SFB 944 Physiology & Dynamics of Cellular Microcompartments

IRTG Integrated Research Training Group of the SFB 944

IRTG Retreat 2022

Castle Rauischholzhausen

24th to 26th November

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Talk Abstracts

Session I

Keynote Lecture

Eva Herker, DRUID Professur Molecular Virology
Institute of Virology, Philipps-University Marburg

Lipids go viral: the role of lipid droplets in virus infection

Capsid proteins of several viruses of the *Flaviviridae* family localize to lipid droplets. Most prominently, the hepatitis C virus (HCV) capsid protein core localizes to lipid droplets to initiate virion assembly. Infectious low-density HCV particles are characterized by their association with host cell apolipoprotein E (ApoE). Importantly, expression of ApoE in cells types other than hepatocytes enables them to produce infectious viral particles. Here, we used fluorescent protein-tagged ApoE in microscopy experiments to visualize ApoE trafficking in HCV-infected cells to decipher this step of HCV maturation.
Flavivirus particles are not lipidated and do not associate with ApoE. Still, first microscopy studies on dengue virus (DENV) and Zika virus (ZIKV) capsid protein expression in cultured cells indicated that lipid droplet localization might be conserved among *Flaviviridae*. We utilized expression constructs carrying only the capsid sequence of different members of the flavivirus family. We observed localization of the capsid proteins to cytoplasmic lipid droplets with all tested capsid proteins. Interestingly, in addition to this phenotype, ZIKV capsid protein strongly localized to nuclear lipid droplets. Infection with two different strains of ZIKV (historical vs. new isolate) showed strain-, as well as time-dependent localization to cytoplasmic, nuclear, or both types of lipid droplets. Given that ZIKV is a neurotropic flavivirus, we analyzed capsid localization during infection in a microglial, a neuronal, and an astrocytoma cell line and we were able to confirm the localization patterns seen in Huh7 cells. Thus, while capsid localization is conserved in *Flaviviridae*, the localization patterns differ between viruses.

Characterization of a sphingomyelin-dependent lysosomal repair pathway (Poster Index: 13)

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

Damaged lysosomes threaten cell viability if their contents leak into the cytosol. ESCRT machinery can repair damaged lysosomes, which are particularly vulnerable to injuries. We recently uncovered an ESCRT-independent, sphingomyelin (SM)-based lysosomal repair pathway (1). Perturbations in lysosomal integrity inflicted by bacterial pathogens or lysosomotropic drugs are tightly coupled to a rapid, Ca2+-activated scrambling of SM across the bilayer. Live cell imaging approaches revealed a critical role of SM in the recovery of lysosomes from acute, potentially lethal damage. SM was not required for ESCRT recruitment to damaged lysosomes. Instead, the conversion of SM by neutral SMases on the cytosolic surface of injured lysosomes promoted their repair. Collectively, these results support a model whereby SM scrambling and hydrolysis can clear minor lesions from the lysosome-limiting membrane by driving an inward budding of the damaged membrane area, analogous to - but independent of ESCRT-mediated lysosomal repair. The aim of this project is to validate the proposed model of SM-dependent lysosomal repair and explore its biological relevance. Toward this end, we will set out to identify the scramblase responsible for lysosomal damage-induced SM translocation. As approach, we will conduct a systematic analysis of the TMEM16 family of putative, Ca2+-activated scramblases. We will also analyse the impact of blocking SM scrambling and/or hydrolysis on the lipidome of affinity-purified lysosomes and the ability of bacterial pathogens to perforate the vacuolar membrane and translocate to the host cytosol. Furthermore, we will explore the functional crosstalk between SM-dependent lysosomal repair and a recently identified phophoinositide signalling pathway that controls an ER-mediated lipid flow to damaged lysosomes (2,3).

References

(1) Niekamp, P., Scharte, F., Sokoya, T. et al. (2022). Ca2+-activated sphingomyelin scrambling and turnover mediate ESCRT-independent lysosomal repair. Nat Commun 13, 1875.
(2) Tan, J.X., Finkel, T. A phosphoinositide signalling pathway mediates rapid lysosomal repair. Nature 609, 815–821 (2022).
(3) Radulovic et al. (2022). Lysosome repair by ER-mediated cholesterol transfer. https://www.biorxiv.org/content/10.1101/2022.09.26.509457v1.full.pdf

Deficiency in the mycobacterial acyl-CoA synthetase FACL6 exacerbates membrane damage and phagosome escape *of* *M. marinum* in *Dictyostelium discoideum* (Poster Index: 23)

Sylvana Victoria Hüttel, Division of Molecular Infection Biology & Center of Cellular Nanoanalytics, University of Osnabrück, Germany

Tuberculosis, caused by *Mycobacterium tuberculosis* (*Mtb*), is the second deadliest infectious disease in the world. *Mtb* and other pathogenic mycobacteria cause damage at the membrane of their vacuole to escape into the host cytosol. The bacterial translocation depends on the secreted pore-forming peptide ESAT-6 that cooperates with phthiocerol dimycocerosate (PDIM), a complex mycobacterial membrane lipid that is shed within host membranes, to generate pores and membrane damage. It was hypothesized that *Mtb* uses primarily host-derived fatty acids (FAs) as carbon and energy source but also as building blocks to synthesize membrane lipids including PDIM during infection. To this end, bacterial fatty acyl-CoA synthetases activate imported FAs with coenzyme A. In total, the *Mtb* genome encodes 36 fatty acyl-CoA desaturases (FadDs). One of these enzymes is the peripheral membrane protein FadD6 (also known as long chain fatty acyl-CoA ligase (FACL6)) that was shown to import a wide range of FAs by vectorial acylation when expressed in *E. coli* and is involved in triacylglycerol (TAG) synthesis during *in vitro* dormancy of *Mtb*. Strikingly, the function of FACL6 during infection is still poorly understood.
Using the powerful *Dictyostelium discoideum*/*M. marinum* system, this project aims to characterize the role of FACL6 in lipid acquisition, assimilation and phagosome escape during mycobacteria infection. To this end, we will map alterations in host-to-pathogen FA flows in the *M. marinum* Δ*facl6* mutant with the help of fluorescent lipid probes combined with live cell imaging, flow cytometry and thin layer chromatography (TLC). Interesting, by performing transmission electron microscopy, we observed less mycobacterial lipid droplets in *M. marinum* lacking FACL6 suggesting that FA-assimilation and turnover might be affected during infection. Infections of *Dictyostelium* with the *M. marinum* Δ*facl6* mutant resulted in greater damage at the *Mycobacterium*-containing vacuole (MCV) visualized by expression of fluorescent markers for ESCRT- (ALIX, Vps32/CHMP4B) and autophagy-mediated repair (TrafE (E3 ubiquitin ligase), Atg8a/LC3). This is in line with the observation that bacteria lacking FACL6 escape more efficiently into the cytosol. Surprisingly, the Δ*facl6* mutant is highly attenuated in *Dictyostelium*, however, mycobacteria growth is restored in host cells lacking Atg1/ULK kinase indicating that the Δ*facl6* mutant is successfully detected and cleared by the xenophagy pathway. Strikingly, when immortalized bone marrow-derived macrophages (iBMDMs) were infected with *M. marinum*, many apoptotic and pyroptotic cells were observed in infections with the Δ*facl6* mutant, indicating that in macrophages the increase in membrane damage and/or phagosome escape might trigger cell death pathways to facilitate the spreading of the bacteria into neighbouring cells. When the lipid composition of the *M. marinum* Δ*facl6* mutant was examined using TLC, we discovered an accumulation of PDIMs that might account for the increase in membrane damage. Consequently, we hypothesize that PDIM synthesis serves to balance out potential toxic excessive fatty acids that are usually activated by FACL6 to generate TAGs.

Heterogeneity of intracellular *Salmonella* and the unique cytosolic lifestyle of *S.* Paratyphi *A*

Felix Scharte, Microbiology Division, Department of Biology and Center for Cellular Nanoanalytics, University of Osnabrück

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 self-limiting gastroenteritis. There are key differences between typhoidal (TS) and non-typhoidal (NTS) *Salmonella* in pathogenesis, but research on TS is challenging due to host restriction. Since STM causes a typhoid-like disease in mice, it was widely used as model organism to mimic human TS infection. Although results gained by research on STM could provide major insights in *Salmonella* virulence in general, the specific virulence mechanisms of TS are far from being understood.

We recently described the intracellular transcriptomic architecture and phenotypes of STM and SPA during presence in epithelial cells. Surprisingly, *Salmonella* pathogenicity island 1 (SPI-1) as well as motility and chemotaxis genes showed distinct expression patterns in intracellular SPA vs. STM. We observed that induction of flagella genes by intracellular SPA led to cytosolic motility. We report flagella-dependent cytosolic motility of SPA that neither depends on SPI-2 nor on recruitment of host cell actin. The elevated expression of SPI-1 genes resulted in increased invasiveness of SPA, following exit from host cells.

The triggers and cellular consequences of cytosolic motility are currently under investigation. Live cell imaging (LCI) revealed that SPA invades host cells in a cooperative manner with multiple bacteria per invasion site, possibly leading to error-prone macropinocytosis with increased membrane damage in the early *Salmonella*-containing vacuole. After release into the cytosol, motile bacteria showed reduced decoration with autophagosomal protein LC3 in LCI and ultrastructural analyses.

Our results provide new insights into the virulence profile of SPA by unravelling previously unknown intracellular phenotypes and virulence traits. We propose cytosolic motility as possible xenophagy evasion mechanism and higher invasiveness as contribution to progression of infection and systemic dissemination in the host. Furthermore, we established 3D and 2D intestinal organoid models which offer new tools for analyses of human-restricted pathogens in a more in vivo relevant context.

Session II

Keynote Lecture

Sven Bogdan, Molecular Cell Physiology, Institute of Physiology and Pathophysiology, Philipps-University Marburg

Molecular control of cell shape and motility - insights from the fly

The actin cytoskeleton provides mechanical support for cells and generates forces to drive cell shape changes and cell migration in morphogenesis. Molecular understanding of actin dynamics requires a genetically traceable model system that allows interdisciplinary experimental approaches to elucidate the regulatory network of cytoskeletal proteins in vivo. Here, I will present some examples of how advances in Drosophila genetics and high-resolution imaging techniques contribute to the discovery of new actin functions, signaling pathways and mechanisms of actin regulation in vivo.

