Mechanisms of Localized Mitophagy using Correlative Microscopy

Presenting author: Nicholas Ader

MRC Laboratory of Molecular Biology, Cell Biology, Francis Crick Avenue, CB2 0QH, Cambridge
nader@mrc-lmb.cam.ac.uk

Using correlative light and electron microscopy (CLEM), we address the ultrastructural sequence of events for localized mitophagy in tissue culture cells. The removal of damaged mitochondria through autophagy machinery, or mitophagy, is a key aspect of proper cell health and is one of the ways our cells prevent us from developing Parkinson’s disease. Mitophagy is initiated by the PINK1/Parkin pathway, which detects mitochondrial membrane depolarization or an abundance of misfolded proteins within a mitochondrion. Once marked by Parkin, the damaged mitochondrion is degraded. Recent studies suggest that Parkin activity can also identify damage at a specific spot of an otherwise healthy mitochondrion. Parkin may thus take part in a form of localized mitophagy, facilitating deletion of the damaged portion of the mitochondrion without sacrificing the entire organelle. How the damaged portion of a mitochondrion is removed remains unclear. Here, we present data suggesting a sequence of events for isolation and clearance of mitochondrial fragments. In particular, we observe that isolation of mitochondrial fragments occurs independently of the mitochondrial fission protein, Drp1, and the isolated fragments do not significantly differ in size between Drp1-knockout and Drp1-intact cells. We collected these data using a precise, fiducial-based correlation method to localize fluorescent signals of interest in STEM tomograms of resin-embedded cells.

Authors: Nicholas Ader, Jonathon Burman, Chunxin Wang, Richard Youle, Wanda Kukulski

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Molecular characterization of the matrix protein Z of Lassa virus isolated from the first domestically acquired case in Germany

Presenting author: Ann Kathrin Ahrens

Geschwister-Scholl Straße 5, 35039, Marburg
Ahrensa@students.uni-marburg.de

Since its first discovery in Nigeria in 1969, Lassa virus (LASV), a member of the arenaviridae family, causes annual outbreaks in West African countries. LASV is an enveloped, bi-segmented RNA virus that can cause severe hemorrhagic fever in humans. Due to the lack of effective therapeutics and licensed prophylactic vaccines, LASV has to be handled under biosafety level 4 (BSL-4) conditions. The natural reservoir is the multimammate rat Mastomys natalenis. Humans get primarily infected through exposure to contaminated rodent excreta; however human-to-human transmission can occur during nocosomial outbreaks due to lack of proper hygiene measures and effective barrier nursing. We recently described the isolation and full-genome sequencing of a new LASV isolate, designated Alzey, which was responsible for the first human-to-human transmission outside of Africa. In this work we aim to address its yet unknown molecular characteristics, focusing on the viral matrix protein Z that is the driving force for virus release. Amino acid comparison between isolate Alzey and the reference isolate Josiah revealed a sequence identity of ca. 79%. Using systematic site-directed mutagenisis analysis we will investigate critical amino acid residues that are presumed to be associated with the strong membrane binding abilities of Z.

Authors: Ann Kathrin Ahrens, Sarah Katharina Fehling, Thomas Strecker

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Investigating the role of H2O2 and thiol peroxidases in the yeast metabolic cycle

Presenting author: Prince Saforo Amponsah

Technische Universität Kaiserslautern, Cellular Biochemistry, Erwin-Schrödinger-Straße 13, 67663, Kaiserslautern
amponsah@rhrk.uni-kl.de

The yeast metabolic cycle (YMC) is an intrinsic ultradian clock found in the budding yeast Saccharomyces cerevisiae. The YMC was first identified as a metabolic cycle, during which cells switch between low and high oxygen consumption modes of metabolism. Cyclical changes in the levels of more than 50% of cellular transcripts occur during the YMC. Also, the YMC appears to gate the cell division cycle. Genetic or chemical perturbation of cellular redox processes can strongly disrupt the YMC, however, the mechanistic underpinnings remain unexplored. Hydrogen peroxide (H2O2) is an important cellular redox species, which acts as a second messenger in a variety of cell signaling pathways and can elicit large-scale transcriptional responses. We hypothesized that H2O2 plays an important regulatory role during the YMC. We generated yeast strains with a genomically integrated roGFP2-Tsa2ΔCR sensor to monitor changes in endogenous H2O2 levels during the YMC. We observed cyclical changes in roGFP2 oxidation consistent

with high-amplitude changes in endogenous H2O2 levels. Addition of exogenous H2O2 led to YMC perturbation, with the effect being strongly dependent upon the phase of the YMC at which H2O2 was added. We see that deletion of the cytosolic thiol peroxidases Tsa1 and Tsa2 disrupted both the YMC per se and the response to exogenous H2O2. We speculate that thiol peroxidases may be important for coupling cyclical metabolic changes to the transcriptional cycles observed in the YMC.

Authors: Prince Saforo Amponsah, Bruce Morgan

Multiscale Origami Structures as Interfaces for Cells (MOSAIC) – A novel platform for the investigation of early cell signalling

Presenting author: Alessandro Angelin

Karlsruher Institut für Technologie (KIT), Institut für Biologische Grenzflächen (IBG1), Hermann-von-Helmholtz-Platz 1, 76344, Eggenstein-Leopoldshafen
alessandro.angelin@kit.edu

Surface-based bioanalytical methods are of utmost importance for fundamental research in molecular cell biology. We here describe a novel method for the investigation of early cell signalling, Multiscale Origami Structures As Interface for Cells (MOSAIC),[1] which is based on the site-directed sorting of differently encoded, protein-decorated DNA nanostructures on DNA microarrays. Specifically, DNA origami nanostructures (DON) were used as molecular pegboards for the precise arrangement of ligands for cell surface receptors with a lateral resolution of ~6 nanometers. DON constructs presenting the ligand epidermal growth factor (EGF) were employed to activate EGF receptors in adhered MCF7 cells. To this end, a series of DONs was prepared which contained 4 to 12 EGF entities, arranged either evenly distributed or densely clustered on the DON´s surface. Their immobilization on glass surfaces was followed by adherence of MCF7 cells and immunohistochemical analysis of EGFR activation. We found that the nanoscale architecture of the immobilized EGF-DONs significantly affect EGFR activation depending on both stoichiometry and the spatial arrangement of the EGF ligands. Owing to its modularity, the MOSAIC technology can be used as novel tool for the investigation of EGF, integrin and ephrin signalling pathways.
[1]Angelin, A. et al., 2015, Angew Chem Int Ed 54, 15813

Authors: Alessandro Angelin, Klavdiya Gordiyenko, Alessa Schilling, Christof M. Niemeyer

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Multiple essential roles of mitochondrial fatty acid synthesis and insights into a new protein-protein interaction motif

Presenting author: Heike Angerer

Goethe University Frankfurt, Biozentrum, Institute of Biochemistry II, Max-von-Laue Str. 9, 60438, Frankfurt am Main
Angerer@em.uni-frankfurt.de

The highly conserved mitochondrial fatty acid synthesis (FAS) type II machinery produces acyl groups independent from the cytosolic FAS type I complex (Hiltunen et al., 2010). The mitochondrial acyl carrier protein (ACPM)-dependent system produces C8 acyl groups for the de novo biosynthesis of lipoic acid, the essential cofactor of e.g. pyruvate dehydrogenase and alpha-keto glutarate dehydrogenase; and long-chain acyl groups (C10-C16 groups) with so far unknown functions in mitochondria. ACPM is the central element of this biosynthetic pathway, however additional functions were identified: ACPM is a bona fide subunit of respiratory complex I and of the cysteine desulfurase NFS1 complex a key player of mitochondrial Fe-S cluster biogenesis, respectively (Sackmann et al., 1991, van Vranken et al., 2016). Interestingly, these complexes also contain LYR (leucine/tyrosine/arginine) motif-containing protein (LYRM) subunits. We showed that complex I subunit LYRM6 is essential for complex I activity and anchors an ACPM to the complex. In the recent 3.9 Å atomic structure of mammalian complex I was observed that subunits LYRM6 and LYRM3 tightly bind the acyl group of ACPM subunits (Fiedorczuk et al., 2016). Our present study revealed that LYRM mediated binding of ACPM to different protein complexes was consistently linked with the presence of a C10-C16 acyl chain modification. This suggests a new type of protein-protein interaction motif for the association of ACPM with LYRM proteins.

Authors: Heike Angerer, Stefan Schönborn, Jan Gorka, Ute Bahr, Michael Karas, Ilka Wittig, Juliana Heidler, Jan Hoffmann, Nina Morgner, Volker Zickermann

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Characterisation of Iron-Sulfur Cluster Coordination by Spectroscopy

Presenting author: Dominique F. Bechtel

TU Kaiserslautern, FB Chemie, Biochemistry, Blumenstraße 2, 67655, Kaiserslautern
bechtel@rhrk.uni-kl.de

Iron-sulfur proteins are widely-used in nature and are distributed in all kingdoms of life. These proteins are of key importance for respiratory chain, Krebs cycle, ribosomal maturation, tRNA modification, DNA replication and repair. In contrast to the electron transport and catalytic function, many iron-sulfur clusters are involved in the stabilisation and shaping of proteins.
Generally, there are three different basic types of iron-sulfur clusters, the [2Fe-2S], [3Fe-4S] and [4Fe-4S] cluster. Their coordination in proteins is carried out by amino acids with heteroatoms in their sidechains. Coordinating residues are in most cases cysteines, like in many ferredoxins. In rare cases aspartate, glutamate, histidine, serine or peptide nitrogen can supply one of the ligands, too. However, e.g. in Rieske-type proteins [2Fe-2S] coordination is described by two cysteines and two histidines. The coordination via a non-cysteinyl ligand, especially to a [4Fe-4S] cluster, is not easily detected with common methods, e.g. UV/Vis, CW-EPR and Mössbauer spectroscopies.
Here, we present our bioinformatic findings on novel proteins containing a [4Fe-4S] or [2Fe-2S] cluster coordinated via one or two histidine residues, respectively. For the verification of the coordination by histidine(s) we apply (pulsed) EPR spectroscopy and spin density-modifying chemical derivatization of the imidazole moiety of histidine.

Authors: Dominique F. Bechtel, Kathrin Stegmaier, Antonio J. Pierik

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Separating mitochondrial protein assembly and endoplasmic reticulum tethering by selective coupling of Mdm10

Presenting author: Thomas Becker

University of Freiburg, Institute for Biochemistry and Molecular Biology, Stefan-Meier-Str. 17, 79104, Freiburg
thomas.becker@biochemie.uni-freiburg.de

Lars Ellenrieder1, Lukasz Opalisnki1, Oliver Mirus2, Sebastian Stiller1, Nadine Flinner2, Chris Meisinger1, Nils Wiedemann1, Enrico Schleiff2, Richard Wagner3, Nikolaus Pfanner1, Thomas Becker1 et al.

1Institut für Biochemie und Molekularbiologie, Universität Freiburg
2Molekulare Zellbiologie der Pflanzen, Universität Frankfurt
3Life Sciences & Chemistry, Focus Area Health, Jacobs University Bremen

The mitochondrial distribution and morphology protein 10 (Mdm10) is a central player in mitochondrial biogenesis. Mdm10 is a core component of the endoplasmic reticulum-mitochondria encountering structure (ERMES) that tethers mitochondria to the endplasmic reticulum. Furthermore, Mdm10 associates with the sorting and assembly machinery. Due to its multiple functions deletion of Mdm10 causes a plethora of defects including loss of mitochondrial tubular network, reduced cardiolipin level and impaired formation of the TOM complex. So far, the functions of the individual Mdm10 populations remain unclear. We successfully mapped the interaction sites of ERMES and SAM on the Mdm10 beta-barrel and identified the binding partner of Mdm10 within the ERMES complex. Analysis of side-directed point mutations revealed the specific functions of Mdm10 at the SAM or at the ERMES complex. Altogether, our studies demonstrate how coupling to different partner proteins modifies the functional specificity of Mdm10.

Authors: Thomas Becker

Identification of the ligands of a novel class of [2Fe-2S] proteins via chemogenomics

Presenting author: Catharina Blinn

Blumenstraße 2, 67655, Kaiserslautern
blinn@chemie.uni-kl.de

The genome of Saccharomyces cerevisiae consists of 6,600 genes of which 10 % have not been characterised so far. Identification of the function of these genes is essential for our understanding of intra- and intermolecular processes in eukaryotes.
Eukaryotes contain more than hundred iron-sulfur proteins, which act as catalyst (aconitase), are involved in redox processes (mitochondrial electron transport chain), or play a role in diverse processes like regulation, tRNA modification, DNA replication, transcription and repair. A widespread type of Fe/S center is the rhombic [2Fe-2S] cluster with four cysteine residues as ligands. However, in the protein with a Rieske fold in respiratory (bc1)- and photosynthetic (b6f)- complexes, the apoptosis-inducing factor-like protein, as well as in mono- and dioxygenases,

two histidine and two cysteine residues coordinate the [2Fe-2S] cluster.
We identify two hitherto uncharacterised yeast proteins with a Rieske-type [2Fe-2S] cluster coordination in non-Rieske fold yeast proteins. The putative ligands of the cluster are highly conserved in many thioredoxin-like [2Fe-2S] ferredoxin domains of fungal and other eukaryotic proteins. Via chemogenomic studies, we verified the cluster ligands and could demonstrate their functional role in yeast. pH-dependency of spectral properties, electrochemistry, EPR and Mössbauer spectroscopy of the wildtype protein and His to Cys mutants corroborate our in vivo findings.

Authors: Catharina Blinn, Kathrin Stegmaier, Antonio J. Pierik

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Ferroptosis-like cell death in African trypanosomes

Presenting author: Marta Bogacz

Heidelberg University Biochemistry Center, AG Krauth-Siegel, Im Neuenheimer Feld 328, 69120, Heidelberg
marta.bogacz@bzh.uni-heidelberg.de

Ferroptosis is a form of non-apoptotic cell death recently described in various mammalian cell lines. It is characterized by iron-dependent lipid peroxidation and glutathione peroxidase 4 (GPx 4) has been identified as its main regulator. African trypanosomes possess three well characterized tryparedoxin peroxidases (Px I-III) responsible for the detoxification of lipid hydroperoxides and their closest mammalian homolog is GPx 4. In the procyclic insect (PC) form of Trypanosoma brucei, either the cytosolic or the mitochondrial peroxidases are essential. Flow cytometry analysis of PC Px I-III KO cells using BODIPY 581/591 C11 revealed strong lipid peroxidation that originates in the mitochondrion. Cellular damage and death can be prevented by ferroptosis inhibitors or lipophilic antioxidants, but not by ascorbate. Studies using iron chelators, MitoSOX and overexpression of a mitochondrial SOD showed the role of mitochondria-generated superoxide anions and iron in the induction and progression of the lethal mechanism in Px I-III-lacking cells. This work indicates the occurrence of ferroptosis-like cell death in the early-branched protist class of Kinetoplastida.

Authors: Marta Bogacz, Corinna Schaffroth, R. Luise Krauth-Siegel

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NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation

Presenting author: Markus T. Bohnsack

Göttingen University, Institute for Molecular Biology, Humboldtallee 23, 37073, Göttingen
markus.bohnsack@medizin.uni-goettingen.de

Defects in mitochondrial function caused by mutations in components of the mitochondrial translation machinery, including mitochondrial (mt-)tRNAs and RNA modification enzymes, have been linked to a wide variety of severe clinical disorders. Using a combination of localization analysis, in vivo UV and 5-azacytidine crosslinking and in vitro analyses, we have identified a biosynthetic pathway that introduces RNA modifications at the wobble position of mt-tRNAMet; the RNA methyltransferase NSUN3 first methylates cytosine 34 to produce 5-methylcytosine (m5C), which can then be oxidized to 5-formylcytosine (f5C) by the alpha-ketogluterate and Fe(II)-dependent dioxygenase ALKBH1/ABH1. In vitro codon recognition studies with mitochondrial translation factors reveal that these modifications serve to expand codon recognition by mt-tRNAMet, enabling decoding of the non-conventional mitochondrial genetic code. Consistent with this, depletion of either NSUN3 or ABH1 strongly affects mitochondrial translation in vivo, suggesting that both enzymes are required to install the anticodon modifications necessary for mt-tRNAMet function. Our data further demonstrate that NSUN3 specifically recognises the anticodon stem loop (ASL) of the tRNA and interestingly, pathogenic mutations that compromise basepairing in the ASL reduce C34 methylation, implying that lack of this modification in mt-tRNAMet can lead to disease.

Authors: Markus T. Bohnsack

Interactions and functions of the NF-κB-repressing factor in human ribosome biogenesis

Presenting author: Markus T. Bohnsack

Göttingen University, Institute for Molecular Biology, Humboldtallee 23, 37073, Göttingen
markus.bohnsack@medizin.uni-goettingen.de

The production of eukaryotic ribosomes is one of the most energy-consuming cellular processes during which, a myriad of enzymatic proteins catalyses important maturation events, including ribosomal (r)RNA processing, modification and folding. Such irreversible steps drive the

directionality of the assembly pathway and careful spatial and temporal regulation of the enzymes that catalyse them is required. RNA helicases play major roles in structural remodelling of pre-ribosomal complexes and their activity is often regulated by dedicated cofactors, such as G-patch proteins. Here, we show that the human G-patch protein NF-κB repressing factor (NKRF) forms a nucleolar subcomplex with the DExH-box helicase DHX15 and the 5′-3′ exonuclease XRN2. We demonstrate that NKRF crosslinks to the transcribed spacer regions of the pre-rRNA transcript and that depletion of NKRF, XRN2 or DHX15 impairs an early pre-rRNA cleavage step (A′). The catalytic activity of DHX15, which is stimulated by NKRF, is required for efficient A′ cleavage, suggesting that a structural remodelling event may facilitate processing at this site. Furthermore, depletion of NKRF or XRN2 leads to the accumulation of excised pre-rRNA spacer fragments and we show that NKRF is essential for recruitment of the exonuclease to nucleolar pre-ribosomal complexes. Our findings therefore reveal a novel pre-ribosomal subcomplex that plays various roles in the maturation of pre-rRNAs and the turnover of excised spacer fragments.

Authors: Markus T. Bohnsack

Effect of oxidative stress on ubiquitin homeostasis, mitofusin post translational modifications and mitochondrial morphology

Presenting author: Ira Buntenbroich

CECAD, Cologne University, Institute for Genetics, Joseph-Stelzmann Straße 26, 50931, Cologne
ira.buntenbroich@uni-koeln.de

Mitochondria are dynamic organelles constantly undergoing fusion and fission to maintain their function. We are interested in the process of mitochondrial fusion, which in yeast is mediated by the dynamin-related protein Fzo1. Fzo1 is present at the outer membrane of mitochondria and its function is regulated by ubiquitylation.
Mitochondrial networks spread around the cell and are prone for intrinsic and extrinsic stresses. Especially oxidative stress challenges mitochondrial integrity since reactive oxygen species are a byproduct of oxidative phosphorylation. Oxidation leads to major changes in the morphology of mitochondria. On the other hand, oxidation alters ubiquitin phosphorylation and homeostasis. Consistently, damaged proteins within mitochondria are ubiquitylated and degraded via the ubiquitin proteasome system.
We have investigated the molecular mechanisms affecting Fzo1 and mitochondrial fusion under conditions of oxidation. Our results give new insights that help to understand defects in mitochondrial morphologies and dysfunctions upon oxidative stress.

Authors: Ira Buntenbroich, Mafalda Escobar

Glutathione and H2O2: a cross-talk in cytosol and matrix with two strategies to keep a reducing environment.

Presenting author: Gaetano Calabrese

University of Cologne, Inst. of Biochemistry, Zuelpicher Str. 47A, 50674, Cologne
g.calabrese@uni-koeln.de

A balanced local redox environment is essential for life. Both cytosol and mitochondrial matrix harbor sets of reducing enzymes to counteract oxidizing influences. Some of the enzymes are shared between cytosol and matrix (e.g. glutaredoxins and glutathione reductase). Others are unique to the respective compartment (e.g. peroxiredoxins, thioredoxins and thioredoxin reductase). The likewise involved redox molecule glutathione (GSH, oxidized: GSSG) is present in both compartments but it is initially synthesized in the cytosol and transported into the matrix by a yet unknown mechanism. Apparently, this transport is relatively slow and thus both glutathione pools fluctuate independently from each other. While GSSG can be exported from the cytosol to maintain a reducing environment, this is not possible for the matrix, which has to rely on other mechanisms to cope with overwhelming oxidative stress. Reducing systems ultimately rely on NADPH as electron source and the pathways for its regeneration differ between cytosol and matrix. While we know the components involved in maintaining balanced redox environments in cytosol and matrix, little is known about their dynamic intra- and inter-compartmental cross-talk in the cell. We investigate these dynamics by using genetically-encoded fluorescent sensors for glutathione and H2O2 and redox biochemical methods. Here we present novel insights on how cells handle H2O2 to maintain a reducing mitochondrial matrix under all circumstances.

