Apoptosis (2007) 12:1011–1023
DOI 10.1007/s10495-007-0758-0

The mitochondrial pathway in yeast apoptosis

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Published online: 9 February 2007

Abstract Mitochondria are not only important for the energetic status of the cell, but are also the fatal organelles deciding about cellular life and death. Complex mitochondrial features decisive for cell death execution in mammals are present and functional in yeast: AIF and cytochrome *c* release to the cytosol, mitochondrial fragmentation as well as mitochondrial hyperpolarisation followed by an oxidative burst, and breakdown of mitochondrial membrane potential. The easy accessibility of mitochondrial manipulations such as repression of respiration by growing yeast on glucose or deletion of mitochondrial DNA (rho^0) on the one hand and the unique ability of yeast cells to grow on non-fermentable carbon sources by switching on mitochondrial respiration on the other hand have made yeast an excellent tool to delineate the necessity for mitochondria in cell death execution. Yeast research indicates that the connection between mitochondria and apoptosis is intricate, as abrogation of mitochondrial function can be either deleterious or beneficial for the cell depending on the specific context of the death scenario. Surprisingly, mitochondrion dependent yeast apoptosis currently helps to understand the aetiology (or the complex biology) of lethal cytoskeletal alterations, ageing and neurodegeneration. For example, mutation of mitochondrial superoxide dismutase or *CDC48*/VCP mutations, both implicated in several neurodegenerative disorders, are associated with mitochondrial impairment and apoptosis in yeast.

Keywords Yeast · Apoptosis · Mitochondria · Ageing · Programmed cell death

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Introduction

Saccharomyces cerevisiae has been successfully used to solve complex bioenergetical questions in healthy mitochondria. Recently, fundamental results have been achieved unravelling the deadly function of mitochondria during apoptosis execution in yeast [1].

Like metazoan cells, yeast cells undergo cell death showing characteristic apoptotic markers such as externalization of phosphatidylserine to the outer leaflet of the plasma membrane, DNA fragmentation and chromatin condensation [2]. Moreover, the yeast genome codes for many proteins of the basic molecular machinery executing cell death, including orthologues of caspases [3], apoptosis inducing factor [4], HtrA2/Omi [5], and inhibitor of apoptosis (IAP) proteins [6]. Notably, histone phosphorylation, which is considered to be a universal prerequisite for apoptosis execution [7], was shown to be necessary for cell death induction upon oxidative stress in yeast [8]. Recent evidence suggests a regulated cross-talk between deacetylation and phosphorylation within Histone H2B tails required for apoptosis induction in yeast [9]. Physiological scenarios of yeast apoptosis have been demonstrated during ageing [10–12], the mating process [13], and development of yeast multicellular colonies [14]. Hence, a physiological finality for a unicellular suicide program has emerged (reviewed in Buttner et al. [15]).

The programmed death of yeast has been linked to complex mitochondrial processes, such as cytochrome *c* and AIF

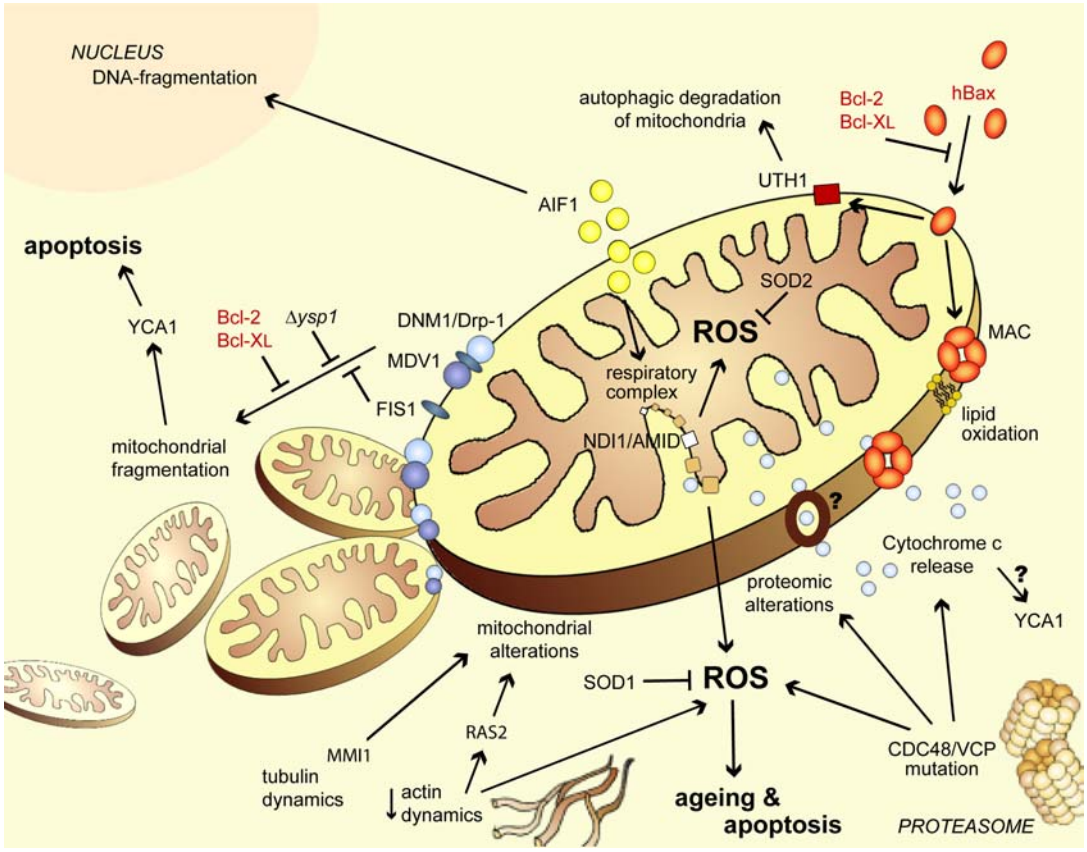

Fig. 1 *Mitochondrial pathways of yeast apoptosis and age induced programmed cell death*. Conserved molecules and processes involved in the regulation of yeast cell death are shown. Heterologous expression of mammalian proteins is depicted by red text colour. Question marks indicate interrelations, which have been hypothesised but yet not clearly demonstrated (for details see text)

release, channel opening upon human Bax expression, depolarisation of mitochondrial membrane potential, and mitochondrial fragmentation. These mitochondrial events are more or less common to various scenarios of yeast apoptosis, namely chronological and replicative ageing, decreased actin dynamics, as well as apoptosis induced by acetic acid, H_2O_2, amiodarone, or α-factor.

