FRIAS Research Focus for the Academic Year 2015/16

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2. Abstract

Membrantransport im Alter und in Krankheit

Alter führt zu einer progressiven Verschlechterung von Geweben und Organen. Dies beeinträchtigt Körperfunktionen, erhöht die Anfälligkeit für Infektionen, und führt letzten Endes zum Tod. Daher wird Altern als ein Krankheitsschlüsselfaktor angesehen. In den alternden westlichen Gesellschaften ist die Verbesserung der Gesundheit im Alter von enormer sozio-ökonomischer Bedeutung.

In der Zellbiologie sind Membranen von essentieller Bedeutung. Sie unterteilen Zellen in funktionale Einheiten und ermöglichen Initiation und Weiterleitung von Signalprozessen. Daher ist es nicht erstaunlich, dass die Deregulierung von Membrantransportprozessen immer mehr als ein Schlüsselereignis im Altern und in Krankheit angesehen wird. Mit Hilfe unterschiedlicher Modelsysteme, molekularbiologischer und proteinbiochemischer Ansätze, und ‚Omics' Methoden untersuchen wir gestörte, krankheitsrelevante Membrantransportprozesse. Ziel ist es, zugrundeliegende biologische Mechanismen zu verstehen und neue Therapieansätze zu entwickeln, die ein gesundes Altern ermöglichen.

Membrane Trafficking in Ageing and Disease

Increasing age causes progressive deterioration of tissues and organs, leading to impaired tissue function, increased organismal vulnerability to infection, and death. Hence ageing is recognized as a prime disease factor. Improving health in the elderly will be crucial to deal with the enormous socio-economic challenges arising as a consequence of increased life expectancy.

Membranes are at the center of cellular biology, compartmentalizing cells into functional distinct sub-compartments and constituting scaffolds for signal initiation and propagation. Hence, it is not surprising that deregulated membrane trafficking emerges also as key processes in ageing and disease. By combining model organisms, such as *C.elegans* and mouse, with mammalian cell culture, advanced molecular biology and protein biochemistry, and 'omics' approaches we aim at characterizing deregulated membrane trafficking in ageing and disease. This will allow a functional understanding of underlying biological processes which can be employed to design strategies promoting healthy ageing.

3. Research proposal and work plan

State-of-the-art

Ageing is the most common risk factor for prevalent diseases in western societies like cardiovascular diseases, neurodegenerative diseases and cancer (Niccoli and Partridge, 2012). In addition, in age increased numbers of infection and impairment of the immune system can be observed. Thus, it is estimated that approximately half of a persons' health care expenses accrue in her/his senior years, being the last third of a persons' lifetime (Alemayehu and Warner, 2004). A common feature of numerous age-related disorders is accumulation of biomolecules and organelles indicating a deregulation of intracellular membrane trafficking and homeostasis (Ravikumar et al., 2010). Membrane trafficking processes such as autophagy take a center stage as they are not only necessary to for survival under stress conditions but also strongly influence lifespan (Niccoli and Partridge, 2012). Autophagy is a catabolic, cellular turnover pathway in which cytoplasm is shuttled by autophagosomes, newly formed double membrane vesicles, to lysosomes for degradation. As such, autophagy is still regarded as unspecific bulk degradation pathway. However, specific autophagy subtypes, such as the selective degradation of mitochondria by mitophagy, have been described. Damaged mitochondria, i.e. the lack of their efficient removal, have also been causatively linked to age-related disorders such Parkinson's disease (Wohlgemuth et al., 2014). Autophagic processes also play a major role during apoptosis and are required for the efficient removal of dying or aberrant cells, which when defective can lead to neurodegenerative diseases and cancer (Thorburn et al., 2014).

Perturbations in cellular membrane trafficking and autophagy cannot only be linked to age-related diseases, but are a common phenomenon in various disease settings. Whereas accumulations of vesicles and organelles are readily observed underlying molecular mechanisms are only understood rudimentary. By a combination of the model systems mouse, *C. elegans*, and mammalian cell culture, and studies of age-related decline in kidney function and mitochondrial homeostasis we aim at characterizing causative molecular mechanism for the observed phenotypes. For this, we will combine electron microscopy, mass spectrometry-based proteomics, genetics and protein biochemistry. The use of bacterial toxins which activate/inhibit Rho proteins, regulators of vesicular transport, give us a unique tool at hand which allows specific perturbation of single reaction steps.

