

### Lectures

#### L16.1

##### Small heat shock proteins in protein aggregation and disaggregation

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Many factors leading to unfolding and misfolding of proteins eventually result in protein aggregation. Stress imposed by high temperature was one of the first aggregation-inducing factors studied, and remains one of the main models in this field. The cell needs chaperone proteins to control and counteract the aggregation process. Elimination of aggregates can be achieved by solubilization of aggregates and either refolding of the liberated polypeptides or their proteolysis. Here we focus on the molecular mechanisms by which small Hsps chaperones and chaperones from Hsp100 family in cooperation with Hsp70 and Hsp40 chaperones liberate and refold polypeptides trapped in protein aggregates.

It has been proposed that small Hsps associate with aggregating polypeptides, thus changing their biochemical properties so that the subsequent Hsp100-Hsp70 disaggregation process becomes much more efficient. Two members of the small Hsp family, IbpA and IbpB, are present in *Escherichia coli*. Their cooperation is crucial for prevention of irreversible aggregation of proteins. We investigated the mechanisms by which these chaperones influence the aggregation process. We concentrated on the importance of the N- and C-terminal regions of IbpA for self-oligomerization and chaperone functions. Our results show that the defect in chaperone function, observed in truncated versions of IbpA, is due to the inability of these proteins to interact with substrate proteins and consequently to change the properties of aggregates.

The subsequent step in disaggregation and reactivation of polypeptides trapped in aggregates depends on the activities of ATP-dependent Hsp100-Hsp70 chaperones. This process requires the substrate threading through the central channel of hexameric Hsp100 chaperone. At that point the small heat shock proteins start to play an inhibitory role in the process. Our results suggest that Hsp70 chaperone system is responsible for the release of small heat shock proteins at the initial step of disaggregation reaction.

#### L16.2

##### Regulation of mitochondrial protein homeostasis

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Mitochondria play an important role in variety of metabolic and regulatory processes and their dysfunction leads to life threatening disorders. About 1000–1500 proteins are needed to build this essential organelle. The large majority of mitochondrial proteins is synthesized on the cytosolic ribosomes, and after completion of their synthesis must be properly targeted to mitochondria. After synthesis, precursors of mitochondrial proteins are directed to the TOM complex and subsequently to specific mitochondrial import machineries that are responsible for their sorting. Proteins destined to the intermembrane space of mitochondria follow the mitochondrial intermembrane space import and assembly (MIA) pathway. The MIA pathway provides an efficient and redox-dependent mechanism for trapping substrate proteins in the intermembrane space of mitochondria. Our findings on dynamic processes that contribute to the regulation of mitochondrial protein homeostasis with the novel role beyond mitochondria will be discussed.

## L16.3

### How information theory can help us to understand biochemical signalling?

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All biological organisms need to sense and respond to their environment. The molecular reaction networks that coordinate the response of an organism to changing environmental conditions are central for the organism survival. At the cellular level such interactions are marshalled by molecular interaction networks. These take up cues from the environment and the cell's physiological state, and transduce this information to the relevant cellular response machinery, which ranges from regulators of protein activity to transcriptional activators and their respective downstream targets. The precise way in which biological information is being processed is still largely unknown: while we have established candidate structures for many signal transduction and stress-response pathways, the details and the dynamics of information flow are only beginning to be understood. For the better understood signal transduction networks we have only sketchy knowledge of they way information is processed, or how information is mapped onto such molecular machineries. In the talk I will explain how conceptual models, which have been formulated based on information theory, can influence the way we think about signalling transduction networks, and how they can change the way we interpret wet-lab experiments. I will argue that computational models of cell signalling, despite their complexity, are valuable complement to experimental research.

## L16.4

### Structural biology of DNA methylation and hydroxymethylation

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DNA methylation and hydroxymethylation introduce small changes in nucleic acid structure that can very effectively prevent protein binding, simply because molecular clashes are associated with very high energetic penalties. However, there are some proteins that only bind to modified DNA, but fail to bind to unmodified DNA. This is much harder to explain, particularly for methylation, since attractive van der Waals interactions are relatively weak. We have studied two model systems to understand specific binding to modified DNA.

