12. Yeast models for human disease and ageing

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12-01

Mitochondria-independent morphological and biochemical apoptotic alterations promoted by the antitumor agent bleomycin in *Saccharomyces cerevisiae*.

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Bleomycin is a highly potent cytotoxic and genotoxic agent used in the chemotherapy of various types of tumors. It is a radiomimetic anticancer drug that produces single- and double stranded DNA breaks in a catalytic way. Using *Saccharomyces cerevisiae* as a model system, here we show that when a high amount of bleomycin molecules was internalized (100 μM) morphological changes identical to those usually associated with apoptosis *i.e.* sub-G_1 region peak, chromatin condensation as well as very rapid DNA fragmentation into oligonucleosomal-sized fragments were observed. The known bleomycin inhibitors cobalt and EDTA were able to prevent bleomycin nucleasic activity and thus apoptotic cell death. However oligomycin, a potent inhibitor of the mitochondial F_0F_1-ATPase or antimycin, a drug affecting mitochondria respiration, were unable to prevent the bleomycin-induced apoptotic-like cell death. These results suggest that high bleomycin concentrations induce an apoptosis-like mitochondria-independent cell death in yeast.

12-02

Role of mitochondrial proteins in yeast aging and apoptosis.

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Deletion of YGR076C (*MRPL25*) caused marked resistance to oxidants and led to a 60% increase in the mother cell-specific lifespan. An investigation of the transcription factor Sfp1p in wild type and mutant under the influence of rapamycin gave us a clue as to how Ygr076cp might influence the TOR signaling pathway which controls growth, and according to our data, yeast mother cell-specific lifespan. Rapamycin, which inhibits growth, leads to inhibition of the *TOR1* kinase and to the shuttling of Sfp1p out of the nucleus. In the YGR076C-Δ strain, which is rapamycin-resistant, Sfp1p remains in the nucleus. We hypothesize that Ygr076cp is a negative regulator of *TOR1*, whose absence leads to active *TOR1* kinase and shuttling of Sfp1p to the nucleus. We propose to name the signaling pathway which we have described here 'generalized retrograde response'. It is one of the pathways by which mitochondrial physiology controls nuclear gene expression. The TOR signaling pathway is a general eukaryotic pathway that has been shown in *C. elegans* and in *Drosophila* to be crucial for the control of lifespan. Mmi1p, the yeast homolog of the highly conserved TCTP protein family, in stressed yeast cells shuttles to mitochondria and prevents apoptosis induced by mammalian Bax. Recently we have identified and characterized the yeast superoxide producing NADPH oxidase (*NOX1*) and are studying the role of this enzyme in aging and apoptosis.

12-03

A putative pathway for Endonuclease G mediated apoptosis.

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Endonuclease G (EndoG) is located in mitochondria, yet translocates into the nucleus of apoptotic cells during human degenerative diseases. Nonetheless, a direct involvement of EndoG in cell death execution remains equivocal and the mechanism for mitochondrio-nuclear translocation is not known. Here, we show that the yeast homolog of EndoG (Nuc1p) can efficiently trigger apoptotic cell death when excluded from mitochondria. We demonstrate that the permeability transition pore, karyopherin Kap123p, and histone H2B interact with Nuc1p and are required for cell death upon Nuc1p overexpression, suggesting a pathway in which mitochondrial pore opening, nuclear import, and chromatin association are successively involved in EndoG mediated death.

12-04

Evolution of prion domains and species barrier in yeast.
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Self-perpetuating amyloid aggregates (prions) cause infectious neurodegenerative diseases in mammals and transmit heritable traits in yeast. The ability of a yeast protein to generate and propagate an amyloid state is controlled by a specific region of the protein termed prion domain (PrD), which is characterized by a modular structure. Although PrD usually evolves faster than the functional region of the same protein, amyloid-forming potential of PrD is maintained in evolution, despite variations in sequence. In some combinations, sequence divergence impairs transmission of the prion state from a prion protein to its homolog, originated from a different species. This phenomenon, termed 'species barrier', is detected even for some closely related prion proteins of the *Saccharomyces sensu stricto* group, which are able to coaggregate. Thus, prion conversion does not represent a mechanical consequence of coaggregation and requires a higher level of sequence identity at certain regions. Moreover, slight sequence differences may result in 'poisoning' of amyloid growth by a divergent protein. Detailed analysis of Sup35 protein revealed that different modules of its PrD are responsible for the species barrier in different cross-species combinations, pointing to the role of both aggregation and prion propagation capabilities in the control of species specificity. Biological implications of the prion domain evolution are being discussed. (Supported by grant MCB-0614772 from NSF.).

