Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1783 (2008) 1354–1368

Biochimica et Biophysica Acta BBA

www.elsevier.com/locate/bbamcr

Reactive oxygen species and yeast apoptosis

Gabriel G. Perrone, Shi-Xiong Tan, Ian W. Dawes *

Ramaciotti Centre for Gene Function Analysis, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

Received 21 December 2007; received in revised form 22 January 2008; accepted 24 January 2008
Available online 11 February 2008

Abstract

Apoptosis is associated in many cases with the generation of reactive oxygen species (ROS) in cells across a wide range of organisms including lower eukaryotes such as the yeast *Saccharomyces cerevisiae*. Currently there are many unresolved questions concerning the relationship between apoptosis and the generation of ROS. These include which ROS are involved in apoptosis, what mechanisms and targets are important and whether apoptosis is triggered by ROS damage or ROS are generated as a consequence or part of the cellular disruption that occurs during cell death. Here we review the nature of the ROS involved, the damage they cause to cells, summarise the responses of *S. cerevisiae* to ROS and discuss those aspects in which ROS affect cell integrity that may be relevant to the apoptotic process.

Keywords: *Saccharomyces cerevisiae*; Yeast; Apoptosis; Programmed cell death; Reactive oxygen species; Oxidative stress; Mitochondria; Endoplasmic reticulum; Actin; Stress responses

1. Introduction

Yeast cells have a range of responses to reactive oxygen species (ROS) that depend on the dose [1]. At very low doses the cells can adapt to become more resistant to a subsequent lethal exposure [2,3]. At higher doses the cells activate various antioxidant functions including a program of gene expression mediated mainly by the Yap1p and Msn2,4p transcription factors [4] and cell-division cycle delay [5,6]. At even higher doses, death of a proportion of cells in the population occurs, initially by apoptosis, but at extreme doses by necrosis. It has been demonstrated that yeast cells ageing either chronologically or replicatively accumulate ROS and undergo apoptotic death [7,8], indicating that oxidative stress defence plays a major role in governing ageing-induced apoptosis. These aspects have been reviewed [9,10].

Yeast apoptosis was initially described in a temperature-sensitive *cdc48* mutant of *Saccharomyces cerevisiae* incubated at the restrictive temperature [11]. *CDC48* is an essential gene involved in the translocation of ubiquitinylated proteins from the endoplasmic reticulum (ER) to the proteasome for degradation. The subsequent finding that a similar form of apoptosis also occurred in *gsh1* mutants depleted for the important cellular redox buffer glutathione, or in cells exposed to moderate levels of H_2O_2, led to the hypothesis that generation of oxygen radicals is a key event in the apoptotic pathway [12]. It was also shown that ROS accumulated at the restrictive temperature in the *cdc48* mutant and that the radical scavenger spin traps PBN and TMPO, as well as anaerobiosis suppressed the DNA fragmentation phenotype seen in this mutant.

Subsequently, apoptosis was found to result from exposure of cells to a wide range of environmental treatments including NaCl stress [13], acetic acid [14], hypochlorite [15], glucose in the absence of other nutrients [16], as well as other forms of starvation seen in chronological ageing experiments [17]. In most of these cases there was concomitant production of ROS as judged by the use of fluorochromes such as dihydroethidium, which is moderately specific for superoxide anion, and dihydrorhodamine 123 and 2,7-dichlorodihydrofluorescein diacetate, which are oxidised by a broader range of ROS. Other examples of mutations leading to ROS generation and apoptotic death include loss of *LSM4* [18,19].

* Corresponding author. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia. Tel.: +61 2 9385 2089; fax: +61 2 9385 1050.
E-mail address: i.dawes@unsw.edu.au (I.W. Dawes).

0167-4889/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.01.023

Similar apoptotic processes have been identified in other lower eukaryotes, including *Aspergillus fumigatus* and *Candida albicans* in response to the antibiotic amphotericin B [20]. In *Schizosaccharomyces pombe*, mutations blocking triacylglycerol synthesis lead to accumulation of fatty acids and lipoapoptosis, and this process is dependent on ROS generation [21].

The question arises of which comes first (apoptotic breakdown or generation of ROS). This is answered to some extent by the findings with the *cdc48* mutant, and also those showing that expression of the human *bax* gene in *S. cerevisiae* leads to accumulation of ROS and an apoptotic phenotype that can be alleviated by trapping of free radicals and anaerobiosis. This indicates that in *S. cerevisiae* ROS generation is an early and possibly regulatory step in many of the apoptotic processes. It is, however, not possible to generalise since in *Kluyveromyces lactis* expression of the anti-apoptotic mammalian gene Bcl-x_L does alleviate the effects of expressing Bax in yeast, but does not prevent the induction of ROS by Bax [22] and much remains to be done to determine the mechanisms whereby ROS are generated and how they become involved in cell death pathways.

2. What are the important ROS and where do they come from in cells?

The emergence of oxygen in the early evolution of organisms led to a variety of stresses due to the reactive nature of molecular oxygen and the diverse reactive species that are produced from its metabolism, or reaction of sensitive metabolites with oxygen. These products, collectively termed ROS, include a range of molecules that are either oxidants (such as H_2O_2) or reductants (such as the superoxide anion $O_2^{\cdot-}$) and all are capable of affecting the redox homeostasis of the cell. In addition to the primary ROS, there are a number of toxic reactive species produced from their reaction with other compounds in cells. While the term oxidative stress has generally been applied to the range of processes involved, it is clear from the differential responses of *S. cerevisiae* mutants to a range of ROS that "oxidative stress" is really a set of different conditions depending on the nature of the redox-active species involved [1,23].

The ROS and their products produced during aerobic metabolism have been discussed extensively in several reviews including [24–26]. The main ROS generated from oxygen in cells include the superoxide anion, $O_2^{\cdot-}$, which is relatively abundant in cells and mainly generated from the leakage of electrons from the mitochondrial respiratory chain as a normal consequence of aerobic respiration [27]. It is also formed in microsomal metabolism, and during the respiratory burst produced by phagocytes. The superoxide anion is not strongly reactive, but can react directly with some proteins [28]. Hydrogen peroxide is produced during the detoxification of superoxide anion catalysed by superoxide dismutases, also in yeast during the oxidation of fatty acids in the peroxisome, various oxidase reactions and also from the protein folding processes in the ER [29–32]. H_2O_2 can readily cross most biological membranes. While it is relatively unreactive, it has deleterious effects through its conversion to the extremely reactive hydroxyl radical. During phagocytosis, neutrophils produce the very reactive hypochlorite from H_2O_2 and chloride ions via the action of myeloperoxidase. Hypochlorite can react with free amines to generate chloramines that are also toxic to cells.

The very reactive hydroxyl radical is formed via the Fenton and Haber–Weiss reactions involving the combined action of superoxide anion and H_2O_2, catalysed by transition metal ions such as Fe^{2+} [33]. Ferrous ions react with H_2O_2 to generate the hydroxyl radical and an OH^- ion with the formation of Fe^{3+}. This can then be reduced to the Fe^{2+} by superoxide anion to reinitiate the process. The hydroxy radical reacts indiscriminately with most metabolites and macromolecules, in many cases generating other radicals in the process [34]. The processes involved in Cu and Fe homeostasis therefore are a very important component of the cellular defences minimising the damage caused by ROS. In addition, in cells that can produce nitric oxide, the nitric oxide radical can react with the superoxide anion to generate a range of reactive nitrogen species including the fairly reactive peroxynitrite ($ONOO^-$) which is more reactive than H_2O_2 in oxidising thiols and damaging some proteins [35]. *S. cerevisiae* has recently been shown to produce NO in mitochondria under hypoxic conditions [36] and NO is formed as part of the apoptotic process induced by H_2O_2, and an inhibitor of NO synthesis leads to decreased damage to the nitrosylation-sensitive protein glyceraldehyde 3-phosphate dehydrogenase, decreased ROS production and increased cell survival [37]. These results indicate that NO may play a significant role in apoptosis in yeast.

From the action of primary ROS a variety of secondary reactive molecules and radicals with varying reactivity are generated and hence identifying the main processes involved in ROS damage to cells (and hence in apoptosis) is not a simple task. One major target of the hydroxyl radical and protonated superoxide anion is unsaturated fatty acids, resulting in autocatalytic formation of lipid radicals and toxic lipid hydroperoxides [38,39]. Reactive carbonyls such as malondialdehyde, acrolein and 4-hydroxynonenal formed from the breakdown of the lipid hydroperoxides, together with methylglyoxal formed non-catalytically as a consequence of glycolysis can lead to carbonylation of proteins and the formation of glycation end products by direct, metal-catalysed, attack on specific amino acid side chains, mainly arginine and proline to form glutamic semialdehyde and on lysine to form aminoadipic semialdehyde [40]. These modified proteins accumulate during chronological ageing when cells are starved for carbon or nitrogen and in mother cells during replicative ageing [41,42], both conditions under which cells undergo apoptosis [8,17].

3. ROS-induced cellular damage

These aspects have been covered extensively in several reviews [26,43,44]. ROS can damage a wide range of molecules, including nucleic acids, proteins and lipids, and with this wide range of targets it is difficult to determine which events

lead to loss of viability of cells following damage, and in any one population there may be more than one cause of death of different individuals. One of the prime targets for ROS-induced damage includes thiols leading to their oxidation to form disulphides or sulphenic or sulphinic acids, and proteomic data are available for which proteins are most susceptible to thiol oxidation [45] as well as those that are *S*-thiolated (glutathionylated) in response to H_2O_2 [46]. Interestingly, these studies have identified similar sets of proteins that function in H_2O_2 and carbohydrate metabolism, translation factors and stress chaperones, and the analysis of oxidised proteins has shown that these target proteins are to some extent oxidised under normal aerobic conditions in the absence of added H_2O_2 [45]. Similar studies have identified the proteins that are carbonylated as cells age [47–50] and again there is an interesting overlap with those that are sensitive to oxidation or *S*-glutathionylation.

Free radical damage can also lead to cross-linking and fragmentation of proteins leading to enhanced proteolysis [51] and hydroxyl radicals oxidise amino acid residues, especially aromatic amino acid residues and cysteine [52]. Reactive protein hydroperoxides formed can decompose leading to free radicals causing modification of amino acids on the protein backbone, fragmentation, cross-linking and unfolding [53]. Hydroxylated derivatives are generated from aliphatic amino acids and oxidation of aromatic amino acid residues can also produce reactive phenoxyl radicals [54,55]. ROS can damage nucleic acids and they have been implicated in mutagenesis and carcinogenesis [56]. Only the hydroxyl radical and singlet oxygen are considered energetic enough to affect DNA directly, and H_2O_2 and superoxide anion cause damage to nucleic acids via the generation of the hydroxyl radical [33,57]. There is some discussion about whether DNA damage is one of the main contributors to cell death caused by ROS, DNA repair functions are not prominent in the mutant strains identified as sensitive to five different ROS-generating agents [23], although some functions relevant to DNA repair and synthesis of nucleotides are induced on exposure to oxidants [4]. DNA damage, or induction of replication stress by mutations affecting replication initiation, has been shown to lead to apoptosis in *S. cerevisiae* and *Schiz. pombe* [58, 59], and this is accompanied by the generation of ROS.

4. Cellular responses to ROS

The diversity of ROS species that can be generated in cells is matched by the variety and complexity of cellular responses leading to detoxification, repair of damage or maintenance of metal ion homeostasis. Cells produce a range of metabolites, either hydrophilic or lipophilic, with antioxidant activity as well as many enzymes with specific antioxidant functions. Some of the major ones are listed in Table 1, together with their cellular location where known. In many cases there are two or more genes encoding these functions, with their products located in different compartments or regulated in different ways. This list is far from comprehensive since at least 450 genes are required to maintain cellular resistance to ROS [23,60]. Overall resistance to ROS attack is mediated by a combination of these factors — deletion of any one does not lead to inviability and in some cases only a mildly sensitive phenotype. However, deletion of the *SOD1* gene encoding the cytosolic Cu/Zn superoxide dismutase leads to auxotrophy for several amino acids [61] due to damage to Fe/S cluster protein integrity, while deletion of *GSH1* required for synthesis of glutathione does lead to a significant shortening of chronological lifespan of the cells and increased apoptosis [12]. This highlights the role of glutathione as a major cellular antioxidant, and the relative importance of reducing superoxide anion levels to maintain redox integrity in cells.