R.DpnI is a restriction endonuclease that specifically cleaves DNA within the Gm6ATC sequence, when the adenines of both strands are methylated. We have solved two structures of R.DpnI in complex with DNA and conclude from these that the enzyme consists of a catalytic and a winged helix domain, which are separately specific for DNA sequence and methylation. In the course of our work, we have noticed that methyl groups grafted on the GATC sequence in DNA with conventional B geometry clash. This implies restraints on DNA conformational flexibility in solution, and suggests that the entropic price that has to be paid for protein binding is lower for methylated than nonmethylated DNA, simply because some conformational freedom is already lost prior to protein binding. We believe that this mechanism, which is specific for adenine methylation, together with more conventional desolvation effects, which operate for both 5-methylcytosine and 6-methyladenine binding, is responsible for the specificity of R.DpnI (Mierzejewska *et al.*, manuscript revision requested by NAR).

PvuRts1I is an endonuclease that is specific for 5-hydroxymethylcytosine. Prior to our work, the structural basis for modification specificity was not known. We have solved a structure of PvuRts1I. Even though the structure does not contain bound DNA, it is informative. First, it confirms the prediction that PvuRts1I belongs to the family of PD-(D/E)XK endonucleases. Second, it reveals the presence of an SRA domain. SRA domains have previously been detected in several proteins such as MspJI, UHRF1 and UHRF2, which are specific for modified DNA and are known to flip a DNA base to read its modification status. Although we could not demonstrate base flipping with fluorescent DNA base analogues, we have performed mutagenesis experiments, which proved consistent with the flipping model (Kazrani *et al.*, 2014, *NAR*, **42**: 5929–5936).

## L16.5

### Nucleoprotein complexes in DNA replication and proteolysis

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Formation of nucleoprotein complexes is an essential element of nucleic acids metabolism. During DNA replication initiation subsequent steps involve specific interactions of replication proteins with DNA. Novel discoveries on structure of the bacterial replication initiator DnaA protein, as well as our recent study (*Nucl Acids Res* first published online May 16, 2014 doi:10.1093/nar/gku453) on plasmid RK2 and plasmid F replication initiation proteins TrfA and RepE, reveal new features which change our understanding on the mechanism of plasmid replication initiation. We demonstrate that plasmid Rep proteins, which are composed of two Winged Helix (WH) domains, specifically bind to one of the strands of ssDNA within the DNA unwinding element (DUE). We described nucleoprotein complexes formed by Rep proteins involving both dsDNA containing the Rep-binding sites (iterons) and the strand-specific ssDNA of the DUE. Our recent data also demonstrate that Rep protein is directly involved in assembly of replisome at replication origin through specific interactions with Polymerase III holoenzyme subunits. Moreover, stability of nucleoprotein complexes formed by replication initiator at replication origin depends on specific protease systems whose activity is strictly dependent on both substrate protein and protease interactions with DNA.

## L16.6

### RNA decay in health and disease

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Ribonucleases are central components of the eukaryotic RNA processing and decay machineries. They participate in processing of stable RNA species and pre-mRNA molecules as well as remove unwanted molecules like: RNA processing by-products and malformed RNA species that are identified by surveillance pathways. Finally, ribonucleases participate in mRNA turnover and regulated mRNA decay pathways. Interestingly many of such enzymes are connected to various human diseases. Dis3 a catalytic subunit of the primary eukaryotic ribonuclease, the exosome complex, is frequently mutated in Multiple Myeloma. Mutations in the Dis3L2 exonuclease cause Perlman overgrowth syndrome.

This talk will provide an overview on our recent progress in understanding the cellular function of the above mentioned enzymes and in case of Dis3 the application of basic biological knowledge obtained by us to putative therapeutic approaches.

**L16.7****Role of antisense transcription in gene regulation in plants**

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Antisense transcription originating from transcription terminators is an abundant feature of eukaryotic genes. In many cases the antisense transcription is tightly linked to level of sense transcription. Using *Arabidopsis* as a model we show that the firing of antisense transcription is linked to termination of sense transcription. Our work revives components of the termination machinery involved and suggests a mechanistic explanation for this crosstalk. In addition our data shows that although the absolute level of antisense transcript is positively correlated with sense transcript, the antisense transcription can be regulated independently of sense transcription extending the repertoire of gene expression regulators. We will present data about some of the molecular players involved in antisense transcription regulation and provide the biological function of this novel ncRNA.