12-05

Block of Endoplasmic Reticulum-Golgi Vesicular Transport by α-Synuclein as an Underlying Cause of Parkinson's Disease.
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Parkinson's disease (PD) is a neurological disorder involving the loss of dopaminergic neurons. α-synuclein has been identified as a central component of PD: gene duplication or mutant alleles of α-synuclein result in familial PD while Lewy bodies, cytoplasmic inclusions consisting primarily of aggregated α-synuclein, are a defining feature of PD. Using a yeast model of PD in which induction of α-synuclein was cytotoxic, we observed the earliest cellular defect following α-synuclein induction to be a block in endoplasmic reticulum (ER) to Golgi vesicular trafficking. A genome-wide screen identified the best suppressor as the Rab GTPase Ypt1p, a highly conserved protein functioning at this same ER-Golgi trafficking step, and which associated with cytoplasmic α-synuclein inclusions (Cooper et al., Science 313:324, 2006). With this strong prediction provided by the yeast PD model, our collaborators were able to demonstrate that elevated expression of Rab1a, the mammalian *YPT1* homolog, was sufficient to protect against α-synuclein induced dopaminergic neuron loss in animal models of PD. We are continuing to utilize this yeast PD model to (i) identify the molecular mechanism by which α-synuclein causes the vesicular trafficking block and (ii) identify genetic and environmental conditions responsible for the transition from non-toxic α-synuclein at the cell periphery to cytotoxic inclusions of α-synuclein that inhibit vesicular transport.

12-06

Expression of *Burkholderia pseudomallei* proteins in yeast can affect vesicle trafficking and vacuolar morphology.
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Burkholderia pseudomallei, the causative agent of meliodosis, is prevalent in tropical regions. Invading bacteria evades the host's immune response by escaping from the phagosomes into the cytosol to evade lysosomal degradation. Such cells unsuccessfully attempt to destroy the cytosolic bacteria by initiation of autophagy to engulf the bacteria. The mechanism by which *B. pseudomallei* subverts these processes is currently unknown. Several *B. pseudomallei* proteins were identified from literature and bioinformatics analysis as being homologous to pathogen effector proteins of other invading bacteria. We characterized the effect of expression of selected proteins by subjecting them to three tests. Application of the PEPSY (Pathogen Effector Protein Screening in Yeast) test (Shody et al., 2005, PNAS, 102, 4866-4871), indicated that some of the proteins tested had a defect in vesicle trafficking to the vacuole. Cells were then grown under autophagic conditions to identify any change in vacuolar morphology. Staining of the vacuoles with FM4-64 revealed multiply-fragmented vacuole morphology is associated with expression of all of the tested proteins. We also determined whether turnover of cytosol within the vacuole by autophagy could be detected using a fluorescent biosensor. In all cases it appears that cytosolic turnover via autophagy was inhibited. These results indicate that the *B. pseudomallei* proteins interfere with aspects of vesicle trafficking relating to vacuolar function.

12-07

Determining the effects of Alzheimer's Aβ in yeast and towards high-throughput assays for compounds that prevent Alzheimer's disease.

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Alzheimer's disease (AD) is the most devastating and major cause of senile dementia. Despite its high prevalence, treatments currently available only alleviate many of the symptoms. As yet the molecular mechanisms to explain the AD biogenesis remain unknown. The 42-amino acid Aβ peptide is a hallmark of AD, being a major component of senile plaques in the AD brain. By implementing yeast molecular biology techniques, we aim to decipher the underlying principles by investigating cellular responses to Aβ, to elaborate Aβ interactions with other molecules,and to develop a cell based assay to screen for therapeutics. Microarray analysis of yeast cells expressing Aβ revealed majority of the significant differentially expressed genes form part of a particular stress response, namely the heat shock response. This enabled us to devise a primary robust yeast assay to simultaneously monitor biological effects of intracellular Aβ and test for inhibitory compounds. The assay has been validated against factors that are thought to be the main culprits in AD (*e.g.* metals, reactive oxygen species, etc.)and is currently being improvised for high-throughput screening of compounds that abrogate the stress caused by Aβ in cells. The assay has many advantages owing to its convenience, robustness, high-throughput and cell based nature, eukaryotic environment and recognition of yeast as a relevant neurobiology model. This assay could thus be of value in identifying effective compounds that prevent AD.

12-08

Yeast isolates from Antarctica adapt to UV stress.

