IRTG Retreat 2024

Youth hostel DJH Dortmund

21 to 23 October 2024

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11:50 – 12.10	**Lena Tveriakhina**: *Visualization and Characterization of Notch Signaling Dynamics*
12:10 – 12:30	**Dmitry Shvarev** (Structural Biology of Photosynthetic Microorganisms): *Multiprotein Complexes Governing Chlorophyll Biosynthesis*
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9:30 – 9:50	**Verena Fritsch** (Microbiology): *Analysis of translocated T3SS effector protein dynamics and their role in endosomal remodeling by intracellular Salmonella enterica*
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11:10 – 11:30	**Kai Jürgens** (Zoology and Developmental Biology): *A Drosophila model for TMEM43-induced human cardiomyopathies reveals ERMCS involvement*
11:30 – 11:50	**Lena Clausmeyer** (Bioanalytical Chemistry): *In vitro characterization of the ceramide synthase in S. cerevisiae*
11:50 – 12:10	**Lea Hoffmann** (Structural Biology): *Activity and Structural Changes of MsbA upon Interaction with LPS and Precursors*
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9:30 – 9:50	**Victor Cornily** (Animal Physiology): *Formation, composition and role of extracellular vesicles in spiny mouse tissues*
9:50 – 10:10	**Katharina Kott** (Molecular Cell Biology): *SGMS2 variants linked to skeletal dysplasia disrupt cellular sphingomyelin gradients*
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14:10 – 14:30	**Arthur Felker** (Biophysics): *Combining single-molecule FRET and GIET for nanoscopic 3D structural analysis of proteins*
14:30 – 14:50	**Christoph Pollmann** (Biophysics): *Mechanism of cytokine receptor activation and dysregulation at single molecule level*
14:50 – 15:10	**Pascal Höhne** (Organelle Communication): *Optogenetic manipulation of organelle contact sites*
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Talk Abstracts

Session I

Stard3 transports sphingosine at lysosome-ER contact sites

Denisa Jamecna

Chemical Biology of Membranes Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The endolysosomal system constantly facilitates the recycling of cellular lipids. Lysosomal transport has so far been mostly elucidated for sterols, as lysosomes are integral to incorporating lipoprotein-derived cholesterol into cellular membranes. In contrast, transport of other lipids such as sphingolipids is much more enigmatic. The major sphingolipid backbone, sphingosine, is generated by catabolism of complex sphingolipids in the lysosome and it is exported (recycled) into the ER for reuse in anabolic pathways. Defects in lysosomal transport of sterols and sphingolipids occur in severe pathologies called lysosomal storage diseases but are also a feature of more common diseases such as neurodegenerative disorders. Taking the advantage of synthetic photoactivatable and clickable lipid analogs with organelle targeting functionality, we recently found that lysosomal sphingosine is exported by a transmembrane sterol transporter STARD3. STARD3 tethers lysosome-ER membrane contact sites (MCS), transports cholesterol from the ER into the lysosome and fuels lysosomal sphingosine towards the synthesis of more complex sphingolipids in the ER and Golgi. This finding adds to the growing list of examples on how sterols and sphingolipids utilize common transport routes in and out of the lysosomes.
In my research group, we intend to elucidate how lysosomes co-regulate the metabolism and distribution of sterols and sphingolipids in the cell. We posit that the endolysosomal system works as a metabolic and logistic center and is instrumental in regulating cellular lipid homeostasis. Specifically, lipid transfer proteins and metabolic enzymes located within MCS between lysosomes and other organelles serve as means of achieving a highly dynamic sterol/sphingolipid co-regulation.

Visualization and Characterization of Notch Signaling Dynamics

Lena Tveriakhina

Department of Biology/Chemistry, University of Osnabrück, Germany

Notch signaling is a fundamental cell signaling pathway that controls a variety of cell fate decisions during development and adult tissue homeostasis and its dysfunction is implicated in many human diseases and several cancers. The signaling is activated through direct interaction of the Notch receptor with its ligand present on the surface of the adjacent cell. Following ligand binding two proteolytic cleavages release the receptors extracellular (NECD) and intracellular (NICD) domains, respectively. NECD is trans-endocytosed into the ligand cell, whereas the liberated NICD translocates into the receptor cell's nucleus and acts as a transcriptional effector.

We use cell and organoid based model systems, CRISPR/Cas9 genome engineering and multidisciplinary approaches such as advanced microscopy, biochemistry, and transcriptomics to study the dynamics of physiological Notch signaling events in different tissue contexts. In recent work, we used real time quantitative live cell microscopy to visualize the spatiotemporal dynamics of cells expressing fluorescently labeled endogenous Notch and its ligand. We showed that at sites of direct cell-cell contact ligands and receptors immobilize and accumulate forming synapses with a 1:1 stoichiometry. Synapses resolve ~15-20 minutes after formation and precede NECD trans-endocytosis and NICD nuclear accumulation1. Our current studies focus on the understanding how the lipid-binding properties of Notch ligands, the identity and dynamic characteristics of membranes as well as how protein partners regulate ligand and receptor trafficking, synapse formation, and signal transduction. With this we aim to identify new modulators of key signaling events both in cells and in vivo.

1. Tveriakhina, L., Scanavachi G., et al. Temporal dynamics and stoichiometry in human Notch signaling from Notch synaptic complex formation to nuclear entry of the Notch intracellular domain. Dev. Cell (2024) doi:10.1016/j.devcel.2024.03.021.

Multiprotein Complexes Governing Chlorophyll Biosynthesis

Dmitry Shvarev

Structural Biology of Photosynthetic Microorganisms Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The process of chlorophyll biogenesis is complex and involves intricate multiprotein complexes. However, the mechanistic understanding of the underlying enzymatic reactions is poor due to a lack of structural information. By using structural biology techniques such as cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) together with functional characterization we will gain mechanistic insights into the process of chlorophyll biosynthesis. We will address different steps of chlorophyll production, from the first committed step catalyzed by magnesium chelatase to the terminal step, which is the incorporation of chlorophyll into chlorophyll-binding proteins. We will also analyze the intermediate steps of the process and investigate its regulatory mechanisms. The results of this project will advance the understanding of chlorophyll biosynthesis and photosynthesis in general.

Session II

Keynote Lecture

Kathrin Thedieck

Department Metabolism, Senescence and Autophagy, Research Center One Health Ruhr, University Alliance Ruhr & University Hospital Essen, University Duisburg-Essen, Germany
German Cancer Consortium (DKTK), partner site Essen, a partnership between German Cancer Research Center (DKFZ) and University Hospital Essen, Heidelberg, Germany
Laboratory of Pediatrics, Section Systems Medicine of Metabolism and Signaling, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
Freiburger Materialforschungszentrum, Stefan-Meier-Straße 21, 79104 Freiburg, Germany

New metabolic signals to mTOR at lysosomes

Amino acid metabolism enhances tumor malignancy and cancer are often deplete of essential amino acids, raising the question how growth is sustained under nutrient stress. We have discovered new metabolic inputs to the tuberous sclerosis protein (TSC) complex, a suppressor of the metabolic master regulator mTOR (mechanistic target of rapamycin) that is often hyperactive in tumors. The stress granule protein G3BP1 anchors the TSC complex to lysosomes in a nutrient sensitive manner, unveiling unexpected vulnerabilities. I will discuss new mechanistic insights, opening new avenues for therapies in ER-positive cancer.

1. G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling. doi: 10.1016/j.cell.2020.12.024

This project has received funding by the European Union (H2020, MESI-STRAT, 754688, https://mesi-strat.eu/; ERC, BEYOND STRESS, 101054429). Views & opinions are those of the author(s).

