Session 9: Proteostasis and stress response

Lectures

L9.1

INMAD: a quality control mechanism at the inner nuclear membrane

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Protein quality control mechanisms protect cells from defective, mislocalized or supernumerary proteins. The clearance of those proteins is achieved by intracellular degradation pathways, among which the ubiquitin-proteasome system is a major player. In this system, proteins to be degraded are labeled through the covalent attachment of ubiquitin. This is achieved through a complex enzymatic network that comprises in human cells ~30 ubiquitin conjugating enzymes (E2s) and more than 500 ubiquitin ligases (E3s). We used bimolecular fluorescence complementation to systematically probe the E2-E3 interaction network in living yeast. This enabled us to identify the E2s Ubc6 and Ubc7 as interaction partners of Asi1 and Asi3, two putative E3s that were previously described to form a complex at the inner nuclear membrane (INM) and to inhibit the activity of the transcription factors Stp1 and Stp2. We demonstrated that Ubc6 and Ubc7 directly interact with the RING domains of both Asi1 and Asi3 and that they are involved in the ubiquitylation of nuclear Stp2. Furthermore, an unbiased microscopy screen revealed that multiple transmembrane proteins of the yeast endomembrane system are stabilized and accumulate at the nuclear rim when the Asi ubiquitin ligase is inactivated. This suggests that the Asi complex primarily targets transmembrane proteins that should normally not be at the INM. We propose that this INM associated degradation (INMAD) is a quality control mechanism which clears proteins mislocalized in the nucleus due to their fortuitous passage through nuclear pores.

L9.2

Heavy metals and metalloids cause widespread protein misfolding and aggregation

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While the toxicity of metals and metalloids, like arsenic, cadmium, mercury, lead and chromium, is undisputed, the underlying molecular mechanisms are not entirely clear. General consensus holds that proteins are the prime targets; heavy metals interfere with the physiological activity of specific, particularly susceptible proteins, either by forming a complex with functional side chain groups or by displacing essential metal ions in metalloproteins. Recent studies have revealed an additional mode of metal action targeted at proteins in a non-native state; certain heavy metals and metalloids including cadmium and arsenic have been found to inhibit the *in vitro* refolding of chemically denatured proteins, to interfere with protein folding *in vivo*, and to cause aggregation of nascent proteins in living cells. Thus, by interfering with the folding process, heavy metal ions and metalloids profoundly affect protein homeostasis and cell viability. My talk will highlight our recent findings on how arsenic and cadmium interfere with protein folding and trigger widespread protein aggregation in living cells. I will also describe mechanisms by which cells regulate quality control systems to protect themselves from heavy metal/metalloid toxicity.

References:

1. Weids AJ, Ibstedt S, Tamás MJ, Grant CM (2016) Distinct stress conditions result in aggregation of proteins with similar features. *Sci Rep* **6**: 24554.
2. Ibstedt S, Sideri TC, Grant CM, Tamás MJ (2014) Global analysis of protein aggregation in yeast during physiological conditions and arsenite stress. *Biology Open* **3**: 913-923.
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4. Jacobson T, Navarrete C, Sharma SK, Sideri TC, Ibstedt S, Priya S, Grant CM, Christen P, Goloubinoff P, Tamás MJ (2012) Arsenite interferes with protein folding and triggers formation of protein aggregates in yeast. *J Cell Sci* **125**: 5073-5083.

L9.3

The role of new homologous recombination mediators in replication stress response in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

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Functional DNA damage tolerance (DDT) pathways are critical for timely completion of DNA replication but must be tightly regulated in order to avoid the formation of deleterious recombination intermediates. Here we show that*S. cerevisiae* Uls1, a member of the Swi2/Snf2 family of ATPases and a SUMO-targeted ubiquitin ligase, as well as its *S. pombe* paralogues, Rrp1 and Rrp2, are involved in this process. Uls1 physically interacts with both PCNA and Srs2, and regulates the balance between SUMOylated and PCNA-bound forms of Srs2. Uls1 presence is beneficial in *rad27*Δ and toxic in *sgs1*Δ mutant backgrounds, pointing to its role in modulating Rad51 dependent recombination. Interestingly, epistasis analysis also placed Rrp1 and Rrp2 together with Srs2 in a synthesis-dependent strand annealing homologous repair pathway of replication fork restart. Strains lacking Rrp1 or Rrp2 are characterized by the increase in the percentage of conversion-type recombinants, observed earlier in mutants devoid of the Srs2 helicase, suggesting a possible function for Rrp1 and Rrp2 with Srs2 in counteracting Rad51 activity.
We thus provide genetic and physical evidence that *S. cerevisiae* Uls1 and *S. pombe* Rrp1 and Rrp2 contribute to DDT pathway choice and thus contribute to genomic stability during DNA synthesis.