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In the current study, yeasts isolated from Antarctic soil and snow, have been exposed to various stress conditions in order to ascertain their response. One of these isolates, was identified as *Rhodotorula mucilaginosa* and this yeast, together with a number of newly identified strains, were examined in relation to heat and UVA-radiation stress. Cells grown at 25°C and subjected to a mild heat shock exhibited heat shock-induced thermotolerance to a normally lethal heat stress. Heat shock induced proteins were identified by ^{35}S-methionine labeling on SDS-PAGE. Response to UVA radiation (355-375 nm) was measured over a time course and cell viability estimated by serial dilution plate count. Essentially 100% cell survival was observed in the case of the Antarctic yeast *R. mucilaginosa*, as compared with 0% survival for the typical mesophilic yeast *Saccharomyces cerevisiae*. The coenzyme Q_8 redox ratio ($CoQH_2/CoQH_2$ + CoQ) was measured during exposure of *R. mucilaginosa* cells to UVA. The reduced CoQ redox ratio increased from 54% at time zero to 90% after a 4 hr exposure to UVA and remained constant during a 2 hr post-exposure recovery period. The observed increase in the reduced CoQ redox ratio indicated an adaptive response to photooxidative stress. Importantly, this profile was not seen in any other Antarctic or mesophilic yeast analysed, suggesting a novel stress response.

12-09

Overexpression of *RAD2* encoding a nucleotide excision repair related endonuclease induces mitotic catastrophe in *Saccharomyces cerevisiae*.

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Nucleotide excision repair (NER) is a major DNA damage repair process. Eight complementation groups are known. Among them, the xeroderma pigmentosum (XP) group G gene (*XPG*) that is essential in the incision step of NER, has a Proliferating Cell Nuclear Antigen (PCNA)-binding motif at its C-terminal region. However, the role of this motif is controversial because its presence does not affect NER. Mutations in the *XPG* gene cause XP and Cockayne syndrome depending on the nature of the mutation. On the other hands, overexpression of *RAD2* gene, a yeast counterpart of human *XPG*, interrupts cell growth. Using *RAD2*, we show that Rad2p interacts with PCNA through its PCNA-binding motif and the Rad2p overexpression causes mitotic catastrophe resulting in cell growth retardation. Mitotic catastrophe resulting from Rap2p induction is expression level dependent. While moderately overexpressed Rad2p does not affect on UV sensitivity, drastically increased Rad2p expression adversely affects on cell survival after UV irradiation. However, the apoptotic markers in the mitotic catastrophe induced cells by Rad2p overexpression were not observed by Annexin-V, TUNEL, and ROS level assays. Since inadequate mitotic catastrophe can cause genomic instability, the drastically increased skin cancer incidence in XP could arise from the synergistic effects between mitotic catastrophe by XPG-PCNA interaction and the accumulation of damaged DNA via defects in DNA damage response.

12-10

Extra-coding functions of ribosomal RNA gene repeats.
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The nucleolus is a region of the nucleus with high protein density and it acts as a ribosome factory. The nucleolus contains a distinct region of the genome, the ribosomal RNA gene repeats (rDNA) that supply ribosomal RNA (rRNA) molecules. The rDNA is the most abundant gene in the cell: for example, there are ~150 copies in yeast *S. cerevisiae*. Interestingly, it is known that only about half of them are transcribed. Moreover, as we previously reported, 20 copies of rDNA are enough for cell growth. Therefore, we speculate that the rDNA has some extra-coding functions. I would like to propose a new model regarding the functions of the rDNA and nucleolus in the nucleus in which they are important to keep genome stability and trigger aging.

12-11

Channel mutations in Hsp104 hexamer distinctively affect thermotolerance and prion-specific propagation.
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The yeast prion *[PSI$^+$]* represents an aggregated state of the translation termination factor Sup35 resulting in the tendency of ribosomes to readthrough stop codons. In this study, we constructed an auxotrophic chromosomal marker, *ura3-197* (nonsense allele), applicable to selection for loss of *[PSI$^+$]* to *[psi$^-$]*. Unlike *[psi$^-$]* yeast strains, *[PSI$^+$]* yeast strains exhibit nonsense suppression of the *ura3-197* allele and are not viable in the presence of 5-FOA that is converted to a toxic material by the readthrough product of Ura3. We selected eleven 5-FOA-resistant, loss-of-*[PSI$^+$]*, mutants spontaneously from *ura3-197 [PSI$^+$]* cells. All of the eleven *[psi$^-$]* isolates were affected in Hsp104, a protein-remodelling factor. Although most of them were disabled in a normal Hsp104 function for thermotolerance, three single mutants, L462R, P557L and D704N, remained thermotolerant. Importantly, L462R and D704N also eliminate other yeast prions [URE3] and *[PIN$^+$]*, while P557L does not, suggesting that Hsp104 harbours a unique activity to prion propagation independent of its function in thermotolerance. The mutations that are specific to prion propagation are clustered around the lateral channel of the Hsp104 hexamer, suggesting a crucial and specific role of this channel for prion propagation.