Especially in the fields of ageing and neurodegeneration, intricate experimental and genetic problems present in higher organisms were addressed in yeast. The discovery of an apoptotic yeast strain carrying a *CDC48* mutation [2] shed light on its mammalian orthologue VCP [16], which is involved in several polyglutamine triggered neurodegenerative disorders. Very recently, a crucial involvement of mitochondria in the *CDC48* connected cell death pathway was observed [17, 18].

In this review we focus on pathways regulating mitochondrial apoptosis and describe sequences of mitochondria-associated events during cell death execution in yeast. Moreover, we summarise the importance of crucial mitochondrial functions like mitochondrial respiration in regulating yeast apoptosis and ageing.

The molecular machinery of mitochondrial apoptosis in yeast

Drug-induced cell death

Several substances have been used to induce apoptosis in wild type yeast cells (reviewed in Madeo et al. [19]), some of which have been shown to act via mitochondrial pathways (Fig. 1). While most of the upstream mechanisms remain still unknown, apoptosis induction by acetic acid, amiodarone, and α-factor has clearly been linked to hyperpolarisation of the mitochondrial membrane potential, reduction of cytochrome *c* oxidase (COX) activity, mitochondrial ROS formation, cytochrome *c* release, and eventually loss of mitochondrial membrane potential and cell death [13, 20–22].

Acetic acid induces yeast apoptosis when applied at low doses [20, 23] leading to mitochondrial fragmentation, cytochrome *c* release accompanied by an increase in mitochondrial membrane potential and the loss of COX activity [21]. Of note, acetic acid does not induce apoptosis in respiratory deficient (rho^0) cells and cell death is at least partially inhibited in cells that are unable to synthesize cytochrome *c*

[21]. Several other studies have linked cytochrome *c* release, ROS formation, and changes in mitochondrial membrane potential to yeast apoptosis, such as heterologous expression of human Bax [24, 25] or human α-synuclein [26], and deletion of the histone chaperone *ASF1/CIA1* [27].

Recently, hyperosmotic stress has been reported to trigger apoptotic death of yeast cells mediated by a caspase-dependent mitochondrial pathway [28]. Incubation in media containing high concentrations of glucose or sorbitol led to mitochondrial swelling and reduction of christae number accompanied by increased caspase activation and cell death. Deletion of the metacaspase *YCA1* largely rescued these phenotypes. In addition, the mutant strains Δ*cyc1*Δ*cyc7* and Δ*cyc3*, both lacking functional cytochrome *c*, displayed a decreased number of cells with activated caspases and increased survival under hyperosmotic stress conditions compared to wild type [28]. These findings support the involvement of mitochondria and cytochrome *c* in caspase activation in yeast. However, it remains elusive if the mechanisms downstream of cytochrome *c* release are related to formation of an apoptosome-like structure and subsequent activation of the yeast caspase Yca1p. Homologues of Apaf, the mammalian apoptosome-associated factor, have not been found and proteins of similar function have not been described in yeast so far.

Nonetheless, a large body of evidence exists pointing to a connection of the yeast caspase and the mitochondrial apoptotic pathway. Cell death induced by deletion of the mitochondrial fission factor *FIS1* is abrogated by additional deletion of *YCA1* [23]. Mitochondrial fragmentation (fission) appears to play a causal role in mammalian programmed cell death [29, 30] and is regulated by the dynamin-related protein 1 (Drp1). A similar pathway was recently discovered in yeast by Fannjiang et al. involving the yeast homologue of human Drp1, Dnm1p [23]. Dnm1p promotes mitochondrial fragmentation, degradation and subsequent apoptotic cell death of yeast treated with hydrogen peroxide or acetic acid. Fis1p, in contrast to its fission function in healthy cells, inhibits Dnm1p-mediated mitochondrial fission-dependent cell death. Consistently, deletion of *FIS1* promotes mitochondrial fission and cell death, which can be rescued by expression of mammalian Bcl-2 or Bcl-X_L [23].

Yeast cells that are exposed to salt (NaCl) undergo cell death accompanied by nuclear markers of apoptosis and can be rescued by expression of human Bcl-2 [31], similarly as it has been found in mammalian cells [32]. In addition, Butcher and Schreiber identified a small molecule suppressor (SFK1) of FK506 (an inhibitor of the mammalian apoptosis regulator calcineurin) promoting salt mediated death: SFK1 improves growth in high concentrations of NaCl in the presence of FK506, but induces death via the mitochondrial pathway in conditions of low salt [33]. The authors suggest that SFK1 targets the mitochondria by interacting directly or indirectly with Por1p (the yeast homologue of mammalian VDAC) and demonstrate that the respiratory function of mitochondria is required for SFK1-mediated death. Furthermore, deletion mutants with impaired mitochondrial function (e.g. Δ*sod2*) were identified as hypersensitive for SFK1-mediated death. Accordingly, deletion of *SOD2* leads to a decrease in life span (see below) and susceptibility to various redox stresses [34].

