I R T G Integrated Research Training Group of the SFB 944

IRTG Retreat 2021

Borkum

03.11.2021 to 06.11.2021

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Program

Wednesday, 3. November 2021

16:45	Departure ferry from Emden harbor to Borkum harbor
18.55	Arrival at Borkum harbor and transfer to Borkum City
19:15	Registration and room distribution at Hotel Kachelot
20:15	Welcome Dinner at Hotel Kachelot

Thursday, 4. November 2021

7:00 - 8:10	Breakfast at Hotel Kachelot
8:20 - 8:30	Opening Remarks
Session I (Chair: Isabelle Watrinet)	
8:30 - 9:10	**Keynote: Sharon Tooze** (Francis Crick Institute, London): *Regulation of Mammalian autophagy: The role of the ULK kinase*
9:10 - 9:30	**Ann-Christin Borchers** (Biochemistry): *Functional analysis of the trimeric Mon1-Ccz1-Bulli Rab7-GEF*
9:30 - 9:50	**Maren Janz** (Zoology/Developmental Biology): *A trimeric Rab7 GEF complex is necessary for endosomal maturation in Drosophila nephrocytes*
9:50 - 10:10	**Eric Herrmann** (Structural Biology, Münster): *Structure of Mon1-Ccz1: differential targeting mechanism for activation of a RabGEF*
10:10 - 10:30	Coffee Break

Session II (Chair: Sophie Grziwa)	
10:30 - 11:10	**Keynote: Dagmar Wachten (**University Bonn): *Shedding light on ciliary signaling and function*
11:10 - 11:30	**Tolulope Sokoya** (Molecular Cell Biology): *Perturbation of sphingomyelin asymmetry and subcellular sterol distribution by pathogenic SMS2 variants*
11:30 - 11:50	**Carolin Körner** (Molecular Membrane Biology): *In vitro characterization of the serine palmitoyltransferase in S. cerevisiae*
12:00 - 13:00	Lunch at the Kulturinsel Borkum
13:00 - 13:25	**Flash Poster Presentations I**

Session III (Chair: Michael Philippi)	
13:25 - 14:05	**Keynote: Aymelt Itzen** (UKE Hamburg): *A biochemical approach to the understanding of post-translational modifications during bacterial infections*
14:05 - 14:25	**Stevanus Listian** (Molecular Infection Biology): *Impact of host cell lipid flows on mycobacterial infection*
14:25 - 14:45	**Aby Anand** (Molecular Infection Biology): *Induction of membrane contact sites and manipulation of host lipid transfer proteins by pathogenic mycobacteria*
15:10 - 16:45	Social Activity: Hiking tour to seals starting at Hotel Kachelot
17:00 - 18:30	**Poster Session I & Beverages**
18.30 - 19:15	**Meet the Speaker** Sharon A. Tooze, Dagmar Wachten, Aymelt Itzen (Speakers and PhD Students only)
19:45	Dinner followed by a Pub-Quiz at Hotel Kachelot

Friday, 5. November 2021

7.00 - 8:20	Breakfast at Hotel Kachelot
Session IV (Chair: Pascal Felgner)	
8:30 - 9:10	**Keynote: Martin Aepfelbacher** (UKE Hamburg): *Live cell and super-resolution microscopy of bacterial protein translocation pores*
9:10- 9:30	**Lena Lüken** (Microbiology): *Uncovering potential new adhesins of Salmonella enterica serovar Infantis*
9:30 - 9:50	**Benedikt Pauli** (Ecology): *Does auxotrophy trigger cell-cell attachment, which facilitates cross-feeding in bacteria?*
10:10 - 10:30	Coffee Break
Session V (Chair: Jimmy Villalta)	
10:10 - 10:50	**Keynote: Margret Bülow** (University of Bonn): *Organelle communication in neuron function and physiology*
10:50 - 11:10	**Marina Rierola** (Neurobiology): *Tau modulates microtubule and transport dynamics in the dendritic compartment of neurons*
11:10 - 11:30	**Daniel Bisinski** (Cellular Communication): *Cvm1, a novel component of multiple vacuolar contact sites*
11:30 - 11:55	**Flash Poster Presentations II**
12:00 - 13:00	Lunch at the Kulturinsel Borkum
13:00 - 14:30	**Poster Session II & Beverages**
14:30 - 16:30	Free time for social activity in small groups

Session VI (Chair: Lioba Winkler)	
16:30 - 17:10	**Keynote: Rifka Vlijm** (University of Groningen): *Live cell STED microscopy*
17:10 - 17.30	**Thomas Meyer** (Biophysics): *Regulation of JAK activation by the membrane environment*
17:30 - 17:50	**Annika Buhr** (Zoology/Developmental Biology): *Neprilysins maintain heart function via cleavage of SERCA-inhibitory micropeptides*
18:00 - 18:40	**Meet the Speaker** Martin Apfelbacher, Margret Bülow, Rifka Vlijm (Speakers and PhD Students only)
19:15	Dinner followed by Get-together at Hotel Kachelot

Saturday, 6. November 2021

7.00 - 8:20	Breakfast at Hotel Kachelot
Session VII (Chair: Samira Klössel)	
8:30 - 9:10	**Keynote: Stefan Linder** (UKE Hamburg): *Compartmentalized adhesion and phagocytosis in human macrophages*
9:10 - 9:30	**Jonas Olbrich** (Zoology/Developmental Biology): *The role of the APC/C adaptor Fzr on the dynamics of my-oblast fusion*
9:30 - 9:50	**Christian Meyer** (Zoology/Developmental Biology): *Membranous cellular compartments in specialized heart cells*
9:50 - 10:10	Coffee Break

Session VIII (Chair: Lara Jorde)	
10:10 - 10:50	**Keynote: Martin Graef** (MPI for Biology of Ageing): *Mechanisms and physiological functions of autophagy*
10:50 - 11:10	**Cilian Kock** (Botany): *The Pivotal Role of Glutathione for Redox Plasticity in Marchantia polymorpha development and stress response*
11:10 - 11:30	**Jens Koczula** (Botany): *MpTGA, together with MpNPR, is essential for induction of sexual tissues in the liverwort Marchantia polymorpha*
11:30 - 12:15	**Meet the Speaker** Stefan Linder, Martin Graef (Speakers and PhD Students only)
12:15 - 12:25	Closing remarks

12:25 - 15:30	**Check out and free time**
15:30	Transfer from Borkum City to Borkum harbor
15:45	Departure ferry from Borkum to Emden
17:40	Arrival at Emden harbor

Poster Index

Flash Poster Presentations I Thursday, 13:00		Flash Poster Presentations II Friday, 11:30	
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Poster Session I Thursday, 17:00		Poster Session II Friday, 13:00	
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Talk Abstracts

Session I

Keynote Talk
Regulation of Mammalian autophagy: The role of the ULK kinase

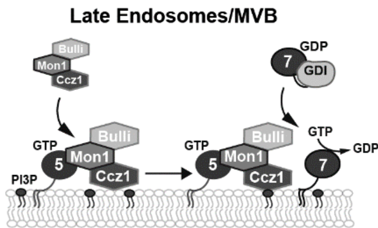
Sharon Tooze, The Francis Crick Institute, London UK

Macroautophagy (commonly called autophagy) is a highly conserved pathway acting to deliver cytosolic components to the lysosome (vacuole in yeast) for degradation. Yeast ATG (autophagy-related) proteins were the first discovered providing the template for identification of the mammalian homologues. Ireegardless of the component (protein, organelle, pathogen) sequestered or the mechanism (non-selective versus selective) the set of core autophagy proteins (ATGs) are required in both yeast and mammals. However, the autophagy-pathway specific membranes formed are different between yeast and mammals. Mammalian cells have a layer of complexity involving subcellular compartments such as the ER, endosomes and plasma membrane which are required for the initiation of the autophagosome and its fusion with lysosomes.
As in yeast, the mammalian ULK kinase complex (ATG1 complex in yeast) is the most upstream component in the autophagy signaling pathway. ULK1 and homolog ULK2, the sole serine/threonine kinases in autophagy transduce an array of autophagy-inducing stimuli to downstream autophagic machinery, regulating autophagosome initiation and fusion of autophagosomes with lysosomes. However, the exact mechanisms by which ULK1/2 regulates these diverse processes remains elusive. Most notably, the number of validated ULK substrates remains surprisingly low. We have identified new ULK substrates from a wide array of protein families and signaling pathways which support an expanded range of physiological roles for the ULKs. We focused on a new substrate, the VPS34 complex subunit VPS15 (p150). We identified sites on VPS15 and could show they regulated VPS34 activity and autophagy. By analysing VPS15-deficient mammalian cell models we discover novel aspects of ULK signaling with potential relevance in selective autophagy.

Functional analysis of the trimeric Mon1-Ccz1-Bulli Rab7-GEF

Ann-Christin Borchers, Department of Biochemistry, Osnabrück University

Small GTPases of the Rab family are key regulatory proteins in membrane trafficking processes by giving membrane identity and provide binding platforms. They act as molecular switches and require guanine nucleotide exchange factors (GEFs) for their activation.



Endocytic cargo marked for degradation reaches the lytic compartment via the endolysosomal pathway, a pathway characterized by the maturation of early endosomes to late endosomes, which then fuse with the lysosome. During this maturation process, early endosomal Rab5 is replaced by the late endosomal Rab7 in a so-called Rab-cascade. The heterodimeric Mon1-Ccz1 complex is the GEF for Ypt7 in yeast and Rab7 in higher eukaryotes. Lately, a third subunit associated with this complex was identified in metazoan, named Bulli in *Drosophila*. *In vivo* studies suggest that this third subunit plays a key role in endosome maturation, however, it does not affect GEF activity[1].

Recently, we showed that membrane associated Rab5 directly promotes Mon1-Ccz1 dependent activation of Rab7[2]. Further analysis revealed that Rab5 interaction and GEF-localization is regulated by kinases, but details and the underlying mechanism of the Rab5-GEF interaction are not yet understood.

We now identified the disordered N-terminal part of Mon1 as a potential regulatory region for this process. Systematic truncations of this region in the *Drosophila* Mon1-Ccz1-Bulli complex reveals enhanced GEF activity. We further show that a hydrophobic motif in the N-terminal part is involved in GEF autoinhibition. This data together with structural information of the complex supports a model in which the Mon1 N-terminal part directly restricts accessibility of the Rab5 or Rab7 binding site. Consequently, this regulatory region controls the Rab5-to-Rab7 transition process.

References:

1. Dehnen, Janz et al., Journal of Cell Science (2020) 133, jcs247080
2. Langemeyer et al., eLife 2020;9:e56090

A trimeric Rab7 GEF complex is necessary for endosomal maturation in *Drosophila* nephrocytes

Maren Janz, Department of Zoology and Developmental Biology, Osnabrück University

Drosophila nephrocytes are specialized cells displaying highest endocytic activity. As a functional adaptation they display enormous cell membrane expansions and membrane tubulation where the endocytic active site is situated. The huge size of the cells (50-100 µm) also ensures enormous storage capacity, e.g. for degraded proteins or metabolic components. Nephrocytes serve as an invertebrate model system for glomerular podocytes. We use Nephrocytes to investigate the trimeric Rab7 GEF (guanine nucleotide exchange factor) complex[1-3].

During endocytosis, Rab5 decorated early endosomes that mature later into Rab7 positive late endosomes. Subsequently, fusion with lysosomes leads to acidification and degradation of the lysosomal content/cargo. To function on membranes, Rab proteins are activated by GEFs and further inactivated by GAPs (GTPase activating proteins). Maturation of early endosomes to late endosomes and the fusion with lysosomes also depend on the two hetero-hexameric tethering complexes CORVET and HOPS. The CORVET complex is an effector of Rab5 whereas HOPS interacts with activated Rab7[4].

In *Drosophila*, a trimeric Rab7 GEF complex, containing Mon1, Ccz1 and Bulli is active. It was shown that the absence of Bulli results in impaired endosomal maturation and enlarged Rab7 positive and negative vesicles with clustered Rab5 inside these vesicles[5].

My aim is to understand the role of the trimeric Rab7 GEF in *Drosophila* nephrocytes and especially the role of the third subunit Bulli. Therefore, I analyzed endosomal maturation in these specialized cells of wild-type and mutant flies.

