

Conference report

## The 1st International Meeting on Yeast Apoptosis, Braga, Portugal, 4-6 October, 2002

*“I know it’s only dying yeast, but I like it”*

Why should a unicellular organism commit suicide? What sense does it make? Is it really apoptosis? Aren’t there enough model organisms for apoptosis research? Finally: Why are you doing this? Questions a researcher working on programmed cell death in yeast has to deal with daily. But not in this meeting. Fifty believers from all over Europe and overseas had gathered to discuss their latest results on this new and fast-growing field. Although its increasing importance in cancer, neurodegenerative disorders and AIDS has led to intensive research and a better understanding of apoptosis, many details of its regulation or the apoptotic phenotypes are poorly understood. It is possible that the power of yeast genetics and proteomics may allow to address conserved aspects of cell death more readily in yeasts than in metazoan systems. During the past two years, scientists have been successful in identifying new cell death regulators of humans, plants and fungi while using *Saccharomyces cerevisiae*.

When Manuela Côrte-Real and Paula Ludovico called me one year before and talked about the idea of such a meeting, I was both enthused and unsure. Will anybody come? It was a very brave initiative from these Portuguese colleagues and it has been rewarded. Citing Michael Breitenbach: ‘One of the best meetings I have ever been to.’ Moreover, the organisation was fantastic, the scientific and social programme very well structured, and the location beautiful.

The talks started with apoptosis-inducing agents and **Manuela Côrte-Real** discussed acetic acid-induced apoptotic markers in yeast. Low doses of acetic acid induced cell death, accompanied by DNA strand breaks, chromatin condensation and externalisation of phosphatidylserine to the outer leaflet of the plasma membrane, whereas higher doses appeared to induce necrosis. All these features are translation-dependent.

**Mark Ramsdale** told us later that *Candida albicans* responds to acetic acid in a manner parallel to that observed in *S. cerevisiae*. *C. albicans* is a pathogenic fungus that causes both irritating infections and life-threatening disease. Resistance to traditional therapies is significantly on the increase. Understanding the molecular basis of apoptosis-like cell death responses in *C. albicans* and sev-

eral other fungi could be of great importance in the search for novel therapies. Alterations in sphingolipid metabolism can result in death of both *Candida* and *Saccharomyces* cells, with phenotypic changes reminiscent of mammalian apoptosis. Signalling via the ras-cAMP pathway appears to be necessary but not sufficient for the apoptosis-like cell death response observed.

Another mode to induce apoptotic cell death in *Candida* is application of garlic, as demonstrated by **Katey Lemar**. A high dose of garlic might cure some human *Candida* infections. Problematic social implications of this therapy have been discussed.

**Gennaro D’Urso** presented evidence that also *Schizosaccharomyces pombe* might undergo apoptotic changes.

**David Granot** showed results about sugar-induced apoptotic death of yeast. Stationary-phase yeast cells incubated in presence of only glucose, in the absence of additional nutrients, lose viability within a few hours, exhibiting apoptotic markers.

Another section of the meeting dealt with the genes involved in yeast apoptosis. **Frank Madeo** reported about YCA1, a caspase-related protease that induces apoptosis in yeast in an oxygen stress-dependent manner. Interestingly, **Wen-Chih Cheng** showed that disturbance of mitochondrial biogenesis leads to apoptosis, which can be rescued by disruption of YCA1.

**Bill Burhans** and his group presented a connection between initiation of DNA replication and apoptosis in yeast. They performed a synthetic lethality screening which showed that the temperature-sensitive *orc2-1* mutation in the Origin Recognition Complex (ORC) confers hypersensitivity to DNA-damaging antitumor drugs. Subsequently, they determined that in both budding yeast and mammals, these drugs induce the proteasome-dependent degradation of Cdc6, which interacts with ORC and modulates its function at origins of replication. Bill Burhans concluded that the *orc2-1* mutation causes apoptosis in cells at a nonpermissive temperature, and this apoptotic response underlies the hypersensitivity of *orc2-1* cells to DNA damage.

**Cristina Mazzoni** and **Claudio Falcone** reported for the first time a connection between mRNA stability and apo-

ptosis. They demonstrated that components of the Lsm complex, which are involved in pre-mRNA splicing and also in mRNA decapping, leads to enhanced mRNA stability and apoptosis in yeast.

It was **David Goldbarb** who surprised us all with beautiful pictures of a completely new autophagic pathway, in which the vacuole constantly degrades portions of the nucleus. He coined the term ‘piecemeal’ microautophagy for it. During piecemeal microautophagy teardrop-like blebs are pinched from the nucleus, released into the vacuolar lumen, and degraded by soluble hydrolases. David found promising connections to yeast apoptosis, which are still under investigation.

