SFB – Retreat 2019

Schloss Etelsen/Langwedel

16.10. – 18.10.2019

Wednesday 16 October 2019

11:45 – 12:30	Check-in & Registration
12:30 – 13:30	Lunch
13:30 – 13:45	Opening Remarks
Session I	Chair: Ronja Schiemann
13:45 – 14:25	**Michael Kolbe** HZI Braunschweig, CSSB Hamburg
14:25 – 14:45	Felix Scharte (Microbiology)
14:45 – 15:05	Nathalie Sander (Microbiology)
15:05 – 15:20	Coffee Break
15:20 – 15:40	Nânci Monteiro Abreu (Neurobiology)
15:40 – 16:00	Piyali Pal Chowdhury (Ecology)
16:00 – 16:20	Caroline Barisch (Molecular Infection Biology)
16:20 – 16:35	Flash Poster Presentations Sebastian Eising, Jan Hänsel, Eric Herrmann, Sergej Limar, Dorothee Merker, Patrick Niekamp, Han Yaping
16:35 – 16:45	Coffee Break & Poster Setup
16:45 – 18:00	Poster Session I
18:30 – 20:00	Dinner and Get Together
20:00	Pub Quiz

Thursday 17 October 2019

Session II	Chair: Cilian Kock
08:45 – 09:25	**Marina Mikhaylova** ZMNH Hamburg, UKE Hamburg
09.25 – 09:45	Maren Janz (Zoology)
09:45 – 10:05	Ayelén González Montoro (Biochemistry)
10:05 – 10:25	Coffee Break
Session III	Chair: Rico Franzkoch
10:25 – 11:05	**Rita Groß-Hardt** Uni Bremen
11:05 – 11:25	Nora Klinke (Zoology)
11:25 – 11:45	Annika Buhr (Zoology)
11:45 – 12:05	Arne Möller (Structural Biology)
12:05 – 13:05	Lunch
Session IV	Chair: Thomas Meyer
13:05 – 13:45	**Sebastian Springer** Jacobs Uni Bremen
13:45 – 14:05	Zilei Chen (Biochemistry)
14:05 – 14:25	Katharina Fitzian (Structural Biology)
14:25 – 14:40	Flash Poster Presentations Maximillian Hafer, Nadia Füllbrunn, Iqra Kasu, Irene Reimche, Marcel Reinhardt, Prado Vargas Duarte
14:40 – 14:50	Coffee Break
14:50 – 16:00	Poster Session II
16:15 – 19:00	Sightseeing Tour Bremen
19:30	Dinner

Friday 18 October 2019

Session V	Chair: Jimmy Villata Villalobos
08:45 – 09:25	**Rainer Kaufmann** Uni Hamburg, CSSB Hamburg
09:25 – 09:45	Hauke Winkelmann (Biophysics)
09:45 – 10:05	Lucia Maß (Botany)
10:05 – 10:25	Katia Cosentino (Molecular Cell Biophysics)
10:25 – 10:40	Coffee Break
Session VI	Chair: Lena Lüken
10:40 – 11:20	**Guntram Graßl** MH Hannover
11:20 – 11:40	Bianca Esch (Molecular Membrane Biology)
11:40 – 11:50	Coffe Break
11:50 – 12:10	Tolulope Sokoya (Molecular Cell Biology)
12:10 – 12:30	Mirsana Kutty (Medical Physics & Biophysics)
12:30 – 12:45	Closing Remarks
12:45 – 13:45	Lunch
14:15	Departure

Poster Index

Poster Session I (Wed)		Poster Session II (Thu)	
1	Sebastian Eising	17	Maximillian Hafer
2	Jan Hänsel	18	Nadia Füllbrunn
3	Eric Herrmann	19	Iqra Kasu
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Speaker Abstracts

Session I

Structure and function of molecules involved in bacterial infections

Michael Kolbe

HZI Braunschweig
CSSB Hamburg

Several species of Gram-negative bacteria infect hundreds of millions of people worldwide and lead to millions of deaths every year. Given the lack of vaccines and the increasing rate of antibiotic resistance for certain pathogenic Gram-negative bacteria, our research draws on the combined efforts of the CSSB and focuses on the discovery of new targets for the treatment of these infections and their translation into clinics. To understand host-pathogen interaction and pathogenesis during infection, we examine the characteristics, functionalities and interactions of nanomolecular structures involved in the survival and multiplication of bacteria within the host. One example of such nanomachine is the type III secretion system (T3SS), a membrane-embedded macromolecular complex that allows the delivery of pathogenicity factors into human host cells and initiates infection by subverting host cell defense mechanisms. The T3SS is a highly conserved virulence machinery of Gram-negative bacteria, and thus represents an attractive target for novel anti-infectives. However, there are currently no efficient T3SS inhibitors. To develop a T3SS blocking strategy, we need to gain knowledge of the molecular function and regulation of the T3SS. Employing molecular genetics, biophysical methodologies and high resolution structural imaging and X-ray technologies we envisage to understand the molecular mechanism for virulence factor secretion, transport dynamics and regulation of the T3SS itself across all resolution levels. The detailed three-dimensional structures of T3SS interacting with small molecules would also allow us to guide the design of new generation of anti-infectives against Gram-negative infections.

Imaging *Salmonella enterica* Infection in 3D and 2D Enteroids

Felix Scharte[1], Nathalie Sander[1], Michael Kim[1], Rico Franzkoch[1], Olympia Ekaterini Psathaki[2], Guntram Graßl[3], Michael Hensel[1]

[1] Department of Microbiology, University of Osnabrueck
[2] Integrated Bioimaging Facility iBiOs, Center for Cellular Nanoanalytics (CellNanOs)
[3] Hannover Medical School

Analyses of host pathogen interactions is a key requirement for the understanding of bacterial virulence mechanisms. Cell culture models are frequently used to study cellular interactions with a high temporal and spatial resolution. However, these models lack the complex tissue architecture of whole tissues. Most recently, organoids have become a promising tool to study host-pathogen interactions in a more *in vivo* relevant setting. Especially the research with human-restricted pathogens like typhoidal *Salmonella* is dependent on human cell culture with a close relation to *in vivo* conditions. In contrast to cell culture with only a single cell population, crypt-derived intestinal organoids (enteroids) mimic tissue characteristics of distinct sections of the gastrointestinal tract. Stem cells of human or murine origin can be differentiated to 3D or 2D tissues with crypt formation, distinct cell populations, polarization and mucus secretion.

We established a human and murine 3D and 2D intestinal organoid model to study virulence mechanisms of the *Salmonella enterica* serovars Typhimurium, Typhi and Paratyphi A regarding invasion and their extra- and intracellular lifestyle that currently remain unknown. Therefore, we adapted our imaging approaches to analyze *Salmonella* infection with confocal spinning disc microscopy, scanning electron microscopy and transmission electron microscopy. Using immunohistochemistry approaches, we aim to identify which cell types are mainly targeted by *Salmonella*, and how mucus production and the overall tissue complexity affect the infection process in different stages of infection.

Previous investigations in cell culture models led to definition of hallmark virulence functions such as invasion with induction of massive membrane ruffles, formation of *Salmonella*-containing vacuoles and remodeling of the endosomal system of host cells, resulting in *Salmonella*-induced filament formation. We will critically analyze if these phenotypes also occur in more tissue-like organoid infection models. The organoid model also offers options to investigate the cellular mechanisms of pathogen exit from infected host cells.

References:

1. Sato, T., et al. (2009). "Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche." Nature 459: 262-265.
2. Lau, et al. (2012). “Peyer’s Patch M Cells Derived from Lgr5$^+$ Stem Cells Require SpiB and Are Induced by RankL in Cultured “Miniguts” MCB
3. Sato & Clevers (2013). “Growing Self-Organizing Mini-Guts from a Single Intestinal Stem Cell: Mechanism and Applications” Science 340 (6137), 1190-1194.
4. Co, J. Y., et al (2019). "Controlling Epithelial Polarity: A Human Enteroid Model for Host-Pathogen Interactions." Cell reports 26: 2509–2520.

Role of the non-canonical membrane proteins SiiA and SiiB for the type I secretion system of *Salmonella enterica*

Nathalie Sander, Dorothee Merker and Michael Hensel

Department of Microbiology, University of Osnabrueck

For adhesion to polarised epithelial cells, *Salmonella enterica* serovar Typhimurium requires the *Salmonella* Pathogenicity Island 4 (SPI4)-encoded type 1 secretion system (T1SS) and its substrate SiiE, mediating the first intimate contact to the apical side of the host cell. Following adhesion, the pathogen can invade the host cell by translocating effector proteins via the SPI1-T3SS, leading to membrane reorganization and subsequent uptake.

The SPI4-T1SS is composed of the ATPase SiiF, the periplasmic adaptor protein SiiD, and the outer membrane protein SiiC. SiiE, the only known substrate, is temporary retained on the surface during secretion. The N-terminal region of SiiE is known to play a key role in retention and secretion, and is suggested to interact with at least one of the other Sii subunits. There are two additional non-canonical proteins, SiiA and SiiB, forming a proton channel and known to be also important for invasion in polarized cells. Recent results indicate a role of SiiA and SiiB for SiiE retention and secretion, suggesting an effect on SiiE function and conformation during invasion process.

Due to structural similarities in the peptidoglycan-binding domains to the flagellar stator complex MotAB, we suppose that SiiAB not only function at the T1SS, but additionally at other membrane complexes like the flagellum.

However, still little is known about the molecular mechanisms and the role of SiiAB for the T1SS and SiiE secretion and retention. By colocalization analyses by 3D dSTORM we attempt to resolve the localization of SiiAB at possible interaction partners like the T1SS or the flagellum. First results now indicated applicability of this method to investigate SiiAB localization in the cytoplasmic membrane.

References:

1. Gerlach *et al.* Cooperation of *Salmonella* pathogenicity islands 1 and 4 is required to breach epithelial barriers (2008), http://doi.10.1111/j.1462-5822.2008.01218.x
2. Wille *et al*. SiiA and SiiB are novel type I secretion system subunits controlling SPI4-mediated adhesion of *Salmonella enterica* (2014), http://doi.org/10.1111/cmi.12222
3. Hizukuri *et al*. The peptidoglycan-binding (PGB) Domain of the *Escherichia coli* Pal Protein can also Function as the PGB Domain in E. coli Flagellar Motor Protein MotB (2009), http://doi.org/10.1093/jb/mvp061
4. Kirchweger *et al.* Structural and functional characterisation of SiiA, an auxiliary protein from the SPI4-encoded type 1 secretion system from *Salmonella enterica* (2019), https://doi.org/10.1101/641415

MEA recordings implicate impaired LTP in a pseudohyperphosphorylated-tau model of Alzheimer's Disease

Nânci Monteiro Abreu, Nataliya Trushina, Roland Brandt and Lidia Bakota

Department of Neurobiology

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills, and eventually the ability to carry out the simplest tasks. One of the key molecules that has been linked to this disease is the microtubule associated protein tau (MAPT) since it is a principal component of the neurofibrillary tangles (NFTs) present in AD. NFTs contain straight and paired-helical filaments that are composed almost entirely of the MAPT in a hyperphosphorylated state. Distribution of the NFTs in the brain correlates with the severity of the disease, suggesting that the hyperphosphorylation of tau plays an important role in this dementia's pathogenesis and progression. Therefore, it is important to understand the effect of hyperphosphorylated tau in the central nervous system. To investigate the role of hyperphosphorylated tau regarding synaptic transmission, extracellular electrophysiological recordings in acute hippocampal brain slices of male mice transgenically expressing pseudohyperphosphorylated-tau (PHP-tau) as a mimic of hyperphosphorylated MAPT were performed by using Micro Electrode Array (MEA) System. Mouse strain of the same genetic background (B6) was used as control. Long-term potentiation (LTP), both early-phase and late-phase, was induced by one train of high-frequency stimulation (HFS - 100Hz). Excitatory postsynaptic potentials (EPSP), within the *stratum radiatum* and compound action potentials (CAP), within the *stratum pyramidale* of the CA1 hippocampal subfield were analyzed. LTP analysis revealed that PHP mice tend to have a lower potentiation of CAPs than control animals. Furthermore, we employed mass spectrometry (MS) assays on PC12 cells expressing either WT or PHP-tau to understand which proteins are influenced particularly by the diseased form of tau. Evaluation of the MS results indicated an increased expression of the tubulin-binding protein stathmin 2 suggesting induction of increased microtubule dynamics. The data suggest that hyperphosphorylated tau modulates microtubule dynamics via stathmin 2 expression thereby influencing synaptic connectivity.