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Control of phosphatidylinositol levels on endosomes and vacuole

Frederik Brinks

Biochemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Phosphatidylinositol phosphates (PIPs) play a crucial role as membrane identity markers by recruiting effector proteins to specific organellar membranes. These PIPs are derivatives of phosphatidylinositol (PI) and are dynamically phosphorylated at three positions. Their production and turnover are tightly regulated by kinases and phosphatases, enabling rapid interconversion between various forms. In this study, we focus on the yeast Fab1 kinase complex (FAB1C), which catalyzes the phosphorylation of PI(3)P to PI(3,5)P_2. The FAB1C comprises the kinase Fab1, the scaffold protein Vac14, and the phosphatase Fig4, which is believed to facilitate the conversion of PI(3,5)P_2 back to PI(3)P. However, the specific cellular sites of PI(3,5)P_2 production, its turnover, and the regulatory mechanisms governing the FAB1C, and are not yet fully understood. To investigate Fab1 activity in vivo, we established the recombinant, fluorescently tagged PI(3,5)P_2 probe SnxA for live-cell imaging in yeast. Surprisingly, we found elevated levels of PI(3,5)P_2 in mutants of the recently described CROP complex, which is closely associated with vacuolar fission processes. Additionally, certain mutations in membrane trafficking pathways resulted in significantly altered levels of PI(3,5)P_2. Using SnxA, we demonstrated that the phosphorylation of Fab1, near its PI(3)P-binding FYVE domain, by the master growth regulator Target of Rapamycin Complex 1 (TORC1), also influences its activity. We propose that, following its activation on the vacuole, the FAB1C cycles between endosomes and the vacuole to: i) enhance catalytic activity at endosomal signaling platforms via TORC1, or ii) regulate the controlled depletion of PI(3,5)P_2 by Fig4.

Vac7- a regulator of Fab1 activity

Annabel Arens

Biochemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Lysosomes have a key function in cellular physiology as a central organelle for protein degradation and organelle quality control, and are tightly connected to autophagy. Yeast vacuoles as the equivalent to lysosomes have on their surface the only phosphatidyl 3-phosphate (PI(3)P) 5-kinase Fab1 (PIKfyve in humans), which generates $PI(3,5)P_2$ from PI(3)P. Fab1 forms a complex with the scaffold protein Vac14 and the lipid phosphatase Fig4. The vacuolar transmembrane protein Vac7 and the β-propeller protein Atg18 were previously described as regulators Fab1 activity. In this study, we show Vac7 regulates more than just Fab1. All subunits of the Fab1 complex, including Atg18 as a regulator, change their vacuolar localization due to a loss of Vac7. In turn, anchoring the cytosolic domain of Vac7 to the mitochondrial outer membrane was sufficient to recruit both Fab1 and Fig4, but not Vac14. Using vacuole morphology as a read-out, we identified important residues in the cytosolic part of Vac7, which are necessary for proper functionality of the Fab1 complex. Intriguingly, Vac7 can dimerize and change its localization during osmotic stress. This suggests that Vac7 acts as a vacuolar sensor, which controls Fab1 activity by changing its oligomerization and localization on the vacuole.

Determining the Effect of Membrane Composition on the Activity of Yeast Serine Palmitoyl-Transferase

Verena N. Wolf

Bioanalytical Chemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Sphingolipids (SLs) are a major lipid class of eukaryotic membranes and function as signaling molecules. The first and rate-limiting step of the SL synthesis is catalyzed by the serine palmitoyl transferase (SPT), organized in a larger protein complex called SPOTS (SPT, ORM, TSC3, SAC1). The SPOTS complex contains the regulatory subunits Tsc3, the negative regulators Orm1 and Orm2 and the phosphatidyl-inositol-4 phosphate (PI4P) phosphatase Sac1. Recently the structure of yeast SPOTS complex has been solved, revealing direct regulation by ceramide. Moreover, the SPOTS complex binds ergosterol, indicating a potential regulatory mechanism that provides a link for the previously observed co-regulation of sphingolipids and sterols. Finally, a negative regulatory function has been observed for the PI4P-phosphatase Sac1, which is directly linked to the ergosterol transfer from the endoplasmic reticulum to the Golgi via the oxysterol-binding protein homolog Osh4. To understand the effect of ER membrane composition on the activity of the SPOTS complex and the difference between Orm1 and Orm2 dependent regulation, we reconstituted the SPOTS complex in liposomes. This *in vitro* system allows us to manipulate both the lipid and SPOTS complex composition to determine their effects on SPT activity.

Adaptation of Plasma Membrane ATPase1 to metabolic stress

Aniket Bandyopadhyay

Institute for Cell Dynamics and Imaging, University of Münster, Germany

Plasma membrane domains, owing to their heterogeneity in lipid and protein composition, have been a point of interest for quite some time. While increased dynamicity of the plasma membrane of mammalian systems have hindered the researchers to explore these domains further, budding yeast with its very stable and dense membrane has emerged as a very powerful model to understand plasma membrane organization. In my project, I am interested in the protein Pma1, which is a major proton pump on yeast plasma membrane. Upon encountering chronic glucose stress, yeast plasma membrane undergoes massive re-organization, with most of the proteins getting endocytosed. Very uniquely, Pma1 does not get internalized, but laterally clusters, forming a network of crystalline arrays on the plasma membrane. By performing whole genome screening, we identified a plasma membrane protein, Mrh1, which co-clusters with Pma1 and perfectly colocalizes with Pma1 clusters. Deletion of Mrh1 blocks Pma1 clustering. Mrh1 has an exceptionally long positively charged C terminal. Modulating these charges have a severe impact on Pma1 clustering. Furthermore, depletion of negatively charged phosphatidylserine (PS) completely blocks Mrh1 clustering and hinders Pma1 clustering, indicating a strong association between Mrh1 and PS. To this end, we have hypothesized that chronic glucose stress leads to decrease in protein density on the plasma membrane, increasing the diffusion rate of the residual proteins, further leading to coalescing of proteins associated with a lipid molecule. Our very recent data has suggested that the clustering phenomena is indeed necessary for the long-term survival of the cells under stress conditions.

Session III

Keynote Lecture

Marianne Grognot

Institute of Medical Microbiology, RWTH Aachen University Hospital, Germany

The Fast and the Furious: A quantitative dive into the motility of gut bacterial pathogens and commensals

An estimated half of all bacterial species can swim by rotating one or more flagella. There are strong incentives to think that motility is important in the early steps of inflammation and/or infection, especially in allowing bacteria to breach through the mucus barrier that protects our intestinal mucosa.
In this talk, I will focus on how we quantitatively assess the motility of gut bacterial pathogens and commensals, to elucidate *if* and *how* motility shapes their interactions with the host environment. How can we track thousands of swimming bacteria in 3D within minutes? How can we analyze their behaviors in liquid, in mucus-mimicking media or in stool samples? How do diverse swimming behaviors change the performances of a bacterial population? How are different bacteria cruising in your gut?
By introducing you to the motivations, challenges, and recent advances in microbial motility, I will highlight why my new group tackles host-microbe interactions through this interdisciplinary lens.

1. Grognot, M. , J. W. Nam, L. E. Elson and K. M. Taute. Physiological adaptation in flagellar architecture improves *Vibrio alginolyticus* chemotaxis in complex environments. *Proc. Natl. Acad. Sci. U.S.A.*, 2023. doi:10.1073/pnas.2301873120
2. Grognot, M., A. Mittal, M. Mah'moud and K. M. Taute. *Vibrio cholerae* run-reverse-flick motility in aquatic and mucus-mimicking environments. *Appl. Environ. Microbiol*., 2021. doi:10.1128/AEM.01293-21

Manipulation of host cell lipidome by translocated effector proteins of intracellular *Salmonella enterica*

Shirley Helms

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Salmonella enterica is a pathogen causing gastroenteritis with more than 1.3 billion cases worldwide and therefore causing major impact on public health. As facultative intracellular pathogen *S. enterica* is capable to modify and remodel the host cell endosomal system to ensure nutritional supply and replication. This endosomal remodelling is mediated by translocation of effector proteins by the *Salmonella* pathogenicity island 2 (SPI2) type 3 secretion system (T3SS). Endosomal remodelling results in biogenesis of the *Salmonella*-containing vacuole (SCV) and formation of *Salmonella*-modified membranes (SMMs).
In this project, we investigate the lipid modification and organization of the SCV and SMMs after infection by *S. enterica* serovar Typhimurium (STM). We hypothesise that certain SPI2-T3SS effector proteins manipulate the lipid transport towards the SCV. There are already some effector proteins like SseJ, SseL and SteA proposed to have impact on the lipid supply to the SCV Currently, the lipid composition of the SCV and SMMs are not known.
We established a purification method by using antibody-conjugated magnetic beads to purify lysosomes/SCVs/SMMs of cells infected by STM for subsequent extraction of lipids for analysis of their composition. With this method, we aim to analyse the impact of specific effector proteins on the SCV/SMM membrane structure, and the different lipid classes located in these membranes. In the next step we will focus on various mutant strains to analyse the contribution of effector protein on the lipid composition. In a second line of investigation, we want to visualize these effects by microscopy. Therefore, we would label lipids like cholesterol and analyse their localization after infection.