Authors: Gaetano Calabrese, Jan Riemer

TIM29 is a subunit of the human carrier translocase required for protein transport

Presenting author: Sylvie Callegari

University Medical Center Göttingen, Department of Cellular Biochemistry, Humboldtallee 23, 37075, Göttingen
sylvie.callegari@med.uni-goettingen.de

The mitochondrial carrier translocase (the TIM22 complex) mediates the insertion of multispanning proteins with internal targeting signals, such as the metabolite carriers, into the inner mitochondrial membrane. The architecture of the TIM22 complex in yeast has been well defined and although the pore-forming unit of this complex, the TIM22 protein, is conserved in higher eukaryotes, additional constituents of the yeast translocase lack human homologs. In fact, very little is known about the mammalian TIM22 complex. We purified the human carrier translocase and, using a proteomics approach, identified a 29 kDa mitochondrial inner membrane protein, TIM29, as a novel, metazoan-specific component of the TIM22 complex. TIM29 is a stoichiometric component of the 440 kDa TIM22 complex and its integration into the complex depends upon the oxidized state of TIM22. Depletion of TIM29 disrupts complex stability and affects the translocation of carrier substrates, resulting in significantly compromised cell growth. The discovery of TIM29 suggests there may be additional metazoan-specific TIM22 associates. Their identification would provide an important insight into the workings of the mammalian TIM22 complex.

Authors: Sylvie Callegari, Frank Richter, Katarzyna Chojnacka, Daniel Jans, Isotta Lorenzi, David Pacheu-Grau, Stefan Jakobs, Christof Lenz, Henning Urlaub, Jan Dudek, Agnieszka Chacinska, Peter Rehling

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Analysis of Mitochondrial-ER associated Membranes (MAMs) Proteome through Quantitative Mass Spectrometry

Presenting author: Giovanna Cenini

University of Bonn, Institute für Biochemie und Molekularbiologie, Nussallee 11, 53115, Bonn
gcenini@gmail.com

In the eukaryotic cells, the mitochondria and the endoplasmic reticulum (ER) form characteristic tubular networks that also promote functional and structural contacts between the two organelles in sub-regions called mitochondria-ER associated membranes (MAMs). The research about mitochondria-ER connections has thrived in the past few years with the discovery of a role of MAMs not only in many physiological pathways such as in lipid metabolism and Ca2+ exchange between mitochondria and ER, but also in the pathology of diseases like Alzheimer disease. Indeed, in this neurodegenerative disease, MAM functions seem up-regulated and it might contribute to the AD pathogenesis.
Apart from the findings about functions and dynamics of this sub-cellular compartment, the detailed protein composition of MAMs is still unclear. For this reason using a powerful analytical tool as quantitative mass proteomics, we determined the relative amount of proteins in MAMs, mitochondria and ER fractions isolated from mouse liver through a Percoll density gradient. Our results not only characterize the protein composition of these specific sub-organelles region, but it will help to shed more light on the functional roles of ER-mitochondria contacts.

Authors: Giovanna Cenini, Wolfgang Voos

Cytosolic factors involved in the biogenesis of mitochondrial tail-anchored proteins

Presenting author: Bogdan Cichocki

University of Tuebingen, Interfaculty Insitute of Biochemistry and Biophysics, Moltkestarsse, 55, 50, 72072, Tübingen
bogcichocki@gmail.com

Unlike other classes of mitochondrial outer membrane proteins, the import pathway of mitochondrial TA proteins is unclear. No known insertases or receptors have been found to participate in the insertion of these TA proteins. Similarly unresolved is the role of cytosolic proteins in the biogenesis of mitochondrial TA proteins. In order to address this issue, different potential yeast cytosolic chaperones have been tested for their effect on the biogenesis of the mitochondrial TA outer membrane proteins: Fis1 and Gem1. For comparison, Atg32 that contains a soluble C-terminal domain in the mitochondrial intermembrane space was included in the assays. We found that the deletion or the dysfunction of cytosolic Hsp70 chaperones and the Sti1 co-chaperone reduce the steady state levels of Fis1 in mitochondria. Another chaperone, the peroxisomal targeting factor Pex19 appears to contribute to the biogenesis of the tested proteins. Deletion of Pex19 caused a reduction in the relative amount of tagged versions of Gem1 and Atg32 in whole cell lysates. These effects seem to occur independently of the protein segment upstream of the TMD or the type of the mitochondrial TA tail. Furthermore the intercellular

distribution of GFP-Fis1 and GFP-Gem1 is changed upon deletion of PEX19. Taken together, our data indicates an involvement of cytosolic chaperones in the biogenesis of mitochondrial TA proteins and shows that Pex19 participates also in the biogenesis of mitochondrial membrane proteins.

Authors: Bogdan Cichocki, Doron Rapaport

Systematic identification of inducers of the mitochondrial unfolded protein response (UPRmt) in C. elegans

Presenting author: Barbara Conradt

LMU München, Biozentrum, Großhaderner Str. 22, 82152, Planegg
conradt@bio.lmu.de

Mitochondrial homeostasis is essential to ensure proper mitochondrial function and the disruption of mitochondrial homeostasis induces various stress responses. For example, the accumulation of unfolded proteins in mitochondria activates a conserved stress response pathway referred to as 'mitochondrial unfolded protein response' (UPRmt). Using a genome-wide RNAi screen, we identified a total of 147 C. elegans genes that when inactivated induce UPRmt and that encode mitochondrial proteins. Interestingly, the majority of these genes have not been identified in previous screens for inducers of UPRmt. Furthermore, some of these genes define mitochondrial protein complexes or processes that have previously not been implicated in the maintenance of mitochondrial homeostasis and/or the induction of UPRmt. Conversely, we demonstrate that the loss of certain mitochondrial processes or aspects of mitochondrial function do not result in UPRmt induction. Finally, we present evidence that all inducers of UPRmt trigger a response that is dependent on the transcription factor ATFS-1 but only partially dependent on the kinase GCN-2. Based on these findings we propose that defects only in specific mitochondrial protein complexes or processes induces UPRmt and that they do so through a common genetic pathway.

Authors: Barbara Conradt, Stephane Rolland

The Peroxisome-ER connection: Identifying the first peroxisome-ER contact site in mammals

Presenting author: Joe Costello

University of Exeter, Biosciences, Stocker Road, EX4 4QD, Exeter
j.costello@exeter.ac.uk

Cooperation between peroxisomes and the ER is critical for a number of lipid-related metabolic processes including synthesis of ether-phospholipids and polyunsaturated fatty acids which are essential for normal brain function. The ER also plays a role in peroxisome biogenesis, the extent of which is controversial but which likely involves supplying phospholipids for peroxisomal membrane expansion. In addition, early ultrastructural studies have shown peroxisomes entwined in ER tubules, suggesting a close, physical interaction. Although potential peroxisome-ER tethers have been described in yeast, the molecular basis and factors involved in mammals remained elusive. Here, we identify the peroxisomal membrane protein ACBD5 as a binding partner for the ER protein VAPB. Both are tail-anchored proteins with a C-terminal transmembrane domain and cytoplasmic N-terminus. We show that the N-termini of ACBD5 and VAPB directly interact via a FFAT-like motif in ACBD5 and that this interaction regulates ER-peroxisome associations. Disruption of the ACBD5-VAPB complex results in loss of peroxisome-ER association, perturbing peroxisomal membrane expansion and increasing peroxisomal movement. Our findings reveal the first molecular mechanism for establishing peroxisome-ER associations in mammals. In addition we have recently identified patients carrying mutations in ACBD5 and the potential links between disrupting the peroxisome-ER connection and disease are currently being investigated.

Authors: Joe Costello, Inês Castro, Christian Hacker,
Tina Schrader, Jeremy Metz, Afsoon Azadi,
Hans Waterham, Markus Islinger, Michael Schrader

Loss of mitochondrial pyruvate carrier function in growing and selectively permeabilized Schizosaccharomyces pombe cells

Presenting author: Vasileios Delis

Biochemical Engineering Institute, Saarland University, Campus A1 5, 66123 Saarbrücken, Germany
vas.delis@googlemail.com

The multimeric mitochondrial pyruvate carrier (MPC) controls the entry of pyruvate into the TCA cycle at the mitochondria-cytosol interface. In the Crabtree positive yeast Schizosaccharomyces pombe, the MPC is inferred from homology to be composed by the monomers Mpc1p and Mpc2p. However, it is not known how the loss of either or both of these monomers influences the distribution of carbon fluxes at the pyruvate node and the mitochondrial activity. We have shown that during pseudo-steady-state growth on glucose, all deletion mutants behaved similarly exhibiting impaired growth, a reduced glycolytic flux, an accumulation of intracellular pyruvate and a redirection of fluxes towards acetate formation. However quantifying the compartmentalized fluxes and the mitochondrial activity in digitonin-permeabilized deletion mutants in a miniaturized reactor system revealed fine differences between the deletion mutants and the wild type. The MPC composition had a varying effect on cytosolic fluxes either towards the cytosolic pyruvate decarboxylase or towards the anaplerosis. At the same time, the P/O ratio for single mutants was significantly reduced whereas it remained unchanged for the double mutant compared to the wild type. As such we hypothesize that the MPC machinery as part of a transmembrane metabolon connects pyruvate transport to oxidative phosphorylation and modulates metabolic pathways in the cytosol and the mitochondrial matrix, possibly regulating the Crabtree effect.

Authors: Vasileios Delis, Konstantin Schneider, Elmar Heinzle

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A mitochondrial ribosome nascent chain complex facilitates translational plasticity

Presenting author: Sven Dennerlein

Universitätsmedizin Göttingen, Institut für Zellbiochemie, Humboldtallee 23, 37073, Göttingen
sven.dennerlein@med.uni-goettingen.de

Four multisubunit enzyme complexes represent the respiratory chain in human mitochondria. The cytochrome c oxidase (COX), as the terminal enzyme, reduces oxygen to water. Eleven of 14 structural COX subunits are nuclear-encoded and need to be posttranslationally imported into mitochondria by the import machineries of the outer and inner mitochondrial membrane. COX contains 3 essential core subunits (COX1, COX2 and COX3), which are encoded by the mtDNA and synthesized within the organelle. COX1 represents the “step stone”, on which the holo-enzyme is assembled. Although an increasing number of COX1-associated factors have been described (Richter-Dennerlein et al., 2015), the molecular mechanisms of how COX1 translation and COX maturation is regulated to adapt to the availability of nuclear encoded COX-subunits remains enigmatic. Here, we demonstrate that a ribosome nascent chain complex selectively translating COX1-mRNA interacts with early assembly factors of the COX enzyme (Richter-Dennerlein et al., 2016). These early assembly factors interact with defined membrane-integrated translation intermediates of COX1 during its translation. A block in early steps of COX assembly stalls translation in an assembly-primed state. Thus, we propose a mechanism of mitochondria translational plasticity that enables a precise control of mitochondrial translation and allows for adaptation to the influx of nuclear encoded subunits.

Authors: Sven Dennerlein, Silke Oeljeklaus, Isotta Lorenzi, Alexander Benjamin Schendzielorz, Cong Wang, Bettina Warscheid, Peter Rehling

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Diversity of mitochondrial protein import

Presenting author: Marcel Deponte

Ruprecht-Karls-Universität Heidelberg, Dept. of Parasitology, Im Neuenheimer Feld 345, 69120, Heidelberg
marcel.deponte@gmx.de

Our current view of mitochondrial protein import is predominantly based on studies in baker's yeast and related opisthokonts. However, in silico data as well as recent wetlab studies on protists uncovered astonishing variations of mitochondrial protein import machineries in eukaryotes. Our group compares the import machineries of the kinetoplastid parasite Leishmania tarentolae and the apicomplexan parasite Plasmodium falciparum with the established machineries in yeast (1,2). The organisms belong to three independent major eukaryotic lineages, have completely different life styles and are, therefore, excellent study objects to decipher evolutionary conserved as well as alternative import mechanisms and protein structure-function relationships. We established L. tarentolae as the first non-opisthokont model system for the comprehensive analysis of protein import into all four mitochondrial compartments. Using this model system, we

could demonstrate that mitochondrial import signals are functionally conserved in the course of evolution despite drastically altered import machineries (1). Furthermore, we characterized mechanistic differences regarding oxidative protein folding in the mitochondrial intermembrane space (3). Recent advances on oxidative protein folding and the optimization of genetic and biochemical tools will be presented.

(1) Eckers E et al. (2012) Biol Chem 393:513-24.
(2) Deponte M et al. (2012) MBP 186:95-116.
(3) Eckers E et al. (2013) JBC 288:2676-88.

Authors: Linda Liedgens, Sandra Specht, Gino Turra, Elisabeth Eckers, Marcel Deponte

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Interaction of Alzheimer´s disease Aβ1-42 peptide with human neuroblastoma cells and their organelles

Presenting author: Tamara Dzinic

Technische Universität Darmstadt, Chemie, Physikalische Biochemie, AG Dencher, Alarich-Weiss-Str.4, 64287, Darmstadt
dzinic.tamara@gmail.com

Monomeric and small oligomeric forms of Alzheimer's disease (AD) Aβ peptide, rather than extracellular Aß plaques, are recently proposed to be involved in damage of neurons but not as sole players. Since ionizing radiation causes oxidative stress and inflammation, occurring in AD as well, there is a concern that radiation exposure may be linked with neurodegeneration.
In order to shed light on the role of oxidative stress in Alzheimer´s disease, we investigate interaction of externally applied disaggregated Aβ peptide with human neuroblastoma (SH-SY5Y) cells and their organelles (i.e. lysosomes, mitochondria and endoplasmatic reticulum). Changes in cellular responses to Aβ peptide and/or to ionizing radiation are investigated under conventional cell culture condition of oxygen (~21%) versus more physiological oxygen (5%). Occurrence of mitochondrial DNA deletion and lysosomal integrity are assayed.
Aβ peptide interacts preferentially with the organelles of SH-SY5Y cells. Oxygen concentration in the cell culture does not affect kinetics of Aβ peptide trafficking. However, lysosomal integrity depends on Aβ peptide treatment, irradiation and oxygen level in the cell culture. MtDNA deletion of 4977 bp specifically occurs upon ionizing radiation. Traffic of Aß peptide toward cellular organelles and its kinetics are demonstrated that might be of relevance for AD pathology.

Authors: Tamara Dzinic, Norbert A. Dencher

Monitoring redox changes in African Trypanosomes by use of genetically encoded biosensors

Presenting author: Samantha Ebersoll

Heidelberg University Biochemistry Center, AG Krauth-Siegel, Im Neuenheimer Feld 328, 69120, Heidelberg
samantha.ebersoll@bzh.uni-heidelberg.de

Trypanosomes lack glutathione reductase and thioredoxin reductase. Instead they rely on the trypanothione/trypanothione reductase system. To analyze this specific thiol redox metabolism, several redox sensors were generated. The parasite specific oxidoreductase tryparedoxin was genetically fused to roGFP2 and purified as His-tagged fusion protein. The in vitro response of this sensor towards GSH/GSSG and $T(SH)_2/TS_2$ was compared with that of hGrx1-roGFP2 and roGFP2. Oxidized hGrx1-roGFP2 and Tpx-roGFP2, but not free roGFP2, were reduced by millimolar concentrations of GSH during the observation time. However, they displayed a remarkably different sensitivity towards $T(SH)_2$. Micromolar concentrations of $T(SH)_2$ resulted in a rapid complete reduction of Tpx-roGFP2, whereas hGrx1-roGFP2 required again millimolar concentrations of the thiol.
To employ these novel tools in living cells, bloodstream and procyclic Trypanosoma brucei were transfected with constructs allowing the inducible expression of the sensors. First experiments showed that under physiological conditions hGrx1-roGFP2 and Tpx-roGFP2 are fully reduced. Upon treating the cells with diamide, the sensors become fully oxidized. Addition of H_2O_2 leads to a rapid reversible oxidation of both sensors, but with different kinetics. These data indicate that the biosensors are well suited to analyze the unique thiol redox system of these parasites under endogenous and exogenous stresses and in different cellular compartments.

Authors: Samantha Ebersoll, Koen Van Laer, Tobias Dick, R. Luise Krauth-Siegel

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Mutations in the accessory subunit NDUFB10 result in isolated complex I deficiency due to incomplete assembly of the complex I holoenzyme

Presenting author: Alican Erdogan

University of Cologne, Institute of Biochemistry, Zuelpicherstr. 47A, 50674, Cologne
a.erdogan@uni-koeln.de

An infant presented with fatal infantile lactic acidosis and cardiomyopathy, and was found to have profoundly decreased activity of complex I in muscle, heart and liver. Exome sequencing revealed compound heterozygous mutations in NDUFB10, an accessory subunit located within the PD part of complex I. One mutation resulted in a premature stop codon and absent protein, while the second mutation replaced the highly conserved cysteine 107 with a serine residue. Protein expression of NDUFB10 was decreased in muscle and heart, and less so in liver and fibroblasts, resulting in perturbed assembly of the holoenzyme at the 830 kDa stage. NDUFB10 was identified together with three other complex I subunits as substrate of the oxidoreductase CHCHD4 in the intermembrane space. We found that during its mitochondrial import and maturation NDUFB10 acquires disulfide bonds. The mutation of cysteine residue 107 in NDUFB10 impaired oxidation and efficient mitochondrial accumulation of the protein and resulted in degradation of non-imported precursors. Taken together, mutations in NDUFB10 are a novel cause of complex I deficiency associated with a late stage assembly defect and emphasize the role of intermembrane space proteins for the efficient assembly of complex I. Interestingly, NDUFB10 levels and the correlating complex I acivities indicate that the IMS redox environment is controlled differently across different tissues.

Authors: Alican Erdogan, Jan Riemer

The activation of the unfolded protein response by lipid bilayer stress

Presenting author: Robert Ernst

Goethe-Universität Frankfurt am Main, Institute of Biochemistry, Max-von-Laue-Str. 15, 60439, Frankfurt
ernst@em.uni-frankfurt.de

Biological membranes are complex assemblies of proteins and lipids and their homeostasis is crucial for life. We are interested in the crosstalk between protein homeostasis and lipid metabolism at the molecular and the cellular level. The significance of this connection is underscored by the fact that most diseases related to protein misfolding also have specific lipid signatures. The role of lipids in the pathogenesis of these diseases and the underlying mechanisms remain poorly understood. The unfolded protein response (UPR) is a central homeostatic program regulating the expression of more than 5% of all genes in S cerevisiae. The UPR of mammals is more complex and active in every secretory cell. Professional secretory cells such as antibody producing plasma cells or insulin producing β-cells are particularly dependent on the UPR to cope with high secretory demand. Originally identified as stress response to accumulating unfolded proteins in the lumen of the ER, it is now accepted that aberrant lipid compositions are equally potent activators of the UPR. Here, we describe the molecular mechanism underlying the activation of the UPR by lipid bilayer stress.

Authors: Robert Ernst

Cdc48 regulates a deubiquitylase cascade critical for ubiquitin homeostasis and mitochondrial fusion

Presenting author: Mafalda Escobar

CECAD, Cologne University, Joseph-Stelzmann-Straße 26, 50931, Köln
mafalda.escobar@uni-koeln.de

Mitochondria are dynamic organelles constantly undergoing fusion and fission events. Due to their pathological relevance, e.g. for Parkinson's disease, these processes are subject to intense investigation. Most fusion processes rely on SNAREs. In contrast, fusion of the endoplasmic reticulum and fusion of mitochondria depend on large dynamin-related GTPases. In mitochondria, they are named mitofusins (Mfn1/Mfn2 in mammals and Fzo1 in yeast). Deficiencies in Mfn2 cause a subset of the Charcot-Marie-Tooth disease (CMT), the most common degenerative disorder of the peripheral nervous system.

Mitochondrial plasticity is modulated by several post-translational modifications. Ubiquitin is a double-faced regulator of mitochondrial fusion. Mitofusins are ubiquitylated by different E3 ligases, capable of either promoting or inhibiting mitochondrial fusion. In yeast, the deubiquitylases Ubp2 and Ubp12 control these opposing pathways. However, the molecular mechanisms remained poorly studied.

Cdc48/p97, a ubiquitin-selective chaperone, orchestrates the function of E3 ligases and deubiquitylases. Here, we identify a new function of Cdc48, as part of a ubiquitin signaling pathway consisting of Ubp12 and Ubp2. Our results reveal a hierarchical organization of Cdc48, Ubp12

and Ubp2, which controls Fzo1 function and cellular ubiquitin homeostasis. This uncovers a new deubiquitylation cascade where Cdc48 synergistically modulates ubiquitylation-dependent processes.

Authors: Mafalda Escobar

Studying the in-vivo client spectrum of the GET pathway via high-throughput screens in S. cerevisiae

Presenting author: Akos Farkas

Georg-August-Universität Göttingen, Dept. of Molecular Biology, Humboldtallee 23, 37073, Göttingen
akos.farkas@med.uni-goettingen.de

The TRC40/GET pathway plays an important role in the biogenesis of tail-anchored proteins, and one of its cytosolic components, Get3 has also been recently demonstrated to function as a chaperone. Due to a lack of systematic in-vivo studies on the client spectrum of the pathway, our understanding of the relevance of the pathway in living organisms has been limited. Therefore, we have set out to investigate the range of proteins that are in-vivo substrates of the GET pathway in S. cerevisiae with the help of high-throughput synthetic genetic array-based screens and automated microscopy, building on the recently published SWAT library of N-terminally GFP-tagged S. cerevisiae strains (Yofe et al., 2016). Furthermore, in order to reduce human bias and error in the evaluation of the results of our screens, we have developed a bioinformatics image analysis tool using the KNIME software suite. We have found that most tail-anchored proteins are unaffected upon the loss of different combinations of GET pathway components (Rivera-Monroy et al., 2016), also confirmed by our KNIME-based automated image analysis tool. In addition, several non-tail-anchored proteins change their localization upon the perturbation of the GET pathway, shedding light on the involvement of the pathway in diverse cellular processes.