Genome-wide microarray analysis of the response of *S. cerevisiae* cells to a wide range of different stresses has shown that there are sets of genes that are coordinately up- and down-regulated across stresses including those caused by heat, oxidants, osmolytes, salt and starvation [4]. This environmental stress response (ESR) was shown to result from a combination of two main regulatory systems: one regulated by the Yap1p transcription factor that mediates response to ROS and xenobiotics; and the other by the homologous Msn2p and Msn4p more general stress response transcription factors. Recently Gasch (2007) has reviewed these responses from the perspective of different yeast species [62]. While the evolutionarily very diverse *Schiz. pombe* and *S. cerevisiae* show a very strong correspondence in their ESR responses at a genome-wide target level, there was less of a common response seen in *Candida albicans* under the conditions examined, possibly due to the specific niche of *C. albicans* as a pathogen and the range of stress conditions tested. Interestingly, in *S. cerevisiae* there is not much correspondence between the genes that are strongly up-regulated in response to ROS and those that are required to maintain full resistance to a range of oxidants [23], presumably since the latter genes need to be constitutively expressed to maintain cellular resistance while those that are needed for repair functions are induced when required.

The Yap1p transcription factor has a major role in regulating responses to ROS and xenobiotics [63,64]. This factor is activated by H_2O_2 via formation of disulphide bonds mediated by a glutathione peroxidase homologue (Gpx3p/Orp1p;[65]). Oxidised Yap1p is transported from the cytoplasm to the nucleus [66] by either a process mediated by Ybp1p [67] or one involving the peroxiredoxin (thioredoxin peroxidase;[66]). The activity of Yap1p has been suggested to be the result of a balance between its oxidation and reduction of the resulting disulphides by thioredoxins [68]. Disulphide formation leads to structural changes in Yap1p, which inhibit its export from the nucleus [69,70]. Recently Okazaki et al. (2007) have shown in an *in vitro* system that oxidation of key cysteines in Yap1p occurs in several steps leading to the formation of inter-domain disulphides and that this leads to an active form of the transcription factor that is more resistant to reduction by thioredoxin [71]. This may explain the observed persistence of adaptation of *S. cerevisiae* cells to low doses of H_2O_2. The resistance imparted by a low dose of H_2O_2 can persist for up to four hours [72] and has also been observed in mammalian cells [73]. A screen of H_2O_2-sensitive mutants for those unable to adapt identified a restricted set of functions including the Yap1p,

Table 1
Important antioxidant defence systems in *Saccharomyces cerevisiae*

Function	Gene(s)	Location*	Role
Antioxidant metabolites			
glutathione		General	Aqueous redox buffer, excretion of xenobiotics
ubiquinone		Mit, ER and Mem	Lipid soluble antioxidant, respiratory chain component
D-erythroascorbate		Cyt?	Water-soluble antioxidant
Enzymes			
catalases	*CTT1*	Cyt	Dismutation of H_2O_2 to H_2O+O_2
	CTA1	Per	
superoxide dismutases	*SOD1*	Cyt, Mit, Nuc?	Dismutation of O_2^- to $H_2O_2+O_2$
	SOD2	Mit	
SOD chaperone	*CCS1*	Cyt	Required for loading copper into Sod1p
thioredoxins	*TRX1*, *TRX2*	Cyt	Deoxyribonucleotide synthesis, sulphate assimilation, cofactor for some peroxidases, redox control
dithiol glutaredoxins	*GRX1*, *GRX2*,	Cyt, Nuc	Overlap with thioredoxins in many enzymatic functions, have glutathione peroxidase activity
monothiol glutaredoxins	*GRX3*, *GRX4*, *GRX5*	Nuc Mit matrix	Protect cells from redox damage Fe/S cluster assembly
peroxiredoxins	*AHP1*, PRX1	Cyt Mit	Reduction of alkyl peroxides, ROOH+R'SH=R'SSR'+ROH
thioredoxin peroxidase	*TSA1*	Cyt	Peroxiredoxin activity, thioredoxin peroxidase activity H_2O_2+ RSH=H_2O+RSSR. Also mediates nuclear localisation of Yap1p
sulfiredoxin	*SRX1*	Cyt, Nuc	Reduces cystein-sulfinic acid residues in Tsa1p, Ahp1p
glutathione peroxidases	*GPX1*, *GPX2*	Cyt/Mem	Reduction of oxidised lipid hydroperoxides
phospholipid hydroperoxide GSH peroxidase	*GPX3/ORP1*	Cyt	Has thioredoxin peroxidase activity. hydroperoxide receptor transducing signal to Yap1p
cytochrome *c* peroxidase	*CCP1*	Mit inner membrane space	Reduction of hydrogen peroxide in mitochondrion
glutathione synthesis	*GSH1*, *GSH2*	Cyt	Stepwise synthesis of GSH
glutathione reductase	*GLR1*	Cyt	Reduction of GSSG to GSH
pentose phosphate pathway enzymes	*ZWF1*, *GND1*, *TAL1*, *TKL1*,*2*, *RPE1*,	Cyt	Generation of NADPH for recycling of oxidised glutathione, glutaredoxins and thioredoxins
	ALO1, ARA2	Mit Outer Mem ?	D-erythroascorbate synthesis
Metal-binding proteins			
metallothioneins	*CUP1*, *CRS5*	Cyt	Copper binding protein, multiple genes at locus, also binds Cd^{2+}
Transcription factors, regulators			
Yap1p	*YAP1*	Cyt/Nuc	Oxidative stress, resistance to xenobiotics, cadmium
Skn7p	*SKN7*	Nuc	Auxiliary transcription factor, functions with Yap1p for some oxidative stress, also acts in osmoregulatin
Msn2/4	*MSN2*, *MSN4*	Cyt/Nuc	General stress responsive transcription factor responding to PKA pathway, responsive to heat, starvation, osmotic and oxidative stress
Yap1-binding protein	*YBP1*	Cyt	Protein involved in one mechanism for nuclear localisation of Yap1p
Haem activated protein	*HAP1*	Nuc	
	HAP2,*3*,*4*,*5*	Nuc	Regulation of respiratory functions
Metal-binding activator	*MAC1*	Nuc	Regulator of genes involved in copper ion homeostasis and H_2O_2-induction of *CTT1*
Cup2p	*ACE1/CUP2*	Nuc	Cu-binding transcription factor activates *CUP1* at high copper

*Cyt: cytoplasm; Mit: mitochondrion; ER: endoplasmic reticulum; Per: peroxisome; Nuc: nucleus; Mem: membrane; ? indicates not known or not certain. Genes encoding the functions are given with cellular location where known or indicated in the SGD database (http://www.yeastgenome.org/).

Skn7p and Gal11p transcription factors and other mutations affecting the capacity of cells to generate NADPH including those affecting the pentose phosphate pathway and *ALD6* and *IDP2* [74]. This is in accord with the view that adaptation to H_2O_2, and probably the maintenance of normal homeostasis of redox status in cells under aerobic conditions, is mainly regulated by the extent of Yap1p activation by oxidation and reduction via thioredoxin and ultimately NADPH required to reduce oxidised thioredoxin. For ROS other than H_2O_2 there are likely to be other systems involved overlapping with the above. In response to low doses of lipid hydroperoxides (which are much more toxic to *S. cerevisiae* than H_2O_2) the cells up-regulate a specific set of genes including those involved in NADPH generation and those for export of toxic compounds under the control of Pdr1p and Pdr3p, which regulate expression of multi-drug resistance transporters in *S. cerevisiae* [75]. Surprisingly, with low doses of linoleic acid hydroperoxide several major antioxidant systems including the catalases, a glutaredoxin and thioredoxin 2 were down-regulated at low doses and these were only up-regulated when a threshold level of cell tolerance was reached at doses that led to cell cycle delay and the onset of some cell death [75]. It was speculated that this threshold may be the redox buffering capacity of the cell and glutathione appears to be the major redox buffer in many

cellular systems. This raises the question of which ROS are involved in apoptosis and what mechanisms and targets are important.

5. Mitochondrial ROS and apoptosis

5.1. Mitochondrial ROS production

Mitochondria are a major source or ROS generation in cells under physiological conditions [76–78]. In humans it has been estimated that ~1–2% of oxygen consumed leads to the production of the superoxide anion. Damage to mitochondrial macromolecules may lead to increased ROS production and further damage to mitochondrial components thereby causing a 'vicious downward spiral' in terms of ROS production and damage accumulation [77]. Mitochondrial mutations that slow metabolism have been shown to result in altered production of ROS in a number of eukaryotic organisms including *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, and *S. cerevisiae* [79].

S. cerevisiae grows fermentatively on glucose even in the presence of air, and there is a reduced rate of respiration. On non-fermentable substrates, including ethanol formed during fermentation, the cell switches to respiratory metabolism and the redox environment of the cell becomes more oxidised [80]. This also leads to an up-regulation of antioxidant functions such as GSH biosynthesis, and catalase and mitochondrial superoxide dismutase (Sod2p) to offset the increased flux of ROS produced. These functions are regulated by the Yap1p, Hap2,3,4,5p and Msn2,4p transcription factors [26]. The Target of Rapamycin (TOR) pathway also plays a role regulating mitochondrial respiratory function and ROS production [81,82]. Increased TOR pathway activity favours fermentation, suppresses mitochondrial respiratory capacity leading to increased ROS production and reduced cell lifespan. Increased ROS production is proposed to occur due to reduced oxygen consumption by the respiratory chain, which is associated with an increase in the availability of intracellular oxygen for ROS production. Activation of the retrograde pathway signalling, a transcriptional response that has evolved to counter/circumvent the consequences of reduced mitochondrial respiratory capacity, antagonises the negative effect of TOR signalling of mitochondrial respiratory capacity, thereby reducing ROS production and increasing lifespan [83,84].

Growth of *S. cerevisiae* cells under conditions of caloric restriction (reduced glucose), or treatment with the mitochondrial respiratory chain uncoupler dinitrophenol (DNP), was found to give rise to extended chronological and replicative lifespan and this was prosed to occur through an increase in mitochondrial respiratory capacity which acted by decreasing the availability of intracellular oxygen for ROS generation [85]. Similarly, treatment of human fibroblasts with low concentrations of DNP led to reduced mitochondrial superoxide production and an extension of replicative lifespan [86]. On the other hand, partial uncoupling of mitochondrial respiration in human fibroblasts or yeast cells with carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) led to an increase in reactive species generation, and in this case a decrease in replicative lifespan [87]. Evidently, mitochondrial respiratory capacity and mitochondrial inner membrane potential are key factors affecting ROS production, accumulation of damage in cells and lifespan.

Mutations that block the respiratory chain have also been associated with increased production of ROS by mitochondria. Barros et al. (2003) demonstrated that the nature of the block strongly influenced the magnitude of ROS production [88]. Mitochondria derived from a *cyc3* mutant with reduced cytochrome *c* activity produced the highest level of H_2O_2, while appreciable levels of H_2O_2 were released from mitochondria isolated from mutants affected in complex III and IV function. Elevated RAS/protein kinase A (PKA) pathway signalling has been shown to lead to increased ROS production and decreased ATP/ADP carrier activity, due to damage accumulation [89]. Over-expression of *SOD1* suppressed these effects, highlighting the role of the superoxide anion in decreased ATP/ADP carrier activity. Despite the effect of *SOD1* the source of ROS production was not pin-pointed. Interestingly, it has previously been shown that hyper-activation of the RAS/PKA pathway in *S. cerevisiae* leads to reduced lifespan and increased ROS production, however elevated ROS appeared to be produced independently of mitochondrial respiration [90]. In this case reduced lifespan and ROS accumulation could be rescued by treatment of cells with 1 mM glutathione.

Further evidence supporting an association between mitochondrial respiratory chain function and ROS production is provided by observations that treatment of *S. cerevisiae* cells with farnesol, which causes hyper-polarisation of the mitochondrial inner membrane, leads to increased ROS production [91]. ROS production caused by exposure to farnesol was abolished by treatment of mitochondria with inhibitors (sodium azide; oligomycin) of the F_1F_0-ATPase.

