatial and temporal resolution. In order to establish generic surface functionalization for the assembly of PSM we synthesized a poly-L-lysine-based copolymer grafted with a polyethylene glycole (PEG) spacer carrying a hydrophobic anchor group. Anchoring groups enable capturing of proteoliposomes, which then can be fused into a membrane on a PEG polymer cushion.[1]

We could demonstrate fast and reproducible formation of a lipid bilayer and functional reconstitution and mobility of transmembrane proteins within the bilayer by solid-phase detection and fluorescence techniques. Fluorescently labelled model receptors will be incorporated into the PSM to address possible conformational changes dependent on the interaction with their respective ligand and on the lipid environment.

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The *Salmonella enterica* giant adhesin SiiE binds to polarized epithelial cells in a lectin-like manner

Britta Barlag*[1], Carolin Wagner*[1], Roman Gerlach[2], Jörg Deiwick[1], Michael Hensel[1]

[1] University of Osnabrück, FB Biology, Department of Microbiology, Osnabrück, Germany
[2] RKI Wernigerode Branch
* authors contributed equally
Correspondence: michael.hensel@biologie.uni-osnabrueck.de

The invasion of polarized epithelial cells by *Salmonella enterica* requires the cooperative activity of the *Salmonella* Pathogenicity Island (SPI) 1-encoded type III secretion system (T3SS) and the SPI4-encoded giant non-fimbrial adhesin SiiE [1]. SiiE is a highly repetitive protein composed of 53 bacterial Ig (BIg) domains and mediates binding to the apical side of polarized epithelial cells [2;3]. We analyzed the binding properties of SiiE and observed lectin-like activity. SiiE-dependent cell invasion can be ablated by chemical or enzymatic deglycosylation. Lectin blockade experiments revealed that SiiE binding is specific for glycostructures with terminal N-acetyl-glucosamine (GlcNAc) and/or α 2,3 linked sialic acid. In line with these data, we found that SiiE-expressing *Salmonella* bind to the GlcNAc polymer chitin. Various recombinant SiiE fragments were analyzed for host cell binding. We observed that C-terminal portions of SiiE bind to the apical side of polarized cells and the intensity of binding increases with the number of BIg domains present in the recombinant proteins. Based on these results, we propose that SiiE mediates multiple interactions per molecule with glycoproteins and/or glycosylated phospholipids present in the apical membrane of polarized epithelial cells. This intimate binding enables the subsequent function of the SPI1-T3SS, resulting in host cell invasion.

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Localization of clathrin at a CNS synapse

Daniel Boening, Juergen Klingauf

University of Muenster, Department of medical physics and biophysics, Muenster

Clathrin mediated endocytosis (CME) regulates many cell physiological processes such as synaptic vesicle recycling during synaptic transmission. Within the presynaptic network clathrin functions as an organizing platform for coated pit assembly. Although it is suggested that CME is the dominant process for endocytosis, it seems to be too slow for fast recycling. Therefore it was suggested that a preassembled pool of clathrin-ligand molecules exists. To look at the existence of these preassembled clathrin we used three dimensional stochastically optical reconstruction microscopy (3D STORM), which offers the possibility to localize proteins in the synapse with nanometer precision. This technique allowed us to even differentiate between different regions within the synapse by localizing specific proteins.

By performing dual colour 3D STORM we localized clathrin in comparison to bassoon, which is an active zone protein . These results confirms the existance of ‘peri-active zone’ (Hua et al., 2011) where clusters of clathrin were localized.

Behavior analysis of the microtubule-associated protein TORTIFOLIA1 using superresolution and single-molecule tracking strategy *in planta*

A. Borchers[1], D. Janning[2], R. Brandt[2], J. Piehler[3], S. Zachgo[1], H. Buschmann[1]

[1]University of Osnabrück, Biology, Department of Botany, Osnabrück, Germany
[2]University of Osnabrück, Biology, Department of Neurobiology, Osnabrück, Germany
[3]University of Osnabrück, Biology, Department of Biophysics, Osnabrück, Germany

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 transport of the plant growth regulator auxin or in cell wall structure. This research aims to better understand how microtubules control cell elongation. To this end leaf growth of *tortifolia1* mutant plants was studied in detail. We show that *tor1* is a tropism mutant and defective in natural plant movements. It was found that these plant movements involve the plant-hormone auxin, however, *tortifolia1* was found to be partially auxin-insensitive. *TORTIFOLIA1* (*TOR1*) encodes a plant-specific microtubule-associated protein, that labels plus-end comets as well as microtubule cross-over sites. Double labeling of TOR1 and microtubules showed that the protein labels microtubule plus-ends during growth and catastrophe. To gain insight into TOR1 behavior on microtubules we initiated a superresolution (SR) and single-molecule tracking strategy using Tobacco Bright Yellow-2 (BY-2) cells. We found that individual TOR1 molecules are relatively immobile despite the apparent translocation of TOR1-containing microcompartments on the microtubule plus-end. This indicates that TOR1 molecules specifically recognize the plus-end tip (presumably the GTP-cap) and do not move by themselves. This suggests a mechanism for plus-end recognition to ENDBINDING1 (EB1).

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Quantification of the Cpx-TCS proteins and their spatial distribution in living cells

Emina Ćudić

University of Osnabrück, Molecular Microbiology
*Correspondence to ecudic@uni-osnabrueck.de

Bacteria rely on two-component systems (TCS) to acclimate in response to environmental changes and therefore to different stimuli [1]. Typically, these systems consist of a transmembrane sensor kinase (SK) and a cytoplasmic response regulator (RR). In case of a suitable stimulus, a conserved histidine residue of the SK becomes autophosphorylated. Subsequently, the phospho-SK serves as a kinase and phosphotransferase leading to the phosphorylation of a conserved aspartate residue of the corresponding RR. As a result, the RR is activated and mediates the intracellular response by differential expression of its target genes. The system is set back to the initial state via dephosphorylation of the RR [2]. The conjugation pilus expression control (Cpx) stress system belongs to the group of TCS and consists of the membrane-bound SK CpxA, the cytosolic RR CpxR and the periplasmic accessory protein CpxP, which inhibits the autophosphorylation activity of CpxA [3]. This envelope stress system responds to different stimuli; e.g. elevated pH, salt, metals, changes in lipid composition and misfolded proteins [4].

Since an enrichment of CpxA in pole-derived membrane fragments of Escherichia coli has been shown [5], one goal is to investigate the spatial distribution of the Cpx-TCS in living cells under different conditions and in dependence of different substances as Cardiolipin [6] or MinD, which was found to interact with CpxA [7]. These investigations will be performed using different microscopy techniques such as total internal reflection fluorescence (TIRF) microscopy.

There is no data available for the cellular determination of all three components of a TCS – SK, RR and accessory protein. Thus, one further goal is to determine the cellular levels of CpxA, CpxR and CpxP under non-inducing conditions. Furthermore we are interested in the stoichiometries between CpxA, CpxR and CpxP under different inducing conditions. For these experiments Mass-Spectrometry will be used.