Authors: Akos Farkas, Anne Clancy, Blanche Schwappach

Evolution of the nucleus: Lamina and the nuclear pore complex in trypanosomes

Presenting author: Mark Field

University of Dundee, Dow Street, DD1 5EH, Dundee
mfield@mac.com

The nuclear envelope (NE) is the defining structure of the eukaryotic cell. Prominent structures of the NE are the nuclear pore complexes (NPC) and the filamentous lamina underlying the nuclear membranes. Until recently, these structures were only characterised in model organisms that belong to a relatively narrow eukaryotic group. Here we provide more insight into the evolution of the NE from pan-eukaryotic homology searches and phylogenetic analyses of the individual NE components and recent experimental data on the composition of the NE from the diverged eukaryotic parasiteTrypanosoma brucei.We found that an NPC very similar to that in humans was already present in the last eukaryotic common ancestor (LECA). Although lamins were assumed a derived feature of animal nucleus, we found lamin homologs with shared domain architecture and sequence motifs in diverse protists. The additional NE components facilitating connections between the nucleoskeleton and the NPC, cytoskeleton and chromatin were likely also integrated into the LECA lamina. Our data further suggest that different nucleoskeletal structures that support the nuclear membranes, organise chromatin and connect nucleus to the cytoskeleton operate at the nuclear periphery of trypanosomes. These findings contribute to the understanding of the origin and evolution of the eukaryotic cell.

Authors: Mark Field

HsAK2 is a substrate of CHCHD4

Presenting author: Yannik Finger

Zülpicher Straße 47a, 50674, Cologne
yfinger1@uni-koeln.de

In a proteomic approach, we identified novel substrates of Mia40/CHCHD4 including the human adenylate kinase AK2. Adenylate kinases are a group of phosphotransferases with high importance for cellular energy balance and signalling, controlling the cellular levels of adenine nucleotides. The human isoform AK2 is found in mitochondria, located in the intermembrane space. The mature AK2 has a length of 239 amino acids and the crystal structure shows a disulfide bond between C42 and

C92. The interaction of AK2 with CHCHD4 and the role of CHCHD4 in AK2 import and maturation is not understood yet. We propose that CHCHD4 uses its redox-active CPC motif to import and oxidize AK2. We found that a redox-inactive variant of CHCHD4 was unable to bind AK2 and depletion of CHCHD4 results in absence of AK2. Moreover, we identified a potential CHCHD4 recognition motif in AK2, confirmed the existence of one disulfide bond in AK2 and that mutation of specific cysteines in AK2 (C42 and C92) results in absence of AK2 from mitochondria. Using an AK activity assay we showed that formation of the disulfide bond is only important for mitochondrial import, but not for AK2 activity.

Authors: Yannik Finger, Jan Riemer

Re-modelling plant mitochondrial respiration during drought

Presenting author: Philippe Fuchs

University of Bonn, INRES - Chemical Signalling, Friedrich-Ebert-Allee 144, 53113, Bonn
s7phfuch@uni-bonn.de

Drought is a stress that can severely limit growth and productivity of plants. To save water plant leaves can close their stomata, which comes at the price of impaired gas exchange. Continuing photosynthesis can then deplete the available CO_2 and risk over-reduction of redox systems with detrimental effects for the cell. In that situation, mitochondrial electron transport can counteract over-reduction by acting as a cellular dumping site for excess electrons. Mechanistically, this is thought to be mediated by flexible uncoupling of electron flux to oxygen from the phosphorylation of ADP. Just the significance, regulation and integration of uncoupling strategies is only rudimentarily understood. To investigate how (un-)coupling impacts in drought acclimation, we have been manipulating and engineering mitochondrial uncoupling capacity, considering multiple organizational levels and mechanisms in combination. We assess the impact of those adjustments by fluorescent biosensors for selected parameters of energy physiology, including pH, ATP or NAD redox status. We aim to map energy dynamics in response to drought with subcellular precision to investigate the specific in vivo impact of tuning (un-)coupling capacity. We will discuss our insights into the flexibility of subcellular energy regulation in plant cells along with recent methodological innovations.

Authors: Philippe Fuchs, Stephan Wagner,
Markus Schwarzländer

A cytosolic peroxiredoxin links mitochondrial H2O2 emissions to stress kinase signaling

Presenting author: Ana Gomes Barata

German Cancer Research Center, Im Neuenheimerfeld 280, 69120, Heidelberg
a.barata@dkfz.de

Mitochondria are increasingly recognized as signaling organelles. Their communication with the rest of the cell is central to homeostasis and stress adaptation. One of the signals emitted by mitochondria is hydrogen peroxide (H_2O_2). Mitochondrial H_2O_2 (mt-H_2O_2) contributes to the activation of adaptive stress response pathways, but the molecular mechanisms remain poorly understood. Using genetically encoded H_2O_2 probes in Drosophila we identified conditions of mild mitochondrial dysfunction which also lead to increased emission of mt-H_2O_2 into the cytosolic compartment. In particular, depletion of specific supernumerary subunits of respiratory complex I triggered mt-H_2O_2 emissions and concurrent activation of the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Here we show that the activation of p38 signaling by mt-H_2O_2 depends on the cytosolic peroxiredoxin Jafrac1. We find that Jafrac1 is oxidized by mt-H_2O_2. Oxidized Jafrac1 in turn interacts with the MAP3 kinase Mekk1, an upstream activator of p38, forming mixed disulfide intermediates in the process. These findings show that a cytosolic peroxiredoxin acts as the primary sensor and signal transmitter for mt-H_2O_2 under conditions of mild mitochondrial dysfunction.

Authors: Ana Gomes Barata, Tobias Dick

The role of phospholipids for mitochondrial protein import

Presenting author: Alexander Grevel

Freiburg University, Institut für Biochemie und Molekularbiologie, Stefan-Meier-Straße 17, 79104, Freiburg
alexander.grevel@biochemie.uni-freiburg.de

Alexander Grevel, Max-Hinderk Schuler, Lena Böttinger, Nikolaus Pfanner, Thomas Becker et al.
Institute for Biochemistry and Molecular Biology, Faculty of Medicine, University of Freiburg, Germany

Mitochondria exhibit essential functions for the survival of the cell like ATP production via oxidative phosphorylation, synthesis of lipids, amino acids and iron-sulfur clusters. To fulfill these various tasks mitochondria need to import 99%

of their proteome. Specific protein translocases sort precursor proteins to the mitochondrial subcompartments. The membrane potential across the inner membrane, generated by the activity of the respiratory chain, drives transport into the mitochondrial matrix and the inner membrane. Recently, much evidence has been found that the correct phospholipid composition of mitochondrial membranes is essential for protein import and assembly pathways as well as for the activity of the respiratory complexes.
As mitochondria are capable to produce cardiolipin and phosphatidylethanolamine, the functionality of protein translocases and respiratory complexes specifically depends on these two phospholipids. Phosphatidylcholine, phosphatidylserine and phosphatidylinositol are further major components of mitochondrial membranes. Whether reduced levels of these phospholipids affect mitochondrial protein import is not known. Here, we provide evidence that individual lipid depletion differentially influences the functions of mitochondrial membrane-bound machineries.

Authors: Alexander Grevel

Cytosolic processes during import of IMS proteins

Presenting author: Markus Habich

University of Cologne, Department of Chemistry, Zülpicher Str. 47A, 50674, Cologne
mhabich@uni-koeln.de

The vast majority of mitochondrial proteins are synthesized on cytosolic ribosomes and become imported into mitochondria. While most of them depend on an N-terminal mitochondrial targeting sequence (MTS) to become imported, members of the twin CX9C protein family contain conserved cysteine residues as targeting information. These cysteines become oxidized during the import into the intermembrane space (IMS) by Mia40/CHCHD4. Inside the IMS, twin CX9C proteins fulfill essential functions such as in the assembly of the respiratory chain and of the MICOS complex. How twin CX9C proteins are handled inside the IMS has been studied intensively. In contrast, only little is known about the cytosolic steps that twin CX9C proteins have to take to reach mitochondria. To investigate these cytosolic processes we established different conditions to prevent twin CX9C protein import/oxidation and to accumulate them in the cytosol. Interestingly, twin CX9C proteins remain stable in the cytosol for an extended time. Conversely, this stability is lost when equipping these proteins with an MTS. From this, we conclude that both targeting pathways have different cytosolic protein sets conveying specificity for their respective client proteins. We determined the interactome of cytosolically accumulated twin CX9C proteins by quantitative proteomics. We thereby identified members of the cytosolic chaperone network. Depletion of these chaperones affects the abundance of cytosolic twin CX9C proteins.

Authors: Markus Habich, Jan Riemer

The J protein Djp1 is involved in the targeting of mitochondrial precursor proteins

Presenting author: Katja Hansen

Erwin-Schrödinger-Str. 13, 67663, Kaiserslautern
khansen@rhrk.uni-kl.de

Cells contain different compartments such as mitochondria, peroxisomes and ER. Almost all proteins are synthesized in the cytosol and have to be targeted subsequently to their cellular destination. While the membrane-located translocation machineries of these compartments were studied extensively in the past, we know only little about factors which help to usher precursor proteins from the ribosome to surface receptors on their target compartments. To identify novel cytosolic components involved in the mitochondrial import process we established a genome-wide screen to select mutants in which mitochondrial precursor proteins accumulate in the cytosol. Two factors found in this screen were Djp1 and Pex21, both proteins involved in the import process of peroxisomal proteins. In the absence of Djp1 or Pex21 we observed defects in the biogenesis of mitochondria and of peroxisomes. Overexpression of the mitochondrial protein Oxa1 in Δdjp1 cells caused a strong accumulation of Oxa1 precursor in the cytosol. Djp1 is an abundant cytosolic DnaJ protein of poorly characterized function. We are currently trying to identify the client proteins bound by Djp1 in order to better understand its role in mitochondrial and peroxisomal biogenesis. Our results point to a role of the cytosolic chaperone network in coordination of the early step of intercellular protein targeting.

Authors: Katja Hansen, Naama Aviram, Janina Laborenz, Maren Meyer, Maya Schuldiner, Johannes Herrmann

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PINK1 mRNA is transported with mitochondria to facilitate mitophagy in axons

Presenting author: Angelika B. Harbauer

Boston Childrens Hospital/Harvard Medical School, F.M.Kirby Center for Neurobiology, Blackfan Circle, 02115, Boston
Angelika.Harbauer@childrens.harvard.edu

PTEN-induced kinase 1 (PINK1) is found mutated in hereditary forms of Parkinson's Disease (PD).
In current models, PINK1 acts as a sensor for depolarized mitochondria as it is constantly synthesized, but then rapidly degraded upon import into mitochondria. Import and degradation of PINK1 is impaired in depolarized mitochondria, which allows the protein to accumulate on the mitochondrial surface and consequently triggers mitophagy of the damaged organelle.
Neurons however, the main cell type affected in PD, face an additional challenge: ribosomes are thought to reside mainly in the cell body and dendrites, but mitochondria are actively transported into axons and can be up to a meter away from the cell body. We therefore hypothesized that there may exist a targeted mechanism to provide axonal mitochondria with the required PINK1 supply via transport of PINK1 mRNA and local protein synthesis.
We find indeed that axonal protein synthesis is a requirement for mitophagy in compartmentalized hippocampal cultures. Live-imaging of PINK1 mRNA showed, that PINK1 mRNA particles were present in axons where they localize to mitochondria. Mitochondria and PINK1 mRNA move together and measures that inhibit mitochondrial transport inhibit PINK1 mRNA transport, suggesting that PINK1 mRNA is probably tethered to the mitochondrial surface. We also find that translating ribosomes appear to be localized to axonal mitochondria and are likely to mediate the local translation of PINK1.

Authors: Angelika B. Harbauer, Ghazaleh Ashrafi, Thomas L. Schwarz

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Propagation of toxic mtDNAs by the UPRmt

Presenting author: Cole Haynes

University of Massachusetts, Molecular, Cell and Cancer Biology, 364 Plantation Street, 01605, Worcester
cole.haynes@umassmed.edu

During development and cell specification, cells acquire the appropriate amount of mitochondrial mass to meet their physiologic needs. In recent years, multiple stress response programs have been shown to respond to reduced mitochondrial activity. One such pathway is the mitochondrial unfolded protein response (UPRmt), which responds to mitochondrial dysfunction by activating a transcriptional response to promote the repair and recovery of dysfunctional mitochondria. While we have found this pathway to be protective during exposure to mitochondrial toxins or pathogenic bacteria, we recently discovered a consequence of prolonged UPRmt activation that may occur in a variety of pathologies. Interestingly, UPRmt activation is both necessary and sufficient to promote the propagation of deleterious mtDNAs at the expense of wildtype mtDNAs. Thus, in an attempt to repair and recover mitochondrial dysfunction caused by the deleterious mtDNA, the UPRmt creates an environment that allows for propagation of the deleterious mtDNA. Our recent data pointing towards a potential mechanism driving this phenomena will be presented.

Authors: Cole Haynes

Membrane protein biogenesis: from extracts to mechanism

Presenting author: Ramanujan S. Hegde

MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH
rhegde@mrc-lmb.cam.ac.uk

Approximately 20-25% of all genes encode integral membrane proteins that must be selectively targeted to and inserted into the endoplasmic reticulum membrane. These insoluble hydrophobic proteins need to transit the aqueous cytosol to the ER, where they are guided into the lipid bilayer. If this process fails, the mislocalized protein must be selectively targeted for degradation to avoid cellular dysfunction and disease. Over the past several years, our group has used biochemical and structural approaches to understand how the critical events during membrane protein biogenesis occur with high fidelity. By investigating the relatively simple case of a tail-anchored

membrane protein, we found a cytosolic factor dedicated to chaperoning the transmembrane domains (TMDs) of this class of proteins to the ER. This led to the discovery, by us and others, of additional TMD-selective chaperones that participate in this and other pathways to maintain cytosolic protein homeostasis. Reconstitution studies with purified factors, combined with structural snapshots of key intermediates, are beginning to reveal a mechanistic framework for both the biogenesis and quality control of membrane proteins.

Authors: Ramanujan S. Hegde

The dual inhibition of the TAP complex by the herpesviral inhibitor ICP47

Presenting author: Valentina Herbring

Goethe-Universität Frankfurt am Main, Biochemistry, Max-von-Laue Straße 9, 60438, Frankfurt am Main
v.herbring@hotmail.de

As a centerpiece of antigen processing, the ATP-binding cassette transporter associated with antigen processing TAP is responsible for the peptide translocation from the cytosol into the ER lumen. There, the peptide loading complex assembles MHC I-peptide complexes, which, if optimally loaded, are allowed to traffic to the cell surface. The antigenic peptides are presented to cytotoxic T-lymphocytes, which trigger a subsequent immune response. This adaptive immune response can be suppressed by the herpesviral inhibitor ICP47, which blocks the TAP function. ICP47 binds with a 50 nM affinity to the cytosolic TAP-interface, arresting the transporter in an open inward-facing conformation, thus prohibiting further peptide translocation. Here, we report on a thermostable ICP47-TAP complex, generated by fusion of different ICP47 fragments. These ICP47-TAP fusion complexes are arrested in a stable conformation, as demonstrated by MHC I surface expression, melting temperature, and the mutual exclusion of herpesviral TAP inhibitors. We unveiled a conserved region next to the active domain of ICP47 as essential for the complete stabilization of the TAP complex. Based on our findings, we propose a dual interaction mechanism for ICP47. A per se destabilizing active domain inhibits the function of TAP, whereas a conserved C-terminal region stabilizes the transporter. These new insights into the ICP47 inhibition mechanism can be applied for future structural analyses of the TAP complex.

Authors: Valentina Herbring, Anja Bäucker,
Simon Trowitzsch, Robert Tampé

Internal presequences in mitochondrial proteins and their role during import into mitochondria

Presenting author: Steffen Hess

TU Kaiserslautern, FB Biologie, Cellular Biology, Erwin-Schrödinger-Straße 13, 67663, Kaiserslautern
hesss@rhrk.uni-kl.de

With the help of the N-terminal matrix targeting sequence (MTS), the so called presequence, mitochondrial precursor proteins are recognized by the import receptors on the mitochondrial surface and sorted to their final destination. Protein localization can be predicted based on their amino acid sequence with algorithms such as TargetP. We used the TargetP algorithm to screen for MTS-like properties in the mature part of mitochondrial proteins. Surprisingly, many matrix proteins contain internal regions that mimick MTS (iMTSs). One of these proteins is Atp1, which, based on the TargetP score contains two iMTS segments. With an affinity assay, we showed significant interaction between the internal presequences and the receptor Tom70. Furthermore, a model protein was designed which contains an N-terminal presequence as well as an internal MTS, followed by a DHFR domain imposing a challenge on the mitochondrial import machinery. Import experiments showed that the iMTS containing construct was imported much more efficiently compared to the construct without the iMTS when the import was performed at lower temperature. These results indicate that the iMTSs facilitate binding to the TOM complex, help in the unfolding of strongly folded domains and maintain the solubility of aggregate-prone substrates, thereby enhancing the import process.

Authors: Steffen Hess

Alternative splicing dynamically controls ER export

Presenting author: Florian Heyd

FU Berlin, Takustr. 6, 14195, Berlin
florian.heyd@fu-berlin.de

The transport of secretory proteins from the endoplasmic reticulum (ER) to the Golgi depends on COPII-coated vesicles. While the basic principles of the COPII machinery have been identified, it remains largely unknown how COPII transport is regulated to accommodate tissue- or activation-specific differences in cargo load and identity. To address this question we have used T cells as a model system that shows strongly increased secretory cargo load upon activation. We have confirmed increased ER-export

efficiency in activated T cells and then used RNA-Seq to uncover the molecular mechanism for this adaptation. While we do not find substantial changes in the abundance of mRNAs encoding for components of the early secretory pathway, we observe a strong change in alternative splicing of Sec16, a protein essential for COPII vesicle generation. This splicing switch controls the number of ER exit sites and transport efficiency, thus providing the first connection between the COPII pathway and alternative splicing and adding a new regulatory layer to protein secretion and its adaptation to changing cellular environments. As a mechanistic basis, we suggest the C-terminal Sec16 domain to be a splicing-controlled protein-interaction platform, with individual isoforms showing differential ability to recruit COPII components. Based on this first example we are now analyzing a broader impact of alternative splicing in controlling the functionality of the early secretory pathway.

Authors: Florian Heyd

Dynamics of H2O2 in mammalian mitochondria

Presenting author: Michaela Hoehne

University of Cologne, Biochemistry, Zuelpicher Straße 47a, 50674, Köln
mhoehne1@smail.uni-koeln.de

Mitochondria are a major source of reactive oxygen species. The respiratory chain produces superoxide anions (O2°-), which are released towards the matrix and the intermembrane space (IMS). O2°- are rapidly disproportionated to hydrogen peroxide (H2O2) by superoxide dismutases. At high concentrations H2O2 is cytotoxic and linked to many diseases. However, at low concentrations H2O2 can serve as signaling molecule. At present, we lack knowledge on the spatiotemporal dynamics of H2O2 during its release from mitochondria. Moreover, to reach the cytosol, H2O2 from the respiratory chain has to pass the IMS, but the influence of IMS-localized redox enzymes on this release has not been explored. Genetically encoded redox-sensitive fluorescent probes targeted to different compartments allow measuring H2O2, glutathione and pH dynamics with high spatiotemporal resolution. Using these tools we currently explore mitochondrial redox dynamics and mitochondrial H2O2 release in mammalian cells.

Authors: Michaela Hoehne, Jan Riemer

The T-tubule system – physiology and pathophysiology of a neglected organelle

Presenting author: Julia Hofhuis

University Medical Center Göttingen, Department of Child and Adolescent Health, Robert-Koch-Str.40, 37073, Göttingen
julia.hofhuis@med.uni-goettingen.de

The T-tubule system is an unusual organelle: it is an extensive intracellular network formed by invaginations of the plasma membrane (PM) accounting for about 80% of the PM surface of striated muscle. It functions in excitation-contraction coupling, muscle fatigue, muscle differentiation, intracellular trafficking, and PM repair. However, the molecular mechanisms required for biogenesis and function of this organelle are only partially understood.

The dysferlin protein contains seven C2 domains and a single transmembrane domain. The C2 domains promote calcium-dependent membrane interaction. Dysferlin localizes to the PM and the T-tubule system in skeletal muscle, but its physiological mode of action is unknown. Dysferlin is involved in the pathogenesis of two clinically distinct muscular disorders.

We show that dysferlin has membrane tubulating capacity and that it directly shapes the T-tubule system. Dysferlin tubulates liposomes, generates a T-tubule-like membrane system in non-muscle cells, and links the recruitment of the membrane lipid PI(4,5)P2 to T-tubule biogenesis. Pathogenic mutant forms interfere with these functions indicating that muscular dystrophy is caused by the mutants' inability to form a functional T-tubule system. We suggest a model for the membrane-shaping function in T-tubule biogenesis, and propose that the ability to shape the T-tubule system is the primary molecular and cellular function of dysferlin.