Own preliminary work

Rho GTPases are molecular “on-off” switches and master regulators of the cytoskeleton and actin dynamics. They effect numerous cellular processes, including vesicle trafficking (Heasman and Ridley, 2008). The **Aktories** group studies bacterial protein toxins that target Rho proteins. The protein toxins are pivotal pathogenetic factors, which activate or inactivate the GTPases in a highly specific manner (Aktories, 2011). Therefore, the toxins can be used as extremely potent pharmacological tools to study Rho-dependent cellular functions. The group characterized the structure-function relationship of these toxins, discovered novel types of Rho modification by toxins and analyzed the functional consequences of toxins’ actions (Jank et al., 2013; Lang et al., 2010). The group of Jörn **Dengjel** employs mass spectrometry-based proteomics approaches to study the plasticity and dynamics of the autophagosome. They could show that classical stress induced autophagy is not an entirely unspecific bulk degradation process. The subcellular localization of proteins influences their degradation by autophagy (Kristensen et al., 2008). Cytosolic proteins are degraded rapidly in contrast to organellar proteins which are spared from degradation. This is also mirrored by the protein composition of the autophagosome. Stress stimuli as well as timing influence the organellar proteome reflecting the specific cellular needs (Dengjel et al., 2012). Recently, they could show that autophagy is blocked in primary skin fibroblasts in an age dependent manner (Dumit et al., 2014). The **Eimer** lab uses the nematode *Caenorhabditis elegans* as a model system to study the regulation of intracellular membrane trafficking events mainly in neurons. One main focus is the mechanisms how vesicular transport carriers such a synaptic vesicles (SV) and dense core vesicles (DCV) are generated, trafficked within the cell and subsequently released. A particular interest is how these processes are coordinated and regulated by the Rab family of small GTPases (Hannemann et al., 2012; Sasidharan et al., 2012; Sumakovic et al., 2009). The second focus of the Eimer lab are the questions how neuronal trafficking processes change during ageing and how loss of genes involved in trafficking can lead to neurodegeneration. In particular, the Eimer group is interested to understand how alterations in intracellular trafficking trough loss-of-function of Parkinson’s disease genes influences mitochondrial function and integrity (Kamp et al., 2010; Karpinar et al., 2009), largely studied by using high resolution and electron microscopy techniques (Kittelmann et al., 2013a, 2013b). The team of Tobias B. **Huber** utilizes a translational research program involving model organisms, transgenic mouse models, high throughput screenings, systems biology and high resolution imaging

approaches to elucidate kidney signaling programs in disease and aging. Specifically, this work identified signaling programs that regulate renal epithelial cell survival, endocytosis, cytoskeletal organization and polarity, providing novel insight of how specialized renal cells contribute to kidney diseases. Recently, Huber's team established a role of MTOR and autophagy in progressive kidney disease and kidney aging (Canaud et al., 2013; Gödel et al., 2011; Hartleben et al., 2010; Liu et al., 2012). These studies have broad clinical implications and imply novel therapeutic strategies.

Aims

Combining interdisciplinary backgrounds we aim at deciphering causative molecular mechanisms being deregulated in ageing and disease and involved in perturbed membrane trafficking focusing on autophagy. In **Aim 1** (Aktories) we will study the trafficking of bacterial toxins and will use these toxins as tools to decipher the influence of the cytoskeleton and Rho GTPases on autophagosomal vesicle trafficking. **Aim 2** (Dengjel) addresses the crosstalk of retrograde Golgi/ER vesicle and autophagosome trafficking by proteomics approaches and its deregulation in ageing. The role of SCYL1, a member of the Scy1-like family of catalytically inactive protein kinases, will be the focus. **Aim 3** (Eimer) employs the nematode *Caenorhabditis elegans* to study the function of Rab GTPases in mitophagy. Using electron microscopy the function of the Rab GTPase GLO-1 will be investigated. In **Aim 4** (Huber) the autophagosomal-mitochondrial crosstalk is studied in kidney ageing using mouse genetics, optical markers and proteomics. Hence, the proposal is characterized by a high degree of interdisciplinarity and interdependency. Molecular targets identified in the specific aims will be investigated by the other researchers on their functions in the respective model systems. Thus, by using discrete biological and technical approaches we will comprehensively study vesicular trafficking mechanisms and their regulation in physiology and pathology.