Transition of a basement membrane into the cardiac specific ECM by Lonely heart and Pericardin in *Drosophila*

Marcel Reinhardt, Department of Zoology and Developmental Biology

Basement membranes (BM) are specialized forms of extracellular matrix (ECM). They play essential roles in multicellular organisms by mediating e.g. cell migration, cell polarity as well as tissue integrity and function. BMs constitute a highly conserved toolkit of core components. The BM can be further specialized by additional proteins depending on the necessity of tissues such as the cardiac ECM (cECM). The cECM is specialized by incorporation of two unique proteins: Lonely heart (Loh), an ADAMTSL adaptor protein, and Pericardin (Prc), a collagen IV like protein (reviewed in Reinhardt *et al*.). Both proteins are essential for heart function and integrity, 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 *et al*., 2018). The current work focusses on understanding the transition from a canonical BM into the highly specialized cardiac matrix. Especially, the temporal assembly hierarchy of the cECM. Thus, to elucidate the time point at which Lonely heart and Pericardin were incorporated. Furthermore, we are interested in the protein dynamics within the cardiac matrix. We utilized fly lines containing fluorescently labeled ECM proteins to follow their expression throughout *Drosophila* cardiogenesis. Especially, establishing an endogenously labeled Lonely heart revealed a fibrous protein localization comparable to Pericardin fibers. Furthermore, our current data helped us to understand the temporal assembly hierarchy of the cECM proposing the necessity of an intact and fully functional BM for Lonely heart anchoring and subsequent recruitment of Pericardin. Additionally, analysis of the cECM protein mobility by FRAP measurements within the heart matrix was performed. In detail, despite being a structural component, Pericardin is more mobile and behaves more like a linker protein. In contrast, Lonely heart as an anchor protein seems to be rather immobile.

References:

(1) Reinhardt M, Drechsler M, Paululat A (in revision). *Drosophila* collagens in specialised extracellular matrices. Biological Chemistry.

(2) Drechsler M, Schmidt AC, Meyer H, Paululat A (2013). The Conserved ADAMTS-like Protein Lonely heart Mediates Matrix Formation and Cardiac Tissue Integrity. PLoS Genet 9(7): e1003616.

(3) Wilmes AC, Klinke N, Rotstein B, Meyer H, Paululat A (2018). Biosynthesis and assembly of the collagen IV like Protein Pericardin in *Drosophila melanogaster*. Biol Open (2018) 7 (4): bio030361.

(4) Rotstein B, Post Y, Reinhardt M, Lammers K, Buhr A, Heinisch JJ, Meyer H, Paululat A (2018). Distinct domains in the matricellular protein Lonely heart are crucial for cardiac extracellular matrix formation and heart function in *Drosophila.* J Biol Chem (2018) May 18;293(20):7864-7879

The influence of the APC/CFzr on the actin dynamics during Drosophila myoblast fusion (Poster Index: 16)

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

In Drosophila, founder cells (FC) and fusion competent myoblasts (FCM) form multinucleated somatic muscle cells by fusion. The asymmetric nucleation of F-actin at the contact site between both cell types, and the subsequent formation of podosome-like structures, which invade the founder cell and drive the fusion of the opposing cell membranes, are crucial for successful cell-cell fusion. Previously, we found that the Anaphase-promoting complex (APC/C) adaptor protein Fizzy related (Fzr) plays a vital role during myoblast fusion1. Because APC/C is a key regulator for the mitotic exit, we found by studying the genetic interaction of Fzr with CyclinB, that increased proliferation of myoblasts is not the cause of the fusion defect. Additional genetic interactions with APC/C components showed, that the main reason for the fusion defect is the inhibition of the activity of the APC/C complex and not an undescribed function of Fzr. Furthermore, the size of actin foci between fusing myoblasts is reduced and the fusion-specific proteins Duf and Rols are accumulated in the FC. Characterization of the dynamic changes of actin during myoblast fusion in Fzr mutants, led to the identification of a novel kind of actin aggregation between adhering FC and FCM. This type of accumulation has a significantly increased lifetime, and a drastically reduced amount of actin compared to the WT. Applying HPF/FS, and using TEM could further show that these accumulations are characterized by PLS with significantly reduced length and width, corresponding to the reduced amount of actin. Additionally, we used FRAP analysis to explore the actin exchange at these mutant fusion sites and discovered, that the recovery kinetics are comparable to the WT, but the total amount of recovery is increased significantly. We now hypothesize that Fzr functions as a key regulator for F-actin nucleation during myoblast fusion. We could reinforce this assumption by dual-color live-cell imaging of F-Actin and WASp, a nucleation-promoting factor, which did not localize properly at the fusion site. This results in a lack of Arp2/3 activity, which is needed for PLS establishment, invasion of the FCM, and subsequently fusion itself.

References:

(1) Drechsler M, Meyer H, Wilmes AC, Paululat A. APC/CFzr regulates cardiac and myoblast cell numbers, and plays a crucial role during myoblast fusion. J Cell Sci. 2018 Jul 17;131(14):jcs209155. doi: 10.1242/jcs.209155. PMID: 29898917.

Endosomal maturation in *Drosophila* nephrocytes depends on a trimeric Rab7 GEF complex

Maren Janz, Department of Zoology and Developmental Biology

Drosophila nephrocytes are specialized cells displaying highest endocytic activity and serve as an invertebrate model system for glomerular podocytes. 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 also ensures enormous storage capacity, e.g. for degraded proteins or metabolic components[1]. Nephrocytes. We use nephrocytes to investigate the trimeric Rab7 guanine nucleotide exchange factor (GEF) complex Mon1-Ccz1-Bulli.
During endocytosis, Rab5 decorated early endosomes mature 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 GTPase activating proteins (GAPs). Maturation of early endosomes to late endosomes and the fusion with lysosomes also depend on the two heterohexameric tethering complexes CORVET and HOPS. The CORVET complex is an effector of Rab5 whereas HOPS interacts with activated Rab7[2].
In *Drosophila*, a trimeric Rab7 GEF complex, containing Mon1, Ccz1 and Bulli is active. We have shown that the absence of Bulli results in impaired endosomal maturation and enlarged Rab7 positive and negative endosomes with clustered Rab5 inside[3]. Furthermore, the lack of either Mon1 or Ccz1 reveals defective Rab5 and Rab7 localization and internalization of Rab5 into endosomes. Bulli has been identified in insects[3] and mammalian cells[4], indicating that the trimeric GEF is common in metazoan.

Overall, our data demonstrate the importance of the trimeric Rab7 GEF complex for proper localization of Rab5 and Rab7 in *Drosophila*. Our work aims to provide fundamental insight into the regulation of the endocytic pathway via this complex in nephrocytes. Especially the mechanism leading to Rab5 clustering in mutant flies will be investigated in future studies.

References
(1) Weavers *et al*., 2009, *Nature* 457, 322-326.
(2) Huotari & Helenius, 2011, *EMBO J.* 30, 3481-3500.
(3) Dehnen *et al.,* 2020, *J Cell Sci* 133.13.
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Session III

Keynote Lecture

Inga Hänelt, Membrane Biochemistry, Institute of Biochemistry, Goethe-University Frankfurt

Potassium transporters and channels in bacterial survival

Potassium ion homeostasis is essential for bacterial survival, playing roles in osmoregulation, pH homeostasis, regulation of protein synthesis, enzyme activation, membrane potential adjustment and electrical signaling. I will discuss the physiological roles of the three major bacterial potassium uptake systems, the ion channels TrkAH and KtrAB, the proton-coupled potassium transporter KUP and the potassium pump KdpFABC based on their molecular structure and function. In particular, I will highlight the transport mechanism of KdpFABC and how its activity is regulated by a serine phosphorylation mediated by the sensor kinase KdpD.

Exchange of hydrophobic for polar amino acids in the Tom20 substrate binding domain has no effect on function

Niklas Webeling, Faculty of Biology, Institute of Molecular Cell Biology, University of Münster,

Mitochondria are the eukaryotic organelle of oxidative phosphorylation and ATP generation. During evolution the progenitor of eukaryotic cells established a symbiotic relationship with the progenitor of mitochondria, which nowadays, leads to a high demand of maintenance and import of proteins into mitochondria. While most protein complexes which are involved into the import are known, the mechanism how hundreds, if not thousands of proteins are recognized is still elusive. One major import pathway for proteins, with a so called preprotein sequence is the Tom complex, especially the Tom20 subunit. Preprotein sequences shares only a low sequence homology, but still they are recognized by Tom20 through an amphipathic alpha helix structure. Here, we attempt to modify this binding domain to generate an import deficient or impaired variant and examine the effects through live cell imaging on the import rate, mitochondrial morphology and cellular survival.

Support layer for cryo-electron microscopy grids

Lara Jorde, Structural Biology Division, University of Osnabrück

Cryo-electron microscopy (cryo-EM) enables the 3D-structure determination of macromolecules at near-atomic resolution by averaging thousands of particles in various orientations. However, sample preparation is expensive and challenging for many macromolecules leading to insufficient particle concentration inside the holes of a cryo-EM grid. Moreover, particle interaction with the air-water interface during vitrification results in a preferred orientation and consequent difficulties in the structure determination. To overcome these limitations, a hydrophilic support layer is created to cover the holes inside a cryo-EM grid which leads to adsorption of the target particle onto the support layer, away from the air-water interface. Since particle adsorption also results in an increased particle concentration in the image region, macromolecules with a low expression yield can be analyzed easier and in a less time-consuming manner.
By using a 2D-crystal of a class II hydrophobin as a support layer, the necessary target sample concentration can be decreased drastically and the support layer can be identified by a hexagonal pattern in the fast fourier transformation. Another advantage lies in the possibility to provide the hydrophobin with a tag, which could enable even further decreasing of sufficient target concentrations in addition to selective binding. Therefore, this support system provides the opportunity to improve the structure determination of macromolecules and complexes in cryo-EM without the addition of a non-erasable background signal.

The role of membrane composition in JAK binding
(Poster Index: 24)

Isabelle Watrinet, Biophysics Division, University of Osnabrück

Janus kinase (JAK) family proteins are associated to Class I and II cytokine receptors (CR) to mediate ligand induced CR signaling via the JAK/STAT pathway. Malfunction in JAK activation can lead to severe diseases including abnormal immune responses and various types of cancer. By all-atom molecular dynamic simulations of JAK2 and the Thrombopoietin Receptor (TpoR), we found a membrane interacting region in the JAK2 FERM-SH2 domain consisting of a conserved lysine residue and a patch of positively charged residues. To pinpoint the contribution of this interaction between the kinase and lipid environment, we built up a minimal receptor-kinase-complex *in vitro*. Reconstitution of this complex into polymer supported lipid systems enabled us to investigate the JAK FERM-SH2 (FS) association to CRs. In addition, this approach opens an avenue to study the influence of lipid composition on the stability and conformation of the receptor-kinase-complex. To this end, we purified a truncated version of the TpoR and the FS domain of JAK2 wildtype including a covalent modification for stabilization and labelling.
The truncated version of the TpoR was reconstituted into small-unilamellar vesicles followed by addition of fluorescently labelled JAK2 FS. Fluorescence recovery after photobleaching (FRAP) allowed us to quantify the complex stability of JAK2 FS to TpoR in a defined lipid environment. We developed surface capturing of the receptor-containing liposomes onto trisNTA functionalized substrates. Thereby we eliminated bilateral diffusion of the transmembrane protein which otherwise could interfere with our FRAP readout. With respect to our dynamic simulations, the composition of the vesicles was altered in terms of charge. Adding negative charges (DOPS, PIP2) prolonged the interaction time of JAK2 FS with TpoR significantly, especially PIP2 did show a drastic effect. The resulting changes in complex stability suggest, that the interaction of the FS domain with negatively charged lipids, particularly PIP2, plays an important role in receptor binding.