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Chs3 topology and the role of oligomerization in chitin extrusion

Simon Gohlke[1], Hans Merzendorfer[1*]

[1]University of Osnabrück, FB Biology, Osnabrück, Germany
*Correspondence to Hans.Merzendorfer@Biologie.Uni-Osnabrueck.DE

The chitin synthase III complex in *S. cerevisiae* consists of the catalytic subunit Chs3 and the regulatory subunit Chs4. The topology of Chs3 is discussed controversial and computational prediction of transmembrane helices (TMHs) yields unambiguous results with regard to the orientation of the *C*- and *N*-termini and the number of TMHs. Therefore, we examined Chs3 topology using protease accessibility assays. We provide a topological model of Chs3, which is in line with a homology model based on the crystal structure of the bacterial cellulose synthase complex, BcsA-BcsB [1]. An important characteristic of the cellulose synthase is a narrow channel formed by six conserved TMHs which conserved also in chitin synthases and involved in the extrusion of the sugar polymer. However, there are only 4 TMHs in Chs3, a number which is presumably too low for pore formation.

Therefore, we proposed that pore formation in Chs3 could involve oligomerization of two or more individual molecules. To test Chs3 oligomerization *in vivo*, we used bimolecular fluorescence complementation. We detected oligomeric complexes in the plasma membrane at the bud neck and in membranes of Golgi vesicles. Using this method, we additionally showed an interaction of Chs3 with Chs4 at the ER and the bud neck, supporting previous data on Chs4 localization [2].

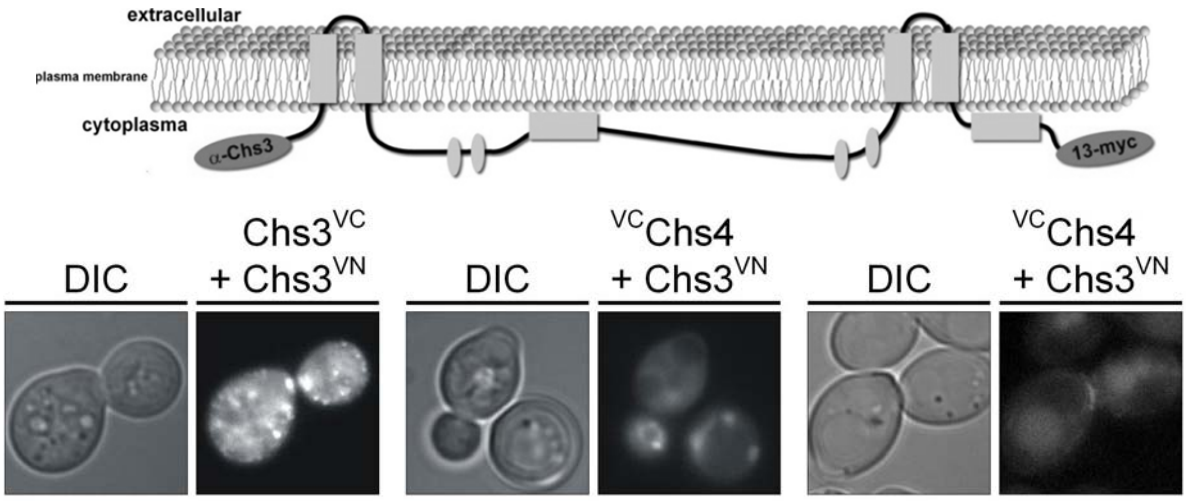

Fig. 1 Topology model for Chs3 and detection of oligomeric complexes. Bimolecular Fluorescence complementation demonstrates oligomerization of Chs3 at the bud neck and at the membranes of intracellular (presumably Golgi) vesicles, and possibly interaction between Chs3 and Chs4 at the bud neck and at the endoplasmic reticulum, which is in line with a previous model on CaaX processing of Chs4 [2].

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

Benjamin Hallier[1], Mareike Panz[1], Arne Jendretzki[2], Jürgen Heinisch[2], Achim Paululat[1] and Heiko Harten[1*]

[1]University of Osnabrück, Department of Biology, Section of Zoology, Osnabrück, Germany
[2]University of Osnabrück, Department of Biology, Section of Genetics, Osnabrück, Germany
*Correspondence to Harten@Biologie.Uni-Osnabrueck.de

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. Significantly, in the affected tissue Nep4A co-localizes with SERCA at distinct subcompartments of the sarcoplasmic reticulum rather than being plasma membrane bound. This result is novel since so far neprilysins were considered to be present exclusively at the cell surface with their active site facing the extracellular space. By expressing several mutated and truncated forms of Nep4 in transgenic animals, we show that the intracellular domain is responsible for the observed degeneration phenotype 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 Nep4A. However, recent findings indicate that SERCA may also play a critical role in manifesting the phenotype. Our data represent the first report of an intracellular neprilysin domain being involved in muscle integrity.

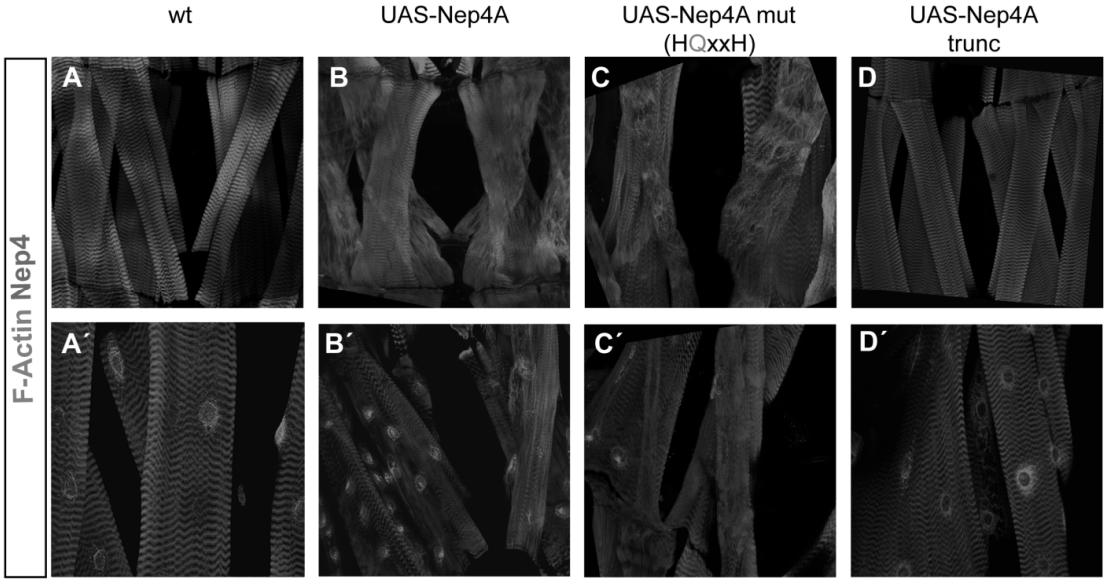

Figure 1: Altering Nep4A expression levels severely impairs muscle integrity
Overexpression of wild type (B, B´) but also of catalytically inactive Nep4A (C, C´) causes severe muscle atrophy. By contrast, overexpression of truncated Nep4A lacking the N-terminal cytoplasmic domain, but retaining its transmembrane as well as extracellular domains, does not induce the atrophy phenotype (D, D´).

Endozytic origin of giant vesicles in the Drosophila intracardiac valve cells establish functional cell shape

Abeln B.[1*], Hüsken M.[1], Paululat A.[1]

[1]University of Osnabrück, FB Biologie, Osnabrück, Germany
*Correspondence to abeln@biologie.uni-osnabrueck.de

Two types of valves regulate hemolymph flow in the *Drosophila* heart. Ostia confine the entry of hemolymph into the heart, whereas intracardiac valve, build by only two cells, control hemolymph flow within the heart tube. A unique feature of intracardiac valve cells is the presence of very large intracellular membraneous vesicles that define the shape of the cells. Our high-speed video microscopy analysis revealed that the valve cells in wildtype animals display a biphasic shape change causing an alternate open and closed state of the heart tube. This implicates a passive mode of action, which is driven by the helical myofibers in cardiomyocytes [1] that cause cell shape changes upon contraction. We assume that in functional valve cells the characteristic large vesicles provide a unique structural element, which allows the cells to become easily flattened upon contraction, thereby opening the luminal space in the heart tube. Furthermore, the high flexibility provided by the large vesicles is presumably a prerequisite for sustaining the strong mechanical stress that is applied to the respective cells in the course of a heart cycle.