Severin and Hyman demonstrated that mating-type pheromone (α-factor) induces apoptosis in yeast, thus connecting the mating process of yeast to cell-death mechanisms [13]. Indeed, unsuccessful mating attempts lead to yeast apoptosis, not due to sexual frustration, but probably as a cleaning mechanism to eliminate unfertile cells. The authors suggest a mitochondrial death pathway with the necessity of respiration, based on the observations that α-factor fails to induce death in cells lacking cytochrome *c* (Δ*cyc1*Δ*cyc7*) or mitochondrial DNA (rho^0) [13]. Also during sporulation yeast cells have been shown to undergo apoptotic cell death when respiration is enhanced through pre-culturing the cells in glycerol media [35]. To the contrary, Zhang et al. proposed a rather necrotic type of death upon pheromone treatment of yeast cells [36].

In a further study, Severin and colleagues elegantly extended their findings towards a timeline of events, proposing a scheme of the mitochondrial death cascade in yeast [22] (Fig. 2). In summary, treatment of yeast cells with α-factor or amiodarone leads to an intracellular rise in Ca^{2+} concentration. In turn the strong boost in cytosolic [Ca^{2+}] acts on mitochondrial respiration by stimulating the activity of respiratory enzymes and by increasing energy coupling, which is followed by hyperpolarisation of the mitochondrial membrane potential ($\Delta\Psi_M$). Elevation of $\Delta\Psi_M$ promotes ROS production, which then initiates the mitochondrial thread-grain transition (mitochondrial fragmentation) and de-energisation. This process was shown to be dependent on a mitochondrial protein named yeast suicide protein 1 or Ysp1p [22].

The mitochondrial de-energisation finally results in loss of $\Delta\Psi_M$ accompanied by the release of cytochrome *c*. Disruption of cytochrome *c* does not affect mitochondrial ROS production in response to α-factor or amiodarone but the rate of cell death is significantly reduced, suggesting cytosolic cytochrome *c* as one of the downstream death executioners of yeast mitochondria [13, 22].

Of note, the proposed mitochondrial death pathway by Severin and collegues goes in line with the findings obtained through stimulation with various drugs (reviewed above). It is well conceivable that several distinct inducers of yeast apoptosis acting via the mitochondrial pathway trigger a similar chain of events.

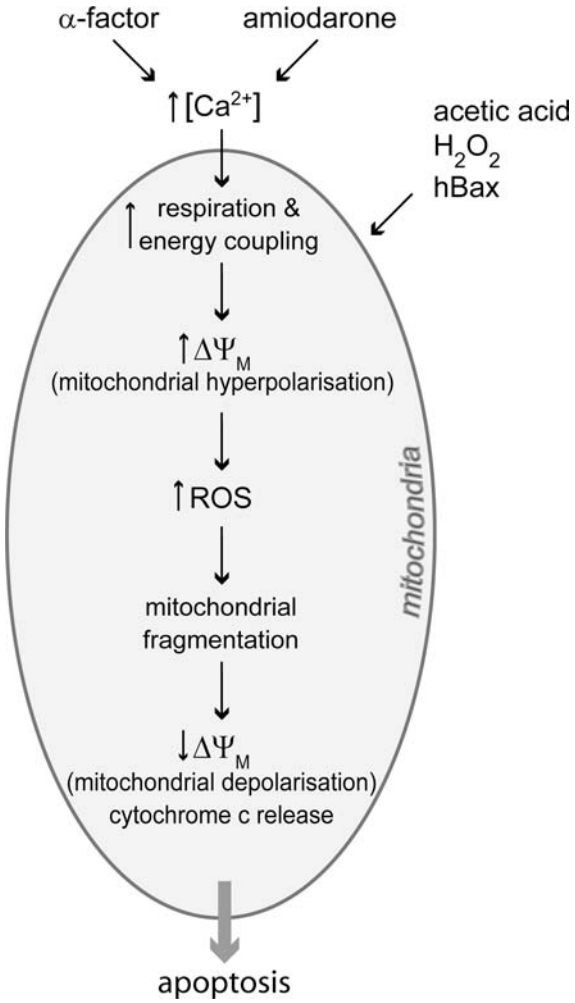

Fig. 2 *Schematic time lapse of mitochondrial events during apoptosis execution in yeast*. The illustrated chain of events has been elucidated by apoptosis induction with α-factor or amiodarone. Treatment of yeast cells with acetic acid, low doses of peroxide (H_2O_2) or human Bax-expression (hBax) leads to similar mitochondrial changes. Though, the precise sequence of events has not been addressed so far under the latter conditions. ROS, reactive oxygen species; $\Delta\Psi_M$, mitochondrial membrane potential

Endogenously induced cell death

Similar to its human orthologue [37], the yeast apoptosis inducing factor Aif1p has been reported to be released from mitochondria and might account for cytochrome *c* and caspase independent apoptotic pathways in yeast [4]. Aif1p translocates from the mitochondria to the nucleus upon apoptosis induction with hydrogen peroxide or during chronological ageing and facilitates degradation of nuclear DNA. While overexpression of Aif1p sensitises yeast cells to peroxide induced death in dependency of *CYP1* (the yeast cyclophilin A), disruption of *AIF1* largely inhibits cell death [4]. The mechanism of Aif1p translocation and its mitochondrial regulation are not known in yeast. Apoptosis inducing factor has also vital functions as it is responsible for efficient respiration in mammals and yeast [38]. Until recently, it was not clear whether the deadly effects of AIF translocation to the nucleus arose from inhibition of the respiratory chain or from apoptotic DNA degradation in the nucleus [39]. However, Cheung et al. could solve the latter problem proving that during neurodegeneration the presence of AIF in the nucleus is the reason for its lethality, dissociating the pro-apoptotic from the mitochondrial function [40]. In this study, additional expression of a non-releasable AIF mutant anchored to the mitochondrial inner membrane and therefore maintaining its mitochondrial function failed to protect from AIF-mediated neuronal cell death in mice [40].