Oral presentations

O9.1

The ubiquitin-proteasome system mediated control of mitochondrial intermembrane space proteins

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The mitochondrial proteome comprises of around one thousand different proteins. Nearly all mitochondrial proteins are synthesised by cytoplasmic ribosomes in a precursor form and require active translocation to their final localization. Several specialised protein import and folding pathways act in parallel to allow biogenesis of mitochondrial proteins. Importantly, synthesis, transport and folding mechanisms must be accompanied by the quality control machinery that degrades misfolded or mislocalised proteins to assure the proteostasis.
The major cytosolic system for specific protein degradation – the ubiquitin-proteasome system (UPS) – has a limited access to mitochondrial proteins. Only the proteins exposed on the outer mitochondrial membrane (OM) appear to be within reach of the UPS. However increasing evidence accumulates on the role of the UPS in surveillance of the mitochondrial proteome, which is not limited to the OM proteins. Our results demonstrate an important role of the UPS in regulation of the proteome of the intermembrane space of mitochondria (IMS). This role is especially robust during IMS protein import failure, which results in protein mislocalisation to the cytosol. Furthermore, proteasomal degradation also impacts IMS proteins under normal physiological conditions. By continuous removal of a fraction of newly synthesised IMS precursor proteins the proteasome competes with mitochondrial protein import. This process likely establishes a fast-acting mechanism allowing for adaptation to changes in supply and demand for IMS precursor proteins. Interestingly , a backward movement of proteins from the IMS to the cytosol is also possible. This additionally increases the significance of the UPS system for the IMS proteins.
In the current study we focus on gaining the mechanistic insight on the specific recognition and targeting of IMS proteins for the proteasomal degradation. Using genetic screen in yeast model we aim to uncover specific proteins regulating stability of IMS proteins. We also attempt to understand the internal features of the IMS proteins that are important for the specificity of their recognition.

Acknowledgements:

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O9.2

Two faces of one disease – difference in cell cycle regulation and apoptotic response between lymphocytes from familial and sporadic Alzheimer's disease patients

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Alzheimer's disease (AD) was first described over 100 years ago. It is the most common cause of dementia with an estimated prevalence of 30 million people worldwide. A growing body of data has shown that AD is characterized by complex alterations in cellular processes that occur not only in neurons, but also in peripheral cells such as lymphocytes. Recently we have demonstrated that lymphocytes from the sporadic form of AD (SAD) show G1 phase arrest and increased levels of protein p21, the key regulator of apoptosis and the G1/S cell cycle checkpoint. Since it is known that p21, besides controlling the G1/S checkpoint, can regulate apoptosis, we conducted studies to determine if p21 levels play a role in the cellular response to an oxidative stress challenge like 2d-ribose (2dRib) treatment. Apoptosis was measured using flow cytometry (SubG1 level assessment, Annexin V staining and mitochondrial membrane potential measurement). Cell viability after 2dRib and pifitrin (PFT-a) treatment was measured using MTT assay, mRNA levels were evaluated using real-time PCR, and protein levels by immunoblotting. p21 levels in nuclear and cytosolic cellular compartments were visualized using confocal laser scanning microscopy and analyzed by cell fractionation.

We report here that cells from familial AD (FAD) are more resistant to 2dRib-induced cell death than control or SAD cells. p21 mRNA and protein levels significantly increased in FAD cells in response to 2dRib. In addition, we found a higher cytosolic accumulation of p21 in FAD cells. Transcriptional activation of p21 was shown to be dependent on p53, as it can be blocked by PFT-a and was correlated with phosphorylation of p53. Thus in human B-lymphocytes under oxidative stress evoked by 2dRib, 7 PS1 mutants seem to strongly exacerbate phosphorylation of p53 exhibiting a gain of function effect over wtPS1. These activities of mutPS1 seem to represent a compensatory mechanism against acute oxidative stress, preventing depolarization of the mitochondrial membrane and apoptosis in human FAD lymphoblasts. Altogether, our results showed that the mechanism of apoptotic response to acute oxidative stress distinguishes cells from SAD and FAD patients. Thus, caution should be taken in extrapolating data obtained from cellular or animal models based in FAD mutations, as they may not be relevant in SAD. Consistently, therapeutic designs should take into account the possible effect variability in SAD *versus* FAD cells.

Posters

P9.1

The role of methyltransferase Dnmt2 during stress-induced premature senescence in NIH3T3 mouse fibroblasts

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The physiological role(s) of methyltransferase Dnmt2 in biological systems is(are) enigmatic and far for being understood. Dnmt2 has been suggested to be implicated in both DNA methylation as well as RNA methylation, and involved in the regulation of fundamentally different cellular pathways modulating genomic stability, organ development, metabolic processes and aging. In the present study, we have analyzed the effects of lack of Dnmt2 during hydrogen peroxide-induced premature senescence in the mouse fibroblast model. *Dnmt2* gene was silenced using shRNA technology that was confirmed at mRNA and protein levels using RT-PCR and Western blotting, respectively. *Dnmt2*-silenced NIH3T3 cells were stimulated with hydrogen peroxide and changes in the levels of metabolically active cells (MTT assay), Annexin V-positive cells, Ki67-based proliferation and p53/p21 pathway-mediated cell cycle were revealed. Hydrogen peroxide was also considered as an inducer of stress-induced premature senescence in *Dnmt2*-silenced fibroblasts (SA-beta-gal activity). Moreover, redox imbalance was documented in hydrogen peroxide-treated *Dnmt2*-silenced fibroblasts (augmented production of total reactive oxygen species and total and mitochondrial superoxide and protein carbonylation). DNA double and single strand breaks (comet assay) were also observed that was accompanied by affected 53BP1 foci formation as judged by single cell analysis using imaging cytometry. We postulate that Dnmt2 may be implicated in oxidant-based DNA damage response in mouse fibroblasts.