**L16.8****New roles of the Grainyhead-like family of transcription factors in cancer**

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The Grainyhead-like (GRHL) family of transcription factors has been highly conserved in the course of evolution of multicellular organisms. There are three mammalian members of this family, termed GRHL1-3. All of them are highly expressed in the epidermis and are crucial for the maintenance of this organ. The aim of our research was to investigate the roles of GRHL proteins in cancer. In this study we employed mouse models, and we also examined material obtained from human patients.

The loss of *Grhl3* is lethal in mice, therefore for the purpose of our study we engineered a mouse strain with a skin-specific ablation of *Grhl3*. These mice display increased susceptibility to chemically-induced skin carcinogenesis. Deletion of *Grhl3* evokes loss of expression of a known tumor suppressor *Pten*, a direct target of GRHL3 regulation, resulting in aggressive squamous cell carcinoma (SCC) induced by activation of PI3K/AKT/mTOR signaling.

In human patients we detected reduced levels of *GRHL3* and *PTEN* in SCC of the skin. This reduction was associated with increased expression of microRNA miR-21, which targets both tumor suppressors. This is the first example of coordinated miRNA-mediated regulation of both transcription factor and its direct target gene for signal amplification.

The *Grhl1*-null mice also exhibit altered susceptibility to skin carcinogenesis, but their cancer phenotype is more complex. These mice develop fewer papillomas than wild type control animals, but with a rate of conversion to SCC that is strikingly higher than in normal littermates. The underlying molecular mechanism differs from *Grhl3*-deficient mice, as GRHL1 does not regulate the expression of *Pten*. Accordingly, the levels of *PTEN* are normal in the *Grhl1*-null epidermis, and the PI3K/AKT/mTOR signaling is not perturbed. Instead, the GRHL1 transcription factor regulates the expression of desmosomal cadherin desmoglein 1 (*Dsg1*) in suprabasal layers of the epidermis. As a consequence, the epidermis of *Grhl1*-null mice displays fewer desmosomes that are abnormal in structure. These mice also exhibit mild chronic skin barrier defects as evidenced by altered keratinocyte terminal differentiation, increased expression of inflammatory markers and infiltration of the skin by immune cells. All these phenomena contribute towards a tumor promoting microenvironment of the skin, which explains their cancer phenotype.

## L16.9

### Tryptophan-derived metabolites in the immunity of model Brassicaceae species

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One of the evolutionary conserved responses of flowering plants to pathogen attack involves biosynthesis and secretion of antibiotic secondary metabolites. Model plant *Arabidopsis thaliana* accumulates upon infection Trp-derived metabolites, including indole-3-carboxylic acids (I3CAs) and phytoalexin camalexin. In addition, pathogen intrusion attempts trigger in this plant species a pathway for metabolism of indole glucosinolates (IGs). As indicated by the analysis of susceptibility of mutants defective in distinct branches of Trp- and IG-metabolism respective end products are critical for *A. thaliana* immunity against a number of fungal and oomycete pathogens. Interestingly, particular compounds provided by these metabolic pathways play independent roles in the pre- and post-invasive stages of defence.

Concerning the high structural diversification of plant secondary metabolites in the plant kingdom we investigated conservation of pathogen-inducible Trp metabolism in *A. thaliana* relatives on the metabolic and genomic level. Our survey revealed a surprising conservation of the pathogen-triggered IG metabolic pathway between the tested plant species, suggesting an ancient and important function of this metabolic branch in Brassicaceae pre-invasive defence responses. In contrast, I3CA and camalexin biosynthesis appeared to represent clade-specific innovations within the conserved framework of pathogen-inducible Trp metabolism that exemplify relatively recent manifestations of the plant-pathogen arms race in the Brassicaceae family.