Analysis of translocated T3SS effector protein dynamics and their role in endosomal remodeling by intracellular *Salmonella enterica*

Verena N. Fritsch

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Salmonella enterica Typhimurium (STM) relies on the translocation of effector proteins by two type III secretion systems (T3SS) encoded by genes located on *Salmonella* pathogenicity islands 1 (SPI1) and 2 (SPI2). While SPI1-T3SS is required for the invasion of non-phagocytic cells, SPI2-T3SS allows the formation of a replication-permissive niche within host cells. By means of SPI2-T3SS, a massive endosomal remodeling is induced, leading to the formation of an extended interconnected network of *Salmonella*-induced filaments (SIF). Our initial analyses of STM-infected host cells revealed that the endosomal remodeling creates novel membrane contact sites (MCS) between different host cell organelles, such as mitochondria and ER, and the SIF network. Using volumetric electron microscopy (VEM), we aim to analyze the 3D organization of MCS in the context of the entire host cell at the ultrastructural level. By applying SPLIT-GFP-based contact site sensors (SPLICS), we can monitor and quantify MCS formation between various organelles over time in living cells. Our further analyses are directed to assess the biological function of these novel MCS, and to determine the individual contribution of key SPI2-T3SS effector proteins to MCS formation during SIF biogenesis. Additionally, we employ genetically encoded self-labeling enzyme (SLE) tags to investigate the translocation, subcellular localization, and interaction partners of SPI1-T3SS and SPI2-T3SS effector proteins in living cells. Our aim is to develop improved tools to study the function and translocation dynamics of STM effector proteins *in vivo* in real-time during infection. To enable analyses of effector translocation with high spatial and temporal resolution, we identified HaloTag variants with improved T3SS translocation and high signal intensity after ligand binding. The fusion proteins were functional as effectors after translocation into host cells, allowing the analysis of T3SS effector translocation early during infection and monitoring of effector fate.

Manipulation of VAP-mediated membrane contacts by pathogenic mycobacteria

Anna-Carina Mazur

Host-Microbe Interactome Division, Centre for Structural Systems Biology, Hamburg, Germany

Tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), remains the deadliest bacterial infectious disease. Once inside macrophages, Mtb resides in a vacuole and manipulates the host lipid metabolism to create a nutrient-rich environment. Mycobacteria utilize outer cell wall protein complexes to take up lipids, such as sterols, which serve as carbon and energy sources. In studying tuberculosis, the *Dictyostelium discoideum*/*Mycobacterium marinum* infection model reveals sterol accumulation within the *Mycobacterium*-containing vacuole (MCV), hinting at a lipid supply route for mycobacteria. Lipid transfer proteins (LTPs) facilitate non-vesicular lipid transport at membrane contact sites (MCS). Tether proteins like the ER-resident (VAMP) associated protein (VAP) stabilize MCS by interacting with proteins containing FFAT motifs. Some pathogens, like *Chlamydia*, exploit VAP to create lipid pipelines, expanding their vacuoles. Our research identified the recruitment of the LTP oxysterol binding protein 8 (OSBP8) to the infection site with *M. marinum* due to membrane damage, forming an ER-MCV MCS. We also noted OSBP8 at ER-cytosolic bacteria MCS in later infection stages, possibly due to interactions with the bacterial cell wall. VAP was discovered on the surface of cytosolic mycobacteria, emphasizing its role in MCS formation. We plan to further explore these interactions in the *D. discoideum*/*M. marinum* system and infected macrophages.

Session IV

Keynote Lecture

Stefan Raunser

Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, 44227 Dortmund, Germany

Unlocking the secrets of heart muscle structure

Sarcomeres are force-generating and load-bearing devices of muscles. A precise molecular picture of how sarcomeres are built underpins understanding their role in health and diseases. We determined the molecular architecture of native skeletal and cardiac sarcomeres and structures of sarcomeric proteins using cryo-focused-ion-beam milling (cryo-FIB) and electron cryo-tomography (cryo-ET). Our three-dimensional reconstruction of the sarcomere reveals molecular details in the A-band, I-band and Z-disc and demonstrates the organisation of the thin actin and thick myosin filaments and their cross-links [1,2]. Our reconstruction of the thick filament reveals the three-dimensional organization of myosin heads and tails, myosin-binding protein C (MyBP-C) and titin, elucidating the structural basis for their interaction during muscle contraction [2]. Using sub-tomogram averaging, we determined an in situ structure of the thinfilament at 4.5 Å, revealing the structure of nebulin and the molecular mechanism underlying its role as a "molecular ruler", in stabilising thin filament and in regulating myosin binding [3]. We also determined single particle cryo-EM structures of F-actin at unprecedented resolutions, offering a direct visualization of water molecules and allowing atomic insight into ATP hydrolysis and phosphate release [4,5]. In our latest study, we elucidated the molecular mechanism of actin filament elongation by formins [6]. Taken together, our cryo-EM studies provide deep insights into muscle structure, actin filament aging and the interaction between actin and myosin and their associated proteins, which form the basis of muscle contraction and regulation.

1. Wang Z, Grange M et al. (2021), Cell. 184, 2135-2150.613
2. Tamborrini D et al. (2023), Nature, 623(7988):863-871
3. Wang Z, Grange M et al. (2022), Science. 375, eabn1934
4. Oosterheert W et al. (2022), Nature, 611(7935):374-379
5. Oosterheert W, Blanc F et al. (2023), Nat Struct Mol Biol. 30(11):1774-1785
6. Oosterheert W, Boeiro Sanders M et al. (2024), Science, 384(6692):eadn9560

A Drosophila model for TMEM43-induced human cardiomyopathies reveals ERMCS involvement (Poster Index: 18)

Kai Jürgens

Zoology and Developmental Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

TMEM43 encodes a membrane protein with four transmembrane helices located in the outer ER membrane. A point mutation, p.S358L, causes heritable cardiomyopathies, characterized by sudden cardiac death in young adults, particularly in men aged 20-40. Despite its crucial role in cardiac health, details about TMEM43's molecular mechanisms, subcellular localization, functions, and interaction partners have remained largely elusive.

In our study, we established Drosophila as a model organism for investigating TMEM43-induced cardiomyopathies, utilizing genetic engineering to generate knock-in, knock-out, and tagged transgenic fly models. This approach not only mirrors the human disease phenotype but also provides an innovative platform for in-depth functional analyses.

Our investigations revealed that flies carrying the dTMEM43p.S333L point mutation parallel human pathologies, displaying arrhythmias, altered energy metabolism, and premature death. This phenotypic similarity underscores the evolutionary conservation of TMEM43's role in cardiac function.

A recent advancement in our research was uncovering the role of the Endoplasmic Reticulum-Mitochondria Contact Sites (ERMCS) in relation to the TMEM43 mutation. We have gained new insights into potential mechanisms by comparing human TMEM43 data with our fly model findings. Employing a combination of proteomic analyses, DNA-Paint, pulldowns, and electron microscopy, we have delineated the impact of TMEM43 on energy metabolism in detail.

We will unveil these findings, highlighting the functional role of dTMEM43 and its broader implications for the human system. This research aims to accelerate our understanding of cardiac health and disease, bridging model organism studies with human clinical data to unravel the complexities of genetic heart diseases.

1. Klinke, N., Meyer, H., Ratnavadivel, S., Reinhardt, M., Heinisch, J.J., Malmendal, A., Milting, H. and Paululat, A. (2022) A Drosophila melanogaster model for TMEM43 related Arrhythmogenic right ventricular cardiomyopathy type 5. Cell Mol Life Sci, 79(8):444. DOI: 10.1007/s00018-022-04458-0

In vitro characterization of the ceramide synthase in *S. cerevisiae*

Lena Clausmeyer

Bioanalytical Chemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The ceramide synthase (CerS) catalyzes the N-acylation of a sphingoid base and a CoA-activated fatty acid to produce ceramide, a bioactive molecule, and the backbone of all complex SL. Accumulation of ceramide is toxic for cells and lead to cell death. Various diseases such as neurodegenerative disorder, diabetes and cardio vesicular disease are linked to elevated ceramide levels. In yeast, the CerS consists of three subunits Lac1, Lag1, and Lip1. While Lac1 and Lag1 are non-essential and able to compensate for the loss of each other, Lip1 is essential for CerS activity, but its molecular function remains unknown.
Here, we purified the yeast ceramide synthase complex and solved its structure by cryo-electron microscopy in an active and a fumonisin B1 inhibited state. We provide structural insights into the assembly of the complex indicating a hetero-tetramer with Lip1-Lip1 as dimerization interface. The catalytic subunits Lac1 and Lag1, harbor a hydrophobic crevice with an opening to the cytosol and to the intermembrane space of the endoplasmic reticulum. Within this cavity, we identify an acyl-chain indicating a pre-loaded state.

