Mitochondrial dynamics in yeast cell death and aging

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Abstract

Mitochondria play crucial roles in programmed cell death and aging. Different stimuli activate distinct mitochondrion-dependent cell death pathways, and aging is associated with a progressive increase in mitochondrial damage, culminating in oxidative stress and cellular dysfunction. Mitochondria are highly dynamic organelles that constantly fuse and divide, forming either interconnected mitochondrial networks or separated fragmented mitochondria. These processes are believed to provide a mitochondrial quality control system and enable an effective adaptation of the mitochondrial compartment to the metabolic needs of the cell. The baker's yeast, *Saccharomyces cerevisiae*, is an established model for programmed cell death and aging research. The present review summarizes how mitochondrial morphology is altered on induction of cell death or on aging and how this correlates with the induction of different cell death pathways in yeast. We highlight the roles of the components of the mitochondrial fusion and fission machinery that affect and regulate cell death and aging.

Introduction

Mitochondria are semi-autonomous organelles that contain their own genome [mtDNA (mitochondrial DNA)], encoding a small subset of mitochondrial proteins. They are bounded by two membranes: the outer and the inner membrane. Mitochondria are essential for various metabolic processes, including oxidative phosphorylation, amino acid metabolism and the formation of iron–sulfur clusters. In addition, mitochondria play crucial roles in the regulation of cell death and aging in various organisms, including humans and yeast [1–4]. In the budding yeast *Saccharomyces cerevisiae*, mitochondrial dysfunction, mutations in mtDNA and ROS (reactive oxygen species) produced by mitochondria have been proposed to determine both chronological lifespan (i.e. the survival time of a post-mitotic yeast culture) and replicative lifespan (i.e. the number of cell divisions of an individual mother cell) [3–5]. In particular, production of detrimental ROS by the NADH:ubiquinone oxidoreductase Ndi1 and the cytochrome bc_1 complex of damaged mitochondria was shown to be an important factor promoting cell death [6–9]. Mitochondrial permeabilization and the release of mitochondrial proteins into the cytosol define distinct mitochondrion-dependent cell death pathways [10–13]. Release of cytochrome *c* correlates with activation of the cell death protease Yca1 (yeast caspase 1), which ultimately executes cell death [13,13a]. In Yca1-independent pathways, Aif1 (apoptosis-inducing factor 1) or endonuclease G (Nuc1) are released from mitochondria and translocate into the nucleus, resulting in the fragmentation of the nuclear genome [10,12]. Mitochondria are also involved in cell death pathways mediated by the alternative cell death protease Kex1 or Ysp1 (yeast suicide protein 1) and Ysp2 [9,14–16]. However, the molecular mechanisms of these cell death scenarios remain poorly understood.

Mitochondria are highly dynamic organelles that continuously fuse and divide [17]. In many organisms, including worms and mammals, mitochondrial division promotes the release of cytochrome *c* to trigger apoptosis [1]. Furthermore, mitochondrial dynamics is thought to counteract aging and constitute an organellar quality control mechanism. Fusion allows complementation and repair processes of damaged mitochondria, whereas fission separates defective organelles from the mitochondrial network, which are then subjected to degradation through mitophagy [17]. In addition to their role in apoptosis and mitochondrial quality control, mitochondrial fusion and fission are needed to optimally adapt mitochondria to the metabolic needs of the cell [17]. In yeast, inhibition of fusion leads to damage and loss of mtDNA [18–20]. In contrast, mitochondrial fusion increases mtDNA copy number and ensures high accuracy of the encoded genetic information [21]. While respiratory-active yeast cells harbour an extended tubular mitochondrial network, resting non-dividing cells contain mostly fragmented mitochondria [22].

A conserved molecular machinery mediates mitochondrial fusion and fission in humans and yeast [17]. In yeast, the large GTPase Fzo1, a member of the mitofusin protein family, promotes fusion of the mitochondrial outer membrane, whereas the dynamin-related large GTPase Mgm1 enables fusion of the inner membrane [18,19,23]. Outer and inner membrane fusion is co-ordinated by the outer membrane protein Ugo1, which physically interacts with both Fzo1 and Mgm1 [24]. Division of the outer membrane is performed by the dynamin-related large GTPase Dnm1, which binds via

Key words: aging, cell death, mitochondrial dynamics, mitochondrial fission, mitochondrial fusion, *Saccharomyces cerevisiae*.

