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Minireview

Apoptosis in yeast – a monocellular organism exhibits altruistic behaviour

Kai-Uwe Fröhlich*, Frank Madeo[1]

Physiologisch-chemisches Institut, Universität Tübingen, Hoppe-Seyler-Straße 4, 72076 Tübingen, Germany

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Abstract Apoptosis is a highly regulated form of programmed cell death crucial for life and health in metazoan animals. Apoptosis is defined by a set of cytological alterations. The recent discovery of these markers in yeast indicates the presence of the basic mechanisms of apoptosis already in unicellular eukaryotes. Oxygen radicals regulate both mammalian and yeast apoptosis. We suggest that apoptosis originated in unicellular organisms as an altruistic response to severe oxidative damage. Later, cells developed mechanisms to purposely produce reactive oxygen species as a regulator of apoptosis. Yeast may become an important model to investigate the conserved steps of apoptosis.

Key words: Apoptosis; Evolution; Reactive oxygen species; Yeast

1. Introduction

Apoptosis has been defined as a highly regulated form of programmed cell death in metazoan animals resulting in the removal of mutated, infected or simply dispensable cells. While accidental cell death (necrosis) is usually accompanied by cell breakage and neighbouring inflammation, apoptosis avoids rupture of the dying cell, preventing spillage of cell contents.

The term apoptosis was coined by Kerr, Wyllie and Currie [1] in the early 1970s. They also described characteristic events which are still used today to recognise apoptosis such as chromatin condensation, nuclear fragmentation and formation of apoptotic bodies. Nevertheless, there was little research on the subject in the following years.

When its crucial role in development, homeostasis, and the prevention and the promotion of different diseases was recognised, apoptosis suddenly became a hot topic. Failure of apoptosis can result in cancer, autoimmune diseases and spreading of viral infections, while neurodegenerative disorders, AIDS and ischaemic diseases are caused or enhanced by apoptosis [2].

Apoptosis has been found throughout the animal kingdom. In vertebrates, it is controlled by a complex regulatory network which can be activated by toxins or external signals (e.g. ethanol, reactive oxygen species (ROS), receptor ligands) and internal processes (e.g. mitotic catastrophe, replication failures or developmental programmed cell death). Regulatory pathways and inducers vary depending on tissue, developmental state, or host organism, resulting in diverse and sometimes contradictory models for the regulation of apoptosis. The regulatory mechanism seems to be simpler in *Caenorhabditis elegans* or *Drosophila melanogaster* [2].

Despite these differences, the late events of apoptosis downstream of the complex regulatory network seem to be shared between all metazoan animals as many of the typical markers, DNA fragmentation, chromatin condensation, membrane blebbing, externalisation of phosphatidylserine and formation of apoptotic bodies, can be observed in most cases of apoptosis. The mechanisms directly responsible for these features are poorly understood [3].

2. Is yeast a suitable model for investigation of metazoan apoptosis?

Yeast comprises a phylogenetically heterogeneous group of ascomycetic fungi. Two yeast species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, have been investigated intensively as they have simple, well understood genetics and are amenable to modification by gene technology. They serve as simple yet powerful models of common eukaryotic processes such as cell division or intracellular transport. For apoptosis research however, yeasts have not appeared to be suitable model organisms. Being monocellular, a suicide programme would not seem to offer the advantage inherent in multicellular systems. In addition, the complete genomic sequence of *S. cerevisiae* lacks any obvious homologue of major apoptotic regulators described in metazoan organisms (*bax*/*bcl-2* family, caspases, Apaf-1/CED-4, p53).

3. Apoptotic genes kill yeast

Therefore, yeast was used as a 'clean room' system to study interactions between heterologously expressed components of apoptotic pathways. Unexpectedly, expression of some apoptotic inducers such as Bax [4–6], caspases [7,8], p53 [9,10], or CED-4/Apaf-1 [11] resulted in cell death of the yeast host in both *S. cerevisiae* and *S. pombe*. Simultaneous expression of Bcl-2 or Bcl-x_L which inhibit apoptosis in animals prevented Bax-induced cell death [4–6,12]. In animals, Bax or Bak proteins are not cytotoxic per se, as inhibition of the caspases acting downstream of Bax can prevent cell death [13]. Therefore, *bax*-induced death of yeast cells seems not to be the

*Corresponding author. Fax: (49)-7071-295565.
E-mail: kaifr@uni-tuebingen.de

[1] Also corresponding author. Fax: (49)-7071-295565; E-mail: frank.madeo@uni-tuebingen.de

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result of simple cytotoxicity, but is rather caused by the activation of some sort of death programme. In fact, the structural requirements for proteins of the *bax/bcl-2* family for killing or rescuing appear to be the same in animals and yeast. The dimerisation-mediating BH3 domain of Bax and its targeting to mitochondrial membranes are both essential for killing mammalian as well as yeast cells [14]. Furthermore, mutant forms of Bcl-x_L, an anti-apoptotic Bcl-2 family member, lacking Bax binding activity can prevent Bax-induced death in yeast [15,16]. This indicates that Bcl-x_L acts downstream of Bax, perhaps by competing for binding to a common target, which may be part of the conserved apoptotic machinery.