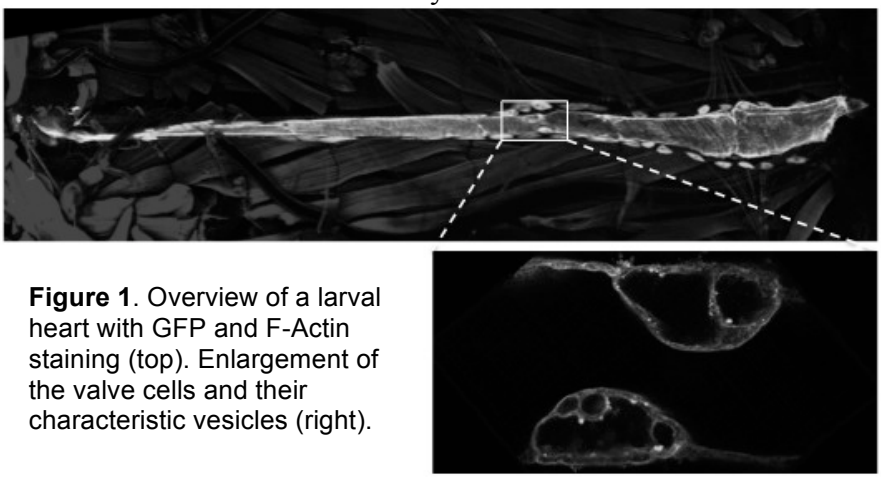

Figure 1. Overview of a larval heart with GFP and F-Actin staining (top). Enlargement of the valve cells and their characteristic vesicles (right).

Live imaging using a GFP reporter line and TEM analysis furthermore showed, that the intracardiac valves differentiate during larval development. As in the vertebrate system, the PDGF/VEGF receptor (Pvr) is necessary for adult [2] and larval valve differentiation in *Drosophila*. We could show that Pvr and its ligand (Pvf2) are expressed at the same time in the valve cell, indicating autocrine signalling. To unravel the origin of the unique morphology of the valve cells, we performed an RNAi mediated candidate screen. This led to the conclusion that endocytosis and vesicle fusion processes are crucial for valve cell development.

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Analysis of functional domains regulating tau-microtubule interaction in living cells

Maxim Igaev, Jörg Brühmann, Dennis Janning and Roland Brandt*

University of Osnabrück, Department of Neurobiology, Osnabrück, Germany
*Correspondence to roland.brandt@biologie.uni-osnabrueck.de

The microtubule-associated phosphoprotein tau has been a subject of neurobiological research for a long time, to greater extend due to its role in Alzheimer's disease (AD), where the protein is believed to get hyperphosphorylated and form neurofibrillary tangles. However, further insight into pathological behaviour of tau requires understanding of the physiological tau-microtubule interaction. The aim of the current work is to gain an idea of how reaction-diffusion properties of tau and the structure of its MT binding domains are interrelated and whether hyperphosphorylation provokes alteration in tau's binding to MTs. For this purpose, a htau441wt construct containing four MT binding repeats was tagged with photoactivatable GFP (PAGFP). The influence of the domain configuration was tested employing a panel of carboxyterminal deletion constructs with different numbers of the repeats, whereas the effect of hyperphosphorylation was mimicked by a pseudohyperphosphorylated tau construct (PHP-tau). Thereafter, focal activations of a fraction of the PAGFP-tau constructs in the middle of cellular processes were performed. The fluorescence decays after the photoactivation (FDAPs) were gathered, averaged, normalized and fit to theoretically predicted scenarios based on a two-component reaction-diffusion model. Our results show that the reaction-diffusion properties of tau correlate both with the number of repeats and with the introduction of the pseudohyperphosphorylation sites. Consecutive deletion of the repeats as well as mutating the corresponding phosphorylation sites reveal a reduction of the binding ability of tau molecules to MTs. The results also suggest that the photoactivation approach combined with the appropriate theoretical techniques is a flexible and suitable tool for extracting general information concerning pathological changes in the tau-MT interactions.

Manipulation of intracellular ceramide flows and its impact on cell fate and organization

Amrita Jain[1], Katharina Ebell[1,2], Oliver Beutel[2], Jacob Piehler[2], Joost C.M. Holthuis[1]

[1] Molecular Cell Biology Division, University of Osnabrueck, 49076 Osnabrueck, Germany
[2] Biophysics Division University of Osnabrueck, 49076 Osnabrueck, Germany
*Correspondence to Amrita.Jain@Biologie.Uni-Osnabrueck.DE

Ceramides are central intermediates of sphingolipid metabolism with critical functions in cell organization and survival. They are synthesized on the cytosolic surface of the endoplasmic reticulum (ER) and then transported by ceramide transfer protein CERT to the Golgi for conversion to sphingomyelin (SM) by SM synthase SMS1. We previously identified SMS1-related protein SMSr as a critical regulator of ceramide levels in the ER. Disruption of SMSr function causes a rise in ER ceramides and their mistargeting to mitochondria, triggering mitochondrial apoptosis. How ER ceramides reach mitochondria is not known. Their delivery may rely on intimate membrane contacts between the two organelles, a cytosolic transfer protein acting at the ER-mitochondrial interface, or both. To explore the molecular principles by which ER ceramides are delivered to mitochondria, we here use constitutive and drug-inducible tethers that allow a manipulation of contact surface area and gap-width at ER-mitochondria junctions. In addition, we engineered CERT proteins to redirect the biosynthetic ceramide flow from the Golgi to mitochondria and will investigate their impact on cell fate and organization.

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Functional analysis of peritrophic matrix proteins in *Tribolium castaneum*

Marco Kelkenberg[1*], Lea Steinfeld[1], Subbaratnam Muthukrishnan[2] and Hans Merzendorfer[1]

[1]University of Osnabrück, FB Biology/Chemistry, Osnabrück, Germany
[2]Kansas State University, Department of Biochemistry, Manhattan, Kansas, USA
*Correspondence to marco.kelkenberg@biologie.uni-osnabrueck.de

The peritrophic matrix (PM) is an extracellular barrier that lines the midgut epithelium of most insects and protects the intestine from invasion by microorganisms and parasites. It consists of chitin fibrils that are embedded in a matrix of proteins and glycoproteins. Some of them have CBM14-type chitin-binding domains and are referred to as peritrophic matrix proteins (PMPs). To examine the function of individual PMPs in the midgut of the red flour beetle, *Tribolium castaneum*, we established a permeability assay based on fluorescein isothiocyanate (FITC)-dextrans. FITC-dextrans of defined sizes were added to a wheat flour-based diet and fed continuously to larvae. Next, cryosections were prepared to analyze the distribution of fluorescence signals in the larvae. While the PM of the anterior midgut has a high permeability for FITC-dextrans of even 2 MDa, the PM of the median midgut is almost impermeable for FITC-dextrans larger than 150 kDa.

