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Meeting Report

Killing and chilling in Graz

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'7th International Meeting on Yeast Apoptosis' in Graz, Austria, 9–13 September 2009

From 9 to 13 September 2009, more than 100 scientists from 22 different countries came together in the lovely city of Graz in Styria/Austria to share information and experiences, as well as to discuss their data on 'killing' yeast at the 7th International Meeting on Yeast Apoptosis. This year's meeting was organised by Frank Madeo and colleagues, who did not miss the chance to create a chilling atmosphere accompanied by wine tasting, folk music and traditional food in the typical Austrian countryside, as well as on a viewpoint in the old city centre of Graz, to make their stay as glamorous as possible and share their two passions, science and dissoluteness.

This year, the participating groups reported great deliverables from their recently published and ongoing work on cell death, stress defence and ageing in yeast. Several researchers presented their work with humanised yeast models to investigate cell-death pathways and possible drug therapies for treating neurodegenerative diseases and cancer.

The opening lecture was given by the most cited cell-death researcher Guido Kroemer (Paris, France) who presented an overview into the progress of cell-death research in the last 20 years, including the turnabout from a rather 'nucleocentric system' to the 'mitochondriocentric system' of programmed cell death (PCD) in multiple organisms. In his lecture, he focused on the evolutionary development of PCD and on the emerging concept that any kind of protein that has previously been specifically implicated in cell-death pathways has a phylogenetically conserved apoptosis-unrelated function. Consequently, he hinted at the increasing importance of the yeast system to clarify pathways and discover factors, which would support current views and generate new views on ageing, cell death and diseases.

Cell-Death Pathways and Key Players

As apoptotic markers were described in yeast for the first time, a dozen years ago,[1] several yeast key players of cell death and their mode of action have been characterised.[2,3] In addition, yeast is a useful tool for investigating mammalian cell death proteins, and their regulation, functions and interactions. Manuela Côrte-Real (Braga, Portugal) presented a study of mammalian protein kinase C (PKC). Distinct isoforms of PKC specifically modulate the anti-apoptotic effect of Bcl-xL in yeast, and are currently investigated for their role in the regulation of pro-apoptotic Bax.

Esther Owsianowski (Basel, Switzerland) addressed the regulation of Bir1p. Her data indicated that SUMOylation regulates the stability of Bir1p. An *in vitro* interaction of Bir1p with the anti-apoptotic E3 ubiquitin and SUMO ligase Bre1p could be demonstrated, although the role of SUMOylation of Bir1p by Bre1p remains elusive.

Karin Thevissen (Leuven, Belgium) hinted at the physiological involvement of *IPT1* and *SKN1*in the biosynthesis of sphingolipid mannosyl diinositolphosphoryl ceramide, and their role in triggering autophagy in response to nutrient starvation. Her results demonstrated a role of Ipt1p and Skn1p in the negative regulation of autophagy with a putative involvement of sphingoid bases in this process.

Renée Guérin (Montréal, Canada) demonstrated in the fission yeast *Schizosaccharomyces pombe* that the ER chaperone calnexin regulates an ER stress-triggered apoptotic pathway, independently of the metacaspase Pca1p and the pro-apoptotic protein Bap31p. New data suggest that calnexin also participates in apoptosis initiated by inositol starvation and that the cleavage of calnexin has regulatory roles.

Tomas Grousl (Prague, Czech Republic) presented new insights into the assembly of stress granules (SGs) in heat-shocked cells of *Saccharomyces cerevisiae* and illustrated that different scaffolding proteins are required to induce SGs.

In the last few years, RNA polymerase II also turned out to transcribe, besides its well-known mRNA-generating activity, non-coding RNAs with potential regulatory roles. These transcripts are degraded by various different mechanisms. Cristina Mazzoni (Rome, Italy) demonstrated that yeast mutants in genes of the mRNA-decapping pathway exhibit increased mRNA stability, premature ageing and undergo apoptosis by a *YCA1*-dependent pathway.

Ana Kitanovic (Heidelberg, Germany) presented a novel high-throughput method to examine the influence of extracellular parameters, such as culture conditions on cellular sensitivity to DNA damage using a computer-controlled robot system. This systematic and comprehensive assessment enabled the generation of a large detailed data set for several different strains.