Ref: Hofhuis et al., J Cell Sci. 2017, in press.

Authors: Julia Hofhuis, Kristina Bersch, Jutta Gärtner, Lars Klinge, Sven Thoms

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The coupling between ribosome biogenesis and nucleo-cytoplasmic transport

Presenting author: Ed Hurt

Ruprecht-Karls-Universität Heidelberg, Biochemie-Zentrum (BZH), Im Neuenheimer Feld 328, 69120, Heidelberg
ed.hurt@bzh.uni-heidelberg.de

Ribosomes consisting of ribosomal RNA and ribosomal proteins are the machines that synthesize the cell proteins. In eukaryotes, the two ribosomal subunits (60S and 40S subunit) are assembled in the nucleolus before export to the cytoplasm. Ribosome biogenesis is not only complicated but the most energy consuming process in growing cells, and thus requires extensive regulation and coordination. Eukaryotic ribosome synthesis is initiated by transcription of a large rRNA precursor, which is subsequently modified and processed to 25S, 5.8S and 18S rRNA with a concomitant assembly of the ribosomal proteins. At the beginning of ribosome synthesis, a huge (90S) precursor particle is formed that is subsequently split to induce the formation of the pre-60S and pre-40S particles, which each follow separate biogenesis and export routes. During ribosome synthesis about 200 non-ribosomal factors and 100 small non-coding RNAs (snoRNAs) transiently work on the evolving ribosomal subunits to facilitate their assembly, maturation and transport. We study ribosome formation in vivo in the yeast Saccharomyces cerevisiae and exploit a eukaryotic thermophile, Chaetomium thermophilum, for structural studies. In my talk I will summarize our recent findings on the mechanism of ribosome biogenesis and its link to nucleocytoplasmic transport, which were obtained from in vitro assays combined with genetic investigations and structural studies including electron microscopy and x-ray crystallography.

Authors: Ed Hurt

Henipavirus glycoproteins induce the unfolded protein response

Presenting author: Julian Hüther

Philipps-Universität Marburg, Institut für Virologie, Hans-Meerwein-Straße 2, 35043, Marburg
jjhuether@gmail.com

To assess the infectious potential of Henipaviruses we study the biological activity of individual viral genes. We focus on the Henipavirus G and F proteins which are essentially needed for virus entry and cell-to-cell fusion in any host cell. Our previous studies revealed that, in contrast to the functional glycoproteins of pathogenic Nipah virus (NiV), the F and G proteins of the African bat Henipavirus prototype GH-M74a (GH) were poorly expressed on the cell surface and had an impaired ability to mediate cell-cell fusion.
As there is strong evidence that GH glycoproteins accumulate in the ER, we wanted to analyze their ability to induce the unfolded protein response (UPR) in comparison to the non-ER retained NiV glycoproteins.
Individual expression of any of the GH or NiV glycoproteins led to a prominent UPR induction. Compared to the F proteins, both Henipavirus G proteins caused a more pronounced UPR upregulation. Interestingly, co-expression of NiV-F and NiV-G, which resulted in the formation of large syncytia, did no longer induce the UPR. This effect was not observed upon co-expression of fusion-defective GH-G and -F proteins, suggesting that cell-cell fusion prevents the UPR induction by Henipavirus glycoproteins. Supporting this idea, the combined expression of NiV-G with a cleavage- and fusion-defective NiV-F efficiently triggered the UPR. The molecular basis for this difference in single and syncytial cells is currently under investigation.
jGBM-Poster

Authors: Julian Hüther, Laura Behner, Cornelius Rohde, Verena Krähling, Marc Ringel, Andrea Maisner

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The dual role of hGBP1's C-terminus - switchable membrane anchor and regulator of the second GTP hydrolysis step

Presenting author: Semra Ince

Ruhr University Bochum, Physical Chemistry I, Universitätsstraße 150, 44801, Bochum
semra.ince@rub.de

Human guanylate binding protein 1 (hGBP1) is an interferon-inducible member of the dynamin surperfamily of large GTPases with antimicrobial activity. A GTP-controlled farnesyl anchor at the C-terminus mediates hGBP1's translocation from cytosolic to membranous compartments within the cell. However, the molecular mechanism of how the farnesyl anchor becomes released is not yet understood. Therefore, this work focuses on GTP-driven intramolecular interactions between the C-terminal GTPase effector domain (GED) and the catalytic large GTPase (LG) domain of hGBP1.
Intramolecular Förster Resonance Energy Transfer suggested that not GTP binding but hydrolysis is required to release the GED, in turn allowing the subdomain to

interact with the GED of another molecule. However, the release became restricted when the GED of the interacting molecule was either restricted to open or deleted, as shown by a set of point- and deletion-mutants that particularly affect LG:GED interactions. By these mutants, we further identified a crucial effect of the GED on the unique GTP hydrolysis mechanism of hGBP1, which in two successive phosphate cleavage steps yields both GDP and GMP as product. Accordingly, tightening LG:GED interactions almost abolished the second hydrolysis step, whereas weakening of the same enhanced the step yielding GMP as predominant product. Altogether, these data emphasize the important role of the GED in subcellular translocation of hGBP1 as well as its enzymatic activity.

Authors: Semra Ince, Ping Zhang, Miriam Kutsch,
Sergii Shydlovskyi, Christian Herrmann

Uptake and toxicity of copper oxide nanoparticles in C6 glioma cells

Presenting author: Arundhati Joshi

University of Bremen, FB02/ Neurobiochemistry, Leobener Straße 5/ NW2B, 28359, Bremen
arujoshi@uni-bremen.de

The C6 glioma cell-line is frequently used as a rat model for gliomas. To investigate the potential use of copper oxide nanoparticles (CuO-NPs) as a therapeutic drug for glioma treatment, the toxicity and uptake of CuO-NPs by C6 glioma cells was investigated. CuO-NPs were synthesized by a wet-chemical method and were coated with dimercaptosuccinic acid and bovine serum albumin to improve colloidal stability in physiological media (pCuO-NPs). Application of pCuO-NPs to C6 cells caused a strong time-, concentration- and temperature-dependent copper accumulation. This cellular copper accumulation was accompanied by severe toxicity as indicated by the loss in cellular MTT-reduction capacity and cellular LDH activity, and by an increase in the number of propidium iodide-positive cells. Toxicity of pCuO-NPs to C6 cells was only observed for incubation conditions that increased the specific cellular copper contents above 20 nmol copper per mg protein. Despite severe toxicity, no obvious formation of reactive oxygen species was found. Unexpectedly, C6 glioma cells were less vulnerable to pCuO-NPs compared to cultured primary brain astrocytes. Both cellular copper accumulation and CuO-NP-induced toxicity in C6 cells were prevented by application of copper chelators, but not by endocytosis inhibitors. These data suggest that liberation of copper ions from the pCuO-NPs is the first step that leads to the observed toxicity of pCuO-NP-treated glioma cells.

Authors: Arundhati Joshi, Wiebke Rastedt, Kathrin Faber, Aaron Schultz, Felix Bulcke, Ralf Dringen

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Insights into transport mechanisms in the secretory pathway using the secretion inhibitor FLI-06

Presenting author: Christoph Kaether

Leibniz Institut für Alternsforschung, Fritz Lipmann Institut, Beutenbergstr. 11, 07745, Jena
ckaether@fli-leibniz.de

The secretory pathway orchestrates fundamental processes of eukaryotic cells, among them the synthesis, modification and transport of most transmembrane and secreted proteins, both of which comprise more than a third of all proteins. Export out of the ER is initiated at specialized membrane domains in the ER, the ER export sites (ERES). The well-characterized SarI/COPII machinery is involved in ER export, but whether and how cargo proteins are concentrated upstream of SarI/COPII is unclear.
Two cargo concentration models are conceivable, a recruitment model, where cargo is actively transported via a transport factor and handed over to the Sar1/COPII in ERES, and a capture model, where cargo freely diffuses into ERES and it is captured there by the Sar1/COPII.
Using the novel secretion inhibitor FLI-06, which acts at a very early transport step, before or at ERES, we show that recruitment of the cargo VSVG to ERES is an active process upstream of Sar1 and COPII.
We also investigated if there are common mechanisms of cargo selection and concentration at different transport steps along the secretory pathway. Our data show that FLI-06 also inhibits export out of the TGN, but not autophagosome biogenesis and the ER-peroxisomal transport route, suggesting that common mechanisms must exist between export out of the ER and out of the TGN, but not other ER export routes to peroxisomes and autophagosomes.

Authors: Yoji Yonemura, Hans-Dieter Arndt,
Koret Hirschberg, Christoph Kaether

Evolutionary divergent organelles provide novel anti-parasite drug targets

Presenting author: Vishal Kalel

Ruhr-Universität Bochum, Systems biochemistry, Universitätsstr. 150, 44780, Bochum
vishal.kalel@rub.de

Eukaryotic pathogens harbor unique or divergent organelles. Glycosomes are divergent peroxisomes in Trypanosomatid parasites which compartmentalize glycolytic enzymes inside organelle. As trypanosomal glycolytic enzymes lack feedback regulation, unique compartmentation of ATP-consuming steps inside glycosomes with limited ATP and net ATP-generating steps in the cytosol is necessary for the regulation of glycolysis. Since glycolysis is the sole source of ATP for trypanosomes in the host bloodstream, disruption of glycosome biogenesis is an attractive drug target. We are characterizing proteins required for glycosome biogenesis called peroxins (PEX). We identified trypanosomal PEX16 involved in glycosomal membrane protein import and show its essentiality for glycosome biogenesis and parasite survival, thereby providing a novel drug target. Using structure-based drug design, we developed high-affinity small molecule inhibitors of the glycosomal matrix protein import by targeting PEX5-PEX14 interaction. The inhibitors disrupt glycosomal matrix protein import leading to mislocalisation of glycosomal enzymes to the cytosol which makes glucose toxic to trypanosomes. Unregulated glucose phosphorylation in the cytosol depletes cellular ATP, accumulates glucose metabolites to toxic levels and kills the parasites. We show that better understanding of organelle biogenesis in pathogens enables identification of new drug targets and design of new drugs which are urgently needed.

Authors: Vishal Kalel, Wolfgang Schliebs, Ralf Erdmann

Organellar gene expression and retrograde signaling

Presenting author: Tatjana Kleine

, Biologie 1/Botanik, Großhaderner Straße 2, 82152, Planegg-Martinsried
tatjana.kleine@lmu.de

Retrograde signals originating from chloroplasts and mitochondria modulate nuclear gene expression (NGE). Arabidopsis thaliana PRORS1 encodes a prolyl-tRNA synthetase, which is targeted to both plastids and mitochondria. Work on the prors1 mutant showed that disturbances of organellar gene expression (OGE) in both chloroplasts and mitochondria cooperate to trigger retrograde signaling. The prors mutant was identified in a screen for photosynthesis affected mutant lines – just as was the mterf6 mutant. Interestingly, prors and mterf6 mutants show very similar NGE profiles, attracting our attention to this mutant.
Arabidopsis mTERF6 (mitochondrial Transcription Termination Factor6) is dually targeted to plastids and mitochondria, and its knockout perturbs plastid development and results in seedling lethality. In the leaky mterf6-1 mutant, a defect in photosynthesis is associated with reduced levels of photosystem subunits, although corresponding messenger RNA levels are unaffected, whereas translational capacity and maturation of chloroplast ribosomal RNAs are perturbed. Bacterial one-hybrid screening, electrophoretic mobility shift assays, and coimmunoprecipitation experiments reveal a specific interaction between mTERF6 and an RNA sequence in the chloroplast isoleucine transfer RNA gene (trnI.2). We show that mTERF6 is required for the maturation of trnI.2. Thus, similar defects in OGE (in prors and mterf6 mutants) are translated into similar NGE changes.

Authors: Tatjana Kleine

A pull-down procedure for the identification of unknown GEFs for small GTPases

Presenting author: Daniel Koch

Max Planck Institute for molecular physiology, Structural Biochemistry, Otto-Hahn-Strasse 11, 44202, Dortmund
Daniel.Koch@mpi-dortmund.mpg.de

Members of the family of small GTPases regulate a variety of important cellular functions. In order to
accomplish this, tight temporal and spatial regulation is absolutely necessary. The two most
important factors for this regulation are GTPase activating proteins (GAPs) and guanine nucleotide
exchange factors (GEFs), the latter being responsible for the activation of the GTPase downstream
pathways at the correct location and time. Although a large number of exchange factors have been
identified, it is likely that a similarly large number remains unidentified. We have therefore
developed a procedure to specifically enrich GEF proteins from biological samples making use of
the high affinity binding of GEFs to nucleotide-free GTPases. In order to verify the results of these
pull-down experiments, we have additionally developed two simple validation procedures: An in

vitro transcription/translation system coupled with a GEF activity assay and a yeast two-hybrid screen for detection of GEFs. Although the procedures were established and tested using the Rab protein Sec4, the similar basic principle of action of all nucleotide exchange factors will allow the method to be used for identification of unknown GEFs of small GTPases in general.

Authors: Daniel Koch, Amrita Rai, Imtiaz Ali, Nathalie Bleimling, Timon Friese, Andreas Brockmeyer, Petra Janning, Bruno Goud, Aymelt Itzen, Matthias Müller, Roger Goody

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Role of the plant Oxa proteins in mitochondrial biogenesis

Presenting author: Renuka Kolli

LMU München, Plant Sciences, Großhadernerstr. 2-4, D-82152, Planegg-Martinsried
renuka.137548@gmail.com

The mitochondrial protein import is achieved by a number of multi-subunit protein complexes that recognize, translocate and assemble mitochondrial proteins into functional complexes. The YidC/Oxa1/Alb3 family of proteins functions in the insertion and folding of proteins into the inner membrane of bacteria and mitochondria as well as the thylakoid membrane of chloroplasts. Due to independent gene duplications, most organisms possess two isoforms, Oxa1 and Oxa2 (Cox18), except Gram negative bacteria, which have only a single YidC-like protein. However, in the plant mitochondrial inner membrane, four such isoforms - Oxa1a, 1b, 2a and 2b are present. Interestingly, Oxa2a and 2b have a predicted TPR domain which is unique to plants. Oxa1a, 2a and 2b were identified to fulfill essential roles in embryo development, but the exact function of all four plant Oxas is unknown. Although the oxa1b homozygous mutant plants display no obvious phenotype, our initial biochemical experiments suggest that Oxa1b is either directly involved in the insertion of F_o subunits or acts as a regulatory protein during the assembly process of the ATP synthase complex. Complementation of oxa2a and oxa2b knockout mutant plants with the corresponding proteins lacking the C-terminal TPR domain resulted in plants with severely retarded growth phenotypes in comparison to those complemented with the full sequence. Hence Oxa2 function is extremely important for normal mitochondrial biogenesis and physiology.

Authors: Renuka Kolli, Chris Carrie, Jürgen Soll

Human hereditary disorders due to dysfunction of the Golgi compartment

Presenting author: Uwe Kornak

Charité Universitätsmedizin Berlin, Institute of Medical Genetics and Human Genetics, Augustenburger Platz 1, 13353, Berlin
uwe.kornak@charite.de

The Golgi compartment is best known as a central hub for intracellular trafficking and post-translational modification, but is also involved in other cellular processes. To fulfill its role the typical stack-like organization of cisternae, appropriate intra-Golgi trafficking and a certain intraluminal ion composition are essential. Depending on the nature of the genetic defect and the affected cell type Golgi dysfunction can lead to different phenotypes ranging from hematological and neurological problems to dermatological and musculoskeletal changes. An important group of Golgi-related pathologies are the congenital disorders of glycosylation (CDGs) affecting N- and O-glycans to different degrees. Typically, these diseases show musculoskeletal and neurological symptoms, and some are characterized by a progeroid appearance. The underlying pathomechanisms comprise mainly defects in glycosylation enzymes, transporters, or trafficking. These findings will be discussed in the light of Golgi changes described in neurodegenerative disorders and aging.

Authors: Uwe Kornak

Mechano-Induced Mitochondrial Fission

Presenting author: Benoît Kornmann

ETH Zurich, Institute of Biochemistry, Otto-Stern-Weg 3, 8093, Zürich
benoit.kornmann@bc.biol.ethz.ch

Organelles form highly extensive and dynamic networks in the extremely crowded cytoplasmic space. This causes many collisions and entanglements. In addition to physiologically-important membrane contact sites, this situation leads to unwanted collisions and entanglements that needs to be resolved.
We show that, in the case of mitochondria, these collisions and entanglements can be resolved by mitochondrial fission. Mechanical forces applied to mitochondrial tubules lead to the recruitment and activation of the mitochondrial fission machinery, leading to the resolution of entanglements. This results imply that a biochemical response can be triggered by a mechanical stimulus, and

that force fields within the cells participate in the shaping of organelle networks.

Authors: Sebastian Helle, Qian Feng, Benoît Kornmann

Phospholamban targeted proximity labeling for proteomic mapping of sarco/endoplasmic reticulum subdomains

Presenting author: Daniel Kownatzki

University Medical Center Göttingen, Cellular Biophysics and Translational Cardiology, Robert-Koch-Straße 42a, 37075, Göttingen
daniel.kownatzki@med.uni-goettingen.de

Introduction: Phospholamban (PLN) is essential for normal heart function. Whereas dephosphorylated PLN inhibits the sarco/endoplasmic reticulum (SER) calcium ATPase SERCA2a, phosphorylation increases cardiac contractile function. To elucidate PLN interactions in signalosome subdomains, we developed a PLN-specific proximity assay.
Methods: APEX2 was fused N-terminally to full-length V5-tagged PLN. Following expression, intact HEK293 and neonatal rat cardiomyocytes (NRCM) were used for PLN-restricted proximity labeling. APEX2-biotinylated proteins were enriched by pulldown, processed and analyzed by mass spectrometry. A truncated APEX-Δ(1-31)-PLN construct (no cytosolic domain) was used as SER-targeted control.
Results: APEX2-PLN was successfully expressed in HEK293 and NRCM as evidenced by Western Blot (WB), and confocal co-immunofluorescence of V5-PLN and SERCA2a. APEX2-biotinylated proteins were shown by streptavidin-RD680 WB (n=4) and in situ confocal immunofluorescence. For global quantitation experiments, NRCM were stable isotope labeled with heavy amino acids (SILAC) resulting in >95% incorporation rate.
Conclusions:
A PLN-specific strategy for proximity labeling was successfully developed in HEK293 and transferred to NRCM including SILAC. Preliminary data suggest that APEX2-PLN expression in cells labels proteins in proximity to PLN. Hence, future experiments will exploit NRCM as model of pathological cell growth to elucidate signalosome changes in PLN-regulated subdomains.

Authors: Daniel Kownatzki, Christof Lenz, Henning Urlaub, Stephan E. Lehnart

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Mitochondrial dynamics during the infection with intracellular pathogens

Presenting author: Vera Kozjak-Pavlovic

University of Wuerzburg, Microbiology, Am Hubland, D-97074, Wuerzburg
vera.kozjak@uni-wuerzburg.de

Simkania negevensis and Chlamydia trachomatis are obligate intracellular bacteria and as such depend on metabolites of the host cell for their survival and propagation. S. negevensis infect mammalian cells and amoeba. The bacteria replicate within a membrane-bound vacuole forming ER contact sites that are associated with mitochondria. During the course of infection with S. negevensis, we observe progressive fragmentation of mitochondria and loss of mitochondrial mass, mediated by a so far unknown mechanism. On the other hand, C. trachomatis, the causative agent of trachoma and the sexually transmitted disease, maintains mitochondrial integrity during ROS-induced stress that occurs during infection. Significant elongation of mitochondrial fragments and loss of Drp1 fission rings take place in infected cells. We could show that C. trachomatis require mitochondrial ATP for normal development and postulate that they preserve mitochondrial integrity through inhibition of Drp1-mediated mitochondrial fission. Although biologically closely related, S. negevensis and C. trachomatis affect mitochondria in opposite ways, which opens many mechanistic questions and emphasizes the importance of host-cell mitochondria in the life cycle of intracellular pathogens.

Authors: Suvagata Roy Chowdhury, Anastasija Reimer, Jo-Ana Herweg, Thomas Rudel, Vera Kozjak-Pavlovic

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Mimivirus-encoded nucleotide carrier VMC1: Import into the inner mitochondrial membrane

Presenting author: Sebastian Kreimendahl

Ruhr-Universität Bochum, Institute of Biochemistry and Pathobiochemistry, Department of Cellular Biochemistry, Universitätsstr. 150, 44801, Bochum
Sebastian.Kreimendahl@rub.de

The Mimiviridae mimivirus was first identified as a possible pathogen in Acanthamoeba polyphaga. Its viral 1,2 Mbp genome contains 911 genes, of which approximately 10% encode proteins of known function [1], including a functional homologue of the mitochondrial carrier family (MCF), the viral mitochondrial carrier 1 (VMC1). Members

of the MCF enable the transport of metabolites and cofactors across the inner membrane of mitochondria [2]. Given the ability of VMC1 to transport dATP and dTTP in artificial membrane studies [3], we raised the question if and how VMC1 interacts with mitochondria. We found that the VMC1 is indeed imported into the inner membrane of isolated mitochondria in a membrane potential-dependend fashion. Its initial recognition is mediated by receptors Tom70 and Tom20. After binding to the import receptors, the VMC1 is translocated across the outer mitochondrial membrane through the general import pore Tom40, following a distinct route for carrier proteins. The aim of our project is to investigate a possible role of the VMC1 in the reproduction of the mimivirus.

