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## Mini Review

### Caspase-dependent and caspase-independent cell death pathways in yeast

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Yeast (*Saccharomyces cerevisiae*) can undergo cell death accompanied by diagnostic features of apoptosis, such as phosphatidylserine externalization, DNA fragmentation, chromatin condensation, cytochrome *c* release from mitochondria, and dissipation of the mitochondrial transmembrane potential. Both caspase-dependent and caspase-independent cell death executors participate in yeast cell death. On one hand, a yeast caspase-like protease appears to be essential for approximately 40% of the investigated cell death scenarios. On the other hand, factors like the mitochondrially located nucleases AIF (apoptosis-inducing factor) and endonuclease G, can execute caspase-independent cell death in yeast. Furthermore, complex correlates of mammalian cell death are observed in yeast. This applies to cell death-associated cytochrome *c* release, mitochondrial fragmentation, cytoskeletal alterations, generation of reactive oxygen species and epigenetic histone modifications. Hence, yeast constitutes an excellent model organism to delineate phylogenetically conserved pathways leading to apoptotic or necrotic cell death. Moreover, yeast can be used to identify pharmacological and genetic modulators of cell death pathways that are relevant for human disease.

Twelve years ago, apoptotic markers were described in yeast for the first time [1,2], challenging the idea that apoptosis would be exclusively executed in multicellular organisms. Ever since, convincing evidence has been added to that initial description of yeast apoptosis. Thus, several yeast counterparts of crucial mammalian apoptosis regulators have been characterized. Conserved proteasomal, mitochondrial, nuclear and epigenetically-regulated cell death pathways have been profiled. Physiological death scenarios such as viral infections, chronological and replicative aging have been described in yeast [3–7]. Moreover, assays for apoptotic and/or necrotic cell death such as viability, DNA fragmentation, exposition of phosphatidylserine, cell integrity or ROS accumulation have been established and are routinely used in the field of yeast programmed cell death (PCD), both on the qualitative (microscopical) and the quantitative (cytofluorometric) levels [1,2,8].

What is the need for yet another model system for cell death research in view of the existence of established animal systems for the exploration of lethal signaling pathways, including rodents (*Mus musculus* and *Ratus norvegicus*), nematodes (*Caenorhabditis elegans*) and flies (*Drosophila melanogaster*)? The answer resides in the exclusive advantages offered by yeast for apoptosis research. For instance, the genetic tractability of yeast is unique and allows, for example, the simultaneous overexpression of one gene and the knockout of another gene, helping to establish hierarchies among cell death executors. In addition, yeast avoids one of the technical problems that affect mammalian cell death research: the detection of cell death mostly relies on apoptotic markers, which however

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can yield false positive results. For example, neither caspase activity nor phosphatidylserine externalization is a sufficient criterion for mammalian cell death, because caspase activation can occur without cell death and cell death can occur without caspase activation [9]. In yeast, the precise measurement of actual cell death, i.e. the number of dead versus living cells, is easily and routinely accomplished by the use of plating assays. The combination of such a clonogenic cell death assay with the analysis of apoptotic markers allows for the discrimination between apoptotic and non-apoptotic forms of cell death. Moreover, there is no other organism where mitochondrial functions can be more easily manipulated than in yeast. Given the fact that mitochondria are decisive for cell death execution [10,11], such manipulations are of special importance. Deletion of mitochondrial DNA or growth on fermentable carbon sources can suppress mitochondrial functions in yeast, whereas the switch to non-fermentable carbon sources strongly boosts respiration, increases mitochondrial mass and enhances the propensity to undergo mitochondria-mediated cell death. Finally, yeast is an ideal tool for high-throughput screenings. The availability of a complete collection of yeast knockout strains render genome-wide yeast screens fast and inexpensive, at least in comparison to other model organisms. Altogether, these factors have led to the recent explosion of research on yeast apoptosis—the topic of this minireview.

### ROS: a major cause of yeast apoptosis

A simple and experimental convenient way to induce yeast apoptosis is the induction of reactive oxygen species (ROS). The first evidence that ROS can trigger unicellular programmed death came from experiments exposing yeast (which were grown at a low cell density) to mild doses of  $H_2O_2$ . While high doses of  $H_2O_2$  lead to a necrotic phenotype, low doses induced apoptosis, which could be genetically mimicked by deletion of glutathione-generat-