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Sergio Giannattasio (Bari, Italy) elucidated the time course of features of the cell death programme and their mutual interrelationships with an experimental system, in which cell death is induced by acetic acid. *En route* to acetic acid-induced cell death, he observed the early production of ROS, the subsequent release of cytochrome *c* from coupled mitochondria into the cytosol, where it can function as an electron donor and as a ROS scavenger, and the later increase in caspase-like activity. His data suggested that alternative acetic acid-induced cell-death pathways exist, which can be either yeast caspase 1 (Yca1p) dependent or Yca1p independent.

Oxidative Stress and Cell Death

Under various stress conditions and during ageing, cell death is often accompanied by strong production of ROS within cells.[4] These oxygen radicals are mostly key elements of cell-death execution. Conversely, faced with life-extending scenarios, such as caloric restriction, cells show an increase in antioxidant defence mechanisms and thus an increased resistance against oxidative stress, as well as decreased levels of intracellular ROS. By stimulating cell death using exogenous oxidants, such as hydrogen peroxide, the decisive proteins and organelles involved in oxidative stress-related apoptotic pathways, can be identified and further characterised.

Using *in vitro* cleavage assays, Paula Ludovico (Braga, Portugal) provided an insight into the role of the Yca1p in proteolytic degradation of the glycolytic enzyme GAPDH and other metabolic enzymes during oxidative stress-induced apoptosis. *In silico* analysis of amino-acid sequences revealed a possible common cleavage site for Yca1p. Nitric oxide (NO) has been shown to have an important signalling role for GAPDH, and a function of GAPDH in the stimulation of autophagy has been demonstrated.

Zhaojie Zhang (Laramie, WY, USA) delineated how hydrogen peroxide causes interruption of the cell cycle and consequently triggers cell death by the activation of the anaphase-promoting complex. Bir1p, a chromosomal passenger protein, and an inhibitor of yeast apoptosis, might have an important role in linking cell-cycle misregulation to cell death under oxidative stress.

Pepijn De Snijder (Leuven, Belgium) analysed the role of the protein kinase Sch9 in oxidative stress defence and mitochondrial functionality. He showed that *SCH9*-deficient cells display different sensitivity against hydrogen peroxide treatment depending on their growth phase. Sch9p appears to affect nucleocytoplasmic localisation of Yap1p, an osmostress and ROS-responsive transcription activator. In Δ*sch9*-cells, ROS levels are drastically decreased during the diauxic shift and during the stationary phase, and a marked reduction in the mitochondrial membrane potential was observed.

Michael Breitenbach (Salzburg, Austria) identified a yeast gene encoding an ER-located superoxide-generating NADPH oxidase (*NOX1*) and characterised its activity both *in vivo* and *in vitro*. Yeast cells, overexpressing Nox1p, display a growth delay, increased ROS production and a significantly larger budding index than the corresponding wild type.

Harald Klinger (Salzburg, Austria) analyzed the function of Mmi1p, a homologue of the human anti-apoptotic protein TCTP. The cytoplasmic protein Mmi1p translocates to the outer surface of the mitochondria under stress conditions, such as heat, oxidative stress, or ageing and *CDC48* mutation. Mmi1p relieves yeast apoptosis upon expression of murine bax, proving an anti-apoptotic function of Mmi1p.

The essential protein Tah18p was shown by Laurence Vernis (Orsay, France) to translocate from the cytosol to the mitochondria controlling mitochondrial integrity and cell death upon exposure to lethal doses of hydrogen peroxide. Under non-apoptotic conditions, Tah18p interacts with the anti-apoptotic Dre2p. To force their interaction, a fusion of *TAH18* and *DRE2* was expressed. The fusion protein does not translocate to the mitochondria upon hydrogen peroxide treatment and leads to a better survival of cells. The human homologue of Dre2p, the anti-apoptotic Ciapin1, replaces Dre2p *in vivo* and physically interacts with Tah18p.

Cao Ying Ying (Shanghai, China) presented the work on the *YCA1* homologue in *Candida albican*s metacaspase 1 (CaMCA1). Deletion of CaMCA1 leads to resistance to oxidative stress-induced cell death, decreased caspase activity, higher accumulation of trehalose, decreased ATP content and mitochondrial membrane potential, delayed cell growth and less ROS production.

Elisa Cabiscol (Lleida, Spain) placed emphasis on the fork-head transcription factor Hcm1p that shifts from the cytoplasm to the nucleus upon oxidative stress in a Sir2p-dependent manner and is involved in oxidative stress resistance and mitochondrial biogenesis.