It was recently shown that disruption of mitochondrial citrate synthase (Cit1p) catalysing the first step of the tricarboxylic acid cycle, leads to a temperature-sensitive growth defect. Growth at elevated temperature led to rapid loss of cell viability accompanied by the appearance of many hallmark traits associated with apoptosis, including nuclear fragmentation, DNA breakage, phosphatidylserine translocation and ROS production [92]. The apoptotic phenotypes associated with *CIT1* deletion were rescued by addition of exogenous glutathione or glutamate, indicating that supply of glutamate for glutathione biosynthesis was likely to be a factor affecting ROS accumulation and cell death.

5.2. Mitochondrial ROS and apoptosis in yeast

The role of mitochondria in ROS production and apoptosis in mammalian cells is well established and has been reviewed extensively [76,93]. The role of mitochondria in yeast apoptosis has recently been comprehensively reviewed by Eisenberg et al. [94] and as a consequence only a limited number of aspects relating to key differences/similarities in mitochondrial ROS and apoptosis in yeast versus mammalian cells are addressed here. In mammalian cells the anti-apoptotic proteins Bcl-2 and Bcl-x_L and pro-apoptotic protein Bax can strongly influence the

progression of cells towards death via apoptosis. *S. cerevisiae* cells do not appear to encode any obvious homologues of Bcl-2, Bcl-x_L, or Bax. Despite this it was demonstrated that expression of Bax in yeast is lethal and this effect is suppressed by co-expression of Bcl-2 or Bcl-x_L [95], or expression of BFL-1 [96]. Expression of Bax in yeast leads to release of cytochrome *c* from mitochondria to the cytosol and it was proposed that Bax-induced release involves a general change in outer mitochondrial membrane permeability and not induction of a permeability transition of the inner mitochondrial membrane [97]. It was subsequently shown that expression of Bax in *S. cerevisiae* induces mitochondrial membrane hyper-polarisation, increased production of ROS, growth arrest and cell death, however under these conditions cytochrome *c* release from mitochondria was not detected [98]. Bax-induced growth arrest was independent of components of the mitochondrial outer/inner membrane including the voltage-dependent anion channel (VDAC), β or δ subunits of the F_1F_0ATPase and mitochondrial cyclophilin and proteins encoded by the mitochondrial genome, while Bax-induced death was dependent on mitochondrial DNA and the β subunit of the F_1F_0ATPase [98]. The mitochondrial TOM complex has recently been implicated in Bax-induced cytochrome *c* release [99] but there is a conflicting report on this involvement [100]. Expression of Bcl-x_L suppressed all Bax-induced effects. Expression of nematode *ced-9*, human *bcl-2* or chicken *bcl-xl* genes in *S. cerevisiae* protects cells from oxidant and heat shock-associated 'apoptotic-like' death [101]. Polcic and Forte (2003) proposed that the protective effect of Bcl-x_L stemmed from the ability of the protein to disrupt interaction of Bax with mitochondrial target that is required for Bax action in *S. cerevisiae* [102]. These studies indicate that although *S. cerevisiae* lacks certain anti- and pro-apoptotic proteins found in mammalian cells that the mechanism through which these proteins (including Bcl-2, Bcl-x_L and Bax) act is, at least conserved to some extent in this yeast.

5.3. Mitochondrial morphology and ROS

An emerging area of interest is the role of mitochondrial morphology in mitochondrial ROS production, apoptosis and/or cell ageing. The morphology of mitochondria is diverse and dynamic and mitochondria in cells may vary greatly in shape and size [103]. Changes in mitochondrial morphology can influence ROS production by the organelle and cell death via apoptosis. In *Podospora anserina* and *S. cerevisiae* deletion of dynamin-related GTPase Dnm1p (or *PaDnm1*), which functions in mitochondrial fission and maintenance of mitochondrial morphology, influenced a number of phenotypes depending on the organism. These included: extension of lifespan; reduced release of H_2O_2 from senescent cells; and, increased tolerance to an exogenous H_2O_2 insult [104]. Fannjiang et al. (2007) subsequently demonstrated that Dnm1p promotes mitochondrial fragmentation and cell death in response to death stimuli [105]. From this study it was also found that Fis1p, an outer mitochondrial membrane protein involved in membrane fission, antagonises the effects of Dnm1p, and that the effect of Fis1p on mitochondria fragmentation associated with Dnm1 function could be replaced by human Bcl-2 and Bcl-x_L. This led to the proposal that humans and yeast possess a conserved programmed cell death pathway regulated by Dnm1p (Drp1 in humans) that is inhibited by a Bcl-2-like function. It has also been reported that Fis1p influences ethanol-induced mitochondrial fragmentation, ROS production and apoptosis [106].

6. ER stress, ROS and apoptosis

6.1. ER stress and apoptosis

The endoplasmic reticulum (ER) provides an environment that is optimised to facilitate synthesis, folding and maturation of resident ER and secretory pathway proteins [107,108]. Oxidative protein folding in the ER is facilitated by a number of factors. These include the activity of the protein disulphide bond-forming machinery (PDBFM) which is comprised of numerous proteins including the flavin-containing oxidoreductase Ero1p, protein disulphide isomerase Pdi1p and other disulphide isomerases, Mdi1p, Mdi2p, and Eug1p [32,109]. Appropriate disulphide bond formation is essential for proteins to achieve their native conformation, and this leads to transfer of two electrons to Pdi1p/Ero1p. Under aerobic conditions oxygen acts as the terminal electron acceptor of the disulphide bond formation in the ER [30,31,110] and formation of H_2O_2 and/or other ROS has been proposed as a deleterious consequence of oxidative protein folding in the ER [32]. *In vitro* Ero1p-mediated oxidation of thioredoxin generates a molecule of H_2O_2 for each disulphide bond formed [30]. Despite the lack of direct *in vivo* data demonstrating H_2O_2 generation by the PDBFM it is likely that under physiological conditions the ER constitutes a source of ROS in cells, and the level of ROS produced would reflect the flux of electrons from reduced sulphydryls to Pdi1p/Ero1p. Import of reduced glutathione (GSH) from the cytosol to the ER modulates the redox state of the ER, by interaction of GSH with ER substrate proteins and/or the PDBFM to minimise genesis of hyper-oxidising conditions, to facilitate disulphide bond isomerisation and activation of Ero1p activity [32,111–114].

ER stress is any change that decreases the capacity of the ER to fold, modify or traffic proteins from the organelle. Eukaryotic cells have evolved the unfolded protein response (UPR) to alleviate ER stress by promoting transient attenuation of translation, increased abundance of ER chaperones and other components of the PDBFM, proliferation of the ER membrane system, and stimulation of ER-associated protein degradation [115–117]. Although activation of the UPR facilitates adaptation to ER stress, depending on the nature, severity and duration of ER stress the UPR can also contribute to cell death through activation of apoptotic signalling in yeast and mammals [117,118]. 'Futile' cycling reactions between ER substrate proteins, the PDBFM, and glutathione have been proposed to give rise to elevated ROS formation [118,119]. In *S. cerevisiae*, severe ER stress caused by over-accumulation of mis-folded proteins in the ER was shown to cause induction of the UPR and elevated ROS production by the PDBFM and mitochondria [118]. ER stress, elevated ROS production and changes in glutathione redox state were associated with appearance of some programmed cell death

markers (DNA fragmentation, phosphatidylserine externalisation), which were manifested through an UPR-dependent and Yca1p-independent mechanism. Glutathione supplementation led to decreased ROS production and an increase in cell survival.

The link between ER stress and induction of apoptosis in yeast is further supported by observations that defective *N*-linked glycosylation caused by mutations in components of the oligosaccharyltransferase glycoprotein complex (OST), including Ost2p [120], the yeast homologue of mammalian Defender Against Apoptosis protein (Dad1p) [121] or Wbp1p [122], the beta subunit of OST, or, exposure of cells to the *N*-linked glycosylation inhibitor tunicamycin, led to nuclear condensation, DNA fragmentation, externalisation of phosphatidylserine and induction of caspase-like activity [122]. The ability of *N*-acetylcysteine to confer partial protection from this apoptosis indicates a likely role of altered redox homeostasis in cell death associated with defective *N*-linked glycosylation and elevated ROS production. Although the source of ROS production was not ascertained, it was found that deletion of *YCA1* did not influence the appearance of apoptosis markers [122].

6.2. Possible source(s) of ROS generated during ER dysfunction, and apoptosis

Mitochondria are proposed to be a source of ROS during ER stress since deletion of mitochondrial DNA reduced ROS accumulation and increased cell survival, however the site(s) of ROS production was not elucidated [118]. The site of mitochondrial ROS production has been ascertained for at least one case involving ER stress, through the study of Cdc48p and its human homologue, valosin-containing protein p97 [108]. As indicated above, apoptosis in yeast was first observed in the *cdc48*^{S565G} mutant [12]. *CDC48* encodes an AAA-ATPase localised to the ER membrane, which together with Ufd1p and Npl4p, forms part of the endoplasmic reticulum-associated degradation (ERAD) pathway that facilitates extraction of ubiquitinated lumenal proteins targeted for proteasomal degradation [123,124]. In human cells, reduction of p97 causes increased ER stress, induction of transcripts involved in the response to ER stress and triggering of apoptosis [125]. In *S. cerevisiae*, growth of the *cdc48*^{S565G} mutant at the restrictive temperature is associated with elevated mitochondrial damage, release of cytochrome *c* to the cytosol, and increased ROS production [126]. In this case a prominent site of ROS production was the bc_1 complex (complex III) of the mitochondrial respiratory chain. An important question raised by these data is what is the identity of the signalling molecule(s) that trigger mitochondrially-induced apoptosis during prolonged ER stress?

In higher eukaryotes the role of Ca^{2+} homeostasis, and ER and mitochondria function in apoptosis has been reviewed extensively [93,103] and the comments herein will only touch on a limited number of aspects relating to ER stress, Ca^{2+} homeostasis and ROS production by mitochondria. In mammalian cells, ER stress is often associated with increased release of Ca^{2+} from the ER to the cytosol, and subsequent uptake into the mitochondrial matrix [103]. Close proximity of sections of the ER and mitochondrial membranes facilitates signalling (including Ca^{2+} signalling) between these organelles. Mild increases in matrix Ca^{2+} can stimulate activity of the tricarboxylic acid cycle enzymes pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase and NAD^+-isocitrate dehydrogenase [127] leading to increased respiratory energy production. Increased matrix Ca^{2+} can also promote elevated ROS production by the respiratory chain through a number of mechanisms, including stimulation of permeability transition pore opening and release of cytochrome *c* [93,128]. Perturbations to respiratory chain function can promote formation of radical species including the superoxide radical anion due to electron 'leakage' components of the respiratory chain.

Ca^{2+} is therefore a potential signalling species linking ER stress to apoptosis in yeast. Although the ER of yeast cells appears to contain 10–100 times lower Ca^{2+} than mammalian cells [129], maintenance of appropriate calcium signalling is vital for cell survival during prolonged ER stress [130]. In *S. cerevisiae* it is yet to be established directly whether induction of ER stress leads to elevated mitochondrial matrix Ca^{2+} levels, elevated ROS and promotion of apoptosis. A consideration of the response of yeast cells to the drug amiodarone appears pertinent to this issue [131]. Amiodarone blocks L-type Ca^{2+} in mammalian cells [132,133], while in *S. cerevisiae* it leads to an increase in cytosolic Ca^{2+} derived from extracellular and intracellular stores [134,135]. In yeast cells, amiodarone triggers a chain of events leading to programmed cell death including an increase in mitochondrial respiration, increased membrane potential, a burst of ROS production by the *o* centre of complex III followed by loss of inner mitochondrial membrane potential, release of cytochrome *c* to the cytosol, and cell death [131]. Furthermore, catalase, and glutathione and thioredoxin peroxidases are required to minimise Ca^{2+}-induced ROS-dependent damage to mitochondria and mitochondrial membrane permeabilisation [136,137]. These findings indicate that changes associated with altered Ca^{2+} homeostasis in yeast lead to mitochondrial ROS production, which in the case of amiodarone was shown to be from complex III of the respiratory chain. Complex III is also the proposed site for ROS production following disrupted *N*-linked glycosylation [122]. Ca^{2+} may therefore serve as the signal triggering apoptosis in response to defective *N*-linked glycosylation in yeast [122]. The role of altered Ca^{2+} signalling in apoptosis in response to defective *N*-linked glycosylation is supported by studies of hamster fibroblast cells where it was found that treatment of cells with type II pyrethroids, which perturb Ca^{2+} signalling, blocks mitochondrial-dependent apoptosis caused by defective *N*-linked glycosylation[138]. Analogous studies in yeast may help to further delineate the role of altered Ca^{2+} in induction of mitochondrial ROS production and apoptosis during prolonged ER stress.