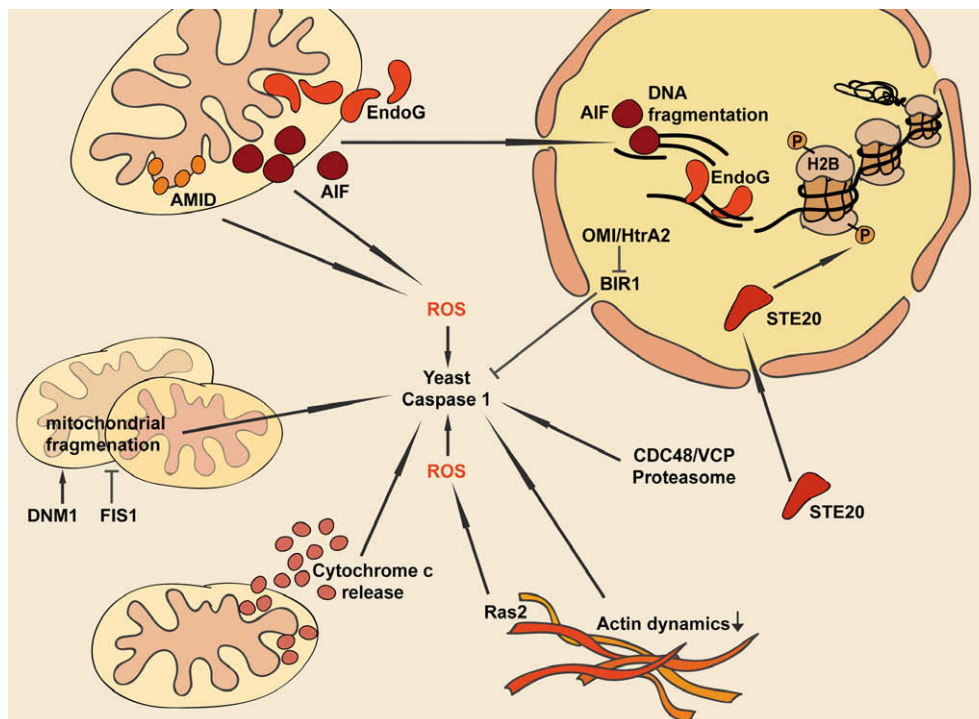
ing enzymes [2]. Subsequent studies revealed several core apoptotic executioners that are involved in ROS-mediated cell death, including the yeast caspase *YCA1* [12] or the apoptosis-inducing factor *AIF1* [13] (see Fig. 1).

### Caspase-dependent yeast apoptosis

Apart from ROS-mediated cell death, deletion of the yeast meta-caspase *YCA1* can protect yeast cells against multiple distinct forms of lethal insult [12] (see Table 1). For instance, yeast cells exposed to salt (NaCl) [14] or low doses of valproic acid, a short chained fatty acid with anti-tumor activity, undergo *YCA1*-dependent apoptosis [15,16]. Excessive iron causes *YCA1*-dependent cell death [17–19], presumably through a pathway in which cardiolipin activates the neutral sphingomyelinase *ISC1*, which in turn generates ceramide. However, deletion of *ISC1* has been shown to shorten chronological lifespan and to enhance  $H_2O_2$  sensitivity, which is *YCA1*-dependent and can be suppressed by iron chelation. Of note, *ISC1* deletion is connected to an upregulation of the iron regulon that increases iron levels, which are known to catalyze the production of the highly reactive hydroxyl radicals (Fenton reaction) [19]. Besides iron, further metals like manganese and cadmium have been shown to induce *YCA1*-dependent apoptosis in yeast [20,21].

Exposure to toxins produced by virus-carrying killer strains also leads to PCD in yeast [3,22,23]. Deletion of *YCA1* in the attacked strain leads to reduced toxin sensitivity [3]. Similarly, heterologous expression of expanded polyglutamine domains, which cause protein aggregation and neurodegeneration in human Huntington's disease, leads to PCD in yeast [24], and this is again inhibited by *YCA1* deletion [25].

Finally, yeast death triggered by defects in ubiquitination, defective DNA replication initiation, reduced mRNA stability, mitochondrial fragmentation or aging can occur at least partly in a caspase-dependent fashion [6,26–31]. During chronological aging (see below), deletion of *YCA1*, initially ameliorates the survival in the



**Fig. 1.** The molecular machinery of yeast apoptosis. Exogenous and endogenous induction of yeast apoptosis leads to the activation of the basic molecular machinery of cell death, which is configured by conserved apoptotic key players such as the yeast caspase Yca1p, the yeast homolog of mammalian HtrA2/OMI (Nma111p) or the apoptosis-inducing factor Aif1p. Furthermore it involves complex processes like histone modification, mitochondrial fragmentation, cytochrome *c* release, and cytoskeletal perturbations.

**Table 1**

Caspase-dependent and -independent cell death in yeast. This table displays where deletion of the yeast caspase was successful in protecting (at least in part or in a time-dependent manner) against a deadly insult—and where not.