Recently, Li et al. described another yeast homologue of a mammalian pro-apoptotic factor, namely Ndi1p, the homologue of AIF-homologous mitochondrion-associated inducer of death (AMID) [41]. Ndi1p is localised to the inner mitochondrial membrane, is part of the yeast respiratory chain, and displays NADH dehydrogenase activity. Overexpression of Ndi1p induces apoptosis with increased ROS production under respiratory-restricted conditions. The authors hypothesise that excessive ROS generation might be the mediator of Ndi1p-induced apoptosis. Consistently, disruption of *NDI1* inhibits ROS formation and extends yeast chronological life span [41].

Yeast cells subjected to DNA damage due to mutation of *CDC13*, a gene which encodes an essential telomere binding protein, display an apoptotic phenotype related to mitochondria [42]. In fact, the mitochondrial proteins Ydr333p and Cyc8p have been identified as multicopy suppressors of cell death caused by *CDC13* mutation. Both Cyc8p and Ydr333p are directly or indirectly related to the mitochondrial COX complex, whose inhibition is known to trigger apoptosis in mammalian cells [43] and to accompany yeast apoptosis induced by various drugs (see above). Consistent with observations during acetic acid treatment, deletion of mitochondrial DNA suppresses the apoptotic phenotype of *CDC13* mutation [42].

The yeast homologue Mmi1p of human TCTP, the translationally controlled tumor protein, has recently been associated with mitochondria and yeast apoptosis. Upon induction of apoptosis by mild oxidative stress, replicative ageing, or mutation of *CDC48*, Mmi1p translocates from the cytoplasm to the mitochondria, stably attached to the outer surface [44]. Moreover, deletion of *MMI1* confers resistance to H_2O_2 induced apoptosis and prolongs yeast replicative life span [44]. The authors further suggest that Mmi1p interacts with the microtubule network. Of note, tubulin dynamics have been implicated in yeast apoptotic processes [45].

Mitochondrial cell death regulation by heterologous expression of Bcl-2 proteins

The yeast system has been validated as an excellent model to study the anti- and pro-apoptotic functions of various mammalian Bcl-2 proteins. The complex interplay of Bax or Bax mutants with other Bcl-2 proteins (e.g. Bid, Bcl-2 and Bcl-X_L) and the mechanism and nature of Bax forming pores have been addressed in numerous studies [46–50].

Heterologous expression of the human key apoptotic inducer Bax causes rapid cell death in the yeast *Saccharomyces cerevisiae* [51, 52] and *Candida albicans* [53]. Manon and colleagues demonstrated that Bax expression induces the release of cytochrome *c* from mitochondria—a hallmark of mammalian Bax action [25]. Ligr et al. [54], and Madeo et al. [55], and others observed the generation of reactive oxygen species and other apoptotic features (e.g. DNA fragmentation, phosphatidylserine externalisation) in Bax expressing yeast cells. The cell-death inhibitor BI-1 (Bax inhibitor 1), which is conserved in mammals, plants, and fungi, can suppress apoptosis induced by Bax or hydrogen peroxide in yeast [56]. Furthermore, heterologous expression of Bcl-2 or Bcl-X_L prevents the cytotoxic effect of Bax [24, 51, 57, 58] and at the same time causes enhanced resistance to H_2O_2 [59] and acetic acid [60]. These observations together with the occurrence of typical apoptotic markers argue for a specific action of Bax in activating mitochondrial cell death pathways rather than a mere perforation of cells through pores formed by Bax.

In line with a highly regulated form of cell death, Manon and colleagues have shown that mitochondrial lipid oxidation is required for Bax-induced lethality and for optimal insertion of Bax into the outer mitochondrial membrane [47, 61].

Specifically cardiolipin, a mitochondrial lipid localised in the mitochondrial inner membrane, is discussed to play an important role in the regulation of Bax mediated cell death and cytochrome *c* release, though it was demonstrated that cardiolipin is not essential for Bax insertion into mitochondria and cytochrome *c* release [62]. However, the pro-apoptotic human truncated Bid (tBid) is suggested to bind to the outer mitochondrial membrane in yeast in a cardiolipin dependent manner, pertubate mitochondrial bioenergetics and thereby enhance Bax action [46]. The authors suggest that oxidation of cardiolipin to monolysocardiolipin might enforce this process. In addition, it was shown that lack of cardiolipin enhances cytochrome *c* release upon co-expression of tBid and Bax in yeast [46]. These data support views suggesting a dual function of cardiolipin in mitochondria-dependent apoptosis and demonstrate that a very complex and sophisticated system exists for the regulation of Bcl-2 proteins during apoptotic events. The underlying mechanisms are functional in yeast and therefore this research field will benefit from the yeast system.

Mitochondrial respiration and yeast components of the permeability transition pore in Bax-induced cell death

Similar to treatment with acetic acid, Bax expression comes along with changes in mitochondrial membrane potential [24] and reduction of COX activity [25], probably due to a reduced amount of Cox2p [63]. It was demonstrated that the mitochondrial protease Yme1p is responsible for the cleavage of Cox2p and that deletion of *YME1* delays Bax-induced cell death [63]. The requirement of oxidative phosphorylation and cytochrome *c* in Bax mediated cell death in yeast has frequently been addressed. Bax-induced death was demonstrated to be increased under respiratory conditions (lactate media) and delayed in cytochrome *c* deficient cells [58]. Consistently, Bax toxicity was reduced in strains lacking mitochondrial DNA [51, 64] or the mitochondrial F0F1-ATPase [65, 66] and therefore deficient in oxidative phosphorylation. Deletion of mitochondrial proteins in general did not affect Bax toxicity as long as the ability to perform oxidative phosphorylation was maintained [64], indicating that indeed oxidative phosphorylation is necessary for Bax mediated killing. It was also observed, that insertion of Bax into the mitochondrial outer membrane requires the presence of ATP [67]. Somehow contradictory, it was reported that mitochondrial deficiency resulting from deletion of mitochondrial DNA increased the sensitivity to Bax in *Kluyveromyces lactis*, a yeast closely related to *S. cerevisiae* [68].