Another possible link between ER stress and mitochondrial ROS production may involve perturbed TOR pathway signalling and a concomitant decrease in mitochondrial respiratory capacity. *PMR1* encodes a Golgi-located Ca^{2+}/Mn2+ ATPase whose deletion leads to defective *N*-linked and *O*-linked glycosylation, and other phenotypes associated with ER dysfunction [139,140]. Interestingly, *pmr1* mutants also exhibit a poor growth phenotype under respiratory conditions [141]. The respiratory growth defect of the *pmr1* mutant may simply be attributable to the effects of

cytosolic Ca^{2+} and/or Mn^{2+} over-accumulation on mitochondrial function [142–144]. However it was recently shown that Pmr1p acts as a negative regulator of TOR pathway signalling [145,146], and as indicated above TOR pathway signalling is a negative regulator of respiratory metabolism [81]. Despite this link there is no direct evidence demonstrating that the respiratory growth defect of *pmr1* cells is mediated through altered TOR pathway signalling, nor whether deletion of *PMR1* leads to increased ROS production and apoptosis. It remains to be determined whether TOR pathway is affected by operation of other ER functions.

7. The actin cytoskeleton as a target for ROS

Altered actin cytoskeleton dynamics has been strongly implicated in yeast apoptosis, as has been reviewed previously [147,148] and discussion here is limited to the influence of ROS on actin dynamics and vice versa. The actin cytoskeleton can be damaged through various conditions including oxidative stress. In *S. cerevisiae* old yellow enzyme (Oye2p), an NADPH-dependent oxidoreductase, plays an important role in preventing formation of a Cys285–Cys374 disulphide in actin [149]. In *oye2* mutants under physiological conditions, the actin cytoskeleton is organised into stabilized actin filaments, and these cells exhibit increased accumulation of ROS, nuclear DNA fragmentation and degradation, and premature chronological ageing [150]. The *oye2* mutant also exhibits increased sensitivity (relative to the wild-type) to H_2O_2, menadione and diamide and this sensitivity can be rescued by introducing mutations into actin preventing formation of the Cys285–Cys374 disulphide, or by treatment of cells with the reductant *N*-acetylcysteine. Although actin dynamics and organisation are subject to change in response to oxidative damage, stabilisation of actin can itself promote elevated ROS production. Disruption of proteins involved in cytoskeletal organisation, including Sla1p and End3p (forms complex with Sla1p and Pan1p), that reduce actin dynamics lead to altered mitochondrial membrane potential, elevated ROS production, nuclear DNA fragmentation and cell death [151,152]. The effects associated with deletion of *SLA1* and *END3* are mediated via activation of the RAS/cAMP pathway and the Tpk3p subunit of protein kinase A [151]. Over-expression of genes encoding the ubiquitin ligase Rsp5p, or the phosphodiesterase Pde2p, restores actin dynamics and rescues the oxidant sensitivity of the *end3* mutant [152]. Importantly, ROS generated as a consequence of actin stabilisation, and which are hypothesised to be generated by mitochondria [151], may in turn promote formation of intramolecular disulphide bonds in actin molecules and promote a downward spiral with respect to cell survival.

8. Metals/Metalloids, ROS production and apoptosis

Exposure of yeast cells to metals (Cu, Mn, Cd) and metalloids (As) has also been associated with ROS production and appearance of apoptotic markers [153–155]. Although copper and manganese are essential for viability of yeast it was recently demonstrated that these metals induce apoptosis or necrosis when present at moderately toxic or highly toxic levels respectively [153]. Disruption of genes encoding components of the mitochondrial respiratory chain, or deletion of mitochondrial DNA, reduced the appearance of apoptotic markers and cell death upon treatment with either metal ion. Distinct phenotypes were also reported for cells exposed to each metal. Copper toxicity was associated with heightened ROS production, was alleviated by over-expression of *SOD2*, encoding the mitochondrial manganese superoxide dismutase, and was associated with induction of apoptosis in a Yca1p- and Aif1p-independent manner. In contrast exposure to manganese did not lead to ROS accumulation, and apoptosis was dependent on Yca1p and Aif1p.

Although the identity of ROS generated during copper toxicity was not determined, the ability of *SOD2* over-expression (Mn-SOD) to confer protection indicates that mitochondrial superoxide is likely to have been formed. As indicated above, transition metal ions including Cu^{+}, and Fe^{2+} can facilitate hydroxyl radical formation from H_2O_2 via the Fenton reaction and this is exacerbated by the presence of superoxide anion, which can recycle the oxidised metal ion to the reduced form. Hydroxyl radicals damage cellular components including peroxidation of lipids and DNA damage, and lipid-peroxidation and/or DNA damage have been shown to trigger apoptosis in both mammalian and yeast cells [58,156–158], suggesting a conserved ROS-dependent mechanism in activating the apoptotic pathway.

Cadmium ions and arsenite have also been shown to trigger apoptosis in yeast [154,155]. Cd^{2+}-induced apoptosis was linked to disruption of cellular GSH:GSSG balance [154]. Exposure of yeast cells to these heavy metals/metalloids leads to up-regulation of glutathione biosynthesis via the Yap1p transcription factor [159,160]. Exposure of yeast cells to arsenite may cause glutathione depletion [161] which may induced apoptosis through a similar mechanism to that seen in the *gsh1* mutant [12]. Exposure of yeast cells to arsenite is also likely to promote superoxide anion formation since mutants defective in Sod1p, or its copper ion chaperone Ccs1p, are hypersensitive to the metalloid [161]. Arsenite also affects vicinyl cysteine residues and its targets therefore include many proteins with oxidoreductase activity and heat shock proteins (similar to those that are sensitive to H_2O_2 as discussed previously). The ability of arsenite to trigger apoptosis may be due to its interaction with cysteine residues on a protein or proteins that influence cell fate via apoptosis. Since glutathione is the major cellular redox buffer in most cells [162] and exposure of cells to arsenic may cause glutathione and/or increased generation of superoxide it is likely that arsenite exposure (and possibly other heavy metal/metalloids) triggers apoptosis by impacting one or more cellular processes including those involved in generation or detoxification of ROS.

9. Possible roles of yeast apoptosis factors in defence against ROS

9.1. Aif1p

Given that there are a number of known anti- and pro-apoptotic functions that have been identified in yeast, it is relevant to ask

whether any of these has an effect on ROS generated in cells. One possibility is Aif1p, the *S. cerevisiae* homologue of human AIF, which like mammalian AIF is located in mitochondria under non-apoptotic conditions, but under conditions that trigger apoptosis it translocates to the nucleus resulting in DNA fragmentation [163,164]. The primary amino acid sequence of Aif1p, especially the oxidoreductase domain, shows homology to mammalian AIF which has been proposed to have ROS-scavenging activity since over-expression of *AIF1* reduces peroxide-mediated cell death in cerebellar granule cells and cells deleted for AIF display increased sensitivity to peroxide stress [165]. Furthermore, based on *in vitro* studies, it was proposed that the NADH-oxidoreductase functions in the mitochondria [166]. Both recombinant AIF and purified AIF were able to oxidise NADH by reducing tetrazolium salt NBT or cytochrome *c in vitro* [166]. H_2O_2 and superoxide anion were generated during this process and removal of superoxide anion via the addition of superoxide dismutase prevented reduction of cytochrome *c* and NBT [166], indicating that the superoxide anion was involved in the electron-donation process. AIF also contains a FAD-binding moiety, which is proposed to accept an electron from NADH for transfer to another redox substrate. This proposed electron transfer would allow AIF to detoxify H_2O_2 via redox cycling [166,167]. The role of yeast Aif1p in terms of its oxidoreductase activity or ROS-scavenging properties has not been investigated in detail. Over-expression of Aif1p in the presence of an apoptotic level of H_2O_2 (0.4 mM) triggers massive apoptotic cell death and deletion of *AIF1* results in increased survival of cells at the same dose of H_2O_2 [164], indicating that Aif1p in yeast cells does not seem to play a role in reducing ROS, at least in terms of H_2O_2. However it has been suggested that in mammalian cells AIF plays a dualistic role and can be both pro- and anti-apoptotic [167–169]. The ROS-scavenging activity of AIF may function under low levels of oxidative stress and when ROS overwhelms the cell, AIF may switch to its pro-apoptotic form. The dose of H_2O_2 used to treat *aif1* yeast cells may be at the higher end of apoptotic treatments, thus only the pro-apoptotic function of Aif1p was observed. Further analysis of the response of *aif1* and *AIF1*-over-expressing strains to various types and doses of ROS-generating agents would elucidate if Aif1p is involved in oxidative stress defence.

9.2. AIF-homologous mitochondrion-associated inducer of death (AMID/Ndi1p)

In mammalian cells AMID, like AIF is a mitochondrial flavoprotein that triggers caspase-independent apoptosis. The closest homologue of AMID in *S. cerevisiae* was identified to be *NDI1*, which encodes the mitochondrial internal NADH dehydrogenase Ndi1p [170]. Ndi1p and the external NADH dehydrogenase (encoded by *NDE1*) transfer electrons from NADH to ubiquinone in the respiratory chain. In yeast, over-expression of either *NDI1* or *NDE1* results in increased ROS production in glucose-rich media, *sod1* cells transformed with *NDI1* grow poorly and *sod2* mutants could not be transformed with the over-expression plasmid, indicating that over-expression of Ndi1p leads to increased ROS production [170]. The ROS produced is likely to be superoxide anion generated from the mitochondria. Intriguingly, only over-expression of *NDI1* but not *NDE1* resulted in hallmarks of apoptosis [170], indicating that ROS alone may not be the sole factor in determining apoptosis in the AMID-mediated pathway in yeast. This notion is further supported by the fact that the oxidoreductase activity of AIF in mammals can be dissociated from its apoptotic role [166]. It remains to be seen if Ndi1p mediates apoptosis independently of Yca1p in a manner similar to its mammalian counter-part, AMID. Consistent with their hypothesis, this group also showed that *ndi1* and to a lesser extent *nde1* strains had increased chronological lifespan, but reduced fitness during recovery and proposed that this increase in chronological lifespan is due to reduction of ROS production.

9.3. Does Yca1p have a role in oxidative stress defence?

S. cerevisiae does not have an aspartate caspase, but does encode a metacaspase (Yca1p) related to the plant family of metacaspases [171]. Yca1p has a definite role in some, but not all yeast apoptotic processes [122,153,171,172]. Mutant *yca1* strains undergo mainly necrotic instead of apoptotic cell death and, under normal growth conditions, have increased levels of protein carbonylation and methionine oxidation compared to the wild-type [173]. This increase in protein carbonylation and methionine oxidation is exaggerated in the *yca1* strain following H_2O_2 treatment. These data indicate that Yca1p has an additional role in protecting cells from ROS accumulation under non-apoptotic conditions, although it may be just that, in the population of cells lacking Yca1p, "damaged" cells do not undergo apoptosis and these cells accumulate oxidised proteins.

Intriguingly, the amount, type and sources of intracellular ROS generated from *yca1* strains were not clear from the available data. For example, wild-type cells treated with an apoptotic dose of H_2O_2 showed increased DHR staining whereas *yca1* cells showed no increase in DHR staining [174]. However, valproic acid-treated *yca1* showed similar DHE staining to wild-type cells despite their reduced apoptotic death as compared to wild-type cells treated under the same condition [172]. On the other hand, cells deleted for *YCA1* were shown to be more resistant to H_2O_2 compared to wild-type cells [171], indicating that Yca1p is may not be required for H_2O_2 tolerance. A more detailed analysis of ROS production needs to be performed in *yca1* strains under different conditions triggering oxidative stress to explain the observed increase in protein oxidation and clarify the possible role of Yca1p in oxidative stress defence.