Abbreviations used: Aif1, apoptosis-inducing factor 1; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; Yca1, yeast caspase 1; Ysp, yeast suicide protein.

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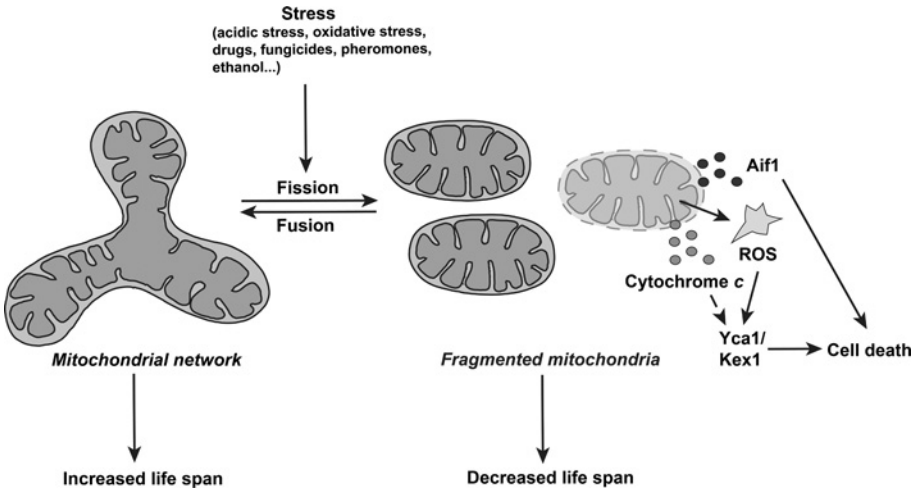
Figure 1 | **Mitochondrial fragmentation and aggregation during programmed cell death and aging in yeast**
See the main text for details.



the adaptor proteins Mdv1 or Caf4 to the outer membrane receptor Fis1 and forms a contractile ring that eventually promotes outer membrane division [25–27]. An increasing number of accessory and regulatory components are being identified to regulate the fusion and fission processes. These include the rhomboid-related membrane protease Pcp1, which processes Mgm1 [28], and the F-box protein Mdm30, a ubiquitin ligase subunit mediating the turnover of Fzo1 [29].

In the present review, we summarize abnormal mitochondrial morphologies that were observed in yeast models for programmed cell death and that link these morphologies with different cell death pathways. We focus on the effect of the mitochondrial fusion and fission balance on yeast cell death and aging, and we outline how these processes may be relevant under deleterious conditions.

Changes in mitochondrial morphology during yeast cell death and aging

Fragmentation of the mitochondrial network into multiple small organelles has been observed on treatment of yeast cells with multiple different stressors inducing programmed cell death (Figure 1; Table 1). Mitochondrial fragmentation occurs on (i) acidic stress, including acetic acid [16,30,31], propionic acid [16] and formic acid [32], (ii) oxidative stress (H_2O_2) [33], (iii) treatment with drugs and fungicides (amiodarone [9,15,16], bostrycin [34] and trichothecene [35]), (iv) yeast pheromones (α-factor) [9] and (v) ethanol [36]. A stringent correlation between mitochondrial fragmentation and yeast cell death was further observed in a variety of mutant yeast strains (Table 1), including strains with mutations in (i) rRNA genes (HsTnII) [37], (ii) mRNA turnover genes (*lsm4*) [38,39], (iii) genes involved in glycoprotein biosynthesis (*wbp1-1*) [14] and (iv) stress response genes (Δ*whi2*) [40]. Furthermore, cell death concomitant with mitochondrial fragmentation was observed in yeast cells overexpressing signalling kinases (*TPK3*) [41], sphingolipid-metabolizing enzymes (*YDC1*) [42] or overexpressing human proteins, including the pro-apoptotic protein BAX [43], and a Huntington's disease-causing variant of huntingtin [44]. Finally, yeast cells that enter the stationary phase and undergo chronological aging contain fragmented mitochondria [22,45]. The wide variety of death-inducing conditions that correlate with disruption of the mitochondrial network indicates that mitochondrial morphology is strongly influenced by the state of health of the cell. Conversely, conditions that trigger mitochondrial fragmentation frequently result in a decreased chronological lifespan [37–39,42].