David Walter and Anja Matter (Basel, Switzerland) delineated the role of histone modifications in apoptosis regulation. Bre1p, an E3 ubiquitin protein ligase, is required for the ubiquitination of histone H2B at lysine 123. Enhanced levels of Bre1p protect from hydrogen peroxide-induced cell death, whereas deletion of *BRE1* enhances cell death. Accordingly, cells lacking Bre1p show decreased lifespan during chronological ageing, a physiological apoptotic condition in yeast. Furthermore, they showed that the death of *BRE1*-deleted cells depends on Yca1p. The ubiquitination of H2B is a prerequisite for the subsequent methylation of histone H3 on lysine residues K4 and K79, mediated by the two methyltransferases, Set1p and Dot1p, respectively. Unlike Δ*dot1* cells, Δ*set1* cells exhibit apoptosis sensitivity during chronological ageing similar to Δ*bre1* cells.

Ida van der Klei (Groningen, The Netherlands) presented her work on the role of peroxisomes in viability of the yeast *Hansenula polymorpha*. Besides the mitochondria, peroxisomes produce ROS as a by-product of oxidative metabolism. The absence of the peroxisomal peroxiredoxin Pmp20p results in enhanced ROS formation and cell death. Δ*pmp20* cells display permeabilisation of peroxisomes, leading to necrotic cell death possibly induced by toxic oxidised lipids.

Within yeast colonies, cells can interact, communicate and synchronise their development. They change the pH level of their surroundings from acidic to alkaline and *vice versa*, and produce ammonia as a signalling molecule inducing extensive changes in gene expression. Zdena Palkova (Prague, Czech Republic) assessed possible roles of the oxidative stress-defence system in yeast colony development, ageing and

survival. Colonies deficient in mitochondrial superoxide dismutase Sod2p and the cytosolic catalase Ctt1p exhibit a reduced ability to produce ammonia and ROS in the centre of the colony, which was suggested to be important for ROS homeostasis and survival of the population.

Fedor Severin (Moscow, Russia) addressed the question of whether ROS generation in response to DNA damage serves to trigger cell-death cascade or to activate cell defence systems. His data revealed that ROS function as a fast signalling intermediate to activate the DNA repair system. Hyperactivation of this protection mechanism possibly leads to cell death when the DNA is heavily damaged.

Alternative Inducers of Cell Death

Besides the widely used 'classical' inducers of yeast cell death, such as hydrogen peroxide or acetic acid, the role of different chemicals, such as copper ions, fatty acids, as well as dehydration and rehydration of yeast cells, in triggering yeast cell death has been introduced.

Bing Zhou (Beijing, China) performed a genetic screen to identify genes involved in copper-induced cell death. Mitochondrial *CPR3*, genes involved in polyamine metabolism, and genes involved in autophagic pathways are implicated in cell death triggered by copper.

Patrick Rockenfeller (Graz, Austria) dealt with lipid-induced cell death using two model systems: diacylglycerole- and fatty acid-induced cell death in *ARE1*, *ARE2*, *LRO1* and *DGA1* quadrupel knockout mutants, which are devoid of lipid particles. With his experiments, he characterised the type of cell death for both model systems.

As ammonium-induced cell death is involved in several human disorders that are accompanied by hyperammonaemia, Maria João Sousa (Braga, Portugal) tried to clarify the mechanisms triggering this type of cell death. Sousa and colleagues showed that ammonium can cause cell death in *S. cerevisiae* and reported on cellular changes and on some of the genes involved. These findings prove that yeast can be used as a valuable model to identify signalling pathways and new therapeutic targets for these disorders.

Boris Rodríguez-Porrata (Tarragona, Spain) realised the importance of yeast cells to survive both dehydration and rehydration during food technology. Therefore, he dehydrated and rehydrated 4850 mutants, of which $\sim$0.2% showed improved tolerance. Among them are Δ*aif1*, Δ*cpr3*, Δ*nuc1* and Δ*qcr7*. As the deletion of *YCA1*, *OXA1* or *MGM1* did not improve resistance, they suggested that cell death triggered by dehydration stress is neither respiratory nor caspase dependent.

Age and Cell Death

Yeast is an excellent model to study the chronological and replicative ageing process. During this meeting, several findings dealing with the interconnection of growth signalling, cell cycle and longevity were presented, and molecular players and chemicals promoting longevity were introduced.