Activity and Structural Changes of MsbA upon Interaction with LPS and Precursors (Poster Index: 14)

Lea Hoffmann

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Membrane proteins, particularly ATP-binding cassette (ABC) transporters, play a crucial role in various biological processes. ABC transporters utilize ATP to translocate a wide range of compounds across lipid bilayers. One notable example is the ABC transporter MsbA, which transports the lipopolysaccharide (LPS) precursor Lipid-A across the inner membrane of gram-negative bacteria. This process enables the protein LptB to incorporate Lipid-A into the outer leaflet of the outer membrane. The transport of LPS involves large-scale conformational changes and is highly dynamic. It is known that the addition of LPS to purified MsbA stimulates its activity. This presentation aims to investigate how the addition of LPS and its precursors affects MsbA's activity and the associated structural changes. The focus will be on the stimulation or inhibition of activity as well as the distribution of particles in different conformational states.

Structure and Function of Tri-Longin-Domain-Rab GEFs

Stephan Wilmes

Biochemistry and Structural Biology Division, Institute for Biochemistry, University Münster

Small GTPases of the Rab family play 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). The Tri-Longin-Domain-Rab (TLDR) GEFs have been identified as a sub-family of GEFs based on bioinformatic analysis. The TLDR GEF family comprises the heterodimeric complexes Mon1-Ccz1, BLOC-3 and Inturned-Fuzzy in metazoans, and the Mon1-Ccz1 complex is conserved all the way to yeast. Based on structural and biochemical data a conserved Y(K/R) motif critical for the nucleotide exchange mechanism could be identified (Figure 1).

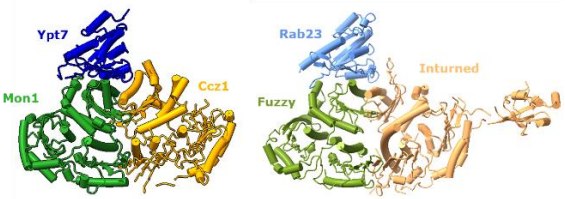

Figure 1: Structures of the TLDR GEFs MC1 and Inturned-Fuzzy in complex with their cognate substrates Ypt7 (Rab7 in metazoans) and Rab23 determined by complementary cryo-EM and X-ray crystallography studies.

Currently, we decipher how specificity and plasticity is achieved in between the different complexes but also along their different signalling pathways, e.g. endosomal maturation, autophagy, ciliogenesis and planar cell polarity. To this end, we investigate the molecular function of membrane interactions, recruiter proteins, auxiliary subunits and unique additional domains like the PDZ domain of Inturned.

1. Kiontke, Langemeyer, Kuhlee, Schuback, Raunser, Ungermann Kümmel., *Nat Comm*, 2017
2. Klink, Herrmann, Antoni, Langemeyer, Kiontke, Gatsogiannis, Ungermann, Raunser, Kümmel, *PNAS*, 2022
3. Herrmann, Schäfer, Wilmes, Möller, Kümmel, *PNAS*, 2023
4. Herrmann, Langemeyer, Auffarth, Ungermann, Kümmel, *J Biol Chem*, 2023
5. Wilmes & Kümmel, *Curr Opin Cell Biol*, 2023

Session V

Keynote Lecture

Ralf Erdmann

Ruhr-University Bochum, Medical Faculty, System Biochemistry, D-44780 Bochum, Germany

Biogenesis of Peroxisomes: From Yeast to Man

The peroxisomal protein import machinery differs from most other translocons as it allows the membrane passage of folded, even oligomerized proteins, and the requirement for cycling import receptor that shuttle between the peroxisomes and the cytosol. These import receptors recognize and bind peroxisomal targeting signals (PTS) of newly synthesized cargo proteins in the cytosol and target them to a docking- and translocation-machinery at the peroxisomal membrane. After import of the proteins in a folded manner, the import receptors are ubiquitinated and released from the membrane in an ATP-dependent manner. Here I will report on the molecular mechanism of peroxisomal protein import and the identification of components of the RADAR pathway, which is the peroxisomal quality control system that removes accumulating receptors from the membrane upon a defect in recycling.
In the second part, I will present our attempts to generate new therapies against parasite diseases. Human Trypanosoma parasites causing sleeping sickness, Chagas disease and Leishmaniasis can be selectively killed by blocking peroxisomal/glycosomal protein import with small molecules that efficiently disrupt interaction of peroxins. In search for new druggable binding interfaces, we identified two new peroxins of the glycosomal protein import machinery. First, PEX15, which functions as a glycosomal docking protein for the export machinery that is responsible for receptor recycling. Second, PEX38, which is a co-chaperon that is required for topogenesis of peroxisomal membrane proteins.

Isolating intact lysosomes from *Drosophila melanogaster*

Franziska Flottmann

Zoology and Developmental Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

We are utilizing Drosophila as a model system for investigating endomembrane-related disorders, e.g. lysosomal storage diseases, endosomal maturation failure and others. We focus on the whole animal to cover the systemic relevance and on specific organs and tissues, such as adipocytes and nephrocytes and aim to analyze the content and composition of intracellular membrane compartments in clinically relevant genetic, dietary or ageing conditions in the Drosophila system.
Here, we present our most recent progress in establishing a protocol to isolate intact lysosomes from *Drosophila* tissue by affinity tag-mediated pull-down. We, therefore, tested two lysosome-specific bait proteins – LAMP1::HA and RpH-ILV-ALFA, a synthetic construct based on TMEM192 (Cheetham-Wilkinson et al., in revision). RpH-ILV-ALFA failed to serve as tool to isolate lysosomes. However, it turned out to be a novel minimally-interactive ratiometric lysosomal pH sensor targeted to intralumenal vesicles that works in mammalian cells and *Drosophila*.
Using a human LAMP1::HA bait protein however, resulted in a significant enrichment of intact lysosomes from tissue. The enriched samples proved to be of high quality and valuable for reproducible protein identification by mass spectrometry.
Our studies were done in collaboration with the Bioanalytical Chemistry division (Osnabrück) and the Molecular and Integrative Biology division (Liverpool).

A chemical strategy for imaging ER-derived lipid flows to damaged lysosomes (Poster Index: 3)

Christian Schröer

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

Cells are frequently exposed to stress that compromises lysosomal membrane integrity. To counteract this, cells utilize repair mechanisms like the sphingomyelin (SM)-dependent repair pathway, which operates independently of ESCRT. This pathway involves the Ca^{2+}-induced scrambling and subsequent hydrolysis of SM by neutral sphingomyelinases, producing ceramide-rich microdomains that promote the inward budding of damaged lysosomal membranes. The endoplasmic reticulum (ER) also plays a crucial role by transferring bulk lipids to damaged lysosomes, at damaged-induced ER-lysosomal contact sites. To explore lipid-based repair mechanisms, we conducted a proteome-wide screen to identify proteins enriched on LLOMe-damaged lysosomes, focusing on those with disrupted translocation in SM-deficient SMS1/2-DKO cells. We found the lipid transfer protein VPS13C being significantly enriched on damaged lysosomes in WT but not in SM-deficient cells, suggesting its role in lipid transfer from the ER to lysosomes. Live-cell microscopy further confirmed that VPS13C removal delays lysosomal repair. To investigate the role of VPS13C in lipid transport, we adapted a novel approach using azido-modified choline, which incorporates into endogenous lipids. By using an ER-targeted fluorescent click reagent, we can selectively label choline-containing lipids within the ER, allowing us to visualize their transport to lysosomes. Our findings indicate that LLOMe-induced damage accelerates the movement of bulk lipids from the ER to lysosomes. Moving forward, we will use this method to quantitatively assess the effect of VPS13C depletion on lipid transport, using lysosomes labeled with pH-sensitive fluorescent aminobeads.