While these results suggest similar mechanisms for the downstream events of bax-induced cell death in yeast and animals, some reports indicate that the morphological features of death differ. *S. pombe* cells expressing *bax* show no DNA laddering and no shrinkage of the cell. Cell death in *S. pombe* is neither accompanied by caspase-like activities, nor can it be prevented by caspase inhibitors [12]. However, other researchers report a chromatin condensation accompanying the expression of bax [17] or of CED-4 (Apaf-1, [11]) as an indication of apoptosis. They also observe as a result of *bax* expression in *S. pombe* the formation of a large DNA fragment, derived from genomic DNA, but no DNA ladder, thus showing the occurrence of DNA cleavage. Furthermore, Zhao et al. [18] observed the typical margination of chromatin against the nuclear envelope in *S. pombe* killed by the expression of pro-apoptotic HIV-1 Vpr protein.

In *bax*-expressing *S. cerevisiae*, even more markers of apoptosis have been detected. Cells show margination of chromatin at the nuclear envelope, extensive DNA cleavage demonstrated by the TUNEL test, membrane blebbing, and externalisation of phosphatidylserine at the cytoplasmic membrane. Simultaneous expression of Bcl-x_L prevents these effects [19].

4. Intrinsically triggered apoptosis in yeast

All previously described examples of yeast apoptotic cell death involved heterologous gene expression. In 1997, we found that a point-mutated *CDC48* (*cdc48*^{S565G}), a *S. cerevisiae* gene belonging to the AAA family and involved in homotypic membrane fusion, results in cells dying with a typical apoptotic phenotype: exposition of phosphatidylserine, margination of chromatin, DNA fragmentation and formation of cell fragments [20]. In spite of massive DNA breakage, demonstrated by the TUNEL test, no DNA ladder could be observed. Nucleosome linkers appear not to be preferred targets of DNA cleavage in yeast, probably due to their short length [21].

The co-ordinate occurrence of these events at different locations within the cell having no obvious connection except their relation to apoptosis indicates the presence of a machinery performing the basic steps of apoptosis already in yeast.

5. Programmed cell death in other monocellular eukaryotes

Yeast is not the only or even the first monocellular eukaryote for which programmed cell death has been described. *Trypanosoma cruzi* and *T. brucei rhodesiense*, *Dictyostelium discoideum*, and *Tetrahymena thermophila* show some apoptotic features such as cytoplasmic blebbing and vacuolisation, DNA fragmentation and chromatin condensation in response to environmental stress or extracellular signals [22]. These organisms are evolutionarily more distant from mammals than yeast is, dating the origin of programmed cell death yet further back in evolution. Indeed, higher plants, which are about as closely related to mammals as yeast is, exhibit characteristic apoptotic features, such as DNA strand breaks and exposition of phosphatidylserine [23], and can be protected from cell death by expression of the anti-apoptotic genes, *bcl*-x_L or *ced*-9 [24].

6. ROS are endogenous regulators of apoptosis in yeast

As the basic apoptotic machinery seems to be present in yeast, the question arises how the apoptotic programme is regulated when all crucial regulator genes known from the mammalian system are missing.

However, regulators of apoptosis exist which are not proteins and therefore cannot be deduced from the genomic sequence. ROS (such as $O_2^{-\bullet}$, H_2O_2 and $OH^{\bullet}$) are a well characterised class of apoptotic inducers. Intracellular ROS accumulate in neural cells deprived of nerve growth factor or potassium as a late step of the apoptotic pathway, downstream of the action of bax and caspases [25,26]. This accumulation is a prerequisite for the ensuing cell death. In addition, exogenous oxygen stress triggers the apoptotic cascade by itself [27–30].