William C Burhans (Buffalo, NY, USA) reported the connection between growth signalling and longevity. If yeast cells arrest in the S phase, instead of the G_0/G_1 phase, DNA replication stress occurs, resulting in genome instability and more frequent deaths of stationary-phase cells. Oxidative stress inhibits G_0/G_1 arrest in the stationary phase, thus inducing replication stress. By monitoring ROS accumulation and G_1 arrest, he compared long- and short-living yeast models, showing that factors contributing to growth signalling-induced ROS accumulation, including high glucose and low pH, might be relevant to natural ageing yeast cells.

Heinz D Osiewacz (Frankfurt a.M., Germany) recently demonstrated that the filamentous ascomycete *Podospora anserina* has potential key players of PCD. Until now, he characterised two metacaspases, which if deleted led to longevity, five potential homologues of apoptosis-inducing factor and a putative cyclophilin D, which reside in the mitochondria of senescent cultures.

Using the *SCH9* deletion mutant to study age-dependent DNA damage and genomic instability, Valter Longo (Los Angeles, CA, USA) demonstrated the interplay between Sod2p and Rev1p expression levels, and the effect on DNA oxidation and mutagenesis. He suggested that Sch9p, a homologue of the human Akt and S6K proto-oncogenes, promotes the accumulation of superoxide-dependent DNA damage in non-dividing cells. This requires the error-prone Rev1/Polζ-dependent trans-lesion repair, to avoid chromosomal rearrangements and cell death during the first round of replication.

Tobias Eisenberg (Graz, Austria) introduced spermidine as a biological drug that promotes longevity by enhancing autophagy and decreasing necrotic cell death in yeast. Intriguingly, he validated these data in higher model organisms and demonstrated that spermidine also prolonged the lifespan of flies, worms and human immune cells.

The Ras protein, a member of the GTP-binding protein family, involved in PKA pathways, was shown to be the main research topic of three groups. Sonia Colombo (Milano, Italy) introduced an inventive way to investigate the localisation of active Ras2p (Ras2-GTP) by monitoring a Ras2p-binding domain fusioned with GFP. Her data suggest that the localisation and the function of Ras2p are dependent on the abundance and quality of the available carbon sources.

Alena Pichova (Prague, Czech Republic) showed interesting results of her work with the permanently active RAS2val19 protein, a mutation corresponding to the oncogenic version of Ras2p in higher eukaryotes. Besides the reduced lifespan and apoptotic phenotype of yeast strains expressing this protein, another hallmark is the high occurrence of spontaneous reversion, which suppresses the oncogenic phenotype.

Campbell W Gourlay (Kent, UK) investigated the regulation of localisation, trafficking and degradation of Ras2p. Gourlay and colleagues revealed that upon shift from fermentation to respiration, mitochondrial biogenesis is highly dependent on the relationship between actin and Ras–cAMP–PKA signalling. The failure to manage Ras2p activity results in ROS accumulation and cell death. Vassilios N Kotiadis (Kent, England), from Gourlay's group went into more detail of an actin-regulatory protein, the highly conserved Cofilin. This protein has been shown to influence mitochondrial function and induce apoptosis in higher eukaryotes. He presented

Cofilin to be a link between actin dynamics, mitochondrial function and cell death in yeast, and demonstrated that the Ras2p signalling pathway is implicated in exhibiting high ROS levels in Cofilin mutant strains.

Yeast Cell Death Models for Human Diseases

In recent years, yeast has been successfully established as a model to study the mechanisms of cell-death pathways.[5] This provides the opportunity to address complex questions with direct relevance to human diseases, such as neurodegeneration, cancer and AIDS. In the first of two sessions, concentrating on this new field, Joris Winderickx (Leuven, Belgium) presented a yeast model for human tauopathies. Hyperphosphorylation of the tau protein is believed to contribute to the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, frontotemporal dementia or Parkinsonism. In the yeast model system, several fundamental aspects of tau-related disorders, such as the phosphorylation–conformation–aggregation cascade is present. On the basis of these typical tau conformations purified from yeast, highly specific antibodies have been constructed, which proved to be very useful for the detection of tau tangles in the blood samples obtained from Alzheimer's patients.

Ralf Braun (Graz, Austria) used the 'neurotic' yeast model (heterologous expression of human TDP-43) to study neurodegenerative disorders underlying TDP-43 pathology. TDP-43 forms aggregates in the brains of patients suffering from amyotrophic lateral sclerosis and frontotemporal lobar degeneration. With powerful genetics and survival assays (clonogenicity), the yeast system will help in identifying the central regulators and cell-death pathways underlying TDP-43 proteinopathies.

