Apoptosis in yeast: a new model system with applications in cell biology and medicine

Abstract Apoptosis is a highly coordinated cellular suicide program crucial for metazoan health and diseases. Although its increasing importance in cancer, neurodegenerative disorders and AIDS led to intense research and a better understanding of apoptosis, many details of its regulation or the apoptotic phenotypes are poorly understood. The complex regulatory network and the often contradictory results obtained with human cell lines made application of an easier model system desirable. Apoptosis in yeast promises to provide a better understanding of the genetics of apoptosis. During the past 2 years, scientists were successful in identifying new cell-death regulators of humans, plants and fungi using \textit{Saccharomyces cerevisiae}. The finding of apoptotic phenotypes, even in protists, suggests that apoptosis developed in unicellular organisms long before the evolutionary separation between fungi, plants and metazoan animals occurred.

Introduction

Apoptosis is a form of programmed cell death with a central role in the homeostasis and maintenance of metazoans. It is required for the removal of autoreactive immune cells, virus-infected cells and cells with unreparable genetic damage posing the risk of a cell transformation. Constant turnover of cells driven by apoptosis is important for homeostasis in tissues and organs. A central feature of apoptosis is its effectiveness.

Removal of cells happens rapidly and without damage to the environment. Because it acts so inconspicuously, it was only discovered in 1972 by Kerr, Wyllie and Currie (Kerr et al. 1972). In contrast, the markers of inflammation indicating an accidental, necrotic cell death have been known for millennia. The realization of the enormous impact of apoptosis on human health took another 20 years. AIDS, cancer, autoimmune and neurodegenerative disorders and even late effects of ischemia can be linked to a misregulation of apoptosis (for a review, see Uren and Vaux 1996). During the past 10 years, apoptosis has become one of the most intensely researched topics in medical science.

Apoptosis is coordinated by a complex network of regulators and effectors, which can be triggered by various toxins or external signals [e.g. ethanol, reactive oxygen species (ROS), receptor ligands] and internal processes (e.g. mitotic catastrophe, replication failure, developmentally programmed cell death). Independently of the regulatory pathway, a characteristic phenotype emerges at the end of almost all apoptotic scenarios: phosphatidylserine flips to the outer leaflet of the membrane, chromatin condenses, DNA is cleaved into small fragments and the cell breaks up into membrane-enclosed vesicles, the so-called apoptotic bodies. Regulatory pathways and inducers vary depending on tissue, developmental state and host organism, resulting in diverse and sometimes contradictory models for the regulation of apoptosis. A simple model system, such as yeast, would be useful to clarify the situation. However, apoptosis had been assumed to be confined to multicellular animals. For a unicellular organism like yeast, a suicide mechanism seemed useless, since it results in the death of the whole organism. When the complete genome sequence of \textit{Saccharomyces cerevisiae} became available in 1997, no relatives of the most central players in apoptosis – e.g. the caspases, members of the Bel-2/Bax family or Apaf-1 – were found, emphasizing the idea of a purely metazoan apoptosis.

It was a surprise, therefore, when cell death with the characteristics of apoptosis was first described in
unicellular organisms (Madeo et al. 1997). Since then, yeast has been used as a model system for apoptosis by several research groups. Genes functioning in the cell death of yeast have been confirmed as apoptotic regulators of metazoans, indicating that the basic machinery of apoptosis is indeed present and functional in unicellular organisms.

**Endogenically triggered yeast apoptosis**

In 1997, we found a yeast mutant dying with a typical apoptotic phenotype: exposition of phosphatidylserine, margination of chromatin and formation of cell fragments (Madeo et al. 1997). While the TUNEL test (O’Brien et al. 1997) indicated massive DNA breakage, no DNA ladder was observed. Nucleosome linkers appear not to be preferred targets of DNA cleavage in yeast, probably due to their short length (Lowary and Widom 1989).

The apoptotic phenotype was caused by a point-mutation of CDC48 (cdc48S565G), coding for a *S. cerevisiae* protein belonging to the AAA family and involved in vesicle fusion.

Recently, Granot and colleagues (Levine et al. 2001) were able to show that Bax-triggered apoptosis in yeast can be blocked by enhancing vesicle trafficking. Moreover, a downregulation of vesicular transport enhances the susceptibility of yeast cells to apoptosis, providing an explanation for the cdc48S565G-mediated apoptosis.