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Authors: Sebastian Kreimendahl, Kathrin Günnewig, Jan Schwichtenberg, Dina Sträter, Joachim Rassow

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Taking Apart the Nuclear Envelope during Open Mitosis

Presenting author: Ulrike Kutay

ETH Zurich, Otto-Stern-Weg 3, 8093, Zurich
ulrike.kutay@bc.biol.ethz.ch

NE breakdown (NEBD) is a major event during the drastic intracellular reorganization in preparation of cell for mitosis. Disassembly of the nucleus directly exploits the activity of protein kinases involved in mitotic entry and is supported by microtubule-dependent restructuring of the NE. In my talk, I cover two different aspects of NEBD, namely NPC disassembly and the removal of membranes from chromatin. To study NPC disintegration, we had previously established a visual in vitro assay relying on semi-permeabilized cells. Exploiting this system, we have now reconstituted the initial steps of mitotic NPC disassembly using purified soluble factors. We show that hyperphosphorylation of both the gate-keeper nucleoporin Nup98 and the central scaffold component Nup53 is required for timely NPC disassembly. The combined action of multiple mitotic kinases appears sufficient to drive mitotic NE permeabilization. Further, we I will discuss the importance of membrane dissociation from chromatin for chromosome segregation and cell division.

Authors: Ulrike Kutay, Monika Linder, Lysie Champion

Prenylation-type of hGBP-1 influences membrane binding but not polymerization

Presenting author: Miriam Kutsch

Ruhr-University Bochum, Physical Chemistry I, Universitätsstr. 150, 44801, Bochum
miriam.kutsch@rub.de

Antibacterial and antiviral effects of human guanylate-binding proteins (hGBPs) depend on the proteins' interaction with cellular and phagosomal membranes. Like for other proteins interacting over a lipid-anchor with membranes, binding of farnesylated hGBP-1 is a dynamic process which was shown most recently (1). Moreover, by establishing a GUV-based assay we showed that beside binding to membranes farnesylated hGBP-1 also tethers GUVs in a fanesyl- and GTP-dependent fashion. In addition, GTP-dependent polymerization of the protein was observed competing for membrane binding. These polymers were shown to be similar to polymers formed by other members of the dynamin superfamily (1). In this work, we investigated binding of hGBP-1 to GUVs dependent on the protein's prenylation-type and the GUV's membrane-composition using fluorescence microscopy. Interestingly, one additional isoprene unit let the protein beside localizing to cytoplasmic membranes also localize to membranes with a Golgi-like composition. Additionally, using a spectroscopic FRET-based assay revealed more information about hGBP-1's polymerization.

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Authors: Miriam Kutsch, Sergii Shydlovskyi, Semra Ince, Adai Colom, Aurélien Roux, Christian Herrmann

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Viral inhibition of trafficking of the innate immune response protein, RAE-1

Presenting author: Natalia Lis

Jacobs University Bremen, Life Sciences, Campus Ring 1, 28759, Bremen
n.lis@jacobs-university.de

Retinoic acid early-inducible protein 1 (RAE-1) is expressed on stressed and virus-infected cells as an ligand for Natural Killer (NK) cells. The murine cytomegalovirus (MCMV) glycoprotein gp40 (encoded by the m152 gene) downmodulates RAE-1 cell surface expression and thus limits the immune response against the virus. The molecular mechanism of action of this downmodulation is unknown. We are investigating whether gp40 and RAE-1 interact, whether gp40 retains RAE-1 in the endoplasmic reticulum or triggers its degradation, and whether there is an additional cellular interaction partner required for RAE-1 downmodulation. The results of our work will enable better understanding of protein maturation, the effect of MCMV genes on cell surface proteins, and the influence of infection on the morphology of the cell.

Authors: Natalia Lis, Venkat Raman Ramnarayan, Swapnil Ghanwat, Sebastian Springer

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Intracellular mechanisms of aging and cell death

Presenting author: Frank Madeo

University of Graz, Institute of Molecular Bioscience, madeo@uni-graz.at

Spermidine is a ubiquitous polycation that is synthesized from putrescine and serves as a precursor of spermine. Putrescine, spermidine and spermine all are polyamines that participate in multiple known and unknown biological processes. Exogenous supply of spermidine supresses necrosis and prolongs the life span of several model organisms including yeast (Saccharomyces cerevisiae), nematodes (Caenorhabditis elegans) and flies (Drosophila melanogaster) and significantly reduces age-related oxidative protein damage in mice, indicating that this agent may act as a universal anti-aging drug. Spermidine induces autophagy in cultured yeast and mammalian cells, as well as in nematodes and flies. Genetic inactivation of genes essential for autophagy abolishes the life span-prolonging effect of spermidine in yeast, nematodes and flies. Recently, we found that spermidine supplementation has a strong cardioprotective effect of diastolic parameters. These findings complement expanding evidence that autophagy mediates cytoprotection against a variety of noxious agents and can confer longevity when induced at the whole-organism level. We hypothesize that increased autophagic turnover of cytoplasmic organelles (in particular Mitochondria) or long-lived proteins is involved in many age associated diseases.

Authors: Frank Madeo

Proteins that shift from the endoplasmic reticulum to lipid droplets

Presenting author: Markus Maniak

Univ. Kassel, Cell Biology, Heinrich-Plett-Str. 40, 34132, Kassel
maniak@uni-kassel.de

The short-chain dehydrogenase-reductase (Sdr) enzymes represent a protein superfamily, with members involved in lipid-, amino acid-, carbohydrate-, and cofactor-, to hormone- metabolism. All enzymes contain a dinucleotide binding domain (Rossmann Fold), an active center and a short tail, mostly at the N-terminus of the protein, often containing a transmembrane domain.
If the amoeba Dictyostelium discoideum is fed with free fatty acids it forms a specialized organelle for storage of neutral lipids like triacylglycerols, etherlipids or sterolesters, the so-called lipid droplet. The coalescence of neutral lipids starts between the two leaflets of the ER membrane. Later, the surface of lipid droplets consists of a phospholipid monolayer and a specific coat of proteins. Five of the Dictyostelium Sdr enzymes, SdrB-SdrF, change their localization from the ER surface to lipid droplets, if fatty acids are supplied to the media. To study this trafficking event, we used different methods, like GFP fusions to different parts of the proteins, followed by confocal microscopy, as well as biochemical membrane association assays, to compare the mechanisms of Sdr anchoring to ER and lipid droplet. GFP variants, in which the fluorescence depends on the absence of a glycosylation reaction, provide topological information for the Sdr proteins on each organelle. From the results we develop a simple model for the localization shift.

Authors: Thomas Gottlieb, Markus Maniak

Quality control during mitochondrial inheritance in budding yeast

Presenting author: Martina Mazurova

University of Bayreuth, Cell Biology, Universitätsstr. 30, 95440, Bayreuth
martina.mazurova@uni-bayreuth.de

Budding yeast reproduces by asymmetric cell division. Since mitochondria cannot be made de novo, they have to be inherited from the mother cell. We hypothesize that this inheritance is regulated by quality control mechanisms to prevent daughter cells from inheriting damaged mitochondria. One of these quality control mechanisms is the ubiquitin-proteasome system (UPS) at the mitochondrial outer membrane. Therefore, we expressed the stress-related UPS component Vms1 targeted to the mitochondrial outer membrane and analyzed its effect on the whole proteome. Vms1 localizes to damaged mitochondria and recruits Cdc48. Vms1 plays an important role in maintaining mitochondrial function. A variant of Vms1, Vms1ΔVIM, does not recruit Cdc48 and thereby decreases mitochondrial protein degradation and declines the respiratory function.
In our study, Vms1 is C-terminally tagged with GFP and the mitochondrial anchor of the Fis1 protein. We performed a SILAC experiment to analyze the effect of Vms1-GFP-fis1 expression on the protein levels. We compared a culture expressing the Vms1-GFP-fis1 with two controls – with a culture containing the empty vector and with a culture containing the Vms1ΔVIM-GFP-fis1 vector. We repeated this experiment and then compared the proteome differences in both analyses. Among the identified proteins are several mitochondrial proteins and proteins involved in stress response. We are currently analyzing the role of these proteins in mitochondrial quality control.

Authors: Martina Mazurova, Verónica I. Dumit, Benedikt Westermann, Ralf J. Braun

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Establishment of an in vivo and cell-specific method for the identification of palmitoyltransferase-interacting proteins in Drosophila using BioID

Presenting author: Christoph Metzendorf

Heidelberg University, BZH, INF 328, 69120, Heidelberg
christoph.metzendorf@bzh.uni-heidelberg.de

We are interested to identify palmitoylated transmembrane proteins (PTMPs) that cause branching of membrane protrusions. One strategy to obtain candidates is to identify PTMPs in highly branched cells, such as neurons. However, no methods exist to extract PTMPs cell-specifically. To overcome this shortcoming, we are establishing the proximity biotinylation system (BioID) in the fruit fly to then identify neuron specific PTMPs in vivo, by expressing palmitoyltransferase-BioID fusion proteins cell-specifically.
Here we present the current state of establishing this method. Of the 22 different palmitoyltransferases (DHHC protein family) in Drosophila, we focused on a subset of 10 DHHCs that show neuronal or ubiquitous expression. As proof of principle in cell culture we find that DHHC-BioID is able to identify the specific interaction between the DHHC Hip14 and its known ligand SNAP25, and the highly similar SNAP24 in a co-overexpression system. Furthermore, by mass-spectrometric identification of biotinylated proteins we also found an interaction of endogenous SNAP24 in Hip14-BioID expressing cells. Applying Bioinformatics we are presently analyzing mass-spectrometric data generated in DHHC-BioID cell culture for the subset of 10 DHHCs mentioned above. First experiments in transgenic Hip14-BioID flies are promising: Hip14-BioID can be expressed and results in a moderately strong biotinylation pattern.

Authors: Elena Porcellato, Mahmoud Elsakka, Felix Wieland, Christoph Metzendorf

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A Novel Repeated Motif of Cyanobacterial Origin Is Required for Plastid mRNA Stabilisation

Presenting author: Jörg Meurer

University of Munich (LMU), Dep. Biology I, Großhadernerstr. 2-4, 82152, Planegg-Martinsried
meurer@bio.lmu.de

We identified the HCF145 gene, which encodes a plant-specific, chloroplast-localized, modular protein containing two homologous domains of cyanobacterial origin and related to the polyketide cyclase family comprising 37 annotated Arabidopsis proteins of unknown function. Two further highly conserved and previously uncharacterized tandem repeat motifs at the C terminus, herein designated the transcript binding motif repeat (TMR) domains, confer sequence-specific RNA binding capability to HCF145. Homologous RNA-binding TMR motifs with up to ten repeats are found in quite diverse proteins of green and red algae and in the cyanobacterium Microcoleus sp PCC 7113, all with unknown function. HCF145 represents the only TMR protein found in vascular plants. Detailed analysis of hcf145 mutants in Arabidopsis and Physcomitrella patens as well as in vivo and in vitro RNA binding assays indicate that HCF145 has been recruited in embryophyta for the stabilization of the psaA mRNA via

specific binding to its 5′ untranslated region. PsaA forms the reaction center of photosystem I. The polyketide cyclase-related motifs support association of the TMRs to the psaA RNA, presumably pointing to a metabolic role in adjusting psaA mRNA and thus photosystem I levels according to the requirements of the plant cell.

Authors: Jörg Meurer

Clathrin coat controls vesicle acidification by mechanical inhibition of vacuolar ATPase

Presenting author: Ira Milosevic

European Neuroscience Institute (ENI), Synaptic Vesicle Dynamics, Griesbachstrasse 5, 37077, Goettingen
imilose@gwdg.de

The vacuolar H+-dependent ATPases are highly conserved proton pumps that use the energy from ATP hydrolysis to produce an electrochemical gradient (ΔμH+) across membranes and acidify numerous organelles, enabling their function. The vATPases are also essential for endocytosis and intracellular membrane traffic, including the synaptic vesicle (SV) recycling. Newly formed SVs are rapidly acidified by vATPases, generating ΔμH+ and subsequently using it to uptake neurotransmitter molecules. To date, it is unclear how vATPase is regulated during the endocytic process, and whether vesicle uncoating is a prerequisite for the vATPase to operate. Here, we developed assays to monitor pH and electrical gradients at the single clathrin-coated vesicle (CCV) level, and used it to provide unequivocal evidences that the ATP-induced acidification of CCVs isolated from the mammalian brain is blocked while vesicles are coated (the isolated SVs acidified under same conditions). Both Vo an V1 domains were present at CCVs, and the pump was fully functional: once the coat was removed from CCVs, uncoated vesicles acidified rapidly. Based on biochemical, imaging and functional studies, we propose a model where formation of clathrin coat around vATPase inhibits it mechanically. These findings resolve the longstanding controversy in the field and provide mechanistic insights into the regulation of vATPase by the clathrin coat which might apply to other clathrin-coated organelles/structures as well.

Authors: Ira Milosevic

The respiratory chain regulates cell growth in response to changing amino acid availability

Presenting author: Bruce Morgan

University of Kaiserslautern, Cellular Biochemistry, Erwin Schrödinger Strasse 13, 67663, Kaiserslautern
morgan@bio.uni-kl.de

Changes in amino acid handling have been observed in a wide-range of human pathologies including diabetes and cancer. We used Saccharomyces cerevisiae as a model to investigate how changes in amino acid availability influences cell growth and fitness. Intriguingly, we observe that increasing the general availability of amino acids relative to the availability of leucine leads to striking growth defects on glucose containing media. Surprisingly, the amino acid-dependent growth phenotypes are completely absent when cells are grown in media containing non-fermentable carbon sources. We found that deletion of the mitochondrial external NADH dehydrogenase-1 (Nde1) in combination with Cox6 (an essential component of complex IV) partially rescued amino acid-dependent growth phenotypes. However, deletion of the Nde1 homolog, Nde2, in combination with Cox6 had the opposite effect, further decreasing growth rate. Furthermore, in all cells grown in conditions of increased amino acids/normal leucine we observe a 10-fold increase in oxidized glutathione levels, strongly increased sensitivity to exogenous H_2O_2 and increased endogenous H_2O_2 levels. We speculate that amino acid availability can severely impact upon cellular redox homeostasis and that respiratory chain components may play an important role in ‘buffering’ cells against these changes.

Authors: Gurleen Kaur Khandpur

Deep characterization of the yeast mitochondrial proteome

Presenting author: Marcel Morgenstern

, Functional Proteomics, Schänzlestr. 1, 79104, Freiburg
marcel.morgenstern@biologie.uni-freiburg.de

Mitochondria play important roles in numerous cellular processes including energy metabolism and iron-sulfur protein biogenesis. For a comprehensive understanding of mitochondrial functions, detailed knowledge of the mitochondrial proteome is essential. Previous studies targeting the mitochondrial proteome of the eukaryotic model organism Saccharomyces cerevisiae compiled a

valuable, first mitochondrial protein inventory. However, new mitochondrial proteins are still being identified indicating that current knowledge of the mitochondrial proteome is still incomplete.
We here present an integrative, quantitative proteomic study of yeast mitochondria. To characterize the mitochondrial proteome, we analyzed fractions of different purity using SILAC-MS and quantified 3,500 proteins of mitochondria and mitochondria-associated fractions. By global absolute quantification, we obtained a quantitative understanding of the mitochondrial proteome and its regulation in cells grown under different conditions. We extend current knowledge of the mitochondrial proteome by identifying more than 60 new mitochondrial proteins including proteins with multiple localizations. The mitochondrial localization of many of these proteins was confirmed by biochemical or fluorescence microscopy analyses. We further studied their submitochondrial localization and revealed for selected new mitochondrial proteins their integration in large membrane protein assemblies with central functions in mitochondria.

Authors: Marcel Morgenstern, Sebastian Stiller, Philipp Lübbert, Christian Peikert, Stefan Dannenmaier, Friedel Drepper, Uri Weill, Maya Schuldiner, Conny Schütze, Silke Oeljeklaus, Nikolaus Pfanner, Nils Wiedemann, Bettina Warscheid

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MIPEP/OCT1 recessive variants cause a syndrome of left-ventricular non-compaction resulting in infantile death

Presenting author: Patrycja Mulica

Institute of Biochemistry and Molecular Biology in Freiburg, Stefan-Meier-Straße 17, 79104, Freiburg
patrycja.mulica@biochemie.uni-freiburg.de

Mitochondrial presequence proteases perform crucial functions as they process about 70% of all mitochondrial preproteins that are encoded in the nucleus and imported posttranslationally. The mitochondrial intermediate presequence protease MIP/Oct1, which executes precursor processing, has not yet been reported to have a role in human disease. In our study we identified four unrelated patients with left ventricular non-compaction (LVNC), developmental delay (DD), seizures, and severe hypotonia. Biallelic single nucleotide variants (SNVs) or copy number variants (CNVs) in MIPEP, which encodes MIP, were present in all four patients, three of whom died in the early childhood or infancy. All amino acids affected in the patients' missense variants are highly conserved from yeast to human and consequently model organism S. cerevisiae was employed for functional analysis (for p.L71Q, p.L306F, and p.K343E). The mutations p.L339F (human p.L306F) and p.K376E (human p.K343E) resulted in a severe decline of Oct1 protease activity and accumulation of non-processed Oct1 substrates and therefore impaired viability under respiratory growth conditions. Our findings reveal for the first time the role of the mitochondrial intermediate peptidase in human disease.

Authors: Patrycja Mulica, Mohammad Eldomery, Zeynep Akdemir, James Lupski, Reid Sutton, Chris Meisinger, Nora Vögtle

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Overlapping Role of Rcf-Proteins in the regulation of Respiratory supercomplexes

Presenting author: Tobias Müller

Universitätsmedizin Göttingen, Cellular Biochemistry, Humboldtallee 23, 37073, Göttingen
tobias.mueller2@med.uni-goettingen.de

The mitochondrial electron transport chain consists of individual
protein complexes arranged into large macromolecular structures, termed respiratory chain supercomplexes or respirasomes.
In the yeast Saccharomyces cerevisiae, respiratory chain supercomplexes form by association of the bc1 complex with the
cytochrome c oxidase. Formation and maintenance of these
assemblies are promoted by specific respiratory supercomplex
factors, the Rcf proteins. A lack of Rcf2 and Rcf3 increases oxygen flux
through the respiratory chain by up-regulation of the cytochrome
c oxidase activity. A double gene deletion of RCF2 and RCF3 affects cellular survival under non-fermentable growth
conditions, suggesting an overlapping role for both proteins in
the regulation of the OXPHOS activity.

Authors: Tobias Müller, Katharina Römpler, Lisa Juris, Mirjam Wissel, Milena Vukotic, Kay Hofmann, Markus Deckers

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Disulfide bond formation in chloroplasts

Presenting author: Lena Murschall

University of Cologne, Institut of Biochemistry, Zülpicher Str. 47a, 50674, Cologne
lena.murschall@uni-koeln.de

Disulfide bonds are often essential for protein folding, structural integrity and as regulators of protein functions. Oxidative protein folding is confined to few compartments like the endoplasmic reticulum, the mitochondrial intermembrane space and the bacterial periplasm. In all compartments the machineries follow similar functional principles: An oxidoreductase oxidizes reduced cysteine motifs in its substrates, thereby becoming itself reduced. A dedicated thiol oxidase then mediates reoxidation. Electrons are subsequently transferred onto a final electron acceptor like oxygen.[1,2]

In chloroplasts, disulfide bond formation likely occurs in the thylakoid lumen by a rarely studied protein named lumen thiol oxidase 1 (LTO1), consisting of a soluble LTO1-DsbA/Trx-like domain fused to a membrane-embedded LTO1-VKOR domain, homologous to the catalytic subunit of mammalian vitamin K1 epoxide reductase (VKOR). Mammalian VKOR is an integral membrane protein in the ER, involved in the recycling of vitamin K1 (phylloquinone), an essential cofactor for γ-carboxylation of certain glutamate residues.[3,4] In silico analyzes revealed eight conserved cysteines in Chlamydomonas reinhardtii (Cr) LTO1, four in the CrLTO1-DsbA domain and four in the CrLTO1-VKOR domain. We present here the biochemical and cell biological characterization of CrLTO1, and its physiological role in Chlamydomonas reinhardtii.

[1]Kojer and Riemer, 2014; [2]Hermann et al., 2009; [3]Karmaoko et al., 2011; [4]Li et al., 2010

Authors: Lena Murschall, Jan Riemer

Minimal Machinery Required for Unconventional Secretion of Fibroblast Growth Factor 2

Presenting author: Walter Nickel

Heidelberg University Biochemistry Center, Im Neuenheimer Feld 328, 69120, Heidelberg
walter.nickel@bzh.uni-heidelberg.de

Fibroblast Growth Factor 2 (FGF2) is a direct mediator of tumour cell survival inhibiting apoptosis by an autocrine secretion-signalling loop. Despite being localized to the extracellular space, FGF2 lacks a signal peptide and was shown to be secreted by an unconventional, ER/Golgi-independent pathway. The molecular mechanism of FGF2 secretion was proposed to be mediated by direct translocation across the plasma membrane and a number of cis-elements and trans-acting factors required for this process were identified. Here, we define the minimal molecular machinery required for FGF2 membrane translocation in a fully reconstituted system using purified components. Our findings establish a novel type of self-sustained protein translocation across membranes that is the molecular basis for unconventional secretion of FGF2 from tumour cells.