Session VI

Keynote Lecture

Thomas Klein

Institute for Genetics, Heinrich-Heine-University Düsseldorf, Germany

Making sense of the tumorsuppressor Lethal (2) giant discs (Lgd) and its interaction partner Shrub/CHMP4

The tumoursuppressor Lgd was discovered through its mutant phenotype, which is over-proliferation of the epithelial imaginal disc cells. We could show that this over-proliferation is caused by the uncontrolled ligand-independent activation of the Notch signalling pathway in all cells. Further analysis revealed that Lgd is acting in the endosomal pathway and is required for the proper function of the ESCRT machinery. This machinery is the only known device that pinches of membrane vesicles away from the cytosol and is operating in a great variety of processes that demand this kind of membrane abscission, e. g. formation of intraluminal vesicles (ILVs) of maturing endosomes and cytokinesis. The talk will describe the results of the ongoing analysis of the function of Lgd and its interaction partner Shrub/CHMP4, which is a core component of the ESCRT machinery.

Unraveling the working mechanism of a tumor suppressor lipid

Sebastian Bieker

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

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 Bax-mediated cell death, molecular details of the underlying mechanism are scarce. Combining a computational approach with functional studies in cancer cells, we previously identified the voltage-dependent anion channel VDAC2 as a direct effector of ceramide-induced apoptosis. VDAC residues involved in ceramide binding are also required for mobilizing hexokinase type I (HKI) to mitochondria, a condition promoting cell growth and survival in hyperglycolytic tumors. Coarse-grain molecular dynamics simulations and localization studies in cells revealed that HKI residues 1-15 are both necessary and sufficient to mediate VDAC-HKI binding. Importantly, a membrane-buried glutamate essential for ceramide binding by VDACs also establishes a direct and critical contact with Met1 of HKI. Based on these data, we postulate that ceramides exert their tumor suppressor activities in part by acting as modulators of VDAC-based platforms to control mitochondrial recruitment of pro-apoptotic Bax and anti-apoptotic HKI. Our ongoing efforts focus on challenging this model using switchable ceramide transfer proteins and mitochondria-specific release of photocaged ceramides in live cell imaging and functional studies.

Formation, composition and role of extracellular vesicles in spiny mouse tissues

Victor Cornily

Animal Physiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The spiny mouse (Acomys cahirinus) uniquely regenerates tissues without scarring and is an amazing research model for the study of regeneration. A key aspect of regeneration is cellular communication, with extracellular vesicles (EVs) playing a significant role. These nanoscale vesicles serve as molecular couriers, transporting a diverse cargo of proteins, lipids, and nucleic acids between cells. Even though EVs were once considered a cell's way of disposing waste, today, EVs are acknowledged as vital elements of homeostasis. EVs carry specific molecules that can stimulate cell proliferation, migration, and differentiation, which are essential processes for tissue repair. Additionally, EVs possess immunomodulatory properties, influencing the behaviour of immune cells in response to injury, dampening excessive inflammation and promoting a regenerative microenvironment. Apart from scar-free skin regeneration, Acomys has been reported to recover from hypoxia-induced cardiac damage. That holds tremendous potential for treating post-infarction patients, as the human heart does not fully recover from a heart attack. Instead, a scar is formed, which impedes cardiac muscle contraction and contributes to complications. Understanding the composition and behaviour of EVs in the spiny mouse is essential for deciphering the mechanisms behind its amazing regenerative abilities.

My project aims to investigate how injury influences the production and composition of EVs in Acomys and their potential to modulate regeneration. I will focus on exosomes (EVs <130 nm) derived from dermal and cardiac fibroblasts, key cell types in injury response. I also plan to explore the role of macrophage-derived exosomes in collagen turnover, particularly in the disposal of degraded collagen.

I am currently shaping a workflow using a well-studied model such as Mus musculus. That includes a successful establishment of an exosome isolation method from cell culture media that balances two aspects: purity and biological functionality. All the commonly used isolation techniques increase either the former or the latter characteristic at the expense of the other. Next, the implementation of LC-MS/MS, TEM, WB and NTA will provide information about the physical properties and lipid and protein composition of the exosomes. After shifting to the organism of interest, activity assays will be performed to gain insight into the immunomodulatory effects of EVs, as well as their effect on injury-induced processes, such as scar formation, angiogenesis or cell proliferation.

SGMS2 **variants linked to skeletal dysplasia disrupt cellular sphingomyelin gradients (Poster Index: 11)**

Katharina Kott

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

Sphingomyelin (SM) is a core component of mammalian cell membranes and preferential binding partner of cholesterol. A widely held view is that bulk production of SM in the *trans*-Golgi provides a sink for cholesterol synthesized in the ER to create a SM/sterol gradient along the secretory pathway. This gradient marks a fundamental transition in physical membrane properties that segregates early from late secretory organelles, allowing an adaptation from biogenic to barrier functions. We recently identified mutations in sphingomyelin synthase SMS2 that cause a rare form a skeletal dysplasia. Strikingly, all pathogenic SMS2 variants retain enzymatic activity but mislocalize to the *cis*-Golgi and/or ER. Combining organellar lipidomics with the use of lipid biosensors, we show that cells harbouring pathogenic SMS2 variants accumulate SM in the ER and display a disrupted transbilayer SM asymmetry, presumably owing to a constitutive SM scrambling across the ER bilayer. These aberrant SM distributions are accompanied by imbalances in cholesterol organization, glycerophospholipid profiles and lipid order along the secretory pathway. In parallel, we uncovered Ca^{2+}-activated SM scrambling and turnover as a novel mechanism to drive an outward budding of cellular bilayers. Besides interfering with a timely release of bone critical proteins by undermining the biogenic function of the ER, we envision that pathogenic SMS2 variants may affect the formation of matrix vesicles by osteogenic cells, a process critical for bone mineralization. Our ongoing efforts focus on challenging these models.

Session VII

Keynote Lecture

Peter Bieling

Department of Systemic Cell Biology, Max Planck Institute of Molecular Physiology, 44227 Dortmund, Germany

Mechanistic principles of Rho GTPase patterning

Rho GTPases organize into plasma membrane-associated patterns that control the cytoskeleton during cell division, morphogenesis, migration, and wound repair. Patterning depends on transitions between inactive cytosolic and active membrane-bound states, regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). However, the relationships between these transitions and the role of regulators in them have been unclear. Through in vitro reconstitution, we show that GTPase dissociation from RhoGDI is the rate-limiting step for membrane association. Activation occurs after membrane insertion, which is unaffected by GEF activity. Activation promotes membrane retention through effector interactions, which are crucial for Rho GTPase enrichment at activation sites. Thus, high cytosolic levels of RhoGDI-bound GTPases ensure a steady supply of inactive GTPases to the membrane, where GEF-mediated activation and effector binding stabilize them. These results delineate the route by which Rho GTPase patterns are established.

Combining single-molecule FRET and GIET for nanoscopic 3D structural analysis of proteins (Poster Index: 1)

Arthur Felker

Biophysics Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Single-molecule fluorescence imaging has become an indispensable tool for almost all fields of life science research. The integration of advanced techniques has revolutionized our ability to analyze molecular dynamics, molecular interactions and structural alterations with nanometer resolution. However, methods to quantify intramolecular conformational changes in all three dimensions with nanometer precision remain challenging due to the lack of appropriate tools.
We introduce an innovative approach that combines single-molecule Förster resonance energy transfer (smFRET) with single-molecule graphene-induced energy transfer (smGIET) to measure comprehensive three-dimensional structural changes of proteins at the nanoscale. For this purpose, graphene substrates were coated with a silica spacer to generate a solid supported lipid bilayer in which tris-NTA-functionalized lipids are embedded, enabling the specific binding of His-tagged proteins. After verifying the mobility and the required distance by FRAP and smGIET, we applied this method to quantify structural changes of a switchable cytokine. The protein was mutagenized for site-specific labeling with FRET pairs, facilitating precise distance measurements. Our analysis revealed significant lateral and axial conformational changes of the cytokine after peptide binding and provides detailed understanding into its three-dimensional behavior. The combination of smFRET and smGIET offers a powerful tool to study structural changes at the single-molecule level, paving the way for new insights into the mechanisms of biomolecular functions. This approach not only demonstrates the feasibility of using graphene as a versatile platform for single-molecule studies but also highlights the potential of integrating multiple advanced techniques to overcome the limitations of individual methods.