A hallmark of age-related neurodegenerative disorders is the accumulation of damaged proteins and protein aggregates within the cell, which are not 'cleaned' by the proteasome. Marija Cvijovic (Göteborg, Sweden) presented a mathematical model to explain the role of aggregate formation and accumulation in yeast. Her simulation model, which was also confirmed experimentally, was to follow a cell during replicative ageing with different rates of protein damage and aggregation. Her results indicate that the fitness of a yeast population requires the segregation of aggregated damaged proteins of mother cells and a beneficial retention to achieve fitter daughter lineages.

Using phenylbutyrate (PB), a chemical chaperone and histone deacetylase inhibitor with neuroprotective effects, Uros Petrovic (Ljubljana, Slovenia) performed a chemogenomic screen using the single deletion collection of *S. cerevisiae* to elucidate the biological activity of PB. He identified members of the Hsp40/DnaJ family as putative targets of PB, and thus substantiated the potential of the yeast system to search for new drug targets that are used for the treatment of neurodegenerative diseases.

With the frataxin-deficient strain $\Delta yfh1$, Renata Santos (Paris, France) presented a yeast model for Friedreich's ataxia (FA). Hallmarks of FA include mitochondrial iron accumulation, iron–sulphur cluster and haeme deficiency, and a higher sensitivity to oxidative stress. With her yeast model, showing a similar phenotype, she could previously demonstrate that oxygen causes a permanent oxidative stress shown by a loss of aconitase activity, the accumulation of oxidised modified proteins, the activation of mitochondrial protease Pim1p and decreased activity of the proteasome. In her ongoing work, she uses anaerobiosis-to-aerobiosis transition as an inducer of oxidative stress and show that a misfunctional or non-functional oxidative stress response is responsible for ROS accumulation and cell death in a *YCA1*-independent manner.

Richard Y Zhao (Baltimore, MD, USA) established a fission yeast model for studying HIV-1 protease activities, enabling both the mechanistic characterisation of the protease activities and high throughput screening of potential protease inhibitors. This is of particular importance, because HIV multi-drug resistance has become a huge problem.

J Marie Hardwick (Baltimore, MD, USA) prompted us to think about the evolution of cancer-like mutations and the yeast knockout collection. After screening $\sim$5000 strains in the BY4741 single deletion collection under three different stress-inducing conditions, she found several hundred strains with the same three phenotypes. A further characterisation revealed that more than 100 deletion mutants carried the same secondary mutation, which was responsible for the survival phenotype showed in the stress screen. Concluding that selection of genetic variants is driven by metabolic defects, these findings support the observation that yeast can be used as a model for tumourigenesis.

While working on the interactions between microbes, Reiko Ikeda (Tokyo, Japan) revealed that the pathogenic yeast *Cryptococcus neoformans* was killed by the bacterial adherence of *Staphylococcus aureus*. Measuring actin dynamics, levels of ROS and DNA fragmentation, an apoptotic hallmark, it could be shown that *C. neoformans* can undergo cell death upon stress in an apoptotic-like manner.

Concluding Remarks

Ten years ago, it was the common goal of a small yeast cell death community to provide evidence that yeast apoptosis is (at least to some extent) comparable with mammalian apoptosis.[1] At present, an increasing number of researchers all over the world apply yeast as a well-established cell death model, and meanwhile focus on giving new insights into cell-death research as a whole, already providing new concepts that were validated later on in higher organisms. Noticeably, methods for the investigation of cell-death markers and survival assays have been improved. Besides growth curves and spot assays that provide hints on the potential toxicity of heterologously expressed proteins, clonogenic assays are now widely used in the field to precisely determine rates of cell death. Measurements of ROS accumulation are accompanied by tests to discriminate between apoptotic and necrotic death, such as Annexin V/PI and TUNEL staining or detection of the release of the nuclear protein Nhp6ap into the cytoplasm upon necrotic cell death. It is making the scientists, who had been in the community from the very first beginning very proud, to see this field blossom out, but it is also very exciting for the students

and newcomers to work on cell death using yeast as a model, with its powerful genetics and easy handling. Therefore, we are all looking forward to the next meeting, organised by Campbell Gourlay in May 2011 in Canterbury, the garden of England.

Conflict of interest

The authors declare no conflict of interest.

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