Authors: Walter Nickel

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tRNAs and proteins use the same import channel for translocation across the mitochondrial outer membrane of trypanosomes

Presenting author: Moritz Niemann

University of Bern, Dept. Chemistry and Biochemistry, Freiestrasse 3, 3012, Bern
niemann@dcb.unibe.ch

Mitochondrial tRNA import is widespread, but the mechanism by which tRNAs are imported remains largely unknown. The mitochondrion of the parasitic protozoan Trypanosoma brucei lacks tRNA genes and thus imports all tRNAs from the cytosol. Here we show that in T. brucei in vivo import of tRNAs requires four subunits of the mitochondrial outer membrane protein translocase but not the two receptor subunits, one of which is essential for protein import. The latter shows that it is possible to uncouple mitochondrial tRNA import from protein import. Ablation of the intermembrane space domain of the translocase subunit, ATOM14, on the other hand while not affecting the architecture of the translocase, impedes both protein and tRNA import. A protein import intermediate arrested in the translocation channel prevents both protein and tRNA import. In the presence of tRNA, blocking events of single channel currents through the pore formed by recombinant ATOM40 were detected in electrophysiological recordings. These results indicate that both types of macromolecules use the same import channel across the outer membrane. However, while tRNA import depends on the core subunits of the protein import translocase, it does not require the protein import receptors indicating that the two processes are not mechanistically linked.

Authors: Moritz Niemann

The behavior of mitochondria

Presenting author: Jodi Nunnari

University of California Davis, Molecular and Cellular Biology, One Shields Avenue, 149 Briggs Hall, 95616, Davis
jmnunnari@ucdavis.edu

Mitochondria are double membrane-bounded organelles that perform a myriad of diverse and essential functions in cells. These functions are dependent on the collective intracellular behavior of the organelle. We have characterized key features of mitochondrial behaviors. Our work has addressed the physiological functions and mechanisms of mitochondrial division and fusion, which are important determinants of overall mitochondrial shape and distribution. We have characterized contact sites that intimately link mitochondria with the ER and determined their roles in mitochondrial positioning and dynamics. We have also addressed the fundamental question of how mitochondrial membranes are sub-compartmentalized to reveal how the complex internal architecture of the organelle is generated. Our current challenge is to determine how mitochondrial behaviors are integrated with one another and physiologically regulated within cells and organisms.

Authors: Jodi Nunnari

Nuclear bromodomains regulate amyloid-like protein aggregation upon aberrant lysine acetylation

Presenting author: Heidi Olzscha

University of Oxford, Department of Oncology, Roosevelt Drive, OX3 7DQ, Oxford
heidi.olzscha@oncology.ox.ac.uk

Lysine acetylation is becoming increasingly recognized as a general biological principle which underpins many different aspects of cellular homeostasis, and is subject to aberrant control in different human pathologies. Lysine acetylation is regulated through the concerted action of acetyltransferases and deacetylases, where bromodomain-containing proteins read the acetylation mark and effect the functional consequences. Here, we describe amyloid-like protein aggregation in cells that result from aberrant lysine acetylation. The protein aggregation disturbs proteostasis by causing abnormalities in the ubiquitin proteasome system (UPS) and protein translation, resulting in decreased cell viability. Nuclear bromodomain proteins are involved in forming the aggregates and, using a chemical biology-led approach to probe their composition, we establish p300/CBP proteins as necessary to form the aggregates. Further, small molecule inhibitors of bromodomain recognition impede aggregate formation, which coincides with enhanced UPS function and increased cell viability. Significantly, amyloid-like protein aggregates formed by a pathologically relevant form of huntingtin protein, causally involved with Huntington's disease, are similarly susceptible to intervention by bromodomain inhibition. Since the aggregates are susceptible to small molecule intervention, our results have implications for treating proteinopathies with bromodomain inhibitors.

Authors: Heidi Olzscha, Oleg Fedorov, Benedikt M. Kessler, Stefan Knapp, Nicholas B. La Thangue

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Chloroplast development: Role of MV-1 and MV2 proteins

Presenting author: Manali Patil

LMU-Munich, Dept. Biologie I, Großhadernerstr. 2-4, 82152, Planegg-Martinsried
manalikp88@gmail.com

Chloroplast development is a complex process. It involves differentiation of mature chloroplast with extensive thylakoid membrane network from immature proplastids that contain very little internal membrane structure. Though microscopic studies suggest that tubules and vesicles emerging from chloroplast inner envelop contribute to thylakoid biogenesis, the molecular mechanisms involved in this transition remain elusive. The objective of this study is to identify and characterize the players involved in chloroplast development.
Using several approaches, we identified MV-1 and MV-2 as potential candidate proteins that might play a role during chloroplast development. The two homologs of MV-1 protein (MV-1A and MV-1B) and MV-2 were found to be localized in the chloroplast using GFP localization and in-vitro import of radio labeled protein into chloroplast. Mv-1 plants are retarded in growth with crumpled leaves. MV-2 knock-out plants have pale green leaves with delayed flowering. Ultra structural analysis of chloroplasts revealed altered thylakoid morphology. This was corroborated with reduced amount of photosystem II super-complexes and slightly higher photoinhibition observed in mv-2 mutants. Further characterization of MV-1 and MV-2 proteins is being done so as to elucidate their exact role in chloroplast development.

Authors: Manali Patil, Serena Schwenkert, Jürgen Soll

Haploinsufficient loss of Endosomal Sorting Complex Required for Transport (ESCRT) components as new drivers of cancer

Presenting author: Arnim Pause

McGill University, Biochemistry, Goodman Cancer Research Centre, 3655 Sir William Osler Promenade, H3G1Y6, Montreal
arnim.pause@mcgill.ca

Receptors located at the surface of the cell integrate the extracellular environment towards
cellular functions such as cell survival, proliferation and migration. The Endosomal Sorting Complexes Required for Transport (ESCRT) efficiently controls cell surface receptor levels and turnover. Impairment in ESCRT function leads to receptor stabilization, persistent signalling, and potentially cancer promotion. We recently established a potent haploinsufficient TSG function of the ESCRT member, HD-PTP/PTPN23. Hemizygous deletion of HD-PTP predisposes mice to spontaneous lung adenoma, B-cell lymphoma and promotes oncogene-driven lymphoma onset and dissemination. Importantly, HD-PTP is frequently deleted in many human tumours including lung and lymphoma, which correlated with poor survival. HD-PTP depletion affects every step of tumorigenesis, including tumour initiation, progression, dissemination and modulation of the tumour microenvironment. Interestingly, we have shown that UBAP1 and Endofin, two novel ESCRT components and HD-PTP binding partners, share HD-PTP functions. Therefore, we hypothesize that HD-PTP and its binding partners exert tumour suppressor functions. Moreover, since ESCRTs play a central role in a wide range of signalling pathways activated downstream of numerous cell surface receptors this pathway might have significant implications in the development of new therapeutic strategies that would be applicable to a wide range of cancers.

Authors: Arnim Pause

Role of astacin-like proteinases in wound healing and scarring with regard to collagen VII maturation

Presenting author: Florian Peters

University of Kiel, Biochemical Institute, Otto-Hahn-Platz 9, 24118, Kiel
fpeters@biochem.uni-kiel.de

Physiological wound healing is a highly regulated process including the expression and activity of proteases. A dysregulation could lead to enhanced extracellular matrix generation and fibrotic diseases as well as chronic wounds. Meprin α, meprin β and BMP-1 are members of the astacin family of metalloproteases and expressed in different compartments of the skin. Dysregulation of meprins is associated with increased collagen deposition in several fibrotic conditions. Aim of this study is to investigate the roles of astacin-like proteases in wound healing and scarring.
For epidermal effects, the HaCaT keratinocyte cell line was used for initial in vitro studies. Treatment with TGFβ1, an important cytokine for cell proliferation and differentiation, resulted in upregulation of all three astacins on mRNA level. Immunofluorescence staining of scratched cells showed meprins and BMP-1 localization at the border of migrating cells indicating a role for ECM modulation at the ‘wounded’ area. Interestingly, reduced ‘wound closure’ was observed when using meprin inhibitors as well as recombinant active proteases revealing a tight regulation of proteases’ activities during epidermal regeneration. In vivo wound healing of meprin α and meprin β knockout mice and inhibition studies are under investigation to ascertain their role in physiological wound healing to develop new therapeutic strategies in disorders.

Authors: Florian Peters, Christoph Becker-Pauly

A versatile method to monitor lipid flux in vivo

Presenting author: Carmelina Petrungaro

ETH Zürich, Biology, Otto-Stern-Weg 3, 8093, Zurich
carmelina.petrungaro@gmail.com

Every organelle is surrounded by membranes composed of an organelle-specific set of lipids. This determines the biophysical properties of the membrane and thereby also the organellar function. The main site of lipid synthesis is the endoplasmic reticulum (ER) from where lipids are transported to different organelles. This transport is either vesicle or protein-mediated. However, the relative contribution of these pathways for specific organelles and if this contribution is possibly altered in response to cellular stress or disease is only poorly understood. One reason is the lack of experimental procedures to monitor lipid fluxes in vivo. Here, we present a method to analyze lipid fluxes between organelles based on an enzymatic lipid modification in an donor organelle and the mass spectrometric detection of these modifications in the acceptor organelle. This allows to dissect the network of lipid fluxes between organelles and enables to investigate potential re-routing of lipid fluxes in response to cellular

stress. This can not only be performed on a cellular level but can also be applied on entire organisms.

Authors: Carmelina Petrungaro, Benoît Kornmann

Role for formin-like 1-dependent acto-myosin assembly in lipid droplet dynamics and lipid storage

Presenting author: Simon Pfisterer

University of Helsinki, Anatomy, Haartmaninkatu 8, 00290, Helsinki
simon.pfisterer@helsinki.fi

Lipid droplets (LDs) are cellular organelles specialized in triacylglycerol storage undergoing homotypic clustering and fusion. In non-adipocytic cells with numerous LDs this is balanced by poorly understood droplet dissociation mechanisms. We identify non-muscle myosin IIa (NMIIa/MYH-9) and formin-like 1 (FMNL1) in the LD proteome. NMIIa and actin filaments concentrate around LDs, and form transient foci between dissociating LDs. NMIIa depletion results in decreased LD dissociations, enlarged LDs, decreased hydrolysis and increased storage of triacylglycerols. FMNL1 is required for actin assembly on LDs in vitro and for NMIIa recruitment to LDs in cells. We propose a novel actomyosin structure regulating lipid storage: FMNL1-dependent assembly of myosin II-functionalized actin filaments on LDs facilitates their dissociation, thereby affecting LD surface-to-volume ratio and enzyme accessibility to triacylglycerols. In neutrophilic leukocytes from MYH9-related disease patients NMIIa inclusions are accompanied by increased lipid storage in droplets suggesting that NMIIa dysfunction may contribute to lipid imbalance in man.

Authors: Simon Pfisterer, Gergana Gateva, Peter Horvath, Juho Pirhonen, Veijo Salo, Leena Karhinen, Markku Varjosalo, Samppa Ryhännen, Pekka Lappalainen, Elina Ikonen

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Protein transport across and into the mitochondrial outer membrane: Regulation by phosphorylation

Presenting author: Daniel Poveda Huertes

Institut für Biochemie und Molekularbiologie, Biochemestry, Stefan-Meier-Straße 17, 79104, Freiburg
danipoveda91@gmail.com

The mitochondrial protein import machinery has been considered for long time as static and constitutively active. However, we have recently shown that phosphorylation of the TOM-complex, which serves as central protein entry gate into the organelle, upon different metabolic conditions or cell cycle phases can regulate mitochondrial biogenesis. Upon profiling of highly purified mitochondrial outer membranes for further phosphorylation sites as regulatory elements we identified > 130 different P-sites within > 50 outer membrane proteins. This includes P-sites in proteins from the SAM pathway and the ERMES complex that may have also a role in mitochondrial biogenesis upon different cellular conditions.

Authors: Daniel Poveda Huertes, Daniel Poveda Huertes, Corvin Walter, Despina Mikripoulou, Oliver Schmidt, Angelika Harbauer, Sanjana Rao, Magdalena Opalinska, Carolin Gerbeth, Albert Sickmann, René Zahedi, Nikolaus Pfanner, Chris Meisinger

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Mitochondria-lysosome crosstalk is a key pathological mechanism relaying mitochondrial and lysosomal stress

Presenting author: Nuno Raimundo

University Medical Center Göttingen, Cellular Biochemistry, Humboldtallee 23, room 01.423, 37073, Goettingen
nuno.raimundo@med.uni-goettingen.de

Mitochondria and lysosomes were long considered as independent entities in the cell. Recently, evidence was reported that defects in one of these organelles result in perturbations of the other. The mechanisms underlying the mitochondria-lysosome crosstalk remain unclear. We use mouse models of mitochondrial and lysosomal diseases to explore these mechanisms.
Acute inhibition of the mitochondrial respiratory chain in wild-type MEF results in the activation of lysosomal biogenesis, dependent on AMPK and on the transcription factors TFEB and MITF. However, in chronic mitochondrial deficiency (prolonged treatments, mouse models, patient fibroblasts) lysosomal biogenesis is severely repressed. Thus, while acute mitochondrial stress activates lysosomal biogenesis, chronic mitochondrial stress represses it. Furthermore, lysosomal function is strongly impaired under chronic mitochondrial deficiency, due to a dysfunction in lysosomal Ca^{2+} homeostasis. When this dysfunction is corrected the lysosomal function is rescued.
Reciprocally, mitochondria are also impaired in lysosomal storage diseases, by unresolved mechanisms. We identified a "lysosomal stress signal" and the mechanism by which it impacts mitochondria. Ablation of that signal rescues mitochondrial function, while its introduction in normal

cells recapitulates the effects caused by lysosomal dysfunction on mitochondria.
The cross-talk between mitochondria and lysosomes is key to understand the role of organellar signaling.

Authors: Lorena Fernandez Mosquera, King Faisal Yambire, Nuno Raimundo

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The murine cytomegalovirus immunoevasin m152/gp40 uses the p24 family of transmembrane proteins to retain MHC class I molecules in the early secretory pathway

Presenting author: Venkat Raman Ramnarayan

Jacobs University Bremen, Life Science and Chemistry, Campus ring 1, 28759, Bremen
ramnarayan.venkat.raman@gmail.com

The murine cytomegalovirus (MCMV) immunoevasin m152/gp40 retains fully folded and assembled major histocompatibility complex (MHC) class I molecules in the early secretory pathway and prevents peptide presentation to CD8+ T cells. gp40 also subverts natural killer cell activation by retaining RAE-1, a ligand of the NKG2D receptor, inside the cell. We show that a 43 amino acid sequence of gp40, which connects the lumenal and the transmembrane domains of this protein, is required to retain gp40 in the ER/Golgi. We show here that the linker interacts with TMED10 (a.k.a Tmp21, mp24δ1), a member of the p24 family of ER/Golgi transmembrane proteins. Presence, and ER/Golgi retention, of TMED10 itself is required for gp40 to retain class I in the early secretory pathway. Surprisingly, the downregulation of RAE-1 is independent of both the gp40 linker and TMED10. Our data reveal the first viral client of the p24 family, and support a chaperone-like role of the p24 proteins.

Authors: Venkat Raman Ramnarayan, Linda Janssen, Zeynep Hein, Swapnil Ghanwat, Natalia Lis, Sebastian Springer

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Crosstalk between MICOS and the ATP synthase

Presenting author: Heike Rampelt

University of Freiburg, Institute of Biochemistry and Molecular Biology, ZBMZ, Stefan-Meier-Str. 17, 79104, Freiburg
heike.rampelt@biochemie.uni-freiburg.de

Mitochondria are essential organelles whose complex membrane morphology is crucial for their role in oxidative phosphorylation. The inner mitochondrial membrane is compartmentalized into the flat inner boundary membrane and invaginations called cristae. They are the site of respiration, and their morphology, which is dynamically regulated by the metabolic state, supports the optimal functioning of the respiratory chain. Cristae are connected to the inner boundary membrane by crista junctions that constitute a diffusion barrier between the two membrane compartments. Several protein complexes of the inner membrane are involved in shaping cristae. The conserved MICOS complex (mitochondrial contact site and cristae organizing system) is required for the maintenance of stable crista junctions and anchors the inner membrane to the outer mitochondrial membrane. Mic10, one of its two core components, forms the oligomeric membrane-shaping scaffold of MICOS. Generation of positive curvature, such as in cristae tubules and rims, requires the F1Fo-ATP synthase or complex V of the respiratory chain. Its dimers and oligomers are intrinsically tilted and thereby impose curvature on cristae membranes. Thus, both MICOS and F1Fo-ATP synthase play crucial roles in cristae biogenesis. We have discovered that Mic10 interacts with dimeric F1Fo-ATP synthase, providing the basis for a functional crosstalk between the two machineries that may coordinate distinct aspects of cristae formation.

Authors: Heike Rampelt, Maria Bohnert, Ralf M. Zerbes, Nikolaus Pfanner, Martin van der Laan

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Exploring MARS, a collection of Mutants Affecting chloroplast-to-nucleus Retrograde Signaling

Presenting author: Silvia Ramundo

UCSF, Biochemistry and Biophysics, 600, 16TH ST, N316 3rd floor, the Walter Lab, 94110, San Francisco
silvia@walterlab.ucsf.edu

Chloroplasts are plant cell organelles specialized in harvesting light energy via photosynthesis. Although chloroplasts have their own genome, the large majority of chloroplast proteins are transcribed from nuclear genes and imported post-translationally. Thus, the nucleus controls the majority of chloroplast processes and must rely on chloroplast signals that monitor the status of the organelle. Recently, we discovered a chloroplast-to-nucleus signaling pathway in Chlamydomonas reinhardtii, a unicellular green algae, which allows to sense and respond to perturbations in chloroplast protein homeostasis (1, 2). In this work, the ClpP protease, which degrades damaged chloroplast proteins, was depleted. The cellular changes that ensued, including protein aggregation, induction of

chaperones, proteases, and proteins involved in lipid trafficking and membrane homeostasis, emerged as telltale signs of an unfolded protein response (UPR). To identify factors that mediate this signal transduction, we carried out a genetic screen that identified mutants that are either i) defective in inducing the cpUPR (silencing mutants) or ii) constitutively in a cpUPR induced-state (activating mutants). I will report on MARS1, an uncharacterized protein with a predicted kinase domain, which potentially acts as signal transmitter of this pathway.

Authors: Silvia Ramundo, Karina Perlaza, Mable Lam, Martin Jonikas, Peter Walter

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Synthesis of fluorescent iron oxide nanoparticles for cellular uptake studies

Presenting author: Wiebke Rastedt

University of Bremen, AG Dringen, Leobener Strasse, 28359, Bremen
wiebke.rastedt@uni-bremen.de

To investigate the cellular uptake and the intracellular trafficking of iron oxide nanoparticles (IONPs), we have synthesized fluorescent IONPs by using as coating material dimercaptosuccinate (DMSA) which had been covalently modified with the fluorescence dyes BODIPY (BP), Oregon Green (OG) or Tetramethylrhodamine (TMR). Dispersed in physiological media, the synthesized IONPs did not differ in hydrodynamic diameter, ζ-potential and colloidal stability compared to non-fluorescent IONPs. Also the accumulation of the IONPs by C6 glioma cells was not affected by the additional functionalization. The cellular iron accumulation during exposure of C6 cells to IONPs at 37°C was lowered by around 30%, if the cells were incubated with IONPs at 4°C, and by 85%, if the cells had been incubated in the presence of serum. Fluorescence microscopy of the cells exposed to fluorescent IONPs confirmed these results and revealed a perinuclear doted fluorescence staining of the cells after exposure to fluorescent IONPs. These data demonstrate that C6 cells efficiently accumulate DMSA-coated IONPs in a time- and concentration-dependent manner by a mechanism which is strongly affected by the temperature and the presence of serum. The newly synthesized fluorescent D-IONPs appear to be suitable to investigate the cellular uptake mechanism and the intracellular trafficking of D-IONPs by fluorescence microscopy.

Authors: Wiebke Rastedt, Karsten Thiel, Ralf Dringen

Mitophagy is dynamically regulated by an interplay of ubiquitylation and deubiquitylation in Saccharomyces cerevisiae

Presenting author: Andreas Reichert

Heinrich-Heine-Universität Düsseldorf, Institute of Biochemistry and Molecular Biology I, Universitätsstraße 1, 40225, Düsseldorf
reichert@hhu.de

Mitophagy, the selective degradation of mitochondria, is an evolutionarily conserved process. There is cumulating evidence that this process is linked to numerous human disorders as the removal of damaged mitochondria is impaired. This autophagic process can be mechanistically divided into two principal ways: a receptor -mediated and an ubiquitin- mediated pathway. Mutations in genes such as PINK1 or PARKIN that are essential for the ubiquitin-mediated pathway in mammalian cells are known to cause Parkinson's disease. Recent research suggest that an ubiquitin-mediated mitophagy pathway may also exist in baker’s yeast. We addressed the role of all three non-essential ubiquitin genes (UBI1, UBI2, UBI4) in different types of selective autophagy in Saccharomyces cerevisiae. We show that mitophagy depends on the only polyubiquitin encoding gene, UBI4, but not on UBI1 or UBI2. It further depends on K48-linkage of ubiquitin. Induction of mitophagy led to a marked alteration of the overall ubiquitylation of mitochondrial proteins which was dependent on Ubp3. We identified an outer membrane protein as a novel player required for mitophagy which depended on its ubiquitylation. In summary, we identified a mitophagy pathway in yeast which depends on mitochondrial protein ubiquitylation and is dynamically regulated by UBI4-dependent ubiquitylation and Ubp3-dependent deubiquitylation.