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P9.2

Rrp1 and Rrp2 overexpression affects chromosome segregation and leads to genome instability

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Rrp1 and Rrp2 are involved in the Swi5-Sfr1 branch of Rad51 dependent homologous recombination pathway. After MMS treatment Rrp1 and Rrp2 form foci co-localizing with Rad52 and Rrp1 is enriched at HO-induced double strand breaks. Rrp1 and Rrp2 also form spontaneous foci in the nucleus partially associated with centromere and rDNA regions. Prolonged overexpression of *rrp1+* and *rrp2+* results in the increase of the number of Rad11 and Rad52 foci. It causes nuclear morphology aberrations and growth defect accompanied by the increase in the level of mini chromosome loss. We examined the influence of homologous recombination proteins on the phenotypes induced by overproduction of Rrp1 and Rrp2 and their localization.

P9.3

Investigating the ubiquitylation network of Irc20 and Tfb3 ubiquitin ligases needed upon DNA damage

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Protein ubiquitylation is a post-translational modification that plays a crucial role in regulating many cellular functions. Defects in this control mechanism are associated with various diseases, including cancers, neurodegenerative or metabolic disorders and some viral infections. In this process a small protein called ubiquitin is covalently attached to a lysine residue of a substrate protein, marking it either for degradation or for having other functional consequences for the cell. In order to control protein activity, interactions, localization and degradation, ubiquitin encodes complex molecular signals and is aided by a large network of structurally-related enzymes. The ubiquitylation process involves a cascade of enzymatic reactions catalyzed by ubiquitin activating enzymes (E1s), ubiquitin conjugating enzymes (E2s) and ubiquitin ligases (E3s). E2s and E3s are at the centre of the ubiquitylation cascade. These enzymes need to physically interact with one another in order to ubiquitylate their substrates. To date, only a small fraction of all possible E2-E3 pairs have been investigated, mainly using biochemical and *in vitro* approaches that often do not accurately reflect the conditions that occur in living cells. Using Bimolecular Fluorescence Complementation (BiFC), we identified the E2 interaction network of some of the ubiquitin ligases in living yeast *Saccharomyces cerevisiae*. The efficacy of BiFC technique has already been demonstrated in the search for potential E3 ubiquitin ligases interacting with Ubc6 and Ubc7 ubiquitin conjugating enzymes. This led us to the discovery of a novel E3 ubiquitin ligase, the Asi-complex, and its involvement in a protein quality control pathway at the inner nuclear membrane (Khmelinskii *et al.*, 2014). Here we aim to focus on two E3s needed upon DNA damage, namely Irc20 and Tfb3. One of our goals is to analyze whether their E2 interaction network changes under conditions of the genotoxic stress. We also aim at identifying specific substrates of Irc20 and Tfb3. We are planning to use molecular biology and biochemical approaches as well as novel proteomic approaches to explore the function of these important ubiquitin enzymes.

Reference:

Khmelinskii A, Blaszczak E *et al* (2014) *Nature* **516**: 410-413.

P9.4

Polyhydroxyalkanoates genes expression changes in the response to the environmental stress in *Pseudomonas putida* KT2440

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Medium-chain-length polyhydroxyalkanoates (mcl-PHAs) are an interesting class of biodegradable polymers that can be obtained from renewable resources. Mcl-PHAs, environmentally friendly materials, are considered as potentially useful for replacing petroleum-based polymers. These biopolymers are synthesized by various bacteria as intracellular carbon and energy storage materials under nutrient-limited conditions and in the presence of excess of carbon sources. In spite of existing knowledge about mcl-PHAs synthesis by *Pseudomonas* species, there is a lack of information about linkage between nitrogen starvation and molecular regulation of this process. Therefore, the main aim of this work is to find the relation between nitrogen limitation and expression of key genes involved in the mcl-PHAs synthesis. In this study *Pseudomonas putida* KT2440 and its two mutants lacking *rpoN* and *relA* genes were examined under optimal and nitrogen limiting conditions using oleic acid as the only carbon source. The highest mcl-PHAs concentration was observed in the wild strain and *rpoN* mutant under nitrogen deficiency. Whereas, *relA* mutant accumulated biopolymers at the same level in both conditions. Reverse-transcription real-time PCR analysis showed the differences in the expression of mcl-PHA related genes. Nitrogen limitation stimulated positively the expression of *phaD*, *phaI*, *phaF*, and *phaG* genes in the wild strain. The obtained results revealed that nitrogen limitation did not influence on the analyzed genes expression in the mutants. It could be concluded that both nitrogen regulon and stringent response could be involved in the process of mcl-PHAs synthesis.

P9.5

Stimulating effects of low doses of UVA

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UV is an electromagnetic radiation with wavelengths of 100-400 nm, naturally emitted by the Sun and divided into three main fractions: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm). Most solar UVB and UVC radiation is blocked by the atmosphere, and about 95% of UV which reaches the Earth is UVA.

We have studied the response of human cells in culture to UVA at different doses. Using the clonogenic survival test, we found that some low doses, different for different cell lines, can stimulate cell proliferation. The stimulating effect was observed in human cancer (HCT116, ME45) cells and in normal fibroblasts (NHDF). For HCT116 cells the dose stimulating clonogenicity was 10 kJ/m^2. Stimulation of proliferation was also observed for low doses of UVB radiation in NHDF and Me45 cells.