Nanci.MonteiroAbreu@biologie.uni-osnabrueck.de

Session I

Cell adhesiveness is key for establishing nanotube-mediated cross-feeding interactions

Piyali Pal Chowdhury and Christian Kost

Department of Ecology

In their natural habitats, bacteria live in multispecies communities, in which metabolic cross-feeding interactions are commonplace. By deriving costly metabolites such as amino acids, vitamins, or even nucleotides from other bacterial cells, auxotrophic bacterial genotypes can gain a significant fitness advantage relative to metabolically autonomous cells[1]. Recently it has been discovered that cells of *Escherichia coli* that were unable to produce a certain amino acid used intercellular nanotubes to derive this metabolite from the cytoplasm of other bacterial cells[2].However, given that in this case the survival of an auxotrophic cell depends primarily on the identification and attachment to a suitable host, the question arises how amino acid-starved cells manage to find their partner. In order to determine the molecular underpinnings of partner choice and nanotube formation, genes related to motility, chemotaxis, adhesiveness, and quorum sensing were individually deleted from the genome of amino acid cross-feeding mutants to verify their role for the establishment of nanotube-mediated interactions. Our results clearly revealed that neither quorum sensing nor active motility or chemotaxis are involved in finding the cognate partner, because deleting the corresponding genes did not impair growth of the respective cross-feeding consortia in an agitated liquid environment. Instead, deleting gene essential for producing components of the bacterial extracellular matrix such as flagella, fimbriae, curli, or poly N-acetylgluscosamine strongly compromised growth of auxotrophic consortia. These findings suggest that passive attachment through adhesive structures on the cell surface are likely sufficient to form intercellular connections. Moreover, we also discovered extracellular DNA– a known structural element of bacterial biofilms - was one of the main components of the extracellular matrix of nanotube-forming bacteria. This finding indicates that eDNA might also play a role in the establishment of nanotube-mediated interactions. Surprisingly, also lipopolysaccharide (LPS) was required to successfully establish intercellular connections. This finding corroborates previous observations, namely that nanotubes are primarily composed of lipids. Together, our results clarify that the production of adhesive structures on the surface of auxotrophic bacterial cells are necessary and sufficient to establish contact-dependent interactions with other metabolically complementary bacterial cells.

References:

1. D'Souza, G., Shitut, S., Preussger, D., Yousif, G., Waschina, S., and Kost, C. (2018). Ecology and evolution of metabolic cross-feeding interactions in bacteria. Nat. Prod. Rep. 35, 455-488.
2. Pande, S., Shitut, S., Freund, L., Westermann, M., Bertels, F., Colesie, C., Bischofs, I.B., and Kost, C. (2015). Metabolic cross-feeding via intercellular nanotubes among bacteria. Nat. Commun. 6, 1-13.

Functional impact of lipid logistics on mycobacterial infection in *Dictyostelium*

Barisch, Caroline (caroline.barisch@biologie.uni-osnabrueck.de)

Molecular Infection Biology, Fachbereich Biologie

Tuberculosis (Tb) is a wide-spread infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). The high lipid content of this pathogen accounts for many of its unique clinical manifestations. One of the main characteristics of Tb is the formation of lipid-loaded, foamy macrophages during chronic infection. A growing body of evidence indicates that *Mtb* mobilizes lipid droplets (LDs) to scavenge lipids from their host cell. However, how this pathogen remodels the lipid metabolic network of the host to support its persistent lifestyle is so far poorly understood. Using *Dictyostelium* as experimental model for foamy macrophages in *Mtb* infections, I found that mycobacteria accesses host LDs to build up their own lipid storage organelles and exploits ER-derived phospholipids when LDs are lacking (Figure 1, steps 1 and 2 (1, 2)).

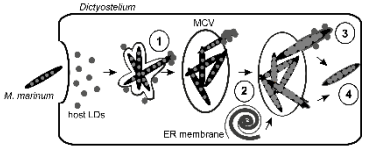


Figure 1: Dynamics of LDs and LD proteins at the MCV and cytosolic M. marinum.

Moreover, I observed that mycobacteria that escaped from the mycobacterium vacuole (MCV) to the cytosol recruit LD-derived enzymes (1, 2) and regulatory proteins on their hydrophobic surface (Figure 1, steps 3 and 4). Previous work indicated that these pathogens not only hijack LDs, but also lipid metabolic enzymes and various components of the lipid trafficking machinery. However, a comprehensive understanding of the underlying molecular principles and their relevance for mycobacterial infection is missing at present. We will launch a first systematic effort to unravel the molecular mechanisms by which mycobacteria acquire and exploit host lipids using the *Dictyostelium*/*Mycobacterium marinum* infection model. To this end, we will chart the lipid flows between mycobacteria and their host that are potentially relevant for infection and identify lipid species acquired by intracellular mycobacteria using metabolic tracing studies and mass spectrometry lipidomics. In addition, we plan to disrupt lipid flows from the host to the pathogen during infection using genetics or drugs. With the help of fluorescent and clickable lipid probes, we will first analyze the impact of these disruptions on host-to-pathogen lipid flows. Next, we will determine the consequences of blocking specific lipid supply routes on various stages of the mycobacterial infection course. Collectively, these efforts may uncover novel therapeutic targets to fight mycobacteria infection.

References:

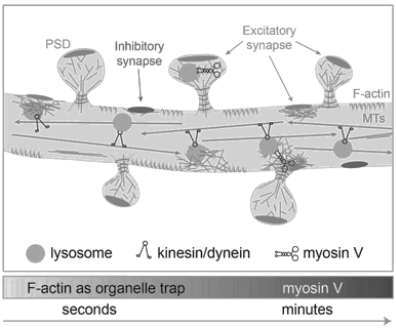
1. Barisch C, Lopez-Jimenez AT, Soldati T. Live Imaging of Mycobacterium marinum Infection in Dictyostelium discoideum. In: Parish T, Roberts D, editors. Mycobacteria Protocols (Methods Mol Biol). 1285: Humana, NYC; 2015. p. 369-85.
2. Barisch C, Soldati T. Mycobacterium marinum Degrades Both Triacylglycerols and Phospholipids from Its Dictyostelium Host to Synthesise Its Own Triacylglycerols and Generate Lipid Inclusions. PLoS pathogens. 2017;13(1):e1006095.

Glutamatergic shaft synapses - regulators of organelle trafficking in dendrites?

Marina Mikhaylova

DFG Emmy Noether Group 'Neuronal Protein Transport', Center for Molecular Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

In early neuronal development, the majority of glutamatergic synapses are located on dendritic shafts. Following maturation, they are gradually replaced by excitatory spine synapses. In adult pyramidal neurons in hippocampus about 10% of glutamatergic synapses are still located on the shaft, in the cortex this number can reach up to 30%. In contrast to spine synapses, there is much less known about their structure and function. In ongoing work, we are addressing the stability and plasticity of shaft synapses, testing their role in synaptic transmission and performing detailed characterization of the molecular components. Interestingly, we discovered that shaft synapses play an important role in regulation of dendritic organelle and membrane protein trafficking which in turn could be critical for dendritic compartmentalization. We show, that in contrast to inhibitory shaft synapses, the post-synaptic density of the excitatory shaft synapse is surrounded by a dense mesh of F-actin filaments. Using lysosomes as an example, we show that the presence of actin patches has a strong impact on dendritic organelle transport, as lysosomes frequently stall at these locations. We provide mechanistic insights on this pausing behavior, demonstrating that actin patches form a physical barrier for kinesin-driven cargo. In addition, we identify myosin Va as an active tether which mediates long-term stalling[1, 2]. This correlation between the presence of actin meshes and halting of organelles could be a generalized principle by which synapses control organelle trafficking[3].



Synaptic control of organelle trafficking in dendrites.

References:

1. van Bommel B, et al. F-actin patches associated with glutamatergic synapses control positioning of dendritic lysosomes. EMBO J. (2019) 1; 38(15).
2. Konietzny A, et al. Characterization of neuronal synaptopodin reveals a myosin V-dependent mechanism of synaptopodin clustering at the post-synaptic sites. J of Cell Sci. (2019).
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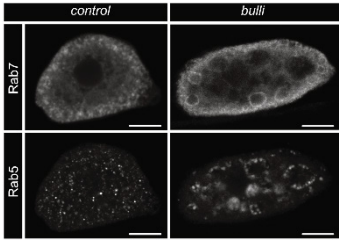
Endosomal maturation in nephrocytes depends on the trimeric metazoan Mon1-Ccz1-Bulli complex

Maren Janz* (maren.janz@biologie.uni-osnabrueck.de), Lena Dehnen*, Jitender Kumar Verma, Olympia Ekaterini Psathaki, Lars Langemeyer, Florian Fröhlich, Jürgen J. Heinisch, Heiko Meyer, Christian Ungermann, and Achim Paululat (*equal contributions)

Department of Zoology and Developmental Biology

The nephrocytes in insects are huge cells optimized for endocytic uptake of signaling peptides, membrane proteins or by-products from various chemical pathways that are no longer needed in the animal. Surface expansion and huge storage capacity are features of the cell to allow endocytosis to its extreme. This renders nephrocytes an excellent model for investigating the endosomal maturation (Das et al. 2008, Weavers et al. 2009, Ivy et al. 2015, Psathaki et al. 2018). Early endosomes are initially associated with Rab5, which is then replaced by Rab7 during endosome maturation, resulting in acidified late endosomes/multi vesicular bodies (MVBs). Recruitment of Rab7 to the maturing endosomes depends on the Mon1-Ccz1 complex, which serves as a GEF to Rab7.

We identified a new protein, named Bulli (CG8270) as being essential for endosomal maturation in metazoans. Bulli serves as the third constituent of the Mon1-Ccz1 complex (pull down data). In the absence of Bulli, endocytosis occurs at a reduced efficiency. In CRISPR-Cas9-generated new *bulli* mutants, we observed a block of endosomal maturation, resulting in artificially enlarged giant endosomal compartments. We found, that Rab5 accumulates at and in these enlarged endosomes. Furthermore, Rab7 is poorly localised to late endosomes and endosomal maturation in general is strongly affected in *bulli* mutants. Our results indicate a critical role for Bulli in endosomal maturation and physiology.



Immunostaining of Rab5 and Rab7 in pericardial nephrocytes. In the control, Rab7 is located at the periphery of the cell and Rab5 shows a spotted staining pattern. In bulli mutants, Rab5 signals cluster and appear internalized into enlarged vesicular structures, which are partially Rab7 positive. Scale bar 10 µm.

References:

1. Ivy, J.R., Drechsler, M., Catterson, J.H., Bodmer, R., Ocorr, K., Paululat, A., and Hartley, P.S. (2015a). Klf15 is critical for the development and differentiation of *Drosophila* nephrocytes. PLoS ONE *10*, e0134620.
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4. Psathaki, O.-E., Dehnen, L., Hartley, P.S., and Paululat, A. (2018). *Drosophila* pericardial nephrocyte ultrastructure changes during ageing. Mechanisms of ageing and development *173*, 9-20.

Session II

Membrane contact sites of the vacuole

Ayelén González Montoro

Membrane contact sites are regions where specific tethers establish proximities between the membranes of two organelles. These structures constitute direct communication platforms between the organelles, allowing the exchange of metabolites mediated by channels or transporters located there. Additionally, these contacts can exert forces on organelles, influencing their positioning and dynamics.

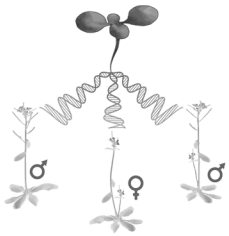
We are interested in the mechanisms that allow the establishment and regulation of membrane contact sites, as well as their molecular functions. Additionally, we would like to understand the interplay among the different contacts of an organelle, and how they collectively affect its identity and function. To address these questions, we use the lysosome-like vacuole from yeast as a model organelle. The vacuole has degradative and detoxification functions, it is connected to several vesicular trafficking pathways and is an important signaling hub as the place of action of the TORC1 complex. It has been reported that the vacuole forms membrane contact sites with the mitochondrial network, the plasma membrane, the endoplasmic reticulum, peroxisomes and lipid droplets. However, for most of these contact sites the molecular machinery and function is unknown. I will present our recent advances on understanding the function of the vacuolar-mitochondria contact site, and how it cooperates with the vacuolar-endoplasmic reticulum contact site to ensure the functionality of the vacuole. Additionally, I will present our work on a novel proteins that mediates the formation of vacuolar membrane contact sites with several different organelles.

Building and bypassing plant polyspermy barriers

Thomas Nakel*[1], Dawit G Tekleyohans*[1], Yanbo Mao*[1], Golo Fuchert[2], Dieu Vo[1], Rita Groß-Hardt[1]

[1] University of Bremen, Department of Biology and Chemistry, Bremen, Germany,
[2] Max-Planck-Institute, Plasma Physics, Greifswald, Germany
* equal contribution

The ultimate goal for the survival of all species on earth is to reproduce. This uncompromising principle has triggered the evolution of numerous adaptations. One strategy commonly employed by sexually reproducing eukaryotes is the production of tremendous amounts of sperm to maximize the likelihood of an egg becoming fertilized. High sperm to egg ratios are, however, associated with an increased risk of polyspermy. To avoid potential harmful or deleterious consequences of supernumerary sperm fusion, many eukaryotes have evolved polyspermy barriers, which are implemented at different levels in the reproductive process. Here, I will focus on polyspermy preventing mechanisms in flowering plants and discuss the developmental consequences associated with their failure.



Establishment of a *Drosophila* model for arrhythmogenic cardiomyopathie (ARVC 5)

Nora Klinke, Marcel Reinhardt, Heiko Harten and Achim Paululat

Department of Developmental Zoology

Applying pedigree analyses and gene sequencing, a point mutation in the human *TMEM43* gene was identified in families in which sudden cardiac death occurs frequently in men between the ages of 20-40 years. *TMEM43* encodes a membrane protein with four transmembrane domains. It localizes to the ER- outer membrane compartment. The point mutation in TMEM43 results in arrhythmogenic right ventricular cardiomyopathy (ARVC) type 5[1,2,3]. Characteristics of ARVC-5 are ventricular tachycardia, heart attacks, and sudden cardiac death[4]. The identified point mutation is a missense mutation in the human *TMEM43* gene that results in incorporation of the amino acid leucine rather than serine at position 358 of the protein, pS358L.

Although the exact molecular function of TMEM43 is still unknown, recent studies have provided evidence that TMEM43 regulates nuclear shaping. Cells carrying the point mutation in TMEM43 display altered biomechanical properties of cell nuclei, including an increased nuclear stiffness[3].