Authors: Christina Behrendt, Andreas Reichert

Coupling of protein kinase maturation to the interaction dynamics of chaperone complexes

Presenting author: Klaus Richter

TU München, Chemie, Lichtenbergstr. 4, 85748, Garching
klaus.richter@tum.de

Hsp90 is a highly conserved molecular machine, which performs ATP-driven movements to support conformational changes in its client proteins, including protein kinases and several transcription factors. More than 20 diverse cofactors define the substrate spectrum of this

machine and it is of importance to understand how these cofactors regulate the movements and the ATP-dependency to adjust this machine to its highly diverse clients.

We here investigate the interaction with the cofactor Cdc37, which is the protein kinase-specific cofactor of Hsp90 and highly conserved from metazoan to humans. Using fluorescently tagged Cdc37 and analytical ultracentrifugation we study the complex affinity and the hydrodynamic properties of this 200kDa Hsp90-Cdc37 complex. We realize that several interaction sites exist on Hsp90 and that Cdc37 slows down the closing reaction of the two subunits of Hsp90. We further find that the protein kinase interacts with Cdc37 alone prior to being bound in a ternary complex and we find that these complexes are sensitive to ATP-binding to the kinase. Mass spectrometric identification of peptides close to the interaction sites between Cdc37 and sB-Raf and between Cdc37 and Hsp90 help to define the complex formation properties. Using specifically-tagged proteins we thus develop ways to biochemically characterize this important chaperone complex, which is known to be a powerful modulator of cancerous growth, when the Raf-protein kinase is mutated in the cell.

Authors: Klaus Richter, Siyuan Sima, Julia Eckl

Clathrin adaptors, coat evolution, and endocytosis

Presenting author: Margaret S. Robinson

University of Cambridge, CIMR, Hills Road, CB2 0XY, Cambridge
msr12@cam.ac.uk

Eukaryotic cells use vesicles to transport macromolecules from one membrane compartment to another. The formation of these vesicles and the selection of the right cargo requires coat proteins. Clathrin coats, which are probably the best understood, consist of two major components: clathrin itself and heterotetrameric adaptor protein (AP) complexes. Part of the COPI coat is distantly related to the APs, and there are two additional AP complexes identified in the 1990s by sequence-based searches, AP-3 and AP-4. More recently, we have identified a fifth AP complex and a more distantly related coat, TSET. All of these coats must have been present in the last eukaryotic common ancestor, because they are found throughout the diversity of eukaryotes. Other coat components are more recent innovations. For instance, PTB domain-containing adaptors have so far been found only in metazoans. We are currently investigating whether PTB domain adaptors may also be present in other holozoans, in particular Capsaspora owczarzaki. These studies may help to shed light on the co-evolution of endocytosis and signalling in animals.

Authors: Margaret S. Robinson

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deMorphogenesis of the stalked budding bacterium Hyphomonas neptunium

Presenting author: Sabine Rosskopf

Philipps-Universität Marburg, Microbiology, Hans-Meerwein-Str. 4, 35043, Marburg
rosskopf@students.uni-marburg.de

The spatial and temporal regulation of peptidoglycan biosynthesis and its role in cell morphology has been studied intensively in well-characterized model organisms such as Escherichia coli, or Caulobacter crescentus, which divide either by symmetric or asymmetric binary fission. To broaden our knowledge of the mechanisms governing bacterial morphogenesis, we have started to investigate the dimorphic marine alphaproteobacterium Hyphomonas neptunium as a new model organism. This species is characterized by a unique mode of proliferation, that is budding.

The main focus of our studies was the identification of cell wall biosynthetic enzymes and regulatory factors enzymes that are critically involved in stalk and bud biogenesis. To this end, we comprehensively analyzed the localization patterns of all the proteins with cell wall-related functions encoded in the H. neptunium genome. Moreover, we probed the essentiality of these candidate proteins by deletion analysis. Furthermore, we could show that peptidoglycan hydrolases play a prominent role in cell division. Most likely not all have a function in active cleavage but are involved in the regulation of other proteins. These results for the first time provide insight into the mechanisms of morphogenesis in stalked budding bacteria, thus setting the stage for an in-depth analysis of the regulatory mechanisms that control the spatiotemporal dynamics of the peptidoglycan biosynthetic machinery in these organisms.

Junior GBM Poster

Authors: Sabine Rosskopf, Emöke Cserti,
Martin Thanbichler

Post-transcriptional regulation of mitochondrial function

Presenting author: Elena Rugarli

University of Cologne, Joseph-Stelzmann Str. 26, 50931, Köln
elena.rugarli@uni-koeln.de

Mitochondria contain more than 1,500 proteins, most of which are encoded in the nucleus, translated in the cytosol, and subsequently imported into the mitochondrion. It is still poorly understood whether expression of mitochondrial proteins in mammals is regulated post-transcriptionally, by controlling stability, translation rate or localization of their transcripts. CLUH is an evolutionary conserved protein, the deletion of which leads to mitochondrial clustering next to the nucleus in several species. Recently, we demonstrated that CLUH is an RNA-binding protein that binds specifically a subset of mRNAs encoding mitochondrial proteins. I will present novel findings on how CLUH coordinates the expression of a mitochondrial protein network, which supports reprogramming of mitochondrial metabolism under different conditions.

Authors: Elena Rugarli

Functionalizing the unannotated mitochondrial proteome

Presenting author: Jared Rutter

University of Utah/HHMI, 15 N. Medical Dr. E, 84108, Salt Lake City
rutter@biochem.utah.edu

Mitochondria are dynamic and complex organelles that play a central role in all aspects of biology, including energy production, intermediary metabolism, and apoptosis. These broad cellular functions also place mitochondria as a central player in human health. Mitochondrial dysfunction is associated with a wide range of diseases, including cancer, type 2 diabetes, and most neurodegenerative disorders. As a result of these wide-ranging critical activities, many efforts have focused on identifying and characterizing the mitochondrial proteome, with over 1,000 proteins identified to date in mammals. Remarkably, however, roughly one-quarter of these proteins remain essentially uncharacterized. These include many proteins that are highly conserved throughout eukarya, a strong indication that they perform a fundamentally important function. The overall goal in this research is to provide a new understanding of the biochemical and cellular function of each conserved uncharacterized mitochondrial protein, determine how they contribute to normal mitochondrial activity and human disease. Our studies of a handful of these uncharacterized mitochondrial conserved proteins have revealed new roles for these proteins in critical aspects of mitochondrial function, including mitochondrial protein quality control, lipid synthesis and mitochondrial ETC complex and supercomplex assembly.

Authors: Jared Rutter

Unraveling the role of p1, a yeast AMID homolog, as a key regulator of apoptosis in Saccharomyces cerevisiae

Presenting author: Sreedivya Saladi

University of Kaiserslautern, cell biology, Erwin-Schrodinger str. 13, 67663, kaiserslautern
saladisreedivya1@gmail.com

Apoptosis is a highly regulated programmed cell death essential for development and tissue homeostasis of multicellular organisms. It is a genetically controlled and highly conserved process even occurring in unicellular organisms such as baker's yeast. Due to its conserved apoptotic core machinery, yeast is a convenient model organism to understand human pathologies such as cancer, aging and neurodegeneration. Mitochondria harbour factors like AIF or yeast AMID that upon cellular stress turn into proapoptotic agents and execute cell death. The closest homologs of yeast AMID are p1 and p2. In yeast, the molecular mechanism of AMID in apoptosis is well studied. However, the mechanism and functional significance of its other two homologs in regulating apoptosis are poorly understood. In an unbiased screen employing mass spectrometry based proteomics we identified p1 as the mitochondrial protein with highest turnover. Our initial findings show that the deletion of p1 makes yeast resistant to treatment with proapoptotic compounds such as H2O2 and acetic acid. Moreover, p1 mutant show considerably reduced levels of cytosolic H2O2. Interestingly, under apoptosis-inducing conditions degradation of p1 is heavily reduced indicating that stabilization of p1 may trigger apoptosis. Together, our findings suggest that p1 serves as a novel proapoptotic mitochondrial protein which apparently is tightly controlled by mitochondrial proteases.

Authors: Sreedivya Saladi, Felix Boos, Michael Poglitsch, Frank Madeo, Johannes Herrmann

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Characterization of extracellular vesicles purified from HIV-1 Nef overexpressing HEK293 cell supernatants

Presenting author: Julia Sanwald

Forschungszentrum Jülich, ICS-6, Wilhelm-Johnen-Str. 1, 52428, Jülich
jsanwald1@gmx.de

HIV-1 protein Nef provokes many pathogenic effects during AIDS progression and induces extensive (unconventional) secretion activities. Distribution of Nef via extracellular vesicles (EVs) is regarded as crucially pathogenesis-relevant, yet knowledge about the secretion machinery is insufficient.
We demonstrate that Nef secretion depends on the human GABARAPs, a protein family involved in vesicle transport and autophagosome formation. All GABARAPs exhibit direct Nef interaction for which W13 of Nef is essential. Here, we characterize EV pools obtained from cells expressing Nef(WT), the GABARAPs-binding deficient variant Nef(W13A) or another secretion relevant variant, Nef(SMR5A). EV count and size distribution were determined by nanoparticle tracking analysis. Consistent with Nef's function as secretion inducer, an increase in vesicle count was observed. By FM1-43 staining we found extensive, Nef containing bleb-like membrane patches in Nef(WT) expressing cells. Nef(W13A) expressing cells produced smaller vesicles that possibly passed the plasma membrane in a more scattered manner, hinting towards the existence of more than one secretion pathway. As we found the GABARAP-binding deficient Nef(W13A) in EVs, too, obviously not each Nef secretion path depends on the direct Nef-GABARAP interaction. Separation of EV subpopulations specific for the different Nef variant expressing cells by size or density was not feasible. Proxitome based techniques may help to overcome this issue.

Authors: Julia Sanwald, Alexandra Boeske, Andreas Weber, Payam Akhyari, Silke Hoffmann, Dieter Willbold

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Analysis of S-palmitoylation in U937 cells

Presenting author: Vinzenz Särchen

Kiel University, Institut of Immunology, Michaelisstraße 5, 24105, Kiel
vinzenz.saerchen@uksh.de

Protein fatty acylation is an emerging field in post-translational modification. The major form of fatty acylation (74%) is the reversible S-palmitoylation. Its reversibility has regulatory functions and mediate various processes such as membrane association of peripheral proteins, targeting to membrane subdomains, protein stability and protein and vesicle trafficking. DHHC palmitoyltransferases catalyze the attachment of palmitate to a cysteine residue. Thioesterases cause the depalmitoylation of proteins. Disruption of both types of enzymes is associated with human disorders, i.e. Huntington's disease, infantile neuronal ceroid lipofuscinosis and various types of cancer. The cytokine Tumor Necrosis Factor (TNF) has pleiotropic effects on cells ranging from induction of inflammation and cell proliferation to cell death. We here describe a palmitoylom analysis of the monoblastoid cell line U937. We established the acyl-RAC (resin assisted capture) method and analyzed changes in the palmitoylation pattern upon TNF stimulation. Palmitoylated proteins of untreated and TNF-treated wild type cells were enriched. The robustness and purity of the method was verified, followed by a mass spectrometric analysis. Roughly 600 palmitoylated proteins were identified. Among those, new previously undescribed proteins could be identified as being palmitoylated, i.e. Cathepsin G, Multivesicular body subunit 12, and TRAF2.

Authors: Vinzenz Särchen, Timo Glatter, Philipp Zingler, Stefan Schütze, Jürgen Fritsch

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Cytosolic protein aggregates interfere with the biogenesis of mitochondria

Presenting author: Anna Schlagowski

TU Kaiserslautern, Cell Biology, Erwin-Schrödinger-Str. 13, 67663, Kaiserslautern
schlagow@rhrk.uni-kl.de

Mitochondrial biogenesis depends on the translation of hundreds of precursor proteins in the cytosol which have to be targeted to mitochondria and threaded through the TOM and TIM23 translocases of the organelle. In my work I use the accumulation of mitochondrial precursor proteins in the cytosol of yeast cells as a marker for the functionality of the mitochondrial import process. To investigate the connection between protein aggregation in the cytosol and mitochondrial biogenesis, I examine mitochondrial import in the presence of cytosolic aggregates as they appear in neurodegenerative diseases such as Huntington's disease or amyotrophic lateral sclerosis. Applying an in vivo reporter assay which monitors the accumulation of mitochondrial precursor proteins in the cytosol by a growth test, I was able to show that in the presence of protein aggregates, mitochondrial precursor proteins accumulate in the cytosol. Furthermore, the expression of protein aggregates shows a strongly

increased toxicity in mutants of mitochondrial import components. These observations indicate that cytosolic protein aggregates negatively interfere with the posttranslational targeting of proteins into mitochondria which might contribute to their inherent toxicity. At present we try to understand which mitochondrial precursor proteins are particularly vulnerable and to identify the specific steps in their biogenesis that are affected by the cytosolic aggregates.

Authors: Anna Schlagowski, Ralf Braun,
Johannes Herrmann

A membrane fusion protein inside chloroplasts and cyanobacteria

Presenting author: Dirk Schneider

Johannes Gutenberg-University Mainz, Pharmacy and Biochemistry, Johann-Joachim-Becher-Weg 30, 55128, Mainz
dirk.schneider@uni-mainz.de

Thylakoid membranes are unique cellular membranes in cyanobacteria and chloroplasts. However, despite their apparent importance, remarkably little is known about the biogenesis, dynamics and maintenance of thylakoids. The "inner membrane-associated protein of 30 kDa" (IM30), also known as the "vesicle inducing protein in plastids 1" (Vipp1), was known to be involved in the biogenesis of the thylakoid membrane system, although the exact physiological function of IM30 remained mystic for a long time. We have identified IM30 as being a thylakoid membrane fusion protein, which turns out to be a key player for thylakoid membrane formation, dynamics and maintenance in chloroplasts and cyanobacteria (Hennig, R., et al. (2015), Nat Commun. 6, 7018). While IM30 monomers interact and spontaneously assemble into large oligomeric ring complexes, we could show that ring formation is not crucial for, but even counteracts membrane interaction of IM30 (Heidrich J. et al. (2016) JBC 291, 14954ff). Membrane interaction of IM30 rings is mediated by different protein domains, with the C-terminal domain being of special importance (Hennig R. et al., (2017) BBA 1858, 126ff).
In summary, we propose that IM30 establishes contacts between internal membranes and promotes membrane fusion to enable regulated exchange of proteins and/or lipids in cyanobacteria and chloroplasts.

Authors: Jennifer Heidrich, Raoul Hennig, Benedikt Junglas, Michael Saur, Jürgen Markl, Dirk Schneider

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The diversity of iron-sulfur cluster containing dehydratases

Presenting author: Monika Schneider

Heinrich-Lorenz-str. 2a, 67487, St. Martin
mo_schne@rhrk.uni-kl.de

Various dehydratase (hydrolyase) enzymes employ an iron-sulfur cluster to stereospecifically remove water from a broad range of hydroxyacid compounds. For aconitase it is known that a [4Fe-4S]2+ cluster coordinated by three cysteine residues acts as catalytic center. In other dehydratase families, e.g. serine dehydratase and fumarate hydratase, a [4Fe-4S]2+ cluster has also been crystallographically demonstrated. Astonishingly, for dihydroxyacid dehydratases some members contain a [2Fe-2S]2+ cluster. The key enzyme of the Entner-Doudoroff-pathway (6-phosphogluconate dehydratase) has a [4Fe-4S]2+ cluster, whereas the dihydroxyacid dehydratase IlvD (part of the valin, leucin and isoleucin synthesis) contains a [2Fe-2S]2+ as cofactor.
The application of phylogenetic analysis has been used to give a first idea about the distribution of the cluster and enzyme types in this class of dehydratases. We anaerobically purified several dihydroxyacid dehydratases and characterized enzyme kinetics. UV-Vis, Mössbauer and EPR spectroscopy allowed us to identify the cluster type. Indeed some highly active IlvD enzymes have a [2Fe-2S]2+ cluster, but most dihydroxyacid dehydratases have a [4Fe-4S]2+ cluster. This poster gives an overview of our data on the different enzymes.

Authors: Monika Schneider, Thérèse Pambe, Ingo Kouker, Dominique F. Bechtel, Hendrik Auerbach, Christina Müller, Volker Schünemann, Antonio J. Pierik

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Mitochondrial biogenesis in trypanosomes - Expect the unexpected

Presenting author: André Schneider

University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, 3012, Bern
andre.schneider@dcb.unibe.ch

Trypanosomes have emerged as a novel system to study mitochondrial biogenesis that is essentially unrelated to yeast and mammals. Recently we have characterized the protein complexes in the outer and the inner membrane that mediate protein import. They are very different from the TOM and TIM complexes of yeast and mammals. The trypanosomal TOM complex analogue, termed ATOM

complex, contains only two proteins that show homology to yeast and mammalian Tom40 and Tom22, respectively. All other ATOM subunits, including two protein import receptors, are unique to trypanosomatids. For the integral membrane subunits of the trypanosomal TIM complex the situation is even more extreme. While yeast and mammals have two TIM complexes that are specialized for different substrates. Trypanosomes contain a single TIM complex only. Moreover, only one of its subunits shows homology to Tim subunits of yeast and mammals. - The inheritance of the single unit mitochondrial DNA of trypanosomes is also unusual. It is physically linked, across both membranes, to the basal body of the flagellum in the cytosol. This linkage is termed tripartite attachment complex (TAC) and mediates the segregation of the replicated mitochondrial genomes. Recently we have characterized an OM protein complex, that is essential for TAC function. It contains two alpha-helically anchored membrane proteins as well as two beta-barrel proteins. The biogenesis pathway of this membrane complex is currently being investigated.

Authors: André Schneider

Stress responses in chloroplasts

Presenting author: Michael Schroda

TU Kaiserslautern, FB Biologie, Molecular Biotechnology & Systems Biology, Erwin-Schrödinger-Strasse 70, 67663, Kaiserslautern
schroda@bio.uni-kl.de

In contrast to the cytosol, the ER and mitochondria of eukaryotic cells, relatively little is known about how molecular chaperones maintain protein homeostasis in the chloroplast. We used the unicellular green alga Chlamydomonas reinhardtii as a model organism and the chloroplast-targeted small heat shock proteins HSP22E/F as an entry point to shed more light on this issue. We found that HSP22E/F accumulate rapidly and strongly in cells exposed to heat stress. This response depends on the sole heat shock transcription factor (HSF1) present in this alga. By IP-MS we could identify 37 high-confidence proteins that interact with HSP22E/F in heat stressed cells, among them Rubisco activase. The idea will be discussed that Rubisco activase and other HSP22E/F substrates are thermolabile on purpose to serve as switches allowing cells to rapidly acclimate to heat stress. HSP22E/F accumulate with much slower kinetics than upon heat stress also in cells exposed to high light intensities, which can be mimicked by treating cells with hydrogen peroxide. This response is independent of HSF1 and is also observed in mutants with defects in processes associated with thylakoid membranes. Our data suggest that HSP22E/F expression is regulated by at least two signaling pathways and that HSP22E/F play roles in maintaining protein homeostasis under heat stress and under conditions leading to thylakoid membrane stress.

Authors: Michael Schroda

Transversing boundaries - contact sites between organelles

Presenting author: Maya Schuldiner

Weizmann Institute of Science, Molecular Genetics, Herzl 234, 7610001, Rehovot
maya.schuldiner@weizmann.ac.il

The flow of solutes and information within the eukaryotic cell requires inter-organelle communication. Membrane contact sites between two organelles can facilitate both signaling and the passage of ions and lipids from one cellular compartment to another. To uncover the extent of contact site formation between the cellular organelles we created a split fluorescence sensor of contact sites in yeast. In our sensor, one part of a fluorophore is fused to the outer membrane of each organelle in one mating type while the other is fused to the outer surface of the membrane of all organelles in the other mating type. Yeast can then be mated in all possible pairwise combinations and a fluorescent signal reports on the formation of a contact site between two organelles. We extensively validated that the sensor reports on bona fide contact sites and have discovered novel contact sites. I will discuss how these are now being used in high content screens to uncover novel tethers and regulatory molecules as well as the physiological roles of the contacts.