We silenced the expression of three genes encoding *TcPMP3, TcPMP5-B* and *TcPMP9* by systemic RNA interference (RNAi). While injection of dsRNA specific for *TcPMP9* did not affect growth and development of the insects, those for *TcPMP3* and *TcPMP5-B* caused growth reduction, fat depletion due to starvation and lethality either at the larval-pupal molt or at the pupal-adult molt when dsRNA was injected into larvae or pre-pupae, respectively. The results indicate that the properties of the PM significantly vary in different midgut regions, and that some PMPs are required for PM integrity leading. Their loss leads to altered permeability, which in turn may compromise nutrition and fecundity. This study demonstrates for the first time the essential nature of PM proteins and sheds light on the mode of action of this class of proteins.

Analysis of subnuclear interactions between ROXY1 and PAN

Shutian Li*, and Sabine Zachgo

University of Osnabrück, FB Botanik, Osnabrück, Germany
*Correspondence to shutian.li@biologie.uni.osnabrueck.de

ROXY1 encodes a land plant-specific CC-type GRX and is known to regulate petal primorida initiation and further petal morphogenesis in *Arabidopsis thaliana*. Yeast 2-hybrid assays and BiFC experiments revealed nuclear interactions of ROXY1 with floral TGA transcription factors, including PAN [1,2]. Deletion experiments of ROXY1 coupled with complementation analysis of *roxy1-2* mutants demonstrate that this interaction is a prerequisite for ROXY1 to exert its activity required for Arabidopsis petal development [1,2]. To ascertain where in the nucleus ROXY1 associates with TGA proteins to regulate petal development, confocal microscopy has been employed to examine subnuclear distribution and interactions of ROXY1 and PAN. Preliminary data indicate that both proteins were detectable in distinct subnuclear foci. Next, we will further explore the identities and dynamics of these foci to gain further insight into the molecular mechanism by which ROXY1 regulates petal development.

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Identification of Formiminotransferase Cyclodeaminase as a Binding Partner of Golgi-resident Sphingomyelin Synthase 1

Mirjana Nordmann[1*], Edo Elstak[2], Joost C. M. Holthuis[1]

[1]University of Osnabrück, Department of Biology and Chemistry, Osnabrück, Germany
[2]Galapagos BV, Leiden, Netherlands
*Correspondence to mirjana.nordmann@biologie.uni-osnabrueck.de

Sphingomyelin (SM) is an essential component of cellular membranes. Its synthesis involves enzymatic transfer of a phosphocholine head group from phosphatidylcholine to ceramide, yielding diacylglycerol (DAG) as a side product. This reaction is catalysed by sphingomyelin synthases (SMS) and occurs within the Golgi complex and at the plasma membrane.

In a GST-pull down experiment we recently identified formiminotransferase cyclodeaminase (FTCD) as a binding partner of the Golgi-resident sphingomyelin synthase 1 (SMS1). FTCD is a microtubule-binding bifunctional enzyme involved in histidine degradation and folate metabolism. Interestingly, both proteins co-localize at the Golgi complex. Therefore we assume that SMS1 might be required for recruitment of FTCD to the Golgi surface where it participates in the interaction of this organelle with the vimentin intermediate filament cytoskeleton.

To test this hypothesis, we will investigate the impact of SMS1 deletion on FTCD Golgi localization in KBM7 cells by fluorescent microscopy. In addition, distribution of FTCD will be followed by separation of membrane and cytosolic fractions from these cells and subsequent Western blot analysis. To further confirm SMS1 interaction with FTCD we will perform pull down experiments using cell-free expressed proteins.

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Functional characterization of yeast BLOC-1 complex, a new player in yeast endocytic pathway

Meenakshi Rana[1], Arun Thomas John Peter[1], Christian Ungermann[*1]
Christian.Ungermann@Biologie.Uni-Osnabrueck.DE

[1]University of Osnabrück, FB Biochemie, Osnabrück, Germany
*Correspondence to christian.ungermann@biologie.uni-osnabrueck.de

In metazoan cells, three BLOC complexes namely BLOC-1, 2, and 3 are involved in sorting of cargo to lysosomal related organelles (LRO) and mutations in subunits of any of these complexes lead to a hypopigmentation and bleeding disorder called Hermansky Pudlak Syndrome (HPS) [1]. Recently, in a bioinformatic screen a heterohexameric putative BLOC-1 complex has also been identified in yeast [2], which suggests a more general role of this complex in endosomal sorting and trafficking since yeast cells do not contain LROs. According to a recent finding of our lab, BLOC-1 is an effector of endosomal Rab5 (Vps21) and acts as an adapter for Rab5 GAP (GTPase activating protein) Msb3, thus providing an intriguing connection between a Rab and its own GAP. We postulated that in absence of BLOC-1 subunits Rab5 is hyperactive (as in msb3Δ) and displaced to the vacuole [3]. In this study we are trying to decode the sequence of events which facilitates the Rab5-Rab7 conversion and how does BLOC-1 controls this process. The central question of this project is when and what triggers the Msb3 recruitment to endosomes by BLOC-1 complex.

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Software for Localization-based Imaging in Matlab (SLIMfast)

Christian P. Richter, Stephan Wilmes, Changjiang You and Jacob Piehler*

University of Osnabrück, FB Biology/Chemistry, Division of Biophysics, Osnabrück, Germany
*Correspondence to piehler@uos.de

Over the last decade single-molecule localization microscopy (SMLM) has rapidly developed into a versatile toolbox to study cellular processes at the nanometre scale. To achieve complete mapping of the molecules' spatio-temporal distribution from recorded images with sub-diffractive resolution, SMLM requires the fitting of individual point spread functions (PSF). Stochastic photo-switching or sub-stoichiometric labelling can be applied to generate such sparse subsets although much effort is made to extent the accessible range of molecular densities by application of more sophisticated techniques like Gaussian-mixture models or compressed sensing. To process the growing amount of generated data, use of software-assisted analysis becomes more and more mandatory. Access to these algorithms is unfortunately restricted or inhibited by lack of proper implementation. Therefore we aim to create an integrated environment that facilitates access to state-of-the-art computational approaches in localization-based imaging. Our environment is designed to be open and modular so its functionality can be easily adapted or expanded by software developers. Further we aim to provide a simple and intuitive yet powerful graphical interface for scientists to profit from state-of-the-art analysis of their experimental data.