To understand better the molecular basis of this effect, HCT116 cells were exposed to three different doses of UVA radiation which gave different responses in clonogenic tests (stimulating, suppressing, or no effect) and examined the influence on cell cycle parameters and cellular levels of reactive oxygen species (total ROS, O_2^-, and NO) at different times (1, 6, 12, 24 h) after irradiation. The levels of ROS and NO differed significantly in cells irradiated with doses stimulating or suppressing clonogenic proliferation, and cells exposed to $10kJ/m^2$ showed an negative correlation between viability measured at different time points and the levels of NO, suggesting a role of NO in the regulation of cell survival.

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P9.6

The influence of α-lipoic acid and extract of garlic on the morphology of selected organs of rabbits fed a diet complete in oxidised rapeseed oil

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Introduction: Fat-rich diet and the consumption of oxidized oils are factors that play an important role in the development of atherosclerosis and nonalcoholic steatohepatitis (NASH). Oxidized oils are the source of reactive oxygen species (ROS) which have a negative effect on health. A protective factor in diseases caused by ROS is the use of substances having antioxidative properties. One of it is α-lipoic acid (ALA), which helps to discard or dispose of ROS. **Aim**: The purpose of these studies was to investigate the influence of α-lipoic acid (ALA) and garlic on the morphology of aorta and liver in rabbit fed with oxidized rapeseed oil. **Material and methods**: Three months old, Chinchilla rabbits were taken into experiment. Animals were divided into six groups of six animals each. The control group was fed a basal diet. „I" experimental group was given α-lipoic acid in the dose of 10mg/kg of body mass. „II" experimental group was given garlic extract in the dose of 4mg/kg of body mass. „III" group had 10% oxidized rapeseed oil added. „IV" group was fed with 10% oxidized rapeseed oil and α-lipoic acid in the dose of 10mg/kg of body mass. „V" group was given 10% oxidized rapeseed oil and garlic extract in the dose of 4mg/kg of body mass. The presented study lasted six months. During necropsy aorta and liver tissue were collected. The presence of patomorphological lesions was assessed using a standard paraffin technique. Slides were stained by the standard H-E method. Additionally Sudan II stain was used to show the presence of neutral lipids. **Results**: The studies show that diet which contains oxidized oil leads to fatty changes in hepatocytes and presence of necrotic lesions in 2 rabbits. In aortas of 2 rabbits, which were given oxidized oil, atherosclerotic plaques with fatty infiltrateand and fatty cells were observed. The addition of ALA and garlic extract has the positive influence on lesions caused by oxidized rapeseed oil however, this influence was not fully successful. **Conclusion**: Presented studies confirmed that examined antioxidants have protective effects on pathomorphological changes in rabbits given oxidized oils

Key words: α-lipoic acid, garlic extract, rabbit, reactive oxygen species, oxidized rapeseed oil, liver, aorta

P9.7

Distributive phosphorylation of yeast Spl2 protein engaged in phosphate starvation in *Saccharomyces cerevisiae*

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A comprehensive mechanism of regulation of phosphate signal transduction pathway (PHO) assumes that decrease in the concentration of phosphate ions is a signal to switch the PHO pathway between functional Pho87/Pho90 transporters and activation of transcription factor Pho4 and Pho84 transporter. It is known that functions of Pho87/Pho90 and Pho84 are mutually exclusive. Transporters of low affinity contain in their sequence SPX domain, which allows their negative regulation by binding protein Spl2. When phosphate is abundant Spl2 is responsible for endocytosis of Pho87 and dislocation of this transporter to the vacuole.
Protein kinase CK2 is an enzyme engaged in many processes fundamental to the functioning of a living cell. Since many years CK2 is an object of intensive research in the field of molecular biology and medicine, particularly in the aspect of regulation of its activity in the cell and finding specific inhibitors of CK2 that may act as potential therapeutic agents.
Results presented in this report improve knowledge about the role of CK2 in the stress response in yeast *Saccharomyces cerevisiae*. Furthermore, obtained results expand the list of protein kinase CK2 *in vitro* substrates on yeast protein Spl2 which exhibit some homology, in amino acid sequence, with inhibitors of Cdk kinases (especially Pho81 inhibitor). Site-directed mutagenesis assays of Spl2 protein, confirmed that only Ser142 and Ser143 are phosphorylated by CK2. Spl2 multisite phosphorylation has a distributive manner. This type of phosphorylation occurs when each phosphorylation requires a separate binding event between the kinase and substrate. In the case of Spl2, Ser142 is phosphorylated first, then CK2 dissociates and phosphorylation of Ser143 takes place after another enzyme-substrate association. Probably CK2-mediated phosphorylation of Spl2 affects its activity against Pho87 and Pho90 proteins andadjusts response to phosphate concentration in the environment.

P9.8

Characterization of the Spen expression after heat shock

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SPEN protein, known also as MINT (MSX2-Interacting Nuclear Target) or SHARP (SMRT/HDAC1-associated repressor) belongs to a protein family characterized by a SPOC (Spen Paralog and Ortholog C-terminal) domain at the C-terminus, and four RNA recognition motifs at the N-terminus.This large (~400 kDa) transcriptional repressor may bind to DNA and RNA and plays an important role in the regulation of transcription. Therefore, we postulate that altered expression of SPEN could be a reason of general transcriptional repression observed during the heat shock in heat-sensitive cells.