Session IV

Keynote Lecture

Annette Borchers, Department of Biology, Molecular Embryology, Philipps-University Marburg

Of frogs and men: *Xenopus* neural crest development and human disease

Neural crest (NC) cells are an excellent in vivo model system to study questions of cell-cell communication. NC cells are a transient embryonic cell population that migrates extensively throughout the embryo contributing to various tissues. After induction, cranial NC cells undergo an epithelial to mesenchymal transition and migrate – first as a cell collective and later as single cells – on precise pathways. In Xenopus, NC migration can be analyzed in vivo for example by tracking of transplanted NC cells. Furthermore, explant cultures allow for a detailed in vitro analysis of protein localization and cell-cell communication at high resolution. During the last decade, we have identified adhesion and ligand/receptor complexes affecting planar cell polarity, cell-cell communication and motility of migrating NC cells. Currently, we are using these tools to analyze the molecular mechanisms of human diseases, which are likely caused by defects in NC development. In this talk, I will focus on the PTK7 receptor complex, which is dynamically localized in migrating NC cells and relevant for cell-cell communication. In addition, I will take a closer look at Kabuki-syndrome, an autosomal dominant disorder with high similarities to CHARGE syndrome, which is characterized by a typical facial gestalt indicative of defects in neural crest migration.

Generation of a vacuolar interactome map by cross-linking mass spectrometry and characterization of the role of TLDc domain proteins on V-ATPase function

Samira Klössel, Cellular Communication, University of Osnabrück

The vacuole of *S. cerevisiae,* equivalent to the lysosome in higher eukaryotes, functions as the final destination of several vesicular trafficking pathways and degrades macromolecules for re-use of building blocks by the cell. Additionally, it is an important cellular signaling hub and a storage compartment for ions and amino acids.
In this work, we have established protocols for the use of cross-linking mass spectrometry to map protein-protein interactions on isolated vacuoles. This allowed us to generate a map of vacuolar protein complexes. Our map reproduced most known complexes and molecular interactions and identified novel ones. From the identified interactions, we focus on the characterization of a novel binding partner of the vacuolar V-ATPase, the protein of unknown function Rtc5. V-ATPases are conserved protein complexes throughout eukaryotes and are the main source of acidification of intracellular organelles. They play important roles in trafficking through the endocytic and secretory pathways, as well as in signaling of nutrient availability.
We show that Rtc5 localizes to the vacuole membrane *in vivo*, depending on the presence of an assembled the V-ATPase. We find that Rtc5 is N-myristoylated, and this modification is necessary for membrane association. Rtc5 contains a conserved TLDc domain, which was recently identified to be present in several interactors of the V-ATPase of different organisms. Yeast contains a second protein with a TLDc domain, called Oxr1. This protein has been recently reported to interact with the V-ATPase and promote the disassembly of the complex *in vitro*. For both Oxr1 and Rtc5 we observe an effect on V-ATPase assembly *in vivo.* Additionally, the absence of Oxr1 causes the Golgi-localized isoform of the V-ATPase to relocalize to the vacuole.
Altogether, our work has generated a map of the interactions of vacuolar proteins. Furthermore, we have characterized a novel interactor of the V-ATPase and found a role for TLDc domain-containing proteins in V-ATPase assembly and subcellular localization.

Tethering proteins of the same contact site affect the localization and mobility of each other

Lucia Amado, Cellular Communication, University of Osnabrück

The membrane-bound organelles of eukaryotes allow the existence of different biochemical environments tailored for specific processes within one cell. However, this also presents the challenge of coordinating these compartments for the cell to function as a unit. One mode of communication among organelles is through specialized platforms, called membrane contact sites. These are regions where the membranes of the organelles come into a very close apposition, without fusing. Within contact sites, channels allow the exchange of luminal metabolites, and lipid transfer proteins allow the exchange of membrane lipids. These structures can also apply mechanical forces on the organelles, influencing their positioning and movement within the cell[1].
Contact sites have a specific proteome that determines the functionality of the structure[2]. These proteins achieve their localization at contact sites by interacting with both membranes, thus contributing to their tethering, or by interacting with tethering proteins[3]. In many cases, multiple tethers co-exist within one contact site. In this work, we use artificial drug-inducible tethers in vivo to address how different tethers influence each other. We find that the establishment of a region of membrane proximity can recruit tethers, influencing their distribution between different localizations or protein complexes. In addition, restricting the localization of one tether to a subdomain of an organelle causes other tethers to be restricted there. Finally, we show that there is a wide distribution in the mobility of contact site tethers and that other tethers of the interface can influence this mobility. Overall, our results show that the presence of other tethers at contact sites is an important determinant of the behavior of tethering proteins. This suggests that contact sites with multiple tethers are controlled by the interplay between specific molecular interactions and the cross-influence of tethers of the same interface.

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(2) Scorrano, L. et al. Coming together to define membrane contact sites. Nat. Commun. 10, 1–11 (2019).
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Molecular dissection of ceramide-induced apoptosis using photo-caged ceramides (Poster Index: 20)

Christian Schröer, Molecular Cell Biology, Universiy of Osnabrück

Ceramides are central intermediates of sphingolipid metabolism that can activate a variety of tumor suppressive cellular programs, including cell cycle arrest, senescence and apoptosis. Consequently, the potential of ceramide-based therapeutics in the treatment of cancer has become a major focus of
interest. While a growing body of evidence indicates that ceramides can act directly on mitochondria to trigger apoptotic cell death [1,2], molecular details of the underlying mechanism are scarce. Combining a computational approach with functional studies in cancer cells, we recently identified the voltage-dependent anion channel VDAC2 as a direct effector of ceramide-mediated apoptosis. These findings support a sophisticated molecular framework in which ceramides serve as critical modulators of VDAC-based platforms to control mitochondrial recruitment of pro- and anti-apoptotic machinery [3]. Our current efforts are focused on challenging this model in model membranes as well as in living cells. The latter requires experimental approaches that enable robust control over mitochondrial ceramide levels. Toward this end, we synthesized short- (C6) and long-chain (C16) ceramides that carry a coumarin photocage modified with a mitochondria-targeting triphenylphosphonium (TPP) group. Live cell fluorescence microscopy revealed that photocaged ceramides readily and selectively accumulate in mitochondria. A brief (30 sec) flash of UV light was sufficient to release the photocage. Photo-release of C6-ceramide triggered mitochondrial apoptosis, as evidenced by proteolytic cleavage of Casp9 and the caspase substrate PARP1.
In contrast, photo-release of C16-ceramide failed to trigger an apoptotic response, possible due to a reduced transfer of the lipid from the inner to the outer mitochondrial membrane. These findings establish photocaged ceramides as novel tools to dissect the mechanism underlying ceramide-induced apoptosis using the unmatched spatiotemporal precision of light. Our ongoing efforts focus on the application of photocaged ceramides to challenge a role of mitochondrial ceramides as modulators of VDAC-dependent recruitment of pro- and anti-apoptotic proteins in living cells.

References:

(1) Jain A et al. (2016) *J Cell Sci* 130, 360-371
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Session V

Keynote Lecture

Zoe Köck[1], Roman Levin[1], Dovile Januliene[2], Kilian Schnelle[2], Utz Ermel[3], Daniel Hilger[4], Achilleas Frangakis[3], Arne Möller[2], Frank Bernhard[1], Volker Dötsch[1]

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[4] Institute for Pharmaceutical Chemistry, Philipps University Marburg

Combination of cell-free protein expression, liquid state NMR spectroscopy and Cryo EM for the structure determination of membrane proteins

Cell-free expression systems are ideal for the production of proteins that cannot be overexpressed in E. coli cells. For NMR spectroscopy, cell-free expression provides major advantages since it allows for amino acid type selective labeling without metabolic scrambling. We have developed several labeling protocols that allow us to obtain the backbone assignment of membrane proteins in an efficient way. Using these methods we have obtained the backbone assignment for the retinal binding protein Proteorhodopsin and have determined it's structure. Cell-free expression is also very well suited for the production of samples for CryoEM investigations. The main advantage is that it is possible to directly express the membrane protein of interest into preformed nanodiscs of varying size and composition. In addition, complexes with ligands or other proteins can be also formed co-translationally by adding these components to the reaction mixture. Using this method we have determined the structure of the Hβ1AR and the H2R GPCRs in complex with G-protein and a stabilizing nanobody.

Regulation of a lipid phosphatase through the autophagy specific kinase Atg1

Katharina Olschewski, Department of Biology/Chemistry, Biochemistry Division, Osnabrück University

Autophagy is a conserved catabolic pathway in eukaryotic cells, where a newly formed organelle, the autophagosome, transports cytoplasmic material, organelles, pathogens, or aggregates to the vacuole/lysosome for degradation and reuse. During autophagy, a membrane disc forms, expands by lipid import, thereby surrounding its cargo, and finally forms a double membrane structure, called autophagosome. After maturation, the autophagosome fuses with the vacuole/lysosome, where the engulfed material is degraded. During the autophagosome formation, the Vps34 kinase complex generates phosphatidylinositol-3-phosphate (PI3P) on the membrane, which can recruit distinct effector proteins to the autophagosome. Finally, after closure, the clearance of PI3P by the PI3P-specific phosphatase Yeast Myotubularin Related 1 (Ymr1) is necessary for fusion. Deletion of Ymr1 leads to a strong delay in autophagy flux and the effector proteins are not completely released from the membranes. Thus, it was proposed that the clearance of the PI3P-specific effectors is essential for the maturation of the autophagosome and consequently for fusion. How the Ymr1 activity is controlled during autophagy is still and open question that we aim to address in this work.
First, we established the purification of yeast Ymr1 and two *in vitro* assays to follow its activity. For this, we fused a PI3P specific-binding domain PX domain to GFP, and used it to track the turn-over of PI3P on model membranes. We observed PI3P specific phosphatase activity of Ymr1 *in vitro*. We then established an *in vitro* kinase assay to show that Ymr1 is a substrate of the autophagy specific kinase Atg1. We mapped the phosphorylation sites by mass spectrometry and built the phosphomimetic and non-phosphorylated versions of the protein for the *in vitro* and *in vivo* characterization.
Our results demonstrate that the phosphatase activity of Ymr1 towards PI3P is regulated by Atg1, suggesting a coordination of autophagosome maturation with fusion.