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.
2. Weavers, H., Prieto-Sanchez, S., Grawe, F., Garcia-Lopez, A., Artero, R., Wilsch-Brauninger, M., Ruiz-Gomez, M., Skaer, H., and Denholm, B. (2009). The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. Nature *457*, 322-326.
3. 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.
4. Huotari, J. and Helenius, A. (2011). Endosome maturation. EMBO J. 30, 3481-3500.
5. Dehnen, L., Janz, M., Verma, J.K., Psathaki, O.E., Langemeyer, L., Fröhlich, F., Heinisch, J.J., Meyer, H., Ungermann, C., and Paululat, A., A trimeric metazoan Rab7 GEF complex is crucial for endocytosis and scavenger function. Journal of Cell Science 133, jcs247080.

Structure of Mon1-Ccz1: differential targeting mechanism for activation of a RabGEF

Eric Hermann, Department of Biochemistry, University of Münster
Poster Session II, No. 17

Rab GTPases are peripheral membrane Proteins and act as molecular switches to give certain organelles certain identities. They can alternate between an inactive GDP (guanosine diphosphate) bound state and an active GTP (guanosine triphosphate) bound state. While in the inactive state Rab GTPases remain cytosolic, but after activation by Guanine nucleotide Exchange Factors (GEFs) they are recruited to membrane environment.
We solved the structure of the heterodimeric GEF Mon1-Ccz1 (MC1), which activates the Rab GTPase Rab7 (Ypt7 in yeast), via cryo electron microscopy and reveal a pseudo C2 symmetric protein complex, which has a unique domain architecture. In addition, we investigated the membrane association of MC1 with proteoliposome binding assays in vitro and complementation studies in yeast. While the binding to negative charged lipids like Phosphatidylinositol phosphates (PIPs) was described before, we were able to pinpoint the interaction site of a positively charged patch on the surface of Mon1. In addition, we reveal an amphipathic helix in Ccz1, which can sense lipid-packing defects by insertion into membranes
Thus, we reconstituted synergistic binding of MC1 to model membranes. The complex uses multiple lipid and protein interaction sites that are collectively required for membrane recruitment. Our data supports a model where MC1 integrates different inputs for proper spatiotemporal coordination in vivo.

Session II

Keynote Talk
Shedding light on ciliary signaling and function

Dagmar Wachten, Institute of Innate Immunity, Medical Faculty, University of Bonn

Cilia are membrane protrusions, emerging from the surface of most eukaryotic cells. Cilia are either motile, like the sperm flagellum, or immotile and named primary cilia. Primary cilia dysfunction causes severe human diseases, called ciliopathies. However, the underlying molecular mechanisms are ill-defined. Each cell has only one primary cilium, which is supposed to function as cellular antenna that senses extracellular stimuli and transduces this information into an intracellular response. Cilia constitute a unique microdomain, containing not only a certain protein repertoire and organization, but also forming a distinct membrane domain, whose lipid composition is different from the cell body. My lab has developed tools to specifically analyze and manipulate ciliary signaling and investigate the regulation by the lipid environment to understand how primary cilia control cell fate and function under physiological and pathological conditions.

Perturbation of sphingomyelin asymmetry and subcellular sterol distribution by pathogenic SMS2 variants

Tolulope Sokoya, Molecular Cell Biology Division, Osnabrück University

Sphingomyelin (SM) is a vital component of mammalian cell membranes that contributes to mechanical stability, signaling and sorting. Its production is catalyzed by Golgi-resident SM synthase (SMS)1 and Golgi/plasma membrane-resident SMS2. A widely held view is that bulk production of SM in the Golgi provides a sink for sterols synthesized in the ER to help create a SM/sterol gradient along the secretory pathway. This gradient marks a fundamental transition in physical membrane properties such as bilayer thickness and lipid packing density that segregates early from late secretory organelles [1,2]. The contrasting features of these properties likely provide specific cues for peripheral and integral membrane proteins that operate along the secretory pathway. However, this fundamental concept in cell biology awaits experimental validation. In collaboration with others, we identified mutations in SMS2 as the underlying cause of a congenital form of osteoporosis and found that these mutations enhance *de novo* SM synthesis by blocking export of a functional enzyme from the ER [3]. Using organellar lipidomics and fluorescent lipid reporters, we here report that expression of pathogenic SMS2 variants disrupts the SM gradient, breaks SM asymmetry and alters the subcellular distribution of sterols. Strikingly, SM accumulation in the ER does not affect the organelle's sterol content but causes a rise in phosphatidylethanolamine levels. By systematically probing the impact of pathogenic SMS2 variants on the protein and lipid landscapes of ER and PM, we aim to gain further insight into the functional implications of, and compensatory cellular responses to disease-induced imbalances in organelle-specific lipid codes.

References:

1. Bigay, J. & Antonny, B. Curvature, Lipid Packing, and Electrostatics of Membrane Organelles: Defining Cellular Territories in Determining Specificity. Developmental Cell vol. 23 886–895 (2012).
2. Holthuis, J. C. M. & Menon, A. K. Lipid landscapes and pipelines in membrane homeostasis. Nature vol. 510 48–57 (2014).
3. Pekkinen, M. et al. Osteoporosis and skeletal dysplasia caused by pathogenic variants in SGMS2. JCI Insight vol. 4 (2019).

In vitro characterization of the serine palmitoyltransferase in *S. cerevisiae*

Carolin Körner, Molecular Membrane Biology, Osnabrück University

Sphingolipids (SPs) are essential components of cellular membranes, which are predominantly found in the plasma membrane. While they play a significant role as structural components, they also act as signaling molecules in different pathways. The synthesis of sphingolipids starts in the ER with the condensation of L-serine and palmitoyl-CoA generating 3-ketodihydrosphingosine catalyzed by the serine palmitoyltransferase (SPT). In yeast, the SPT consists of two catalytic subunits Lcb1 and Lcb2 and the small regulatory subunit Tsc3.1 Additionally, the SPT forms a complex with the PI4P phosphatase Sac1 and the negative regulators Orm1 and Orm2, known as the SPOTS complex. The homeostatic regulation of the SPT involves the phosphorylation of Orm1/2 via the signaling cascade Slm1/2-TORC2-Ypk1/2.2 However, the exact inhibitory mechanism of Orm1/2 in yeast or the direct role of Sac1 in SP homeostasis is still unknown. Here we establish a protocol for the purification of the SPOTS complex from yeast cells and set up an in vitro SPT activity assay measuring free CoA released during the SPT-catalyzed reaction enabling the characterization of the SPOTS complex in vitro. Furthermore, we generated orm1∆ orm2∆ cells overexpressing a SPOTS complex with Orm1/2 phosphomutants preventing their dissociation and thus, allowing structural analysis of the entire SPOTS complex.

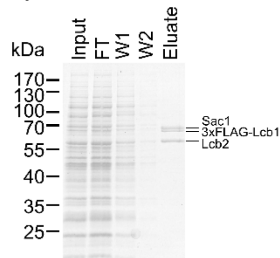


Figure 1. FLAG-based purification of the SPOTS complex with 3xFLAG-tagged Lcb1 subunit.

References:

1. Hanada, Kentaro (2003): Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. In Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1632 (1-3), pp. 16–30. DOI: 10.1016/S1388-1981(03)00059-3
2. Breslow, David K.; Collins, Sean R.; Bodenmiller, Bernd; Aebersold, Ruedi; Simons, Kai; Shevchenko, Andrej et al. (2010): Orm family proteins mediate sphingolipid homeostasis. In Nature 463 (7284), pp. 1048–1053. DOI: 10.1038/nature08787

Session III

Keynote Talk

A biochemical approach to the understanding of post-translational modifications during bacterial infections

Aymelt Itzen, Institute of Biochemistry and Signal Transduction, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Post-translational modifications (PTMs) are covalent alterations of proteins and are involved in the modulation of diverse cellular processes. Many pathogenic bacteria use a number of enzyme-mediated PTMs to ensure their survival, proliferation and virulence during infection. In this context, AMPylation is a PTM that is apparently used by many bacteria to modify host cell proteins. Adenosine monophosphate (AMP)-transferring enzymes catalyze the attachment of an AMP moiety from the co-substrate adenosine triphosphate (ATP) to threonine, tyrosine, or serine side chains. For example, the causative agent of Legionnaires' disease – the bacterium Legionella pneumophila – appears to manipulate vesicular trafficking by AMPylating the small GTPase Rab1.
However, understanding the substrate profile of AMP-transferases and biological impact has been hampered by the lack of general tools for enriching AMPylated target proteins. Furthermore, due to the low affinity of AMP-transferases for their respective substrates, obtaining mechanistic insights using structural biology methods is challenging. In my presentation, I will discuss our efforts in establishing tools to study AMP-transferases. Additionally, I will critically evaluate the significance of AMPylation in health and disease.

References:

1. Du J, Wrisberg MV, Gulen B, Stahl M, Pett C, Hedberg C, et al. Rab1-AMPylation by Legionella DrrA is allosterically activated by Rab1. Nat Commun 2021, 12(1): 460.
2. Fauser J, Gulen B, Pogenberg V, Pett C, Pourjafar-Dehkordi D, Krisp C, et al. Specificity of AMPylation of the human chaperone BiP is mediated by TPR motifs of FICD. Nat Commun 2021, 12(1): 2426.
3. Barthelmes K, Ramcke E, Kang HS, Sattler M, Itzen A. Conformational control of small GTPases by AMPylation. Proc Natl Acad Sci USA 2020, 117(11): 5772-5781.
4. Ernst S, Ecker F, Kaspers MS, Ochtrop P, Hedberg C, Groll M, et al. Legionella effector AnkX displaces the switch II region for Rab1b phosphocholination. Sci Adv 2020, 6(20): eaaz8041.
5. Gulen B, Rosselin M, Fauser J, Albers MF, Pett C, Krisp C, et al. Identification of targets of AMPylating Fic enzymes by co-substrate-mediated covalent capture. Nat Chem 2020, 12(8): 732-739.
6. Hopfner D, Fauser J, Kaspers MS, Pett C, Hedberg C, Itzen A. Monoclonal Anti-AMP Antibodies Are Sensitive and Valuable Tools for Detecting Patterns of AMPylation. iScience 2020, 23(12): 101800.

Impact of host cell lipid flows on mycobacterial infection

Stevanus A. Listian, Division of Molecular Infection Biology, Osnabrück University
Poster Session II, No. 29

Tuberculosis caused by *Mycobacterium tuberculosis* (Mtb) is responsible for 1.2 million deaths every year. Although it is well established that Mtb relies on host lipids during infection, the molecular mechanisms of how this pathogen remodels the lipid metabolic network of its host to support its persistent lifestyle are so far poorly understood. My key objective is to use the *Dictyostelium discoideum/M. marinum* model system to identify lipid species acquired by mycobacteria.
Since the lipid composition of *D. discoideum* is mostly unknown, we aim to determine all lipid classes synthesized by the organism using a mass spectrometry-based lipidomics approach. To verify this method, we performed lipid profiling of *D. discoideum* grown in the presence of fatty acids (FAs) to induce LDs. In line with previous TLC experiments, cells treated with FAs showed a significant increase of triacylglycerols (TAGs). In cells lacking both diacylglycerol acyltransferase (dgat1&2 DKO), i.e. enzymes that catalyze the last step of TAG synthesis, TAG levels were significantly decreased. Next, we will use LC-MS/MS to monitor changes in the *D. discoideum* lipidome during different stages of mycobacterial infection. To this end, infected cells will be enriched by FACS-sorting. These efforts will provide a first guide to lipid metabolic pathways of the host that are potentially exploited by mycobacteria. In addition, I aim to disrupt host-to-pathogen lipid flows using genetics or drugs to monitor the impact of specific lipid supply routes on mycobacteria infection. Interestingly, mycobacteria get access to host sterols by co-opting oxysterol-binding protein (OSBP) 8 that is localized at ER-MCV-contact sites during infection and presumably transfers sterols from the ER to the MCV in exchange for PI4P. When OSBP8 is depleted, the distribution of sterols and PI4P is completely altered: Whereas PI4P is redistributed to the Golgi apparatus, sterols are transferred to endosomes. During infection PI4P accumulates at the MCV indicating that the OSBP8-mediated ER-MCV sterol transport is blocked. Since in OSBP8- cells mycobacteria growth is increased, we hypothesize that the apparent block in sterol trafficking is compensated by the accumulation of sterols in endosomes that finally fuse with MCV. In the future we plan to use the established lipidomics pipeline to analyze the lipid composition of infected OSBP8- cells and cells treated with OSW-1, a drug that blocks the lipid-binding pocket of OSBPs and consequently inhibits sterol trafficking. We believe that these efforts may lead to new starting points for anti-Tb therapies by disclosing how mycobacteria exploit host lipids at the molecular level.