Mechanism of cytokine receptor activation and dysregulation at single molecule level

Christoph Pollmann

Biophysics Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Cytokines of the class I/II family are small, secreted messenger proteins that serve as pivotal mediators of hematopoiesis and immunity, regulating cell proliferation, differentiation, and activity. They transmit signals by binding receptors on the cell surface and subsequently forming dimeric or higher oligomeric receptor complexes. Downstream signaling is mediated by non-covalently associated, cytosolic Janus family tyrosine kinases (JAKs), which phosphorylate diverse effector proteins, most prominently members of Signal Transducer and Activator of Transcription (STAT) family. JAK activation requires complex interplay of subtle interactions, which are dysregulated in diverse cancers and immune deficiencies. Furthermore, specificities of cytokine receptor signaling can be tuned by engineered agonists, opening exciting possibilities for pharmaceutical targeting. We have developed multicolor single-molecule imaging techniques to observe and quantify cytokine receptor complex formation on the plasma membrane of live cells. This allows us to investigate the mechanistic principles underlying activation, dysregulation, and potential therapeutic manipulation of JAK/STAT signaling.

Optogenetic manipulation of organelle contact sites

Pascal Höhne

Organelle Communication Division, Institute for Cell Dynamics and Imaging, University of Münster, Germany

Contact sites are pivotal structures for inter-organellar communication, lipid metabolism, calcium homeostasis, structural integrity of organelles and stress-related responses. Currently, they are increasingly characterized as highly dynamic structures, reacting quickly to changes in metabolic conditions such as nutrient availability. However, tools to investigate and manipulate contact site formation and physiology with a high spatiotemporal resolution are rare.
Therefore, we took an optogenetic approach, by tagging organelle membrane proteins with the optogenetic dimer pair iLID and nano. With one half of the opto-tether system linked to one organelle and the second half on another organelle, we are able to reversibly induce tethering, similar to what we would observe at a contact site, via blue light illumination. We are currently creating and characterizing an optogenetic toolkit for the manipulation of contact sites between different organelles, tuned to match the specific requirements of the respective interfaces.

Poster Abstracts

Poster Session I

Poster numbers 1 - 10

21 October, 16:10 – 17:50

Characterization of a sphingolipid-operated lysosomal repair pathway (Poster Index: 2)

Elisabeth Südhoff

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

Lysosomes are vital organelles vulnerable to injuries from diverse materials. Failure to repair or sequester damaged lysosomes poses a threat to cell viability. We previously identified a sphingomyelin (SM)-based lysosomal repair pathway that operates independently of ESCRT to reverse potentially lethal membrane damage. Pathogen or drug-induced perturbations in lysosome integrity trigger a rapid Ca^{2+}-activated scrambling of SM across the bilayer. Cytosolic SM exposure is not required for ESCRT recruitment to damaged lysosomes. Instead, metabolic conversion of SM by neutral SMases on the cytosolic surface of injured lysosomes promotes their repair, also when ESCRT function is compromised. Conversely, blocking the turnover of cytosolic SM renders cells hypersensitive to lysosome-damaging drugs. We postulate that Ca^{2+}-activated SM scrambling and hydrolysis help 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. To challenge this model, we set out to analyse the impact of scramblase inhibitors on lysosomal repair, conduct a functional screen to identify the damage-induced lysosomal scramblase, and visualize SM-dependent restoration of injured lysosomes using correlative 3D electron microscopy. Moreover, a proteome-wide search for proteins undergoing SM-dependent translocation to damaged lysosomes revealed multiple lipid transfer proteins, consistent with the idea that efficient lysosomal repair relies on a non-vesicular and phosphoinositide-dependent supply of ER-derived lipids. By exploring potential crosstalk between sphingolipid- and phosphoinositide-operated pathways of lysosomal repair, we hope to gain crucial insights into the lipid-based mechanisms by which cells preserve the structural integrity of their lysosomes.

Biochemical characterization of the Rab23 GTPase, its GEF IntuFy and the formin-related protein multiple wing hairs (Poster Index: 4)

Nada Elagouri

Biochemistry and Structural Biology Division, Institute for Biochemistry, University Münster

The Rab family of small GTPases plays an important role in the regulation of intracellular trafficking and membrane organization. They are activated by guanine nucleotide exchange factors (GEFs). Previously, it was found that the ciliogenesis and the planar cell polarity (PCP) effector proteins Inturned and Fuzzy (IntuFy) form a Rab23 GEF complex [1]. Rab23 was also shown to be required for the cilium formation and membrane trafficking at mature cilia [1].To understand the distinct molecular functions of the Rab23/IntuFy module, we aim to investigate the structure and interaction networks of Rab23 and its GEF Inturned-Fuzzy (IntuFy) in mammalian and files. Therefore, hTERT-RPE1 stable cell lines expressing Rab23, the active mutant Rab23Q68L and the non-canonical PDZ domain of Intu were generated and subsequent proteomics approaches were performed. The proteomics data did not reveal any specific interactors of Rab23 and IntuPDZ, but we suspect an implication of Rab23 in the exocytic pathway. Furthermore, the subcellular localization of Rab23 and Rab23Q68L mutant upon ciliogenesis induction was investigated using immunofluorescence. A specific localization of the protein and the mutant at the cilium was not observed.
In addition to the function of IntuFy as a GEF for Rab23, IntuFy regulates the localization of the atypical formin-related protein multiple wing hairs (Mwh) [1], which in turns regulate the actin polymerization in *Drosophila* wing cells [2]. To investigate the molecular function of Mwh in the actin polymerization, the formin homology domain (FH3) of Mwh was purified for subsequent actin polymerization assays.

1. A. Gerondopoulos *et al.*, “Planar Cell Polarity Effector Proteins Inturned and Fuzzy Form a Rab23 GEF Complex,” *Current Biology*, vol. 29, no. 19, pp. 3323-3330.e8, Oct. 2019, doi: 10.1016/j.cub.2019.07.090.
2. Q. Lu, D. A. Schafer, and P. N. Adler, “The Drosophila planar polarity gene multiple wing hairs directly regulates the actin cytoskeleton,” *Development (Cambridge)*, vol. 142, no. 14, pp. 2478–2486, Jul. 2015, doi: 10.1242/dev.122119.

Fate of Cytosolic *Salmonella* and Role of Apoptotic Bodies for *Salmonella* Release and Infection of New Host Cells (Poster Index: 5)

Angelina Hübner

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Salmonella enterica serovar Typhi (STY) and Paratyphi A (SPA) are invasive intracellular human-restricted pathogens that cause a number of systemic infectious diseases. *S. enterica* serovar Typhimurium (STM) is a less harmful pathogen, and frequently used model system to study the mechanisms of pathogen-host interactions. After oral ingestion, primarily gastrointestinal epithelial cells are infected. Within host cells, *Salmonella* form so-called *Salmonella*-containing vacuoles (SCV), in which they multiply, protected from host defence mechanisms. We observed that SPA frequently escapes into the host cytosol and exhibits flagella-mediated cytosolic motility, which was also observed for STM, and contributes to partially evade host cell defences. We propose that cytosolic *Salmonella* are detected and modified by host cell immune mechanisms and that we can analyse host factors on bacterial surfaces of isolated *Salmonella* by mass spectrometry. We currently establish differential isolation of SCV-bound and cytosolic *Salmonella* using a dual fluorescence reporter system designed by us, which is based on the lack of glucose-6-phosphate in the SCV. The results may reveal known and novel mechanisms of host cell immunity against intracellular pathogens.
Furthermore, we are interested in the escape of *Salmonella* and spread of infection. We have data indicating that during infection-induced cell death in macrophages, apoptotic bodies (AB) form vehicles for the protected release of STM. We intend to show that this process accelerates the infection of further host cells. We currently work on the isolation and enrichment of AB+STM to analyse the cellular environment and to track the fate of AB+*Salmonella* after uptake by naïve host cells. For this purpose, we plan to use state-of-the-art techniques such as fluorescence-activated cell sorting, electron microscopy and high-resolution fluorescence microscopy. Since the gallbladder is a hiding place for persistent STY and SPA infections, we establish not only human colon organoids but also human bile duct epithelial organoids as infection models to investigate cellular escape via AB and thus reinfection of host cells such as macrophages. We propose that *Salmonella* masked in AB represent a protected mode of *Salmonella* dissemination in infected hosts, and that in this work we describe a previously unknown model of *Salmonella* exit strategies.