Functional and structural analysis of the HOPS tethering complex

Caroline König, Department of Biology/Chemistry, Biochemistry Division, Osnabrück University

The HOPS tethering complex is a heterohexamer consisting of the subunits Vps11, Vps16, Vps18, Vps33, Vps39 and Vps41 in *Saccharomyces cerevisiae*. For membrane tethering HOPS binds active, GTP-loaded Ypt7 and for subsequent fusion assembles SNAREs from opposing membranes. Vps41 and Vps39 at opposite ends of the complex bind to the Rab7-like Ypt7 protein. Vps33 is crucial for binding to SNAREs and is structural related to members of the Sec1/Munc1 (SM) protein family. The other five subunits (Vps11, Vps16, Vps18, Vps39, and Vps41) are predicted to share a similar architecture, comprising an N-terminal β-propeller and a C-terminal α- solenoid domain.

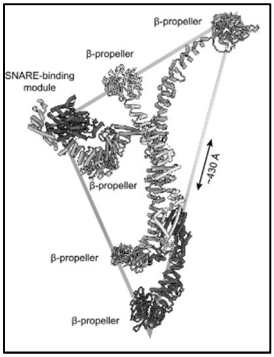

Figure 1: Overall architecture of the HOPS tethering complex.

Despite a thorough biochemical characterization, it is mostly speculative how the HOPS complex mediates tethering and fusion and what is necessary for the complex to accomplish its role during these processes. Previous structural studies were limited in possibilities to obtain high resolution of particles due to low stability and high flexibility of the complex. Here we show a high-resolution structure of the HOPS complex using cryo-electron microscopy.

HOPS forms a largely extended, triangular structure of approximately 430 Å in height and 130 Å in width (Figure 1). Vps11 and Vps18 align antiparallel through their elongated α-solenoids, establishing a large interface. The two core subunits create a central assembly area for the four other subunits that are needed for specific functions and localize to the periphery of the complex. Functional analysis of N-terminal deletions of Vps11 and Vps18 showed that deficient HOPS complexes are still able to function as tethers, whereas the fusion activity is impaired. We hypothesize that the deletions result in this deficiency due to a less stable backbone, where SNARE assembly may be impaired. Our data suggest a model of how the stable backbone and the Rab binding sites at the flexible ends of the HOPS complex fulfil an essential role in its dual functionality of tethering and fusion.

Determining the cargo spectrum of yeast SNX-BAR sorting complexes by vacuolar proteomics

Julia Seimert, Bioanalytical Chemistry, University of Osnabrück

The endovacuolar system is involved in the cellular supply, degradation, and recycling of proteins and other molecules from various sources. Thereby, plasma membrane turnover, vacuolar upkeep and protein degradation are mediated. For this purpose, the endovacuolar system utilizes different interconnected pathways and transport complexes. Newly synthesized hydrolases can either take a pathway from the Golgi via endosomes or a direct pathway to the vacuole, the AP-3 pathway. A third pathway traffics cargo to the and utilizes the autophagy machinery. Retrograde pathways that direct proteins from the endosome to the Golgi are dependent on the sorting nexins (SNX BAR proteins). The correct sorting of cargo in this interwoven system is crucial for cellular homeostasis. One underlying regulatory mechanism for this is the phosphatidylinositol (PI)-coding of organelle membranes, which defines their identity. This lipid species-specific coding is utilized by SNX-BARs that contain a PX domain which is able to recognize PI(3)P, the defining lipid of endosomal membranes. Additionally, SNX-BAR proteins have dimerizing BAR domains that are able to sense and stabilize membrane curvature. Thereby, sorting nexins combine the selection of cargo with the deformation of membranes to form membrane carriers for transport. In yeast, they include the proteins Snx4, Snx41, Snx42, the retromer components Vps5, Vps17 as well as the proteins Snx3 and Mvp1. However, the cargo spectrum of the different sorting nexins in yeast remains largely unknown.
To overcome this challenge and systematically determine the cargo spectrum of yeast sorting nexins we used quantitative proteomics of isolated vacuoles (Q-Prevail). We had previously shown that Q-Prevail can be used to define the cargo spectrum of protein transport pathways to the yeast vacuole.
Here we show that QPrevail is a reliable method to analyse retrograde trafficking pathways and PI-dependent sorting events in the endovacuolar system. We show that Ptm1 is a previously undescribed cargo of the Mvp1 dependent retrograde transport pathway. Its function is so far unknown, but we confirmed its dependence on the transport protein Mvp1 and the Golgi-resident Gga1/Gga2 complex by fluorescence microscopy, which indicates a shuttling of Ptm1 between the Golgi and the endosome. In addition, we found a connection to the sorting of the Golgi-associated Rab5-GEF Muk1. We also confirmed earlier suggested interactions of SNX-Bars in other pathways, such as a function of SNX4 complexes in the CVT pathway. We also confirmed the role of the two PI-kinases Vps34 and Vps38 for cargo sorting. Here we showed that Vps34 is responsible for the correct transport of proteins of different pathways. The retrograde trafficking map provides a larger-scale understanding of the relationships between trafficking factors and cargo. Since many of the studied pathways are evolutionary conserved, our studies will help to understand neurodegenerative diseases, since Alzheimer's disease, Parkinson's disease, and Down syndrome have been linked to retromer and sorting nexin dysfunction.

Session VI

Structure and regulation of GSDMD pores at the plasma membrane of pyroptotic cells.

Shirin Kappelhoff, Molecular Cell Biophysics, University of Osnabrück

Pyroptosis is a highly inflammatory form of regulated cell death implicated in the host response to pathogen infection and in inflammatory diseases. Pyroptosis is executed by the Gasdermin (GSDM) family member GSDMD upon cleavage by inflammatory caspases. The active GSDMD-N domain translocates from the cytosol to the plasma membrane (PM), where it oligomerizes and forms pores that allow the release of cytokines and promote cell lysis.

Resolving the structure of GSDMD pores at the PM of cells is key to understand the role of GSDMD in modulating inflammation by the release of inflammatory molecules. So far, the supposedly small size of GSDMD pores, the strong cytosolic background and the morphological PM changes during pyroptosis prevented resolving GSDMD pores in their native PM environment. In order to resolve the structure of GSDMD at the PM of pyroptotic cells, we combined DNA-PAINT super-resolution microscopy with a newly developed technique called polymer-supported membranes (PSPMs) (Figure 1). PSPMs are plasma membrane sheets generated by removing the cell body from cells tethered to a polymer-coated surface. This strategy preserves membrane topography and integrity while removing any cytosolic fluorescence contribution.

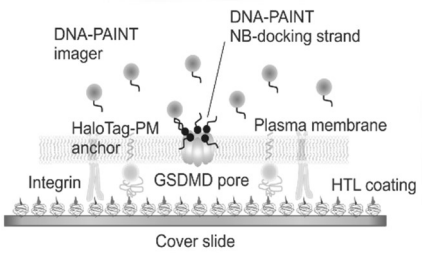

Figure 1: DNA-PAINT on GSDMD pores in polymer-supported plasma membrane generated from a pyroptotic cell.

Using this powerful approach, we have been able to resolve GSDMD structures at the nanoscopic level directly at the PM of pyroptotic cells. We revealed the presence of heterogeneous structures in form of rings and arcs of variable size and stoichiometry. At this point, we started exploring the role of the membrane environment in modulating GSDMD pore formation in cells. Specifically, we observed the conversion of $PI(4,5)P_2$ to PIP_3 during pyroptosis. Notably, the increase in PIP_3 levels correlated with faster pyroptosis kinetics and the formation of larger ring-like structures.

GAP together! – Structure and Function of the Heterodimeric RAL-GAP Complexes (Poster Index: 18)

René Rasche, Institute of Biochemistry, University of Münster

The GTPase activating protein (GAP) complexes RalGAP1 and RalGAP2 play a crucial role in regulation of cellular signaling as they turn the small Ras-like GTPases RalA and RalB into their inactive conformation by catalyzing GTP hydrolysis. Those GTPases are involved in controlling of proliferation and regulation of autophagy, endocytosis and exocytosis. An imbalanced regulation of RalGTPases leads to phenotypes like pancreatic inflammation and an enhanced progression of several cancers. Furthermore, some activating RalGEFs are effectors of the Ras oncogene and Ral signaling is one of the key branches of Ras-driven signal transduction. Hence, the RalGAP complexes are crucial tumor suppressors in these processes.

The complexes consist of one of the two RalGAPα1/2 ('catalytic') and the RalGAPβ ('regulatory') subunit. Furthermore, the atypical Ras-like protein κBRas constitutively interacts with RALGAPα. So far, no (sub)structures of these complexes were resolved. Computational analysis leads to the conclusion, that RalGAP harbors an asparagine-thumb GAP domain and that the complex is structurally related to the heterodimeric TSC GAP complex. As a unique feature, RalGAP needs both of its subunits for GAP activity on Ral. Thus, we assume, that the catalytic mechanism of RalGAP differs from other Asn-tumb GAPs. Furthermore, κBRas is required for proper RalGAP activity in vivo, raising the question of its role in Ral/RalGAP regulation.

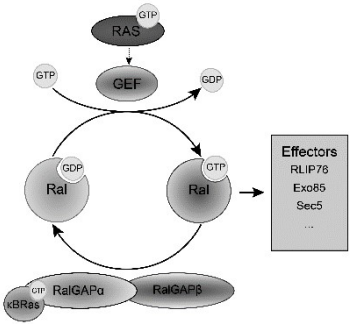

Figure 2: Ral GTPase cycle.

Our first aim is to determine the structure of the RalGAP complexes by combining X-ray crystallography and electron microscopy to understand its function. A low-resolution map could be reconstructed from an initial cryo-EM dataset and further structural experiments are ongoing. Additionally, we isolated subcomplexes from core parts of the RalGAP subunits. With these, we mapped interaction sites of the components and are investigating the catalytic activity by biochemical assays to elucidate molecular mechanisms.

Correlative and volumetric EM imaging of inflammatory dynamics in skin vessels

Leonhard Breitsprecher, iBiOs–integrated Bioimaging Facility Osnabruck, CellNanOs- Center for Cellular Nanoanalytics, University of Osnabruck

Serial block-face scanning electron microscopy (SBF-SEM) is a technique that greatly increases reliability, availability and throughput of 3D-EM. The ability to resolve nanoscales, such as membranes and small vesicles, with a large field of view and great depths, can provide insight to many biological fields. However, analysis of cellular dynamics remains exclusive to fluorescent markers, visualized in light microscopy. Pairing both processes in a complicated, correlative (CLEM) approach is therefore necessary and new reliable workflows have to be established, taking into consideration mismatch of resolution and targeting on nano-scale level.
For instance, tissue homeostasis and its balance upon inflammatory events often lead to severe changes regarding permeability of blood vessels and transmigration of neutrophils and platelets. Neutrophils may leave the vessel in a controlled process, whereas platelets seal the endothelial barrier, preventing vascular leakage. Interaction dynamics of both have been observed at sites of inflammation but to this day lacked ultrastructural investigation.
This talk unravels the possibility to observe the interaction of platelets and leukocytes following an inflammation stimulus via intravital microscopy (IVM) before fixation and afterwards relocate that specific vessel and region of interest led by artificial and natural landmarks to allow subsequent ultrastructural investigation. It is revealed that neutrophils leave vessels through partial openings of endothelial junctions in response to inflammation stimuli (paracellular transmigration). Additionally, rare occasions of co-interaction in which an increased number of platelets following the transmigration of neutrophils can be observed. SBF-SEM detects complete platelets transferring the basement membrane barrier and allows reconstruction of highly activated vessel regions.