12-12

Effects of antioxidants on yeast cell replicative ageing.
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An imbalance between ROS formation during normal metabolism or as a result of environmental stress and antioxidant detoxification, leads to the accumulation of radicals. According to the free radical theory of ageing, ROS contribute by damaging nucleic acids, proteins and lipids. One form of cell ageing of the budding yeast *Saccharomyces cerevisiae* is replicative ageing, described as a reduction in reproductive capacity of a mother cell. The aim of our study is to evaluate the correlation between oxidative stress and cell ageing, in particular to assess whether an increase in any antioxidant defence level would have a beneficial effect on replicative lifespan. We employed a conditional mutant strain, K6001, to estimate the number of divisions a mother cell undergoes prior to senescence. The growth of this strain is characterised by enrichment of aged mothers because newly formed buds fail to divide. Antioxidants used included α-tocopherol, glutathione, ubiquinone (coenzyme Q10), lipoic acid and L-ascorbate. Here we report the influence of antioxidants on yeast replicative lifespan. Surprisingly it was found that some antioxidants can lead to decrease in yeast replicative lifespan. For α-tocopherol, its effect required the redox activity of the antioxidant and its ability to interact with cellular components, such as the membrane.

12-13

Investigating novel functions of A-type lamins in yeast: Insights into laminopathy-associated dystrophies and progeroid syndromes.

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Nuclear lamins are intermediate-filament proteins unique to metazoans. The two types of lamins in mammalian cells (types A and B) form a network that abuts the inner nuclear envelope. B-type lamins are present in all cells while A-type lamins are only expressed in differentiated cells. Both lamins are important in the maintenance of nuclear shape, the organization of NPCs, and the anchoring of interphase heterochromatin. Mutations in the A-type lamin gene (*LMNA*) have been linked to several dystrophic and progeroid diseases, collectively termed laminopathies. The mechanism by which mutations in *LMNA* result in laminopathies is still unclear. We tested *LMNA-DBD* fusion proteins for transcriptional activity to address if lamins have the ability to regulate gene expression. Our data indicates that lamins possess intrinsic transcriptional repression activity when targeted to a promoter in mammalian and yeast cells. Further, although not found in yeast, lamins localize aptly to the nuclear periphery when expressed. Given the structural and functional similarity in the transcription machineries of yeast and mammalian cells, we used the yeast system to determine regions of A-type lamins that are required for transcriptional repression, as well as to identify yeast genes that aid A-type lamin localization to the nuclear periphery. We are especially interested in testing various laminopathy mutants to determine if they retain the ability to repress transcription and localize correctly.

12-14

Modeling mouse breast cancer in yeast.

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A missense mutation in *Mcm4* causes a high incidence of aggressive mammary adenocarcinomas and no other types of cancers in female mice. The altered residue is located in a conserved region of *Mcm4* at the interface between subunits of the MCM complex. Assuming that the pathway to genetic instability (GI) induced by $Mcm4_{Chaos3}$ is conserved, we use *S. cerevisiae* as a model to investigate the molecular mechanism of $Mcm4_{Chaos3}$-induced cancer. The diploid yeast containing equivalent mutations undergo cycles of chromosome instability, and exhibit a G2/M delay as well as a 100-fold increased rate of mitotic recombination. In contrast, the $mcm4_{Chaos3}$ haploid is largely normal although the gross chromosome rearrangement rate is significantly increased. Further genetic analysis suggests that the ploidy effect is mainly due to the different repair pathways. In haploids, repair is conducted mostly by Mgs1- and Rad6-dependent pathways suggesting that the $Mcm4_{Chaos3}$-induced damages are mainly stalled forks. However, in diploids, the repair is conducted solely by homologous recombination that requires the MRX complex, suggesting that the main damages are DSBs. In conclusion, GI induced in mice by $Mcm4_{Chaos3}$ can be recapitulated in yeast. This study suggests that $Mcm4_{Chaos3}$ causes stalled replication forks, which are repaired by different pathways in haploids and diploids, which may be relevant to the tissue-specificity of cancer caused by $Mcm4_{Chaos3}$ in mammals.

12-15

A longevity network for *Saccharomyces cerevisiae*.

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The Akt/PKB, Ras, and Tor pathways have been implicated in life span regulation in different model systems but the mechanisms by which they regulate life span are poorly understood. Yeast lacking Sch9, Ras2, and Tor1, orthologs of mammalian Akt/PKB, Ras, and mTor respectively, were shown to be stress resistant and long-lived. Here we present gene expression and genetic data indicating that Sch9, Ras2, and Tor1 control a remarkably common set of genes functioning in stress resistance and in a wide variety of metabolic processes. DNA microarray analysis of aging populations of long-lived yeast mutants suggests a cellular state that favors increased glucose metabolism (induced expression of glucose transporters and genes involved in glycolysis), activation of glycerol biosynthesis and autophagy but attenuated mitochondrial function (decreased expression of mitochondrial ribosomal proteins and genes involved in ATP synthesis and TCA cycle). Higher levels of glycerol production are detected in all the long-lived mutants and survival experiments conducted in the presence of glycerol show that this carbon source plays a role in promoting life span extension. The striking similarities between the gene expression profiles of the different long-lived mutants support the hypothesis that the three major longevity pathways so far identified converge on the activation of common down-stream regulatory mechanisms.