10. Conclusions

Fig. 1 illustrates the possible sources of ROS that are relevant to apoptosis in yeast and how they may interact with cellular systems. Based on differences in the response of mutants affected in various components of the apoptotic machinery to different conditions triggering apoptosis, it is clear that there may be more than one route to cell death, and more than one trigger in yeast [175]. It is still not clear in every case what comes first, the generation or accumulation of ROS leading to cell death, or the onset of apoptosis leads to damage that results

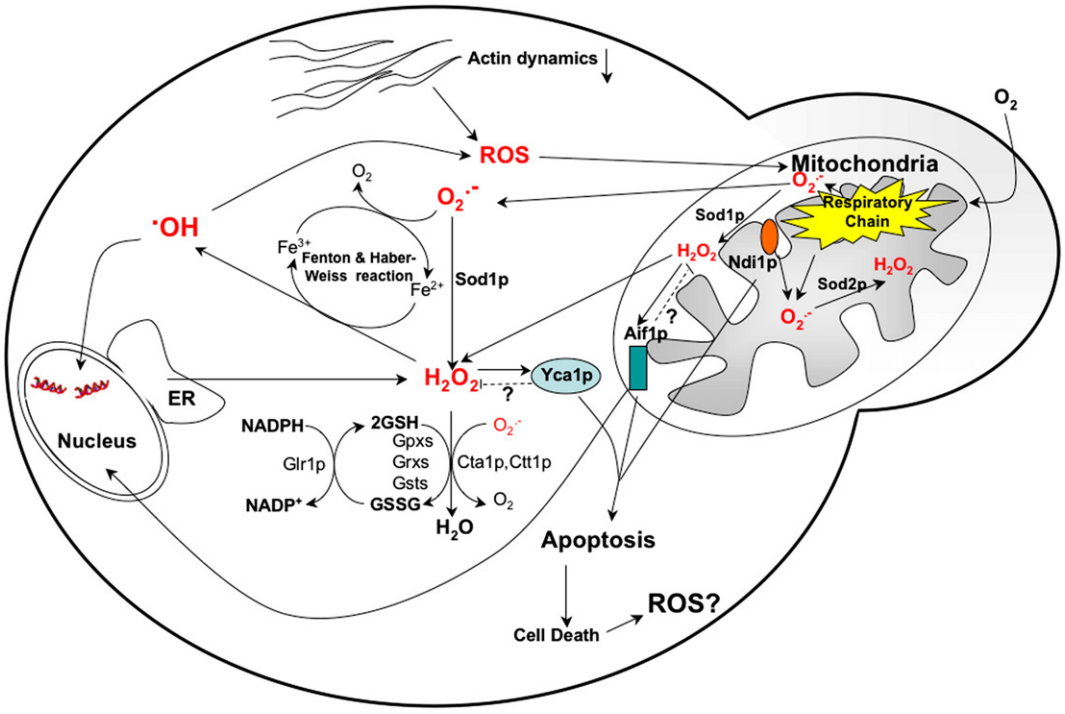

Fig. 1. Potential sources of ROS relevant to apoptosis in yeast. Intracellular sources of reactive oxygen species that are involved in apoptosis are in red. The respiratory chain in the mitochondrion produces superoxide anions which are converted to H_2O_2 by Sod2p in the mitochondria matrix or by Sod1p in the cytosol and the mitochondrial inter-membrane space. Ndi1p represents the internal mitochondrial NADH dehydrogenase which transfers electrons to complex III of the respiratory chain. The apoptosis inducing factor (Aif1p) is normally located in the mitochondrion and may have antioxidant activity. As part of the apoptotic process, Aif1p is translocated to the nucleus where it initiates DNA breakage [159]. Deletion of the yeast metacaspase Yca1p may lead to increased ROS-induced damage [173]. During protein folding, protein disulphide bond formation machinery in the ER produces H_2O_2. Hydrogen peroxide is detoxified to water by catalases (Ctt1p and Cta1p) or glutathione-dependent enzymes including glutathione peroxidases (Gpxs), glutaredoxins (Grxs) and glutathione transferases (Gsts). In the latter processes reduced glutathione (GSH) is converted to oxidised glutathione (GSSG). GSSG is reduced to GSH by glutathione reductase (Glr1p) using NADPH as electron donor. Hydroxyl radical (·OH) is generated via the Fenton and Haber–Weiss reactions as described in the text. Actin dynamics involves changes in the actin cytoskeleton, if converted to stabilized actin filaments, leads to increased accumulation of ROS as indicated in the text.

in augmented ROS production. There is good evidence discussed above from spin-trapping studies and from the suppression of apoptosis by mutations in specific processes linked to ROS production (e.g. mitochondrial respiratory mutants, and actin mutants lacking reactive cysteine residues) that ROS production is upstream of, or an important component in the initiation of a number of apoptotic processes.

Primary sources of ROS appear to be the mitochondrion and ER with some cross talk or interaction between the two, especially in the direction of ER to mitochondrion. Where mitochondria are involved in apoptosis there is the very real opportunity for free radical generation, probably as superoxide, and the main source appears to be complex III of the respiratory chain. Interestingly, from the genome-wide screening of deletion strains, of five oxidants tested, H_2O_2 was that most deleterious to mutants affected in mitochondrial respiration. This was proposed [23] to be due to the defect in mitochondrial respiration leading to production of superoxide radicals, which would, in conjunction with H_2O_2, lead to generation of the very damaging hydroxyl radical via the Fenton reaction. Cellular H_2O_2 may therefore be an important contributor that initiates, or augments, an apoptotic signal in yeast cells when mitochondria are involved. It has recently been shown that treatment of cells with H_2O_2 leads to increased superoxide anion formation [176] — since this is unlikely to be due to reversal of superoxide dismutase activity, it indicates that once H_2O_2-induced damage begins, then the process of superoxide radical production is exacerbated leading to even further damage. One important defence against this ROS-induced cell death is the cellular redox buffering capacity. From the phenotype of *gsh1* mutants blocked in glutathione synthesis and reduction of oxidised glutathione (*glr1*) it is clear that reduced glutathione is a primary defence against ROS insult [177–179]. Interestingly, mutants that have impaired NADPH production overproduce reduced glutathione to maintain cellular redox status [74]. Compared to the wild-type, such mutants were found to have a greater initial resistance to death caused by an acute dose of H_2O_2, which indicates that cells can maintain resistance to a sudden change in ROS flux until the reduced form of the redox buffers are consumed, at higher ROS levels apoptosis ensues.

In those situations in which some other process than ROS generation initiates apoptosis, damage elicited to mitochondria during the subsequent stages would probably also lead to free radical production and this would lead to further mitochondrial damage causing an inescapable path to death. H_2O_2 is probably the product of protein folding in the ER [30,32], and hence this may constitute a link between apoptosis induced by extreme ER stress and the observed role of mitochondria in the process, there are other potential links including altered Ca^{2+}-signalling

as discussed above. Of the oxidants used to stress cells, that which was first reported, and is most reported to initiate apoptosis is H_2O_2 — although this may be merely a result of its being the main choice as a convenient oxidant to use. Clearly there is a need for careful appraisal of which other oxidants, or compounds generating known ROS, cause apoptosis in yeast and to determine the dose/effect relationships for both necrosis and apoptosis. While the above discussion has indicated some of the processes that may be important, the mechanisms whereby ROS initiate apoptosis remain to be fully elucidated.

Acknowledgements

We acknowledge research grant support from the Australian Research Council and the Cancer Council of NSW. S-X. T. is recipient of an Endeavour International Postgraduate Research Scholarship from the Australian Government.

References

[1] M.D. Temple, G.G. Perrone, I.W. Dawes, Complex cellular responses to reactive oxygen species, Trends Cell Biol. 15 (2005) 319–326.
[2] L.P. Collinson, I.W. Dawes, Inducibility of the response of yeast cells to peroxide stress, J. Gen. Microbiol. 138 (1992) 329–335.
[3] D.J. Jamieson, *Saccharomyces cerevisiae* has distinct adaptive responses to both hydrogen peroxide and menadione, J. Bacteriol. 174 (1992) 6678–6681.
[4] A.P. Gasch, P.T. Spellman, C.M. Kao, O. Carmel-Harel, M.B. Eisen, G. Storz, D. Botstein, P.O. Brown, Genomic expression programs in the response of yeast cells to environmental changes, Mol. Biol. Cell 11 (2000) 4241–4257.
[5] J.A. Flattery-O'Brien, I.W. Dawes, Hydrogen peroxide causes *RAD9*-dependent cell cycle arrest in G2 in *Saccharomyces cerevisiae* whereas menadione causes G1 arrest independent of *RAD9* function, J. Biol. Chem. 273 (1998) 8564–8571.
[6] J. Lee, A. Romeo, D.J. Kosman, Transcriptional remodeling and G1 arrest in dioxygen stress in *Saccharomyces cerevisiae*, J. Biol. Chem. 271 (1996) 24885–24893.
[7] P. Fabrizio, L. Battistella, R. Vardavas, C. Gattazzo, L.L. Liou, A. Diaspro, J.W. Dossen, E.B. Gralla, V.D. Longo, Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*, J. Cell Biol. 166 (2004) 1055–1067.
[8] P. Laun, A. Pichova, F. Madeo, J. Fuchs, A. Ellinger, S. Kohlwein, I. Dawes, K.U. Frohlich, M. Breitenbach, Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis, Mol. Microbiol. 39 (2001) 1166–1173.
[9] M. Breitenbach, P. Laun, G. Heeren, S. Jarolim, A. Pichova, Mother cell-specific ageing, in: J.R. Dickinson, M. Schweizer (Eds.), The Metabolism and Molecular Physiology of *Saccharomyces cerevisiae*, CRC, Boca Raton, 2004, pp. 20–41.
[10] V.D. Longo, J. Mitteldorf, V.P. Skulachev, Programmed and altruistic ageing, Nat. Rev. Genet. 6 (2005) 866–872.
[11] F. Madeo, E. Frohlich, K.U. Frohlich, A yeast mutant showing diagnostic markers of early and late apoptosis, J. Cell Biol. 139 (1997) 729–734.
[12] F. Madeo, E. Frohlich, M. Ligr, M. Grey, S.J. Sigrist, D.H. Wolf, K.U. Frohlich, Oxygen stress: a regulator of apoptosis in yeast, J. Cell Biol. 145 (1999) 757–767.
[13] I. Wadskog, C. Maldener, A. Proksch, F. Madeo, L. Adler, Yeast lacking the SRO7/SOP1-encoded tumor suppressor homologue show increased susceptibility to apoptosis-like cell death on exposure to NaCl stress, Mol. Biol. Cell 15 (2004) 1436–1444.
[14] P. Ludovico, M.J. Sousa, M.T. Silva, C. Leao, M. Corte-Real, *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid, Microbiology 147 (2001) 2409–2415.
[15] D.A. King, D.M. Hannum, J.S. Qi, J.K. Hurst, HOCl-mediated cell death and metabolic dysfunction in the yeast *Saccharomyces cerevisiae*, Arch. Biochem. Biophys. 423 (2004) 170–181.
[16] D. Granot, A. Levine, E. Dor-Hefetz, Sugar-induced apoptosis in yeast cells, FEMS Yeast Res. 4 (2003) 7–13.
[17] E. Herker, H. Jungwirth, K.A. Lehmann, C. Maldener, K.U. Frohlich, S. Wissing, S. Buttner, M. Fehr, S. Sigrist, F. Madeo, Chronological aging leads to apoptosis in yeast, J. Cell Biol. 164 (2004) 501–507.
[18] C. Mazzoni, P. Mancini, F. Madeo, V. Palermo, C. Falcone, A *Kluyveromyces lactis* mutant in the essential gene KlLSM4 shows phenotypic markers of apoptosis, FEMS Yeast. Res. 4 (2003) 29–35.
[19] C. Mazzoni, P. Mancini, L. Verdone, F. Madeo, A. Serafini, E. Herker, C. Falcone, A truncated form of KlLsm4p and the absence of factors involved in mRNA decapping trigger apoptosis in yeast, Mol. Biol. Cell 14 (2003) 721–729.
[20] A.J. Phillips, I. Sudbery, M. Ramsdale, Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 14327–14332.
[21] Q. Zhang, H.K. Chieu, C.P. Low, S. Zhang, C.K. Heng, H. Yang, *Schizosaccharomyces pombe* cells deficient in triacylglycerols synthesis undergo apoptosis upon entry into the stationary phase, J. Biol. Chem. 278 (2003) 47145–47155.
[22] D. Poliakova, B. Sokolikova, J. Kolarov, L. Sabova, The antiapoptotic protein Bcl-x(L) prevents the cytotoxic effect of Bax, but not Bax-induced formation of reactive oxygen species, in *Kluyveromyces lactis*, Microbiology 148 (2002) 2789–2795.
[23] G.W. Thorpe, C.S. Fong, N. Alic, V.J. Higgins, I.W. Dawes, Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6564–6569.
[24] B. Halliwell, J.M. Gutteridge, Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy, Lancet 1 (1984) 1396–1397.
[25] B. Halliwell, Free radicals and metal ions in health and disease, Proc. Nutr. Soc. 46 (1987) 13–26.
[26] I.W. Dawes, Yeast stress responses, in: J.R. Dickinson, M. Schweizer (Eds.), The Metabolism and Molecular Physiology of *Saccharomyces cerevisiae*, CRC Press, Boca Raton, 2004, pp. 376–438.
[27] A. Bouveris, E. Cadenas, Production of superoxide radicals and hydrogen peroxide in mitochondria, in: L.W. Oberely (Ed.), Superoxide Dismutases, CRC press, Boca Raton, Florida, 1982, pp. 15–30.
[28] P.R. Gardner, I. Fridovich, Superoxide sensitivity of the *Escherichia coli* aconitase, J. Biol. Chem. 266 (1991) 19328–19333.
[29] B. Halliwell, J.M. Gutteridge, Free Radicals in Biology and Medicine, 4thOxford University press, Oxford, 2007.
[30] E. Gross, C.S. Sevier, N. Heldman, E. Vitu, M. Bentzur, C.A. Kaiser, C. Thorpe, D. Fass, Generating disulfides enzymatically: reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1p, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 299–304.
[31] B.P. Tu, S.C. Ho-Schleyer, K.J. Travers, J.S. Weissman, Biochemical basis of oxidative protein folding in the endoplasmic reticulum, Science 290 (2000) 1571–1574.
[32] B.P. Tu, J.S. Weissman, Oxidative protein folding in eukaryotes: mechanisms and consequences, J. Cell Biol. 164 (2004) 341–346.
[33] B. Halliwell, O.I. Aruoma, DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems, FEBS Lett. 281 (1991) 9–19.
[34] B. Halliwell, The biological significant of oxygen-derived species, in: J.S. Valentine, C.S. Foote, A. Greenberg, J.F. Liebman (Eds.), Active Oxygen in Biochemistry, Blackie Academic and Professional, Glasgow, 1995, pp. 313–335.
[35] D.P. Buchczyk, K. Briviba, F.U. Hartl, H. Sies, Responses to peroxynitrite in yeast: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a sensitive intracellular target for nitration and enhancement of chaperone expression and ubiquitination, Biol. Chem. 381 (2000) 121–126.
[36] P.R. Castello, P.S. David, T. McClure, Z. Crook, R.O. Poyton, Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes, Cell. Metab. 3 (2006) 277–287.