## L16.10

### Structure and mechanism of reverse transcriptases

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Reverse transcriptases (RTs) catalyze the conversion of single-stranded RNA to double-stranded DNA using its two enzymatic activities: DNA- or RNA-dependent DNA polymerase and RNase H. The polymerase activity is required to synthesize new DNA and RNase H is used to degrade the RNA strand in RNA/DNA hybrid intermediates of the reaction. RTs are essential enzymes for proliferation of retrotransposons and retroviruses, such as for example human immunodeficiency virus (HIV). Retroviral RTs are divided into two classes – dimeric (HIV enzyme belongs to this class) and monomeric. The mechanism of monomeric RTs was not clear, so to characterize it, we solved a crystal structure of XMRV RT fragment in complex with RNA/DNA substrate (1). We then used modeling and small-angle X-ray scattering to study the full-length enzyme. Our results showed that the polymerase part of monomeric and dimeric RTs functions in the same manner, but the mechanism of the fine-tuning of the RNase H activity differs. In XMRV the RNase H domain is highly mobile and only occasionally interacts with the substrate to cleave it. In HIV RT the RNase H domain is rigidly placed in the dimeric structure of the enzyme and, as previously shown by others, substrate deformations are required for substrate hydrolysis.

Retroviruses evolved from retroelements, which are also the most potent forces shaping eukaryotic genomes – up to 40% of human genome derives from those elements. In order to understand the mechanism of retrotransposon RT we solved a crystal structure of yeast elements Ty3 RT in complex with RNA/DNA substrate (2). Quite unexpectedly, the structure showed that the enzyme forms a highly asymmetric homodimer. Our structural and biochemical data demonstrated that, unlike in HIV RT, in Ty3 RT the polymerase activity reside in two different subunits of the dimer. Therefore, the function of the dimerization is to position the RNase H to cleave the hybrid. Our results not only show important differences between retrotransposon and retroviral RTs but also provide clues about the evolution of retroviruses from retroelements.

#### References:

- Nowak E. *et al* (2013) *Nucleic Acids Res* **41**: 3874–3887.
- Nowak E. *et al* (2014) *Nat Struct Mol Biol* **21**: 389–396.

## L16.11

### Chaperoning the guardian. Lesson from tumours

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The p53 protein is a key human tumour suppressor known as guardian of the genome. Mutations in *TP53* tumour suppressor gene were identified in most human cancers including Non-Small Cell Lung Cancer (NSCLC). More than 70% of these mutations are missense. Accumulation of point-mutated p53 protein in cancer cells contributes to transformation and metastasis. In this case, mutated p53 protein gains new pro-oncogenic functions. Non-Small Cell Lung Cancer (NSCL) patients with *TP53* mutation showed significant chemoresistance, among them some experienced progressive disease after chemotherapy. Studies in mouse models have demonstrated that the stabilization of p53 R172H (R175H in humans) mutant protein by unknown factors is a prerequisite for its oncogenic gain-of-function phenotype, such as tumour progression and metastasis. Interestingly, knock-in p53 R172H mice do not develop cancer when the heat shock response is blocked (Hsf1-knockout). Recently, we described the molecular mechanism of how Hsp70 molecular chaperone induces oncogenic activity of p53 R175H in lung cancer. Elevated levels of Hsp70 induce transient folding intermediates of p53 R175H, which in the presence of MDM2 form stable amyloid nuclear aggregates. Recurrent interaction of HSP70 with the p53 polypeptide, in an ATP dependent manner, causes transient exposure of its aggregate prone domain(s). Subsequent aggregation of mutant p53 is further augmented by the MDM2-p53 allosteric interaction. This dynamic, irreversible molecular process sequesters p73 tumour suppressor, thus inhibiting its activity. Formation of such nuclear aggregates may lead to chemoresistance in lung cancer cells. Ironically, the evolutionary conserved role played by the heat shock response in helping cells survive, adapt and proliferate is assimilated in malignant cancers. By enabling oncogenesis, the activation of this ancient prosurvival mechanism thereby actually impairs survival of the host.

