MINIREVIEW

Aging and cell death in the other yeasts, Schizosaccharomyces pombe and Candida albicans

Su-Ju Lin1 & Nicanor Austriaco2

1Department of Microbiology and Molecular Genetics, College of Biological Sciences, University of California, Davis, CA, USA; and 2Department of Biology, Providence College, Providence, RI, USA

Correspondence: Nicanor Austriaco, O.P., 549 River Ave., Providence, RI 02918, USA.
Tel.: +1 401 865 1823;
fax: +1 401 865 2959;
e-mail: naustria@providence.edu

Received 10 June 2013; revised 18 September 2013; accepted 10 October 2013.
Final version published online 8 November 2013.
DOI: 10.1111/1567-1364.12113

Editor: Dina Petranovic

Keywords aging; cell death; Saccharomyces cerevisiae; Candida albicans; Schizosaccharomyces pombe; apoptosis.

Abstract

How do cells age and die? For the past 20 years, the budding yeast, Saccharomyces cerevisiae, has been used as a model organism to uncover the genes that regulate lifespan and cell death. More recently, investigators have begun to interrogate the other yeasts, the fission yeast, Schizosaccharomyces pombe, and the human fungal pathogen, Candida albicans, to determine if similar longevity and cell death pathways exist in these organisms. After summarizing the longevity and cell death phenotypes in S. cerevisiae, this mini-review surveys the progress made in the study of both aging and programmed cell death (PCD) in the yeast models, with a focus on the biology of S. pombe and C. albicans. Particular emphasis is placed on the similarities and differences between the two types of aging, replicative aging, and chronological aging, and between the three types of cell death, intrinsic apoptosis, autophagic cell death, and regulated necrosis, found in these yeasts. The development of the additional microbial models for aging and PCD in the other yeasts may help further elucidate the mechanisms of longevity and cell death regulation in eukaryotes.

Introduction

Over the past 50 years, the yeasts have proved to be superb model systems for eukaryotic biology that have yielded seminal insights into a diversity of cellular and molecular processes including cell cycle control, vesicular trafficking, prion biology, and cancer biology, just to name a few (Botstein & Fink, 1988, 2011). In this mini-review, we survey the progress made in the study of both aging and programmed cell death (PCD) in the yeast models. It begins by summarizing the seminal studies performed with the budding yeast, Saccharomyces cerevisiae, before focusing on the biology of the other yeasts, the fission yeast, Schizosaccharomyces pombe, and the human fungal pathogen, Candida albicans. The development of these additional microbial models for aging and PCD may help further elucidate the mechanisms of longevity and cell death in eukaryotes.

Background: aging studies in Saccharomyces cerevisiae

Recent studies in genetically tractable model systems including yeast demonstrate that longevity can be modulated by single gene mutations (Jazwinski, 2000; Kenyon, 2001; Dilova et al., 2007; Longo et al., 2012). In addition to genetic interventions, calorie restriction (CR; or dietary restriction) has also been shown to extend life span in a variety of species, further supporting possible conservation between longevity-regulating pathways in different species (Weindruch & Walford, 1998). CR has also been reported to delay the onset or reduce the incidence of many age-associated diseases such as cancer and diabetes (Weindruch & Walford, 1998; Guarente, 2007). However, the molecular mechanisms underlying these CR-induced beneficial effects are not fully understood. As more longevity genes are identified, it is evident that aging is modulated by a complex interplay of multiple signaling pathways even at the cellular level.

Owing to the short life span and well-established molecular genetic techniques, the budding yeast Saccharomyces cerevisiae has been the most popular yeast model to identify new components in the longevity regulating pathways and to study these factors at the molecular/genetic level. Yeast life span can be studied in two distinct ways: replicative lifespan (RLS) and chronological lifespan (CLS). RLS measures the number of cell divisions, an
The mother-daughter cell asymmetry in *S. cerevisiae* can be easily observed under the microscope, allowing development of the replicative lifespan (RLS) assay (Mortimer & Johnston, 1959). Thus far, budding yeast remains the most efficient model for RLS studies. On the other hand, CLS studies are commonly adopted in other yeast models as CLS can be readily determined by monitoring the viability of nondividing stationary phase yeast cells over time (Fabrizio & Longo, 2003; Longo & Fabrizio, 2012). Although RLS and CLS are addressing two very different forms of longevity, many longevity factors appeared to regulate both CLS and RLS.