TMEM43 is a highly conserved protein throughout different species. Homologous proteins exist in chimpanzees, rhesus monkeys, dogs, cows, mice, frogs and fruit flies. The corresponding homolog in *Drosophila melanogaster* is CG8111. Interestingly, the serine, which is involved in the familial mutation in humans, is conserved in fruit flies (S333) and other species[5]. My major aim is to establish *D. melanogaster* as a model to analyze TMEM43 protein function.

Therefore, the effect of a null mutation (CRISPR) and over expression of wildtype and mutant CG8111 will be investigated in *Drosophila*.

References:

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2. Christensen, et al. „Mutation analysis and evaluation of the cardiac localization of TMEM43 in arrhythmogenic right ventricular cardiomyopathy.“ Clinical Genetics, 2011, vol. 80: 256-264.
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4. Fontaine, G.F., et al. „Arrhythmogenic Right Ventricular Cardiomyopathies - Clinical forms and main differential diagnoses.“ Circulation, 1998, vol. 97: 1532-1535.
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Collaboration partner: Hendrik Milting (Erich und Hanna Klessmann Institut, Bad Oeynhausen)

Neprilysin 4 regulates muscle contraction via cleavage of SERCA inhibitory peptides

Annika Buhr[1], Ronja Schiemann[1], Paola Ferrero[2], Achim Paululat[1], Heiko Harten[1]

[1] Department of Developmental Biology and Zoology, Osnabrück University
[2] National University of La Plata, Argentina

The ability of muscle fibers to contract is based on well characterized molecular processes, with the cytosolic Ca^{2+} concentration representing a crucial parameter. A major player responsible for regulating this concentration is the sarcoplasmic/endoplasmic reticulum calcium ATPase, SERCA, which transports Ca^{2+} from the cytosol of muscle cells into the sarcoplasmic reticulum, thereby reducing cytosolic calcium ion levels and marking the beginning of the muscle relaxation phase. Based on this essential functionality, tight regulation of SERCA-activity is vital to the proper functionality of muscle tissue.

In vertebrates, SERCA activity is regulated by binding of certain peptides, e.g. Phospholamban or Sarcolipin (Payre and Desplan, 2016). In *Drosophila melanogaster,* two functionally homologous peptides are known, Sarcolamban A (SCLA) and Sarcolamban B (SCLB). By binding to SERCA, SCL peptides significantly reduce its activity (Magny et al., 2013). Consequently, SCL loss-of-function mutants exhibit impaired Ca^{2+} transients in heart cells, concomitant with severe heart arrhythmia. We found that increased expression of the peptidase Neprilysin 4 (Nep4) phenocopies these effects on heart physiology. In addition, SERCA-activity is considerably elevated in corresponding animals. Ectopic expression of catalytically inactive Nep4 is without consequences, which confirms that impaired catalytic activity, and thus abnormal peptide hydrolysis, represents a causative factor.

Further experiments showed that Nep4 and SERCA co-localize in membranes of the sarcoplasmic reticulum and co-precipitate in pull-down assays. In order to characterize the molecular mechanism by which Nep4 regulates SERCA activity, we performed cleavage assays, which confirmed that Nep4 can hydrolyze both SERCA-regulatory SCL peptides at their C-terminus. Heart-specific expression of the correspondingly truncated peptides will reveal the physiological relevance of the cleavage event.

To analyze the suborganellar localization of SERCA, Nep4, and SCLA / SCLB in more detail, we will apply Halo-, SNAP-, and CLIP-tagged fusion constructs. Initial experiments indicate co-localization of all three factors is S2 cells. Utilizing the same constructs, interaction dynamics of the three components will be assessed. The detailed analysis of the Nep4 mediated regulation of SERCA activity will significantly advance the current understanding of muscle physiology and functionality.

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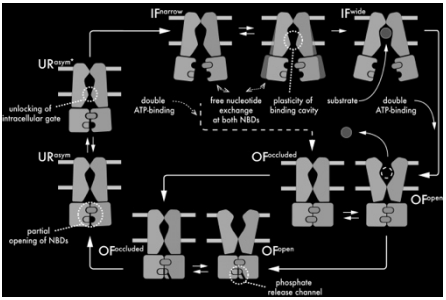
Using electron microscopy to analyze protein dynamics

Arne Möller

Department of Structural Biology

With the advent of direct electron detecting cameras, the structure determination of macromolecular machines to atomic resolution became possible, without the need for crystallization. This initiated a resolution revolution in structural biology[1]. In addition to high-resolution analyses, electron microscopy is a powerful tool to characterize protein behavior and quality. An EM-image often reveals flaws in the sample which hamper biochemical or biophysical experiments. We utilize straight forward negative stain EM and automated protocols to rapidly access protein quality and to routinely guide biochemical optimizations[2]. As sample preparation allows for a broad usage of detergents, and alternative hydrophilic environments, cryo-EM became the prime method to determine the structures of membrane proteins, which are notoriously difficult to crystallize. Furthermore, image processing and in silico sorting of protein conformations allow analyzing macromolecular machines under turnover conditions.



The conformational cycle of the heterodimeric ABC transporter TmrAB.

In my talk, I will introduce the new department of structural biology and highlight different aspects of our research. As such, we have recently used cryo-EM to decipher the entire conformational space of a heterodimeric ABC transporter to high-resolution and were able to explain the structural conversions during the transport cycle[3]. I will present our biological findings and discuss the methodological actions that were necessary to achieve them.

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Empty MHC class I molecules: peptide binding, cell surface interactions, and tumor immunotherapy

Sebastian Springer

Department of Life Sciences and Chemistry, Jacobs University Bremen, Germany

Major histocompatibility complex (MHC) class I molecules are transmembrane receptor proteins that present intracellular peptide antigens to cytotoxic T cells. Understanding peptide binding to class I molecules requires an understanding of the structure of the empty form of the protein, which has only now become experimentally accessible through a stabilizing disulfide bond mutation. The crystal structure of empty HLA-A*02:01 reveals an interesting conformational switch between the peptide-bound and –free states. In our investigation of the cell biology of empty class I molecules, we have found that one of their conformational states forms strong and stable oligomers ('clusters') at the cell surface that may play roles in cell signaling or endocytosis[1]. Finally, I will report on our biotechnological use of recombinant empty class I molecules to make MHC tetramer reagents for the antigen-specific staining of tumor-specific T cells[2].

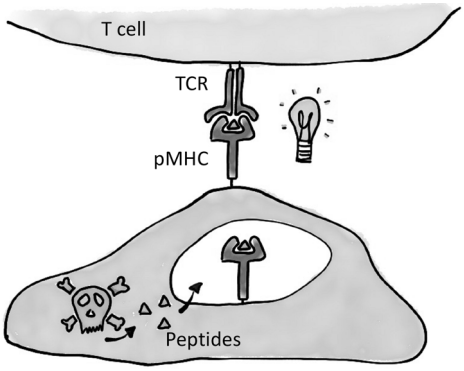

Figure: Antigen presentation. MHC class I proteins present tumor-specific peptides (blue) to activated cytotoxic T lymphocytes. The specific interaction between the T cell receptor and the MHC/peptide complex triggers the T cell to induce apoptosis of the tumor cell.

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Fab1 lipid kinase phosphorylation by TORC1 controls signaling and vacuole membrane homeostasis

Zilei Chen[1], Pedro Carpio Malia[1], Riko Hatakeyama[2], Raffaele Nicastro [2], Zehan Hu[2], Marie-Pierre Péli-Gulli [2], Elja Eskes [3], Joris Winderickx[3] , J oern Dengjel[2] , Claudio De Virgilio[2] and Christian Ungermann[1]

[1]Department of Biochemistry, Osnabrueck University, Germany
[2]Department of Biology, Fribourg University, Switzerland
[3]Functional Biology, KU Leuven, Belgium

Lysosome/vacuole plays a key role in controlling cellular metabolism as a central signaling hub and major organelle for degradation.[1] During yeast growth under different environment conditions, the Fab1 lipid kinase complex and the nutrient-regulated target of rapamycin complex 1 (TORC1), which localize on both vacuole and endosomes, are responsible for endomembrane system homeostasis. Here, we identify Fab1 as a direct target of TORC1 on a specific endosomal population named signalling endosome, and vacuoles and then regulate the functional crosstalk between those complexes. By using in vitro and in vivo analyses, we find that Fab1 is phosphorylated by TORC1 and identify the phosphorylation sites. We generated phosphomimetic and non-phosphorylated variants of Fab1 by CRISPR/Cas9 and demonstrate direct effects on rapamycin sensitivity, TORC1 localization, and vacuole morphology. Our data show that Fab1 complex produces P-I3,5-P_2 on endosomes, which in turn affects TORC1 activity on the vacuole.

Together, we find the Fab1 kinase protein is a substrate of TORC1, which not only influences Fab1 activity but also affects TORC1 activity by a feedback regulation. These data reveal a novel crosstalk between a lipid kinase and the metabolically regulated TORC1.

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TSC1 is an oligomeric scaffold for TSC complex assembly and membrane recruitment

Katharina Fitzian, Reinhard Zech, Claudia Antoni, Stefan Raunser, Daniel Kümmel

Institute of Biochemistry, University of Münster
MPI Dortmund

The lysosomal resident small GTPase Rheb is a crucial regulator of the mechanistic target of rapamycin complex 1 (mTORC1) in growth factor signaling. mTORC1 is considered a master regulator of cellular growth by activation of transcription factors and inhibition of autophagy. Rheb in its GTP-bound form activates mTORC1 by an allosteric mechanism at the lysosomal surface. Rheb is negatively regulated by its corresponding GTPase activating protein (GAP) that mediates GTP hydrolysis and therefore inactivates it. The GAP for Rheb is the Tuberos sclerosis complex (TSC) complex consisting of the three subunits TSC1, TBC1D7 and TSC2. So far, the TSC complex is the only known regulator of Rheb and plays an important role as a tumor suppressor in mTORC1 signaling that is conserved from fungi to humans. A crucial factor for *in vivo* GAP activity is the localization of the complex on the lysosomal surface.

The TSC2 subunit is responsible for GAP function, whereas TSC1 acts as a scaffold for TSC complex assembly and has been proposed to act as chaperone. We have characterized the structure of TSC1 by X-ray crystallography and electron microscopy and found that TSC1 can form dimers, hexamers and dodecamers. The dimer shows N-terminal globular head domains that are linked via long C-terminal coiled-coil domains. A central helical domain is necessary for the formation of oligomers that likely represents the functional state observed in cells. Furthermore, this part is also sufficient to mediate the interaction with TSC2. We also find that TSC1 can bind to membranes in a phosphatidylinositol-3,5- and -3,4-bisphosphate-dependant manner. We and thus describe the first molecular function of TSC1 and suggest that it might play an important role in localization and regulation of activity of the TSC complex.

Correlative Super-Resolution Fluorescence and Electron Cryo-Microscopy

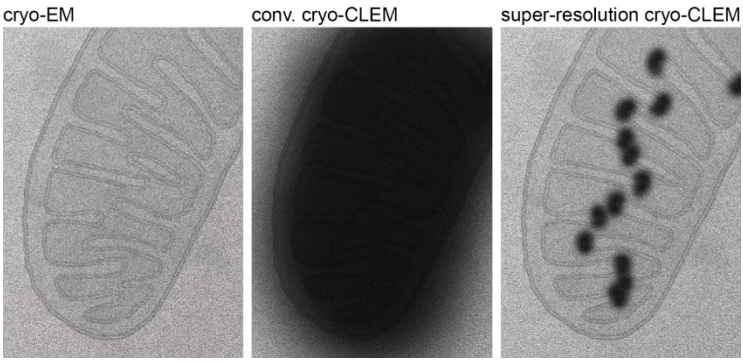
Rainer Kaufmann

Centre for Structural Systems Biology, Department of Physics, University of Hamburg

Super-resolution methods in fluorescence microscopy present a true game changer for the field of correlative light and electron microscopy (CLEM). They allow bridging the big resolution gap between conventional fluorescence microscopy (FM) and electron microscopy (EM). However, resolution improvement is only one side of the story. Equally important is structural preservation of the sample.

At a time when super-resolution FM was just at the very beginning, the EM field was already searching for an alternative immobilization method (in contrast to chemical fixation) that maintains the structural integrity of the sample. The result of this search were fast-freezing techniques, which immobilize the sample in a vitreous (glass-like) state. Electron cryo-microscopy (cryo-EM) has in the meantime evolved into a routine method for structural biology.[1]

On the contrary, super-resolution FM under cryo-conditions is still at a very early and experimental stage. However, the combination of both cryo-microscopy methods (super-resolution cryo-CLEM) has great potential to open up a wide range of new application possibilities in structural and cellular biology.[2]



Schematic illustration of super-resolution cryo-CLEM. Black overlay on top of cryo-EM image represents fluorescence information.