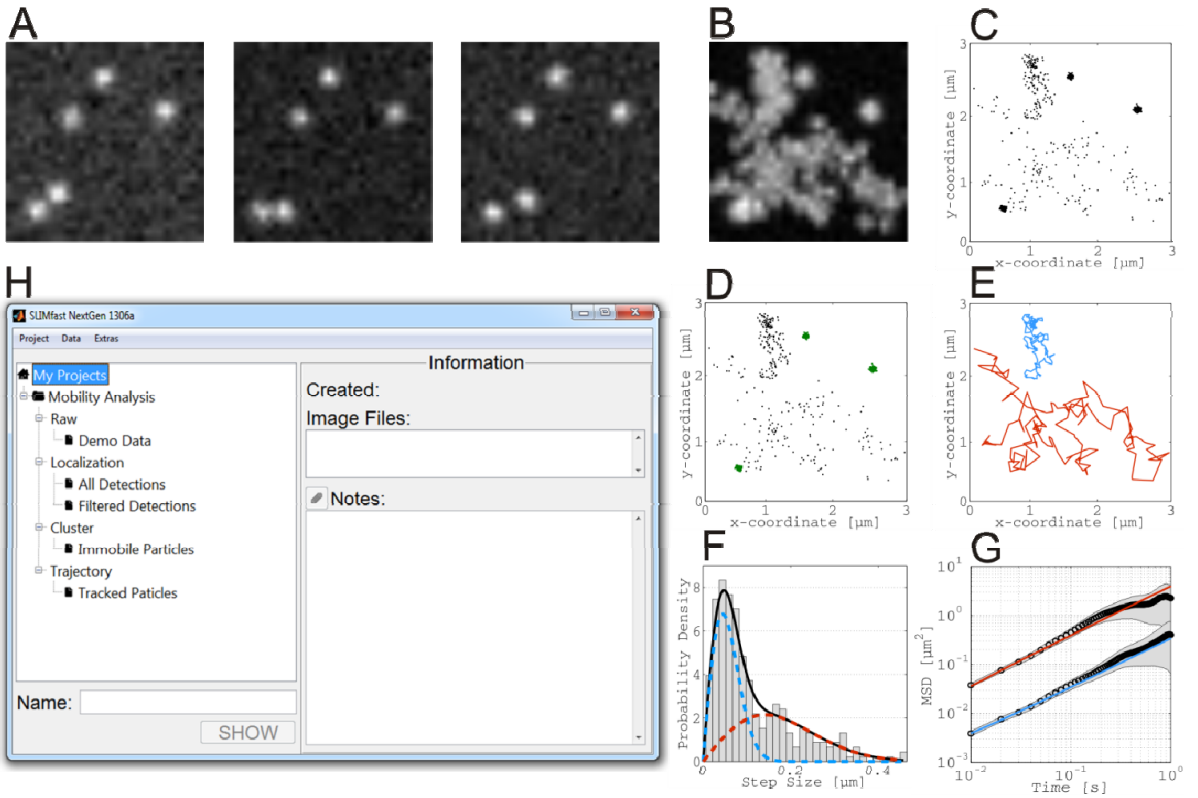

Figure 1: Exemplary data processing steps for single-molecule mobility analysis. (A) Three consecutive frames taken from single-molecule time-lapse movie. (B) Maximum intensity projection. (C) Accumulated single-molecule localizations. (D) Immobile particles (green) detected by density-based clustering and excluded from subsequent tracking. (E) Recovered trajectories revealing the spatio-temporal behavior of individual particles. (F) Observed distribution of frame-to-frame single-molecule displacements and its mixture-model decomposition to extract the average displacements for each mobility subpopulation. (G) Time-dependency of the mean squared displacement with linear fit to estimate the respective diffusion coefficients. (H) Primary user interface of SLIMfast.

Light-Induced Movement of the Transmembrane Helix B in Channelrhodopsin-2

Christian Rickert[1*], Thomas Sattig[2],

Ernst Bamberg[2], Christian Bamann[2] and Heinz-Jürgen Steinhoff[1]

[1]University of Osnabrück, Department of Physics, Osnabrück, Germany
[2]Max-Planck-Institut für Biophysik, Frankfurt a. M., Germany

*Correspondence to *crickert@uos.de*

In the past decade, its versatile usage has established channelrhodopsin-2 (ChR2) [1] as the most prominent optogenetic tool [2]. The precise spatio-temporal control of the activity of a neuron by light allows noninvasive in vitro or in vivo investigations of neural circuits. The key is the heterologous expression of ChR2, a member of the type-I rhodopsins, that enters a photocycle upon light activation starting with the isomerization around the C_{13}-C_{14} bond of its chromophore retinal [3]. The intermediates of the photocycle are linked to a closed and an open state of an ion pore. Cation flux during the open state depolarizes the cell membrane thereby triggering neuronal action potentials.

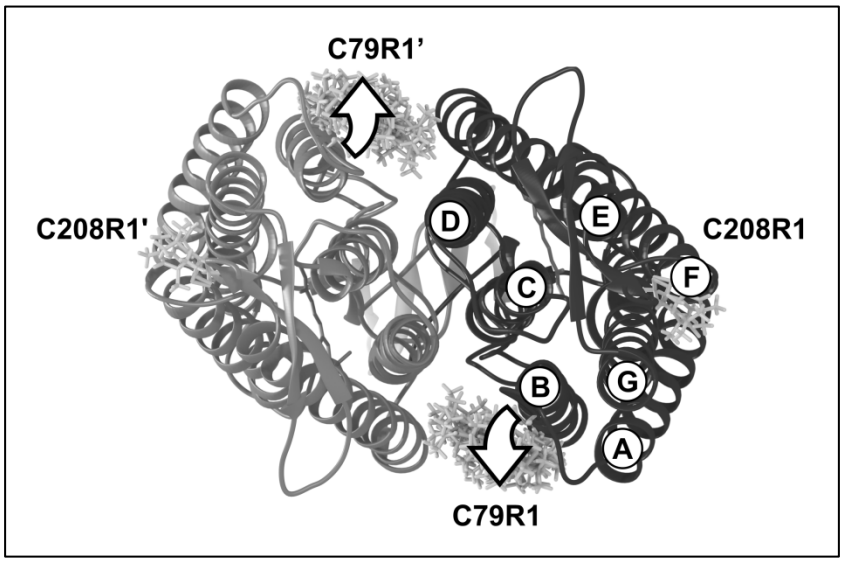

Figure 1: Structural model of a spin-labeled Mut3C dimer.

Calculated spin label rotamers displayed with ball-and-stick models cover 73 % of the total spin populations The seven TMHs are labeled in one of the monomers. The putative TMH B movement following light activation as discussed in the text is indicated by the arrows.

In this study, we followed the relative (re-)arrangements of TMHs B and F by EPR spectroscopy (double electron electron resonance, DEER) on spin-labeled cysteine mutants retaining wild-type (WT) character in ion conductance and photocycle kinetics. By analyzing inter-spin distances we could monitor a conformational change at TMH B or F upon light activation. Because of the unique channel properties of ChR2 and the major structural deviations in TMH A and B compared to other rhodopsins, the movement of TMH B might be a key element for channel opening.

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Investigation of the molecular mechanisms of cytokinesis in the milk yeast *Kluyveromyces lactis*

Dorthe Rippert[1*], Nele Heppeler[1], Hans-Peter Schmitz[1], Jürgen Heinisch[1]

[1]University of Osnabrück, FB Biologie, Osnabrück, Germany
*Correspondence to dorthe.rippert@biologie.uni-osnabrueck.de

In *Saccharomyces cerevisiae* regulatory proteins govern the progress of cytokinesis. The milk yeast *Kluyveromyces lactis* disposes of homologues of many of these components (e.g. *Kl*Cyk3, *Kl*Hof1, *Kl*Inn1, *Kl*Myo1). We found that, unlike *S. cerevisiae*, both *KlCYK3* and *KlHOF1* encode essential functions and null mutants are not viable. A *Klinn1* deletion is lethal in both species. Surprisingly, a deletion of the *KlMYO1* gene, which encodes a central component of the contractile actomyosin ring (CAR), yields viable progeny and lacks obvious phenotypes at 30°C. In *S. cerevisiae* the *myo1* deletion is lethal in most strains 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*, led us to further investigate the role of *Kl*Myo1 in *K. lactis* cytokinesis. TEM images, FACS 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. We also noted a peculiar behaviour of the *K. lactis* actin cytoskeleton, which will be presented.