12-16

Functional expression in yeast of two human chloride channels.
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Chloride is the most abundant extracellular anion, involved in multiple biological processes such as control of intracellular volume, gastric secretion, control of pH, electrolytic balance etc. The pass of chloride to and from the cell plays a fundamental role in the maintenance of the homeostasis for all living systems. This movement of chloride occurs through the activation of different mechanisms that include volume and voltage sensitive channels and co-transporters; all of them represented within the family of ClCs. In mammals nine genes encode for homologous ClC proteins. They are known as *ClC-1* to *ClC-7*, *ClC-Ka* and *ClC-Kb* sharing amino acid sequences of 30-80%. The human channels hClC-1 and hClC-2 are regulated by voltage and share with the ClC of *Saccharomyces cerevisiae* (Gef1p) conserved amino acid sequences, especially those that form the pore. Mutations in *GEF1* induce a petite phenotype which, in some cases is reverted with the expression of homologous proteins such as *ClC5* and *ClC7*. In this study we evaluated the reversion of the petite phenotype of a yeast *gef1* strain after expressing the hClC-1 or hClC-2. In both cases the reversion of this phenotype was determined by the increase in size of the colonies as well cell diameter and the recovery of the oxygen consumption. Acknowledgments: CONACYT-44943, PAPIIT-IN204806 and Dr. Martinez, I and Lopez, A.

12-17

The age old mystery of aging – a genome-wide functional analysis in *Saccharomyces cerevisiae*.
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Aging is a process ascribed as being the exponential decline in vitality of a cell or organism as it matures. Inevitably this decline leads to the death of an organism or cell via the natural decay of molecular processes, cellular integrity and or acquisition of an array of chronological diseases. There have been many theories put forward to define this process including caloric restriction, ROS and genome instability. To gain an insight into these mechanisms on a genome-wide functional level, short-lived phenotypes in *Saccharomyces cerevisiae* were identified with the use of a series of chronological lifespan screens. A large number of these short-lived mutants had genes deleted that encoded for proteins that have a role in the formation and maintenance of the mitochondria. They formed a number of novel sub-families of the mitochondria involved in ribosomal, protein synthesis and ETC processes. Correspondence analysis identified that respiration was highly significant to aging in the first 2 months of the screen; however, it became insignificant after 4 months. A comparison between the aging mutants in this study with mutants sensitive to oxidative stress found that of the oxidants tested, H_2O_2 was the only one that appeared significant. This significance was present over the first 2 months and drastically decreased after 4 months similar to the correlation between the ability to respire and aging mutants.

12-18

Yeast as a model to study genes linked to neurodegenerative diseases and α synuclein toxicity.
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The budding yeast *Saccharomyces cereviseae* has recently become a suitable model to study specific aspects of the molecular mechanisms underlying neurodegenerative disorders like Parkinson's disease due to its unicellular nature, well characterised cellular pathways, availability of complete genome sequences and well established genetic tools. Lewy body formation has been mimicked in yeast by overexpressing human wildtype α synuclein and its variants. Overexpression of wildtype α synuclein and its A53T variant leads to impaired growth of yeast. Large scale screening of different compounds using an automated lab robot has enabled us to identify compounds modulating α synuclein toxicity in yeast. Moreover, several genes linked to human diseases are well-conserved in yeast. A second line of research involves the analysis of yeast homologues of genes implied in Parkinson's disease. Loss of function mutation in the gene encoding HtrA2 or Omi has been identified in patients suffering Parkinson's disease. The mouse knocked out for the gene suffers neurodegeneration due to mitochondrial damage. The yeast orthologue, called Nma111 or Ynm3 shows a dynamic localization pattern in different growth phases with a subset of the population showing colocalization of the protein with mitochondrial markers. It is therefore interesting to understand if this protein has a role in maintaining the health of mitochondria in yeast. We will present the current state of progress of our work.

