Tonight, the same old, deadly programme: BH3-only proteins, mitochondria and yeast

Bjorn Oettinghaus^{1,2}, Stephan Frank² and Luca Scorrano^{1,3,*}

¹Department of Cell Physiology and Metabolism, University of Geneva Medical School, Genève, Switzerland, ²Department of Pathology, University Hospital Basel, Basel, Switzerland and ³Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy

*Correspondence to: luca.scorrano@unige.ch

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Apoptosis is a type of programmed cell death (PCD) conserved among metazoans and crucial for the development and homeostasis of tissues in multicellular organisms. Lower unicellular eukaryotes like yeast would thus have no need for it and they apparently lack the key regulators of mitochondria-dependent apoptosis. In this issue, Büttner *et al* (2011) challenge this view, discovering Ybh3p, a yeast protein homologous to the proapoptotic ‘BH3-only’ members of the mammalian Bcl-2 death regulators. Ybh3p kills yeast and mammalian cells impinging on inner mitochondrial membrane (IMM) proteins. Thus, mitochondria-dependent PCD emerges as an ancestral response of all eukaryotes, including unicellular ones.

Ever since cells interacted to form multicellular organisms, a controlled and programmed form of cellular death also known as ‘apoptosis’ arose, for the benefit of the organism and of its offspring. In its basic mechanisms, apoptosis is conserved in metazoans where it is required for elimination of unneeded cells during development or of cells that are potentially harmful to developed organs, without eliciting inflammation, like necrosis does (Danial and Korsmeyer, 2004). The need of such a process for unicellular organisms is debatable: on one hand, yeast could benefit from the targeted removal of aged cells from their colony, thereby preserving nutrients and hindering genetic conservatism; on the other, the evidence for yeast PCD is controversial (Mazzoni and Falcone, 2008).

In metazoans, the hallmark event of apoptosis is the activation of caspases, proteases that execute death by degrading cytoskeleton, DNA and other cellular components. Caspases can be directly activated by extrinsic stimuli that engage the ‘death receptors’ in the plasma membrane, or by a variety of intrinsic stimuli converging on gateways that control and amplify the signal (Danial and Korsmeyer, 2004). This intrinsic pathway must be tightly regulated to avoid unwanted death and simultaneously to efficiently trigger caspase activation when needed. These key regulators belong to the family of Bcl-2 proteins. The founders of this family are the antiapoptotic proteins Bcl-2 and Bcl-xL, bearing four Bcl-2 homology (BH) domains (BH1–4). Bcl-2 and Bcl-xL are mainly located in the outer mitochondrial membrane (OMM) where they regulate the permeabilization of the organelle followed by the release of proapoptotic factors like

cytochrome *c* from the mitochondrial intermembrane space. Once in the cytosol, cytochrome *c* triggers the activation of caspases by forming a multimolecular complex with the adaptor protein Apaf-1 and pro-caspase 9 (Wasilewski and Scorrano, 2009). Key mediators of OMM permeabilization are the ‘multidomain proapoptotics’ Bax and Bak that harbour BH domains 1–3. Activation of Bax and Bak occurs directly in the OMM by another subset of proapoptotic Bcl-2 family members, known as ‘BH3-only’ proteins that possess only the BH3 domain. In turn, the antiapoptotic Bcl-2-like molecules regulate the whole process by sequestering ‘BH3-only’ proteins (Wasilewski and Scorrano, 2009). Whether yeast can undergo apoptosis has been a matter of intense debate. Yeast cells lack ‘classical’ mammalian-like caspases. They do, however, express Yca1p, which belongs to the family of metacaspases found throughout the plant and fungi kingdoms (Madeo *et al*, 2002). Yca1p is activated following toxic (oxidative) as well as physiological stress (low concentrations of mating pheromone, chronological lifespan), and greatly influences yeast death although its targets or mode of activation remain unknown at present. For example, the role of OMM permeabilization in yeast apoptosis is controversial (Mazzoni and Falcone, 2008), especially since yeast lack Bcl-2-like proteins. Similarities between the death pathways in metazoans and yeast have, however, also emerged. For example, mitochondrial fragmentation, a hallmark of mammalian apoptosis (Wasilewski and Scorrano, 2009), occurs also in yeast and accordingly physiological death is reduced in strains lacking the key fission protein Dnm1p (Fannjiang *et al*, 2004). The emerging consensus is that physiological and morphological changes of mitochondria are key for yeast death, but how these organelles could be recruited in the apoptotic cascade in the absence of proximal BH3-only-like sensors of cellular damage remained unclear.

In the current issue of *The EMBO Journal*, Madeo and colleagues close this gap, by discovering Ybh3p, a proapoptotic yeast BH3-only protein. Ybh3p is a cytoplasmic protein whose overexpression facilitates yeast apoptosis induced by a variety of stimuli, whereas its ablation inhibits death and increases replicative lifespan. In analogy to certain mammalian BH3-only proteins, during apoptosis Ybh3p translocates to mitochondria, triggering their depolarization

Figure 1 The role of mitochondria in yeast and mammalian apoptosis. The cartoon depicts the mitochondrial involvement in the amplification of apoptosis in yeast and mammals. In response to BH3-only proteins, the multidomains Bax and Bak permeabilize mammalian mitochondria, while inner membrane players like Mir1p and Cor1p participate in death by Ybh3p. Question marks indicate unknown mechanisms. The symbols used for the main players are defined in the legend.

and cytochrome *c* release. In this respect, Ybh3p resembles more mammalian Bax that can autonomously permeabilize mitochondria, whereas BH3-only proteins require Bax and Bak to release cytochrome *c* (Wei *et al*, 2001), but not to remodel the IMM (Scorrano *et al*, 2002). Ybh3p can permeabilize murine mitochondria and kill a human lung cancer cell line like a typical mammalian BH3-only protein. Taken together, these data suggest that perhaps BH3-only proteins are the prototypes of the Bcl-2 family regulators of cell death. Antiapoptotic as well as multidomain members could have emerged later in evolution to tighten up the control on mitochondrial damage and apoptosis.

Madeo and colleagues identify two mitochondrial interactors of Ybh3p essential for its killing action: Mir1p and Cor1p, the yeast orthologues of the mitochondrial phosphate carrier and of a core subunit of the ubiquinol-cytochrome *c* reductase (complex III). The link between these two IMM proteins and

Ybh3p suggests that perhaps the most ancestral function of the BH3-like proteins is to trigger changes in function and/or topology of the IMM. In this respect, it would be interesting to explore whether Ybh3p and the fission protein Dnm1p participate in the same pathway of yeast death. Also, it would be worth studying the role of Mir1p and Cor1p in mitochondrial Ybh3p translocation. Notably, the mechanism of killing by Ybh3p seems somehow conserved, since the ablation of the orthologues of Mir1p and Cor1p can blunt apoptosis in human osteosarcoma cells, further pointing to a role for the IMM in mammalian apoptosis (Figure 1).

Taken together, the results by Madeo and colleagues unveil the existence of an ancestral, proapoptotic, BH3-only gene in lower eukaryotes that later in evolution maybe replicated and diversified into multiple genes with one or more BH3 domains to serve specialized functions and to fine-tune death. In this respect, the striking genetic interaction

between Ybh3p and IMM components suggests that OMM permeabilization could have emerged later in evolution. Changes in function and morphology of the IMM that are also observed in mammalian cells could then represent the remnant of the ancestral programme of death, which has subsequently been 'coated' by evolution with an outer membrane to provide a further layer of control to death signalling.

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Conflict of interest

The authors declare that they have no conflict of interest.

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