In *S. cerevisiae*, exposure to low doses of H_2O_2 or accumulation of ROS by depletion of glutathione induces apoptosis as well. Inhibition of translation by cycloheximide prevents the development of apoptotic markers in response to H_2O_2, indicating an active role of the cell in the death process [31]. Furthermore, yeast cell death caused by the expression of *bax* or by *cdc48*^{S565G} is accompanied by an accumulation of ROS. Indeed, these radicals are necessary to induce the apoptotic phenotype [31], putting ROS at a central position in yeast apoptosis.

7. A potential scenario for the development of apoptosis

This important role of ROS in the regulation of apoptosis may indicate the origin and primary purpose of the suicide process. ROS are byproducts of respiration and occur in every aerobic organism. Because ROS are highly reactive and modify proteins, lipids and nucleic acids, ROS-induced cell damage is a frequent event. In cases of potentially fatal damage, rapid suicide will terminate the now futile metabolism of the cell. For a unicellular organism this spares nutrient resources for the surrounding cells – usually cells of its own clone. Its suicide will be of advantage for its clonal relatives – and therefore for the genome of the damaged individual itself. High concentrations of ROS serve as the trigger of the process, possibly because their chemical reactivity made them easy to detect.

In the further development of apoptosis, a suicide mechanism may have proved useful for additional purposes as well. A potential case of cellular suicide in yeast has been described by Longo et al. [32] who observed that stationary cells of *S. cerevisiae* survive for long periods in pure water but quickly lose viability in nutrient-depleted synthetic media. Death of a majority of cells may spare the dwindling resources for the best adapted, healthiest, or – as yeast has a finite lifespan –

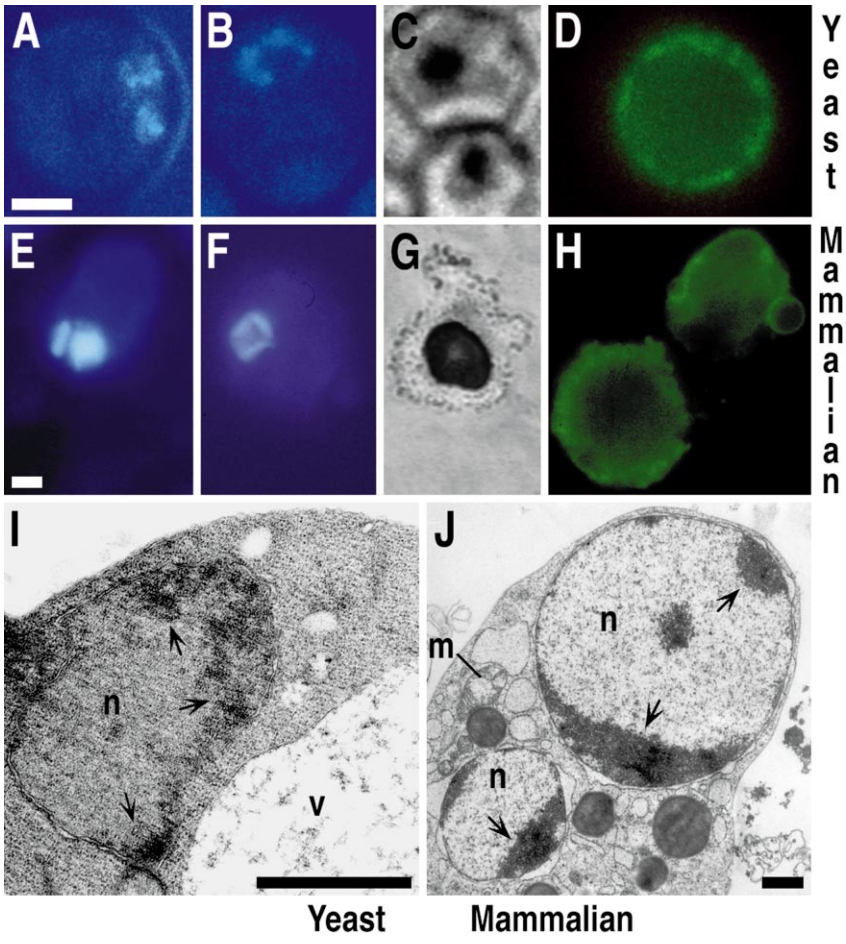

Fig. 1. Cytological markers of apoptosis in yeast and mammalian cells. *S. cerevisiae* treated with 1 mM H_2O_2 (A, B, C, I) or mutated in *CDC48* (*cdc48*^{S565G}, D), porcine thyrocytes treated with 30 μM retinol (E, F, H, J), and human WISH cells treated with 1 mM H_2O_2 (G) stained for chromatin with DAPI (A, B) or bisbenzimide (E, F), for DNA fragmentation with the TUNEL assay (C, G), for the exposition of phosphatidylserine with FITC-labelled annexin V (D, H), or investigated by electron microscopy for chromatin condensation (I, J). Bars 10 μm (A–H), 1 μm (I, J).