Our results indicate up-regulation of the *Spen* transcription after hyperthermia in isolated spermatocytes (which are the most heat-sensitive cells) and whole mouse testes. Using antibodies to different parts of the protein we determined the intracellular distribution of SPEN by immunohistochemistry in control and heat shocked testes. The protein preferentially accumulated in the nuclei of spermatogenic cells and revealed a significant increase of expression after heat shock. Western blot analysis of fractionated testes extracts showed the presence of the full-length protein (which potentially represents a nascent polypeptide precursor) in the cytoplasm, and a shorter, mature forms (created after proteolytical digestion) detected only in the nuclear fractions. Moreover, Western blot analysis of whole testes and nuclear and cytoplasmic fractions revealed an increase of SPEN protein level already 15 min after heat shock, both in testes shocked at 38°C and at 43°C. We also analyzed the *Spen* transcript and protein level in other mouse organs. Our results show that *Spen* expression in somatic tissues is much lower than in testes and it is not uniformly activated by hyperthermia.

Looking for SPEN binding to chromatin in mouse spermatogenic cells we performed ChIP-seq. Using antibody to internal part of SPEN we observed remodeling of the protein binding induced by heat shock both at 38°C and at 43°C. SPEN binding was found preferentially in intergenic and intronic sequences. Among annotation to promoters predominate non-coding genes. Additionally, we found that C-terminal SPEN fragment did not bind to chromatin.

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P9.9

Saccharomyces cerevisiae Uls1 participates in the regulation of DNA damage tolerance pathway choice

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DNA damage tolerance (DDT) pathways function to bypass replication-blocking lesions and ensure completion of DNA replication. However, inappropriate activation of these pathways may lead to increased mutagenesis or formation of deleterious recombination intermediates. Here we show that Uls1, a member of the Swi2/Snf2 family of ATPases and a SUMO-targeted ubiquitin ligase, physically interacts with both PCNA and Srs2, and promotes Srs2 binding to PCNA by downregulating levels of SUMOylated forms of Srs2. We also demonstrate that Uls1 activity contributes to severe growth defect of SGS1 and MUS81 double deletion mutant and propose that deletion of ULS1 in this mutant leads to accumulation of SUMOylated form of Srs2 that is still capable of stimulating Rad5 dependent salvage pathway independently of toxic PCNA association.

P9.10

Glycogen accumulation and stress response in fibroblasts from patients with glycogen branching enzyme deficiency

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Glycogen Storage Disease type IV (GSD IV) is a genetic disease related to deficiency of glycogen branching enzyme (GBE1). It is characterized by intracellular accumulation of structurally abnormal, poorly branched glycogen molecules. Intracellular mechanisms responsible for pathologies observed in GSD IV are poorly characterized and it is not clear whether cell injury results simply from mechanical damage and impairment of intracellular trafficking due to presence of glycogen aggregates, or if some metabolic dysfunctions also contribute to the disease pathogenesis. It is also not clear if glycogen accumulation results from impaired glycogen degradation or from excessive synthesis. To check that, we conducted the studies using primary fibroblast cultures from GSD IV patients and age-matched control subjects.

In fibroblasts with strong GBE1 deficiency excessive glycogen accumulation occurred. The efficiency of glycogen utilization during starvation was similar in control and patient's cells, however upon reintroduction of glucose to the culture medium, glycogen synthesis was more intensive in patient's fibroblasts. Also serum starvation stress led to much stronger glycogen accumulation in patient's cells than in controls. The investigated fibroblasts also differed with respect to response of AMP-regulated protein kinase (AMPK) to starvation and glucose reintroduction. In GBE1-deficient cells upregulation of AMPK levels was significantly stronger and changes in phosphorylation pattern of the kinase were slightly different.

Our results suggest, that in fibroblasts with GBE1 dysfunction, glycogen accumulation may result from improper regulation of glycogen synthesis. Additionally, apart from glycogen accumulation, more complex rearrangements in cellular energy metabolism may contribute to the disease pathogenesis.

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P9.11

The *Saccharomyces cerevisiae* Hog1 regulates G1/S checkpoint adaptation by promoting accumulation of G1/S cyclins during arsenic stress

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The major cellular response to stress conditions is temporal arrest of cell cycle progression.We have previously shown that during long-term exposure to arsenite [As(III)] yeast cells delay cell cycle progression in all phases. However, cells lacking the stress-activated protein kinase (SAPK) Hog1 are permanently arrested in G1 phase. The cell cycle defect of *hog1*D is fully suppressed by deletion the *SIC1* gene encoding an inhibitor of S-phase cyclin-dependent kinase complexes. Here we show that in the presence ofAs(III) *hog1*D exhibited low levels of mRNA and protein of G1/S (Cln2) and S (Clb5) cyclins. Deletion of *WHI5,* an inhibitor of the G1-specific transcription program or overexpression of cyclins did not suppress the G1/S transition defect of *hog1*D. Moreover, Cln2 was still undetectable in the *hog1*D mutant expressing the hyperstable variantof Cln2 ($Cln2^{4T3S}$) under control of the constitutive *TET* promoter. Interestingly, localization of Hog1 exclusively in the cytoplasm was sufficient to promote recovery from G1 arrest in the presence of As(III). This suggests that Hog1 is not involved in activation of transcription of cyclins but contributes to their accumulation probably at the level of mRNA stability and/or regulation of translation initiation. In summary, under long-lasting exposure to As(III) Hog1 promotes accumulation of G1/S and S cyclins that are necessary for induction of degradation of Sic1 inhibitor and replication initiation, respectively. Taken together, our data point to a novel role of Hog1 in regulation of G1/S cell cycle transition during arsenic stress.