MsbA in Inside-Out vesicles (Poster Index: 6)

Alischa Scholz

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

MsbA is an ATP-binding cassette transporter (ABC) found in Gram-negative bacteria. Due to its similarity to the human ABC transporter P-glycoprotein, a multidrug efflux pump, MsbA is intensely researched as a model sytem. MsbA transports a precursor of lipopolysaccharides (LPS) by ATP hydrolysis through the inner membrane of Ecoli.
Previous cryo-EM studies have provided insights into protein structure and conformational changes during transport. In these studies, MsbA either comes into contact with a detergent or is reconstituted in another membrane-like environment such as nanodiscs. As a result, there are differences in the structures and conformations depending on which method is used to characterize the protein. The aim of this project is to avoid changing the environment and to analyze the protein in a near-native environment: in cell-derived vesicles. For this the protein is purified in inside-out vesicles (IOVs) that originate directly from the cell membrane at the original location of the protein. With this method, it is possible to obtain high-resolution structures as previously shown for the protein Slo1. After collecting data via cryo-EM, training an artificial intelligence (AI) to recognize vesicles in the micrographs and picking particles via a Python program, preliminary data shows an inside-out orientation of MsbA and in an open, inward-facing (IF) conformation. Future analyses of membrane composition, structure and conformation in IOVs could provide new insights into MsbA.

Reconstitution of the Mon1Ccz1 Rab GEF on model lipid bilayer (Poster Index: 7)

Jesse Tönjes

Biochemistry and Structural Biology Division, Institute for Biochemistry, University Münster

The structure of the Rab GEF (guanine nucleotide exchange factor) Mon1Cczz1 in solution was determined using cryo-electron microscopy and helped to understand the mechanistic basis for membrane interaction of the complex. Combined with biochemical studies, a model for the Rab7 activation was developed. However, how the complex is oriented on the membrane, how the full-length Rab7 is bound, and how Rab5 binding promotes membrane recruitment remains elusive at a molecular level. To address these questions, we aim for the reconstitution of the tetrameric complex of Mon1Ccz1 with their respective GTPases on membranes for structural analysis by cryo-electron microscopy. As Rab7 has the highest affinity towards Mon1Ccz1 in the nucleotide-free state and Rab5 only interacts with Mon1Ccz1 in the GTP-bound state, getting both Rab-GTPases in their respective nucleotide loading states on one membrane represents the primary challenge. Different approaches to chemically link the Rab-GTPases were tried, including thiol-maleimide-clicking, strain-promoted alkyne-azide cycloaddition (SPAAC), and intein splicing. We successfully employed maleimide-cysteine click chemistry to conjugate *ct*Ypt7 (Rab7 homolog), *ct*Vps21b (Rab5 homolog), and *Sc*Atg8 on Liposomes using MPB lipids. So far, the reconstitution of the *ct*Ypt7/*ct*MC1, *ct*Vps21b/*ct*MC1, and the *Sc*Atg8/*Sc*MC1 complexes on DO/PIP+MPB liposomes was achieved.

Regulatory mechanism of Rab5 dependent Mon1 Ccz1 activation of Ypt7 in endosomal pathway (Poster Index: 8)

Tunde Lawal

Biochemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

In eukaryotic cells, the guanine nucleotide exchange factor (GEF) Mon1-Ccz1 (MC1) activates the Rab GTPase Rab7. The active Rab7 interacts with effector proteins, leading to fusion processes at the vacuole/lysosome. Rab7, alongside its nucleotide-binding domain, has a GTPase-specific linker region known as the hypervariable domain (HVD). The HVD of Rab7 is thought to be involved in its activation by MC1 which is important for directing it to the correct organelle membrane. Some data also suggest a role for the Rab7-HVD in the interaction with Rab7 effector proteins.

Using *S. cerevisiae* as a model organism, we aim to investigate how the HVD of the yeast Rab7-homolog Ypt7 may be involved in the activation and function of this GTPase. We constructed Ypt7 HVD swap mutants that carry the HVD of the endosomal Rab GTPase Vps21 ($Ypt7^{HVD21}$). We tested these and other mutants for functionality in GEF assays *in vitro* and analyzed their phenotypes *in vivo* using fluorescence microscopy of yeast cells expressing $Ypt7^{HVD21}$. Additionally, we also tested the physiological impact of the HVD-swap mutants in growth tests.

Our results suggest that the functionality of Ypt7 along the endosomal pathway depends on its hypervariable domain. Therefore, we seek to gain insights into the mechanistic role and interaction of Ypt7´s HVD during its activation by MC1 and interaction with downstream effector proteins.

Functional architecture of the vacuole - lipid droplet contact site (vCLIP) (Poster Index: 9)

Louis Percifull

Organelle Communication Division, Institute for Cell Dynamics and Imaging, University of Münster, Germany

Adaptation to metabolic changes requires efficient communication between cellular organelles. It is emerging that lipid droplets, the cellular fat storage organelles, communicate mainly via contact sites, regions on the lipid droplet surface that are directly attached to other organelles via tether proteins. We recently identified the protein machinery underlying formation of contact sites between lipid droplets and the vacuole [1]. These vacuole-lipid droplet (vCLIP) contact sites are formed by the lipid droplet surface proteins Ldo16 and Ldo45, and by the vacuolar surface protein Vac8, which together act as organelle tethers. Ldo45 recruits a further protein to vCLIPs, the phosphatidylinositol transfer protein Pdr16. vCLIPs are regulated by the metabolic state of the cell. While these contacts are rare at glucose replete conditions, their formation is strongly promoted during glucose restriction. vCLIP-dependent tethering is a prerequisite for efficient lipid droplet breakdown by lipophagy.
In this project, we explore the structural and functional organization of the novel vCLIP machinery. We employ genome-wide microscopy-based screening approaches to uncover the complete vCLIP proteome, and aim at developing a model for the interplay between lipid droplets and the vacuole across metabolic states.

1. D. Diep et al., Dev. Cell (2024).

It's super effective: Regulation, secretion and mechanisms of effector proteins of *Salmonella enterica* serovars Typhi and Paratyphi (Poster Index: 10)

Jonathan Jansen

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Salmonella enterica Paratyphi (SPA) and Typhi (STY) are two typhoidal serovars of *Salmonella* that have humans, in contrast to non-typhoidal *Salmonella* like *S. enterica* serovar Typhimurium (STM), as their exclusive host and are the causative agent of typhoid fever. As an adaptation to humans, STY and SPA have silenced many viral genes through pseudogenisation (McClelland *et al.*, 2004). These virulence factors include the effector proteins secreted by *Salmonella* during invasion and intracellularly to create the conditions for successful infection and replication. They are secreted via the type 3 secretion system (T3SS) encoded by Salmonella Pathogenicity Island 1 (SPI1) and SPI2. The pseudogenisation of these genes ensures a low immune response of the host, which would be detrimental in systemic infection by *Salmonella*. However, the existence of exclusively typhoidal effectors is possible and may contribute to adaptation to the human host. It has already been shown that the repertoire of effector proteins in different serovars of *S. enterica* is divers (Jennings *et al.*, 2017). Of particular interest are so-called accessory effector proteins that only occur in certain serovars of *S. enterica*.

The aim of the project is to develop and apply high-throughput methods to identify previously unknown effectors using mass-spectrometry based proteomics and bioinformatics tools that search for pattern to already known effectors in genomes. The candidates found by combining these tools will then be characterised. We showed that the secretome of STM effector proteins is resolvable using mass spectrometry allowing for a high resolution of secretomes of STY and SPA during infection experiments and *in vitro*. Data generated by mass spectrometry of STY and SPA secretomes identified proteins of interest synthesised and secreted/translocated under SPI2-T3SS inducing conditions. Further experiments will elucidate the localisation, binding partners in host cells as well as the function in pathogenesis.

The discovery of new effector proteins that are exclusive to typhoidal *Salmonella* could make a significant contribution to the understanding of typhoid fever, immune evasion, and the development of new ways to intercept the virulence of typhoidal *Salmonella* either by antibiosis or deactivation of virulence factors.