Controversial results also exist regarding the involvement of yeast homologues of the mammalian permeability transition pore complex (PTPC) in Bax-induced cytotoxicity. Por1p, the yeast homologue of the voltage dependent anion channel (VDAC), has been reported to affect cell death and cytochrome *c* release caused by Bax-expression in various ways. *POR1* mutant cells exhibited either increased [64] or decreased sensitivity to Bax [69] or showed no effect [24, 48, 67]. However, the most recent study supports the view that VDAC is not required for the functional response of Bax expression in yeast [70]. The requirement of homologues of mammalian ANT's, the yeast ADP/ATP carrier proteins (AAC's), for Bax induced toxicity seems less controversial. At least for yeast, two studies revealed that Bax-mediated cell death is independent of AAC's [58, 71].

As Bax-action might be independent of AAC's and Por1p, it has been speculated that Bax might permeabilise the outer mitochondrial membrane through a PTPC independent mechanism. In accordance to that, Pavlov et al. found a novel high conductance Bax-dependent channel in mitochondria of yeast and mammals [48]. This channel was named the mitochondrial apoptosis-induced channel (MAC) and is a candidate for the outer-membrane pore through which cytochrome *c* and possibly other factors exit mitochondria during apoptosis. Addressing the molecular nature of the pore, oligomeric

Bax was demonstrated to be a structural component of MAC in apoptotic mammalian cells [72].

To summarise, heterologous expression of Bax in yeast leads to regulated cell death, but it is still a matter of considerable uncertainty regarding the necessity of yeast PTPC or mitochondrial respiration. Bax expression in yeast has two major effects: growth arrest and induction of cell death. Some studies do not sufficiently differentiate between these two aspects. Future work should focus on methods determining the precise percentage of cell death using clonogenic assays. Furthermore, time dependency of Bax-mediated killing seems to be very important and should be addressed. Some deletion mutants do not completely rescue but delay cell death induced by Bax expression. This also might account for some inconsistent observations and emphasises the need for more detailed experiments.

Mitochondria and autophagy in Bax-induced cell death

Autophagy is a process that is essential for cellular maintenance, cell viability, differentiation, and development but, conversely, may also be one of the mechanisms of programmed cell death [73]. *UTH1*, a gene that is possibly involved in autophagic death, is needed for efficient Bax-mediated killing in yeast [74]. While deletion of *UTH1* does not prevent the insertion of Bax into the mitochondrial outer membrane or cytochrome *c* release, it inhibits the appearance of mitochondrial lipid oxidation and ROS production [74], suggesting an additional cytochrome *c* independent but autophagy-related pathway of Bax triggering cell death. Furthermore, *UTH1* is involved in various types of stress response and ageing [75, 76] and its deletion confers resistance to rapamycin [74]. Thus, both apoptotic and autophagy-dependend forms of cell death appear to be related upon Bax-action in yeast. Indeed, it was recently shown that yeast cells expressing Bax display typical autophagic features, including accumulation of the protein Atg8p, activation of vacuolar alkaline phosphatase, and the presence of autophagosomes and autophagic bodies [77]. Intriguingly, inactivation of autophagy does not prevent Bax-induced cell death and might therefore point to a rather protective role of autophagy upon Bax expression. Manon and colleagues further demonstrated that deletion of *UTH1*, while delaying Bax-induced death, switches the programmed form of death to a rather necrotic form [77].

The execution of apoptotic and autophagic death can additionally be regulated by intracellular vesicle trafficking in mammals [78, 79]. Accordingly, it was shown that expression of a vesicle-associated membrane protein from Arabidopsis (AtVAMP) leading to enhanced vesicular traffic blocked Bax-induced programmed cell death in yeast downstream of oxidative burst [80]. Furthermore, a screen of yeast mutants interfering with Bax lethality revealed distinct vacuolar and mitochondrial alterations [81] and Bax expression was demonstrated to result in the disintegration and eventual loss of vacuoles as well as the disruption of intracellular protein traffic [82].

Together, these data suggest that Bax not only serves a role in apoptosis but seems to be involved in autophagic protection or an autophagic-controlled form of cell death as well.

Mitochondrion dependent age-induced death

Chronological life span and mitochondrial function

Yeast chronological life-span is the length of time a population remains viable in the post-diauxic and stationary phase on minimal media (scarce nutrition) [83] and is meanwhile an established model for ageing of human postmitotic cells. One of the major theories of ageing, the free radical theory, involves the accumulation of mitochondria-derived reactive oxygen species and subsequent oxidative damage of proteins, DNA, and organelles (e.g. mitochondria) as a cause for senescence in human cells [84]. Consistently, it was shown that chronologically aged yeast cells die exhibiting markers of apoptosis and accumulating oxygen radicals causative for age induced death [10, 11]. In accordance with that, earlier studies revealed that cytosolic and mitochondrial superoxide dismutase (*SOD1* and *SOD2*, respectively) are required for long term survival [85]. Moreover, increased death of *SOD1* or *SOD2* mutants during chronological ageing can be rescued by expression of mammalian Bcl-2 [86]. Also mammalian Bcl-X_L expression counteracts viability loss of yeast cells in late-stationary phase [87], pointing again to a central role of mitochondria during ageing. Fabrizio et al. demonstrated that ageing can be an adaptive process, likely mediated by mitochondrial superoxide [10].

Ageing on minimal media does not induce a kind of sleeping state as cells maintain relatively high metabolic rates [83]. Upon glucose exhaustion, when cells have to switch from fermentative to respiratory metabolism utilizing ethanol, the respiratory competence is critical for survival. Addressing the importance of respiration during ageing, it was shown that cells lacking mitochondrial DNA (rho^0) exhibit decreased survival when grown to late stationary phase (48 h incubation on minimal media) [87]. After three to four days of incubation rho^0 cells completely loose viability (our unpublished observations). Additionally, several mitochondrial mutants, including Δ*atp1*, Δ*qcr7*, Δ*rip1*, Δ*sdh4*, Δ*kgd1*, and Δ*cor1*, are unable to enter the quiescent G0 state, which has been shown to be crucial for survival of stationary phase cultures [88].