Budding yeast has also been a popular model for studying the mechanisms of calorie restriction (CR) induced life span extension. In yeast, moderate CR can be imposed on cells by reducing the glucose concentrations in rich media from 2% to 0.5% (Lin et al., 2000; Easlon et al., 2008; Wei et al., 2008). Under this CR condition, the growth rate remains robust and both RLS and CLS are extended. Variations in CR protocols have also been described in which limitation of amino acids and/or further reduction in carbon sources are employed (Jiang et al., 2000; Kaetherlein et al., 2005a, b; Fabrizio & Longo, 2007; Longo & Fabrizio, 2012). In yeast, moderate CR is suggested to function through reducing the activities of conserved nutrient-sensing pathways to extend life span. Decreasing the activity of the Ras-cAMP/PKA (cyclic-AMP activated protein kinase A) pathway, which regulates cell growth and stress response, extends life span (Lin et al., 2000; Fabrizio et al., 2001). Deleting the nutrient responsive Sch9 (homolog of mammalian S6K kinases) and Tor1 kinases also promotes longevity (Fabrizio et al., 2001; Longo, 2003; Kaetherlein et al., 2005a, b). Both *tor1Δ* and *sch9Δ* mutants have been suggested to be genetic mimics of CR (Fabrizio et al., 2001; Kaetherlein et al., 2005a, b).

In addition to components of the nutrient sensing pathways, other longevity factors (which affect either RLS or CLS or both) have also been identified in the budding yeast, *S. cerevisiae*, some of which have been linked to CR. These factors include proteins that modulate mitochondrial function (Barros et al., 2004; Bonawitz et al., 2007; Scheckhuber et al., 2007; Veatch et al., 2009; Ocampo et al., 2012; Erjavec et al., 2013), stress response/hormesis/mitohormesis (Bonawitz et al., 2007; Mesquita et al., 2010; Li et al., 2011a, b; Pan, 2011; Pan et al., 2011; Longo & Fabrizio, 2012; Ocampo et al., 2012), activity of the NAD⁺-dependent deacetylase Sir2 family (Imai & Guarente, 2010; Lu & Lin, 2010), partitioning of damaged proteins (Erjavec et al., 2007; Erjavec & Nyström, 2007), genome stability (Weinberger et al., 2007; Andersen et al., 2008; Unal et al., 2011), homeostasis of NAD⁺ and other metabolic factors (Lin et al., 2000; Anderson et al., 2002; Belenky et al., 2007; Lu & Lin, 2010; Matecic et al., 2010), vacuolar function (Fabrizio et al., 2010; Hughes & Gottschling, 2012), ribosome biogenesis (Steffen et al., 2012), cell hypertrophy (Bilinski & Bartosz, 2006; Yang et al., 2011; Bilinski et al., 2012), and regulation of proteostasis (Delaney et al., 2013; Schleit et al., 2013), etc. In addition, acetic acid has been suggested to be an extracellular mediator of chronological aging (Burtner et al., 2009). pH neutralization was shown to offset acetic acid induced toxicity and protect CLS (and RLS; Burtner et al., 2009; Murakami et al., 2011, 2012). However, other studies also showed that acetic acid and pH are not the only key determinants of CLS (Longo et al., 2012; Wu et al., 2013). Overall, the contributions of *S. cerevisiae* to the studies of aging and CR have been considerable, and have helped pave way for further research in metazoans and other microbial model organisms. However, certain aspects of *S. cerevisiae* make parallels with metazoans difficult. The molecular mechanisms underlying the life span extension by these longevity factors still remain unclear.