12-19

Young cells of *Saccharomyces cerevisiae* with oncogenic mutation show features of aging.
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Aging and apoptosis, multifactorial processes involving a complex network of regulations, are conveniently studied on single cell models. Yeast, especially *S. cerevisiae*, is suitable for this purpose because it exhibits mother cell specific aging, where the cell divides asymmetrically, giving rise to a next-generation large mother cell and a smaller daughter cell that resets the clock to zero while mother cell accumulates all aging-connected changes. During aging, all types of molecules in the cell are gradually damaged and the cellular homeostasis is thus impaired. Both the nuclear and the mitochondrial genome are prone to progressive instability during aging. The DNA damage involves also the release of extrachromosomal rings (ECR) from rDNA, which further replicate autonomously. *S. cerevisiae* strain $RAS2_{val19}$ with oncogenic mutation exhibits accelerated aging, demonstrated as a dramatic shortening of the life span, higher sensitivity to starvation and heat stress, and as morphological aberration and appearance of apoptotic markers already in separated young cells. Quantitative comparison of the amount of ERC in separated young and old cells of the wild type, $RAS2_{val19}$ mutant and a strain with delayed aging together with other data point to the premature aging being connected with symmetric aging, where the daughter cell is already inheriting some features of aging.
Supported by the Czech Science Foundation grants 301/03/0289 and 301/07/0339.

12-20

Distinct Ca^{2+} or Mn^{2+} Fluxes via the Hailey-Hailey Disease Gene Ortholog Pmr1 are Vital during Ageing and Heat.
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In yeast, Mn^{2+} can substitute for Ca^{2+} in vegetative growth suggesting that some vital cellular process uses interchangeably Ca^{2+} or Mn^{2+} (Loukin & Kung, 1995). SPCA ion pumps transport both cations into the Golgi and appear to be vital, since only heterozygous SPCA defects are found in humans. These individuals eventually develop a skin disorder, called 'Hailey-Hailey disease'. Our study identifies flux of Ca^{2+} or Mn^{2+} through SPCA as a vital part of an essential homeostatic circuit. As we show, yeast cells lacking the SPCA pump Pmr1 face self-induced Ca^{2+}/Mn^{2+} deprivation in stationary phase and initiate apoptosis via the apoptosis-inducing factor Aif1. This apoptotic death is interchangeably suppressed by low doses of Ca^{2+} or Mn^{2+}. During an increased demand for intralumenal ER Ca^{2+} caused by heat stress, Aif1 promotes survival of *pmr1* by suppressing ROS production and subsequent Yca1-dependent apoptosis. This heat-induced apoptotic death is only suppressible by high Ca^{2+}. Our findings show that specific and interchangeable functions of Ca^{2+} and Mn^{2+} within the secretory pathway are under surveillance of Janus-faced Aif1, which reacts upon Golgi Ca^{2+}/Mn^{2+} deprivation as apoptosis executioner, but safeguards during ER Ca^{2+} deficiency against Yca1 meta-caspase-dependent apoptosis. These findings identify a shared role of Ca^{2+} and Mn^{2+} in Golgi function and provide a framework to understand how perturbed Ca^{2+}/Mn^{2+} homeostasis could cause embryonic lethality and human disease.

12-21

Evidence for Amyloid Nature of Aβ-GFP and PrP-GFP Fusion Proteins Expressing in Yeast.
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Baker's yeast *S. cerevisiae* being a genetically and functionally well-defined and easily handled eukaryotic cell is an attractive host for production of mammalian proteins and good tool for analysis of proteins misfolding and searching for therapeutic agents affecting amyloid aggregation and propagation. Fusion proteins Aβ-GFP and PrP-GFP overexpressed in yeast form oligomeres and high-weight aggregates as opposed to cells expressing bare GFP. Cytoplasmic localization of large fluorescent aggregates of the fusion proteins has been proved by staining with fluorescent dyes for particular cellular compartments. Fluorescence recovery after photobleaching (FRAP) examination of PrP-GFP fluorescent fibrils and visible clumps of Aβ-GFP in living cells have revealed significant portion of immobile fraction in the aggregates, which reflects tight stable interactions between individual molecules. These data are well agreed with high resistance of the fusion proteins to SDS treatment as well as yeast proteases and proteinase K digestion. The results suggest amyloid nature of PrP-GFP and Aβ-GFP aggregates in yeast making the system useful for analysis of factors affecting amyloidogenesis. This work is supported by Fogarty (TW006965-01A1), Ministry of Education and Science RF (PHII.2.2.2.3.10047) and CRDF (BRHE Y4-B-12-04).

12-22

Characterisation of Alzheimer's disease Aβ fused to green fluorescent protein.
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We are utilising yeast to understand the neurotoxicity of the Alzheimer's disease (AD) amyloidogenic peptide, Aβ, and to develop high-throughput assays to identify agents that would decrease the amount of Aβ aggregation. Yeast expression vectors for producing N- and C-terminal fusions of Aβ to green fluorescent protein (GFP) were generated for intracellular studies employing flow cytometry and microscopy. The attachment of Aβ to GFP caused its localisation to lipid particles, identified by Nile Red staining. In comparison to GFP producers, there was greatly reduced fluorescence within the population of cells transformed with plasmids directing synthesis of GFP fused to Aβ. We are identifying factors that cause an increase fraction of the cell population to have green fluorescence, indicative of decreased aggregation. The observed effects of Aβ in yeast lead to a tractable system for identifying compounds that may ultimately provide a therapeutic benefit in AD.