In the case of *CDC48*, the yeast model system has already demonstrated its potential to identify new mammalian apoptotic regulators. In 1999, an anti-apoptotic role of the human *CDC48* orthologue *VCP/p97* and a related protein in *Caenorhabditis elegans* was described (Shirogane et al. 1999; Wu et al. 1999). A mutated form of *VCP*, in a manner similar to the cdc48S565G mutation in yeast, dominantly induced apoptosis in B-cells. This makes *CDC48/VCP* the first apoptotic regulator originally discovered by its function in yeast apoptosis. Recently, *CDC48/VCP* was shown to act as a cell-death effector molecule in the brain, suggesting that its concentration is critical for neurodegeneration (Higashiyama et al. 2002). Another AAA protein with a probable role in yeast apoptosis is the mitochondrial protease Yme1p. Expression of Bax in yeast activates Yme1p, resulting in a degradation of cytochrome c oxidase subunit 2 (Cox2p). The absence of Yme1p delays Bax-induced cell death (Manon et al. 2001).

Deletion of *ASF1/CIA1*, a gene coding for a histone chaperone, also results in yeast apoptotic cell death, following an arrest at the G2/M transition. Moreover, a reduction in the mitochondrial membrane-potential, dysfunction of the mitochondrial proton pump and release of cytochrome c into the cytoplasm occur (Yamaki et al. 2001). The human homologue of Asf1p/Cia1p, CIA, interacts with the largest subunit of TFIID, CCG1, which is involved in the regulation of apoptosis (Sekiguchi et al. 1995).

Most recently, we found that a caspase-related protease (YCA1) mediates apoptosis in yeast (Madeo et al. 2002). YCA1 is processed in a caspase-typical manner and has proteolytic activity for caspase substrates. Overexpression of YCA1 in synergy with oxidative stress efficiently triggers yeast cell death, accompanied by markers of apoptosis. Conversely, YCA1 disruption increases tolerance against H2O2. YCA1 belongs to the family of metacaspases described by Uren et al. (2000), which encompass members in fungi, plants and protists. Indeed, observations of caspase activities have also been made in plants and protists. Using camptothecin, a classic apoptosis-inducing drug used in cancer therapy, Woltering and colleagues could induce apoptosis accompanied with a burst of oxygen radicals in tomato cells (de Jong et al. 2002). Both camptothecin-induced cell death and the release of oxygen radicals were effectively blocked by the application of caspase inhibitors (de Jong et al. 2002). H2O2 also induces apoptosis-like death and caspase activity in the protist *Leishmania donovani*. Again, these effects can be efficiently blocked by caspase inhibitors (Das et al. 2001).

Observations pointing to an apoptotic phenotype were also made in *Schizosaccharomyces pombe*: the Rad9 protein (SpRad9) contains a stretch of amino acids with similarity to the Bcl-2 homology 3 death domain, which is required for SpRad9 interaction with human Bcl-2. Overexpression of Bcl-2 in *S. pombe* inhibits cell growth independently of rad9, but enhances the resistance of rad9-null cells to methyl methanesulfonate, ultraviolet (UV) and ionizing radiation. The authors suggest that SpRad9 may represent the first member of the Bcl-2 protein family identified in yeast (Komatsu et al. 2000).

Ceramide is another potential bona fide apoptosis regulator shared by mammals and yeast. Some mammalian growth modulators, including tumor necrosis factor α, induce apoptosis or cell cycle arrest via ceramide, which activates a specific phosphatase (Kishikawa et al. 1999). Ceramide-induced G1 arrest of Saccharomyces cerevisiae is also mediated via activation of a protein phosphatase (Nickels and Broach 1996).

**Apoptosis in yeast by heterologous gene expression**

The two-hybrid system is a popular strategy for the study of protein–protein interactions. Fusions of genes coding for proteins of interest are usually expressed in yeast cells and interactions are detected by restoration of transcriptional activators. When this method was applied to apoptotic genes, an unexpected side-effect occurred. Expression of several pro-apoptotic genes, including Bax (Greenhalf et al. 1996), caspases (Kang et al. 1999) and Apaf-1/CED-4 (James et al. 1997), was found to be lethal for yeast.

Further research indicated that the resulting cell death is indeed of apoptotic nature. Ligr et al. (1998) showed that Bax-mediated cell death in *S. cerevisiae* is accompanied by typical features of apoptosis, such as