In some of the cell death scenarios described above, mitochondrial fragmentation is superimposed by aberrant mitochondrial aggregation [40,41,44], which is an active process requiring the actin cytoskeleton [31,40,41]. Aggregated mitochondrial fragments accumulate on disruption of the mitochondrion degradation pathway [31,43], suggesting that most of these organelles are damaged and destined for degradation. Indeed, fragmented and/or aggregated mitochondria were found to be physically and functionally impaired during cell death (Figure 1; Table 1). Mitochondrial deterioration during cell death is characterized by the following events: loss of the mitochondrial membrane potential [32,37], which can be preceded by a strong increase [9], permeabilization of the mitochondrial outer membrane [31], release of cytochrome *c* [9,31] and loss of mtDNA [40]. As a consequence, cells undergoing cell death frequently become respiratory deficient [37,41]. Notably, the accumulation of ROS appears to be a general hallmark of cell death in cells with fragmented and/or aggregated mitochondria [9,14–16,32,34,36,38,39,41,44]. This suggests that damaged mitochondria are a major source of ROS during cell death and thereby actively contribute to the cellular demise (Figure 1).

Cell death correlated with mitochondrial fragmentation either depends on the activity of the yeast cell death protease

Table 1 | **Yeast cell death and aging associated with abnormal mitochondrial morphologies**

	Inducers of cell death	Mitochondrial morphology	Yeast cell death pathway	Reference(s)
Stressors	Acetic acid	Fragmentation; *FIS1* is not required for fragmentation; actin-dependent formation of mitochondrial aggregates in Δ*pep4* and Δ*aac1* Δ*aac2* Δ*aac3*	Mitochondrial outer membrane permeabilization; cytochrome *c* release; *YCA1*, *DNM1* and *MDV1* promote cell death; *FIS1* relieves cell death	[30,31]
	α-Factor, amiodarone, acetic acid, propionic acid	Fragmentation; fragmentation depends on Ysp1and Ysp2	Cell death depends on *YSP1* and *YSP2*; increased mitochondrial membrane potential; ROS accumulation; cytochrome *c* release	[9,15,16]
	Bostrycin	Fragmentation	*AIF1*-dependent and *YCA1*-independent cell death; ROS	[34]
	Ethanol	Fragmentation; fragmentation depends on *FIS1* but is independent of *DNM1* and *MDV1*; at high concentrations, *FIS1* is not required	ROS accumulation depends on *FIS1*; cell death is independent of *YCA1*, *AIF1*, cytochrome *c*, *MDV1* and *DNM1*	[36]
	Formic acid	Fragmentation	Decreased mitochondrial membrane potential; *YCA1*-independent cell death; ROS accumulation	[32]
	H_2O_2	Fragmentation	Decreased viability	[33]
	Trichothecene	Fragmentation	Mutants of the mitochondrial fusion machinery are resistant to trichothecene-triggered growth deficit	[35]
Gene deletion/mutation	HsTnII	Fragmentation	Decreased mitochondrial membrane potential; respiratory deficiency; decreased chronological lifespan	[37]
	lsm4	*YCA1*-dependent fragmentation	*YCA1*-dependent cell death; ROS accumulation; decreased chronological lifespan	[38,39]
	wbp1-1	*KEX1*-dependent fragmentation on abnormal N-glycosylation	*KEX1*-dependent and *YCA1*-independent cell death; ROS accumulation	[14]
	Δ*whi2*	Fragmentation and aggregation; actin-dependent mitochondrial aggregation; mtDNA loss	Deletion of *WHI2* results in apoptosis triggered by actin aggregation	[40]
Overexpression	BAX	Fragmentation in wild-type strain; aggregation in mitophagy-deficient Δ*uth1* strain	*AIF1*- and *YCA1*-independent clonogenic cell death; plasma membrane permeabilization on *UTH1* deletion	[43]
	Disease-associated human huntingin	Fragmentation and aggregation	ROS accumulation; caspase activation; *YCA1*-dependent nuclear localization of huntingtin aggregates	[44]
	TPK3	Aggregation	Respiratory deficiency and ROS accumulation depends on Tpk3 activity	[41]
	YDC1	Fragmentation	Decreased chronological lifespan and increased apoptosis	[42]