It has further been shown that several mutants of yeast mitochondrial RNA polymerase exhibit reduced chronological

life span [89]. One of these mutants (*rpo41*-R129D) displays imbalanced mitochondrial translation, conditional inactivation of respiration, and ROS accumulation. Overexpression of Sod1p or Sod2p rescues the mutant phenotypes. The authors propose that the extend of ROS generation can depend on the precise nature of mitochondrial gene expression defects [89]. Thus, survival of yeast during chronological ageing largely depends on mitochondrial function. Intriguingly, a loss of mitochondrial capability to produce ATP accompanies ageing in mammals [90]. A triple mutant of the ADP/ATP carrier (Δ*aac1-3*) showed increased ROS production in late stationary phase on respiratory-media, pointing to the importance of maintaining ATP levels for mitochondrial function during ageing [87].

Replicative life span and mitochondrial function

Yeast replicative life-span is defined as the number of divisions an individual cell undergoes before dying (for a review see Bitterman et al. [91]) and is a well established model for human ageing. For example the sirtuins are ageing regulators functional in all phyla and have been discovered in the yeast replicative ageing model. Replicative old cells die showing an accumulation of ROS and exhibiting characteristic apoptotic phenotypes [12]. The replicative life span of chronologically aged mother cells decreases progressively with age, suggesting that chronological and replicative ageing might be connected to some extend [92].

Mitochondrial metabolism and respiration are thought to play a significant role in the regulation of replicative life span, but it has been controversial if respiratory deficiency affects life span. Even though, an early mortality of rho^0 cells during replicative ageing was observed in the yeast strain PSY316 and was first suggested to be due to the respiratory deficiency, Kaeberlein et al. could demonstrate that the disadvantage of rho^0 cells (background PSY316) during replicative ageing originates from the lack of mitochondrial DNA rather than from the inability to respire [93]. The authors showed that a nuclear mutation (Δ*cyt1*), which prevents respiration, results in a life span comparable to that of wild type cells. On the other hand, it was reported that abrogation of mitochondrial DNA increases replicative life span in the yeast strain YPK9 [94]. The observed life span extension depends on the activation of the retrograde response, an intracellular signalling pathway triggered upon mitochondrial dysfunction. Deletion of *RTG2*, the key regulator of the retrograde response, abolishes the life span extension of YPK9 rho^0 yeast [94].

It has been speculated that more efficient electron transport may lead to lower levels of mitochondrial derived ROS, presumably through reduced leakage of electrons from the respiratory chain [95, 96]. Overexpression of *NDE1* or *NDE2*, both members of the yeast electron transport chain, extends yeast replicative life span [97], suggesting the importance of efficient electron transport. It was further shown that Nde1p or Nde2p overexpression lowers NADH levels, which in turn might lead to Sir2p activation [97]. The NAD$^+$-dependent deacetylase Sir2p is discussed to be critical for life span extension upon caloric restriction [98]. Though, it is not clear if the possible activation of Sir2p or rather the stimulation of the whole respiratory chain contribute to the life span extension upon Nde1p/Nde2p overexpression. In fact, life span extension through calorie restriction goes hand in hand with increased respiration and reduced ROS, which is true for replicative as well as chronological ageing [99, 100].

Supporting the importance of high respiration for survival during ageing, our findings show that ROS accumulation during chronological ageing of yeast is almost completely inhibited, when respiration is enforced through culturing on glycerol instead of glucose media (unpublished data). Analogous effects can be observed by enhancing respiration through mild mitochondrial uncoupling. Under these conditions, mitochondrial ROS production decreases, leading to an extension of both chronological and replicative life span [96, 100]. However, mitochondrial respiration is not essential for replicative life span extension by calorie restriction and can occur even in cells completely lacking mitochondrial DNA [93]. This might indicate that, in respect to replicative ageing, increased respiration and energy coupling is sufficient but not necessary in inhibiting ROS formation and extending life span.

As ROS and oxidative damage increase and might be causative for cell death, mechanisms of oxidative stress resistance gain importance during ageing. Striking differences of chronological versus replicative ageing exist on the impact of antioxidant mechanisms. While mechanisms of oxidative stress resistance, such as Sod1p and Sod2p expression, are critical for yeast longevity during chronological ageing (see above), replicative ageing does not depend on the same mechanisms: For example, during replicative ageing, calorie restricted yeast known to have an extended life span, do not show more antioxidant defences or increased resistance against exogenous oxidants [99]. Sod2p overexpression decreases the budding in old mother cells, possibly by causing a defect in mitochondrial segregation from mother to daughter [101]. Fabrizio et al. further suggest that the transcription factors Msn2p/Msn4p, which are necessary for stress resistance, negatively regulate budding and replicative life span in part by increasing Sod2p expression [102].

Summing up, energy is needed for well aged yeasts as replicative life span extension can be achieved by increased respiration whereas ATP depletion leads to early death. Consistently, enhancement of respiration probably leads to decreased ROS levels and improved longevity during chronological ageing. Thus, the secret of well ageing may lie in the

resolution of a paradoxon: high metabolic activity of mitochondria but low ROS leakage from the respiratory chain.

Ageing and actin dynamics

Interestingly, mutations that increase actin dynamics promote longevity of yeasts, whereas decreased actin dynamics result in enhanced caspase activity and cell death as a result of altered mitochondrial function [103, 104]. Deletion of *SCP1*, a gene coding for an actin-bundling protein, increases actin dynamics, extends replicative as well as chronological life span, and reduces ROS accumulation. On the other hand, cells that lack either of the two actin regulatory proteins, End3p or Sla1p, exhibit decreased actin dynamics during stationary phase causing mitochondrial dysfunction, ROS elevation, and a reduction in longevity [105].