Induction of membrane contact sites and manipulation of host lipid transfer proteins by pathogenic mycobacteria

Aby Anand, Division of Molecular Infection Biology, Osnabrück University
Poster Session II, No. 15

Tuberculosis, caused by *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of death from a single infectious agent. Mycobacteria have perfected many ways to utilize host fatty acids and sterols as carbon and energy source to support their intracellular persistence. To investigate the molecular mechanisms underlying host lipid acquisition by mycobacteria, we use the *Dictyostelium discoideum*/*Mycobacterium marinum* infection system. Previous studies have shown that sterols accumulate in the *Mycobacterium*-containing vacuole (MCV) in *D. discoideum* and four of the twelve sterol transfer proteins were identified in a proteomic analysis of early MCVs. Using advanced imaging techniques, we have evidence that intracellular mycobacteria induce the formation of membrane contact sites (MCS) between host organelles and the MCV to co-opt lipid transfer proteins (LTPs): oxysterol binding protein (OSBP) 8 localized at the perinuclear ER, the Golgi apparatus and in the cytosol in non-infected cells, is recruited to ER-MCV and ER-cytosolic mycobacteria MCS. Strikingly, OSBP8 is not mobilized by bacteria that lack ESAT-6, suggesting that bacterial effector proteins that are secreted via ESAT-6 pores might be involved in OSBP8 recruitment. Many LTPs are targeted to MCS via interaction with phosphoinositides. We have found that the MCV accumulates PI4P and postulate that OSBP8 mediates sterol/PI4P counter transport at ER-Golgi-MCS analogous to yeast Osh4p. Consequently, during mycobacterial infection OSBP8 may deliver sterols from the ER to the MCV in exchange for PI4P providing the bacteria with sterols. Interestingly, mycobacteria growth is accelerated in OSBP8^{-} cells in which the distribution of the sterols and PI4P is completely disrupted. In contrast to OSBP8, OSBP7 localized in the cytosol and in the nucleus is not recruited to the MCV indicating some OSBPs are selectively hijacked by mycobacteria. We are now investigating how the MCV composition is altered in *D. discoideum* and *M. marinum* mutant strains to understand the molecular mechanism underlying LTP recruitment. In the future, we will apply fluorescent sterol probes as well as photoactivatable sterols to monitor sterols dynamics during infection. Hence, our study will provide insights on how the lipid trafficking machinery of the host is manipulated to transfer sterols to the MCV that might be used by the bacteria to drive their central metabolism.

References:

1. Barisch, C. *et al*., (2015). Lipid droplet dynamics at early stages of *Mycobacterium marinum* infection in *Dictyostelium*. Cell. Microbiol. 17, 1332–1349.
2. Guého, A. *et al*., (2019). Proteomic characterization of the *Mycobacterium marinum*-containing vacuole in *Dictyostelium discoideum*, bioRxiv 592717.

Session IV

Keynote Talk

Live cell and super-resolution microscopy of bacterial protein translocation pores

Martin Aepfelbacher, Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

The type 3 secretion system (T3SS)/injectisome is a complex multiprotein machinery of a variety of pathogens, including *Salmonella*, *Shigella*, and *Yersinia*. While the composition and structure of the central part of the injectisome, the needle complex, has been elucidated in great detail and at the highest possible resolution, much less is known about the transiently and dynamically attached parts of the injectisome, i.e., the sorting platform and the translocon/translocation pore. The translocon is formed by two translocator proteins that, upon contact of the bacteria with the host cell, form a pore in the host cell membrane that serves as an entry port for the T3SS effectors.
My group has used super-resolution- (e.g. STED, MINFLUX) and live cell microscopy to visualize the *Yersinia* translocator YopD during assembly and after integration of the *Yersinia* translocon into the host cell membrane. For this we engineered a short peptide ALFA-tag into the YopD protein which for the first time allowed both, to record translocation pore formation in living cells and produce MINFLUX images of YopD in fixed translocons at single molecule resolution.
In my talk, I will present this recent work, which reveals the temporal and spatial sequence of translocon formation in maturing host cell membranes and opens a possibility to observe bacterial structures with a 5-10-fold better resolution (about 5-10 nm) than previously possible with conventional super-resolution techniques.

Uncovering potential new adhesins of *Salmonella enterica* serovar Infantis

Lena Lüken, Department of Microbiology, Osnabrück University

Salmonella enterica serovar Infantis is one of the most commonly isolated serovars of *S. enterica*. Due to adaptive mutations as well as increased tolerance against oxidative stress and further resistance to antibiotics, *S*. Infantis has recently been emerging worldwide. This non-typhoidal *S. enterica* (NTS) serovar can infect a broad range of animal and human hosts. In particular, poultry and pigs are the main animal reservoirs for *S*. Infantis. To colonize and infect the different host species, the pathogen possesses a large set of virulence factors, such as adhesins. Most adhesins have not been fully characterized yet and many are even unknown. Since *Salmonella* has such a variable host spectrum, it is crucial to identify these adhesins and determine their role in the infection in order to inhibit *Salmonella* infections.

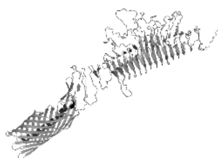


Figure 1: Predicted structure of putative adhesin YaiT from *S*. Infantis using AlphaFold2

We identified YaiT, BigA, RatA, RatB, SinH and SinI, which might function as adhesins, using a prediction algorithm (SPAAN) analyzing the entire *S*. Infantis proteome. Since the native expression conditions were unknown, we used an inducible expression system to initiate the biosynthesis of these putative virulence factors. For further characterization, we analyzed binding specifities, biofilm contribution and their influence in invasion.

In order to identify their function as virulence factors we aim to visualize the structure using cryo-TEM analyses, structure prediction and perform further specific binding assays. Furthermore, to analyze if they are associated with the outer membrane, we want to resolve their secretion mechanisms.

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Does auxotrophy trigger cell-cell attachment, which facilitates cross-feeding in bacteria?

Benedikt Pauli, Department of Ecology, Osnabrück University

A large proportion of microorganisms living in natural communities are auxotrophic, meaning that the corresponding strains cannot produce one or more essential metabolites. These auxotrophic organisms are bound to acquire the necessary metabolites from their surrounding environment. A common way to achieve this is via metabolic cross-feeding with strains in the local vicinity. However, organisms vary in their ability to sustain and support the growth of a respective partner due to their phylogenetic properties and metabolic capabilities. Therefore, partner choice mechanisms are important for determining the fitness of obligately dependent organisms. Moreover, once a suitable partner is found, it is vital to stay in close proximity to ensure a continuous supply with the required metabolites. One way to achieve this is by changing surface properties to increase the cell's stickiness.

However, whether or not auxotrophic bacteria are generally more prone to attach to surrounding bacterial cells than metabolically independent (i.e. prototrophic) cells remains elusive.

I will address this issue by systematically analyzing the clustering behavior of both auxotrophic and prototrophic strains in pairwise coculture experiments between two prototrophs or an auxotroph and prototroph. For this, I will use five different bacterial species (i.e., *Acinetobacter baylyi, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence,* and *Shewanella oneidensis*) that are either prototrophic or auxotrophic for one of three different amino acids (i.e. arginine, histidine, tryptophan).

In a first step, the growth behavior of pairwise intraspecific and interspecific cocultures will be investigated to pinpoint the most appropriate time points for the subsequent clustering analysis. Second, the prevalence and size of clusters will be assessed using a laser diffraction particle size analyzer. Using this approach, it will be possible to dissect the effect of the auxotrophy-causing mutation and species identity on the propensity to form clusters in pairwise cocultures. Third, I will test whether the phylogenetic distance between partners triggers cell clustering or show any species specificity. To achieve this, an assay will be conducted to quantify the cluster formation in cocultures of two auxotrophic strains competing for a wild-type donor. Lastly, it is planned to unravel the molecular processes that determine the increased tendency of auxotrophs to adhere to other bacterial cells.

The planned experiments will provide new insights into the factors regulating clustering in bacterial communities as a mechanism of partner choice and its effect on cross-feeding, a key process governing the fitness and stability of bacterial ecosystems.

Session V

Keynote Talk
Organelle communication in neuron function and physiology

Margret Bülow, University of Bonn, Life & Medical Sciences Institute

No organelle is an island – instead, organelles form dynamic contacts with each other and the plasma membrane to exchange material and signaling cues. One important type of organelle communication exists between mitochondria and peroxisomes. Peroxisomes assist mitochondria in fatty acid oxidation (FAO) and use the same machinery for fission and motility. We found that *Drosophila* mutants for the peroxisome biogenesis factor Pex19 deregulate mitochondrial FAO, which results in depletion of medium-chain fatty acids. This shortage induces a lipolytic program mediated by Hepatocyte nuclear factor 4 (Hnf4) and a Ceramide Synthase (CerS) with transcription factor activity, which leads to the depletion of storage fat and accumulation of free fatty acids (FFA) (Bülow et al. 2018; Sellin et al. 2018). FFA exert lipotoxic effects on mitochondria and lead to disturbed insulin secretion, which alters the nutritional programs in Pex19 mutants.
Contacts between the endoplasmic reticulum (ER) and mitochondria regulate mitochondrial dynamics and allow the exchange of phospholipids. Disturbed ER-mitochondria contacts contribute to failed mitochondrial quality control in Parkinson's disease. We found that Creld, an ER protein previously described in atrioventricular septal defect, is required for the formation of ER-mitochondria contacts in response to low respiratory complex I activity. Under wildtypic conditions, low complex I activity enhances ER-mitochondria contacts to promote phospholipid flux, which supports complex I function. In Creld mutants, constant complex I inactivity reduces H_2O_2 formation in dopaminergic neurons and impairs their function. We show that dopaminergic neuron inactivity drastically impairs locomotion in *Drosophila* and *Xenopus* Creld mutants and thus establish Creld as a novel model for Parkinson's disease.

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Tau modulates microtubule and transport dynamics in the dendritic compartment of neurons

Marina Rierola, Department of Neurobiology, Osnabrück University

The microtubule-associated protein tau has been widely studied in the field of neurosciences due to its relevant role in the development of several neurodegenerative diseases. Alterations in the function of tau have been broadly studied in the axonal process of the neuron, where the protein is highly enriched. Nonetheless, tau has also been localized in the neuronal dendritic arbor under physiological conditions, which makes it an interesting subject of investigation as tau's function might be compartment-specific. Hence, in the present study, we aimed to discern some of the effects of tau in the dendrites of pyramidal hippocampal neurons applying cutting edge techniques. Following Fluorescence Decay After Photoactivation, we observed that tubulin dynamics in dendrites of tau knockout (tau KO) primary neurons was increased compared to controls. In an authentic hippocampal tissue, pyramidal tau KO neurons displayed longer and more branched dendritic arbors. Altogether, this data would indicate that the microtubule array in the dendritic compartment is affected by tau. Mass spectrometry analysis on hippocampal cultured tissue showed the absence of compensatory expression of other structural microtubule-associated proteins that would intertwine previous observations, but revealed an unexpected high increase of α-synuclein expression in tau KO tissue. Using lattice light-sheet microscopy on hippocampal cultured tissue, we observed that microtubule-based transport in dendrites lacking tau displayed higher processivity, and that this event was mimicked by control neurons after overexpressing exogenous α-synuclein.

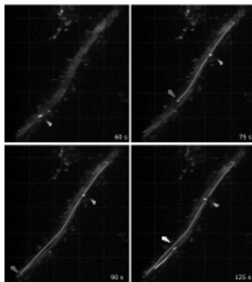

Image 1: Time lapse showing transport dynamics in a dendrite under the lattice light-sheet microscope. Arrows point to kinesin motors, while lines indicate their trajectories.