Table 2 | **Effect of gene deletions on yeast cell death and aging**

	Gene deletion	Inducers of cell death	Cell death pathway on induction	Influence of gene deletion on viability/cell death/lifespan	Reference(s)
Mitochondrial fission	Δ*dnm1*	H_2O_2, acetate, BAR0329, M1 killer virus	*YCA1*-dependent cell death; ROS accumulation	Decreased cytotoxicity	[30,33,46,47]
		Ethanol	*YCA1*- and *AIF1*-independent cell death; ROS accumulation	Unaltered cytotoxicity	[36]
		Aging	–	Increased chronological and replicative lifespan	[33,39,45]
	Δ*fis1* (*whi2*)	H_2O_2, acetate, BAR0329, M1 killer virus	*YCA1*-dependent cell death; ROS accumulation	Increased cytotoxicity; *WHI2* mutation but not *FIS1* deletion confers sensitivity for cell death	[30,33,46–48]
		Ethanol	*YCA1*- and *AIF1*-independent cell death; ROS accumulation	Increased cytotoxicity	[36]
		Aging	–	Increased chronological and replicative lifespan	[33,39,45]
	Δ*mdv1*	H_2O_2, acetate, BAR0329	*YCA1*-dependent cell death; ROS accumulation	Decreased cytotoxicity	[30,46]
		Ethanol	*YCA1*- and *AIF1*-independent cell death; ROS accumulation	Unaltered cytotoxicity	[36]
Mitochondrial fusion	Δ*fzo1*	Aging	–	Increased chronological lifespan	[39]
		Trichothecene	–	Growth deficit relieved	[35]
	Δ*mdm30*	H_2O_2	–	Decreased cytotoxicity	[33]
		Aging	–	Increased chronological lifespan	[33,39]
	Δ*pcp1*	Trichothecene	–	Growth deficit relieved	[35]

Yca1 [30,38,39,44] or is promoted independently of Yca1 by factors including the alternative cell death protease Kex1 [14], the mitochondrial cell death factors Aif1, Ysp1 and Ysp2 [9,15,16,34] or by so far unknown factors. Thus it appears that mitochondrial fragmentation concomitant with mitochondrial dysfunction and ROS accumulation are common and crucial events during cell death, whereas the cell death executing pathways may be very different and specified by the inducer of cell death.

Impact of the molecular machinery of mitochondrial fusion and fission on yeast cell death and aging

The correlation of mitochondrial fragmentation and yeast cell death suggests that preventing fragmentation by inhibiting mitochondrial fission (or increasing mitochondrial fusion) might be beneficial for cell survival. Indeed, deletion of the mitochondrial fission factors Dnm1 or Mdv1 results in increased resistance against various cell death stressors, including acetate [30], H_2O_2 [30,33], the fungicide BAR0329 [46] and the M1 killer virus [47] (Table 2). These observations suggest that Dnm1 and Mdv1 promote cell death. However, deletion of the *FIS1* gene encoding the Dnm1 receptor was surprisingly found to have an opposite effect. Cells lacking Fis1 are highly susceptible to ethanol [36] and all kinds of stressors, against which Δ*dnm1* and Δ*mdv1* mutants are more resistant [30,33,46,47] (Table 2). Intriguingly, later, it was demonstrated that deletion of the *FIS1* gene reproducibly results in the spontaneous acquisition of a secondary mutation in the stress-response gene *WHI2* [48].

Yeast cells lacking functional Whi2 are highly sensitive to undergo cell death [40,48], and their mitochondrial network is prone to fragmentation [40] (Table 1). Δ*fis1* mutants that have acquired secondary *whi2* mutations are as sensitive to stress as *whi2* mutants in a *FIS1* wild-type background, even though they contain a highly interconnected mitochondrial network like Δ*dnm1* or Δ*mdv1* strains [48]. Thus the increased susceptibility of Δ*fis1* cells to undergo cell death is due to mutations in *WHI2* rather than inhibition of mitochondrial fission [48]. Therefore it is reasonable to assume that mitochondrial fragmentation by the fission machinery indeed promotes cell death in yeast.