[37] B. Almeida, S. Buttner, S. Ohlmeier, A. Silva, A. Mesquita, B. Sampaio-Marques, N.S. Osorio, A. Kollau, B. Mayer, C. Leao, J. Laranjinha, F. Rodrigues, F. Madeo, P. Ludovico, NO-mediated apoptosis in yeast, J. Cell Sci. 120 (2007) 3279–3288.
[38] M.V. Evans, H.E. Turton, C.M. Grant, I.W. Dawes, Toxicity of linoleic acid hydroperoxide to *Saccharomyces cerevisiae*: involvement of a respiration-related process for maximal sensitivity and adaptive response, J. Bacteriol. 180 (1998) 483–490.
[39] F.D. Gunstone, Fatty Acid and Lipid Chemistry, Blackie Academic and Professional, London, 1996.
[40] J.R. Requena, R.L. Levine, E.R. Stadtman, Recent advances in the analysis of oxidized proteins, Amino Acids 25 (2003) 221–226.
[41] H. Aguilaniu, L. Gustafsson, M. Rigoulet, T. Nystrom, Protein oxidation in G0 cells of *Saccharomyces cerevisiae* depends on the state rather than rate of respiration and is enhanced in *pos9* but not *yap1* mutants, J. Biol. Chem. 276 (2001) 35396–35404.
[42] H. Aguilaniu, L. Gustafsson, M. Rigoulet, T. Nystrom, Asymmetric inheritance of oxidatively damaged proteins during cytokinesis, Science 299 (2003) 1751–1753.
[43] N. Santoro, D.J. Thiele, Oxidative stress responses in the yeast, in: S.H. W.H. Mager (Ed.), Yeast Stress Responses, Springer, 1997, pp. 171–211.
[44] D.J. Jamieson, Oxidative stress responses of the yeast *Saccharomyces cerevisiae*, Yeast 14 (1998) 1511–1527.
[45] N. Le Moan, G. Clement, S. Le Maout, F. Tacnet, M.B. Toledano, The *Saccharomyces cerevisiae* proteome of oxidized protein thiols: contrasted functions for the thioredoxin and glutathione pathways, J. Biol. Chem. 281 (2006) 10420–10430.
[46] D. Shenton, C.M. Grant, Protein *S*-thiolation targets glycolysis and protein synthesis in response to oxidative stress in the yeast *Saccharomyces cerevisiae*, Biochem. J. 374 (2003) 513–519.
[47] E. Cabiscol, E. Piulats, P. Echave, E. Herrero, J. Ros, Oxidative stress promotes specific protein damage in *Saccharomyces cerevisiae*, J. Biol. Chem. 275 (2000) 27393–27398.
[48] V.M. Costa, M.A. Amorim, A. Quintanilha, P. Moradas-Ferreira, Hydrogen peroxide-induced carbonylation of key metabolic enzymes in *Saccharomyces cerevisiae*: the involvement of the oxidative stress response regulators Yap1 and Skn7, Free Radic. Biol. Med. 33 (2002) 1507–1515.
[49] N. Erjavec, L. Larsson, J. Grantham, T. Nystrom, Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p, Genes Dev. 21 (2007) 2410–2421.
[50] G. Reverter-Branchat, E. Cabiscol, J. Tamarit, J. Ros, Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction, J. Biol. Chem. 279 (2004) 31983–31989.
[51] S.P. Wolff, R.T. Dean, Fragmentation of proteins by free radicals and its effect on their susceptibility to enzymic hydrolysis, Biochem. J. 234 (1986) 399–403.
[52] E.R. Stadtman, Protein oxidation and aging, Science 257 (1992) 1220–1224.
[53] S. Gebicki, K.H. Gill, R.T. Dean, J.M. Gebicki, Action of peroxidases on protein hydroperoxides, Redox Rep. 7 (2002) 235–242.
[54] R. Aeschbach, R. Amado, H. Neukom, Formation of dityrosine cross-links in proteins by oxidation of tyrosine residues, Biochim. Biophys. Acta 439 (1976) 292–301.
[55] S. Fu, L.A. Hick, M.M. Sheil, R.T. Dean, Structural identification of valine hydroperoxides and hydroxides on radical-damaged amino acid, peptide, and protein molecules, Free Radic. Biol. Med. 19 (1995) 281–292.
[56] B.N. Ames, L.S. Gold, Endogenous mutagens and the causes of aging and cancer, Mutat. Res. 250 (1991) 3–16.
[57] M.V. Lafleur, J. Retel, Contrasting effects of SH-compounds on oxidative DNA damage: repair and increase of damage, Mutat. Res. 295 (1993) 1–10.
[58] W.C. Burhans, M. Weinberger, M.A. Marchetti, L. Ramachandran, G. D'Urso, J.A. Huberman, Apoptosis-like yeast cell death in response to DNA damage and replication defects, Mutat. Res. 532 (2003) 227–243.
[59] M.A. Marchetti, M. Weinberger, Y. Murakami, W.C. Burhans, J.A. Huberman, Production of reactive oxygen species in response to replication stress and inappropriate mitosis in fission yeast, J. Cell Sci. 119 (2006) 124–131.
[60] C.L. Tucker, S. Fields, Quantitative genome-wide analysis of yeast deletion sensitivities to oxidative and chemical stress, Comp. Funct. Genomics 5 (2004) 216–224.
[61] E.B. Gralla, D.J. Kosman, Molecular genetics of superoxide dismutases in yeasts and related fungi, Adv. Genet. 30 (1992) 251–319.
[62] A.P. Gasch, Comparative genomics of the environmental stress response in ascomycete fungi, Yeast 24 (2007) 961–976.
[63] K. Hertle, E. Haase, M. Brendel, The *SNQ3* gene of *Saccharomyces cerevisiae* confers hyper-resistance to several functionally unrelated chemicals, Curr. Genet. 19 (1991) 429–433.
[64] S. Kuge, N. Jones, *YAP1* dependent activation of *TRX2* is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides, EMBO J. 13 (1994) 655–664.
[65] A. Delaunay, D. Pflieger, M.B. Barrault, J. Vinh, M.B. Toledano, A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation, Cell 111 (2002) 471–481.
[66] S. Okazaki, A. Naganuma, S. Kuge, Peroxiredoxin-mediated redox regulation of the nuclear localization of Yap1, a transcription factor in budding yeast, Antioxid. Redox Signal. 7 (2005) 327–334.
[67] E.A. Veal, S.J. Ross, P. Malakasi, E. Peacock, B.A. Morgan, Ybp1 is required for the hydrogen peroxide-induced oxidation of the Yap1 transcription factor, J. Biol. Chem. 278 (2003) 30896–30904.
[68] S. Izawa, K. Maeda, K. Sugiyama, J. Mano, Y. Inoue, A. Kimura, Thioredoxin deficiency causes the constitutive activation of Yap1, an AP-1-like transcription factor in *Saccharomyces cerevisiae*, J. Biol. Chem. 274 (1999) 28459–28465.
[69] S. Kuge, T. Toda, N. Iizuka, A. Nomoto, Crm1 (XpoI) dependent nuclear export of the budding yeast transcription factor yAP-1 is sensitive to oxidative stress, Genes Cells 3 (1998) 521–532.
[70] M.J. Wood, G. Storz, N. Tjandra, Structural basis for redox regulation of Yap1 transcription factor localization, Nature 430 (2004) 917–921.
[71] S. Okazaki, T. Tachibana, A. Naganuma, N. Mano, S. Kuge, Multistep disulfide bond formation in Yap1 is required for sensing and transduction of H_2O_2 stress signal, Mol. Cell 27 (2007) 675–688.
[72] J.M. Davies, C.V. Lowry, K.J. Davies, Transient adaptation to oxidative stress in yeast, Arch. Biochem. Biophys. 317 (1995) 1–6.
[73] A.G. Wiese, R.E. Pacifici, K.J. Davies, Transient adaptation of oxidative stress in mammalian cells, Arch. Biochem. Biophys. 318 (1995) 231–240.
[74] C.H. Ng, S.X. Tan, G.G. Perrone, G.W. Thorpe, V.J. Higgins, I. Dawes, Adaptation to hydrogen peroxide in *Saccaromyces cerevisiae*: the role of NADPH-generating systems and the Skn7 transcription factor, Free Radic. Biol. Med. (in press), doi:10.1016/j.freeradbiomed.2007.12.008.
[75] N. Alic, T. Felder, M.D. Temple, C. Gloeckner, V.J. Higgins, P. Briza, I.W. Dawes, Genome-wide transcriptional responses to a lipid hydroperoxide: adaptation occurs without induction of oxidant defenses, Free Radic. Biol. Med. 37 (2004) 23–35.
[76] P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, S.S. Sheu, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, Am. J. Physiol., Cell Physiol. 287 (2004) C817–C833.
[77] A.Y. Andreyev, Y.E. Kushnareva, A.A. Starkov, Mitochondrial metabolism of reactive oxygen species, Biochemistry (Mosc). 70 (2005) 200–214.
[78] E. Cadenas, K.J. Davies, Mitochondrial free radical generation, oxidative stress, and aging, Free Radic. Biol. Med. 29 (2000) 222–230.
[79] M.M. Sedensky, P.G. Morgan, Mitochondrial respiration and reactive oxygen species in mitochondrial aging mutants, Exp. Gerontol. 41 (2006) 237–245.
[80] T. Drakulic, M.D. Temple, R. Guido, S. Jarolim, M. Breitenbach, P.V. Attfield, I.W. Dawes, Involvement of oxidative stress response genes in redox homeostasis, the level of reactive oxygen species, and ageing in *Saccharomyces cerevisiae*, FEMS Yeast Res. 5 (2005) 1215–1228.
[81] N.D. Bonawitz, M. Chatenay-Lapointe, Y. Pan, G.S. Shadel, Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression, Cell. Metab. 5 (2007) 265–277.
[82] S.M. Schieke, T. Finkel, TOR and aging: less is more, Cell. Metab. 5 (2007) 233–235.