## L16.12

### Molecular biology of mind: An introduction

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The Merriam-Webster Dictionary derives term "Mind" from Old English *gemynd*, akin to Old High German *gimunt* meaning memory. Over the last quarter of century we have followed molecular roots of the memory in a hope to identify also building blocks of the mind. Initially, we have identified increased *c-fos* mRNA levels during memory formation, thus discovering phenomenon of gene expression in learning. Following c-Fos protein function as transcriptional regulator, we have focused on its gene targets: TIMP-1 and MMP-9 (tissue inhibitor of metalloproteinases-1 and matrix metalloproteinase-9), composing extracellular proteolytic system that we and others have implicated as a major player in the synaptic plasticity, learning and memory. MMP-9 has been shown first to be activated in dendritic remodeling, accompanying epileptogenesis. Then, functional studies demonstrated MMP-9 role in learning and memory as well as their cellular models and finally epileptogenesis. At the subcellular level, MMP-9 localization and activity helps to explain this role, as the enzyme, its protein and mRNA are all available at the or near excitatory synapses located at the dendritic spines to allow for a rapid, local unleash of the enzymatic activity in response to synaptic stimulation. Furthermore, MMP-9 was shown to directly affect the dendritic spine morphology and excitatory neurotransmitter receptors function and trafficking. In aggregate, the pivotal role of MMP-9 in the synaptic plasticity underlying brain physiology has been firmly established. The present research challenge is to explain possible contribution of the enzyme to such human neuropsychiatric conditions as epilepsy, drug addiction, schizophrenia and autism spectrum disorders to name just those for which such a link has been demonstrated. By these virtues, MMP-9 emerges indeed as a molecular link to the brain molecular underpinning of the mind.

## Poster

### P16.1

#### Co-translational import of protein precursors into mitochondria

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Mitochondria are essential organelles in eukaryotic cells with the bacterial origin. The majority of mitochondrial proteins are nuclearly encoded. They are synthesized by 80S cytosolic ribosomes as precursor form. Each precursor contains a specific targeting signal that supports its transport into the mitochondria in a post-translational manner. However, there is a growing number of evidence suggesting that the synthesis of mitochondria-destined proteins can be coupled with their transport and specifically localized on the mitochondrial outer membrane. Yet the mechanism of the interaction between cytosolic ribosomes and the mitochondrial surface is still unknown.

Our preliminary data confirm the enrichment of ribosomes in the mitochondrial fraction. The cytosolic ribosomes associate with the mitochondrial surface and are co-purified together with the mitochondria. We developed an experimental model to study the mechanism of ribosome-mitochondrion interaction. Our progress in understanding of the mitochondrially-localized translation coupled with the protein transport will be presented.

### P16.2

#### Study of protein translocase MIA40 interactions in human mitochondria

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Mitochondria play a vital role in various cellular functions including ATP synthesis, apoptosis and signalling in eukaryotic cells. The majority of the mitochondrial proteins are nuclear encoded; hence the precursor proteins should be efficiently imported into the mitochondria. The import of mitochondrial proteins has been extensively studied in yeast. Among the various import pathways, the mitochondrial intermembrane space assembly machinery (MIA) pathway is responsible for import of intermembrane space (IMS) proteins [1]. The C-terminal domain of MIA40 that contains the active and structural cysteine residues is conserved in human, but considerably less is known about the MIA pathway in human cells. We are interested to examine the interacting partners of MIA40 to understand the various factors governing the import of IMS proteins in human cell lines. We employ pull down assays to identify regulatory proteins that interact with Mia40. We use mutant forms of MIA40 to stabilize the interaction with various precursor proteins in the IMS of mitochondria.

#### Reference:

1. Chacinska *et al* (2009) *Cell* **38**: 628–644.

## P16.3

### Membrane Palmitoylated Protein 1 (MPP1) as a tuning factor regulating functional microdomains formation in the plasma membrane