Yca1p-dependent scenarios of yeast apoptosis		Yca1p-independent scenarios of yeast apoptosis	
External stimuli/treatment	Reference	External stimuli/treatment	Reference
H <sub>2</sub> O <sub>2</sub>	[12,32,53] [58]*	Formic acid	[37]
Acetic acid	[59,60]	Copper	[20]
NaCl/hyperosmotic stress	[14,61]	Dermaseptins	[62]
Valproic acid	[16]	Spingholipids	[63]
Arsenic	[64]		
Manganese	[20]		
Cadmium	[21]		
Physiological scenarios		Physiological scenarios	
Chronological aging	[4]	Replicative aging	Madeo, Breitenbach, unpublished results
Viral killer toxins	[3,22]	Colony differentiation	[34]
Mutation	Biological process	Mutation/overexpression	Biological process
<i>Δubp10</i>	Deubiquitination [26]	<i>wbp1-1</i>	N-glycosylation [27,36]
<i>orc2-1</i>	DNA replication	<i>ost2-3</i>	N-glycosylation [27]
<i>lsm4Δ1</i> (aging)	mRNA decapping [60]	<i>Δsro77</i> (NaCl stress)	Exocytosis [14]
<i>Δfis1</i>	Mitochondrial fission [6]	<i>NUC1<sup>exp</sup></i> (yeast EndoG)	[8]
<i>Δcit1</i> (heat, aging)	Mitochondrial metabolism [65]	<i>AIF1<sup>exp</sup></i> (yeast AIF)	[13]
<i>Δisc1</i> (H <sub>2</sub> O <sub>2</sub> , aging)	Mitochondrial metabolism/iron homeostasis [20]	Bax, Bid, human caspases	[66,67]
Heterologous expression of proteins associated to human disease		Heterologous expression of proteins associated to human disease	
Expanded poly-Q domain	Huntington's disease, neurodegeneration [24,25]	DR4 expression + DNA damage (MMS treatment)	[68]
Alpha synuclein (mid-log phase)	Parkinson's disease, neurodegeneration [69]	Alpha synuclein (chronological aging)	Parkinson's disease, neurodegeneration [70]

\* *Candida albicans*.

culture. However, at later time points, *YCA1*-deficient cultures accumulate damaged cells that would have been eliminated in wild type cultures. Thus, when aged in a direct competition experiment, the *YCA1* deletion mutant is outlived by the wild type [4]. The importance of Yca1p mediated apoptosis in eliminating damaged cells is corroborated by the finding that *YCA1* disruption increases the level of oxidized proteins [32]. Intriguingly, recent mechanistic of chromatin cohesion have revealed a role for another caspase-like protease in yeast PCD: Eps1p, when released from the anaphase inhibitor Pds1p, acts as caspase-like protease and cleaves Mcd1p, the yeast homolog of human cohesion Rad21. In response to apoptotic stimuli, like H<sub>2</sub>O<sub>2</sub> treatment, the truncated C-terminal fragment of Mcd1p translocates from the nucleus to mitochondria where it causes a decrease of mitochondrial membrane potential and triggers the release of cytochrome c [33].

### Caspase-independent yeast apoptosis

A rough estimation of the published yeast apoptosis scenarios counts approximately 40% as caspase-dependent, meaning that they can be rescued at least in part by deletion of *YCA1* (see Table 1). Thus, in most instances, Yca1p is not necessary for cell death. For example, regulated cell death occurring during long-term development of yeast multicellular colonies [34,35] or apoptosis derived from defective N-glycosylation in cells lacking Ost2p, the yeast homolog of the mammalian defender of apoptosis-1 (*DAD1*) protein [27] are both independent of *YCA1*. Moreover, upon defective N-glycosylation in the temperature-sensitive *wbp1-1* mutant or after treatment with tunicamycin, yeast PCD depends on the protease activity of *KEX1* (but not *YCA1*). This newly identified apoptotic protease also plays a role in cell death induced by acetic acid or

chronological aging [36]. A potential Yca1p-independent extrinsic inducer of yeast apoptosis is formic acid, known also to cause cell death in mammalian ocular cells. While the molecular mechanism in mammalian cells is still unidentified, it was demonstrated in yeast that formic acid induces typical phenotypes of mitochondria-mediated apoptosis (e.g. loss of mitochondrial membrane potential, early ROS burst and mitochondrial destruction) [37].