Remarkably, Dnm1- and Mdv1-promoted cell death depends on Yca1 in all the scenarios tested so far [30,46,47]. Thus it appears that a cell death pathway executed by the dynamin-related mitochondrial fission machinery and caspase-related factors has been conserved from yeast to worms and mammals [1]. On the other hand, stressing yeast cells with high concentrations of ethanol or acetic acid results in mitochondrial fragmentation that is independent of the mitochondrial fission factors Dnm1, Mdv1 and/or Fis1 [30,36] (Table 1). In this pathway, cell death is mediated by alternative cell death factors, such as Aif1, Kex1, Ysp1 and Ysp2 [9,14–16,34] (Figure 1; Table 1). These observations suggest that detrimental environmental conditions may induce mitochondrial fragmentation and cell death in a way that is independent of the known fission machinery and Yca1.

As mitochondrial fragmentation facilitates cell death, it can be expected that inhibition of mitochondrial fusion might have a similar effect (Figure 1). However, a genome-wide screening for resistance against the mycotoxin trichothecene demonstrated that yeast strains with the mitochondrial fusion genes *FZO1* and *PCP1* deleted are highly resistant to the toxin, rather than more susceptible to it [35] (Table 2). Similarly, a yeast strain with *MDM30* deleted demonstrated an increased resistance to H_2O_2 treatment [33] (Table 2). These unexpected phenotypes might be explained by the fact that fusion-deficient yeast strains have a high tendency to lose their mtDNA [18–20]. Since cells depleted of mtDNA were shown to be completely resistant to trichothecene treatment [35], these results suggest that the beneficial effect of the deletion of mitochondrial fusion factors can be ascribed to a loss of mtDNA rather than a blocking of fusion.

Conditions that promote mitochondrial fragmentation and cell death lead to a decreased chronological lifespan [37–39,42] (Table 1). On the other hand, caloric restriction is associated with an increased chronological lifespan in yeast [3,49,50] and leads to the down-regulation of the fission factors Fis1, Mdv1 and Caf4 concomitant with up-regulation of the fusion factor Mgm1 [51]. Thus inhibition of mitochondrial fragmentation may be associated with a prolonged lifespan. Indeed, deletion of the mitochondrial fission gene *DNM1* was shown to significantly increase both the chronological and the replicative lifespan of yeast cells [33,39,45] (Table 2). Consistent with this, deletion of the *FIS1* gene also resulted in increased chronological and replicative lifespan, in spite of the presence of its pro-death secondary mutation in the stress response gene *WHI2* [33,39,45] (Table 2). These observations suggest that during aging the pro-survival phenotype of the Δ*fis1* deletion overrules the pro-death function of the *whi2* mutation that appears to be dominant only in younger cultures.

Surprisingly, deletion of the fusion gene *FZO1* resulted in a slightly prolonged chronological lifespan [33,39], and cells with *MDM30* deleted demonstrated even a 60% increase in chronological lifespan [33,39]. Future experiments will have to show whether the fusion-incompetence or the lack of respiratory capacity and decreased mtDNA stability are responsible for longevity in these mutants.

Conclusions

A variety of different cell death-inducing conditions, including cell stress, gene mutations and overexpression, result in mitochondrial dysfunction accompanied by a boost of ROS and the release of mitochondrial pro-death factors, such as cytochrome *c* and Aif1. In addition to the deterioration of mitochondrial functions, fragmentation of the mitochondrial network appears to be a hallmark of various cell death pathways, including *YCA1*-dependent and -independent pathways. Whereas inhibition of mitochondrial fission was shown to result in increased stress resistance and prolonged chronological and replicative lifespan, the role of mitochondrial fusion is less clear. Future studies are required to determine whether the known components of the mitochondrial fusion and fission machineries play a general role in yeast cell death and aging or whether additional factors exist.

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