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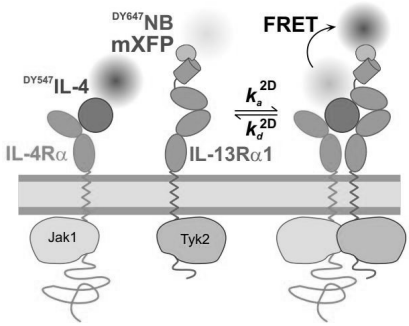
Quantifying cytokine receptor dimerization in the plasma membrane by single molecule FRET

Hauke Winkelmann[1], Maximilian Hafer[1], Stephan Wilmes[1,3], Christian P. Richter[1], David Richter[1], Rainer Kurre[2] & Jacob Piehler[1,2]

[1]Division of Biophysics, Department of Biology, Osnabrück University, Germany
[2]Biophysics, Department of Biology, Osnabrück University, Germany
[3]College of Life Sciences, University of Dundee, Scotland

Class I/II cytokine receptor (CR) signal via the JAK/STAT pathway, which is initiated by protein ligands that simultaneously interact with two or more transmembrane receptor subunits. The spatiotemporal organization and dynamics of CR assembly in the plasma membrane is currently controversially debated. We have recently established dual-color single molecules tracking and co-tracking to quantify CR diffusion and interaction in the plasma membrane at physiological expression levels. We identified ligand-induced dimerization as the key switch of signal activation for various members of the class I and class II families[1,2]. Furthermore, we found weak intrinsic receptor dimerization affinity leading to significant ligand-independent dimerization at artificially elevated receptor expression levels. To pinpoint the interaction dynamics of CR dimers in the plasma membrane with very high spatial and temporal resolution, we have established single molecule FRET (smFRET) imaging in living cells.



Single molecule FRET allows to observe live cell kinetics of cytokine receptors with high spatio and temporal resolution.

For the thrombopoietin receptor, smFRET enabled unambiguous detection of transient ligand-independent dimerization, which was strongly stabilized by constitutively activating receptor and JAK mutations. For the type II interleukin-4 receptor, the dynamics of reversible receptor dissociation and re-association promoted by receptor confinement by the membrane cytoskeleton could be resolved using engineered, site-specifically fluorescent-labeled IL-4.

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Function and Localization of Glutaredoxin ROXY1 in *A. thaliana* Meristem Development

Lucia Maß

Department of Botany

The foundation of multicellular organism development is the balance between cell proliferation and cell differentiation, leading to patterning processes and morphogenesis. Complex genetic networks control ontogenesis, which includes embryogenesis, seed germination, vegetative and reproductive development, seed formation, senescence and death. As all land plants, *Arabidopsis thaliana* is in contrast to animals, capable of postembryonic organogenesis due to the maintenance of undifferentiated stem cell pools in meristems throughout its life.

Reactive oxygen species (ROS) were initially considered to be toxic by-products of aerobic metabolism. As sessile organisms land plants developed a versatile toolbox to cope with variable unavoidable organic - and inorganic stress. These systems ensure a fast response to bring the plant cell back into ROS balance, as well as result in adaption. Recently, accumulating evidence has shown that ROS also act as potential key regulators in the progression of biological processes in the plant shoot and root stem cell maintenance.

One of the open questions still awaiting to be addressed is, how ROS control downstream components, such as transcription factors involved in meristem regulation.

This is where the land-plant specific CC-type glutaredoxin ROXY1 comes into play. Glutaredoxins are ubiquitous, GSH-dependent oxidoreductases belonging to the family of thioredoxin superfold proteins. We have shown a regulatory function of ROXY1 in initiation and differentiation of petals in *A. thaliana* flower development.

The talk will address the question of redox-dependent ROXY1 function in root meristem maintenance and give insight into ROXY1 subnuclear protein localization. Further analysis will focus on the function of ROXY1 in relation to redox regulation of meristem development, as well as downstream targets of redox-associated signaling.

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Molecular mechanisms of membrane pore formation in regulated cell death

Katia Cosentino

Department of Biology and Center for Cellular Nanoanalytics (CellNanOs).
(Katia.cosentino@biologie.uni-osnabrueck.de)

Pore forming proteins (PFPs) comprise different families of proteins, which share the ability to organize into supramolecular complexes that permeabilize cell membranes. Pore opening is usually lethal for the cell and this can have important physiological and pathological consequences, related to infection and immunity, cancer and neurodegeneration. Despite their biological relevance, the mechanisms of assembly and membrane pore formation of many PFPs is not clear1.

Our group combines advanced fluorescence single molecule microscopy and atomic force microscopy (AFM) and spectroscopy to dissect the spatio-temporal assembly of PFPs in cell membranes. Specifically, we focus on PFPs involved in regulated cell death processes, namely, apoptosis and pyroptosis.

Using these approachs, we have characterized the mechanisms of pore formation for the two key executers of the mitochondrial pathway of apoptosis, the Bcl-2 family members Bax and Bak2,3 (Fig. 1).

We are currently expanding our interest to pyroptosis, a highly inflammatory, lytic form of cell death initiated in response to detection of pathogens or other danger signals. Pyroptosis is executed by Gasdermin D (GSDMD) and by other members of the Gasdermin family, by causing plasma membrane permeabilization. We aim to understand the molecular mechanisms governing the assembly and dynamics of GSDMD leading to membrane pore formation and to pinpoint the precise functional role of different members of the Gasdermin family in cell death.

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Intestinal glycans affect the susceptibility to enteric infections

Guntram A. Graßl

Hannover Medical School

Salmonella enterica serovars cause diseases ranging from mild diarrhea to life-threatening systemic diseases such as typhoid fever. Non-typhoidal *Salmonella* species such as *S.* Typhimurium cause intestinal inflammation and diarrhea in humans. Glycans can be used by commensal bacteria and enteric pathogens as carbon and energy sources as well as receptors for attachment and subsequent invasion. Three examples demonstrate how glycans can differentially affect the susceptibility to infections:

1. The *B4galnt2* gene encodes a blood-group-related glycosyltransferase that is subject to strong selective forces in natural house mouse populations, whereby a common allelic variant exists that results in loss of *B4galnt2* gene expression in the gastrointestinal tract. A lack of *B4galnt2* leads to a change in the composition of the intestinal microbiota which is responsible for increased resistance towards *Salmonella* Typhimurium induced colitis.

2. *Salmonella* employs multiple virulence factors such as type 3 secretion systems, flagella, and fimbriae to infect its host. *Salmonella* carries different types of chaperone-usher fimbriae which are involved in receptor binding and are crucial for invasion and pathogenicity of *Salmonella*. The fimbrial operon, *std*, encodes the π-class Std fimbriae, which have been described to bind glycans with terminal α-1,2 fucose residues. The *FUT2* gene encodes the α-1,2-fucosyltransferase which adds fucose to the terminal galactose of a type I chain in an α-(1,2) linkage. This enzyme is responsible for the expression of ABH and Lewis histo-blood group antigens on the gastrointestinal epithelium and in bodily secretions. Our data show that *Salmonella*-triggered intestinal inflammation and colonization are dependent on Std-fucose interaction.

3. *Salmonella* cannot degrade complex glycans itself but can use simple sugars once they are liberated from complex glycans by commensals. The intestine is lined by a heavily glycosylated mucus layer. A commensal mucus degrader that is ubiquitously present in the intestine of humans and other mammals is *Akkermansia muciniphila*. *A. muciniphila* was shown to thrive on glycoproteins in the intestinal mucus and increases the production of short chain fatty acids thereby supporting a healthy epithelial barrier. A decrease in intestinal *A. muciniphila* colonization has been linked to various diseases such as inflammatory bowel diseases, metabolic disorders or diabetes. Using gnotobiotic mice colonized with a defined microbial community we demonstrate that *A. muciniphila* decreases the intestinal burden of *Salmonella* and concomitant intestinal inflammation.

A role for the amino acid permease Gnp1 in serine uptake and sphingolipid homeostasis in yeast

Bianca M. Esch

Molecular Membrane Biology Group

Sphingolipids (SLs) are abundant and essential molecules in eukaryotes that have crucial functions as signalling molecules and as membrane components. Sphingolipids biosynthesis starts in the endoplasmic reticulum with the condensation of serine and palmitoyl-CoA to yield long chain bases, which are further processed to ceramides. Ceramides are transported to the Golgi apparatus where head groups are added to yield complex sphingolipids. They are transported to and enriched in the outer leaflet of the plasma membrane.

The process of sphingolipid biosynthesis is highly regulated in *Saccharomyces cerevisiae* to maintain sphingolipid homeostasis [1,2]. Even though, serine is an essential component of the sphingolipid biosynthesis pathway, its role in maintaining sphingolipid homeostasis has not been precisely studied. Using genetic experiments, we show that the broad specificity amino acid permease Gnp1 is essential for serine uptake and therefore growth in serine auxotroph cells. We confirm these results with serine uptake assays in gnp1Δ cells. We further show that uptake of exogenous serine by Gnp1 is important for maintaining sphingolipid homeostasis. Additionally, we demonstrate that the interaction of serine uptake and sphingolipid homeostasis is solely located at the first, serine-consuming step of sphingolipid biosynthesis by excluding interactions with other parts of the biosynthesis pathway. This underlines the direct dependency of sphingolipid homeostasis to serine availability.

Our results demonstrate that yeast cells rely on the uptake of exogenous serine to regulate sphingolipid biosynthesis according to need and cannot activate intra-cellular serine pools for this task. Our data adds serine as an additional metabolite involved in the feedback regulation of sphingolipid levels in yeast. This can also be a starting point to analyse the role of serine uptake in mammalian sphingolipid metabolism.

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Session VI

Dissecting sphingolipid function with photoswitchable lipid probes

Tolulope Sokoya[1], Matthijs Kol[1], Johannes Morstein[2], James Frank[2,3], Dirk Trauner[2], Joost Holthuis[1]

[1]Molecular Cell Biology Division, Department of Biology/Chemistry, University of Osnabrück, 49076 Osnabrück, Germany; [2]Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003-6699, USA; [3]The Vollum Institute, Oregon Health and Science University, Portland OR 97239-3098, USA

Email address: tosokoya@uni-osnabrueck.de

Sphingolipids are highly versatile membrane components that contribute to mechanical stability, life-death signalling and molecular sorting. Progress in understanding how sphingolipids exert their multitude of tasks is hindered by a limited availability of suitable methods to probe lipid function. Manipulation of subcellular lipid pools through removal or overproduction of metabolic enzymes is slow, allowing cells to mount an adaptive response that dampens functional impact. In this respect, photoswitchable azobenzene-modified lipids hold great promise by allowing translation of optical stimuli into a reversible cellular response. Seeking to control sphingolipid biosynthesis and signalling by light, we synthesized a panel of photoswitchable sphingoid bases and ceramide analogs. In this talk, I will describe how these analogs enabled us to exert optical control over the production of ceramide, glucosylceramide and sphingomyelin both in model membranes and live cells. Our findings open up new avenues for studying sphingolipid function *in vivo*, in particular regarding their dual roles in cell proliferation and apoptotic signalling.

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Selectivity of BAR domains at curved membranes

Mirsana Ebrahim Kutty

Institute of Medical Physics and Biophysics, University of Münster

Curved cellular membranes are ubiquitous in live cells. To properly function, the membrane has to be cordially deformed, creating curvature-dependent signaling sites. Playing a major role in these curvature-induced signaling hubs, is a group of approximately 70 proteins that belong to the BAR superfamily. Phylogenetically, these proteins are classified into three major subgroups – N-BAR, F- BAR and I- BAR – that vary in curvature preference: BAR or N-BAR proteins are recruited to strong positive curvatures (i.e. membrane bend towards the cytoplasm), F- BAR proteins prefer less curved positive membrane regions, and I- BARs enrich at negatively curved membrane sections.

While the curvature-preference has been well studied, the contribution of lipid composition during curvature-dependent protein-membrane interactions has remained largely elusive. In this project, we aim at decoupling the role of curvature vs. lipid composition at the cell membrane. In preliminary studies, we cloned the curvature-sensing domain of all annotated BAR domain family members and probed their subcellular localization. Strikingly, we find for domains with similar intrinsic curvatures a strong variability in their ability to enrich at deformed membrane sections. While tether pulling experiments with optical tweezer in cells and GUVs(Giant unilamellar vesicle) showed a curvature dependent enrichment of the BAR domain in the tether[1,2] similar experiments with Giant Plasma Membrane Vesicles derived from cells didn't show enrichment in the tether, suggesting additional factors guiding BAR domains to the curved surfaces. Additionally, loss of negatively charged lipid phosphatidylinositol bisphosphate, has been observed with the decreased association of a BAR domain to the plasma membrane.

Collectively, our studies suggest that BAR domain proteins, generally discussed as curvature sensing units, should be considered as coincidence detectors computing membrane geometry and lipid composition. These findings are relevant as they posit a novel mechanism how cells achieve selectivity in curvature-dependent signaling hubs with similar geometry that are formed at different endo-membranes.

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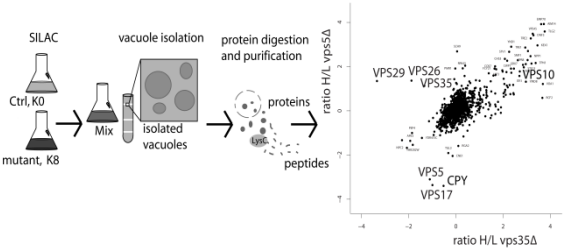
Poster Abstracts

A systematic MS-based approach to identify cargos of trafficking pathways in yeast

Sebastian Eising

Molecular Membrane Biology, Department of Biology/Chemistry, University of Osnabrück

Proteins are constantly recycled and transported between different organelles to maintain the function and flexibility of the cell. The yeast vacuole is a key player in several trafficking pathways, acting as an important organelle for recycling and degradation of proteins. This trafficking has a great impact, because many neurodegenerative disorders like Parkinson´s desease are caused by mutations in proteins in the endo-lysosomal pathway. We have developed tools to systematically study the protein and lipid composition of yeast vacuoles. Here we use this tool to systematically map cargo-protein interactions of trafficking pathways in yeast. Besides the retrograde transport pathway depending on the GARP complex (Golgi associated retrograde protein) and retromer, we are also interested in the trafficking of the vacuolar sterol transporter Npc2. The aim behind this project is to use the MS-based approach to systematically identify trafficking pathways. A great advantage of this method is the possibility to cluster different proteins based on their function without any tagging. I will now use this approach to test how protein complex formation is influenced by mutations, which proteins and lipids are accumulating or lacking at vacuoles and what cargo specificity of the mutant is. While the method is also a screening approach it allows much higher throughput than e.g. visual or genetic screens.