Consequently, our data show that tau protein modulates dendritic microtubule dynamics, and affects the expression of α-synuclein, that in turn alters the microtubule-based transport in dendrites, indicating a clear role of tau in this compartment.

Cvm1, a novel component of multiple vacuolar contact sites

Daniel Bisinski, Cellular Communication Laboratory, Osnabrück University, Germany

Membrane contact sites are areas of close apposition between the membranes of almost every organelle. They are specialized communication platforms with a variety of distinct functions in maintaining cellular homeostasis. Among others, the yeast vacuole forms a contact site with the mitochondrial network, named vCLAMP. Tethered by the interaction of Vps39 with the vacuolar protein Ypt7 and the mitochondrial protein Tom40, the molecular function of the vCLAMP and the existence of similar tethers remain elusive.

We have identified the protein of unknown function Ymr160w (Cvm1, Contact of the vacuole membrane 1) as a novel component and tether of vCLAMPs by the use of a systematic high content microscopy screen in Saccharomyces cerevisiae. We have shown that in addition to the vCLAMP Cvm1 localizes to contacts between the vacuole and peroxisomes (PerVale) as well as the nuclear endoplasmic reticulum (Nucleus-Vacuole Junction). We further characterized Cvm1 and described a role in sphingolipid homeostasis and have shown that the contact sites it forms are induced upon a decrease of sphingolipid levels. In sum, our study presents a novel machinery that forms multiple contact sites at the vacuolar membrane and supports a role of endolysosomal contacts in sphingolipid homeostasis.

Session VI

Keynote Talk
Live cell STED microscopy

Rifka Vljim, Faculty of Science and Engineering, University of Groningen, Groningen, the Netherlands

Cell division is an extremely dynamic process, and errors in this phase of the cell cycle have far-reaching consequences. Studying the protein dynamics at relevant scale is not trivial, as the dynamical interplay is at scales much smaller than the diffraction limit of light, and thus beyond the reach of traditional microscopes. Stimulated Emission Depletion (STED) microscopy on the other hand does allow imaging with a resolution of 30-40 nanometres even in cells.

Where STED has been used in studies with fixed cells for a long time at resolutions of 30nm or even better, live cell imaging for long posed problems as traditional dyes did not enter cells in a non-invasive manner, and fluorescent proteins are not stable enough. However, the development of a broad collection of novel fluorogenic dyes (which only fluoresce when specifically bound) which enter cells in a non-invasive manner now enable multi-colour live imaging. Further important developments also entail adaptive illumination strategies, which significantly reduce the light dose, crucial for light sensitive samples such as living cells.

In my presentation today I will discuss our latest results on improving live-cell STED by non-toxic photostabilizing agents, the possibilities of chemo-genetic tags with probe exchange for live-cell fluorescence microscopy, and our work on heterochromatin compaction states in the presence of HP-1.

Regulation of JAK activation by the membrane environment

Thomas Meyer, Division of Biophysics, Osnabrück University
Poster Session II, No. 21

Class I and II cytokine receptor signaling is activated via Janus family tyrosine kinases (JAK) that are non-covalently associated via membrane-proximal binding motifs of the intracellular receptor domains. Dysregulation of JAK activation is involved in multiple diseases including inflammatory and immune disorders as well as various types of cancer. We have recently developed an atomistic model of JAK2 activation that critically depends on interactions between the regulatory pseudokinase (PK) domains. Interestingly, this model suggested a conserved membrane interaction site within the FERM/SH2 (FS) domains of JAKs that is important for orienting JAK within the signaling complex.
This project aims (i) to uncover the principles of membrane interaction and its role in JAK association with cytokine receptors and downstream signal activation, (ii) to explore the regulation by the different membrane environment encountered during trafficking and (iii) to unravel how these features are impacting oncogenic JAK mutants. Using mutagenesis and live cell micropatterning, we have identified anchoring of JAK2 into the membrane via conserved hydrophobic residues that is supported by positively charged residues in the FERM-SH2 (FS) domain. Strikingly, mutations in the membrane binding site not only strongly reduced JAK2 recruitment, but also compromised its activity, in particular for a subset of constitutively activating, oncogenic mutants. Moreover, fluorescence microscopy revealed a key role of the JAK2 membrane binding site in efficient recruitment to receptors located in the plasma membrane, while much weaker receptor binding was observed in the ER. These results point to a confined JAK activity at the plasma membrane due to an interaction with negatively charged lipids, which is overcome by several, but not all constitutively activating mutations. To shed light into differential JAK2 dysregulation, we probed by flow cytometry their tyrosine kinase activity in the absence of the FS domains. These studies support the existence of at least two different mechanisms of constitutive JAK2 by mutations that differentially dysregulate PK domain interactions.

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Neprilysins maintain heart function via cleavage of SERCA-inhibitory micropeptides

Annika Buhr, Department of Zoology and Developmental Biology, Osnabrück University

Muscle contraction depends on strictly controlled Ca^{2+} transients within myocytes. A major player maintaining these transients is the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, SERCA. Activity of SERCA is regulated by binding of micropeptides and impaired expression or function of these peptides results in cardiomyopathy. To date, it is not known how homeostasis or turnover of the micropeptides is regulated.[1]
We found that the *Drosophila* endopeptidase Neprilysin 4 hydrolyzes SERCA-inhibitory Sarcolamban peptides in membranes of the sarcoplasmic reticulum, thereby ensuring proper regulation of SERCA. Cleavage was necessary and sufficient to maintain homeostasis and function of the micropeptides.
To analyze the consequences of Neprilysin-mediated hydrolysis at molecular level, we expressed SERCA-inhibitory peptides either with or without Neprilysin in Drosophila S2 cells. In line with the localization pattern in *Drosophila* muscle tissue, the peptides localized to ER membranes in absence of Neprilysin, but shifted into the cytosolic fraction if the peptidase was coexpressed. This indicates a reduced membrane anchoring of the hydrolyzed peptides. Analyses on human Neprilysin, sarcolipin, and ventricular cardiomyocytes indicated that the regulatory mechanism is evolutionarily conserved.
By identifying a neprilysin as essential regulator of SERCA activity and Ca^{2+} homeostasis in cardiomyocytes, these data contribute to a more comprehensive understanding of the complex mechanisms that control muscle contraction and heart function in health and disease.

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Session VII

Keynote Talk
Compartmentalized adhesion and phagocytosis in human macrophages

Stefan Linder, Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Macrophages are part of the innate immune system and among the first cells to counter invading pathogens. For this, they have to migrate to sites of invasion, necessitating a tight spatiotemporal control of cell adhesion and extracellular matrix degradation. Both functions are enabled by podosomes, actin-rich adhesion structures that are also sites of matrix metalloproteinase release.
At the site of infection, macrophages internalize pathogens by a tightly orchestrated chain of events, including formation of uptake structures and processing in maturing phagolysosomes.
This talk will give an overview of subcellular regulation of macrophage adhesion and invasion by podosomes, as well as pathogen clearance by phagolysosomes.

The role of the APC/C adaptor Fzr on the dynamics of myoblast fusion

Jonas Olbrich, Department of Zoology and Developmental Biology, Osnabrück University

Poster Session I, No. 30

In *Drosophila*, multinucleated somatic muscle cells are formed by the fusion of founder cells and fusion competent myoblasts. The asymmetric nucleation of F-actin at the contact site between both cell types, and the subsequent formation of a podosome-like structure that invades the founder cell and drives the fusion of the opposing cell membranes, are crucial for successful cell-cell fusion. Previously, we found that the APC/C adaptor protein Fizzy related (Fzr) plays a vital role during myoblast fusion1. However, the mechanism by which Fzr influences this process remains elusive. We now use live-cell imaging and advanced electron microscopy to study the influence of loss of fzr on the dynamics of actin nucleation at the myoblast cell contact sites, as well as the formation of podosomes during fusion, respectively. Preliminary data suggest that actin accumulation at the fusion site exhibits a two-step process. During a first phase we observe a swift accumulation of F-actin, which might not be sufficient to drive the entire process. The second, slower phase of nucleation seems to be vital to drive successful fusion. Prominently, this second wave of F-actin accumulation is lacking in most fusion sites of fzr mutants. Furthermore, actin accumulations in fzr mutants seem extremely stable, and might lack any dynamic changes. In order to visualize the membrane invasion of muscle cells during fusion, we fixed whole mount embryos by high pressure freezing and subsequent electron microscopy. Consistent with the altered actin nucleation dynamic at the fusion site, invading podosome-like fingers were greatly altered in fzr mutants. Compared to wild type, aberrant fusion sites exhibit small and stubby finger-like protrusions, which we think correlate with the stable actin foci found in our live imaging approach.
In the future we are going to use dual-color life imaging to test the dynamic behavior of other fusion relevant proteins at the fusion site in addition to F-actin. This might help us to understand the relation of actin nucleation and assembly of the fusion machinery at the membrane. Additionally, we will implement a genetic screening strategy, to search for interaction partners of Fzr.

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Membranous cellular compartments in specialized heart cells

Christian Meyer, Department of Zoology, Osnabrück University

The *Drosophila melanogaster* larvae possess a simple linear heart built up by two adjacent rows of cardiomyocytes with a total number of 104 cells. The 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 valvosomen that account for the dynamic cell shape changes that occur during heart beating. Using cell-specific RNAi-mediated knockdown we demonstrated that endocytosis plays an important role in valvosome formation as downregulation of the *rab5* gene results in miss-differentiated valve cells. Furthermore, the valvosomal membrane displays a similar PIP2-PIP3 phosphoinositide signature as the plasma membrane and we speculate that it can recognized as a potential target membrane for recycling endosomes to deliver membrane resources and allow valvosomal growth. 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.

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Session VIII

Keynote Talk
Mechanisms and physiological functions of autophagy

Martin Graef, Max Planck Institute for Biology of Ageing, Cologne, Germany

(Macro)autophagy is a highly conserved homeostasis and stress response pathway. Defined by the formation of a transient double-membrane organelle, autophagy displays an unparalleled capacity to degrade a broad spectrum of cytoplasmic substrates in a non-selective or highly targeted manner. The protein machinery and mechanisms underlying non-selective and selective forms of autophagy have been characterized with increasing detail. However, the scope and temporal regulation of the autophagy programs that are elicited by diverse stresses are poorly understood. Here, I will describe our work on the composite autophagy program induced by nitrogen starvation in budding yeast. Interestingly, we found that autophagy plays a multilayered role in maintaining mitochondrial homeostasis, sustaining biogenesis and, importantly, controlling network size providing a rationale for the existence and sequence of bulk and selective forms of autophagy. Our work shows the complexity and multifaceted roles of autophagy in order to maintain cellular function during nutrient stress.

The Pivotal Role of Glutathione for Redox Plasticity in *Marchantia polymorpha* development and stress response

Cilian Kock, Department of Botany, Osnabrück University

We are interested in the evolution of land plant redox processes and investigated the effects of altered GSH levels in the bryophyte model species *Marchantia polymorpha*. EMS point mutations of the *Arabidopsis GSH1*, the first enzyme of the glutathione synthesis, results in mutants with reduced GSH content revealing severe developmental defects.1
Genome editing approaches demonstrated a pivotal role for GSH in Marchantia, as no vital knockout mutants could be generated. Therefore, Mp*GSH1* transcript levels were reduced by an ami-RNA approach. In contrast to *AtGSH1* functional knockdown plants, these Marchantia plants revealed a normal development and can seemingly cope better with low GSH levels than *Arabidopsis*.
The redox sensor roGFP2-hGRX1 was introduced into this plant model system to investigate and visualize the cellular redox-potential during vegetative and sexual development. It could be shown that the redox potential is linked to proliferation and cell cycle processes. Marchantia seems to require a certain, yet unknown, threshold GSH level to maintain normal development, as only KD plants treated with the GSH synthesis inhibitor BSO show a severe phenotype.
Currently, we are addressing the interplay between hydrogen peroxide (H_2O_2) signaling and glutathione in Marchantia polymorpha during development. For that we started to establish the ratio metric H2O2 sensor HyPer7 into Marchantia and compare it with roGFP2.