Gourlay et al. further established an upstream regulatory network controlling actin dynamics and mitochondrial apoptosis [106] (Fig. 3). Mutations that lead to decreased actin dynamics and hence promote actin aggregation were shown to cause hyperactivation of the RAS2 signalling pathway. As a consequence, cAMP levels elevate and stimulate protein kinase A (PKA) activity, mediated primarily through the PKA subunit Tpk3p [106]. The authors suggest Tpk3p to be directly responsible for mitochondrial alterations and dysfunction. Notably, Tpk3p has been shown to be involved in the regulation of mitochondrial enzyme content, in particular affecting COX activity, ATPase activity, and the amount of cytochrome *c* [107]. The mitochondrial alterations caused by actin/RAS/Tpk3p signalling finally initiate the execution of apoptosis, involving depolarisation of mitochondrial membrane potential and ROS production [105]. Antimycin A, an inhibitor of the respiratory chain complex III, was able to prevent ROS generation under these conditions [105]. Thus, the respiratory chain seems to be the major source of ROS during ageing and apoptosis mediated by actin dynamics. Considering the fact that Δ*tpk3* cells exhibit decreased respiratory activity [107], it will be interesting to explore if activation of the RAS/cAMP/Tpk3p pathway during decreased actin dynamics also leads to a primary increase of respiration, initiating the same course of mitochondrial events observed with α-factor or amiodarone treatment (see Fig. 2).

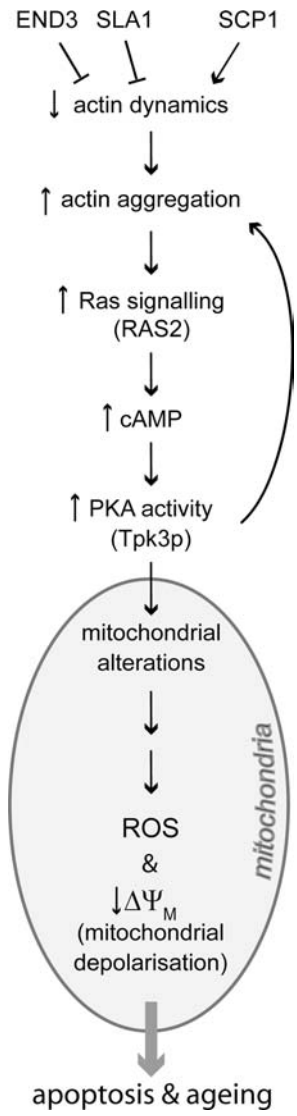

Fig. 3 *Actin-regulatory network upstream of mitochondrial apoptosis*. The control of actin dynamics is crucial for healthy mitochondrial function and integrity. Decreased actin dynamics result in activation of the Ras2/cAMP/PKA signalling pathway, which is amplified by a positive regulation loop upon PKA activity promoting actin aggregation. This in turn causes fatal mitochondrial (enzymatic) changes followed by typical apoptotic events. Conversely, enhancement of actin dynamics leads to a prolonged life span of yeast

Mitochondria controlled yeast apoptosis elucidates neurodegenerative death

During several human neurodegenerative disorders, typical features of mitochondria-dependent cell death have been described [108, 109]. To elucidate mechanistic aspects of these processes, yeast has been proven to be a useful system: Mutation of *CDC48* (*cdc48*^{S565G}) led to the discovery of apoptosis in the unicellular organism *Saccharomyces cerevisiae* [2]. Afterwards, VCP, the highly conserved human orthologue of Cdc48p, has been identified as an apoptosis regulator in mammalian cells, making Cdc48p/VCP the first death mediator originally discovered in yeast [16, 110]. VCP was shown to be a pathological effector for polyglutamine-induced neurodegeneration [110] and mutations in VCP have been connected to inclusion body formation associated with Paget disease, a dominant human disorder [111, 112]. However, the mechanisms underlying VCP-mediated cell death in these human disorders remain elusive.

Recently, a mechanistic explanation with crucial involvement of mitochondria for *CDC48* mediated death was described [17, 44]. In a proteomic approach, subcellular fractions of wild type and *cdc48*^{S565G} mutant cells were compared using two-dimensional gel electrophoresis. Surprisingly, only minimal alterations could be found in the cytosol or the nucleus. Instead, the mitochondrial fraction

was drastically affected upon *CDC48* mutation [17]. Of note, proteins suspected to play a role in cell death were among the 32 deregulated spots found in mitochondria from *cdc48*^{S565G} mutant cells, such as cyclophilin D and C, actin binding proteins, the yeast homologue of translationally controlled tumor protein (MMI1), as well as Mmf1p and Ilv5p, proteins which are necessary for mitochondrial DNA stability and mitochondrial function [17, 44]. Interestingly, seven other proteins associated with the NE-ER network, a continuous membrane system consisting of the endoplasmic reticulum (ER) and the ER-related nuclear envelope (NE), showed enriched levels in the mitochondrial fraction of the *cdc48*^{S565G} strain [17]. Accumulation of these NE-ER-associated proteins could be a result of the ER expansion and the dysfunction in ER-associated protein degradation (ERAD) earlier described in the *cdc48*^{S565G} strain [2] as Cdc48p is an important player in the ubiquitin-dependent ERAD pathway [113]. Upon mutation of *CDC48* (*cdc48*^{S565G}), cells accumulate polyubiquitinated proteins not only in microsomes, but also in a specific subfraction of mitochondria [18]. This probably means that only a subset of the mitochondria is responsible for the lethality of the apoptotic *cdc48*^{S565G} strain. It will be interesting to elucidate the special feature which distinguishes these mitochondria from the rest of the organelle fraction.