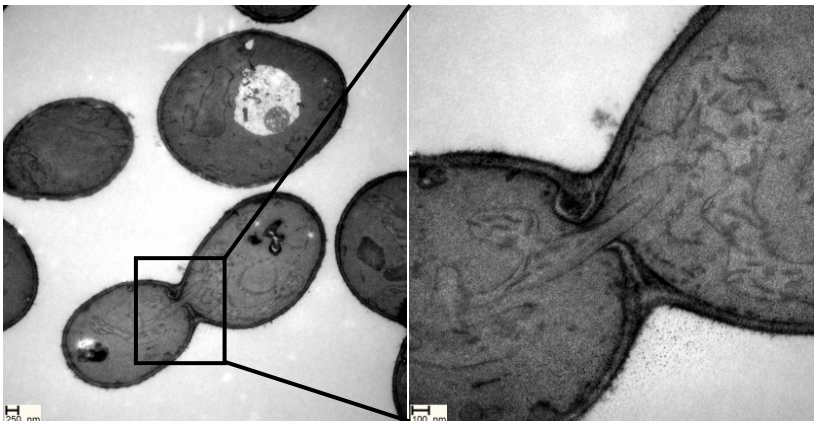

Figure 1: Electron micrographs of a cell lacking *KlMYO1* gene.

The plastid-localized NAD-dependent malate dehydrogenase is crucial for energy homeostasis in developing *Arabidopsis thaliana* seeds

Jennifer Selinski[1], Nicolas König[1], Benedikt Wellmeyer[1], Guy T. Hanke[1], Vera Linke[1], H. Ekkehard Neuhaus[2] & Renate Scheibe[1*]

[1]University of Osnabrück, Department of Plant Physiology, FB 5, Osnabrück, Germany
[2]TU Kaiserslautern, Department of Plant Physiology, FB Biology, Kaiserslautern, Germany
*Correspondence to scheibe@biologie.uni-osnabrueck.de

In the absence of photosynthesis, ATP is imported into chloroplasts and non-green plastids by ATP/ADP transporters or formed during glycolysis, the latter requiring continuous regeneration of NAD^+, supplied by the plastidial isoform of NAD-MDH. During analysis of T-DNA insertion mutants of *A. thaliana* only heterozygous but no homozygous mutants could be identified. These heterozygous plants show higher transcript levels of an alternative NAD^+-regenerating enzyme, NADH-GOGAT, and, remarkably, improved growth when ammonium is the sole N-source. In-situ hybridization and GUS-histochemical staining revealed that plNAD-MDH was particularly abundant in male and female gametophytes. A knockout of plNAD-MDH has a strong effect on pollen tube growth. Knock-out pollen lacking plNAD-MDH do not germinate in vitro, but can fertilize egg cells in vivo. However, young siliques of selfed heterozygous plants contain both green and white seeds corresponding to wild-type/heterozygous (green) and homozygous knock-out (white) mutants in a (1:2):1 ratio. Embryos of the knock-out seeds only reached the globular stage, did not green, and developed to tiny wrinkled seeds, suggesting that a blocked major physiological process in plNAD-MDH mutants stops both, embryo and endosperm development in order to avoid assimilate investment in compromised offspring.

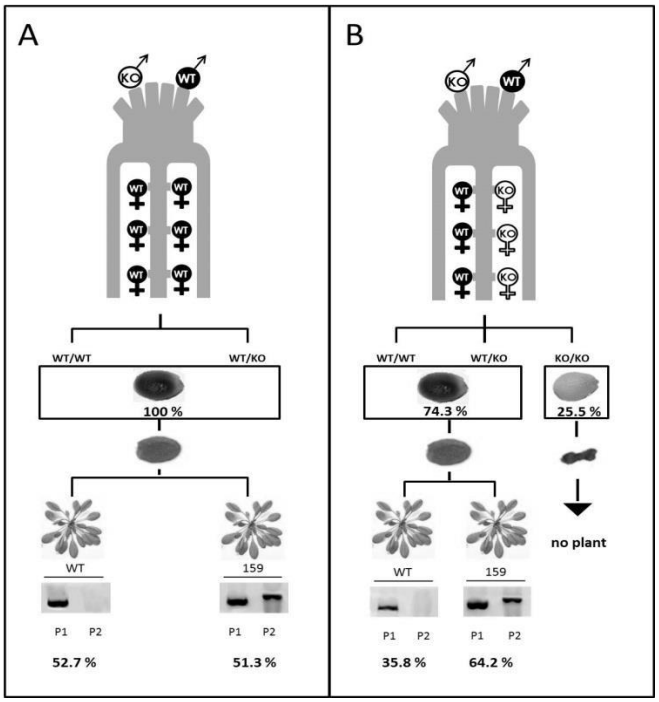

Figure 1.
Crossing experiments and evaluation of progenies.
A, Backcrossing of wild types with pollen of line 159.
B, Self-fertilization of heterozygous plNAD-MDH knock-out plants.

References

Selinski, J., König, N., Wellmeyer, B., Hanke, G.T., Linke, V. Neuhaus, H.E. & Scheibe, R. (2013), Molecular Plant, DOI: 10.1093/mp/sst151

Regulation of the yeast V-ATPase by reversible dissociation of subunit C

Katharina Tabke[1], Andrea Albertmelcher[1], Helmut Wieczorek[1*]

[1]University of Osnabrück, FB Biology/Chemistry, Animal Physiology, Osnabrück, Germany
*Correspondence to Wieczorek@biologie.uni-osnabrueck.de

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-hydrolysing 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*) [1]. In the baker's yeast *Saccharomyces cerevisiae*, dissociation of the V-ATPase holoenzyme is induced by glucose deprivation [2]. The shut-down of this energy consumptive pump appears to be an economic mode during diauxic shift. 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,

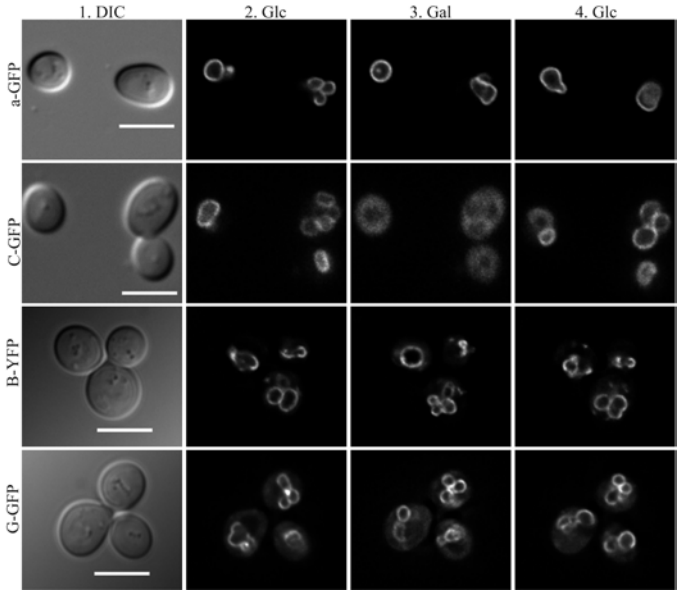

Figure 1. Yeast cells expressing fluorescent protein tagged V-ATPase subunits were immobilized on a cover slip and viewed under differential interference contrast optics (images 1.DIC), Fluorescent signals from subunits a, C, B and G are shown after a 20 min incubation in YNB containing 2% glucose (images 2.Glc), after medium change to 2% galactose followed by a 20 min incubation (images 3.Gal) and after a further medium change back to 2% glucose followed again by a 20 min incubation (images 4.Glc). Calibration bar: 5 µm

we found that only the V_1 subunit C but not the whole V_1 complex dissociates from the membrane under starvation conditions. Furthermore, FRET interaction studies support these observations [3]. We could also show that an increased 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 direct interaction of subunit C with the cytoskeleton, especially with microtubules, seems to be a universal property [3]. 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.