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MpTGA, together with MpNPR, is essential for induction of sexual tissues in the liverwort *Marchantia polymorpha*

Jens Koczula, Department of Botany, Osnabrück University

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 streptophyte 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. In angiosperms such as *Arabidopsis thaliana*, TGAs regulate various developmental and stress-related responses. However, gene duplications and neofunctionalization during land-plant evolution resulted in functional TGA redundancy, hampering their analyses in angiosperms. The liverwort *Marchantia polymorpha* is the ideal model organism to get new insights in the regulatory network of TGA TFs. *M. polymorpha* possesses in contrast to *A. thaliana* (10 TGA TFs) only one single TGA TF, MpTGA and we aim to unravel the function of the sole Marchantia TGA TF in this informative bryophyte.
Interestingly, our Mp*tga* mutant analyses revealed that Mp*TGA* is a key regulator for sexual formation in *M. polymorpha* and that MpTGA exhibits also 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. Moreover, besides a key regulatory role in sexual development, MpTGA exerts an additional function is the response to stress-related processes. This observation was intrigued by the finding that the number of oil bodies is strongly increased in Mp*tga* knockout mutants. RNA seq analyses show an upregulation of terpenoid pathway genes, responsible for formation of these secondary metabolites which serve to deter pathogens and herbivores from liverworts.
Is MpTGA exerting these crucial and diverse regulatory function all alone?
The nonexpressor of pathogenesis-related genes 1 (NPR1) and related NPR1-like proteins are known in angiosperms as interaction partners of TGA TFs. Our expression and interaction studies of Mp*TGA* and the sole NPR gene in *M. polymorpha* (Mp*NPR*) support that both proteins interact *in planta* and *in vitro*. These findings, together with phenotypical analyses of Mp*npr* single and Mp*tga* Mp*npr* double mutants, as well as the respective overexpression mutants, demonstrate that MpTGA, in combination with the coregulator MpNPR, control the induction of sexual tissues in *M. polymorpha*.

Poster Abstracts

Poster Session I

Specific function of yeast Rab5-variants and their regulation via associated GEFs (Poster Index: 2)

Alexandra Nesterova, Department of Biochemistry, Osnabrück University

Rab-GTPases constitute a group of proteins involved in the regulation of endomembranous trafficking processes like the endocytic pathway. During endocytosis extracellular cargo is taken up via endocytic vesicles and transferred to early endosomes which are typically decorated by Rab5-GTPases. Recruitment of Rab5 is thought to be mediated by associated guanine nucleotide exchange factors (GEFs) which catalyze the exchange of GDP for GTP thereby activating and stabilizing the Rabs at the target membrane. Activated Rab5-GTPases associate with different effectors which can modulate the biochemical properties and drive endosomal maturation by recruitment of downstream effectors. Although compared to metazoan organisms *S. cerevisiae* is assumed to have a minimal endocytic pathway four Rab5-homologs, namely Vps21, Ypt52, Ypt53 and Ypt10, were identified[1,2]. Vps21 is assumed as the main Rab5-variant since its deletion leads to most pronounced impairments in cargo sorting for the vacuole[1]. The observed endocytic trafficking defects are further enhanced by co-depletion of Ypt52 and Ypt53, which indicates partial redundancy[1]. Despite this observation, functional differences of the variants are assumed after identification of few isoform-specific effectors. No significant growth or trafficking defects were observed in Ypt10-knockout cells and the function of Ypt10 remains unknown[2]. Furthermore, three Rab5-GEFs (Vps9, Muk1 and Vrl1) are encoded in the yeast genome, which likely regulate the activity of the Rabs. To evaluate the specificity of activation of the Rab5-variants by the different GEFs, this lab established *in vitro* GEF-assays in which GEF-mediated release of fluorophore-labeled GDP is quantified by fluorescence spectroscopy. First assays have been conducted but the purification of the components and the assay conditions require optimization. To increase the biological relevance, the GEF-assays will be modified by addition of liposomes and *in vitro* prenylated and chaperoned Rabs[3]. We plan to gather further cues on the different functions of the Rab5- and Rab5-GEF-isoforms by defining their interactomes via pull-down- and proximity labeling assays followed by mass spectrometry. These results together with the results from planed phenotype screens of deletion mutants and microscopic localization studies will aid deciphering the specific functions of the Rab5-isoforms.

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Spatio-temporal control of sphingolipid metabolism in *Saccharomyces cerevisiae* (Poster Index: 4)

Bianca Esch, Molecular Membrane Biology Group, Osnabrück University

Sphingolipids (SPs) are abundant and essential molecules and have critical functions as membrane components and as signaling molecules. SP biosynthesis begins with the condensation of serine and palmitoyl-CoA. A reaction catalyzed by the conserved enzyme serine palmitoyl transferase (SPT). The SPT is located in the endoplasmic reticulum (ER) and its activity is known to be controlled by multiple pathways including signals form the plasma membrane and the vacuole [1–3]. In yeast, the ER consists of the cortical and the nuclear ER. It was indicated, that the SPT activity varies at the different sites in the ER [2,4]. Although the SPT is a highly regulated protein, the role of its localization within the ER on its activity is still elusive. Here we developed a system to genetically re-wire sphingolipid metabolism in yeast and study its spatio-temporal organization. We successfully recruited the SPT to two different sites of the ER using organelle-targeted nanobodies *in vivo*. We recruited the SPT either to the cortical or to the nuclear ER via its catalytically active subunit Lcb2. We confirmed the recruitment of the SPT with all its subunits by microscopy. Using SP inhibitors in growth assays and in western blots to see differences in the phosphorylation of proteins inhibiting the SPT, we observe first hints for differences in SPT activity at the two localizations. By mass spectrometry-based flux analysis, we established a tool to directly observe differences in the *de novo* synthesis activity of the SPT as a function of its temporal and spatial localization within the ER. Our results demonstrate that yeast cells can be genetically manipulated to control the localization of a specific complex and that this tool can be used to determine its differential regulation within the cell.

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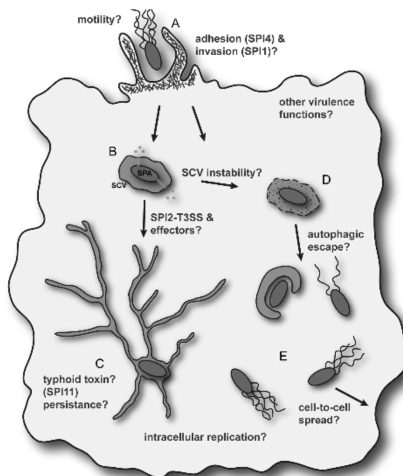
From vacuole to cytosol – Cytosolic motility of *Salmonella Paratyphi A* and its role in autophagy (Poster Index: 6)

Felix Scharte, Department of Microbiology, Osnabrück University

Salmonella enterica is a common foodborne, facultative intracellular enteropathogen. Typhoidal *S. enterica* serovars like Paratyphi A (SPA) are human-restricted and cause severe systemic diseases, while many *S. enterica* serovars like Typhimurium (STM) have a broad host range and in human hosts usually lead to a self-limiting gastroenteritis. There are key differences between typhoidal and non-typhoidal *Salmonella* in pathogenesis, but underlying mechanisms remain largely unknown.



Model of the intracellular lifestyle of SPA. After invasion (A), SPA is present in a *Salmonella*-containing vacuole (SCV) (B). It induces either the formation of *Salmonella*-induced filaments (SIF) in later stages of infection (C) or the SCV ruptures due to unknown membrane damage (D) and the expression of flagella is induced in the host-cell cytosol (E). Subsequent to flagella expression, SPA is capable of movement in the host cell cytosol and thereby may escape from the autophagosomal machinery or spreads to a neighboring cell. It is also possible that SPA remains dormant in one of the stages B, C or D.

We recently described the intracellular transcriptomic architecture and phenotypes during epithelial cell infection. Surprisingly, *Salmonella* pathogenicity island 1 (SPI1) as well as motility and chemotaxis genes showed distinct expression patterns in intracellular SPA vs. STM. Moreover, the induction of flagella genes by SPA lead to cytosolic motility and elevated SPI1 expression results in increased invasiveness of SPA, following exit from host cells.

Our results provide new insights into the virulence profile of SPA by unravelling previously unknown intracellular phenotypes and virulence traits. We report flagella-dependent cytosolic motility of SPA that neither depends on SPI2 nor on recruitment of host cell actin. The cellular consequences are currently under investigation, addressing evasion of xenophagy and cell-to-cell spread. We propose that cytosolic motility as possible autophagy evasion mechanism and higher invasiveness contribute to progression of infection and systemic dissemination in the host.

Quantifying cytokine receptor dimerization dynamics in the plasma membrane by single molecule FRET (Poster Index: 8)

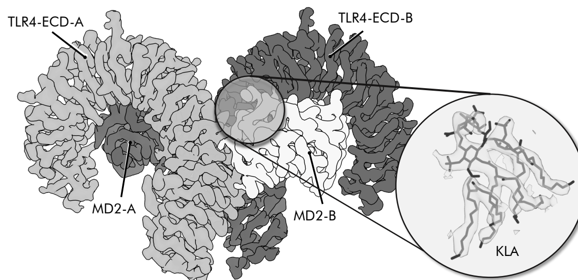
Hauke Winkelmann, Department of Biology and Center of Cellular Nano-analytics, Osnabrück University

Class I/II cytokine receptor (CR) signal via the JAK/STAT pathway, which is initiated by protein ligands that interact with two or more transmembrane receptor subunits. The spatiotemporal organization and dynamics of CR assembly in the plasma membrane (PM) is currently controversially debated. By dual-color single molecule (co-)tracking in living cells, we have identified ligand-induced dimerization as the key switch of signal activation for various members of the class I and class II families. However, we found weak intrinsic receptor dimerization affinity leading to significant ligand-independent dimerization at artificially elevated CR expression levels. Strikingly, ligand-independent dimerization was substantially enhanced by oncogenic mutations in CR or kinase. To pinpoint the interaction dynamics of CR dimers in the plasma membrane, we have established single molecule FRET (smFRET) imaging in living cells. For the thrombopoietin receptor, smFRET enabled unambiguous detection of transient ligand-independent dimerization and increased lifetime for constitutively activating mutations. To extend the potential of smFRET imaging of receptors, we increased the labeling precision by using engineered nanobodies. Combined with alternating laser excitation (ALEX) and homogeneous TIRF illumination we achieved unambiguous detection of CR association and dissociation events in the PM of living cells with high spatial and temporal resolution.

How to sense danger? Understanding Toll-like receptor 4 activation by endogenous and exogenous ligands (Poster Index: 10)

Jan-Hannes Schäfer, Structural Biology Division, Osnabrück University

Toll-Like Receptors (TLRs) are pattern-recognition receptors, sensing tissue damage and pathogen-associated marker molecules. TLR4 in complex with its co-receptor MD2 (Myeloid Differentiation Factor 2) is essential for recognizing lipopolysaccharides (LPS).[1] Despite their medical importance, the mode of pattern recognition and signal transmission is understudied and detailed information is missing. We set out to gain a better understanding of TLR4 signal integration across the plasma membrane for both endogenous and exogenous ligands by using cryo-EM. Here, we present the cryo-EM structure of soluble human TLR4-MD2 with short-chain LPS variant KLA at 3.0 Å resolution (cryo-EM data shown below). Consistent with crystallographic data, LPS-induced TLR4 adopts a characteristic m-shaped multimeric structure.
Amongst a growing number of putative TLR4 agonists, DNA-binding proteins like High Mobility Group Box 1 (HMGB1)[2] has been discovered to act as endogenous TLR4 activator in response to cellular stress. Here, we show experimental evidence of soluble TLR4 oligomerization with HMGB1 by using negative-staining TEM. The resulting oligomers induce the formation of m-shaped multimers, indicative of receptor dimerization and possible downstream immune response. The presented TLR4-MD2 complexes provide new insights into the relevance of native glycosylation in TLR4 activation. Future high-resolution structures of HMGB1-bound TLR4 oligomers may show alternative binding modes.