**Aging studies in *Schizosaccharomyces pombe***

Fission yeast *S. pombe* has been the second most popular microbial aging model. Many conserved longevity factors originally identified in *S. cerevisiae*, such as the progrowth kinases and Sir2 family, also exist in *S. pombe* and have been shown to affect cellular life span. In addition to being a complementary model for *S. cerevisiae*, several characteristics of *S. pombe* make it a unique model for studying certain cellular processes that are conserved in mammalian cells but are absent or different in *S. cerevisiae*. For example, the mRNA splicing and RNA interference machinery are conserved in *S. pombe* and in the metazoas but appear to be lost in *S. cerevisiae* (Aravind et al., 2000; Buhler et al., 2008). In addition, unlike budding yeast, cell division in *S. pombe* is morphologically symmetric giving rise to two almost indistinguishable daughter cells. It has also been shown that *S. pombe* and mammalian cells share a similar mechanism of mitochondrial inheritance (Chiron et al., 2007). These differences make *S. pombe* a valuable model for the studying the mechanisms of cellular aging and CR (Roux et al., 2010a, b).

Replicative life span (RLS) analysis is greatly simplified in *S. cerevisiae* due to gross morphological differences
between mother and daughter cell (Mortimer & Johnston, 1959). On the other hand, RLS measurement in S. pombe, wherein cell division is morphologically symmetric, is more complicated. Despite the difficulty, Barker et al. were able to demonstrate that old mother cells become bigger and rounder after four divisions (Barker & Walmsley, 1999). Therefore, the RLS of S. pombe can be determined and the average RLS range between 9–16 divisions depending on the strain background (Barker & Walmsley, 1999; Erjavec et al., 2008). Interestingly, older S. pombe cells show asymmetric partitioning of damaged proteins (Erjavec et al., 2008), a phenomenon that has also been reported in S. cerevisiae (Aguilaniu et al., 2003). This asymmetric partitioning mechanism requires Sir2 and a functional cytoskeleton in both S. pombe and S. cerevisiae suggesting that Sir2-mediated selective damage partitioning is likely to be a conserved mechanism (Erjavec et al., 2008). To date, very few studies have focused on the identification of genes that affect the RLS of S. pombe. The effects of CR upon the RLS of S. pombe are also unknown. However, the binary fission property of S. pombe is similar to the mechanisms of mammalian cell division, making it a promising model for studying RLS for higher eukaryotes.

Chronological life span (CLS) is more thoroughly characterized in fission yeast. Many genes have been found to extend CLS when deleted or overexpressed (Zuin et al., 2008; Roux et al., 2010a, b; Chen & Runge, 2012; Ohtsuka et al., 2013). Most of these longevity genes have homologs in S. cerevisiae, which contribute to the characterization of their roles in S. pombe CLS. These studies have associated CLS with nutrient signaling (Roux et al., 2006; Ohtsuka et al., 2008; Chen & Runge, 2009; Roux et al., 2009; Ito et al., 2010), mitochondrial activity/maintenance (Zuin et al., 2008; Roux et al., 2010a, b; Azuma et al., 2012; Ohtsuka et al., 2013; Stephan et al., 2013), ROS production/stress resistance (Mutoh & Kitajima, 2007; Zuin et al., 2010a, b; Ohtsuka et al., 2011, 2012), proteasome activity/redistribution, autophagy (Takeda & Yanagida, 2010; Takeda et al., 2010), and vacuolar function (Stephan et al., 2013). Many of these factors also play a role in low glucose CR-induced CLS extension (Ohtsuka et al., 2008; Chen & Runge, 2009; Roux et al., 2009; Zuin et al., 2010a, b).