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Plasma membrane is a site of multiple physiological events (signaling, fusion, fission etc.) often either connected with each other or separated spatially and temporally. The existence of membrane rafts helps to conceptually understand the spatiotemporal organization of membrane associated events, however as rafts themselves are nanoscopic and dynamic assemblies they are impossible to capture in a metabolizing cell by traditional microscopy [1]. The observation of phase separation in plasma membrane-derived vesicles from live cells is a great tool for studying eukaryotic membrane lateral heterogeneity, specifically in the context of membrane rafts [2]. Microscopic phase separation is detectable by fluorescent labelling, followed by cooling of the membranes below their miscibility phase transition temperature. It remains however unclear if this lipid-driven process is tuneable in any way by the interactions with proteins. Here we demonstrate that single protein MPP1 a member of the MAGUK family can directly modulate membrane properties like fluidity and phase separation ability of Giant Plasma Membrane-derived Vesicles. Most importantly we observed the correlation between lateral organization of the membrane and the absence of MPP1. Vesicles isolated from cells with silenced MPP1 gene expression showed decreased membrane order detected by advanced microscopy techniques such as general polarization (GP) measurements via two-photon microscopy [3] or fluorescence lifetime imaging microscopy (FLIM) but also as a significant change in their resulting miscibility transition temperature. Our data suggests that membrane by having a constant set of lipids can modulate domain physicochemical properties by proteins like MPP1 and therefore regulate the functional role of the nanoassemblies, which could be observed in living cells as disruption of signal transduction from RTK receptors dependent on membrane raft assembly. We were able to show that activation of membrane receptors in the absence of MPP1 is strongly impaired strengthening our hypothesis that MPP1 is crucial for proper lateral membrane structure and pointing to physiological importance of observed mechanism of erythroid cell membrane organization.

#### References:

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2. Levental I *et al* (2011) *Proc Natl Acad Sci U S A* **108**: 11411–11416.
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## P16.4

### The interplay between the inner mitochondrial membrane organizing complex MICOS and the MIA pathway responsible for protein transport into mitochondria

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Mitochondria play a crucial role in energy metabolism and regulatory processes in eukaryotic cells. The distinctive morphology of mitochondrial membranes, especially the inner membrane forming cristae, is crucial for their function. We recently discovered MICOS complex (mitochondrial contact site and cristae organizing system), which is responsible for the proper architecture of mitochondria and formation of contact sites between outer and inner membranes. MICOS is an inner membrane complex, which is composed of six proteins. MICOS has been shown to interact with several outer and inner membrane proteins, including protein translocases. The vast majority of mitochondrial proteins are synthesized in cytosol and therefore must be imported into this organelle. All precursor proteins utilize TOM (translocase of the outer membrane) as the main entry gate. Subsequently, the protein precursors directed to the mitochondrial intermembrane space use the specialized MIA (mitochondrial import and assembly) pathway. Mia40, the key component of this pathway, recognizes precursors and specifically transfers disulfide bonds, thus enabling the proteins transport, oxidation and folding. Our studies showed that the protein import activity of Mia40 is regulated by Mic60 protein, a key component of MICOS. We investigate the mechanisms underlying the interplay between the process of inner membrane formation by MICOS and protein sorting by MIA.

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## P16.5

### Modulation of proteasome activity by mitochondrial precursors at early stage of their biogenesis

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Mitochondria are dynamic organelles involved in numerous fundamental cellular processes that include energy production, metabolism of lipids and amino acids, iron-sulfur clusters biogenesis and regulation of apoptosis. These broad functions are executed by 1000–1500 (depending on the organism) different proteins that comprise mitochondrial proteome. Although mitochondria possess their own DNA, only a handful of proteins are translated on mitochondrial ribosomes. The vast majority of mitochondrial proteins (99%) are nuclear-encoded and synthesised as precursors in the cytosol. To reach their final destination and fulfil their functions, precursors are targeted into the mitochondria and sorted within the organelle via specialized import pathways. Given their essential role in cellular homeostasis, it is important to adjust mitochondrial proteome by maintaining the protein import into the organelle well orchestrated. On the other hand, under the conditions of decreased mitochondrial import, the delivery of precursors into the organelle is slowed down and leads to their accumulation in the cytosol. We hypothesize that it is important to clear the cytosol from mislocalized mitochondrial precursors in order to restore cellular balance.

In our studies we concentrated on the group of presequence-containing precursors that are delivered into the mitochondria via the TIM23 pathway. As the pathway depends on energy and electrochemical potential of the inner mitochondrial membrane, we used CCCP or conditional yeast mutant strains to inhibit the protein import into mitochondria. Our results indicate that mitochondrial precursor proteins accumulated in the cytosol regulate activity of proteasome, a major degradation machinery in the cytosol. Modulation of proteasomal activity by the rate of mitochondrial protein import emerges as the mechanism that is triggered to minimize stress at the cellular level.