References

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[2] Kane, P. M. (1995) Disassembly and reassembly of the yeast vacuolar H(+)-ATPase *in vivo*. J. Biol. Chem. **270**, 17025-17032

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Toward understanding the function of AP-3 in fusion and membrane remodelling

Erdal Yavavli, Angela Perz, Christian Ungermann

University of Osnabrück, Division of Biochemistry, Osnabrück, Germany

The AP-3 complex is a heterotetrameric adaptor complex that mediates the cargo-selective transport from the trans-Golgi network (TGN) to the vacuole/lysosome bypassing the endosomal pathway. Whereas homotypic fusion of the yeast vacuole is well understood and needs specific lipids, Ypt7 Rab GTPase, SNAREs and the tethering complex HOPS (homotypic fusion and vacuole protein sorting), less is known about fusion of AP-3 vesicles derived from the TGN. The AP-3 complex is recruited to the TGN by the Arf1 GTPase and sorts cargo (e.g. Vam3, Nyv1, Yck3) to emerging AP-3 vesicles. The molecular mechanism of fusion at the vacuole and the interplay of AP-3 and HOPS are open questions.

The aim of this study is to identify and purify all components to reconstitute this fusion event and get new molecular insights into the AP-3 pathway.

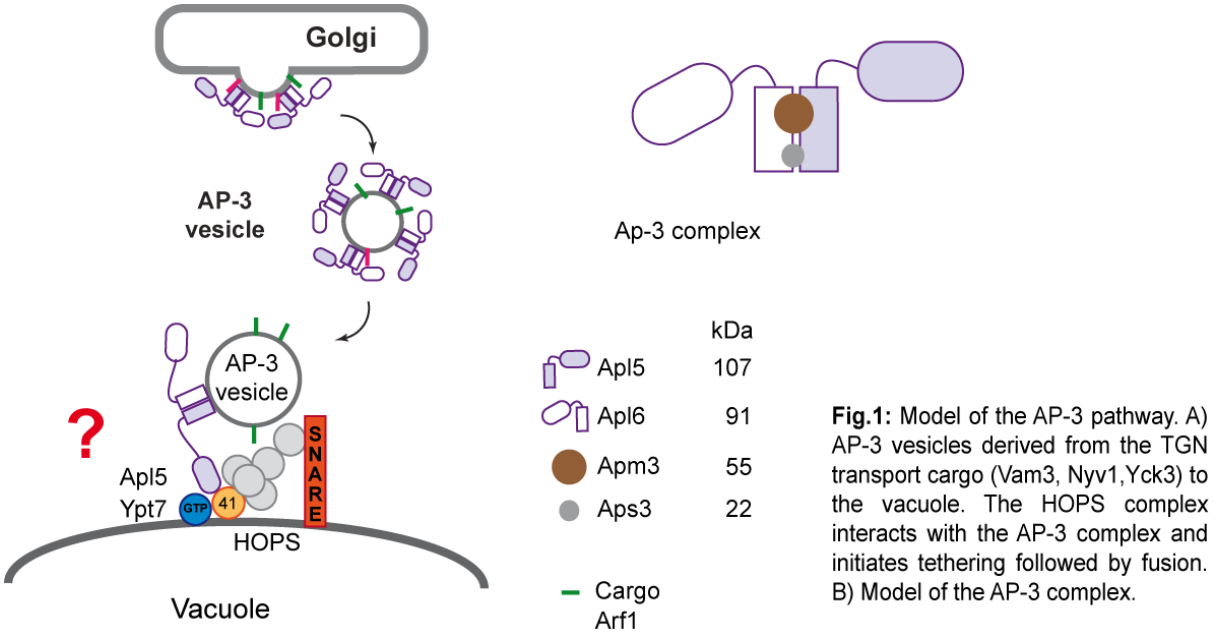


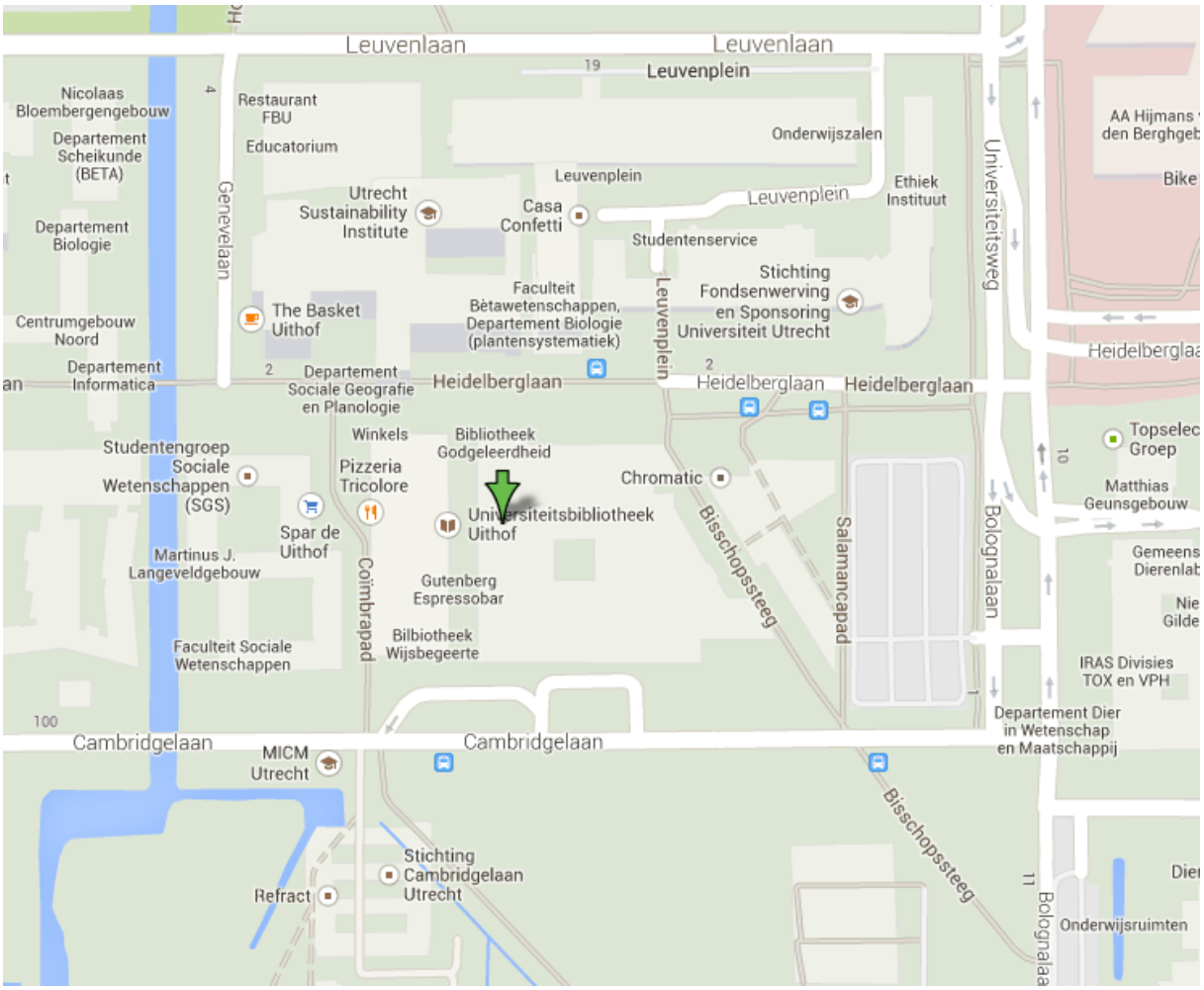
Fig.1: Model of the AP-3 pathway. A) AP-3 vesicles derived from the TGN transport cargo (Vam3, Nyv1,Yck3) to the vacuole. The HOPS complex interacts with the AP-3 complex and initiates tethering followed by fusion. B) Model of the AP-3 complex.