However, the above described proteomic alterations lead to measurabel mitochondrial dysfunction in the *cdc48*^{S565G} strain accompanied by release of cytochrome *c* to the cytosol and mitochondrial swelling. It was also demonstrated that accumulation of ROS, produced predominantly by the cytochrome bc_1 complex of the mitochondrial respiratory chain, are fatal in this strain [2, 17]. Consistently, and in contrast to age-induced death, enhancing mitochondrial respiration dramatically increases death of the *cdc48*^{S565G} mutant, while deletion of mitochondrial DNA (rho^0) abrogates death in clonogenic assays [17]. Summing up, mitochondria are a specific site for qualitative protein alterations in *cdc48*^{S565G} mutant cells, with both "enrichment" and "depletion" of distinct proteins. Consistently, recent transcriptome analysis of *cdc48*^{S565G} cells demonstrated nuclear genes coding for mitochondrial proteins to be the largest group of differentially regulated genes [114]. Thus, mitochondria are a pivotal site of changes on the protein level associated with *CDC48* mutation.

A variety of diseases including Alzheimer's, Huntington's and Parkinson's are linked to ER-stress and the unfolded protein response (UPR). In yeast, mitochondrial respiration was shown to contribute to ROS generation and cell death during ER stress caused by defects of the UPR, as deletion of mitochondrial DNA inhibits the apoptotic phenotype [115]. In a yeast model of Parkinson's disease, heterologous expression of human α-synuclein triggers apoptotic cell death accompanied by the release of cytochrome *c* [26]. Application of a brief heat shock or overexpression of the Hsp70 protein Ssa3p were found to be preventive for α-synuclein toxicity [26], pointing to a critical role of the heat shock response during neurodegenerative disorders. Recently, it has been demonstrated that α-synuclein alters proteasome composition and impairs proteasome-mediated protein degradation, protein synthesis, and the ability to maintain survival during yeast stationary phase [116]. Therefore, it is tempting to speculate that the pathway of α-synuclein toxicity involves the proteasome/CDC48/mitochondria apoptotic axis.

In a model of Huntington's disease, expression of an expanded polyglutamine domain in yeast leads to nuclear aggregates and Yca1p-dependent cell death with typical apoptotic changes in mitochondria [117]. Interestingly, the yeast Ndi1p (homologue of AMID) expressed in the substantia nigra of mice elicited protective effects against neurodegeneration and loss of neuronal function caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment [118, 119], a drug known to trigger Parkinson's disease, arguing for respiration mediated protection against cell loss during this disease.

Discussion

In recent years, yeast apoptosis research has begun to resolve the complex interplay of mitochondrial cell death mediators. It becomes increasingly clear that the connection between mitochondrial respiration and apoptosis is intricate, as suppression of respiration can either be beneficial or detrimental for the cell, strongly depending on the apoptotic scenario (summerised in Table 1). It was likely not by chance that nature has coupled pro-apoptotic potential to many molecules that have a genuine function in the respiratory chain in healthy cells, such as cytochrome *c*, AIF or AMID. Thus, by simply changing the localisation from mitochondria, the daytime place of action, to the cytosol, cell death is executed in a redundant, highly effective manner. As a result, mitochondrial outer membrane permeabilisation is probably the point of no return in cell death execution and thus an excellent target for clinical manipulations of apoptosis [120] and perhaps even necrosis [121]. The question if the yeast homologues of VDAC, ANT and cyclophilin D are involved in formation of the PTPC, which might be connected to yeast apoptotic scenarios similar to mammalian apoptosis, remains to be elucidated. Most studies have focused on the requirement of these proteins during Bax-mediated cell death and cytochrome *c* release. Other scenarios of yeast apoptosis could help resolving this question, for instance apoptosis induction by exogenous stress (acetic acid, peroxide, amiodarone, etc.), ageing, or AIF-mediated cell death. Intriguingly, yeast strains lacking the yeast ANT or VDAC proteins were shown to be less susceptible to cell killing

Table 1 Mitochondrial function can be deleterious or protective for cell survival during different apoptotic scenarios

Apoptotic scenario	Experimental manipulation of respiration	Survival	ROS
Replicative ageing	deficient (rho^0)	no effect[1]	ND
	Increased (CR)	↑	↓
Chronological ageing	Deficient (rho^0)	↓	↑
	Increased (CR)	↑	↓
hBax expression	Deficient (rho^0)	↑[2]	ND
	Increased (lactate)	↓	ND
CDC48 mutation	Deficient (rho^0)	↑	↓
	Increased (glycerol)	↓	ND
CDC13 mutation	Deficient (rho^0)	↑	↓
Hydrogen peroxide	Deficient (rho^0)	↓	ND
Acetic acid	Deficient (rho^0)	↑	ND
Amiodarone/ α-factor	Increased[3]	↓	↑
Sporulation (Meiosis)	Increased (glycerol)	↓	↑

Note. The effects of experimental manipulation of mitochondrial respiration during yeast apoptotic scenarios are outlined. CR, calorie restriction; rho^0, deletion of mitochondrial DNA; ND, not determined.

[1,2]Results of the most convincing studies are depicted, although contradictory effects have been reported (see text).

[3]Increased respiration was observed during α-factor/amiodarone treatment and not experimentally achieved.

induced by heterologous expression of the viral protein R (Vpr) encoded by HIV-1 [122].

The utilisation of yeast has provided new information concerning the molecular aspects and mechanisms of Bax pro-apoptotic function that have been further confirmed in mammalian cells [123–126]. Evidence accumulates that Bax lethality is tightly connected and might even be regulated by autophagic and in particular mitophagic processes. Similar is true for the mitochondrial fission machinery [77].

Fairly well established systems now exist, using yeast to study the mechanisms and pathways of mammalian proteins crucial for neurodegenerative disorders. The intersection of neurodegeneration-related yeast apoptotic scenarios with mitochondria will help to understand the pathologies of these disorders. Hints from genetic and clinical data in humans suggest that excessive mitochondrial fission may mediate some forms of neurodegenerative disease and may contribute to the decline of mitochondrial function during ageing [127]. However, the precise mechanism and role of mitochondrial fission in apoptosis and neurodegenerative diseases remain to be resolved. The increasing understanding of the yeast apoptotic machinery together with the easy feasibility of clonogenic assays, measuring the precise amount of dead versus living cells will help to unravel important questions.

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