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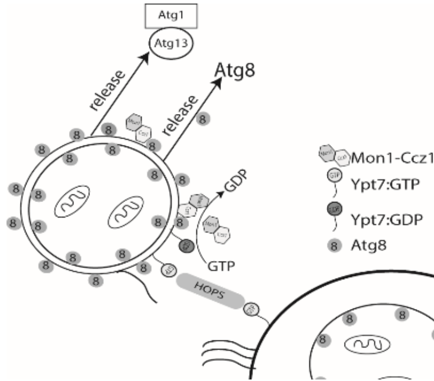
Regulation of the Mon1-Ccz1 GEF complex in autophagosome-vacuole fusion (Poster Index: 12)

Katharina Olschewski, Department of Biochemistry, Osnabrück University

Autophagy is a conserved catabolic pathway in eukaryotic cells. During autophagy, a newly formed organelle called autophagosome transports cytoplasmic material, organelles, pathogens or aggregates to the vacuole/lysosome for degradation (Nobuo *et al*. 2015). It is known that the fusion of autophagosomes with vacuoles requires SNARE proteins, the homotypic vacuole fusion and protein sorting (HOPS) tethering complex, the Rab7-like Ypt7 GTPase, and its guanine nucleotide exchange factor (GEF) Mon1-Ccz1. Recently, we showed that Rab5 on endosomes can directly promote Mon1-Ccz1 dependent Rab7 activation (Langemeyer *et al*. 2020). Mon1-Ccz1 also targets to the pre-autophagosomal structures by binding to Atg8 and phosphatidylinositol-3-phosphate (PI3P) (Gao *et al*. 2018). However, it remains unknown how Mon1-Ccz1 activity is regulated on autophagosomes. Here, we show that the autophagy-specific kinase complex of Atg1 and Atg13 phosphorylates Mon1-Ccz1. We identified phosphorylation sites by mass spectrometry, and generate the corresponding mutants by CRISPR-CAS9 to test their effect on autophagy. To investigate if membrane-bound Atg8 affects Mon1-Ccz1 activity we are currently establishing an *in vitro* GEF assay. We expect that our data reveals how Mon1-Ccz1 is differentially regulated in its activity on endosomes and autophagosomes.



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Functional and structural analysis of the human HOPS tethering complex (Poster Index: 14)

Caroline König, Department of Biochemistry, Osnabrück University

The HOPS tethering complex is a heterohexamer consisting of the subunits Vps11, Vps16, Vps18, Vps33 and the Rab interacting subunits Vps39 and Vps41 (Bröcker et al., 2012). Vps41 and Vps39 locate to opposite ends of the complex and bind to the Rab7-like Ypt7 protein in yeast. Metazoan HOPS does not bind Rab7, but is recruited to the surface of autophagosomes via the interaction of Vps39 with the GTPase Rab2a (Garg et al., 2011, Lund et al., 2018). The metazoan subunit Vps41 binds the Arf-like GTPase Arl8b on the surface of lysosomes (Hofmann and Munro, 2006). According to the prevailing model, the interaction of HOPS via Vps39 and Vps41 with Arl8b and Rab2a tethers autophagosomes with the lysosomes.

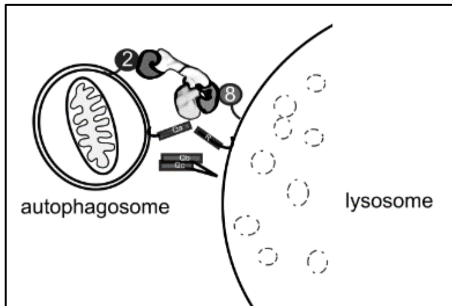

Figure 1: Regulation of fusion in mammalian cells.

So far, it is not understood how the human HOPS complex mediates tethering and fusion and what is necessary for the complex to function on membranes. For this purpose, I established the purification of the human HOPS complex from *Spodoptera frugiperda* cells. To determine whether the complex is well assembled, I analysed the complex regarding correct assembly of the six subunits via mass spectrometry and gel filtration. I now plan to investigate the structure of the holocomplex via electron microscopy in collaboration with the group of Arne Möller (Structure biology, Osnabrück University). To shed light on the activity of human HOPS I will establish *in vitro* tethering and fusion assays. Using a fully reconstituted system I will measure activity of HOPS in liposome tethering and fusion in dependence of GTPases and SNAREs. Our results will elucidate how the autophagosomal and endosomal fusion machinery is coordinated in mammalian cells.

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(Correlative-) Volume Electron Microscopy SBF-FESEM imaging to study rare events in whole cells and tissues (Poster Index: 16)

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Light microscopic visualization of live and fixed cells at low resolution using fluorescent markers, in combination of electron microscopic observation of fixed cells at near atomic-level spatial resolution, provides greater insight into subcellular protein localization and trafficking. Technological advances in regards of LM resolution, as well as volumetric EM methods open up the possibility of functional and ultrastructural analysis in three dimensions.

The Serial Block-Face Scanning Electron Microscope (SBF-FESEM) is an excellent way to visualize tissues and whole cells at high spatial and axial resolution. At nanoscale, complex ultrastructures of samples can be reconstructed and visualized in 3D. The pairing of this technique with light microscopic imaging modalities in the same sample can provide key information to relate cellular dynamics to the ultrastructural environments.

However, analysis of relevant events in biology, or dynamic changes in cellular functions utilizing CLEM approaches need consistent identification, selection and relocalization during all subsequent imaging and preparation steps. Development of reliable workflows utilizing fiducials and gridded dishes to make volumetric, correlative approaches broadly applicable is described and discussed, using different examples of cultured cells in *Salmonella* infection models.

Whereas imaging conditions are of crucial importance, nowadays data processing and subsequent image analysis needs equal amount of attention. In correlative and foremost volumetric approaches with large amounts of data being generated, the downstream workflows are often bottlenecks of entire projects. Prospects of semi-automatic processing and unsupervised machine learning approaches are therefore of special interest to CLEM experiments.

Cooperative interactions at nanoscale patterned signaling complexes in the plasma membranes (Poster Index: 18)

Michael Philippi, Institute for Chemsitry and New Materials, Osnabrück University, iBiOs CellNanOs electron microscopy unit, Osnabrück University

Spatiotemporal organization of signaling complexes at the plasma membrane plays an intricate role in regulating cell signaling. Multivalent interactions and liquid-liquid phase separation (LLPS) of proteins and lipids are emerging as key determinants of efficiency and specificity of downstream signaling. To explore these phenomena under well-defined conditions, we have here established capturing of signaling complexes in the plasma membrane of live cells based on surface functionalization by capillary nanostamping. Exploiting surface biofunctionalization with a bifunctional anti-GFP clamp, capturing of GFP-tagged membrane receptors into high-density nanodot arrays with a characteristic spot diameter of ~300 nm was achieved. This technique was utilized to explore the determinants of Wnt signalosome formation. GFP-tagged Wnt co-receptor Lrp6 expressed at the surface of living cells was successfully assembled into nanodot arrays. Strikingly, the co-receptor Fzd8 as well as the cytosolic scaffold proteins Axin1 and Disheveled2 were spontaneously recruited into the nanodot array to form spatially defined signalosomes in the absence of ligand pointing toward LLPS-driven signalosome assembly. Immunofluorescence staining confirmed ligand-independent Wnt/β-catenin signaling activated the nanodot arrays.

New insights into the role of the yeast casein kinase Yck3 in signaling endosome homeostasis (Poster Index: 20)

Sophie Grziwa, Biochemistry section, Department of Biology/Chemistry, Osnabrück University, Germany.

In eukaryotic cells, cargo endocytosed from the plasma membrane (PM) is transported via early endosomes and multivesicular bodies (MVB) to the lysosome. Recently, we identified a novel type of endosome called signaling endosomes (SE) in yeast, which is distinct from MVBs. SEs harbor the target of rapamycin complex 1 (TORC1), the EGO complex (EGOC) and the lipid kinase Fab1. One additional marker of SEs is the I-BAR protein Ivy1. Importantly, SE are involved in TORC1 signaling and maintaining lysosome integrity.

In yeast, the serine/threonine kinase Yck3 (yeast casein kinase homologue 3) is localized to the lysosome-like vacuole. It is involved in the regulation of several transport pathways, such as the AP-3 pathway and endosome-vacuole fusion events. Here we generate a kinase-dead mutant of Yck3 and identify Ivy1 as a novel target. Ivy1 localizes to endosomal structures and the vacuolar membrane. We now show that the localization of Ivy1 undergoes a dramatic change upon the phosphorylation by Yck3. Unphosphorylated Ivy1 localizes exclusively to signaling endosomes (SE), whereas it localizes mainly to the vacuole in its phosphorylated state. Our study suggests that Yck3 is involved in phosphorylation of the SE marker protein Ivy1 and probably in signaling endosome homeostasis.

Membrane Contact Sites: A study of protein recruitment mechanisms and dynamics (Poster Index: 22)

Lucía Amado, Cellular Communication Lab, Osnabrück University

Coordination and communication between organelles is essential for eukaryotic cells. One type of communication involves structures, called membrane contact sites (MCSs), in which membranes belonging to at least two different compartments of the cell come into a close proximity. Proteins that localize to these structures provide the functionality of the contact, allowing exchange of material between the compartments or attaching the organelles to specific positions in the cell. Many of these proteins achieve this localization by interacting with both membranes, forming a tether between them. Additionally, the membrane regions involved in MCSs formation likely have a differential lipid composition.
In this study, we analyze the recruitment of tethering proteins to contact sites and their mobility. Using a system to generate inducible artificial tethers, we show that the proteins that tether two membranes can affect each other in absence of specific binding. An initial tethering event can cause other tethers to coalesce to this structure, and restricting one tether to a specific subdomain of an organelle causes the restriction of the other tethers. We find that different contact sites contain tethers of different mobility and that this mobility is affected by several factors, including presence of other proteins at the MCSs, and the metabolic state of the cell.

Constructing artificial membrane structures on graphene to resolve membrane protein conformational dynamics
(Poster Index: 24)

Lara Jorde, Structural Biology Department, Osnabrück University

Mechanistic understanding of membrane-based processes requires detailed insights of the membrane environment and its impact on protein structure and function. Membrane properties such as fluidity, curvature, and lipid phase segregation have tremendous influence to membrane protein structure. To explore the conformational spectra of membrane protein on variable membrane structures, we tether liposomes as membrane curvature models and fuse them into planar lipid bilayer on graphene-coated glass substrate. For this purpose, ultra-small unilamellar vesicles (USUVs) are prepared with a mean hydrodynamic diameter of 40±15nm. Using biotin-streptavidin interaction, stable immobilization of the USUVs on graphene-supported lipid monolayer (GSLM) is confirmed by negative staining electron microscopy (EM). The USUVs can thus be used to integrate individual ABC transporter MsbA per liposome on graphene EM grid to determine the correlation of protein structure with membrane curvature. By fusing the USUVs on GSLM on glass substrate, fluorescence recovery after photobleaching reveals a fluidic membrane structure. Calibrating the axial resolution by graphene-induced energy transfer (GIET) using DNA origami confirms tethering of a lipid bilayer on GSLM. Based on these artificial membrane structures on graphene, we aim to combine single molecule fluorescence microscopy (FRET or GIET) and cryo-EM to correlatively investigate the time-resolved conformational dynamics of membrane protein in controlled membrane environment.

Rtc5 is a novel interactor of the vacuolar V-ATPase with a role in lifespan determination (Poster Index: 26)

Samira Klössel, Laboratory for Cellular Communication, Osnabrück University

V-ATPases are the main source of acidification of intracellular organelles, through hydrolyzing ATP and using the released energy for proton transport across membranes. V-ATPases play important roles in trafficking through the endocytic and exocytic pathways, as well as in cell aging and cell signaling via sensing and signaling amino acid availability to mTORC1. In this work, we identify the protein of unknown function Rtc5 as a novel interactor of the vacuolar V-ATPase in *Saccharomyces cerevisiae*, by cross-linking mass spectrometry of isolated vacuoles and co-purification approaches.
We show that the protein is N-myristoylated and that both this post-translational modification and the interaction with the V-ATPase are necessary for its localization to the vacuolar membrane. Strains lacking the *RTC5* gene or encoding for a mutant that cannot be N-myristoylated show altered chronological lifespan.
In summary, our work identifies the TLDc domain-containing protein RTc5 as a novel player in the biology of the vacuolar V-ATPase, possibly linking this complex to lifespan determination. Future directions will address the molecular mechanisms behind this link, and whether lysosomal TLDc containing proteins in higher eukaryotes are also interactors of V-ATPases.