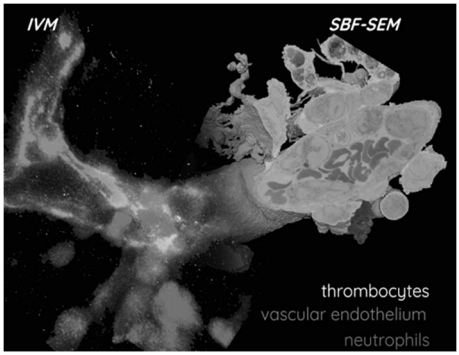

Fig.1: Confocal intravital microscopy (IVM) maximum-intensity-projection of neutrophil recruitment upon inflammatory bleeding event within murine dorsal skin vessel, merged with corresponding volumetric visualization of serial block-face scanning electron microscopy (SBF-SEM) data.

Poster Abstracts

Poster Session I

odd numbers 24th Nov, 16:30

Molecular dissection of ceramide-induced apoptosis: a bottom-up approach (Poster Index: 1)

Milena Wessing, Mollecular Cell Biology, University of Osnabrück

Ceramides attract wide attention as tumor suppressor lipids that can act directly on mitochondria to trigger Bax-mediated cell death [1, 2]. While ceramide engagement in mitochondrial apoptosis is clinically relevant, molecular details of the underlying mechanism are largely unknown. A chemical screen for ceramide binding proteins combined with computer simulations and functional studies in cancer cells previously led us to identify the voltage-dependent anion channel VDAC2 as critical effector of ceramide-induced apoptosis [3]. VDAC residues involved in ceramide binding are also required for mobilizing hexokinase type-I (HKI) to mitochondria, a potential checkpoint in apoptosis. Collectively, these data support a model in which ceramides function as modulators of VDAC-based platforms to control mitochondrial recruitment of pro- and anti-apoptotic machinery. The central aim of the current project is to challenge fundamental aspects of this model using a bottom-up approach. Toward this end, we set out to reconstitute ceramide-induced apoptotic pore formation in synthetic model bilayers. Contrary to previous claims [4], our findings indicate that ceramides on their own are unable to form cytochrome *c*-conducting pores or trigger recruitment of Bax. Moreover, ceramides do not influence the rate by which Bax perforates cardiolipin-containing membranes, indicating that ceramide-induced mitochondrial apoptosis relies on additional machinery. Our ongoing experiments focus on the role of VDACs as molecular platforms for Bax and HKI recruitment. As approach, we monitor binding of recombinant Bax and HKI to proteoliposomes containing wildtype or ceramide binding-defective VDAC channels. Next, we will exploit photocaged ceramides in combination with FRET-based assays to directly challenge a role of ceramides as modulators of VDAC-HKI and VDAC-Bax interactions.

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Repair me if you can: ER-dependent repair by oxysterol binding protein 8 during mycobacterial infection (Poster Index: 3)

Aby Anand, Division of Molecular Infection Biology, Department of Biology & Center of Cellular Nanoanalytics, University of Osnabrück, Germany

Various pathogens including *Mycobacterium tuberculosis*, the causative agent of tuberculosis, have developed sophisticated strategies to remodel the *Mycobacterium*-containing vacuole (MCV) into an optimal niche for their replication and persistence. The type 7 secretion system ESX-1 and its secreted effector protein, the pore forming peptide (ESAT-6), are essential for mycobacteria to inflict vacuolar membrane damage to exit the MCV and to spread to neighboring cells. However, MCV damage triggers the recruitment of repair machineries to contain the bacteria inside the MCV. For example, in *Dictyostelium discoideum*, ESCRT- and autophagy-dependent repair mechanisms cooperate together at the MCV of *M. marinum*. Recently, a new ER-dependent repair pathway has been described in mammalian cells that is involved in lysosomal repair. To this end, lipid transfer proteins of the oxysterol binding protein (OSBP) family are recruited to ER-lysosome membrane-contact sites (MCS) to provide lipids to restore the damage. We have evidence that ER-dependent MCV repair is involved during infection: (i) genes involved in ER-dependent MCS are upregulated at later stages of infection when the MCV is ruptured and (ii) various OSBPs were abundantly found in the proteome of isolated MCVs. In line with that, we have found that OSBP8, one of the twelve OSBPs of *D. discoideum*, is localized in the cytosol, at the perinuclear ER and the Golgi apparatus in uninfected cells. Strikingly, OSBP8 is recruited to lysosomes upon sterile damage and to the MCV upon infection with pathogenic mycobacteria. In addition, lattice light sheet microscopy and expansion microscopy revealed that OSBP8 is localized at ER-MCV MCS. Intriguingly, OSBP8 is not mobilized by mycobacteria which lack ESX-1 or ESAT-6 and are therefore incapable to inflict damage. In cells depleted of OSBP8, the distribution of sterol and phosphatidylinositol 4-phosphate (PI4P) is altered indicating that OSBP8 might be involved in the transport of both lipids. In line with that, OSBP8 depletion caused a drastic accumulation of PI4P at the MCV. Surprisingly, in cells lacking OSBP8, mycobacteria growth is increased. We found that deletion of OSBP8 leads to impaired lysosomes/MCVs with elevated pH and as a consequence reduced proteolytic activity. This suggests that ER-dependent, OSBP8-mediated membrane repair is a key component of the host defense against mycobacteria. We are now investigating how OSBP8 recruitment is affected upon PI4P depletion on lysosomes/MCV and are validating our observations in macrophage/*Mtb* system. Thereby our study may lead to new anti-Tb therapies.

Identification of the IPC synthase of *Dictyostelium discoideum* (Poster Index: 5)

Stevanus A. Listian, Division of Molecular Infection Biology, University of Osnabrück, Germany

The soil-dwelling amoeba *Dictyostelium discoideum* has been used extensively as a model organism for cell biological research. Its relative simplicity and genetic tractability, as well as its unique characteristics (motility, ability to form multicellular aggregates, phagocytic pathway) makes *Dictyostelium* especially attractive to study processes such as chemotaxis, phagocytosis and autophagy. In infection biology, *Dictyostelium* is used as surrogate macrophage to study the pathogenesis of mycobacteria*, Legionella pneumophilla, Pseudomonas aeruginosa* and *Cryptococcus neoformans*. A recent search for genes whose transcript levels are up- or down-regulated during infection of *Dictyostelium* with *Mycobacterium marinum* yielded numerous components of the sphingolipid metabolic network. However, how individual sphingolipid species contribute to this process is largely unexplored.
Strikingly, the lipid composition and the complex sphingolipid profile of *Dictyostelium* are mostly unknown. Consequently, we determined all lipid classes synthesized by this organism using a lipidomics approach. This revealed amongst others that *Dictyostelium* produces inositol phosphorylceramide (IPC). We confirmed this result with the help of a complementary biochemical assay, in which we monitored the turnover of NBD-ceramide to NBD-IPC by *Dictyostelium* lysates by thin layer chromatography.
Surprisingly, the IPC synthase of *Dictyostelium* has not been annotated so far. Using bioinformatics, we identified two potential complex sphingolipid synthases, CSS1 and CSS2. The functional analysis of both candidates using a cell-free assay in a defined lipid environment revealed that CSS2 is an IPC-synthesizing enzyme.
In line with what has been observed for CSS of other organisms, mCherry-CSS2 is co-localizing with markers for the Golgi apparatus/recycling endosomes in *Dictyostelium*. CSS2-mCherry, however, localizes additionally to lysosomes and to the tubular network of the contractile vacuole. When cells were treated with the IPC synthase inhibitor AureobasidinA (AbA), we observed a growth inhibition of *Dictyostelium* indicating that IPC synthesis is blocked. Since the generation of CSS2 knockouts were not successful, we will perform lipidomics of cells treated with AureobasidinA (AbA) to test if this drug changes *Dictyostelium* IPC levels. We will also perform an NBD-ceramide assay with lysates from cells overexpressing CSS2 to investigate if higher CSS2 expression results in higher IPC production. Taken together, this is the first comprehensive effort to define the *Dictyostelium* sphingolipidome and to pinpoint the enzyme that produces IPC in this organism.

Cold case: A new strategy for an ultrastructural analysis of the secretion system of the giant adhesin SiiE (Poster Index: 7)

Pascal Felgner, Division of Microbiology, University of Osnabrück

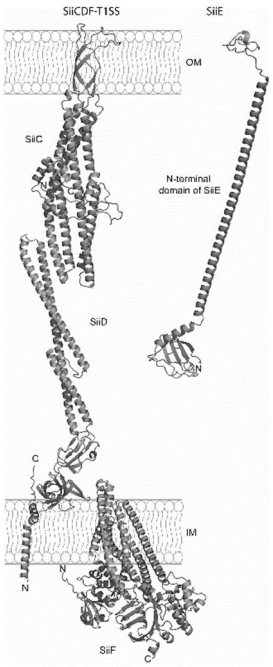

Figure 3: Schematic overview of monomers of modelled SPI4-T1SS subunits SiiCDF and N-terminal part of SiiE. Proteins modelled by trRosetta[3] (TM score > 0.5 = high

The virulence-associated adhesin SiiE of *Salmonella enterica* is secreted by a type 1 secretion system (T1SS) encoded by genes on the *Salmonella* pathogenicity island 4 (SPI4). T1SS are characterised by their structure spanning the Gram-negative cell envelope, consisting of an ABC transporter, a periplasmic adapter protein (PAP), and an outer membrane protein (OMP)[1]. Unique for the SPI4-T1SS are the two accessory subunits SiiA and SiiB that form a proton-conducting channel in the inner membrane of *Salmonella* and share similarities with the MotAB stator complex of the flagellum.[2]. Here, the ultrastructure of this complex can help to receive information about the interaction of the two subunits with the membrane-spanning SiiCDF complex and involvement in proper function of its substrate SiiE. Previous attempts to isolate the fully assembled SPI4-T1SS from *Salmonella* was not fruitful. Therefore, our choice of method for the visualisation of the protein complex is the *in situ* cryogenic electron microscopy tomography (cryo-ET) of the membrane-spanning SPI4-T1SS complex in *Salmonella* minicells. We generated a plasmid-encoded tetracycline-inducible variant to increase the number of complexes in the membrane of *Salmonella* minicells. Additionally, we deleted specific portions of the N-terminal domain of SiiE and analysed the retention and secretion kinetic of these variants. One deletion showed an increased and extended retention of SiiE on the bacterial surface. We used this variant for further analyses of mini-SiiE surface expression by flow cytometry, fluorescence microscopy and negative-stain transmission electron microscopy. After successful pre-tests, first cryo-ET sessions of isolated minicells were performed and tomograms processed. Further steps will be the subtomogram averaging of picked SPI4-T1SS and generation of first initial models.