12-23

Quantitative trait analysis in yeast: lessons from high-temperature growth.
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Quantitative traits are ubiquitous in nature and yeast is an excellent model for studying them. In clinical isolates of yeast, high temperature growth (Htg) is a quantitative trait and by linkage analysis, we mapped two quantitative trait loci (QTL) contributing to the phenotype in two clinical strains. These QTLs were dissected by reciprocal hemizygosity analysis and four genes contributing to this phenotype were identified. One of the QTL had a complex architecture with two alleles coming from the clinical strain and one allele coming from the non-Htg laboratory strain. The second QTL had only one allele from the clinical strain contributing to the phenotype. For all of these alleles, coding and non-coding single nucleotide polymorphisms were identified. Alleles of these genes showed complex genetic interactions which included additive and non-additive interactions. Genetic background in which these alleles exist profoundly influenced their contribution to the phenotype. None of these genes contributing to the phenotype have functions known to influence growth at high temperature and they act in different cellular pathways. Analysis of Htg using genome-wide expression and deletion pool analysis will be discussed. Yeast is helping to uncover complexities and develop techniques for quantitative trait analysis, which will aid to understand and dissect quantitative traits in higher eukaryotes, including humans.

12-24

Depletion of the large ribosomal subunit extends yeast replicative lifespan by a mechanism similar to calorie restriction.
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In yeast, lifespan extension by calorie restriction is mediated by the highly-conserved, nutrient-responsive TOR, PKA and Sch9 kinases. These kinases coordinately regulate various cellular processes including stress response, protein turnover, cell growth, and ribosome biogenesis. To identify which of these processes is responsible for lifespan regulation, we measured the replicative lifespan for 107 deletion strains, each lacking a single ribosomal protein gene. 24 deletion strains were identified as long-lived and, strikingly, every one of these lacks a gene encoding a 60S ribosomal protein. Reduction of 60S subunit levels by deleting genes encoding 60S subunit proteins, by deleting genes encoding 60S-specific processing factors, or by pharmacological intervention is sufficient to increase replicative lifespan by up to 50%. From these data, we conclude that a reduction of 60S subunit levels delays replicative aging in yeast. Depletion of 60S subunits extends lifespan independently of the histone deacetylase Sir2, and calorie restriction fails to further increase the lifespan of cells with reduced levels of 60S subunits. Furthermore, we have determined that Gcn4, a translationally-regulated, nutrient-responsive transcriptional regulator, is required for lifespan extension by depletion of 60S ribosomal subunits. Together our data suggest that depletion of 60S ribosomal subunits is acting through Gcn4 to extend lifespan by a mechanism similar to that of calorie restriction.

12-25

Regulation of mitochondrial activity and genes involved in aluminum resistance in *Rhodotorula glutinis* IFO1125.
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Aluminum ion (Al) severely inhibits plant root elongation in soil, and is known toxic to mammals as well. A red yeast, *Rhodotorula glutinis* IFO1125 is sensitive to Al below pH 4, but the strain acquired the resistance to Al when cultivated in Al-containing medium at pH 4. The resistance was induced by treatment of more than 5 μM Al, and the resistance level increased up to 5 mM Al by repetitive liquid cultivations with stepwise increment of Al concentration. The resultant resistant strains should be good models to identify Al-resistant genes and to clarify cellular responses to Al stress. Al induced stronger mitochondrial membrane potential and concomitant ROS production. The resistant strains contained increased number of mitochondria. The enzyme activities in TCA cycle and respiratory chain were regulated so that the mitochondria produced less ROS. Remarkably reduced activity of complex IV was found in resistant cells, suggesting that the deficiency of the complex would lead to the Al resistance, through producing less ROS and reduced mitochondrial membrane potential. Three genes (laccase, arsenite-translocating ATPase, and calmodulin / Ca^{2+}-dependent protein kinase) were up-regualted in the resistant strain. Introduction of these genes in *Saccharomyces cerevisiae* complemented growth deficiency in Al-containing media. Thus these genes were suggested to be relevant to Al resistance in the *R. glutinis*.