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Poster Session II

Poster numbers 11 - 19

23 October, 10:50 – 12:30

Role of N-terminal arginylation in autophagy (Poster Index: 12)

Jasper Eising

Plant Physiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

N-terminal arginylation is a post-translational modification of proteins by arginyl-tRNA transferases (ATEs) found in multiple eukaryotic species. Arginylation is supposed to be an essential component of the plant N-degron pathway that may lead to lysosomal degradation. The homologue of mammalian p62 (or Nbr1) in Arabidopsis is NBR1, also providing ZZ and a ubiquitin-binding domains. The similarity to p62 put NBR1 in the spotlight as a potential N-recognin for arginylated proteins in the plant N-degron pathway and beyond. The role of NBR1 in lysosomal degradation can be investigated in vitro with described bona finde arginylation targets such as VERNALIZATION2 (VRN2) and LITTLE ZIPPER 2 (ZPR2). These have been shown to depend on ATEs in plants and may represent arginylation targets due to their penultimate Cys residues after the initial Met, which get exposed after processing and are thus potential targets for ATE1 after Met cleavage and oxidation. Both VRN2 and ZPR2 degenerate under normoxia and accumulate under hypoxia. Polyubiquitination is an essential component of the N-degron pathway, leading to protein degradation by proteasomes. Protein ligase complexes consisting of various E1, E2, and E3 enzymes decorate the proteins to be degraded with a specific posttranslational ubiquitination code. This code regulates protein fate and function and is, therefore, crucial for maintaining cellular protein homeostasis.

Quantitative analysis of persister formation of typhoidal, non-typhoidal and invasive non-typhoidal *Salmonella enterica* strains (Poster Index: 13)

Comfort Yeboaa

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Quantitative analysis of persister formation of typhoidal, non-typhoidal and invasive non-typhoidal *Salmonella enterica* strains
Antibiotic resistance poses a significant complication in the treatment of bacterial infections. However, bacterial persister cells, a dormant and tolerant subpopulation, further complicate eradication. This study investigates quantitatively the persister cell formation of intracellular *Salmonella* and explores potential strategies to target these cells.
Infection was done in Murine macrophage cells following the methods outlined in Schulte, M., Olschewski, K. & Hensel, M. (2021). The use of fluorescent reporter systems allowed the determination of the persister status of *Salmonella* on a single cell level.
Strains used in this study were resistant to one or more classes of antibiotics. The strains include standard *S*. Typhimurium (STM) lab strain (SL1344), invasive non-typhoidal *Salmonella* from sub-Sahara Africa (STM ST 313), non-invasive strain from Africa (STM 4/74), epidemic *S.* Infantis from Israel (SIN 119944), pre-epidemic *S.* Infantis (SIN 335-3), and reference strain (STM NCTC 12023 WT). Subsequent tests for the functionality of the reporter in various strains were positive for both DsRed and sfGFP induction (>70%). The stability of test plasmids was positive (>70%). Infection of test strains in murine RAW264.7 macrophage cells was successful. Our results revealed that the frequency of persister cells in vitro was high in stationary phase cultures (≤2%) compared to exponential phase cultures (≤1%) across all strains. The proportion of the intracellular active NR population at the beginning of infection was higher (≥70%) than in the late phase for all strains. Metabolic activities of intracellular NR cells varied across strains at different times post-infection and compared to the WT.

Conclusion:
These findings provide new quantitative insights into *Salmonella's* persistence mechanisms and suggest the need for better therapeutic regimens and treatment options to effectively overcome chronic infections.

Systematic characterization of yeast plasma membrane transporters: localization, turnover and function (Poster Index: 15)

Dekai Dong

Institute for Cell Dynamics and Imaging, University of Münster, Germany

The plasma membrane (PM) of *S. cerevisiae* is segregated into many overlapping but distinct domains that exhibit an astonishing temporal stability. Small molecule transporters constitute a majority of the PM proteome and they are key components for the rapid adaptation to environmental changes and stress conditions. Mechanisms for such adaptations include structural changes and posttranslational modifications that directly modulate transporter function, but also internalization and turnover via endocytosis and changes in lateral distribution within the PM.

In this project, we have started a systematic characterization of PM transporters, focusing on the expression, turnover and segregation at the PM. We will examine these parameters under various conditions such as nutrient starvation, osmotic stress or genetic mutations. We have already screened GFP-tagged transporters from C- and N-terminally tagged libraries using high-throughput fluorescence microscopy and TIRF microscopy. We found widespread internalization of transporters upon glucose or nitrogen starvation, indicating a general reduction of protein density in the PM. Interestingly, we observed cluster formation for some of the transporters that were not or only partially internalized. We now plan to follow up on our findings and explore the importance of protein density for PM patterning and domain formation. Our results will also serve as baseline for future explorations on the effects of lipid composition and protein density on lateral mobility and segregation within the yeast PM.

Structure of a Surface-layer protein covering Anammox cells (Poster Index: 16)

David Dallemer

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The discovery of anammox bacteria in the 1990s changed our understanding of the global nitrogen cycle (1). These extraordinary microorganisms with unusual morphology derive their energy from the oxidation of ammonium coupled with nitrite reduction, which relies on highly toxic intermediates such as hydrazine and nitric oxide (2). Approximately 50% of the dinitrogen gas released is produced by anammox bacteria. In biotechnology, the Anammox process is being used as a sustainable alternative to current wastewater treatment systems for the removal of nitrogen compounds.

We have made significant contributions to elucidating the nature of the catabolic pathway and characterizing the key soluble enzymes. Central to the harvesting of energy from hydrazine is the hydrazine dehydrogenase complex, which converts hydrazine to dinitrogen gas, releasing four extremely low-potential electrons (-750 mV) (3). In addition, anammox bacteria obtain additional reducing equivalents from the oxidation of nitrite to nitrate, catalyzed by a nitrite oxidoreductase (NXR) (4). Currently, using Single-particle cryo-electron microscopy and cryo-electron tomography, we are working on uncovering the complete atomic structure of the Surface-layer protein (SLPs) covering the anammox cells. SLPs are the highest copy number of macromolecules in anammox bacteria and play different roles such as scaffolding membranes and protecting cells from the outside. In the context of anammox bacteria, we believe SLPs also contribute to the global nitrogen cycle by entrapping ammonium ions inside the cells.

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Structural insights of Gasdermin E pores at mitochondria during cell death (Poster Index: 17)

Nadine Gehle

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

The pore-forming protein family of Gasdermins (GSDM) induces pyroptosis by forming pores in the plasma membrane (PM), allowing the release of inflammatory molecules and triggering an immune response. Gasdermin E (GSDME) can be activated by apoptotic caspase-3, switching apoptosis to pyroptosis. Previous research has suggested that during apoptosis GSDME can permeabilize not only the PM but also mitochondria. However, it is unclear whether there is a preference for targeting mitochondria over PM. Furthermore, direct evidence of GSDME pores within cells, particularly within mitochondria, is lacking.
To address this, we investigated the real-time sequence of events of GSDME permeabilization upon apoptosis induction, its preferential membrane localization, and, importantly, the GSDME nanostructures in cells using advanced live-cell and super-resolution microscopy.
Upon apoptosis induction, we observed that mitochondrial fragmentation and permeabilization preceded PM permeabilization. Crucially, we showed that GSDME targets both the outer and inner mitochondrial membranes, suggesting its involvement in the cytosolic release of mitochondrial DNA. Finally, by dual-color DNA-PAINT microscopy, we provided the first evidence of GSDME pore structures in mitochondria at nanometer resolution.
Overall, our work highlights an essential role of GSDME in mitochondrial permeabilization, with significant implications for the inflammatory outcome of apoptosis.

The yeast Rab7 GTPase Ypt7 controls signalling on endosomes and membrane fission processes at the vacuole (Poster Index: 19)

Nadia Füllbrunn

Biochemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Organelles of the endomembrane system contain Rab GTPases as identity markers, which control membrane fusion and intracellular trafficking processes. The activity and localization of Rab GTPases is mainly determined by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). In *Saccharomyces cerevisiae*, the Rab7 GTPase Ypt7 localizes to autophagosomes, late endosomes and the vacuole. Upon interaction with the HOPS tethering complex, Ypt7 mediates all membrane fusion processes at the vacuole. We previously showed that overproduction of the Ypt7 GAP Gyp7 concentrates Ypt7 on endosomes and results in resistance to rapamycin, an inhibitor of the target of rapamycin complex 1 (TORC1), suggesting that Ypt7 affects signalling on late endosomes. Now, we present that the absence of Gyp7 in a recycling mutant concentrates Ypt7 at the vacuole and strongly affects vacuole morphology. The resulting vacuole fragmentation depends on the presence of the Fab1 kinase complex (Fab1C), causing high levels of vacuolar PI-(3, 5)-P_2, as well as on the levels of active Ypt7 at the vacuole and can be clearly distinguished from a vacuole fusion defect. Thus, we postulate that Ypt7 is a master regulator of not only membrane fusion and but also fission processes at the vacuole.