Authors: Maya Schuldiner

Overexpression of Mid51 leads to alteration of mitochondrial morphology and impairment of glucose-stimulated insulin secretion in Beta cells

Presenting author: Julia Schultz

Institute of Biochemistry, University of Rostock,
Schillingallee 70, 18057, Rostock
julia.schultz@med.uni-rostock.de

Mitochondria form a cell-type specific dynamic network that continuously cycle between fusion and fission events. Fission is initiated by the fission protein 1 (Fis1), but only the dynamin-related protein 1 (Drp1) is able to finally complete the mitochondrial division. Drp1 cannot directly interact with Fis1, but recent studies indicate that Mid51 may work as a receptor and initiate mitochondrial fission in mammalian cells. We have previously shown that alterations in the expression of Fis1 and Drp1 affect glucose-stimulated insulin secretion in pancreatic beta cells. Therefore, the aim of this study was to investigate changes in the mitochondrial network structure and cellular function after overexpression of Mid51 in pancreatic beta cells. We found that the mitochondrial membrane potential was significantly reduced in cells overexpressing Mid51. A homogenous mitochondrial network was observed in mouse pancreatic beta cells. Overexpression of Mid51 resulted in fragmentation of mitochondria with cluster formation. Mid51 overexpression resulted in significantly reduced glucose-stimulated insulin secretion in Beta cells compared to control transfected cells. Our results suggest that Mid51 is important for maintaining a proper glucose-stimulated insulin secretion in pancreatic Beta cells. Because the enhanced expression of Mid51 evoked a strong change in mitochondrial morphology, we propose the importance of Mid51 to maintain mitochondrial dynamics and function in beta cells.

Authors: Julia Schultz, Rica Waterstradt, Simone Baltrusch

Phosphorylation in mitochondrial import

Presenting author: Serena Schwenkert

LMU München, Biology, Plant Sciences, Großhadernerstr. 2-4, 82152, Planegg-Martinsried
serena.schwenkert@lmu.de

Mitochondrial localized proteins are mostly synthesized in the cytosol and translocated across the outer mitochondrial membrane via the TOM complex. Although the channel protein is conserved among eukaryotes, the receptor proteins are more divergent and show features specific in the plant lineage as compared to other organisms. OM64, which is a paralogue of the chloroplast docking protein Toc64 is unique to plants. Due to the presence of a cytosolic exposed TPR domain it could functionally replace Tom70, which is not found in plant mitochondria, by interacting with the C-terminal (M)EEVD motif of HSP90 and HSP70. In this study we revealed that OM64 is phosphorylated within its TPR domain on a single serine residue. Using isothermal titration calorimetry it could be demonstrated that phosphorylation reduces the binding affinity of OM64 to HSP90 and the crystal structure has been obtained in complex with the C-terminal HSP90 peptide. Moreover, in vivo expression of OM64 variants in planta could show that phosphorylation of OM64 also impairs the import efficiency of mitochondrial preproteins. In summary, our data provide significant insight into the fine-tuning mechanisms of mitochondrial protein import mediated by phosphorylation via cytosolic kinases.

Authors: Serena Schwenkert

TOM complex-mediated mitochondrial import of Helicobacter pylori VacA toxin causes loss of mitochondrial membrane potential

Presenting author: Jan Schwichtenberg

Ruhr-Universität Bochum, Institut für Biochemie und Pathobiochemie - Abteilung für Zellbiochemie, Universitätstraße 150, 44801, Bochum
jan.schwichtenberg@rub.de

The gram negative bacterium Helicobacter pylori colonizes the mucus layer of human stomach, causing a wide range of gastro-intestinal diseases, such as peptic ulceration, chronic gastritis, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma. The vacuolating cytotoxin A (VacA) is its major secreted toxin [1]. VacA consists of two segments termed p33 and p55. The toxic subunit p33 is able to target mitochondria and to assemble in homohexameric oligomers that act as an anion channel in the mitochondrial inner membrane. Mitochondrial targeting is mediated by an unusual N-terminal presequence, that consists of 32 completely uncharged aminoacids [2,3]. However, the toxin's mitochondrial import mechanism remains enigmatic.
Here, we report that p33 uses the TOM-complex as entry gate into mitochondria. Targeting depends on the three outer membrane receptors Tom20, Tom22 and Tom70. The translocation across the outer membrane is facilitated by internal targeting signals. Insertion of VacA into the inner

membrane entails loss of the mitochondrial membrane potential in a adenocaricoma cellline.

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[2]A. Galmiche et al., EMBO J 2000, 19, 6361-6370.
[3] G. Domanska et al., PLoS Pathog 2010, 6(4).

Authors: Jan Schwichtenberg, Joachim Rassow

Tic110: still the main channel of protein translocation into chloroplasts

Presenting author: Inga Sjuts

Großhaderner Str. 2-4, 82152, Planegg-Martinsried
inga.sjuts@lmu.de

Chloroplasts originated from an endosymbiotic event during which most of the genetic information was transferred to the host cell nucleus. As a consequence, proteins that are destined for the chloroplast have to be imported posttranslationally from the cytosol into the organelle. During import, proteins have to overcome the two barriers of the chloroplastic outer and inner envelope which is facilitated by two distinct multiprotein complexes, designated TOC and TIC.
Whereas the components of the TOC complexes are quite well characterized, much less is known about the TIC apparatus. We are interested in the most abundant TIC component Tic110 which is essential for protein import into plastids. Tic110 has been proposed to be a channel-forming protein, consisting of two N-terminal transmembrane anchors and further four amphiphatic helices that are involved in channel formation. Very recently, the plastid encoded YCF1 was found to be a part of a 1MDa-complex that included Tic20, but neither Tic40 nor Tic110. However, since two out of three lineages of three archaeplastidal lineages whose import apparatus evolved from the same ancestral TOC/TIC as that of chloroplastida lack the YCF1 gene prompted us to speculate about an alternative role of Ycf1.

Authors: Inga Sjuts, Bettina Bölter, Jürgen Soll

Lysosomal pathology by altered chloride transport

Presenting author: Tobias Stauber

Freie Universität Berlin, Institute for Chemistry and Biochemistry, Thielallee 63, 14195, Berlin
tobias.stauber@fu-berlin.de

Lysosomal ion homeostasis is key to the trafficking and function of these organelles. Loss of the Cl- transporter ClC-7 leads to a neurodegenerative lysosomal storage disease and osteopetrosis in mice and humans. We found it involves slowed protein degradation and morphological alterations of lysosomes. Our identification of sorting motifs in ClC-7 allowed cell surface expression and the electrophysiological characterization of ClC-7. This revealed that ClC-7, together with its β-subunit Ostm1, mediates slowly voltage-gated Cl-/H+ exchange. Our knock-in mouse model expressing a purely Cl- conducting ClC-7 mutant demonstrated the physiological importance of this Cl-/H+ exchange. Instead of providing the counterion conductance for lysosomal acidification, ClC-7/Ostm1 rather mediates the pH gradient-driven luminal accumulation of Cl- and changes of the transmembrane voltage –hence of organellar ion homeostasis in general. Surprisingly, we found that some disease-causing ClC-7 mutations do not lead to a loss of function, but accelerate the activation kinetics of ClC-7/Ostm1. We have now generated a mouse model with an 'accelerated' ClC-7. Despite the normal expression and localization of this mutant, the mice are diseased. Expression of another ClC-7 mutant with altered biophysical features results in a drastic enlargement of lysosomes. This mutant and our novel mouse model serve to elucidate the role of slowly activated Cl-/H- exchange in lysosomal ion homeostasis and function.

Authors: Antje Buttgereit, Florian Ullrich, Wing Lee Chan, Uwe Kornak, Thomas Jentsch, Tobias Stauber

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Menadione Reduction in Cultured Brain Astrocytes is Mediated by the Cytosolic NAD(P)H: Quinone Acceptor Oxidoreductase 1 (NQO1)

Presenting author: Johann Steinmeier

University of Bremen, Leobener Strasse / NW 2, 28359, Bremen
johann.steinmeier@uni-bremen.de

Menadione (2-methyl-1,4-naphtoquinone, vitamin K3) is a synthetic derivate of vitamin K1 and an excellent redox cycler. Astrocytes are known to contain a highly efficient

enzymatic antioxidant defence system, which protects them as well as neighbouring cells against adverse effects of oxidants. Cultured primary astrocytes are capable to reduce menadione at an impressive rate. To test whether an enzyme-catalysed process is involved in the menadione reduction we investigated cell lysates of astrocytes and found a menadione reduction that increased with lysate volume and was prevented by heat inactivation (5 min, 90 °C) or filtration (size exclusion: 5 kDa). Determination of the kinetic parameters of the menadione reduction by astrocyte lysates for menadione revealed a Km of 11 ± 4 µM and a Vmax of 213 ± 27 nmol/(min*mg). Suitable electron donors to facilitate menadione reduction were both NADH and NADPH with similar Km- and Vmax-values. After digitonin lysis of astrocyte cultures and subsequent separation of the cytosolic and mitochondrial fractions, the menadione reduction capacity of astrocytes was localised almost exclusively to the cytosol. In addition, the menadione reduction activity of astrocyte lysates was extremely sensitive to the NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1) inhibitor dicoumarol (1.2 ± 0.3 nM). These findings are consistent with and strongly suggest that the cytosolic NQO1 is the enzyme responsible for the reduction of menadione in astrocytes.

Authors: Johann Steinmeier, Eric Ehrke, Ralf Dringen

Mitochondrial genome maintenance and inheritance in yeast

Presenting author: Maria Stenger

University of Bayreuth, Cell Biology, Universitätsstraße 30, 95440, Bayreuth
maria.stenger@uni-bayreuth.de

Mitochondria are semi-autonomous organelles, which contain their own genome. The mitochondrial DNA (mtDNA) exists in several copies per cell, which are arranged in protein containing complexes called nucleoids. The mitochondrial genome in budding yeast encodes 8 mitochondrial proteins, two rRNAs and several tRNAs. All of these genes are required for respiratory chain activity. Therefore, cells without mtDNA cannot grow on non-fermentable carbon sources, like glycerol (YPG), and are called rho^0. When the cells possess wildtype mtDNA they are named rho^+ and when they have nonfunctional mtDNA they are termed rho^-. mtDNA needs to be properly replicated and distributed for its faithful maintenance during a cell's life cycle. So far only few proteins, like the nucleoid protein Abf2 or the DNA polymerase Mip1, have been identified to participate in this process. Several proteins and mechanisms involved in the inheritance of the mitochondrial genome are still unknown. To identify additional proteins, we performed several high-throughput, genome-wide screens. We used the yeast deletion library, which contains about 4,800 strains with deletions of non-essential genes. As a result, we found several genes of unknown molecular function, which could be involved in the inheritance of the mitochondrial DNA. Ongoing experiments address their precise role and whether non-genetic factors, such as growth conditions and extracellular stress, affect maintenance of mtDNA in the mutants.

Authors: Maria Stenger, Benedikt Westermann

From Peroxisomes to Translational Readthrough (with a return ticket)

Presenting author: Sven Thoms

University Medical Center Göttingen, Department of Pediatrics and Adolescent Medicine
sven.thoms@med.uni-goettingen.de

Translation terminates when a stop codon on the mRNA is located in the ribosomal A site. A release factor enters the A site, recognizes the stop codon and initiates release of the newly synthesized protein and dissociation of the ribosome. Due to the natural competition between release factors and near-cognate tRNAs, the stop codon can occasionally be interpreted as a sense codon. In such a case, translation continues until the ribosome encounters the next stop codon, creating an extension to the protein. This process is termed translational readthrough and normally occurs at a frequency of 0.1% or one or two orders of magnitude lower. Using a bioinformatics approach, we identified the first human genes that are controlled by a much higher rate of readthrough (up to 10%) and that possess a functional peroxisomal targeting signal in their readthrough extension. These genes are LDHB and MDH1, encoding the H subunit of lactate dehydrogenase, and malate dehydrogenase, respectively. Between one and five per cent of these proteins are imported into peroxisomes, controlled by functional translational readthrough.

The unusual targeting mechanism of LDH and MDH1 suggests a solution to an old question, that is how peroxisomes recycle their redox equivalents and transport them to the mitochondria. We hypothesize the existence of a peroxisomal lactate/pyruvate and a malate/aspartate shuttle. These intracellular metabolite shuttles mirror the well-characterized metabolite shuttles across the mitochondrial membrane and complement the Cori cycle.

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Authors: Sven Thoms

Using single molecule imaging to study cellular signaling

Presenting author: Maximilian Ulbrich

Universitätsklinikum Freiburg, Internal Medicine IV, Schänzlestr. 18, 79104, Freiburg
max.ulbrich@bioss.uni-freiburg.de

Signal processing through receptors at the plasma membrane is a complex choreography of many interacting proteins. With single molecule imaging of fluorescently tagged proteins it became possible to investigate this process with high temporal and spatial resolution. Lateral interactions of the membrane proteins and recruitment of soluble proteins can only be resolved if nearly complete labeling with the fluorescent marker is achieved.
Our goal is to decipher the molecular events at the plasma membrane during the initial phase of signaling of the ErbB family receptor tyrosine kinases with the epidermal growth factor receptor EGFR as the most prominent member. These receptors are thought to dimerize upon ligand binding to recruit adaptor proteins, and internalize after binding to clathrin coated pits. By quantitative imaging of the receptors and related proteins, we try to understand the temporal and spatial order of these events and in how far they depend on each other.

Authors: Maximilian Ulbrich

Unraveling the mechanism of ER-associated organelle fission

Presenting author: Gia Voeltz

University of Colorado, Boulder, MCD Biology, 347 UCB, 80309, Boulder
gia.voeltz@colorado.edu

ER membrane contact sites (MCSs) define the position where early and late endosomes undergo constriction and fission. ER tubules are recruited to the divide between cargo sorting domains on endosomes that will then constrict and undergo fission. Our aim was to purify ER factors that regulate MCS formation and fission in animal cells. Based on the identity of the candidate proteins, we hope to be able to test hypotheses about the mechanisms used by the ER to drive organelle constriction and division at MCSs. Our strategy to identify MCS proteins was to target a promiscuous biotin ligase (BirA) to ER-associated fission sites. BirA has a modification reach to approximately 30nm, which is ideal for bridging membrane contacts (which have a typical spacer distance of ~5-15nm). Our hybrid construct localizes as predicted and biotinylates ER proteins at MCSs in mammalian cells that we could purify and then identify by mass spec. This strategy has identified ER proteins that regulate ER-associated endosome fission.

Authors: Melissa Phillips, Patrick Chitwood, Gia Voeltz

Mitochondrial calcium in plants

Presenting author: Stephan Wagner

Universität Bonn, INRES - Plant Energy Biology, Friedrich-Ebert-Allee 144, 53113, Bonn
stephan.wagner@uni-bonn.de

Mitochondria are the major cellular source of ATP in most complex life. Respiratory ATP provision needs to match cellular needs and requires tight regulation. Metabolic flexibility is particularly important in plants where respiration needs to be coordinated with photosynthesis. The mechanisms by which respiration is controlled in plants are, however, poorly understood. In mammals, matrix calcium regulates energy metabolism by modulating the activity of key TCA cycle dehydrogenases. The recently identified mitochondrial calcium uniporter mediates import of calcium into the matrix. Plants possess gene homologues of the uniporter but their function is yet unclear. We used GFP-fusions to localize uniporter homologues within plant cells. In conjunction, we worked

with genetically encoded Cameleon sensors in to assess calcium levels and dynamics in living plants by confocal microscopy. We have found uniporter components within Arabidopsis mitochondria and knock-out mutants show altered mitochondrial calcium levels, indicating impaired calcium transport. The presence of a functional uniporter suggests a yet unknown role of calcium in matching the organelle’s function with cellular demands. We now seek to understand the role of calcium in the dynamic control of plant mitochondrial function and will discuss the significance of mitochondrial calcium transport for plant performance.

Authors: Stephan Wagner, Markus Schwarzländer, Philippe Fuchs, Alex Costa

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Charting the Mitochondrial Importome

Presenting author: Bettina Warscheid

University of Freiburg, Faculty of Biology, Biochemistry and Functional Proteomics, Schänzlestr. 1, 79104, Freiburg
bettina.warscheid@biologie.uni-freiburg.de

The majority of eukaryotic proteins are encoded in the nuclear genome and synthesized in the cytosol. To reach their final destination, these proteins need to follow different import pathways into various organelles. The resulting protein inventories define the molecular identity and the specific functions of different organelles in eukaryotic cells. However, methodology to examine whether a protein is transported into an organelle is largely limited to the study of single proteins. This renders our current knowledge about imported proteins incomplete, limiting our understanding of the biogenesis and functions of organelles.
In this work, we report a method that allows for charting an organelle’s importome. Our method relies on inducible RNAi-mediated knockdown of an essential import factor to impair import and quantitative high-resolution mass spectrometry. We demonstrate its high potential by delineating the mitochondrial importome of Trypanosoma brucei, comprising 1,120 proteins with more than 300 novel constituents. Importantly, our new ImportOmics method allows for the exact localization of proteins with dual or multiple locations. To highlight ImportOmics’ specificity and versatility, we targeted different import factors and globally identified the substrates of different protein import pathways into mitochondria.

Authors: Bettina Warscheid, Christian Peikert, Jan Mani, Marcel Morgenstern, Sandro Käser, Bettina Knapp, Christoph Wenger, Anke Harsmann, Silke Oeljeklaus, André Schneider

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The MAT hypothesis of plastid origin through endosymbiosis

Presenting author: Andreas Weber

Heinrich Heine Universität Düsseldorf, Institute of Plant Biochemistry, Universitätsstrasse 1, 40225, Düsseldorf
aweber@hhu.de

Plastids are the defining cellular organelles of photosynthetic eukaryotes, such as plants and algae. Plastids evolved approx. 1.5 billion years ago from free-living cyanobacteria through a primary endosymbiosis. As organelles of primary endosymbiotic origin, they are surrounded by a double membrane envelope, which plays a crucial role in defining plastid identity by delineating it from its surroundings and thereby maintaining its status as a semi-autonomous organelle. As to how the cyanobacterial endosymbiont entered into and evaded digestion by the host cell is not yet understood and to date cannot be experimentally reproduced in the laboratory. Based on phylogenetic and biochemical evidence, the ménage-à-trois hypothesis (MATH) posits that an initial tripartite interaction between a eukaryotic host cell, a cyanobacterium, and a Chlamydia-like intracellular pathogenic bacterium provided the toolkit for the establishment of endosymbiosis. The MATH suggests that inclusion of the emergent endosymbiont in the Chlamydiales parasitophorous vacuole enabled invasion and provided shelter from host defence and, in addition, the Chlamydiales provided critical genes for the integration of the endosymbiont into the hosts metabolic network. The MATH underlines the importance of chlamydial host-pathogen interactions in the metabolic integration of the primary plastid.

Authors: Andreas Weber

Regulation of MICOS activity during mitochondrial cristae remodelling

Presenting author: Florian Wollweber

Saarland University Medical Center, Medical Biochemistry and Molecular Biology, 66421 Homburg
florianwollweber@gmail.com

Mitochondria show a remarkable structural complexity. The mitochondrial inner membrane forms tubular or disc-shaped protrusions, termed cristae, which harbour the OXPHOS machinery for ATP synthesis and are crucial for

many essential mitochondrial functions in energy metabolism and cell-fate decisions. One of the main organisers of cristae structure is the mitochondrial contact site and cristae organising system (MICOS).
MICOS stabilises the membrane curvature at cristae junctions and creates a central hub for mitochondrial biogenesis. Recent studies found that it can be divided into two subcomplexes with distinct functions in membrane-bridging and membrane-shaping. As cristae are highly dynamic structures that have to adapt to cellular demands, the regulation of MICOS activity should play a pivotal role. We are currently investigating the influence of the regulatory subunits Mic19 (a redox regulated component), as well as Mic26 and Mic27 (lipid binding subunits) on modulating and coordinating MICOS activities. In addition, our goal is to understand the role of MICOS-dependent cristae remodelling during cellular adaptation to e.g. altered metabolic demands, since our preliminary observations indicate that MICOS enables efficient transitions between metabolic states.

Authors: Florian Wollweber, Martin van der Laan

Subcellular compartmentalization of TNF receptor-1 (TNF-R1) signaling determines the biological fate of the cell

Presenting author: Philipp Zingler

Kiel University, Institute of Immunology, Michaelisstr. 5, 24105, Kiel
Philipp.Zingler@uksh.de

The activation of many receptors triggers the accelerated endocytosis of ligand-receptor complexes. It is now well established, that endocytic vesicles are important sites for organizing the recruitment of specific components for selective signaling cascades. Our recent findings gave novel insights into the physiological role of TNF-R1 internalization and endosomal trafficking, that lead either to apoptosis or survival of the cell.
We recently identified TNF-induced K63 ubiquitination of TNF-R1, mediated by the ubiquitin ligase RNF8 as an early molecular checkpoint in regulating the decision between cell death and survival. Down-modulation of RNF8 prevented the ubiquitination of TNF-R1, blocked the internalization of the receptor, prevented the recruitment of the "DISC", the activation of caspase-8 and caspase-3/7 and reduced apoptotic cell death. Conversely, recruitment of the adaptor proteins TRADD, TRAF2 and RIP1 to TNF-R1 as well as activation of NF-kB was unimpeded and cell growth and proliferation was enhanced in RNF8 deficient cells.
For the isolation of receptosomes and intracellular organelles we developed a free-flow magnetic separation system with a high gradient up to 3 Tesla permanent magnetic field. This system allows the selective purification of biological materials from cellular lysates after target-specific immunomagnetic labelling, in particular morphological and functional intact endosomes containing activated receptors and receptor-associated proteins.

Authors: Philipp Zingler, Vladimir Tchikov, Jürgen Fritsch, Stefan Schütze

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