Work program

Detailed work programs are part of the respective fellowship applications.

Aim 1 (Aktories): *Role of membrane dynamics, intracellular trafficking and autophagosomal processes in cytotoxicity of Rho-modifying bacterial protein toxins*

Rho proteins are essential GTP-binding proteins, which act as molecular switches in numerous cellular functions. *Clostridium difficile* toxins A (TcdA) and B (TcdB) and *Escherichia coli* Cytotoxic Necrotizing Factor (CNF) 1, which are crucial pathogenicity

factors in colitis and urinary tract infections, inactivate and activate Rho proteins by glucosylation and deamidation, respectively. This alters Rho-dependent signaling and results in disease. To elucidate the precise mode of action of the toxins and to develop new therapeutic strategies, we aim to analyze the cellular toxin trafficking and clarify the role of autophagy in cytotoxicity. Using immunofluorescence with specific marker proteins, we will investigate membrane dynamics, vesicle transport of toxins and toxin translocation into the cytosol. shRNA knock downs and dominant negative and positive mutants of Rho proteins will be employed to delineate the specific functions of Rho, Rac and Cdc42 isoforms in autophagy and to elucidate the functional consequences of their modification by toxins.

Aim 2 (Dengjel): *Analysis of autophagosome Golgi/ER crosstalk*

Autophagosomes are constitutively formed *de novo* in cells and induced under stress conditions and shuttle cytoplasm to lysosomes for degradation. In recent years it has been emerging that membranes from diverse sources are recruited to the nascent autophagosome to form a double membrane pre-autophagosomal structure (PAS). The function of SCYL1, a protein involved in COPI-mediated retrograde Golgi-ER transport and a negative regulator of autophagy, will be studied in primary human cells from donors of different ages. Constitutive and autophagy-dependent protein-protein interactions will be analyzed by affinity purification mass spectrometry (AP-MS). Newly identified interaction partners will be analyzed by shRNA mediated knock downs on their role in autophagy.

Aim 3 (Eimer): *Regulation of mitochondrial integrity by Rab GTPase mediated control of mitophagy*

Small GTPases of the Rab family are master-regulators of intracellular trafficking and sorting events between different membrane domains. In addition to their control of multiple steps during vesicular membrane transport, Rab GTPases also provide identity to intracellular membrane structures and organelles, facilitating directed transport. Through genetic screens in the nematode *Caenorhabditis elegans* we have identified the Rab GTPase GLO-1, which is involved in trafficking to lysosome related organelles, to be required for mitochondrial integrity and function. The role of GLO-1 in mitophagy will be analyzed by electron microscopy and its function in mitochondrial dynamics will be studied. Dowstream effectors will be analyzed by AP-MS.

Aim 4 (Huber): *Autophagy signaling and autophagosmal-mitochondrial crosstalk in kidney ageing*

The kidney glomerulus represents a prime target of age-related tissue damage, which is reflected by the close association of the decline of glomerular filtration with age. Key event in the progressive decline of glomerular function is the loss of highly specialized podocytes that form the outer part of the filtration unit. Interestingly, our previous results indicate that glomerular aging is associated with specific alteration of autophagy and mitophagy. We now aim to uncover the precise role of auto/mitophagy for aging using a complementary set of transgenic mouse cells, synthetic mitochondrion-specific optical markers that allow the 3/4D image analysis of mitophagosomes, and AP-MS to study mitophagy-associated protein complexes and underlying protein dynamics.

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