Scheme of MS-based experiment in combination with vacuole isolation to identify trafficking pathways cargos. Shown is the correlation between the two deletions of Vps5 and Vps35, which are subunits of the retromer complex. The Retromer cargo and CPY receptor Vps10 is enriched in the vacuole because of the missing retromer-dependent recycling route. In contrast there is less CPY in mutant vacuoles combined with the WT.

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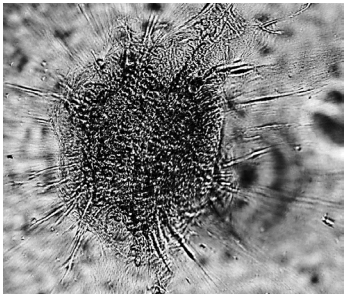
Pancreatic Ductal Adenocarcinoma and its Tumour Microenvironment: Molecular Analysis of novel 3D Co-Culture Spheroids.

Jan Hänsel

Department of Biomedical Sciences

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal and aggressive form of pancreatic cancer, displaying early metastasis and invasion into nearby organs as well as resistance against chemotherapeutic drugs due to its protective tumour microenvironment (TME) and its characteristic fibrotic desmoplasia, which consists of cancer-associated fibroblasts (CAF) and various other recruited cell types (Melstrom et al. 2017; Morris et al. 2010). The formation of the TME is often initialized and accompanied by inflammation (Erez et al. 2010; Chen and Song 2019).



3D-Spheroid (Panc-1 cells + pancreatic stellate cells in 0.1 mg/ml collagen I matrix).

To explore the pharmacological efficacy of drug candidates including natural products from plant and marine sources against PDAC and the formation of its desmoplastic TME, we developed novel 3D-co-culture tumour models. These spheroids are assembled out of PDAC tumour cell lines (e.g. Panc-1, Capan-2) and, most importantly, patient-derived cancer-associated fibroblasts from primary pancreatic tumours or from liver metastases. We are able to create PDAC-stellate cell 3D spheroids that have a highly fibrotic environment. This model focuses on what could be considered some of the most critical early-stage events of metastatic PDAC aiming at exploring the molecular mechanism of communication of tumour cells with stromal cell types from the tumour environment.

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Structural and functional analysis of the Rab7/Ypt7 guanine nucleotide exchange factor Mon1-Ccz1

Eric Herrmann, Lars Langemeyer, Christian Ungermann, Daniel Kümmel

Institute of Biochemistry, University of Münster,
Biochemistry Section, FB Biology/Chemistry, UOS

During endosomal maturation identity of organelles is changed from Rab5- to Rab7-positive membranes (Vps21 and Ypt7 in yeast), which can then fuse with lysosomes/vacuoles. This switch is critically regulated by the Mon1-Ccz1 complex (MC1), which acts as guanine nucleotide exchange factor (GEF) for the GTPase Ypt7/Rab7. We have determined the crystal structure of a catalytic MC1 core complex bound to Ypt7. In combining biochemical studies and mutational analysis in yeast, we identify the determinants that allow for a discrimination of Ypt7 over Vps21, which are both located on the same membrane. Furthermore, we elucidate a novel mechanism of catalytic activity, which involves a conserved lysine residue in Ypt7 to promote nucleotide exchange1.

To address the regulation and molecular basis of membrane recruitment we work towards the structure of the full-length MC1 complex by electron microscopy (EM). In addition, we use localization studies in vivo and investigate membrane association in vitro with liposome binding assays. MC1 contains multiple lipid and protein interaction sites that are collectively required for membrane recruitment, supporting a model where MC1 integrates different inputs for proper spatiotemporal initiation of early-to-late endosome conversion.

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Survival factor 1 as a potential lipid transport protein in *Saccharomyces cerevisiae*

Sergej Limar

Molecular Membrane Biology

Sphingolipids are one of the three major lipid classes in the plasma membrane of all eukaryotic cells [1]. Maintaining sphingolipid homeostasis is crucial for cellular functions and cellular integrity [2]. Sphingolipid biosynthesis starts in the ER. Complex sphingolipids are formed in the Golgi and are transported in a vesicular way to the plasma membrane [3]. How the transport of sphingolipid intermediates is coordinated in yeast and how sphingolipid homeostasis is maintained remains largely elusive.

To bring more light into the understanding of the sphingolipid metabolism, we are studying the yeast protein Survival factor 1 (Svf1). Based on homology models, Svf1 belongs to the family of lipocalins, a class of proteins known for binding small hydrophobic molecules with their hydrophobic binding pocket [4]. Additionally, *SVF1* shows strong genetic interactions with sphingolipid metabolism genes, such as *SUR2* [5]. Together, these data make Svf1 an interesting candidate for a potential sphingolipid binding/transport protein in yeast. Here we show that Svf1 localizes to the cis-Golgi. Svf1 localization is highly dynamic between the cytoplasm and the cis-Golgi. This dynamic localization is necessary for proper Svf1 function. Furthermore, an amphipathic helix at the n-terminus is responsible for Golgi targeting, which is essential for Svf1´s function. Our lipidomic data show an increase of ceramides and a decrease of complex sphingolipid level in the *svf1*Δ cells compared to the WT. Finally, we show that recombinant Svf1 is capable of extracting phytosphingosine (PHS) from membranes, as shown by surface tension measurements. Based on our data we hypothesize that the role of Svf1 in sphingolipid metabolism is based on its capacity to transport sphingolipid intermediates. In the future we will test this model investigating the flux of sphingolipid biosynthesis in *svf1*Δ cells.

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Structural analyses of the *Salmonella* pathogenicity island 4-encoded type I secretion system of *Salmonella enterica*

Dorothee Merker, Nathalie Sander, Jörg Deiwick and Michael Hensel

Department of Microbiology, University of Osnabrück.

The cooperative function of two protein secretion systems is required for adhesion to and invasion of polarized epithelial cells by the pathogen *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium). Before the *Salmonella* pathogenicity island 1-endoced type III secretion system (SPI1-T3SS) mediates invasion, the giant adhesin SiiE, the only known substrate of the SPI4-encoded type I secretion system (SPI4-T1SS), allows adhesion to the apical surface of polarized host cells. While the SPI1-T3SS in *Salmonella* Typhimurium is well characterized, the structure and function of the SPI4-T1SS is not fully understood yet.

The SPI4-T1SS is composed of the canonical subunits SiiF (ATPase), SiiD (periplasmic adapter protein) and SiiC (outer membrane pore). SiiE mediates the adhesion of *Salmonella* to polarized epithelial cells and enables the secretion of T3SS-effector proteins, which leads to an efficient invasion in polarized cells. In contrast to well-studied T1SS of other bacteria, the SPI4-T1SS also features two non-canonical subunits SiiA and SiiB. We suggest that these subunits form a proton-conducting channel that links proton motive force to the release of SiiE.

To uncover the structure and function of the SPI4-T1SS, we enriched the system and quantified its subunits by mass spectrometry, and determined the stoichiometry. We aim to unravel the structure of the T1SS to gain further knowledge of the SPI4-T1SS and its unique structural features. By understanding the interaction between the individual subunits of the SPI4-T1SS and the interplay with the SPI1-T3SS, we get new insights in the invasion process of *Salmonella* Typhimurium.

Dissecting the role of sphingolipids in the phagocytic clearance of pathogens

Patrick Niekamp[1,2], Fikadu Geta Tafesse[2], Joost C. M. Holthuis[1]

[1]Molecular Cell Biology Division, University of Osnabrück, 49076 Osnabrück, Germany
[2]Molecular Microbiology and Immunology Division, Oregon Health and Science University, Portland, OR 97239

The innate immune system is the first line of defence against pathogens such as bacteria and fungi. While a weak immune system is susceptible to infections, chronic immune reactions can lead to autoimmune diseases. Previous work revealed causal roles of sphingolipids in a variety of immune processes.

Using small molecule inhibitors and CRISPR/Cas9-engineered cell lines, we recently showed that an intact sphingolipid biosynthetic pathway is critical for phagocytosis, the mechanism by which innate immune cells like macrophages remove pathogens. Thus, we found that blocking sphingoid base production perturbs the phagocytic clearance of *Candida albicans, Mycobacterium tuberculosis* and *Salmonella enterica*. Live cell imaging of phagocytic uptake of pathogen-mimicking beads revealed a defect in PI3 kinase activation, disrupting internalization by preventing actin disassembly. In addition, we showed that genetic ablation of the Golgi-resident sphingomyelin synthase SMS1 in human monocytes decreases their ability to internalize *Mycobacterium tuberculosis.*

To further dissect the underlying molecular principles, we exploit approaches enabling acute manipulation of the metabolic fate and/or subcellular distribution of individual sphingolipid species in macrophages and dendritic cells. Our efforts should reveal novel mechanistic insights in the causal roles of sphingolipids in innate immunity.

Characterization of human HOPS-mediated membrane tethering and fusion *in vitro*

Yaping Han, Christian Ungermann

Department of Biology/Chemistry, Biochemistry Section, University of Osnabrück

Intercellular organelles exchange lipids and protein contents via membrane fission and fusion processes. Multisubunit tethering complexes (MTCs) are the main coordinators of membrane fusion, which have the combined activity of membrane tethering and fusion. MTCs bring the donor and receptor organelles into close proximity by binding to small GTPases on their membrane surface and assist the assembly of SNARE on both membranes into fusion competent complexes. HOPS is the hexameric complex, consisted of VPS11, VPS16, VPS18, VPS33, VPS39, VPS41, that tethers lysosomes with late endosomes, autophagosomes, and AP-3 vesicles.

In mammalian cells, it has been reported that small GTPases Arl8b recruits HOPS to the lysosome via VPS41[1]. Another small GTPase Rab7, together with its effector RILP, has also been implicated in HOPS membrane recruitment[2]. Meanwhile, it is also found that the Golgi-resident Rab2a plays a role in autophagosome and lysosome fusion[3]. It is also known that the SNAREs Syntaxin7, Vti1b, Syntaxin8, VAMP7/8 are involved in late endosome and lysosome fusion[4], while Syntaxin17, SNAP29 and VAMP7 or Ykt6 are responsible for the fusion between autophagosomes and lysosomes[5].

We want to dissect the functionality and regulation of the human HOPS complex. Our goal is to establish tethering and fusion assay with purified HOPS and liposomes carrying small GTPases and eventually SNAREs. To purify HOPS, all six subunits of human HOPS complex were inserted into one gene expression cassette of the biGBac system, which enables its expression in insect cells[6]. The homogeneity and stability of the complex was confirmed via gel filtration. I detected HOPS interaction with the small GTPases and SNAREs. Further investigation to characterize HOPS functionality regarding different fusion events, like late-endosome and lysosome fusion and auto-lysosome formation, are on-going.

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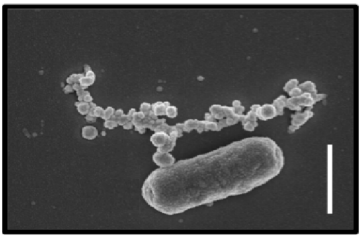
Explosive cell lysis provides the raw material for the establishment of intercellular nanotubes

Shiksha Ajmera[1], Melchior Schröter[1], Shraddha Shitut[1], Muhammand A. Rahman[1], Carina Kommnick[2], Michael Hensel[2], and Christian Kost[1]

[1] Department of Ecology, [2] Department of Microbiology.

In their natural environment, bacteria exist in diverse communities, in which ecological interactions are commonplace. One type of such interactions is the exchange of cytoplasmic materials between different bacteria using nanotubes. These membranous structures are used to transfer nutrients[1] or toxins [2] between interconnected bacterial cells. However, the molecular details of the formation of nanotubes remain elusive. Here, we use a synthetically engineered system consisting of two auxotrophic strains of *Escherichia coli*, which reciprocally exchange essential amino acids via nanotubes, to investigate the molecular basis of nanotube formation.



Scanning electron micrograph of an auxotrophic E. coli cell in coculture, surrounded by vesicles. Scale bar = 1µm.

To identify the key players in nanotube formation, we first performed a transcriptome analysis. This analysis revealed differential regulation of several genes, including some that are known to be involved in vesicle production. To investigate the role of vesicles for the formation of nanotubes, we used hypovesiculating auxotrophic mutants (ΔnlpA). These mutants displayed reduced growth in cocultures with auxotrophs lacking this mutation. Interestingly, this fitness deficit was rescued upon addition of both vesicles isolated from another culture and synthetic lipid vesicles. These results imply vesicals might be involved in nanotube formation, because a reduced number of vesicles apparently impaired nanotube-mediated amino acid cross-feeding. In addition, we observed that vesicles appeared entangled in a mesh-like mass around cells. Staining revealed that these meshes contain eDNA. Testing whether cell death and lysis were the source of eDNA, revealed indeed an increased number of dead auxotrophic cell in cocultures as compared to amino acid-supplemented monocultures. Based in these observations we hypothesise that cell lysis provides eDNA and vesicles; while vesicles are the raw material for nanotube formation, eDNA traps and increases their availability to other cells.

Altogether, this work implicates vesicles in nanotube-mediated bacterial interaction and identifies cell lysis as a likely source of eDNA. Overall, these results advance our limited understanding of nanotubes - a newly identified mode of interaction amongst bacteria.