P9.12

When ribosomes get stressed – how the environment influences yeast translational activity and association between small non coding RNAs and the ribosome

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In the past years it became evident that small non coding RNAs (sncRNAs) play crucial roles in cellular life. Their expression often depends on the environmental conditions, in wchich living organism must struggle to survive. Among such sncRNAs we can find a plethora of tRNA and snoRNA fragments. Transfer RNAs (tRNAs) play their main role as amino acid carriers during protein biosynthesis, while small nucleolar RNAs (snoRNAs) guide enzymatic modifications of other RNAs, mainly ribosomal RNAs (rRNAs). Despite these canonical functions, we and others have recently found that certain stress conditions can induce cleavage of transfer RNAs and snoRNAs in specific sites to stable processing products. Extensively studied tRNA-derived fragments (tRFs) represent a wide functional repertoire, from RNA metabolism and protein biosynthesis regulation through the RNAi mechanisms and apoptosis. Such fragments have been observed in multiple species ranging from mammalian cells to protozoan *Giardia lambia* and yeast *Saccharomyces cerevisiae*. On the other hand, there is a limited number of evidences which clearly show that snoRNA can undergo processing to snoRNA-derived RNAs (sdRNAs). The function of sdRNAs is in most organisms still elusive.
Eukaryotic cells contain robust mechanisms to respond to and mitigate environmental stress. Important component of stress response is regulation of RNA metabolism, which often involves a decrease in general translation and an increase in preferential translation of stress-response genes. We have recently discovered that tRNA-derived fragments preferentially interact with *S. cerevisiae* ribosomes under specific stress conditions. Therefore, the question which we ask now is: Do snoRNA fragments behave in a same manner? Moreover, do such stress conditions influence the translational activity of yeast ribosomes? We believe that answering these questions will reveal a new, yet not investigated stress response mechanism in yeast cells.

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P9.13

Influence of oxidative stress on the expression level of MMP-2, MMP-9 and TIMP-1 in human aortic smooth muscle cells

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Metalloproteinases (MMPs) of extracellular matrix (ECM) belong to a group of zinc-dependent endopeptidases produced and secreted by inflammatory cells and smooth muscle cells, both in physiological and pathological states. They possesses the ability to proteolytic degradation of extracellular matrix proteins. MMPs participate in pathogenesis of many cardiovascular diseases like atherosclerosis or aneurysms. They also play a major role in angiogenesis, cancer development, invasion and metastasis. The cognition of the factors influencing the expression and activity of MMPs and their tissue inhibitors seems to be very important to clarify the pathomechanism of many disorders. One of such factors could be reactive oxygen species (ROS) involved in numerous processes occurring inside the cells – both physiological and pathological.
The aim of the study was to investigate an influence of H_2O_2 exogenously added in various concentration or endogenously generated after TNF-α stimulation on the expression of MMP-2, MMP-9 and TIMP-1in human aortic smooth muscle cells (HASMC). It allowed to compare expression levels and activities of the above-mentioned enzymes under oxidative stress originating from different sources – from outside the body and generated inside the cells.In the experiment, human aortic smooth muscle cells were treated with 10, 50, 150 and 300 μM H_2O_2 or TNF-α in concentration of 50 ng/ml. Additionally, some groups of cells were pretreated with 1mM N-acetylcysteine as an antioxidant in order to determine its contribution to oxidative stress-dependent regulation of investigated MMPs and TIMP-1 expression. The protein level was measured using Western blot analysis and ELISA assay.
The obtained results allow understanding the role of oxidative stress in extracellular matrix remodeling.

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P9.14

The loss of stress response genes results in redox disequilibrium and susceptibility to DNA damage in biotechnologically relevant brewing yeasts

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Industrial yeast strains of the *Saccharomyces sensu stricto* complex are widely used in a plethora of biotechnological processes including brewing, winemaking and distilling. However, their genetic and genomic features are not adequately addressed. In the present study, a selected group of commercially available brewing yeasts both ale top-fermenting and lager bottom-fermenting strains (n=30) was genetically characterized. The diversity of chromosome patterns was observed and four strains (three ale strains and one lager strain) with the most accented genetic variabilities were selected and subjected to genome-wide array-based comparative genomic hybridization (array-CGH) analysis. The differences in the gene copy number were found in five functional gene categories: 1) maltose metabolism and transport, 2) response to toxin, 3) siderophore transport, 4) cellular aldehyde metabolic process and 5) L-iditol 2-dehydrogenase activity (p .05). In the lager strain with the most pronounced differences in the array-CGH profile, the loss of aryl-alcohol dehydrogenase (AAD) genes was detected using both array-CGH and qRT-PCR that was accompanied by redox imbalance, oxidant-based DNA damage and breaks, reduced levels of nucleolar proteins, Nop1 and Fob1, and increased sensitivity to fermentation-associated stress stimuli. We suggest that compromised stress response may promote oxidative stress-mediated changes in the nucleolus state that may affect fermentation performance.