The apoptosis-inducing factor (AIF) was the first one to be implicated in caspase-independent cell death pathways [38]. Yeast bears a bona fide AIF homolog named *AIF1* [13]. Similarly to its mammalian counterpart, yeast *Aif1p* translocates from the mitochondria to the nucleus when challenged by an apoptotic stimulus (H<sub>2</sub>O<sub>2</sub>, acetate, or aging). Once in the nucleus, *Aif1p* mediates chromatin condensation and DNA degradation. Like in mammals, cyclophilin A is required for *Aif1p* induced cell death, and a combination of recombinant cyclophilin A and mammalian AIF (but neither of the two recombinant proteins alone) can mediate DNA degradation in vitro [13,39,40]. Consistently, *AIF1* deletion mutants show better survival during H<sub>2</sub>O<sub>2</sub>- and acetate treatment as well as during chronological aging [13]. Besides its lethal function, *Aif1p* also exhibits vital functions for respiration [41] in equivalence to mammalian AIF, which via its NADH oxidase domain and the resulting redox function is central for optimal oxidative phosphorylation and for an effective anti-oxidant defense [42].

Another *YCA1*-independent cell death effector is *NUC1*, a yeast ortholog of mammalian endonuclease G (EndoG) [8]. As mammalian EndoG, *Nuc1p* is located in mitochondria and translocates to the nucleus upon apoptosis induction [43,44]. Deletion of the mitochondrial localization sequence enhances the pro-apoptotic activity of *Nuc1p*. Interestingly, *Nuc1p*-mediated

death is Aif1p independent but requires yeast homologs of the mammalian permeability transition pore, the karyopherin Kap123p, and phosphorylation of histone H2B [8]. *NUC1* disruption causes an inhibition of apoptotic cell death but only when mitochondrial respiration is increased; in conditions in which oxidative phosphorylation results is inhibited *NUC1* disruption causes enhanced necrotic death. This discriminates between vital and lethal functions of *NUC1*. In fact, a vital role of EndoG has been pinpointed in the maintenance of polyploid cells, which is phylogenetically conserved between yeast and mammals [45].

The yeast nucleus harbors a further caspase-independent death regulator, Nma111p (nuclear mediator of apoptosis), whose overexpression increases cell death upon elevated temperature or H<sub>2</sub>O<sub>2</sub> exposure while its deletion reduces signs of apoptosis [46]. The lethal function of Nma111p is mediated by its serine protease activity and, unlike its mammalian pro-apoptotic homolog (HtrA2/Omi), it does not localize to mitochondria but to the nucleus [46]. Interestingly, one of the substrates for Nma111p is Bir1p, the only known inhibitor-of-apoptosis (IAP) protein in yeast. Upon oxidative stress, *BIR1* disruptants show enhanced apoptosis while overexpression of Bir1p reduces cell death, an effect that can be antagonized by simultaneous overexpression of Nma111p [47]. Along with its function in apoptosis, Bir1p, like its closest metazoan homologs survivin (mammals) and detersin (flies), also plays a role in chromosome segregation and cytokinesis [48,49]. Besides Bir1p, two further antiapoptotic proteins have recently been identified in yeast: Sno1p and Fyv10p. Both proteins show sequence similarity to the mammalian Bax suppressor Tsc22 within its C-terminal domain, and their role as yeast antiapoptotic proteins has been confirmed through overexpression and knockout experiments [50].

A distinct nuclear mechanism of apoptosis control, epigenetic regulation, appears to be conserved from mammals [51] to yeast [52,53]. On prominent pro-apoptotic pathway involves the N-terminal tail of histone H2B, which is deacetylated on lysine 11 by the histone deacetylase (HDAC) Hos3p and the phosphorylated on serine 10 by the Ste20p kinase [53]. Ste20p is also required for pheromone-induced apoptosis in yeast, downstream of the MAP kinase cascade [54].

Summing up, during the last decade evidence has been accumulating to prove that the basal apoptotic machinery including its key regulators are conserved between yeast and mammals, suggesting that the concept of PCD is also applicable to unicellular organisms that exhibit multicellular behavior. This applies at least to the intrinsic (mitochondrial) pathway of cell death regulation/execution. Therefore, yeast may be a useful model organism helping to accelerate the comprehension of human PCD and to identify genetic or pharmacological strategies for its therapeutic modulation. The German poet August Graf von Platen (1796–1835) stated in 1821: “It is uniform, how nature enters life, but there are thousand ways to die”. Although this is undoubtedly true, yeast and human cells have conserved some lethal subroutines. Thus, yeast can undergo cell death that resembles apoptosis, as well as regulated necrosis, a topic that has attracted a great deal of interest during the past two years [55–57]. Hence, we are confident that future research on yeast cell death will identify specific pathways involved in the regulation of caspase-dependent or caspase-independent apoptosis, programmed necrosis or autophagy, and that this information will be invaluable information for future manipulation of pathological cell death linked to lipotoxicity, cardiotoxicity or neurotoxicity.

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