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Xenapses grown on quantifoil-supported TEM grids: towards high-resolution cryo-electron tomography of exocytic and endocytic structures

Junxiu Duan, Philipp Selenschik, Sai Krishnan, Yaroslav Tsytsyura, Nataliya Glyvuk, Ulrike Keller, Jürgen Klingauf

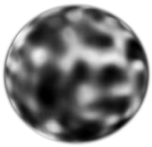
Institute of Medical Physics and Biophysics, University of Münster, Münster, Germany.

For studying quantitative aspects of synaptic exo- and endocytosis at the molecular level, we have developed cultured 'xenapses', TIRFM-amenable purely presynaptic boutons. These are formed by murine hippocampal neurons cultured on microstructured glass coverslips which have been functionalized with synaptogenic proteins. In order to correlate results from fluorescence microscopy (TIRFM and dSTORM/PALM) with the ultrastructure we here developed methods for micropatterning and functionalization of Quantifoil films on TEM grids. While on glass coverslips micropatterning is easily achieved by micro-contact printing of the functionalized polymer, for the thin and brittle Quantifoils we developed a bottom up approach involving click chemistry and UV-photolithography. Xenapses are formed on TEM grids within a few days. Their functionality was confirmed by calcium and pHluorin imaging experiments. At xenapses expressing synptotagmin-pHluorin we observed robust exocytosis and subsequent compensatory endocytosis upon depolarization. Xenapses, cryo-fixed by high-pressure freezing, displayed well-preserved ultrastructure, with large numbers of synaptic vesicles at or near the pre-synaptic membrane. Our results show that structure and function of xenapses on TEM grids do not differ from their counterparts on glass coverslips.
Thus, xenapses grown on SiO2 coated Quantifoil-supported TEM grids enable high-resolution cryo-electron tomography of xenapses.

Cellular and molecular principles of lateral segregation in the plasma membrane

Annegret Elting

Institute of Cell Dynamics and Imaging, Muenster



The „patchwork" membrane
Spira F. et al. (2012) Nat. Cell Biol.

The plasma membrane (PM) of the budding yeast *Saccharomyces cerevisiae* is organized into a patchwork of overlapping domains. To unravel the molecular principles of PM organization, we study the lateral segregation of yeast PM components using several fluorescence microscopy techniques including TIRF. In particular, we are interested in how lateral segregation and turnover of PM components are coordinated through protein-protein and protein-lipid interactions. In the presented project, we are systematically investigating how different lipid perturbations affect PM organization and turnover. Using specific yeast mutants we can deplete entire lipid classes, acutely alter lipid composition via temperature sensitive alleles or control the saturation level of acyl chains. For assessment of altered lipid composition in the lipid mutants, we are collaborating with F. Froehlich (University Osnabrueck) to perform lipid mass-spectrometry (MS). With respect to organization and lateral segregation of PM components, the lipid composition of the PM-fraction is most important. When yeast cells are disrupted mechanically membrane vesicles are formed which can be analysed for their composition on the microscope to subsequently develop strategies to specifically purify PM-derived vesicles for MS. Thus, two main questions arise: (1) Do PM-derived vesicles form distinctly of other membrane-derived vesicles from cellular organelles and (2) if so, how can we extract the PM-derived vesicles to gain pure samples for lipid MS? Using fluorescently labelled marker-proteins for different organelles and PM domains, we found that PM-derived vesicles are formed separately from other membrane-derived vesicles. The next step will be to establish the enrichment of PM-derived vesicles for lipid MS. Moreover, we were able to differentiate vesicles that derive from separate PM domains. This opens up additional options to assess PM domain formation in an efficient and simple manner. In parallel we are screening different PM proteins in various lipid mutants to find biological relevant effects and the functional link between PM lipid composition, protein activity and membrane turnover.

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Nuclear activities of MpTGA: A key regulator of sexual formation in the liverwort *Marchantia polymorpha*

Jens Koczula, Nora Gutsche, Michael Holtmannspötter, Sabine Zachgo

Department of Botany – Zachgo Lab

The evolution of land plants at the beginning of the Ordovician period required various adaptations to the new terrestrial lifestyle. In periodically dry areas, abiotic and biotic environmental factors have probably driven adaptive processes of an ancestral charophycean algae concerning vegetative and sexual development as well as stress tolerance for a successful conquest of land. bZIP TGA transcription factors (TFs) are already present in charophycean algae and regulate in higher plants such as *Arabidopsis thaliana* various developmental and stress-related responses. Due to gene duplications and neofunctionalization during land-plant evolution and the resulting redundancy, it's difficult to draw conclusions about the ancestral role of TGA TFs in developmental and stress-related responses. The liverwort *Marchantia polymorpha* is the ideal model organism to get new insights in the ancestral regulatory network of TGA TFs. *M. polymorpha* possesses in contrast to *A. thaliana* (10 TGA TFs) only one single TGA TF, MpTGA. To analyze the function of MpTGA in developmental and stress-related responses we used CRISPR induced knockout lines and overexpression studies of Mp*TGA*. Recent results indicate that MpTGA is a key regulator for sexual formation in *M. polymorpha*, but also has a function in vegetative development. The response of *M. polymorpha* to red and far-red light conditions plays probably a significant role concerning the regulatory function of Mp*TGA* during vegetative development as well as sexual transition. An additional function is the response to stress-related processes like plant-pathogen or plant-herbivore interactions which is mediated via terpenes stored in oil bodies. In *A. thaliana* ROXYs are known as interaction partners of TGA TFs. ROXYs are glutaredoxins, which evolved exclusively in land plants and regulate like TGA TFs various developmental and stress-related responses. Complementation studies of the *roxy1 A. thaliana* flower phenotype with the two Mp*ROXYs* indicate a highly conserved biochemical function. The DNA-binding of MpTGA alone and also together with MpROXYs is redox-dependent and occurs only under reducing conditions in vitro indicating a potential redox-regulated function of MpTGA in the nucleus. Our mutant analyses will increase the knowledge about the potential ancestral functions of TGA TFs and regulatory network of stress-related processes, vegetative development and sexual formation in *M. polymorpha*..

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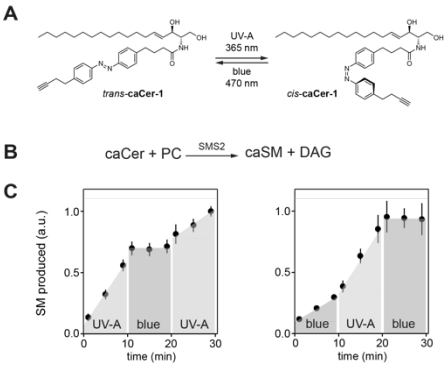
Gaining optical control over sphingolipid metabolism and signalling with photoswitchable lipid probes

Tolulope Sokoya[1], Matthijs Kol[1], Johannes Morstein[2], James Frank[2,3], Dirk Trauner[2] and Joost Holthuis[1]

[1]Molecular Cell Biology Division, Department of Biology/Chemistry, University of Osnabrück, 49076 Osnabrück, Germany [2]Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003-6699, USA. [3]The Vollum Institute, Oregon Health and Science University, Portland OR 97239-3098, USA

Sphingolipids are highly versatile membrane components that contribute to mechanical stability, life-death signalling and molecular sorting. Where and how sphingolipids exert their cellular activities is unclear owing to limited availability of suitable experimental tools. Manipulation of subcellular lipid pools through removal or overproduction of metabolic enzymes is slow, allowing cells to mount an adaptive response that dampens functional impact. In this respect, photoswitchable azobenzene-modified lipids hold great promise by allowing translation of optical stimuli into a reversible cellular response.



A) Photoswitchable & clickable ceramide analogue can be reversibly switched into two conformations by UV and blue light. B) SMS2 produces sphingomyelin from ceramide. C) SMS2-mediated SM production controlled by light.

Seeking to control sphingolipid biosynthesis and signalling by light, we synthesized a panel of photoswitchable sphingoid bases and ceramide analogues. We have demonstrated that these analogues enable optical control over production of sphingomyelin and ceramide both in model membranes and live cells (1). Our results open up new avenues for studying sphingolipid function *in vivo*, in particular regarding their dual roles in cell proliferation and apoptotic signalling.

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Unraveling the molecular and cellular determinants of type I IFN signal activation by engineered receptor chimeras

Thomas Meyer, Division of Biophysics, Department of Biology

Type I interferons (IFNs) are key mediators involved in various cellular communication processes and immune responses. These cytokines bind a common cell surface receptor comprised of the subunits IFNAR1 and IFNAR2 and thus activate signaling via the Janus tyrosine kinases (JAKs)/signal transducers and activators of transcription (STATs) pathway. Phosphorylated pSTAT1 and pSTAT2 form transcription factors, which regulate the expression of more than 300 IFN-stimulated genes and thus coordinate a large regulatory network, which is critically responsible for coordinating innate and adaptive immune responses. However, the detailed synergistic contribution of the involved cytosolic proteins, including JAKs, STATs and the key negative feedback regulator USP18 remains largely elusive. All these players interact with receptor subunit IFNAR2 involving both phosphorylation-dependent and –independent interactions. The aim of this project is to decipher the interplay of molecular and cellular determinants of this regulatory network by means of receptor chimeras using cytokine receptors with different molecular architectures and cellular trafficking properties.

To this end, we selectively transferred the intracellular segment of IFNAR2 comprising the constitutive and phosphorylation-dependent STAT bindings sites, which in our current model is fully responsible for effector activation and regulation by USP18, to different related cytokine receptor systems. For proof-of-concept experiment, we generated a chimera with IL-4Rα, which together with IL-13Rα1 provides a very similar architecture of the signaling complex involving the same JAK members as IFNAR1/IFNAR2. Furthermore, we used the homodimeric cytokine receptors gp130 and erythropoietin receptor (EpoR) as templates for signaling complexes with altered stoichiometry and JAK identities. Functional assembly of these chimeric receptors was confirmed by live cell micropatterning to monitor involved protein interactions. Phospho-flow cytometry and confocal fluorescence microscopy served to quantify STAT phosphorylation and regulation by USP18. Preliminary studies confirm functionality of the IFNAR signaling hub and suggest that both, unique molecular and cellular determinants provided by the IFNAR signaling complex are required to achieve full receptor activity.

Mass spectrometry analysis of pseudohyperphosphorylated tau cell model of Alzheimer's disease reveals changes in factors responsible for cytoskeleton dynamics

Nataliya Trushina[1], Nânci Monteiro Abreu[1], Stefan Walter[2], Daniel Villar Romero[1], Lidia Bakota[1], and Roland Brandt[1,2,3]

[1]Department of Neurobiology, [2]Center for Cellular Nanoanalytics, [3]Institute of Cognitive Science, University of Osnabrück, Osnabrück, Germany.

Microtubule-associated protein tau (MAPT) in a hyperphosphorylated state is a principal component of the neurofibrillary tangles (NFTs) in patients with Alzheimer's disease (AD). The spreading of NFTs across defined brain regions corresponds to the observed level of cognitive decline during AD development. Using mass spectrometry we analyzed changes in the proteome of pseudohyperphosphorylated (PHP) tau expressing rat PC12 cells as a model for tau hyperphosphorylation compared to wildtype (wt) human tau expressing cells.

Comparative proteomics analysis from three sets of experiments with neuronally differentiated PHP tau and wt tau expressing cells revealed proteins that are differentially expressed in the cells. We observed changes in proteins regulating microtubule dynamics, plasma membrane components, vesicle formation and endocytosis, calcium signaling and apoptosis. One of the most upregulated protein, serine protease HTRA1, is known to clear tau aggregates.

Gene Ontology (GO) term enrichment analysis was performed for up-regulated proteins. Enriched GO terms associated with molecular function included: "microtubule polymerization/depolymerization".

Proteins associated with microtubule cytoskeleton whose abundance significantly changed in PHP tau versus wt tau expressing cells (fold change ≥1.5) include microtubule associated proteins (MAP1s↑ and MAP6↓), microtubule-severing proteins (Stmn2↑) and tubulin isoforms (Tubb3↓). Annexin 2A and some S100 family associated proteins that have been implicated in regulating the neuronal membrane skeleton were also shown to have higher abundance in PHP tau expressing cells. These changes may be in line with disturbance of tau interaction with plasma membrane.

Interestingly, alpha-synuclein that forms pathological aggregates in Parkinson's disease (PD), was more than twice less abundant in PHP tau expressing cells in contrast to the frequently observed co-morbidity of AD and PD in patients.

Comparative phosphoproteomics for the same cell lines revealed differentially regulated phosphorylation sites in some microtubule cytoskeleton proteins. For instance, some MAPs whose phosphosites were exclusively present in PHP tau or wt tau expressing cells (MAP2 and MAP1a) are subjects for further analysis.

Thus, our data implies different regulation of microtubule cytoskeleton dynamics on the proteome and possibly phosphoproteome level. We plan to further analyze changes in microtubule dynamicity via live cell imaging techniques to correlate the proteomics data with functional changes.