youngest isogenic relatives. Production of ROS as an endogenous signal to trigger the suicide programme autonomously became desirable. As oxygen radicals are normal byproducts of respiration, a specific modulation of the respiratory chain may have been developed to increase the output of ROS as needed. The release of cytochrome *c* may be a result of that modulation. 'Petite' yeasts lacking functional mitochondria survive *bax* expression [6] consistent with the necessity of an active respiratory chain in the suicide mechanism.

During a further refinement in the regulation of apoptosis, released cytochrome *c* itself became used as an apoptotic signal, perhaps in order to make the regulatory cascade less dependent on the redox state of the cell. With the development of multicellular organisms, a more flexible regulation of apoptosis became necessary, including responses to various external signals, resulting in additional regulatory steps upstream, downstream, or instead of ROS.

Recent observations concerning programmed cell death in the dinoflagellate *Peridinium gatunense* support this hypothesis. Cell death is induced by oxidative stress [33], confirming the evolutionarily ancient link between ROS and cell suicide. *P. gatunense* exhibits typical markers of apoptosis, DNA fragmentation and protoplast shrinkage. A cysteine protease inhibitor prevents cell death indicating that a protease participates in the suicide programme, similar to the situation in higher animals.

8. A comparison of cell death between yeast and mammals

Is it really apoptosis that has been observed in yeast? If apoptosis is defined as autonomous cell death in connection with certain morphological and cytological markers, yeast certainly performs apoptosis (Fig. 1). If the definition demands the involvement of specific proteins such as caspases or bcl-2 relatives, yeast does not. However, the similar response of yeast and animals to oxygen stress and to the expression of several pro- and anti-apoptotic genes argues strongly in favour of a common origin of the processes.

Yeast has already shown its value as a model for apoptosis research (for review, see [34].) Reed and coworkers used the cytotoxicity of *bax* on *S. cerevisiae* to select anti-apoptotic effectors from a human gene library [35] and to identify yeast mutations preventing cell death [36]. Both strategies were successful, and the results could be extended to mammalian apoptosis. A mutated gene preventing yeast cell death was identified as mitochondrial F_0F_1-ATPase. Inhibition of the enzyme by oligomycin prevents cell death in both *S. cerevisiae* and mammalian cells [36]. A novel anti-apoptotic factor, BI-1,

was identified which is located at the endoplasmic reticulum and interacts with Bcl-2, but not Bax or Bak. When overexpressed in mammalian cells, it suppresses apoptosis induced by Bax, growth factor withdrawal or various drugs, but not by Fas [35]. Additional anti-apoptotic genes functional in yeast and mammals have been identified by a similar approach [37]. Recently, an anti-apoptotic function of the mammalian *CDC48* orthologue VCP has been described [38], confirming the effects of the *cdc48*^{S565G} mutation in yeast as bona fide apoptosis.

In the near future, yeast promises the identification of components of the basic, evolutionarily ancient stages of apoptosis. Yeast offers the opportunity to easily screen for substances acting directly on these basic components without being diverted by a complex upstream network. This may result in 'universal' activators or inhibitors of apoptosis which would be helpful for research, and potentially for medical applications as well.