Are mitochondria needed for yeast apoptosis? A question that has been discussed controversially for years. To my knowledge, this meeting gave for the first time a coherent answer, which was shared by all the participants. There clearly exists a mitochondrial-dependent apoptotic pathway, but there must be other pathways which are independent of mitochondria. It is, for example, accepted now that the human proapoptotic protein Bax can kill yeast cells independently of mitochondrial function. This conclusion has been reached in discussions between two pioneers in the mitochondria/cell death field: Jordan Kolarov and Stéphen Manon.

**Jordan Kolarov** presented evidence that in the yeast *Kluyveromyces lactis* the antiapoptotic protein Bcl-xL is localized exclusively to the mitochondria, and prevents the cytotoxic effect of Bax, but does not eliminate the oxidative stress induced by Bax. He demonstrated that phenotypes characteristic for yeast expressing Bax can be mimicked by combinations of mitochondrial inhibitors, mutations and growth conditions affecting mitochondrial functions. Expression of Bcl-xL causes a substantial improvement in viability of the cells possessing defective mitochondrial ADP/ATP transport.

**Stéphen Manon** reported that Bax-induced release of cytochrome *c* in yeast is not due to an unselective permeability transition of mitochondrial membranes. Electrophysiology of mitochondrial outer membrane isolated from Bax-expressing yeast showed that Bax created a large channel, termed Mitochondrial Apoptosis-induced Channel (MAC), having the characteristics required for transport of cytochrome *c*. Before creating a channel, Bax is inserted into the mitochondrial outer membrane. Stéphen Manon and his group studied variants of Bax in the C-terminal and N-terminal ends of the protein, to determine the molecular features of the protein involved in this crucial step. Moreover, they showed that Bax is also able to activate a death pathway related to autophagy in yeast, which also exhibits typical hallmarks of apoptosis, revealing a possible dual function of Bax in both types of death.

**Paula Ludovico**, one of the organizers, presented exciting evidence that mitochondria are required in the previously described programmed cell death process induced by acetic acid in *Saccharomyces cerevisiae*. After treatment

with acetic acid, translocation of cytochrome *c* to the cytosol and reactive oxygen species production, two events known to be proapoptotic in mammals, were observed. Moreover, disruption of the cytochrome *c* gene leads to resistance against low doses of acetic acid.

**Patrice Petit** found that cardiolipin is essential for tBid binding to mitochondrial contact sites and the inhibition of state-3 respiration in yeast. tBid did not bind to cardiolipin null mutant mitochondria and did not alter the ADP-stimulated NADH-sustained respiration. In addition, tBid inhibited ATP production by wild-type yeast mitochondria while it had no action on cardiolipin-deficient mitochondria.

One of the major goals in yeast apoptosis is the question of the purpose. What sense does an altruistic suicide programme make for a unicellular organism? After all, the cell is dead! An intriguing answer was given by **Zdena Palková**. She discussed a role for apoptosis in long-term development of multicellular yeast structures. It is conventional to divide organisms into unicellular and multicellular groups. This distinction does not take into account that microorganisms in nature almost never exist exclusively as individuals or as pure exponentially growing suspension cultures, prevalently used in laboratory investigations. In contrast, natural ‘unicellular’ microorganisms often create multicellular communities attached to solid surfaces (e.g. fruiting bodies, colonies and microbial biofilms). Volatile ammonia, which is produced by colonies in pulses, functions as a signal regulating long-term colony development.

**Michael Breitenbach** then showed a physiological role for yeast apoptosis during the elimination of over-aged cells. He and his group showed impressive evidence that individually aged yeast mother cells die apoptotically. Moreover he investigated the role of oxidative stress and apoptosis in the ageing process of yeast. He found that senescent mother cells contain oxygen radicals in their mitochondria, which makes yeast a useful model to study the oxygen theory of ageing. In conclusion, the experiments confirmed the strong correlation between oxidative stress, apoptosis and cellular ageing that had also been found in other model systems. In addition, they developed a screening which allows for the selection of long-lived yeast mutants.

In *S. cerevisiae*, Ras2p plays a role upstream of several important pathways which integrate metabolism and cell cycle events in response to environmental signals. **Alena Pichová** reported that it also affects the replicative ageing of the cell. The RAS2Val19 allele, a homologue to the mammalian H-RAS-V12 oncogenic allele, greatly reduces replicative ageing, sensitivity to nitrogen starvation, and causes different apoptotic morphological changes.

This was the strong finish of a very exciting meeting. ‘This conference is going to be so crowded in a couple of years’, said Bill Burhans. Indeed, the next two meetings are already being planned. Jordan Kolarov has rented a

whole castle in the Little Carpathian mountains at Smolenice near Bratislava. This beautiful location will be convenient to host the community when the 2nd International Meeting on Yeast Apoptosis will take place from 17-20 September, 2003. Many of the invited speakers have already confirmed to come, please have a look at: [www.fns.uniba.sk/~kbi/imya/](http://www.fns.uniba.sk/~kbi/imya/).

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