Synthetic control of lateral compartmentalization in the yeast plasma membrane using micro patterned surfaces

Julia Wittmar (wittmarj@uni-muenster.de), Prof. Dr. Roland Wedlich-Söldner and Prof. Dr. Jacob Piehler

Institute for Cell Dynamics and Imaging, Münster, Germany Department of Biology/Chemistry, University of Osnabrück, Germany

The plasma membrane (PM) is the primary interface between a cell and its environment and plays a central role in numerous biological functions. To ensure an efficient coordination and regulation of all of these processes, the PM is laterally segregated into micro- and nanodomains with specific PM protein and lipid compositions. The budding yeast *Saccharomyces cerevisiae* is a well-studied model organism and offers manifold applicable genetic tools, making it therefore a perfect system to study the complex correlations and mechanisms of PM domain formation. Total internal reflection microscopy (TIRFM) allows to visualize the segregation of PM proteins in yeast cells into distinct domains.[1,2]

In experiments with HeLa cells chemically functionalized micro patterned surfaces have successfully been used to study the interaction and signaling transduction of transmembrane receptors in living cells. [3]

We aim to synthetically generate protein patterns at the yeast PM via micro patterned surfaces. The lateral segregation of PM proteins is artificially controlled by extracellular tags (His-Tag, Halo-Tag), anchoring them to chemical recognition units on functionalized surfaces (trisNTA, Halo-Tag-Ligand (HTL)). Here, removal of the yeast cell wall could be necessary for providing easier access to expressed tags and increasing the mobility of tagged PM proteins. In the following, the influence of different artificially induced protein clusters on the PM will be investigated, for example their impact on local lipid composition. Furthermore, the yeast polarity marker Cdc24, the guanosine triphosphate exchange factor (GEF) of the Rho-type GTPase Cdc42,[4] can be artificially concentrated in subdomains of the PM. Cdc24 recruits and activates Cdc42, which defines the future bud site of yeast cells, thus allowing detailed investigations of mechanisms in yeast cell polarization.

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Molecular mechanism of cytokine receptor activation and dysregulation by oncogenic mutations

Maximillian Hafer[1], Stephan Wilmes[1], Ignacio Moraga[2,3,4], Joni Vuorio[5], Julie Tucker[6], Sara Löchte[1], Stevan Hubbard[7], Ilpo Vattulainen[5], K. Christopher Garcia[3,4], Ian Hitchcock[6] and Jacob Piehler[1]*

[1] Division of Biophysics, University of Osnabrück, 49076 Osnabrück, Germany. [2]Division of Cell Signalling and Immunology, University of Dundee, UK. [3] Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, USA. [4] Department of Molecular and Cellular Physiology, and Department of Structural Biology, Stanford University School of Medicine, Stanford, California, USA. [5] Department of Physics, University of Helsinki, Finland. [6] Department of Biology, University of York, UK. [7] Skirball Institute & Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, US

The mechanism of cytokine receptor activation and its dysregulation by mutations has remained controversially debated. Paradigmatic members of homodimeric class I cytokine receptor family such as growth hormone (GHR), erythropoietin (EpoR) and thrombopoietin (TpoR) are currently believed to be pre-dimerized and activated by ligand-induced conformational changes. However, the precise mechanism, which triggers Jak activation of pre-formed dimers, has remained speculative. We have tackled this fundamental question by quantitative single molecule imaging in combination with mutational studies and molecular dynamics simulation, yielding a consistent molecular mechanism of cytokine receptor activation by ligand-induced dimerization. In order to resolve the spatiotemporal organization of homodimeric cytokine receptors at physiological expression levels, we have established dual-color single molecule imaging techniques. Using highly efficient posttranslational labeling techniques, monitoring diffusion and interaction of individual receptors in the plasma membrane of live cells with high spatial and temporal resolution. Receptor dimerization was reliably quantified by dual color co-tracking-analysis and complemented by single molecule FRET. Diffusion and interaction analysis at single molecule levels revealed that GHR, EpoR and TpoR are not pre-dimerized. Upon ligand stimulation, efficient receptor dimerization was detectable. Detailed quantification of receptor dimerization revealed stabilization of ligand-induced dimers by Jak2 that is mediated by its pseudokinase (PK) domain. Strikingly, the constitutively active Jak2-V617F mutation located within the PK domain stimulated ligand-independent receptor dimerization of GHR, EpoR and TpoR. Likewise, the constitutively active TpoR mutant W515L was pre-dimerized in absence of ligand. A comprehensive energy landscape obtained from these quantitative dimerization analyses revealed multiple, synergistic, weak interactions within receptor dimers that are readily triggered by the binding energy of the ligand to assemble the signaling complex pinpoint a molecular model of Jak activation by allosteric interactions between the PK domains. By mutational analysis we have identified the interface of PK-PK interactions in the signaling complex. Molecular dynamics simulation of the entire transmembrane and cytosolic part of the signaling complex pinpoint allosteric interactions between the PK domains. Our quantitative measurements and molecular models reconcile previous data to yield the firs conclusive molecular model of Jak activation by cytokine receptors and explain how individual disease-related mutations can constitutively activate signaling.

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Functional orientation of Rabs and effectors relative to membranes

Nadia Füllbrunn[1], Lars Langemeyer[1], Jacob Piehler[2], Changjiang You[2] and Christian Ungermann[1]

[1]Biochemistry Division, Department of Biology/Chemistry, University of Osnabrück
[2]Biophysics Division, Department of Biology/Chemistry, University of Osnabrück

Rab GTPases are one of the key proteins determining organelle identity[1]. All Rab GTPases cycle between an inactive GDP-loaded and an active GTP-loaded form. Specific guanine nucleotide exchange factors (GEFs) trigger the activation of Rabs by favoring the exchange of GDP for more abundant GTP. Once activated at their specific membrane, Rabs are able to interact with effectors, such as tethering factors, to promote fusion. In order to get inactivated, Rabs need GTPase activating proteins (GAPs) promoting GTP hydrolysis[2]. Furthermore, Rabs require C-terminal prenylation, which enables cycling between the cytosol and membranes. In the cytosol, a GDP-loaded Rab is chaperoned by the GDP Dissociation Inhibitor (GDI). At membranes, the Rab can be released, and GEF-triggered GTP-loading mediates stable membrane association[3]. Upstream of the prenylation site, Rabs have, in addition to their N-terminal GTPase-domain, a hypervariable domain (HVD), which differs in length between different Rabs, and is thought to be involved in association with the correct membrane[4].
While the overall machinery that regulates the activity and turnover of the late endosomal Rab7-like Ypt7 in yeast is well known, detailed understanding, how active Ypt7 and its effector HOPS are positioned relative to membranes during tethering, is lacking. To monitor prenylated Ypt7 on membranes, I generated functional, prenylated mNeon-Ypt7:GDI complex, which associates with membranes upon activation. By using an assay, which translates fluorescent lifetime into distance measurements, I first observed that the membrane distance of Ypt7 changes upon interaction with its GEF Mon1-Ccz1, possibly due to the flexibility of its hypervariable domain. Secondly, I found that GFP-tagged HOPS complex associates with membrane-bound Ypt7 in a preferred, upright position. Hence, my work provides a starting point to understand the functional orientation and structure of Rabs, regulators and effectors towards organelle membranes.

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Role of cell lysis in metabolic cross-feeding interactions in bacteria

Iqra Kasu, Piyali Pal Chowdhury and Christian Kost

Bacteria are commonly involved in cooperative metabolic interactions with other members of the microbial community. The exchange of nutrients between bacterial cells can either be mediated via diffusion through the extracellular environment, or, alternatively, involve contact-dependent mechanisms, such as the formation of membrane vesicles or intercellular nanotubes. Recent studies suggest bacterial cell-lysis might be one mechanism to form membrane vesicles, which in turn might be the building blocks for the lipid-based nanotubes. However, the molecular underpinnings of nanotube formation remain unclear. In order to understand the role of cell-lysis for the formation of nanotubes, we used a synthetically engineered model system, in which auxotrophic strains of *E. coli* are cocultured together with donor strains, from which they derive the required amino acid via nanotubes. A previously conducted transcriptome analysis, in which amino acid cross-feeding cells cultivated under nanotube-forming and non-forming conditions were compared, revealed five different genes with a known involvement in cell lysis were significantly up-regulated in nanotube-forming cells. Subsequently, these genes were individually deleted in the auxotrophic background to test whether cell-lysis plays any role in nanotube formation. Preliminary results show differential growth rates and fitness differences in lysis gene mutants relative to the auxotroph not carrying these mutations. These findings provide first evidence that the identified toxin-producing genes may indeed be required for establishing functioning nanotubes. However, further investigations are needed to establish the role of these genes for mediating cross-feeding interactions among bacteria.

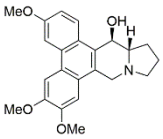
Targeting the Tumor Stroma of Triple Negative Breast Cancer

Irene Reimche

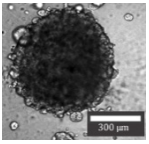
Department of Biomedical Sciences, Faculty of Human Sciences, University Osnabrück

Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer and is characterized by lacking expression of Her2, estrogen as well as progesterone receptor.[1] In TNBC, NFκB is constitutively activated[2] and the inflammatory tumor microenvironment is supported by tumor associated macrophages and cancer associated fibroblasts (CAFs).[3] Their secreted factors, *e.g.* IL-6 and IL-8, are crucial in the crosstalk between cancer and stromal cells.[4]

The molecule of the class phenanthroindolizidine alkaloids, tylophorinine, was found to be antiproliferative *in vitro* against pancreatic and against hepatocellular cancer cells, mainly depended on the suppression of NFκB signaling and the downregulation of cyclin D1.[5] In a TNBC model, inhibition of HIF has been observed[6], whereas, to date, the principle mode of action against breast cancer is still unknown.



Tylophorinine



3D TNBC monoculture

Thus, we further explored the biological activity of tylophorinine against TNBC based on (i) NFκB signalling in 2D TNBC monoculture using luciferase reporter assay, (ii) IL6 and IL8 expression in a 2D co-culture model composed of TNBC and PBMCs (peripheral blood mononuclear cells) performed by RT-qRT-PCR and (iii) growth evaluation in a 3D TNBC monoculture spheroid model. Our results revealed NFκB inhibitory capacity at the low nanomolecular range and showed inhibition of intercellular crosstalk between MDA-MB231 and PBMCs based on IL6 and IL8 secretion. In 3D monoculture, growth was inhibited in a dose-dependent manner. For further testing, we are currently reconstituting a 3D co-culture model of TNBC cells and primary CAFs.

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Understanding ECM assembly

Marcel Reinhardt, Yanina Post, Julia Heckmann and Achim Paululat

Department of Zoology and Developmental Biology

The extracellular matrix (ECM) plays a key role in multicellular organisms by mediating e.g. cell adhesion, cell communication and differentiation. The cardiac ECM harbors two unique proteins: Lonely heart (Loh), an ADAMTSL protein, and Pericardin (Prc), a collagen IV like protein. Both proteins are essential for proper heart development, as absence of the proteins is causing heart failure and cardiac collapse.

We have shown that Lonely heart recruits Pericardin towards the heart where Pericardin assembles into a preformed matricellular network (Drechsler *et al*., 2013). Presence and correct incorporation of Pericardin accounts for the correct balance of ECM stiffness versus elasticity, generating a heart tube that withstands lifelong heartbeats (Rotstein and Post *et al*., 2018, Wilmes et al. 2018). To investigate in depth, on how Lonely heart recruits Pericardin and how Pericardin establishes distinct ECM properties, I will use imaginal discs as an *in vivo* model. Imaginal discs represent larval tissue that develops into organs of the adult fly, e.g. the wing. I will express Lonely heart specifically in distinct areas of the wing discs, followed by a subsequent in-depth analysis of Pericardin recruitment. The advantage of this model is the intrinsic negative control (adjacent Loh-free tissue areas). The wing disc platform can unravel the mechanistic recruitment process and the self-assembly capability of Pericardin.

To better understand the dynamics of ECM assembly *in vivo*, I will continue initial experiments (Post, 2019) using live imaging. Aim is, to utilize lattice light-sheet microscopy tracking individual ECM proteins over time in living *Drosophila* embryos.

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Email: Marcel.Reinhardt@Biologie.Uni-Osnabrueck.DE

Molecular function of the BEACH domain containing protein Bph1p in the endosomal pathway

Prado Vargas Duarte

Department of Biology/Chemistry Biochemistry section

The Chediak Higashi syndrome is a rare autosomal recessive disorder which affects many tissues of the body. Patients with this disorder exhibit hypopigmentation of the skin, eyes and hair, prolonged bleeding times, recurrent infections, abnormal natural killer cell function and peripheral neuropathy. This disorder is caused by mutations in the LYST gene, which encodes for a proteins with a BEACH domain of unknown function. Proteins containing this domain have been identified in all eukaryotic species, suggesting a high degree of evolutionary conservation.

Bph1p is the *Sacharomyces cerevisae* ortholog of BEACH domain containing proteins. Unlike in higher eukaryotes, deletion of *BPH1* does not affect the morphology of organelles of the yeast endolysosomal pathway. However, the functionality of the pathway is affected, as the hydrolase Carboxypeptidase Y is secreted from the cell instead of being properly delivered to the vacuole[1]. In addition, cells lacking *BPH1* are hypersensitive to the drug calcofluor white and to the arginine analogue canavanine, which could be indicative of defective cell wall synthesis and/or recycling or endocytosis of plasma membrane proteins. However, the function of Bph1 at the molecular level remains unknown.

I will present evidence that Bph1 is found at endosomes, and requires its WD40 and BEACH domain for functionality. First insights into a possible function are discussed. My work provides a starting point to understand BEACH domain proteins at endosomes and their role in endosome and lysosome biogenesis in general.

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Mp*TCP1* controls cell proliferation and redox processes in *Marchantia polymorpha*[1]

Marek Deckena, Andrea Busch, Sabine Zachgo

Botany Department, Sabine Zachgo

TCP-transcription factors regulate plant growth and architecture through the modulation of cell proliferation and participate also in the control of several hormone signaling pathways in higher plants. In *Arabidopsis thaliana*, 24 representatives of this family are present, which can be grouped into the TCP-C (11) and the TCP-P (13) clade based on sequence analysis of the name-giving TCP-domain. While TCP-P genes are currently considered as positive regulators of cell division, TCP-C genes often repress proliferation. Opposing functions of the two clade members in the proliferation control have been deduced from angiosperm TCP analyses.