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Generating a lysosomal biogenesis map using mammalian cells (Poster Index: 9)

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Lysosomes are central and dynamic organelles that are also an established degradation endpoint of the endocytic pathway. They are acidic organelles involved in the degradation of macromolecules by means of the soluble hydrolases and lysosomal membrane proteins (LMPs). Lysosomal hydrolases are involved in the degradation of target specific substrates, in addition to, pro-protein processing, antigen processing, degradation of extracellular-matrix and initiation of apoptosis. LMPs are permeases mostly found in the lysosomal limiting membrane and play a major role in lysosomal luminal acidification, membrane fusion, import of proteins and export of degradation products from and to cytosol respectively. For the biogenesis of lysosomes, both the acid hydrolases and membrane proteins are transported either directly via the biosynthetic pathway from the trans-Golgi network (TGN) to the endosomal system, or indirectly, from TGN to the plasma membrane and subsequent endocytosis. The direct intracellular transport of lysosomal protein involves a complex system of sorting signals and recognition proteins. The transport of lysosomal hydrolases through the mannose-6-phosohate receptor (M6PR) is one of the widely understood direct pathway. In contrast, little data is available about the structural and molecular mechanisms of the LMPs transport to lysosomes. The most abundant LMPs are lysosome-associated membrane protein 1 (LAMP1), LAMP2, lysosome integral membrane protein 2 (LIMP2), and the tetraspanin CD63. The significance of the lysosomal hydrolases and membrane permeases are highlighted by the increasing number of mutations in the encoding genes of these proteins, which leads to a family of diseases known as lysosomal storage disorders (LSDs). The accumulation of degradation products, such as lipids, amino acids, sugars and nucleotides within the lysosomal lumen characterize these disorders and most of the LSDs primarily affect the central nervous system. In order to examine the mechanism and the major players in these disorders, it is important to systematically understand the biogenesis of lysosomes.

Here, we established a system to isolate intact lysosomes from mammalian cells and systematically study their protein composition by mass spectrometry-based proteomics. This method is based on the isolation of lysosomes from cells that where fed with small super-magnetic particles. This allows the rapid isolation of lysosomes via magnetic affinity purification. We show that the method can be used to compare the protein composition of lysosomes from WT cells and cells lacking important transport factors, such as the AP-3 complex. In the future, the established protocols will allow me to systematically study lysosomal biogenesis in mammalian cells. I will therefore compare the lysosomal protein composition of a defined set of gene knockdowns with a known function in endo-lysosomal trafficking. Therefore, my work will lay the foundation to understand cargo-receptor relationships in the lysosomal transport pathway which will be crucial to understand the complex phenotypes of lysosomal storage disorders.

Metazoan TLD-RabGEFS and their interactions (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 analysis [1]. The TLDR GEF family comprises the heterodimeric complexes Mon1-Ccz1, BLOC-3 and Inturned-Fuzzy in metazoans, and the Mon1-Ccz1 complex is also conserved in yeast. We previously determined the mechanism of nucleotide exchange by Mon1-Ccz1 from the structural and biochemical characterization of a catalytic core complex [2] and derived a model for membrane recruitment of the complex based on the structure of the full complex [3]. Currently, we are establishing the similarities and differences between Mon1-Ccz1 and the related BLOC-3 and Inturned-Fuzzy complexes on a structural and functional level.

A unique feature within the TLDR GEF family is the N-terminal PDZ domain of Inturned. PDZ domains are a common protein-protein interaction motif and facilitate target binding with affinities in a micromolar range. By structural, bioinformatic and biochemical approaches we have determined that the Inturned-PDZ domain represents a novel non-canonical PDZ-like fold. We are currently working towards identifying its binding targets and functional roles

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Fluorescent dyes for in-resin correlative light and electron microscopy with conventional EPON embedding (Poster Index: 15)

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Correlative light and electron microscopy (CLEM) of biological samples combines the possibilities of observing rare and dynamic processes in living cells via light microscopy (LM) with the high resolving power of electron microscopy (EM). Due to unavoidable changes induced to the sample during preparation for EM the correlation accuracy of such approaches is often poor. For in-resin CLEM on the other hand the same sections are imaged in the EM and LM, facilitating a high-precision registration of the fluorescence signal upon the EM image. Current approaches most often employ acrylic resins, such as Lowicryl HM20, in complicated workflows requiring special instruments for preserving fluorescence while compromising the ultrastructural preservation and contrast (Buerger et al., 2021). Epoxy resins, such as EPON 812, on the other hand are the optimal choice for preserving the ultrastructural features of samples while generating high contrast images and are used in many rapid and robust protocols. However, embedding specimens in epoxy resins in combination with osmium tetroxide staining and dehydration leads to a reduction or complete abolishment of antigenicity and fluorescence signal. Therefore, it has been a longstanding dogma, that LM is not feasible after such treatments. Nevertheless, it was recently demonstrated that in-resin CLEM following a conventional sample preparation as mentioned above is indeed possible with partly specifically adapted fluorescent proteins or protein tags labelled with certain dyes (Müller et al., 2017; Peng et al., 2022).
Therefore, we set out to further test the broad applicability of different fluorescent dyes in a conventional in-resin CLEM workflow with embedding in epoxy resin. We demonstrate the robustness of this by application to a set of different cellular organelles, which allows us to achieve high-precision correlation with a minimally complicated sample preparation.

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Lack of tau in hippocampal CA1 neurons - what is altered? (Poster Index: 17)

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Alzheimer's disease is a progressive brain disorder that slowly destroys memory and thinking skills, and eventually the ability to carry out the simplest tasks. One of the key molecules that has been linked to the disease is the microtubule-associated protein tau since it is a principal component of the neurofibrillary tangles, which are present in neurons of the diseased brain. However, it is unknown how a protein that is primarily located in the axon shaft is involved in a disease that is believed to have a synaptic origin. In this regard, the function and morphology of CA1 neurons from tau knock out (tauKO - B6.129X1-Mapt$^{tm1Hnd/J}$) male mice were studied.
Analysis of potential functional alterations in synaptic transmission was performed by microelectrode array (MEA) system. Induction of LTP by high-frequency stimulation (HFS) leads to a higher potentiation of the population spikes and fEPSP in 12-months-old tauKO mice. Interestingly, 3-months-old tauKO mice lose the ability of a potentiated response in the stratum radiatum over time, but not at the site of the generation of the action potential. To understand the potential reason for these differences, both the length and distance of axonal initial segments (AIS) were analyzed, revealing that tauKO mice generally have shorter AIS and in particular, AIS in the hippocampi of the 3-months-old mice are located further away from the soma than in control mice. The signal propagation down the dendritic branch appears unchanged in 12-months-old mice, because no difference was found in the length of dendrites or branching number between the two genotypes. However, tauKO mice at that age are possessing a higher fraction of mushroom spines on their dendrites. On a molecular level, mass spectrometry data showed that in tauKO mice the amount of $GABA_A$ receptor subunit is decreased.
The data indicate that tau has a complex effect on synapse function, which is dependent on the developmental stage of the animals. This is of particular importance because current therapeutic strategies aim to reduce tau level in the brain of diseased patients.

Specific function of yeast Rab5-variants and their regulation via associated GEFs (Poster Index: 19)

Alexandra Nesterova, Department of Biochemistry, Osnabrück University

Members of the Rab-family of GTPases act as important regulators of endomembrane trafficking processes. Different endomembrane vesicles or organelles carry specific Rab-GTPases, which is why Rabs are recognized as marker proteins of organelle identity. After their synthesis, a geranylgeranyl-anchor is attached to the C-terminus of the Rabs which enables membrane association upon GTP-binding but which is shielded by a chaperone (GDI) in the GDP-bound state. Localization of Rab-GTPases is thought to be determined by specific guanine nucleotide exchange factors (GEFs) which catalyze the exchange of GDP for GTP, thereby inducing a conformational change. This "activation" of Rabs favors their insertion into target membranes and binding of effectors. Recruited Rab-effectors then modulate biochemical properties of the organelles or induce fission- or fusion processes.
Early endosomes which are formed shortly after endocytosis are typically decorated by Rab5-GTPases. Although compared to metazoan organisms *Saccharomyces cerevisiae* is assumed to have a minimal endocytic pathway, four Rab5-homologs, namely Vps21, Ypt52, Ypt53 and Ypt10, were identified. Vps21 is assumed as the main Rab5-variant since its deletion leads to most pronounced impairments in cargo sorting to the vacuole. The observed endocytic trafficking defects are further enhanced by co-depletion of Ypt52 and Ypt53, which indicates partial redundancy. Despite this observation, we assume functional differences between the variants and few isoform-specific effectors (e. g. Bph1 as effector of Ypt52) were already identified. No significant growth or trafficking defects were observed in Ypt10-knockout cells and the function or specific localization of Ypt10 remain unknown. Furthermore, three Rab5-GEFs (Vps9, Muk1 and Vrl1) are encoded in the yeast genome, which likely regulate the activity of the four Rab5-homologs. All three comprise a Vps9-domain responsible for GEF-activity. To evaluate the specificity of activation of the Rab5-variants by the different GEFs, we established *in vitro* GEF-assays in which GEF-mediated release of fluorophore-labeled GDP is quantified by fluorescence spectroscopy. Besides the GEF-assays conducted with soluble components, we attempt to reconstitute Rab5-activation on liposomes using *in vitro* prenylated and chaperoned Rabs. Preliminary results indicate differences in activation kinetics between Vps9 and Muk1 towards the different Rab5-homologues. We plan to gather further cues on the different functions of the Rab5- and Vps9-domain-GEFs by defining their interactomes via pull-down- and proximity labeling assays followed by mass spectrometry. These results together with the results from planed *in vivo* studies will aid deciphering the specific functions of the Rab5-isoforms.