Mitland Hotel Utrecht BV
Ariënslaan 1
3573 PT Utrecht

Stadskasteel Oudaen
Oudegracht 99
3511 AE Utrecht

Djakarta
Lucasbolwerk 19
3512 EH Utrecht

Boothzaal, Universiteitsbibliotheek
Heidelberglaan 3,
De Uithof, Utrecht



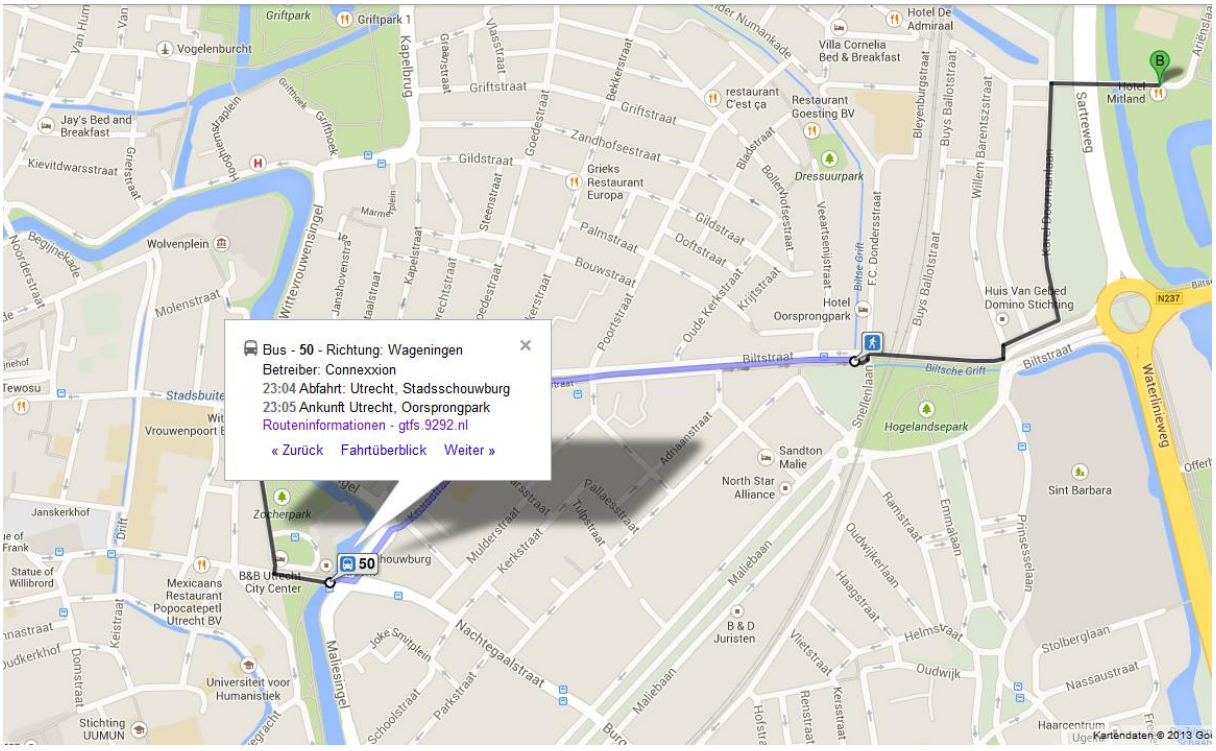
How to get to Hotel Mitland by public transport

From Utrecht Central Station

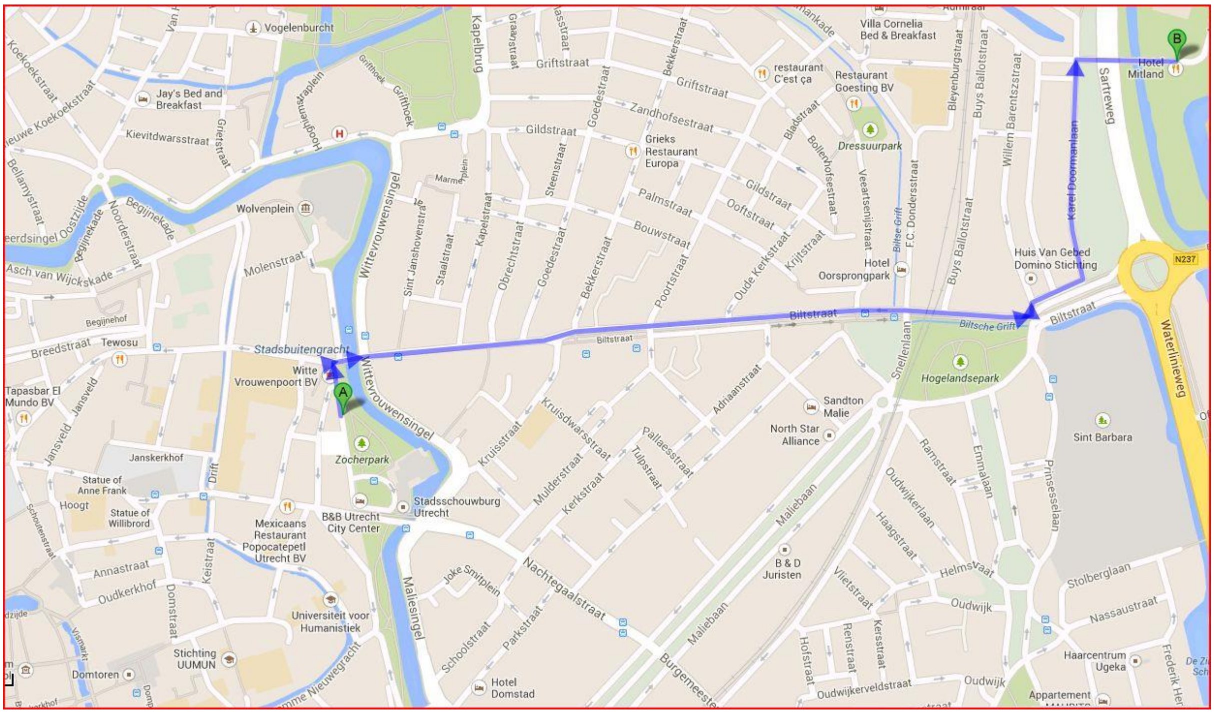
- Bus number **4** direction: F. **Andrealaan** *or*
- Bus number **11** direction: **De Uithof** / AZU; departure time: every 10 minutes between 07:00 a.m. and 12:00 p.m.
- Bus-stop **Oorsprongpark***
- Cross the railway crossing
- Fourth street to the left (Karel Doormanlaan)
- Turn first right to go under the fly-over (Ariënslaan)
- Mitland Hotel Utrecht is on the right after 50 meters

*From bus-stop Oorsprongpark it is about 10 minutes walking.

Restaurant Djakarta → Hotel Mitland (bus, 13 min)



Restaurant Djakarta → Hotel Mitland (1,7 km, 21 min 🚶)



Stadskasteel Oudaen → Restaurant Djakarta