12-26

Yeast as a model for *in vivo* functional characterization of human *MLH1* gene variants.
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Loss of DNA mismatch repair (MMR) in human, mainly due to mutations in *hMLH1* gene, is linked to hereditary non-polyposis colorectal cancer (HNPCC). Not all *MLH1* polymorphisms result in loss of MMR function, therefore accurate characterization of genetic variations is essential for reliable genetic testing and effective treatment. Yeast *Saccharomyces cerevisiae* represents a perfect model for *in vivo* characterization of MMR variants, since mechanism has conserved through the evolution. Mlh1 interacts with Pms1 and acts as a matchmaker, involving other proteins in the MMR complex and thus enabling downstream reactions. Human Mlh1 alone is not functional in yeast. Due to the fact that the conserved ATP domain of the human Mlh1 protein functions properly in yeast, we speculated that its carboxy-terminal PMS1-binding domain is responsible for disabling the MMR activity. For this reason we replaced yeast *MLH1* and *PMS1* genes by their human orthologs directly on yeast chromosomes. By this intervention human orthologs were functional in yeast. HNPCC-related *MLH1* variants 274G→C and 278A→G were subsequently introduced into prepared yeast model and their effect on MMR function was determined. Our model showed to be operational for functional characterization of *MLH1* variants found elsewhere within the coding region of the gene in cancer patients. Analysed *MLH1* variants were shown be polymorphisms.

12-27

Use of yeast to study biological effects of statins (HMG-CoA reductase inhibitors).
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Statins lower cholesterol levels through their action on 3-hydroxy-3-methyglutaryl-CoA reductase in the mevalonate pathway. Yeast HMG-CoA reductases are also inhibited by statins, resulting in lower ergosterol levels (the yeast equivalent of cholesterol) plus several additional effects. Highly noticeable was the inhibition of respiratory growth and in the yeast *C. glabrata*, statins increased the petite frequency during culture. In addition statins affected cell size, cell segregation and cell permeability. *C. glabrata* treated with statins for 48 hours showed clump formation due to the lack of cell segregation. The clump formation was observed in a time- and dose-dependent manner. Parallel studies carried out in other yeasts like *S. cerevisiae* also showed similar clump formation and semi permeability of the cells. As well, propidium iodide (PI) staining showed that statin-treated cells were permeable to the dye after exposure to statins for 48 hours. However, upon re-culture in fresh statin-free media normal cells budded off from the clumps, demonstrating that clumped cells were still viable. These observations in yeast can provide further insights as to the effect of statins in humans. In addition if *C. glabrata* is a valid model to study statin effects then it can be used for preliminary screening of cholesterol lowering agents.

12-28

Alzheimer's Beta Amyloid Fusion Protein Kills *Saccharomyces cerevisiae*.
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The beta amyloid (Aβ) peptide is a major component of senile extracellular plaques found in brains of patients suffering from Alzheimer's disease. It is thought to be responsible for the progressive cognitive decline associated with Alzheimer's disease. Although the Aβ peptide has been shown to be toxic in neuronal cells and mammalian models, the nature of the toxic Aβ species and its precise mechanism of action remain to be determined. The variable nature of the Aβ peptide and the complex nature of mammalian models have been the major obstacles in understanding the mechanism of Aβ peptide toxicity. We constructed a stable maltose binding protein (MBP)-Aβ fusion protein, which served to be a more economical and physiologically adaptive model for studying Aβ toxicity. MBP-Aβ fusion protein caused ~50% decreased viability in *Saccharomyces cerevisiae*. The cells treated with MBP-Aβ fusion protein also showed significant nuclear fragmentation and increased ROS production suggesting apoptosis as the major cause of cell death.

12-29

Microtubule instability increases α-synuclein toxicity in a yeast model of Parkinson's disease.
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The accumulation of fibrillar form of α-synuclein in neuronal Lewy bodies has been implicated in Parkinson's disease. However, the mechanisms for aggregation and toxicity of α-synuclein still remain obscure. Here we used *Saccharomyces cerevisiae* as a model system to uncover molecular mechanisms triggering α-synuclein-mediated cellular toxicity. Studies with truncated α-synuclein mutants showed that the N-terminal membrane binding domain of α-synuclein is necessary for cellular toxicity. In order to identify molecular chaperones regulating the toxicity of α-synuclein, we screened 57 non-essential chaperone gene deletion mutants for increased toxicity upon α-synuclein overexpression from a high copy number plasmid. The toxicity of α-synuclein increased in deletion mutants of the prefoldin complex subunits (*GIM1*, *GIM2*, *GIM3*, *GIM4*, *GIM5* and *GIM6*), which play an important role in folding of actin and tubulin. In addition, deletion of tubulin folding cofactor *RBL2* and *ALF1* increased α-synuclein toxicity. Expression of α-synuclein from a low copy plasmid did not inhibit cell growth. However, cellular toxicity was observed even in cells expressing low levels of α-synuclein upon treatment of microtubule depolymerizing drug benomyl, or overexpression of *TUB1* gene encoding α-tubulin. Taken together, these results suggest that microtubule instability can be one of the cellular factors that trigger α-synuclein-mediated cellular toxicity.