[83] S.M. Jazwinski, Yeast longevity and aging–the mitochondrial connection, Mech. Ageing. Dev. 126 (2005) 243–248.
[84] Z. Liu, R.A. Butow, Mitochondrial retrograde signaling, Annu. Rev. Genet. 40 (2006) 159–185.
[85] M.H. Barros, B. Bandy, E.B. Tahara, A.J. Kowaltowski, Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in *Saccharomyces cerevisiae*, J. Biol. Chem. 279 (2004) 49883–49888.
[86] J.F. Passos, G. Saretzki, S. Ahmed, G. Nelson, T. Richter, H. Peters, I. Wappler, M.J. Birket, G. Harold, K. Schaeuble, M.A. Birch-Machin, T.B. Kirkwood, T. von Zglinicki, Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence, PLoS Biol. 5 (2007) e110.
[87] P. Stockl, C. Zankl, E. Hutter, H. Unterluggauer, P. Laun, G. Heeren, E. Bogengruber, D. Herndler-Brandstetter, M. Breitenbach, P. Jansen-Durr, Partial uncoupling of oxidative phosphorylation induces premature senescence in human fibroblasts and yeast mother cells, Free Radic. Biol. Med. 43 (2007) 947–958.
[88] M.H. Barros, L.E. Netto, A.J. Kowaltowski, H(2)O(2) generation in *Saccharomyces cerevisiae* respiratory pet mutants: effect of cytochrome *c*, Free Radic. Biol. Med. 35 (2003) 179–188.
[89] L. Hlavata, L. Nachin, P. Jezek, T. Nystrom, Elevated Ras/PKA activity in *Saccharomyces cerevisiae* reduces proliferation rate and lifespan by two different ROS-dependent routes, Aging Cell (in press), doi:10.1111/j.1474-9726.2007.00361.x.
[90] G. Heeren, S. Jarolim, P. Laun, M. Rinnerthaler, K. Stolze, G.G. Perrone, S.D. Kohlwein, H. Nohl, I.W. Dawes, M. Breitenbach, The role of respiration, reactive oxygen species and oxidative stress in mother cell-specific ageing of yeast strains defective in the RAS signalling pathway, FEMS Yeast Res. 5 (2004) 157–167.
[91] K. Machida, T. Tanaka, Farnesol-induced generation of reactive oxygen species dependent on mitochondrial transmembrane potential hyperpolarization mediated by F(0)F(1)-ATPase in yeast, FEBS Lett. 462 (1999) 108–112.
[92] Y.J. Lee, K.L. Hoe, P.J. Maeng, Yeast cells lacking the *CIT1*-encoded mitochondrial citrate synthase are hypersusceptible to heat- or aging-induced apoptosis, Mol. Biol. Cell 18 (2007) 3556–3567.
[93] P. Kakkar, B.K. Singh, Mitochondria: a hub of redox activities and cellular distress control, Mol. Cell. Biochem. 305 (2007) 235–253.
[94] T. Eisenberg, S. Buttner, G. Kroemer, F. Madeo, The mitochondrial pathway in yeast apoptosis, Apoptosis 12 (2007) 1011–1023.
[95] W. Tao, C. Kurschner, J.I. Morgan, Bcl-xS and Bad potentiate the death suppressing activities of Bcl-xL, Bcl-2, and A1 in yeast, J. Biol. Chem. 273 (1998) 23704–23708.
[96] H. Zhang, S.W. Cowan-Jacob, M. Simonen, W. Greenhalf, J. Heim, B. Meyhack, Structural basis of BFL-1 for its interaction with BAX and its anti-apoptotic action in mammalian and yeast cells, J. Biol. Chem. 275 (2000) 11092–11099.
[97] M. Priault, B. Chaudhuri, A. Clow, N. Camougrand, S. Manon, Investigation of bax-induced release of cytochrome *c* from yeast mitochondria permeability of mitochondrial membranes, role of VDAC and ATP requirement, Eur. J. Biochem. 260 (1999) 684–691.
[98] A. Gross, K. Pilcher, E. Blachly-Dyson, E. Basso, J. Jockel, M.C. Bassik, S.J. Korsmeyer, M. Forte, Biochemical and genetic analysis of the mitochondrial response of yeast to BAX and BCL-X(L), Mol. Cell. Biol. 20 (2000) 3125–3136.
[99] M. Ott, E. Norberg, K.M. Walter, P. Schreiner, C. Kemper, D. Rapaport, B. Zhivotovsky, S. Orrenius, The mitochondrial TOM complex is required for tBid/Bax-induced cytochrome *c* release, J. Biol. Chem. 282 (2007) 27633–27639.
[100] L.K. Sanjuan Szklarz, V. Kozjak-Pavlovic, F.N. Vogtle, A. Chacinska, D. Milenkovic, S. Vogel, M. Durr, B. Westermann, B. Guiard, J.C. Martinou, C. Borner, N. Pfanner, C. Meisinger, Preprotein transport machineries of yeast mitochondrial outer membrane are not required for Bax-induced release of intermembrane space proteins, J. Mol. Biol. 368 (2007) 44–54.
[101] S.R. Chen, D.D. Dunigan, M.B. Dickman, Bcl-2 family members inhibit oxidative stress-induced programmed cell death in *Saccharomyces cerevisiae*, Free Radic. Biol. Med. 34 (2003) 1315–1325.
[102] P. Polcic, M. Forte, Response of yeast to the regulated expression of proteins in the Bcl-2 family, Biochem. J. 374 (2003) 393–402.
[103] P. Pizzo, T. Pozzan, Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics, Trends Cell Biol. 17 (2007) 511–517.
[104] C.Q. Scheckhuber, N. Erjavec, A. Tinazli, A. Hamann, T. Nystrom, H.D. Osiewacz, Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models, Nat. Cell Biol. 9 (2007) 99–105.
[105] Y. Fannjiang, W.C. Cheng, S.J. Lee, B. Qi, J. Pevsner, J.M. McCaffery, R.B. Hill, G. Basanez, J.M. Hardwick, Mitochondrial fission proteins regulate programmed cell death in yeast, Genes Dev. 18 (2004) 2785–2797.
[106] H. Kitagaki, Y. Araki, K. Funato, H. Shimoi, Ethanol-induced death in yeast exhibits features of apoptosis mediated by mitochondrial fission pathway, FEBS Lett. 581 (2007) 2935–2942.
[107] M. Boyce, J. Yuan, Cellular response to endoplasmic reticulum stress: a matter of life or death, Cell Death Differ. 13 (2006) 363–373.
[108] M.M. Kincaid, A.A. Cooper, ERADicate ER stress or die trying, Antioxid. Redox Signal. 9 (2007) 2373–2387.
[109] C.S. Sevier, C.A. Kaiser, Formation and transfer of disulphide bonds in living cells, Nat. Rev., Mol. Cell Biol. 3 (2002) 836–847.
[110] B.P. Tu, J.S. Weissman, The FAD- and O(2)-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum, Mol. Cell. 10 (2002) 983–994.
[111] M.G. Pollard, K.J. Travers, J.S. Weissman, Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum, Mol. Cell. 1 (1998) 171–182.
[112] J.W. Cuozzo, C.A. Kaiser, Competition between glutathione and protein thiols for disulphide-bond formation, Nat. Cell Biol. 1 (1999) 130–135.
[113] A.R. Frand, C.A. Kaiser, Ero1p oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum, Mol. Cell. 4 (1999) 469–477.
[114] C.S. Sevier, H. Qu, N. Heldman, E. Gross, D. Fass, C.A. Kaiser, Modulation of cellular disulfide-bond formation and the ER redox environment by feedback regulation of Ero1, Cell 129 (2007) 333–344.
[115] K.J. Travers, C.K. Patil, L. Wodicka, D.J. Lockhart, J.S. Weissman, P. Walter, Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation, Cell 101 (2000) 249–258.
[116] L. Ellgaard, A. Helenius, Quality control in the endoplasmic reticulum, Nat. Rev., Mol. Cell Biol. 4 (2003) 181–191.
[117] D.T. Rutkowski, R.J. Kaufman, That which does not kill me makes me stronger: adapting to chronic ER stress, Trends Biochem. Sci. 32 (2007) 469–476.
[118] C.M. Haynes, E.A. Titus, A.A. Cooper, Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death, Mol. Cell. 15 (2004) 767–776.
[119] H.P. Harding, Y. Zhang, H. Zeng, I. Novoa, P.D. Lu, M. Calfon, N. Sadri, C. Yun, B. Popko, R. Paules, D.F. Stojdl, J.C. Bell, T. Hettmann, J.M. Leiden, D. Ron, An integrated stress response regulates amino acid metabolism and resistance to oxidative stress, Mol. Cell 11 (2003) 619–633.
[120] M. Sugiura, H. Takagi, Yeast cell death caused by mutation of the *OST2* gene encoding the epsilon-subunit of *Saccharomyces cerevisiae* oligosaccharyltransferase, Biosci. Biotechnol. Biochem. 70 (2006) 1234–1241.
[121] S. Silberstein, P.G. Collins, D.J. Kelleher, R. Gilmore, The essential *OST2* gene encodes the 16-kD subunit of the yeast oligosaccharyltransferase, a highly conserved protein expressed in diverse eukaryotic organisms, J. Cell Biol. 131 (1995) 371–383.
[122] P. Hauptmann, C. Riel, L.A. Kunz-Schughart, K.U. Frohlich, F. Madeo, L. Lehle, Defects in N-glycosylation induce apoptosis in yeast, Mol. Microbiol. 59 (2006) 765–778.
[123] E. Rabinovich, A. Kerem, K.U. Frohlich, N. Diamant, S. Bar-Nun, AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation, Mol. Cell. Biol. 22 (2002) 626–634.
[124] Y. Ye, H.H. Meyer, T.A. Rapoport, The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol, Nature 414 (2001) 652–656.
[125] C. Wojcik, M. Rowicka, A. Kudlicki, D. Nowis, E. McConnell, M. Kujawa, G.N. DeMartino, Valosin-containing protein (p97) is a regulator of endoplasmic reticulum stress and of the degradation of N-end rule and