Dissecting an N-Degron pathway in chloroplasts
(Poster Index: 28)

Lioba Winckler, Department of Plant Physiology and Protein Metabolism Laboratory, University of Osnabruck; Center of Cellular Nanoanalytics (CellNanOs), University of Osnabruck

The protein content of a cell needs to be constantly readjusted in order to preserve cell functions under changing environmental conditions. This is achieved by continuous protein synthesis and degradation along with mechanisms, which strictly regulate those processes. One pathway that mediates selective protein degradation is the N-degron pathway (previously known as the N-end rule pathway), which relates the in vivo half-life of a protein to the identity of its N-terminal amino acid (reviewed in [1]). Distinct versions of this pathway are present in the cytosol of prokaryotes and eukaryotes. In addition, organelle-specific pathways might also exist. Since chloroplasts are bacterially derived, it seems reasonable to speculate that chloroplasts contain an N-degron pathway similar to the one in prokaryotes.
Recent discoveries indicated that the N-terminal region affects protein stability in chloroplasts and provided support for an N-degron pathway in plastids (reviewed in [2]). Yet, the substrate selection and delivery mechanisms, as well as the factors that determine protein stability remain unidentified.
The aim of my PhD thesis is to investigate the impact of different N-terminal amino acids on chloroplast protein stability. Studying the stability of a protein dependent on its N-terminal residue requires a mechanism, which selectively exposes the amino acid at the N-terminus. In our case, a tobacco etch virus (TEV) protease-based approach will be used to activate the dormant N-degron of a reporter protein. This internal signal for both protein–protein interaction and degradation has to be exposed after translation from the precursor protein, which is per se not a target of destruction. Plastid transformation in tobacco will be used to generate plants with a plastid-encoded TEV protease. The reporter protein will be introduced into the transformed plants using a transient transformation approach. Following translocation into the chloroplast, the plastid localized TEV protease can cleave the reporter protein and expose the desired N-terminal residue. The outlined reporter system will be used to, for the first time, systematically and comprehensively challenge the effect of specific N-terminal amino acids on protein stability in this organelle.

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Poster Session II

The cardiac ECM assembly hierarchy (Poster Index: 1)

Marcel Reinhardt, Department of Zoology and Developmental Biology, Osnabrück University

The basement membrane (BM) is a specialized form of the extracellular matrix (ECM). It plays an essential role in multicellular organisms by mediating e.g. cell adhesion, cell communication and differentiation. The cardiac ECM is specialized by incorporation of 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 (Drechsler et al., 2013; Wilmes et al., 2018). Our former studies have shown that Lonely heart recruits Pericardin to the heart, or ectopically to other tissues, allowing for Pericardin network formation (Drechsler et al., 2013; Rotstein and Post et al., 2018).
The current work focusses on understanding the specialization and temporal assembly hierarchy of the cardiac ECM. Thus, our aim is to determine the time point at which Lonely heart and Pericardin were incorporated into the already canonical basement membrane containing Laminin, Nidogen, Collagen and Perlecan.
We utilized fly lines containing fluorescently labeled ECM proteins to follow their expression throughout Drosophila cardiogenesis. In particular, tagging Lonely heart on endogenous level, using CRISPR/Cas9, with the fast folding mKate2 protein became a promising tool.
Our data helped us to better understand the temporal assembly hierarchy proposing the necessity of an intact and fully finished basement membrane for Lonely heart anchoring and further recruitment of Pericardin. Additionally, FRAP analysis of the cardiac ECM revealed interesting new insights regarding Pericardin. Despite being a structural component Pericardin is more mobile and behaves more like Laminin, a linker protein.

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Function of the endosomal Rab-GAPs Gyp7 and Msb3
(Poster Index: 3)

Nadia Füllbrunn, Biochemistry Division, Osnabrück University

Rab GTPases are key regulatory proteins that determine organelle identity. Rabs cycle between an inactive GDP-bound and an active GTP-bound form and possess a C-terminal prenyl anchor, which enables cycling between the cytosol and membranes. 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 interact with effectors, such as tethering factors, to promote membrane fusion. For inactivation, Rabs need GTPase activating proteins (GAPs) promoting GTP hydrolysis. Inactivation results in extraction from membranes and chaperoning of Rabs in the cytosol by the GDP Dissociation Inhibitor (GDI). Along the endolysosomal system, Rab5 on early endosomes and Rab7 on late endosomes are highly conserved. During endosome maturation, Rab5 is exchanged for Rab7. In yeast, the Rab5-like GTPase Vps21 and the Rab7-like GTPase Ypt7 are in the center of this process termed as a Rab cascade. After fusion of mature endosomes with the lysosome-like vacuole, Ypt7 is present on the vacuolar membrane. Mon1-Ccz1 as the specific GEF activates Ypt7 on endosomes, while Gyp7 as the identified GAP inactivates Ypt7.[1] In addition, the GAP Msb3 inactivates Vps21 and Ypt7.[2]Tight regulation of Rabs, such as Ypt7 and Vps21, is of great importance to prevent premature activation and delayed inactivation on target membranes. While the activation of Ypt7 by Mon1-Ccz1 is well characterized, detailed understanding about the spatiotemporal inactivation of Ypt7 and Vps21 is lacking. To investigate the functional mechanism of the Ypt7 GAP Gyp7, I purified the protein from *E. coli* and used a liposome-based *in vitro* GAP assay. By monitoring the inactivation of Ypt7 and subsequent extraction from membranes by GDI, I first observed that Gyp7 has high GAP activity towards membrane-associated Ypt7. Secondly, I found that Gyp7 strongly binds to membranes and membrane composition plays a crucial role for the association. *In vivo*, Gyp7 localizes to endosomes, while Msb3 is found at the plasma membrane and in the cytosol. Importantly, relocalization experiments indicate that both GAPs can act on a Ypt7 pool at the vacuole, which results in vacuole fragmentation. This suggests that their activity at endosomes must be tightly regulated. Hence, my work provides the starting point to understand the spatiotemporal regulation of the Rab-GAPs Gyp7 and Msb3 during endosome maturation.

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Physiological phosphorylation of tau at disease-relevant sites is required for dynamic microtubule interaction (Poster Index: 5)

Nataliya I. Trushina, Department of Neurobiology, Osnabrück University

Ample evidence indicates that the hyperphosphorylation of tau at selected sites is associated with the development of tauopathies. However, it is less clear whether physiological phosphorylation at the same sites is required for normal tau function in the cell.
To address this question, we have produced and tested a phosphor-blocking tau construct (ALA tau) in which ten major phosphorylation sites, previously identified in PHFs from patients with Alzheimer's disease, were mutated from serine and threonine residues to alanine residues.
We performed fluorescence decay after photoactivation (FDAP) experiments in model neurons to determine the effect of the mutations on tau-microtubule (MT) dynamics. We observed that ALA tau diffused significantly slower with a drastically reduced dissociation rate of tau from MT compared to that of wild-type tau (WT). To study the effect on MT dynamics, we performed additional FDAP experiments with inducible expression of alpha-tubulin and co-expression with the respective tau construct. We did not see major changes in polymerized tubulin fraction.
To study functional consequences of the change in dynamic tau-MT interaction, we analyzed axonal transport in model neurons expressing WT or ALA tau. The mean speed did not show major changes. However, the processivity of transport in cells expressing ALA tau was increased compared to WT tau expressing neurons. Thus, phosphoblocking mutations influence transport properties.
To determine the potential influence of observed characteristics on neurons in an authentic CNS environment, we analyzed neuronal morphology upon viral-based expression of ALA or WT tau in CA1 and CA3 regions of mice hippocampi. We observed that ALA tau reduced the length of the apical dendritic tree in CA3 neurons and decreased spine density in CA1 neurons, indicating that neuron morphology is affected in a region-specific manner.
Since GSK3β is a kinase, which modulates tau phosphorylation at physiological and pathological sites, we determined the effect of its inhibition on tau-MT interactions. WT tau diffusion was significantly reduced upon GSK3β inhibition, similar to the effect of the ALA mutation.
The data indicate that physiological phosphorylation is required to ensure dynamic tau-MT interaction and suggest that hypophosphorylation can adversely affect the physiological function of tau.

High-pressure freezing and freeze substitution for correlative light and electron microscopy (Poster Index: 7)

Rico Franzkoch, Department of Microbiology, Osnabrück University, iBiOs – integrated Bioimaging Facility Osnabrück, CellNanOs – Center of Cellular Nanoanalytics Osnabrück

Conventional sample preparation for transmission electron microscopy (TEM) can induce a number of artefacts into the ultrastructure of the sample. Slow penetration of fixatives based on diffusion, selectivity of the cross-linking reactions and dehydration can lead to autolysis, cell structural changes, extraction of cellular material and shrinkage of the sample.[1] A step to overcome such drawbacks was the idea of using high-pressure freezing (HPF) and freeze substitution (FS). Cryo-fixation by HPF takes place within milliseconds and allows vitrifying biological samples of about 200 nm in thickness in their native state.[2] The following FS combines the improved preservation of cryo-fixation with resin embedding.[3] However, HPF/FS require expertise to obtain high quality uniform results in different biological specimens.

To correlate ultrastructural information with live-cell light microscopy (LM), correlative light and electron microscopy (CLEM) workflows including different LM and EM imaging modalities are necessary. CLEM allows location of rare and/or dynamic events by fluorescently labelled molecules within the ultrastructural context.[4]

In this study, we established advanced (3D)-CLEM workflows such as HPF/FS-CLEM of Salmonella infected HeLa cells and conventional CLEM with lattice light sheet microscopy. We strive to establish such advanced (3D)-CLEM methods for different model organisms of the SFB944. To achieve this, we need to optimize protocols for each of these model organisms to obtain high quality uniform ultrastructural preservation. We already established such advanced HPF/FS and/or CLEM workflows for investigation of *Salmonella* and *Salmonella* infected epithelial cell lines, *Dictyostelium discoideum*, *Drosophila melanogaster* and *Saccharomyces cerevisiae*.

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Identifying the rules that determine the establishment of metabolic cross-feeding interactions in bacteria (Poster Index: 9)

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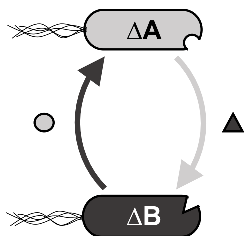
In their natural environment, bacteria commonly engage in ecological interactions, in which they exchange essential metabolites with other members of their community. Some bacteria obligately depend on such a metabolite exchange, as they are unable to autonomously produce certain metabolites and are auxotrophic for them. Interestingly, reciprocally exchanging metabolites among these auxotrophic bacteria can significantly benefit the whole consortium, relative to strains that are capable to autonomously producing these metabolites. However, the factors that determine whether or not a cross-feeding interaction between two bacterial cells can successfully establish remain elusive.



In our project, we aim at addressing this issue. For this, we use a synthetically designed system, where two auxotrophic genotypes of the bacterium *Escherichia coli* can only grow, when exchanging the required metabolite (here: amino acids) with their corresponding partner.

We hypothesize that the auxotrophy-causing mutation itself affects the spectrum and amount of amino acids produced by the corresponding mutant. To test this idea, we use mass spectrometry to profile the amino acid production of 11 auxotrophic mutants of *E. coli* when provided with the amino acid they require to grow. This dataset will help us generate theoretical expectations for how well this auxotroph can support the growth of other cocultured amino acid auxotrophs. These predictions will then also be experimentally tested by coculturing different auxotrophs and studying their growth patterns. In the future, we will also attempt to identify the metabolic principles dictating the growth of these auxotrophic partners when cross-feeding. For this, we look at different biosynthetic costs (e.g., glucose, high energy phosphate bond costs, etc.) for the production of each amino acid to explain the growth of the corresponding cocultures. In addition, we will also test if the metabolic distance (the distance between the biosynthetic steps of the amino acids that the two partners are auxotrophic for) between both interaction partners can explain the efficiency of cross-feeding.

Taken together, this study will help to uncover the basic principles governing the establishment of metabolic cross-feeding interactions in bacteria. The gained insights will contribute to a better understanding of the mechanisms that determine the assembly of natural bacterial communities as well as aid the design of synthetic microbial communities for biotechnological applications (e.g. bioremediation).