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P9.15

Metal binding by cystatin C and its C-terminal fragment

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In recent years, the hypothesis concerning the significant role of metal ions in the development of neurodegenerative diseases such as Alzheimer's or Parkinson's gains growing interest. Disturbances in the homeostasis of metal ions such as zinc, copper and, especially, iron were shown to play an important role in the misfolding of several amyloidogenic proteins, like α-synuclein, or peptides, like amyloid beta (Aβ), directly involved in the etiology of these disorders [1]. This has drawn the attention of scientists to search for new targets with similar properties, especially among those, for which oligomerization leads to a disease state. Human cystatin C (hCC) could be one such target. Physiologically it is an important inhibitor of cysteine proteases and modulator of many physiological and pathological states [2]. Native hCC, present at particularly high concentrations in cerebrospinal fluid (CSF) was shown to possess both neurodegenerative and neuroprotective propensities [2]. Its L68Q variant is associated with a hereditary form of cerebral amyloid angiopathy (Icelandic type) [3] and the wild type protein was shown to modulate the oligomerization and toxicity of amyloid-β peptide [4], as well as to be involved in the response to oxidative stress of neuronal cells [2]. These activities of cystatin C directed our interests towards studying the factors that could modulate its oligomerization state and ligand binding properties in more detail. One of these factors could be metal ions.

In our studies, we have focused on the interaction between hCC and two metal ions: copper and zinc. We have designed and obtained a synthetic peptide encompassing the potential metal binding region in the hCC sequence. Complex formation was studied by mass spectrometry, ITC and NMR. The impact on hCC dimerization and oligomerization was studied by gel filtration and spectroscopic methods.

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P9.16

DNA repair and cell cycle checkpoints are not affected during cell cycle re-initiation

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In proliferating cells the DNA damage response (DDR) is controlled by the cycle checkpoints, which give a cell additional time to repair the damage. However, in cells residing outside the cell cycle – in G0 phase – the activation of canonical G/S, intra-S and G2/M checkpoints is limited. In this research we evaluated the role of quiescence induction and cell cycle re-initiation on the kinetics of DNA repair and distribution of cells in the cell cycle in response to oxidative stress.
Breast cancer cell line MCF-7 was used to model quiescent cells and cells that re-initiate cell cycle by blocking or stimulating estrogen receptor, respectively. The cells were subjected to acute or chronic oxidative stress and the level of intracellular ROS and DNA damage, the kinetics of DNA damage repair and the distribution of cells in the cell cycle were quantified.
Upon cell cycle re-entry the intracellular level of ROS and DNA damage increased to that typical for cells with unperturbed cell cycle in response to oxidative stress. Neither DNA repair nor the cell cycle were affected in quiescent cells, cells that re-initiated cell cycle and cycling cells subjected to oxidative stress.
These data suggest that the increased sensitivity to oxidative stress-induced DNA damage upon cell cycle re-entry does not result from a change in DNA repair nor in the activation of cell cycle checkpoints.

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P9.17

Preliminary analysis of transcriptome of the MCF7 cells with HSF1 knockdown after heat shock or estrogen exposure

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Heat Shock transcription Factor 1 (HSF1) is an evolutionarily well-conserved transcription factor, which is activated under stress conditions. It is considered as primary regulator of heat shock genes expression, encoding for Heat Shock Proteins (HSPs). HSPs act as molecular chaperones, which assist in protein folding during synthesis and repair or contribute to protein degradation under proteotoxic stress. The function of HSF1 as the cytoprotective factor and activator of the heat shock response is well described in experiments utilizing severe hyperthermia. Additionally, HSF1 plays a key role in tumor biology in many cancer cell lines, supporting the malignant transformation as well as tumor progression.
Estrogen (E2) is an important mitogenic factor which enhances cancer cells proliferation. Since we found that E2 treatment leads to activation of HSF1 (phosphorylation on Serine 326) in human breast adenocarcinoma MCF7 cells, we aimed to study whether HSF1 can support genomic action of the estrogen receptor. We silenced HSF1 in MCF7 cells using specific lentiviral shRNA. To find out the changes in the whole transcriptome we performed the RNA-Seq. Expression of typical heat shock genes was strongly induced in cells after hyperthermia (e.g. *HSPA6*, *HSPA1*, *HSPH1*, *DNAJA4*), while after estrogen exposure the highest induction was observed in case of *CYP1A1*, *SLC7A5*, *BIRC3*, *EGR3*.
The *Gene to GO BP* (Gene Ontology Biological Process) analysis revealed that silencing of HSF1 resulted not only in downregulation of genes involved in response to stress and protein folding, but also in deregulation of genes involved in signal transduction. Looking for differences in response to estrogen treatment we found that among activated genes, those involved in cell adhesion were overrepresentedin cells with silenced HSF1, but not in control ones. This findings suggest that in the presence of estrogen HSF1 could change the ability of cells to contact with extracellular matrix and other cells influencing their motility as well as their metastatic potential.