ubiquitin-fusion degradation pathway substrates in mammalian cells, Mol. Biol. Cell 17 (2006) 4606–4618.
[126] R.J. Braun, H. Zischka, F. Madeo, T. Eisenberg, S. Wissing, S. Buttner, S.M. Engelhardt, D. Buringer, M. Ueffing, Crucial mitochondrial impairment upon *CDC48* mutation in apoptotic yeast, J. Biol. Chem. 281 (2006) 25757–25767.
[127] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[128] J.D. Malhotra, R.J. Kaufman, Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid. Redox Signal. 9 (2007) 2277–2293.
[129] J. Strayle, T. Pozzan, H.K. Rudolph, Steady-state free Ca(2+) in the yeast endoplasmic reticulum reaches only 10 microM and is mainly controlled by the secretory pathway pump pmr1, EMBO J. 18 (1999) 4733–4743.
[130] M. Bonilla, K.K. Nastase, K.W. Cunningham, Essential role of calcineurin in response to endoplasmic reticulum stress, EMBO J. 21 (2002) 2343–2353.
[131] A.I. Pozniakovsky, D.A. Knorre, O.V. Markova, A.A. Hyman, V.P. Skulachev, F.F. Severin, Role of mitochondria in the pheromone- and amiodarone-induced programmed death of yeast, J. Cell Biol. 168 (2005) 257–269.
[132] G. Varbiro, A. Toth, A. Tapodi, B. Veres, B. Sumegi, F. Gallyas Jr., Concentration dependent mitochondrial effect of amiodarone, Biochem. Pharmacol. 65 (2003) 1115–1128.
[133] G. Varbiro, A. Toth, A. Tapodi, Z. Bognar, B. Veres, B. Sumegi, F. Gallyas Jr., Protective effect of amiodarone but not *N*-desethylamiodarone on postischemic hearts through the inhibition of mitochondrial permeability transition, J. Pharmacol. Exp. Ther. 307 (2003) 615–625.
[134] W.E. Courchesne, S. Ozturk, Amiodarone induces a caffeine-inhibited, *MID1*-depedent rise in free cytoplasmic calcium in *Saccharomyces cerevisiae*, Mol. Microbiol. 47 (2003) 223–234.
[135] S.S. Gupta, V.K. Ton, V. Beaudry, S. Rulli, K. Cunningham, R. Rao, Antifungal activity of amiodarone is mediated by disruption of calcium homeostasis, J. Biol. Chem. 278 (2003) 28831–28839.
[136] A.J. Kowaltowski, A.E. Vercesi, S.G. Rhee, L.E. Netto, Catalases and thioredoxin peroxidase protect *Saccharomyces cerevisiae* against Ca(2+)-induced mitochondrial membrane permeabilization and cell death, FEBS Lett. 473 (2000) 177–182.
[137] G. Monteiro, A.J. Kowaltowski, M.H. Barros, L.E. Netto, Glutathione and thioredoxin peroxidases mediate susceptibility of yeast mitochondria to Ca(2+)-induced damage, Arch. Biochem. Biophys. 425 (2004) 14–24.
[138] K.E. Niederer, D.K. Morrow, J.L. Gettings, M. Irick, A. Krawiecki, J.L. Brewster, Cypermethrin blocks a mitochondria-dependent apoptotic signal initiated by deficient N-linked glycosylation within the endoplasmic reticulum, Cell. Signal. 17 (2005) 177–186.
[139] G. Durr, J. Strayle, R. Plemper, S. Elbs, S.K. Klee, P. Catty, D.H. Wolf, H.K. Rudolph, The medial-Golgi ion pump Pmr1 supplies the yeast secretory pathway with Ca2+ and Mn2+ required for glycosylation, sorting, and endoplasmic reticulum-associated protein degradation, Mol. Biol. Cell 9 (1998) 1149–1162.
[140] S. Vashist, C.G. Frank, C.A. Jakob, D.T. Ng, Two distinctly localized p-type ATPases collaborate to maintain organelle homeostasis required for glycoprotein processing and quality control, Mol. Biol. Cell 13 (2002) 3955–3966.
[141] K.S. Dimmer, S. Fritz, F. Fuchs, M. Messerschmitt, N. Weinbach, W. Neupert, B. Westermann, Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*, Mol. Biol. Cell 13 (2002) 847–853.
[142] R. Kellermayer, D.P. Aiello, A. Miseta, D.M. Bedwell, Extracellular Ca(2+) sensing contributes to excess Ca(2+) accumulation and vacuolar fragmentation in a pmr1Delta mutant of *S. cerevisiae*, J. Cell Sci. 116 (2003) 1637–1646.
[143] P.J. Lapinskas, K.W. Cunningham, X.F. Liu, G.R. Fink, V.C. Culotta, Mutations in *PMR1* suppress oxidative damage in yeast cells lacking superoxide dismutase, Mol. Cell. Biol. 15 (1995) 1382–1388.
[144] S.Y. Park, S.B. Seo, S.J. Lee, J.G. Na, Y.J. Kim, Mutation in PMR1, a Ca (2+)-ATPase in Golgi, confers salt tolerance in *Saccharomyces cerevisiae* by inducing expression of *PMR2*, an Na(+)-ATPase in plasma membrane, J. Biol. Chem. 276 (2001) 28694–28699.
[145] G. Devasahayam, D.J. Burke, T.W. Sturgill, Golgi manganese transport is required for rapamycin signaling in *Saccharomyces cerevisiae*, Genetics 177 (2007) 231–238.
[146] G. Devasahayam, D. Ritz, S.B. Helliwell, D.J. Burke, T.W. Sturgill, Pmr1, a Golgi Ca2+/Mn2+-ATPase, is a regulator of the target of rapamycin (TOR) signaling pathway in yeast, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17840–17845.
[147] C.W. Gourlay, K.R. Ayscough, The actin cytoskeleton: a key regulator of apoptosis and ageing? Nat. Rev., Mol. Cell Biol. 6 (2005) 583–589.
[148] C.W. Gourlay, K.R. Ayscough, The actin cytoskeleton in ageing and apoptosis, FEMS Yeast Res. 5 (2005) 1193–1198.
[149] B.K. Haarer, D.C. Amberg, Old yellow enzyme protects the actin cytoskeleton from oxidative stress, Mol. Biol. Cell 15 (2004) 4522–4531.
[150] M.E. Farah, D.C. Amberg, Conserved actin cysteine residues are oxidative stress sensors that can regulate cell death in yeast, Mol. Biol. Cell 18 (2007) 1359–1365.
[151] C.W. Gourlay, K.R. Ayscough, Actin-induced hyperactivation of the Ras signaling pathway leads to apoptosis in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 26 (2006) 6487–6501.
[152] C.W. Gourlay, K.R. Ayscough, Identification of an upstream regulatory pathway controlling actin-mediated apoptosis in yeast, J. Cell Sci. 118 (2005) 2119–2132.
[153] Q. Liang, B. Zhou, Copper and manganese induce yeast apoptosis via different pathways, Mol. Biol. Cell 18 (2007) 4741–4749.
[154] D.S. Gomes, M.D. Pereira, A.D. Panek, L.R. Andrade, E.C. Eleutherio, Apoptosis as a mechanism for removal of mutated cells of *Saccharomyces cerevisiae*: The role of Grx2 under cadmium exposure, Biochim. Biophys. Acta (in press), doi:10.1016/j.bbagen.2007.09.014.
[155] L. Du, Y. Yu, J. Chen, Y. Liu, Y. Xia, Q. Chen, X. Liu, Arsenic induces caspase- and mitochondria-mediated apoptosis in *Saccharomyces cerevisiae*, FEMS Yeast Res. 7 (2007) 860–865.
[156] W. Jian, J.S. Arora, T. Oe, V.V. Shuvaev, I.A. Blair, Induction of endothelial cell apoptosis by lipid hydroperoxide-derived bifunctional electrophiles, Free Radic. Biol. Med. 39 (2005) 1162–1176.
[157] R. Reekmans, K. De Smet, C. Chen, P. Van Hummelen, R. Contreras, Old yellow enzyme interferes with Bax-induced NADPH loss and lipid peroxidation in yeast, FEMS Yeast Res. 5 (2005) 711–725.
[158] B. Zhivotovsky, G. Kroemer, Apoptosis and genomic instability, Nat. Rev., Mol. Cell Biol. 5 (2004) 752–762.
[159] M. Thorsen, G. Lagniel, E. Kristiansson, C. Junot, O. Nerman, J. Labarre, M.J. Tamas, Quantitative transcriptome, proteome, and sulfur metabolite profiling of the *Saccharomyces cerevisiae* response to arsenite, Physiol. Genomics 30 (2007) 35–43.
[160] A.L. Wu, W.S. Moye-Rowley, GSH1, which encodes gamma-glutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation, Mol. Cell. Biol. 14 (1994) 5832–5839.
[161] P.J. Dilda, G.G. Perrone, A. Philp, R.B. Lock, I.W. Dawes, P.J. Hogg, Insight into the selectivity or arsenic trioxide for acute promyelocytic leukemia cells by characterizing *Saccharomyces cerevisiae* deletion strains that are sensitive or resistant to the metalloid, Int. J. Biochem. Cell Biol. (in press), doi:10.1016/j.biocel.2007.11.002.
[162] F.Q. Schafer, G.R. Buettner, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple, Free Radic. Biol. Med. 30 (2001) 1191–1212.
[163] S.A. Susin, H.K. Lorenzo, N. Zamzami, I. Marzo, B.E. Snow, G.M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D.R. Goodlett, R. Aebersold, D.P. Siderovski, J.M. Penninger, G. Kroemer, Molecular characterization of mitochondrial apoptosis-inducing factor, Nature 397 (1999) 441–446.
[164] S. Wissing, P. Ludovico, E. Herker, S. Buttner, S.M. Engelhardt, T. Decker, A. Link, A. Proksch, F. Rodrigues, M. Corte-Real, K.U. Frohlich, J. Manns, C. Cande, S.J. Sigrist, G. Kroemer, F. Madeo, An AIF orthologue regulates apoptosis in yeast, J. Cell Biol. 166 (2004) 969–974.
[165] J.A. Klein, C.M. Longo-Guess, M.P. Rossmann, K.L. Seburn, R.E. Hurd, W.N. Frankel, R.T. Bronson, S.L. Ackerman, The harlequin mouse mutation downregulates apoptosis-inducing factor, Nature 419 (2002) 367–374.

[166] M.D. Miramar, P. Costantini, L. Ravagnan, L.M. Saraiva, D. Haouzi, G. Brothers, J.M. Penninger, M.L. Peleato, G. Kroemer, S.A. Susin, NADH oxidase activity of mitochondrial apoptosis-inducing factor, J. Biol. Chem. 276 (2001) 16391–16398.
[167] S.A. Lipton, E. Bossy-Wetzel, Dueling activities of AIF in cell death versus survival: DNA binding and redox activity, Cell 111 (2002) 147–150.
[168] S. Krantic, N. Mechawar, S. Reix, R. Quirion, Apoptosis-inducing factor: a matter of neuron life and death, Prog. Neurobiol. 81 (2007) 179–196.
[169] N. Vahsen, C. Cande, J.J. Briere, P. Benit, N. Joza, N. Larochette, P.G. Mastroberardino, M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schagger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation, EMBO J. 23 (2004) 4679–4689.
[170] W. Li, L. Sun, Q. Liang, J. Wang, W. Mo, B. Zhou, Yeast AMID homologue Ndi1p displays respiration-restricted apoptotic activity and is involved in chronological aging, Mol. Biol. Cell 17 (2006) 1802–1811.
[171] F. Madeo, E. Herker, C. Maldener, S. Wissing, S. Lachelt, M. Herlan, M. Fehr, K. Lauber, S.J. Sigrist, S. Wesselborg, K.U. Frohlich, A caspase-related protease regulates apoptosis in yeast, Mol. Cell. 9 (2002) 911–917.
[172] K. Mitsui, D. Nakagawa, M. Nakamura, T. Okamoto, K. Tsurugi, Valproic acid induces apoptosis dependent of Yca1p at concentrations that mildly affect the proliferation of yeast, FEBS Lett. 579 (2005) 723–727.
[173] M.A. Khan, P.B. Chock, E.R. Stadtman, Knockout of caspase-like gene, *YCA1*, abrogates apoptosis and elevates oxidized proteins in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 17326–17331.
[174] B. Fahrenkrog, U. Sauder, U. Aebi, The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis, J. Cell Sci. 117 (2004) 115–126.
[175] F. Madeo, E. Herker, S. Wissing, H. Jungwirth, T. Eisenberg, K.U. Frohlich, Apoptosis in yeast, Curr. Opin. Microbiol. 7 (2004) 655–660.
[176] G.W. Thorpe, On the cellular mechanisms required for survival of oxidative stress in *Saccharomyces cerevisiae*, PhD thesis, University of New South Wales, Kensington, 2006, p. 172.
[177] S. Izawa, Y. Inoue, A. Kimura, Oxidative stress response in yeast: effect of glutathione on adaptation to hydrogen peroxide stress in *Saccharomyces cerevisiae*, FEBS Lett. 368 (1995) 73–76.
[178] C.M. Grant, L.P. Collinson, J.H. Roe, I.W. Dawes, Yeast glutathione reductase is required for protection against oxidative stress and is a target gene for yAP-1 transcriptional regulation, Mol. Microbiol. 21 (1996) 171–179.
[179] C.M. Grant, F.H. MacIver, I.W. Dawes, Glutathione is an essential metabolite required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*, Curr. Genet. 29 (1996) 511–515.