Structural and Biochemical Characterization of the Tri-Longin-Domain-Rab GEF Inturned:Fuzzy (Poster Index: 11)

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The Rab family of small GTPases plays a pivotal role in the organization of intracellular trafficking and maintenance of organelle identity. The spatiotemporal control of GTPase activity requires guanine nucleotide exchange factors (GEFs), which act as the activators of small GTPases. Recently, the Tri-Longin-Domain-Rab (TLDR) GEFs has been identified as a sub-family of GEFs based on bioinformatic analysis1+2. The TLDR GEF family comprises the heterodimeric complexes Mon1-Ccz1, BLOC-3 and Inturned-Fuzzy in metazoans, and the Mon1-Ccz1 complex is conserved all the way to yeast. We previously determined the unique mechanism how nucleotide exchange is facilitated by Mon1-Ccz1 from structural and biochemical insight into a catalytic core complex3.
Continuing along these lines, we are currently establishing the similarities and differences between Mon1-Ccz1 and the related BLOC-3 and Inturned-Fuzzy complexes on a structural and functional level. We ask whether the catalytic mechanism is conserved between them and explore the interactome of the complexes to understand how they participate in distinct cellular processes.
A unique feature within the TLDR GEF family is the N-terminal PDZ domain of Inturned (figure 1).

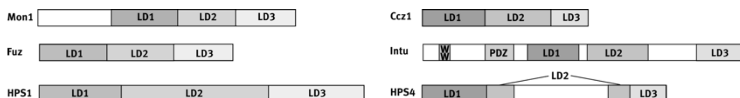

Figure 2: Domain architectures of TLDR GEFs

PDZ domains are a common protein-protein interaction motif and facilitate target binding with affinities in a micromolar range. To determine whether or not the Inturned-PDZ belongs to one of the three canonical classes of PDZ-domains, we are investigating it’s structure and work towards identifying binding targets by structural, bioinformatic and biochemical approaches.

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Role of mycobacterial (membrane) lipids in phagosome escape of Mycobacterium marinum in Dictyostelium discoideum
(Poster Index: 13)

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, causes 1.7 million deaths every year. Its complex cell wall lipids are crucial for the survival and persistence of the bacteria in professional phagocytes. Recently, it was shown that phthiocerol dimycocerosate (PDIM), a lipid of the outer mycobacterial membrane, is involved in phagosomal escape of bacteria. To synthesize membrane lipids during infection, Mtb utilizes mainly host-derived free fatty acids (FFA). However, FFAs need to be activated by fatty acyl-CoA ligases (FACLs) and fatty acyl-AMP ligases (FAALs) to provide activated FAs for protein acylation, energy generation, phospholipid and membrane lipid synthesis, respectively. FA metabolism of Mtb plays a key role during infection, however, it remains poorly understood how Mtb exploits and activates FAs. Interestingly, a deficiency of FACL6 was recently observed to severely attenuate intracellular growth of *M. marinum* in *Dictyostelium*. We hypothesize that an inhibition in FACL6 leads to earlier phagosomal escape of the bacteria caused by upregulated membrane lipid synthesis (including PDIM). Consequently, my project aims to characterize the function of FACL6 in lipid synthesis using the accepted *Dictyostelium*/*M. marinum* model system. I will investigate the impact of the depletion of FACL6 on the infection cycle as well as on the lipidome of *M. marinum* under in vitro and in vivo conditions.
As it was recently observed that the *Dictyostelium* enzyme long-chain fatty acid synthetase (LC-FACS) 1 is recruited to the Mycobacterium-containing vacuole (MCV), I will also investigate the role of host FA-activating enzymes during infection. Strikingly, absence of LC-FACS 1 and 2 affects the synthesis of intracytosolic lipid inclusions, i.e. lipid storage compartments, in *M. marinum*.
To characterize FA flow and metabolism in mutants of host and bacteria depleted in FA-activating enzymes during infection, I will establish a protocol that combines the use of bifunctional FA probes with expansion microscopy and lipidomics.

The role of membrane composition in JAK binding (Poster Index: 19)

Isabelle Watrinet, Department of Biophysics, Osnabrück University

Janus kinases (JAK) family proteins are associated to intracellular binding motifs in membrane proximity of Class I and II cytokine receptors mediating ligand induced signaling via the JAK/STAT pathway. Malfunction in JAK activation can lead to severe diseases including abnormal immune responses and various types of cancer. This project aims (i) to establish an *in vitro* essay to investigate JAK association to cytokine receptors reconstituted in polymer supported membranes (PSM) and (ii) to study the influence of charged lipids on the stability of the receptor-kinase-complex. Recent all-atom MD simulations of JAK2 bound to TpoR predicted a membrane interacting region consisting of a conserved Lysin residue and a batch of positively charged residues in the FERM-SH2 domain (FS) that seems to be important for orientation of the kinase within the complex. [1] Live cell micropatterning experiments with mutations in this region especially introducing charge-reversed residues revealed drastic reduction in kinase recruitment. Reconstitution in PSMs enables tight control of lipid composition as well as regulated protein concentrations. For this purpose, we purified the transmembrane and intracellular domain of TpoR as well as the FS of JAK2 aiming to determine proper parameters for *in vitro* reconstitution and binding measurements in different membrane environments.

References:

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Conserved secretion mechanisms of giant adhesins SiiE of *Salmonella enterica* and LapA of *Pseudomonas* spp. (Poster: 23)

Pascal Felgner, Division of Microbiology, Osnabrück University

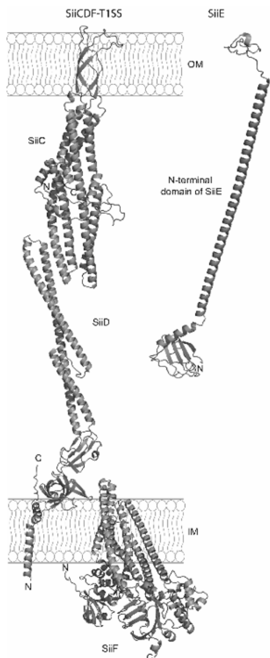

Figure 1: Schematic overview of modelled SPI4-T1SS subunits SiiCDF and N-terminal part of SiiE. Proteins modelled by trRosetta3 (TM score > 0.5 = high confidence).

Type 1 secretion systems (T1SS) are characterized by their structure, consisting of an ABC transporter, a periplasmic adapter protein (PAP) and an outer membrane protein (OMP). Some functions and mechanisms are homologous to each other and conserved. Most T1SS secrete their substrates in one-step, but also two-step secretion is described for substrates like biofilm-associated adhesin LapA of *Pseudomonas* spp1. The virulence-associated adhesin SiiE of *Salmonella enterica* encoded by genes on *Salmonella* pathogenicity island 4 (SPI4) is known to be retained on cell surface during secretion. SiiE mediates first close contact to the host cell membrane, essential for successful invasion of the pathogen, representing a special microenvironment2. However, the detailed mechanisms for retention and release of SiiE are still not known: Are there conserved steps in secretion comparable to other known T1SS? We showed by various overexpression experiments, 3D modelling, Cryo-TEM analyses and outer membrane (OM) isolation that SiiE is retained in the OM and that the SPI4-T1SS dissociates during secretion process. Furthermore, we found a potential retention domain in the N-terminal part of SiiE that seems not to be secreted. Additionally, the accessory proteins SiiAB are involved in steps before retention in the OM. With these results, we gained new insights in SiiE secretion process and can limit possible modes of action of SiiE during adhesion and invasion process.

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Molecular dissection of a sphingomyelin-dependent lysosomal repair pathway (Poster Index: 25)

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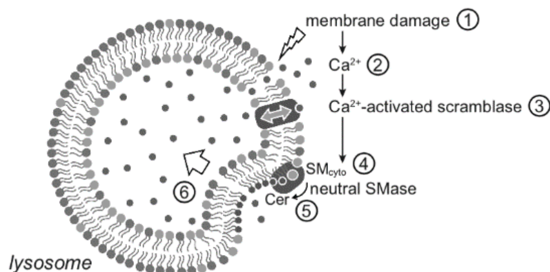
Lysosomes are vital organelles vulnerable to injuries from diverse materials. Failure to repair or sequester damaged lysosomes poses a threat to cell viability. We recently found that cells exploit a sphingomyelin-based lysosomal repair pathway that operates independently of ESCRT to reverse potentially lethal membrane damage[1]. Various conditions perturbing organelle integrity trigger a rapid calcium-activated scrambling and cytosolic exposure of sphingomyelin. Subsequent metabolic conversion of sphingomyelin by neutral sphingomyelinases on the cytosolic surface of injured lysosomes promotes their repair, also when ESCRT function is compromised. Conversely, blocking turnover of cytosolic sphingomyelin renders cells more sensitive to lysosome-damaging drugs. Our data indicate that calcium-activated scramblases, sphingomyelin, and neutral sphingomyelinases are core components of a previously unrecognized membrane restoration pathway by which cells preserve the functional integrity of lysosomes. Using photoactivatable lipid probes, organellar lipidomics and advanced live cell imaging approaches, we now aim to uncover how sphingomyelin-dependent lysosomal repair is accomplished at the mechanistic level.



Model illustrating how Ca^{2+}-activated sphingomyelin scrambling and turnover may promote restoration of damaged lysosomes.

Reference:

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Mitochondrial heterogeneity during differentiation (Poster Index: 27)

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Eukaryotic, non-green cells are able to generate ATP by glycolysis and oxidative phosphorylation electron transport chain (OXPHOS-ETC) of the inner mitochondrial membrane. Mitochondria are essential organelles with multiple functions, especially in energy metabolism where the five complexes (CI-CV) and the two mobile electron carriers (coenzyme Q and cytochrome c) of the OXPHOS-ETC system work together to synthesize ATP, and it is commonly accepted that they are not equivalent in their function and activity within cells[1]. In differentiated cells, they have to meet different requirements depending on their localization[2]. In the present study we investigated parameters of mitochondrial bioenergetics and how those change during the process of differentiation. As model cell lines we used human induced pluripotent stem cells (hiPSC)-derived neurons and murine epidermal keratinocytes (MEKs). For this, the polarized neurons measured in several stages of differentiation were segmented into three cellular compartments- perinuclear, axonal and growth cone. Furthermore, we investigated changes in gene expression as well as mitochondrial function using extracellular flux analysis. We found a drastic decrease in mitochondrial mass in differentiated MEKs, accompanied by the observation that they rely mostly on glycolysis. The differentiated neurons displayed remarkable heterogeneity in all measured parameters, however, gene expression analysis suggested an increase in mitochondrial biogenesis as well as in formation of respiratory supercomplexes of the oxidative phosphorylation system. Membrane potential levels showed no spatial heterogeneity, indicating that there were no differences between the three cellular regions. However, throughout the differentiation process, changes were observed, suggesting temporal heterogeneity. Due to the lack of mitochondrial function, studies in the MEKs will be discontinued. The following investigations will focus on supercomplex formation in differentiating neurons using molecular biosensors and Fluorescence Lifetime Imaging Microscopy (FLIM).

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Membrane assembly of GSDMD pores by single molecule imaging analysis (Poster index: 31)

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Proteins of the gasdermin (GSDMs) family are involved in inflammatory diseases and in cancer, and are promising therapeutic targets. The Gasdermin D (GSDMD) family member is characterized by a C-terminal (CT) auto-inhibitory domain and an N-terminal (NT) functional domain. The NT domain is released upon processing at the NT-CT linker by inflammatory caspases and assembles at the cell membranes creating pores. GSDMD pore structural analysis reveals a 33-subunit ring forming a negatively charged conduit through the membrane promoting cytokines release and cell lysis during pyroptosis. Although a few structural studies suggested a transition from intermediate states, such as arcs, slits and pre-pores, to pore formation, the precise mechanism of pore assembly is still unclear.
Here, we investigate the molecular mechanisms of GSDMD pore assembly by stoichiometry analysis of GSDMD oligomers by single molecule TIRF microscopy. To this aim, we reconstituted mouse GSDMD-mGFP oligomers on supported lipid bilayers with specific lipid composition. Our data reveal a preferential sequential insertion of odd basic units, as well as a protein concentration dependency of the degree of oligomerization. Stoichiometry results have been also correlated with a structural analysis of the oligomers by DNA-Paint super resolution microscopy.

Notes