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P9.18

Transmembrane topology of the arsenite permease Acr3 from *Saccharomyces cerevisiae*

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Acr3 is a plasma membrane transporter, a member of the bile/arsenite/riboflavin transporter (BART) superfamily, which confers high-level resistance to arsenicals in the yeast *Saccharomyces cerevisiae*. We have previously shown that the yeast Acr3 acts as a low affinity As(III)/H^+ and Sb(III)/H^+ antiporter. We have also identified several amino acid residues that are localized in putative transmembrane helices (TM) and appeared to be critical for the Acr3 activity. In the present study, the topology of Acr3 was investigated by insertion of glycosylation and factor Xa protease cleavage sites at predicted hydrophilic regions. The analysis of the glycosylation pattern and factor Xa cleavage products of resulting Acr3 fusion constructs provide evidence supporting the ten-transmembrane topology model of Acr3 with its N- and C-terminus facing the cytoplasm. Next, we investigated the role of large insertion of 28 residues in the cytoplasmic loop connecting TM8 and TM9 that is present only in yeast members of the Acr3 family. We found that this region is not important for Acr3 folding, trafficking, substrate specificity, or transport activity. We also constructed the homology structural model of Acr3, based on the *S. cerevisiae* Acr3 transmembrane topology and the structure of *Yersinia frederiksenii* homologue of the human bile acid sodium symporter ASBT.

P9.19

Ubc9 fusion-directed SUMOylation of human transthyretin

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Transthyretin (TTR) transports thyroid hormones in the serum and brain. TTR protects the brain against insults, such as Alzheimer's disease or ischaemia. However, misfolded TTR results in amyloid deposits in senile and familial amyloidopathies. To resolve this apparent contradiction, we used a SUMO-conjugating enzyme (Ubc9) fusion-directed SUMOylation system, to investigate the SUMOylation of TTR, one of the post-translational modifications modulating protein structure and function. We found that TTR is not simply a substrate for Ubc9 because the SUMOylation of TTR is dependent on and affects the SUMOylation of Ubc9, which is known to alter the specificity of Ubc9 towards a subset of cellular proteins. These findings suggest that complex cross-talk occurs between TTR and Ubc9, regulating the general SUMOylation of cellular proteins.

P9.20

Translocated FGF1 and FGF2 protect the cell against apoptosis independently of FGF receptor activation

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Fibroblast growth factors 1 and 2 (FGF1 and FGF2) bind to specific cell-surface tyrosine kinase receptors (FGFRs) and activate intracellular signaling that leads to proliferation, migration or differentiation of many cell types. Besides this classical mode of action, under stress conditions FGF1 and FGF2 are translocated through the endosomal membrane into the cytosol and nucleus of the cell in a receptor dependent manner. However, despite many years of research, the role of translocated FGF1 and FGF2 inside the cell still remains unclear. Here, we reveal the anti-apoptotic activity of intracellular FGF1 and FGF2, which is independent of FGFR activation and downstream signaling. We observed the inhibition of cell apoptosis induced by serum starvation, staurosporine or p53 activators upon treatment with exogenous FGF1 or FGF2, despite the presence of highly potent FGFR inhibitors. Similar results were found when tyrosine kinase of FGFR was completely blocked by specific mutation, showing that anti-apoptotic activity of translocated FGF1 and FGF2 is separated from the receptor activity. Moreover, the anti-apoptotic effect of growth factors was abolished in the presence of known inhibitors blocking the translocation of FGF1 and FGF2 from the endosomes to the interior of the cell. We also found that endogenous FGF1 and FGF2 expressed by transiently transfected cells were able to decrease the staurosporine-induced apoptosis. Taken together, our data indicate that the translocation of FGF1 and FGF2 protects cells against apoptosis and promotes cell survival.

P9.21

A. thaliana ALKBH6 dioxygenase may play an important role in ABA mediated stress response

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AlkB protein, primary discovered and studied in *Escherichia coli*, is a non-heme iron (II) and α-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in nucleic acids recovering natural bases A and C, respectively. Our and others bioinformatic analysis showed the presence of *E. coli* AlkB homologs in almost all organisms. In *Arabidopsis thaliana* we identified 13 potential homologs of bacterial AlkB protein. Multiplication of ALKBHs in *Arabidopsis* may correspond to their functional diversification and possible involvement in various cellular processes.

In our previous study we have found that after MMS treatment, *A. thaliana* ALKBH6 changes its subcellular localization from cytoplasmic to nuclear, indicating possible role of the protein in alkyl lesions repair or general stress response. In order to decipher its exact function in stress response, we checked the expression level of *ALKBH6* gene before and after treatment with alkylating agents: MMS, CAA, and MNNG, and potent DNA crosslinker – Mitomycin C. We stated that there are no changes in the *ALKBH6* expression level after this treatment. Subsequently, we found an insertion mutant in *ALKBH6* gene showing decreased level of *ALKBH6* and we exposed the homozygous mutant to different stress stimuli like alkylating agents, salt, cold and osmotic stress, and plant hormones or their inhibitors. We observed no phenotype differences in root or hypocotyl length after neither stress nor hormone stimuli with exception of abscisic acid (ABA) treatment. We proved that the germination ratio of mutant seeds on medium containing ABA was decreased in comparison to wild type seeds. Our results indicate that ALKBH6 may have no function in alkyl lesions repair, but possibly plays a role in ABA mediated plant responses to environmental stress or plant pathogens.

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