Super-resolution imaging and quantitative analysis of neuronal microtubule lattice in 3D (Poster Index: 21)

Nataliya Trushina, Department of Neurobiology, Osnabrück University, Osnabrück, Germany

Microtubules (MTs) are essential for the development of neurons and maintaining their structure. MTs serve as a central system for the long-distance transport of cargo. The organisation and dynamics of neuronal MTs are influenced by various factors, and disturbance of their regulation can contribute to neurodegenerative diseases.
Imaging MT filaments in densely packed neuronal processes is challenging. However, the development of super-resolution techniques combined with the use of nanobodies offers new possibilities to visualise and quantitatively analyse neuronal MT organisation.
We implemented DNA-PAINT (Point Accumulation in Nanoscale Topography), a single-molecule localisation microscopy (SMLM) technique, that allowed us to acquire 3D arrays of the MT lattice in axons of model neurons and dendrites of primary neurons.
As a first approach, for the quantitative analysis of MTs, we used the SMLM image filament extractor (SIFNE) [1]. 2D projections of optical sections with 100 to 150 nm thickness having optimal resolution were used to infer various parameters of the MT lattice. For validation of the method, we performed MT lattice analysis upon stabilising and destabilising conditions. We found significant changes in MT organisation concerning the length of individual MTs, filament straightness, and polymer density in neuronal processes affecting the frequency of intersecting filaments. Changes in MT mass were consistent with measurements of ensemble MT dynamics.
To use the full potential of our 3D imaging strategy, we established a protocol to assess MT structure in 3D. We used “Polyphorm”, a versatile visualisation and model fitting tool [2]. The generated trace field enabled 3D data segmentation, which was further used to analyse the orientation and assign the associated filaments in the vicinity.
This new technique will be presented, offering the possibility of improved data analysis and future semi-automated processing to quantitatively assess the effect of conditions that modulate neuronal MT organization.

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Molecular determinants of protein half-life in chloroplasts (Poster Index: 25)

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Proteolysis is an essential process to maintain cellular homeostasis. One pathway that mediates selective protein degradation is the N-degron pathway, formerly called the “N-end rule” (reviewed in [1]). In the cytosol of eukaryotes and prokaryotes, N-terminal residues can be major determinants of protein stability. While the eukaryotic N-degron pathway depends on the ubiquitin proteasome system, the prokaryotic counterpart is driven by the Clp protease system. Plant chloroplasts also contain such a protease network, which suggests that they might harbor an organelle specific N-degron pathway similar to the prokaryotic one.
While recent discoveries indicate that the N-terminal region of proteins affects their stability in chloroplasts and provides support for a Clp-mediated entry point in an N-degron pathway in plastids (reviewed in [2]), direct *in vivo* evidence is still missing. The proteins involved in this pathway, details about the protease substrate selection and the factors that determine protein stability need to be further investigated.
The aim of my PhD thesis is to investigate the impact of different N-terminal amino acids on chloroplast protein stability. Testing for an N-degron pathway in chloroplasts requires an *in vivo* reporter system, which allows the generation of a specific N-terminal amino acid in the stroma of the chloroplast and the quantification of the reporter protein half-life dependent on the identity of its N-terminal residue. In our case, a tobacco etch virus (TEV) protease-based approach is used to activate the dormant N-degron of a reporter protein. Plastid transformation in tobacco was used to generate plants with a plastid-encoded TEV protease. The reporter protein is introduced into the transformed plants using a transient transformation approach and following translocation into the chloroplast, the plastid localized TEV protease cleaves the reporter protein and expose the desired N-terminal residue. The outlined reporter system is used to systematically and comprehensively challenge the effect of specific N-terminal amino acids on protein stability in this organelle.

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Lipidomic analysis of cells infected with human pathogenic flaviviruses (Poster Index: 27)

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Flaviviruses are emerging arthropod-borne pathogens that are phylogenetically closely related to the hepatitis C virus (HCV). It is known that the flavivirus replication cycle is tightly connected to host lipid metabolism. These human pathogenic flaviviruses can hijack metabolic pathways for replication and persistent infection. Previous shotgun lipidomic studies from our group demonstrated that HCV infection profoundly impacts the host cell lipidome. Following up on these results, we performed shotgun lipidomic experiments from cell homogenates in cell culture infection models of the most prevalent human pathogenic flaviviruses (dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV), yellow fever virus (YFV), and tick-borne encephalitis virus (TBEV)). We first established efficient infection models with similar infection kinetics in Huh7 cells. We then determined the host cell lipidome by shotgun lipidomics during the course of infection. We calculated changes of lipid abundances normalized to protein content using lipid standards. Early in infection, some lipid class abundances increased, e.g. triglycerides (TAG) and cholesterol esters (CE) in ZIKV-infected cells and certain lyso-phospholipids in ZIKV, WNV, and YF-17D infections. We additionally analyzed the relative levels of all lipid species. Here, we observed a striking remodeling of the lipidome during infection, with a strong increase of ceramide and hexosylceramide levels. In addition, there are changes in the acyl chain composition of several lipid classes, most prominently in WNV- and DENV-infected cells. Using bioinformatics, we found that glycerolipid and glycerophospholipid metabolism are significantly altered in infected cells. We probed these pathways for relevance using shRNA-mediated knockdown approaches followed by infection with the different flaviviruses. Depletion of proteins that are involved in glycerolipid and glycerophospholipid metabolism strongly reduced DENV and WNV titers, whereas ZIKV, YFV, and TBEV replication were only marginally affected. Further studies are needed to determine which step of the viral life cycle is affected by these proteins.

Poster Session II

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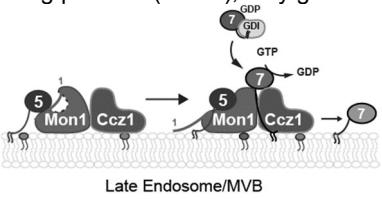
Molecular mechanism of Rab5-dependent Mon1-Ccz1 activation (Poster Index: 2)

Ann-Christin Borchers, Osnabrueck University, Biochemistry Division

Endocytic cargo is taken up via the endolysosomal pathway, a pathway characterized by the maturation of early endosomes to late endosomes,which finally fuse with the lysosome. Small GTPases of the Rab family are key regulatory proteins in this and other membrane trafficking processes by providing binding platforms and giving membrane identity. They act as molecular switches and require guanine nucleotide exchange factors (GEFs) for activation. In their active GTP-bound form, Rabs stably associate with their target membrane. Upon inactivation by GTPase activating proteins (GAPs), they get extracted from membranes again.



During the endosomal 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[1] and Rab7 in higher eukaryotes. Lately, a third subunit of this complex, called Bulli or Rmc1, was identified in metazoan. This protein also functions in endosomal maturation, but does not directly affect GEF activity[2].

Recently, we showed that membrane-associated Rab5 directly promotes Mon1-Ccz1-dependent activation of Rab7[3]. However, the Rab5-binding site in the complex and the mechanistic consequences of this interaction remain unknown.

Here we provide insights on this activation process. We identified the disordered N-terminal part of Mon1 as a regulatory region involved in GEF function. *In vitro* reconstitution of the Rab5-to-Rab7 cascade reveals that N-terminal truncation of Mon1 results in enhanced Rab5-dependent GEF activity. We further show that a conserved hydrophobic motif in the Mon1 N-terminal part is involved in GEF autoinhibition. Using structure prediction and modelling, we mapped the Rab5-binding site in Mon1. We further showed that corresponding mutations of a conserved Tryptophane abolishes the Rab5-dependent GEF activation without affecting the general GEF activity.

This data supports a model in which the Mon1 N-terminal domain restricts accessibility of the Rab5 binding site and therefore directly controls the Rab5-to-Rab7 transition process in endosomal maturation.

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Structural and functional determinants governing constitutive dimerization of an oncogenic GP130 mutant (Poster Index: 4)

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Class I cytokine receptors (CR) have important functions in the regulation of haematopoiesis, immunity and inflammation, and their dysregulation has been implicated in several types of cancer. A number of related mutations and deletions in the ectodomains (ECD) of the shared CR glycoprotein 130 (GP130) have been identified in patients with hepatocellular carcinoma (HCC), a prevalent form of liver cancer and one of the most common types of malignant tumours worldwide. These GP130 variants constitutively activate downstream signalling via Janus family tyrosine kinases (JAK) and signal transducer and activator of transcription (STAT), but the mechanistic bases of GP130 dysregulation are largely unclear. To tackle this challenge, we have here explored the role of receptor dimerization in signal activation and its dysregulation of GP130 in vitro and in live cells. To this end, we have employed dual-color single-molecule total internal reflection fluorescence (TIRF) microscopy in live cells to quantify receptor dimerization in the plasma membrane by co-tracking analysis. These studies revealed ligand-induced dimerization of wildtype (wt) GP130, but very strong constitutive dimerization of the oncogenic GP130 variant “deltaYY”. This truncation lacks Y186-Y190 in the fibronectin III (FNIII)-type D2 domain of GP130, which is critically involved in ligand binding and therefore explain the loss in ligand binding in the mutated receptor chain. Cotransfection assays investigating the influence of associated JAK enforced the hypothesis of a dysregulation solely driven by the extracellular domain. Interestingly, deletion of D1 largely abrogated constitutive dimerization of GP130 deltaYY, suggesting that this domain is involved in the dimerization interface. This could be explained by domain-swapping that is frequently observed for proteins with β-sheet secondary structure such as FNIII, which was confirmed by modelling the dimer by AlphaFold. The purified ectodomains of GP130 wt and deltaYY produced in insect cells also behaved as monomers and dimers, respectively, when analyzed by size exclusion chromatography. Label-free analysis by mass photometric assays clearly identified a monomer-dimer equilibrium for GP130 deltaYY with an equilibrium dissociation constant of ~30 nM. Initial structural analysis by electron microscopy (cryo-EM) confirmed dimer formation, but also indicated loss of a rigid structural organization of the six subdomains that was observed for wt GP130.

Spatiotemporal dynamics of IL-17 family receptor complex formation (Poster Index: 6)

Christoph Pollmann, Divison of Biophysics, Department of Biology, University of Osnabrück, Osnabrück, Germany

IL-17 family cytokines have emerged as central mediators of inflammatory and autoimmune conditions. While most family members remain understudied, therapeutic antibodies targeting IL-17A or IL-17 Receptor A have demonstrated high clinical efficacy in psoriasis, psoriatic arthritis and ankylosing spondylitis patients, indicating the high potential of IL-17 cytokines in the treatment of various diseases. A common feature of the IL-17 family cytokines is that all cytokines recruit the shared receptor subunit IL-17RA and a second chain that is assumed to mediate ligand specificity to initiate signaling. Despite being a critical step for the activation of signaling, receptor complex assembly mediated by the IL-17 cytokine family is poorly understood so far. In order to exploit the full medical potential of IL-17 cytokines, a precise characterization of this step is required. On the basis of recent advances regarding the structural determination of IL-17 receptor complexes, the spatiotemporal dynamics of IL-17RA, IL-17RB and IL-17RC that guide the formation of receptor complexes in response to their corresponding ligands IL-17A and IL-25 were studied *in vivo* in this work. Therefore, dual-color single-molecule TIRF microscopy in combination with nanobody-based labeling was implemented to analyze co-localization and co-diffusion of individual receptor subunits. Furthermore, targeted mutagenesis was utilized to gain insights on a receptor-receptor interaction site, that mediates complex formation spatially distant from the ligand-binding site. Strikingly, our results strongly contradict the prevailing paradigm of heterodimeric receptor complexes in the IL-17 family and rather suggest the formation of higher-order complex stoichiometries, whose formation is orchestrated by distinct extracellular receptor interfaces.