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References

[1] Kerr, J.F., Wyllie, A.H. and Currie, A.R. (1972) Br. J. Cancer 26, 239–257.
[2] Steller, H. (1995) Science 267, 1445–1449.
[3] Chinnaiyan, A.M., Orth, K., O'Rourke, K., Duan, H., Poirier, G.G. and Dixit, V.M. (1996) J. Biol. Chem. 171, 4573–4576.
[4] Sato, T. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 9238–9242.
[5] Hanada, M., Aimé-Sempé, C., Sato, T. and Reed, J.C. (1995) J. Biol. Chem. 270, 11962–11969.
[6] Greenhalf, W., Stephan, C. and Chaudhuri, B. (1996) FEBS Lett. 380, 169–175.
[7] Kang, J.J., Schaber, M.D., Srinivasula, S., Alnemri, E.S., Litwack, G., Hall, D.J. and Bjornsti, M.A. (1999) J. Biol. Chem. 274, 3189–3198.
[8] Ryser, S., Vial, E., Magnenat, E., Schlegel, W. and Maundrell, K. (1999) Curr. Genet. 36, 21–28.
[9] Bischoff, J.R., Casso, D. and Beach, D. (1992) Mol. Cell. Biol. 12, 1405–1411.
[10] Nigro, J.M., Sikorski, R., Reed, S.I. and Vogelstein, B. (1992) Mol. Cell. Biol. 12, 1357–1365.
[11] James, C., Gschmeissner, S., Fraser, A. and Evan, G.I. (1997) Curr. Biol. 7, 246–252.
[12] Jürgensmeier, J.M., Krajewski, S., Armstrong, R.C., Wilson, G.M., Oltersdorf, T., Fritz, L.C., Reed, J.C. and Ottilie, S. (1997) Mol. Biol. Cell 8, 325–339.
[13] Kitanaka, C. et al. (1997) Oncogene 15, 1763–1772.
[14] Zha, H., Fisk, H.A., Yaffe, M.P., Mahajan, N., Herman, B. and Reed, J.C. (1996) Mol. Cell. Biol. 16, 6494–6508.
[15] Tao, W., Kurschner, C. and Morgan, J.I. (1997) J. Biol. Chem. 272, 15547–15552.
[16] Minn, A.J. et al. (1999) EMBO J. 18, 632–643.
[17] Ink, B., Zörnig, M., Baum, B., Hajibagheri, N., James, C., Chittenden, T. and Evan, G. (1997) Mol. Cell. Biol. 17, 2468–2474.
[18] Zhao, Y., Yu, M., Chen, M., Elder, R.T., Yamamoto, A. and Cao, J. (1998) Virology 246, 266–267.
[19] Ligr, M., Madeo, F., Fröhlich, E., Hilt, W., Fröhlich, K.-U. and Wolf, D.H. (1998) FEBS Lett. 438, 61–65.
[20] Madeo, F., Fröhlich, E. and Fröhlich, K.-U. (1997) J. Cell Biol. 139, 729–734.
[21] Lowary, P.T. and Widom, J. (1989) Proc. Natl. Acad. Sci. USA 86, 8266–8270.
[22] Ameisen, J.C. (1996) Science 272, 1278–1279.
[23] O'Brien, I.E.W., Reutelingsperger, C.P.M. and Holdaway, K.M. (1997) Cytometry 29, 28–33.
[24] Mitsuhara, I., Malik, K.A., Miura, M. and Ohashi, Y. (1999) Curr. Biol. 9, 775–778.
[25] Schulz, J.B., Weller, M. and Klockgether, T. (1996) J. Neurosci. 16, 4696–4706.
[26] Schulz, J.B. et al. (1997) J. Neurochem. 69, 2075–2086.
[27] Hockenbery, D.M., Oltvai, Z.N., Yin, X.-M., Milliman, C.L. and Korsmeyer, S.J. (1993) Cell 75, 241–251.
[28] Kane, D.J., Sarafian, T.A., Anton, R., Hahn, H., Gralla, E.B., Valentine, J.S., Örd, T. and Bredesen, D.E. (1993) Science 262, 1274–1277.
[29] Greenlund, L.J.S., Deckwerth, T.L. and Johnson Jr., E.M. (1995) Neuron 14, 303–315.
[30] Slater, A.F.G., Stefan, C., Nobel, I., van den Dobbelsteen, D.J. and Orrenius, S. (1995) Toxicol. Lett. 82–83, 149–153.
[31] Madeo, F., Fröhlich, E., Ligr, M., Grey, M., Sigrist, S.J., Wolf, D.H. and Fröhlich, K.-U. (1999) J. Cell Biol. 145, 757–767.
[32] Longo, V.D., Ellerby, L.M., Bredesen, D.E., Valentine, J.S. and Gralla, E.B. (1997) J. Cell Biol. 137, 1581–1588.
[33] Vardi, A., Berman-Frank, I., Rozenberg, T., Hadas, O., Kaplan, A. and Levine, A. (1999) Curr. Biol. 9, 1061–1064.
[34] Shaham, S., Shuman, M.A. and Herskowitz, I. (1998) Cell 92, 425–427.
[35] Xu, Q. and Reed, J.C. (1998) Mol. Cell 1, 337–346.
[36] Matsuyama, S., Xu, Q., Velours, J. and Reed, J.C. (1998) Mol. Cell 1, 327–336.
[37] Greenhalf, W., Lee, J. and Chaudhuri, B. (1999) Yeast 15, 1307–1321.
[38] Shirogane, T., Fukada, T., Muller, J.M., Shima, D.T., Hibi, M. and Hirano, T. (1999) Immunity 11, 709–719.