However, functional studies are hampered in Arabidopsis by redundancy effects. Compared to all sequenced embryophytes, the novel bryophyte model organism *Marchantia polymorpha* comprises the lowest number of *TCP* genes, namely only one *TCP-P* (Mp*TCP1*) and one *TCP-C* (Mp*TCP2*) gene[2]. Here, we investigate the ancestral function of the TCP-P-clade in *Marchantia polymorpha* applying a combination of genetic, biochemical, expression as well as DNA-binding studies revealing a function in cell proliferation and redox-regulation.

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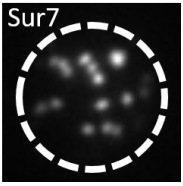
The role of tetraspanners in yeast plasma membrane organization

Daniel Haase

Institute of Cell Dynamics and Imaging, Muenster

Our understanding of plasma membrane (PM) organization has evolved substantially during the last decades. Many different models have been proposed to explain the mechanism of lateral segregation of lipids and proteins into distinct PM domains. Prominent examples include the fluid-mosaic model, the lipid-raft hypothesis and the picket-fence model. Many of the underlying studies on membrane organization have focused on *in vitro* systems. However, PM organization *in vivo* has still not been fully deciphered[1,2]. Therefore, we use yeast as a model to study fundamental mechanisms and principles of PM organization.

The yeast PM harbours at least five well known PM domains that are functionally, spatially and temporarily separated. The membrane compartment of Can1 (MCC) represents a very stable domain (image), which is associated with PM invaginations, furrow-like structures, termed eisosomes. Several metabolite permeases cluster within the MCC and their function and turnover are tightly linked to lateral segregation within the membrane[3]. Six members of a group of integral membrane proteins with four transmembrane domains (tetraspanners) are also concentrated in MCC patches. The proteins Sur7, Fmp45, Pun1, Ynl194c, Fhn1 and Nce102 have been linked to cell wall synthesis, sporulation as well as PM stress and lipid composition. However, deletion of individual tetraspanner genes did not result in strong phenotypes, so the biological functions of these proteins remain poorly understood. Here, we investigate the impact of tetraspanners on PM organization. We combine fluorescence microscopy (TIRF-Microscopy, Super-Resolution-Microscopy), electron microscopy, genetics and biochemistry to study the role of tetraspanners in *S. cerevisiae.* Our data suggest that these proteins limit and restrict the size and distribution of the MCC and help to establish specific subdomains of the MCCs. Furthermore, we want to elucidate how lipid segregation contributes to PM domain formation.



Membrane compartment of Can1 (MCC)

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Unraveling the working mechanism of a tumor suppressor lipid

Dina G. Hassan, Shashank Dadsena, Sergei Korneev, Markus Schneider, Joost C.M. Holthuis

Molecular Cell Biology Division, Department of Biology/Chemistry, University of Osnabrück, 49076 Osnabrück, Germany

Ceramides draw wide attention as tumor suppressor lipids that act directly on mitochondria to trigger Bax-dependent apoptotic cell death [1]. However, the underlying molecular mechanism is poorly understood. Combining photoaffinity labeling experiments with molecular dynamic simulations, we identified the voltage-dependent anion channels VDAC1 and VDAC2 as mitochondrial ceramide binding proteins [2]. Both channels harbor a membrane-buried glutamate that mediates direct contact with the ceramide head group. Loss of VDAC2 or substitution of its membrane-facing glutamate rendered human colon cancer cells largely resistant to ceramide-induced apoptosis. Intriguingly, association of VDAC1 or VDAC2 with hexokinase I, a protein with a pivotal role in promoting cell growth and survival of hyperglycolytic tumors, critically relies on the membrane-facing glutamate. Collectively, our data support a role of VDAC2 as direct effector of ceramide mediated-cell death and establish a novel molecular framework for unraveling how ceramides exert their anti-neoplastic activities. Our current efforts are focused on experimentally addressing this framework.

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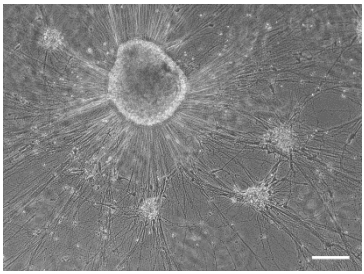
Human synapses on artificial substrate

Aleksandr Ivanenko, Nataliya Glyvuk, Henrik Renner, Jan Bruder, Jürgen Klingauf

Institut für Medizinische Physik und Biophysik

Presynaptic boutons induced on artificial substrate (Xenapses[1)], allow detailed investigation of synaptic transmission using TIRF (Total Internal Reflection Fluorescence) microscopy. Originally the technique of Xenapse induction was developed for mouse primary neurons. However, using only primary neuron cultures imposes a limitation on neuronal phenotypes being used: many neuronal phenotypes highly demanded in translational biomedical research (e.g. dopaminergic and serotonergic neurons) are notoriously difficult to isolate due to their low abundance in the brain.



Human stem cells derived neurons aggregate on artificial substrate. Scale bar: 100µm.

We extended the Xenapse induction technique to human neurons derived from induced pluripotent stem cells (IPSCs). Stem cell reprogramming methods allow derivation of many neuronal phenotypes[2] thus removing the limitation imposed by the usage of primary neuronal cultures. Moreover, applying Xenapse induction to patient-specific neurons enables translational research of human neurological diseases related to synaptic dysfunction (e.g. Alzheimer desease). Additionally, for the neurons releasing oxidizible neurotransmitter (e.g. dopamine) TIRF imaging can be combined with amperometric detection of exocytic events.

IPSCs are first converted to neuronal precursor cells(NPCs) and after a period of predifferentiation are transferred to glass coverslips, functionalized with synaptogenic proteins. Further maturation and Xenapse development are controlled by small molecules and neurotrophic factors.

By means of immunocytochemistry and electron microscopy we show that differentiated NPCs can form GABAergic Xenapses on the functionalized substrate. The protocol can be optimized to increase Xenapse formation efficiency, to shorten neuron maturation and to diversify the final neuron phenotypes.

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RUTBC1- an effector protein of Rab9a that activates GTP-hydrolysis by Rab32

Sabrina Kattelmann, Anna Lürick and Daniel Kümmel

University of Münster, Institute of Biochemistry

Ras-like Rab GTPases are small proteins that acts as molecular switches in cells. They can bind the nucleotides GTP as well as GD, which represents the active or inactive state of the protein, respectively. Their interconversion between active and inactive state is regulated by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP). In the GTP-bound state, Rabs can bind to effector proteins that include vesicle coats, adaptors for motor proteins or tethering factors, which comprises the molecular machinery of each trafficking step.

In humans, over 40 different TBC containing proteins are known that likely represent RabGAP enzymes. *In vitro* studies have shown that RUTBC1 is a GAP for Rab32 and 38. Both GTPases are cell type-specific and have been proposed as regulating the trafficking of melanogenic enzymes to melonosomes in melanocytes. Furthermore, RUTBC1 also binds as an effector to Rab9a in a nucleotide-dependent manner *in vitro* as well as in cultured cells. The Rab 32/38 GEF BLOC-3 also represents an effector of Rab9.

Based on structural prediction, we cloned and expressed the C-terminal TBC domain of human RUTBC1 in *E.coli*. To test whether the protein is still active, we performed a GAP assay and could observe moderate GTP hydrolysis with Rab32. Furthermore, we did a thermal shift assay to determine buffer conditions that lead to the highest protein stability in solution and will be tested for crystallization trials. Our goal is to determine the crystal structure of human RUBTC1 alone and in complex with Rab32 to get insights in the function as a GAP protein. We also investigated the measured activity of the Rab 32 GEF BLOC-3, which belongs to the family of longin heterodimer GEFs, revealing mechanistic similarity to the Mon1-Ccz1 complex.

How to make large vesicles

Christian Meyer, Kay Lammers, Franz Kahlich and Achim Paululat
(Christian.Meyer@Biologie.Uni-Osnabrueck.de)

Department of Zoology

The *Drosophila melanogaster* larvae possess a simple linear heart built up by two adjacent rows of cardiomyocytes with a total number of 104 cells with very little variation in cell number. The fly heart is subdivided into a posterior heart chamber and an anterior aorta section, separated by a single intracardiac valve formed by only two cells. The histology of this valve is characterized by the presence of large membranous vesicles in the 3rd instar larvae. Ultrastructural analysis revealed that these vesicles mature during development from embryo to the adult stage (Figure 1). Furthermore, preliminary studies point to the involvement of *rab* genes in this process as downregulation of the *rab5* gene results in miss-differentiated valve cells (Figure 2). In addition, we found that the expression of *rab* genes in the intracardiac valves differs from all other heart cells. Since they are key regulators of vesicle trafficking, participation of Rab family members in the formation of these vesicles appears likely.

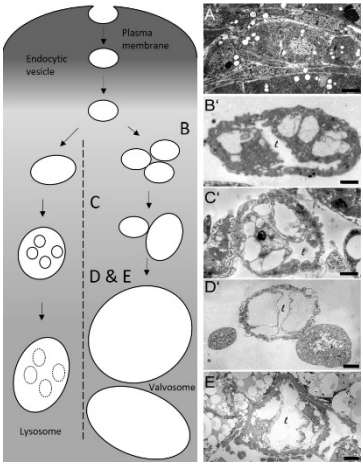

Figure 1: Simplified scheme of the endosomal-lysosomal pathway (left). Working model of vesicle formation in the intracardiac valve cells (right). 20h AED (after egg depositon) valves are indistinguishable from adjacent cardiomyocytes (A'). In the first- and second-instar larvae vesicle arise and occupy most of the cell volume in the third-instar (B'-D'). Valve cells of the adult display the same histology (E').

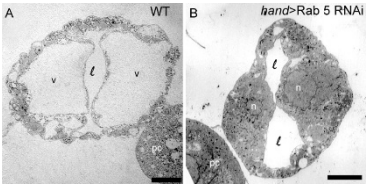

Figure 2: TEM cross-sections of intracardiac valve cells. A: Wildtype valve cells with large vesicles (v). B: Heart specific downregulation of rab5 leads to malformation of valve cells. Heart lumen (l)

References:

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The Intracellular Lifestyle of the Typhoidal *Salmonella enterica* Serovar Paratyphi A

Felix Scharte and Michael Hensel

Department of Microbiology, University of Osnabrueck, Germany

The *Salmonella enterica* serovars Typhi (STY) and Paratyphi A (SPA) are human-restricted pathogens that cause a severe systemic disease known as typhoid fever. Although studies using the broad-host serovar Typhimurium (STM), which causes a self-limiting gastroenteritis, provided insights in the understanding of the molecular pathogenesis of *Salmonella* in general, key differences between typhoidal and non-typhoidal *Salmonella* (TS and NTS) regarding their specific virulence mechanisms remain unknown. Rising infection rates in Asia and Africa and the emergence of multidrug-resistant TS emphasize the importance of basic research to understand TS biology and the development of anti-TS compounds.
Both TS and NTS are able to invade and replicate within host cells, including epithelial cells and macrophages. After successful invasion or phagocytic uptake, *Salmonella* resides in a membrane-bound compartment, the *Salmonella*-containing vacuole (SCV). The subsequent intracellular lifestyle is dependent on the translocation of effector proteins via a type 3 secretion system (T3SS) which is encoded on the *Salmonella* pathogenicity island 2 (SPI2). During the intracellular lifestyle, host cell membranes are manipulated by effector proteins of the SPI2-T3SS and *Salmonella*-induced filaments (SIFs) are formed. A unique characteristic of TS is the presence of many pseudogenes in their genome. The loss of gene function through pseudogene formation may affect various virulence functions. It is currently unknown, if observations made for STM, regarding the molecular pathogenesis, are applicable to the typhoidal serovars SPA and STY.

We were able to provide insights into the virulence profile of the typhoidal serovar SPA in unravelling unknown intracellular phenotypes in comparison with STM. SPA also resides in an SCV and shows SIF biogenesis. However, in contrast to STM, SPA is capable of intracellular movement in the host cell cytosol. The SPI2-T3SS is not necessary for this process, rather the intracellular expression of flagella. Furthermore, this study aims to clarify if intracellular flagella-dependent movement is crucial for evasion of the autophagosome machinery. The SPI2-T3SS of SPA is functional in the translocation of the investigated SPI2-T3SS effector proteins. However, further ultrastructural analyses and studies with a larger set of mutants are needed to enlight the specific virulence mechanisms of TS.

References:

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Notes

Notes

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Wed 16.10.		**Thu 17.10.**		**Fri 18.10.**	
11:45 – 12:30	Check-in & Registration	08:45 – 10:05	Session II	08:45 – 10:25	Session V
12:30 – 13:30	Lunch	10:25 – 12:05	Session III	10:40 – 12:30	Session VI
13:30 – 13:45	Opening Remarks	12:05 – 13:05	Lunch	12:30 – 12:45	Closing Remarks
13:45 – 16:20	Session I	13:05 – 14:25	Session IV	12:45 – 13:45	Lunch
16:20 – 16:35	Flash Talks I	14:25 – 14:40	Flash Talks II	14:15	Departure
16:45 – 18:00	Poster Session I	14:50 – 16:00	Poster Session II		
18:30 – 20:00	Dinner	16:15 – 19:00	Sightseeing Tour Bremen		
20:00	Pub Quiz	19:30	Dinner		