A versatile toolbox for constructing nanoscale signaling platforms in live cells (Poster Index: 8)

Arthur Felker, Divison of Biophysics, Department of Biology and Center for Cellular Nanoanalytics, University of Osnabrück

Nanopatterning approaches for enriching transmembrane receptors with high-density have been used for triggering cell signaling. Such spatially-controlled signaling platforms on bio-chips are powerful tools for exploring the molecular mechanisms of signal transduction. Here, we develop a versatile toolbox for high-contrast nanopatterning of proteins in *vitro* and in live cells. We devised capillary nanostamping of biofunctional proteins and polymers onto glass substrate with optimized surface chemistry for stable nanostamping. To the end, we obtained close-packed biofunctional nanodot arrays (bNDAs) with ~500nm in diameter and 0.8µm spacing. In combination with engineered covalent and non-covalent binding pairs, orthogonal immobilization and dimerization of proteins into bNDAs with a high contrast was achieved. We used this approach to enrich receptor tyrosine kinases TrkB1 in the bNDAs for triggering signalling in live cells. Recruitment of signalling-specific effector Grb2 and the guanine nucleotide exchange factor SOS1 were successfully observed. These results allowed us to spatiotemporally trigger key events of TrkB signalling for quantitative analyses. Furthermore, combining bNDA approach with PI3K and PTEN in a binary patterning format generated PIP3 nanodomains. The obtained nanoscale signaling platform therefore paves the way for high-throughput quantification of protein-protein and protein-lipid interactions in cell signaling pathways.

Structural basis of TIRAP assembly revealed by cryo-EM
(Poster Index: 10)

Jan-Hannes Schäfer, Structural Biology Division, Osnabrueck University

Toll-like receptors (TLRs) activate the innate immune response during pathogen infection. Upon ligand-induced receptor dimerization, intracellular adapter proteins, like TIRAP (TIR-domain containing adaptor protein), initiate the assembly of large signaling complexes. In resting TLR-positive cells, TIRAP multimerizes into filaments at the plasma membrane and is subsequently converted into dimers during TLR signaling. However, the exact process of TIRAP filament formation and interaction with TLR dimers remains unknown.
Here, we utilize negative-stain and cryo-EM to investigate the mechanism of TIRAP multimerization. We demonstrate that the full-length human TIRAP forms filaments in a temperature-dependent and reversible manner in the absence of TLRs. Our 3.2 Å cryo-EM structure, together with the negative-stain analysis, indicates a sequential filament formation, starting from stable dimers that polymerize into thin filamentous intermediates and tubes with a diameter of about 30 nm. Together, our data support the presence of TIRAP filaments in resting TLR-positive cells. Furthermore, filamentous TIRAP may provide a pool of required adapter proteins to stimulate TLR-based signal transduction.

A cross-platform application for cryo-EM and laboratory management (Poster Index: 12)

Kilian Schnelle, Structural Biology Division, Osnabrueck University

Laboratory journals are essential and mandatory for performing reproducible research and documenting experiments. While handwritten lab books are still the most frequently used form of documentation, the development, and usage of digital alternatives are gaining increased popularity amongst researchers. Digital lab books enable cleaner documentation, provide searchability of notes and associated databases, as well as, the possibility to share and disseminate content. A huge drawback, however, resides in the need to document the work twice: initially as notes in the lab, while the experiment is performed, and later, in detail, on stationary computers.
Here we describe the design and development of a cross-platform application, enabling easy-to-use documentation with mobile devices and personal computers. Adding the ability to use phones or tablets enables new documentation methods like scans or photos with the device's camera making the need for hand written notes obsolete. Since our laboratory is focused on cryo-EM and image processing we will combine our lab book with access to processing software and laboratory management tools like databases, and booking systems. Making use of the widespread availability of mobile phones we will include communication and sharing features.

Neprilysins maintain heart function via cleavage of SERCA-inhibitory micropeptides (Poster Index: 14)

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

Muscle contraction is based on a well-characterized molecular mechanism and depends on strictly controlled Ca^{2+} transients within myocytes. The sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, SERCA, is a major player maintaining these transients by transporting Ca^{2+} from the cytosol of muscle cells into the sarcoplasmic reticulum (SR). SERCA activity is regulated by binding of SR membrane integral micropeptides and altered expression or function of these peptides impairs SERCA activity and causes cardiomyopathy. To date, it is not known how homeostasis or turnover of the micropeptides is regulated.
We found that the *Drosophila* endopeptidase Neprilysin 4 (Nep4) hydrolyzes SERCA-inhibitory Sarcolamban (SCL) peptides. Moreover, increased *nep4* expression phenocopied the effects of *scl* knockout on heart physiology. Respective phenotypes strictly depended on the catalytic activity of the peptidase, thus identifying Nep4-mediated hydrolysis as a critical factor for peptide homeostasis and SERCA regulation.
To analyze the consequences of the Neprilysin-mediated hydrolysis at molecular level, SERCA-inhibitory peptides were expressed either with or without Neprilysin in *Drosophila* S2 cells. In accordance with the localization pattern in *Drosophila* muscle tissue, the peptides localized to ER membranes in absence of Neprilysin, but shifted to the cytosolic fraction if the peptidase was co-expressed. This indicates a reduced membrane anchoring of the hydrolyzed peptides. In addition, Nep4-mediated hydrolysis affected the amount and oligomerization state of SCL peptides in muscle cells of transgenic *Drosophila* animals. Full-length peptides were present as monomers and as oligomers, while increased *nep4* expression resulted in absence of such oligomers. Thus, Nep4-mediated cleavage also impaired oligomerization of the SCL peptides. Notably, oligomerization represents a well-known and physiologically highly relevant characteristic of vertebrate SERCA-inhibitory 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.

The Role of TSC1 in TSC complex function (Poster Index: 22)

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Cell metabolism highly depends on growth factor signalling. mTORC1 is one of the master regulators in cells which integrates growth factor signals into cellular growth. In its GTP-bound form the small GTPase Rheb activates mTORC1 at the lysosomal membrane. TSC is the only known regulator of Rheb and inactivates Rheb by catalysing GTP hydrolysis. However, TSC only localizes to the lysosomal membrane upon growth factor starvation. If Rheb is present in its GDP-bound form mTORC1 and the downstream transcription processes are downregulated.
The TSC complex consists of three subunits, TSC1, TSC2 and TBC1D7. Mutations in the *TSC1* and/ or *TSC2* genes cause Tuberous Sclerosis Complex, a disease where patients suffer from benign tumours in multiple organs thus TSC has a crucial tumour suppressor function. The catalytically active GTPase activating protein (GAP) domain is located on the C-terminal part of TSC2[1]. The N-terminal domain of TSC1 contributes to the lysosomal localization of the complex, as it is interacting with $PI3,5P_2$[2]. TSC1 C-terminal coiled-coil region is binding the N-terminal HEAT repeat domain of TSC2. Additionally, TSC1 favours complex formation assembling into dodecamers[2].
The aim of this study is to analyse the role of TSC1 in function and regulation of the complex. We will investigate the contribution of individual TSC1 domains in complex formation as well as modulation of GAP activity. Our findings will also provide a better understanding of the contribution of TSC1 in the development of tuberous sclerosis complex.

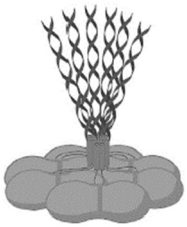

Figure 4: Proposed model of the TSC1 dodecamer built up from TSC1 dimers. Under starvation conditions the N-terminal domain is located at the lysosomal membrane. The coiled-coil region interacts with TSC2[2].

References:

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The Rho GEF Trio is dynamically localized at microtubules and required for neural crest migration (Poster Index: 26)

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Directional cell migration is controlled by various external signals leading to a precisely orchestrated intracellular signaling response that converges at the level of small Rho GTPases. The Rho guanine nucleotide exchange factor (GEF) Trio features distinct catalytical domains to control the activity of Rac1 and RhoA and is therefore well suited to coordinate signaling pathways leading to directional cell movement. Recently, we showed in *Xenopus* that Trio is required for coordinated cranial neural crest (NC) cell migration and formation of the craniofacial cartilage. Furthermore, Trio is important for protrusion formation and Trio morphant NC cells show a blebbing phenotype. As expected, the Trio morphant phenotype can be rescued by low concentrations of constitutively active Rac1 and RhoA but not Cdc42. Interestingly, the Trio GEF2 domain, which activates RhoA, is sufficient to rescue the Trio morphant phenotype, while the Trio GEF1 domain, which activates Rac1, is not. In addition, the GEF2 domain, but not the GEF1 domain, colocalizes with EB3 at microtubule plus-ends in *Xenopus* NC cells. Low concentrations of a Trio GEF2 mutant, lacking the SxIP-motif responsible for microtubule plus-end binding, is no longer able to restore the Trio loss-of-function phenotype, suggesting that the colocalization of Trio to microtubule plus-ends is required for its function. Furthermore, Trio loss of function seems to disrupt the microtubule network as well as focal adhesion assembly in NC cells. Currently, we are investigating the dynamic intracellular transport of Trio and its consequences for microtubule organization and focal adhesion formation leading to coordinated NC cell migration.

Ptk7 is dynamically localized at NC cell-cell contact sites and interacts with the RhoGEF Trio (Poster Index: 28)

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Neural crest (NC) cells are highly migratory cells contributing to a broad range of vertebrate tissues and their migration behavior resembles cancer cell invasion. The directional migration of NC cells is controlled by various mechanisms including information exchange via dynamic NC cell-cell contacts. A transmembrane protein that is likely involved in this process is PTK7 (protein tyrosine kinase 7), an evolutionary conserved Wnt/PCP co-receptor, which is required for *Xenopus* NC migration. Our data demonstrate that Ptk7 is dynamically localized at NC cell-cell contacts and plays a role in contact inhibition of locomotion (CIL), a phenomenon whereby NC cells change their polarity and directionality upon cell-cell contact. We found that loss of Ptk7 results in disturbed CIL behavior. Conversely, non-NC cells were protected from NC invasion by ectopic expression of Ptk7, but not by a Ptk7 deletion construct lacking the extracellular domain. These data suggest that Ptk7 is not only necessary but also sufficient for CIL. Recently, we identified the Rho guanine exchange factor (GEF) Trio as an interaction partner and possible downstream effector of Ptk7 during NC cell migration. Trio is especially well suited to relay signals, as it features two GEF domains, which specifically activate Rac1 and RhoA. Like Ptk7, Trio is also required for NC migration and ectopic expression of Trio rescues the Ptk7 morphant phenotype. Currently, we investigate the dynamic subcellular interaction of Ptk7 and Trio and the molecular mechanisms by which they control NC migration.

Notes