Deletion of the nutrient sensing protein kinase A (Pka1) – which is not lethal in S. pombe (Roux et al., 2006; Ohtsuka et al., 2008) – or the AKT homolog Sck2p (Roux et al., 2006; Chen & Runge, 2009) resulted in an increased CLS. Deletions of both Pka1 and Sck2 extended CLS longer than either of deletion alone (Roux et al., 2006), suggesting these two kinases function in complementary or partially overlapping pathways to regulate CLS. In fact, the pka1Δ mutant showed increased stress resistance but the sck2Δ mutant did not (Roux et al., 2006). Another AKT homolog sck1Δ appeared to play a minor role in CLS as Δsck1 only marginally extended CLS, however, the sck1Δsck2Δ double deletion mutant showed longer CLS than either of the single mutant (Chen & Runge, 2009). TOR signaling has been suggested to play a role in S. pombe CLS (Roux et al., 2010a, b). A recent study confirmed that deletion of the Tor1 kinase indeed extended CLS (Ohtsuka et al., 2013). Deletion of the Gt3 glucose receptor (GPCR) has also been reported to extend CLS, further attesting the role of glucose signaling in CLS (Roux et al., 2009).

Several factors have been reported to be required for maintaining CLS because deletions of these genes shorten CLS. Cells lacking left1Δ which encodes a long-chain fatty acyl-CoA synthetase, showed decreased CLS (Oshiro et al., 2003). Accelerated chronological aging was also observed in a strain lacking the stress attenuation proteins glutathione (Gsh1) and copper/zinc cytosolic superoxide dismutase (Sod1), deletion of Sir2 further reduced CLS (Mutoh & Kitajima, 2007). This suggests that Sir2 may function in regulating certain aspects of the stress response, which is important for CLS in S. pombe. Several mutants with defects in the mitochondrial respiratory chain activity were reported to have decreased CLS (Zuin et al., 2008). These mutants also showed higher intracellular ROS levels and that their short CLS can be rescued by supplementing antioxidants to the growth media, suggesting the short CLS was mainly caused by increased ROS. Another study also showed that mitochondria are the key sources of ROS production and mitochondrial dysfunction lead to decreased viability (Takeda & Yanagida, 2010; Takeda et al., 2010). In this study, cells were induced to quiescence state G0 by nitrogen starvation. Inactivation of the proteasome activity in G0 results in accumulation of ROS, autophagy-mediated destruction of mitochondria, and rapid loss of viability. This study suggests that proteasome function is essential for G0 survival and that the degradation of mitochondria (the primary source of ROS) by autophagy during proteasome dysfunction is a defense mechanism of G0 cells against ROS accumulation-induced toxicity (Takeda & Yanagida, 2010; Takeda et al., 2010).

Calorie restriction (CR) extends CLS in S. pombe in certain growth media (Chen & Runge, 2009; Roux et al., 2009, 2010a, b; Chen & Runge, 2012). Similar to S. cerevisiae, the most common CR intervention is to reduce glucose concentration from the standard concentration of 2% to a lower concentration of 0.5–0.05%. This regimen, however, only works in yeast extract medium (YE; Roux et al., 2009; Zuin et al., 2010a, b) and synthetic dextrose (SD) medium (Chen & Runge, 2009; Stephan et al., 2013). CR in synthetic minimal media like EMM or
EMM supplemented with complete amino acids (SDC, synthetic dextrose completed) failed to extend CLS (Chen & Runge, 2009). It was suggested that in minimal EMM media, cells are already under some form of glucose-independent CR, and therefore, further reduction of glucose might be detrimental to cells. In fact, cells grown in EMM showed much longer CLS than cells grown in YE or SD (Chen & Runge, 2009; Roux et al., 2009). It has also been shown that in S. cerevisiae, CR-induced CLS extension is dependent on specific nutrient composition (Wu et al., 2013). Variations in CR protocols have also been studied in S. pombe. Shifting stationary phase cells originally grown in SD medium to sterile water is considered as ‘severe calorie restriction’, and it greatly enhanced CLS (Ohtsuka et al., 2009). Cells grown in SDC using glycerol as the sole carbon source also showed extended CLS (Roux et al., 2009). However, the molecular mechanisms underlying these two extreme yet robust forms of CR remain unclear. Studies in glucose reduction-mediated CR have linked CR to the glucose sensing Glt3 pathway (Roux et al., 2009). Interestingly, CR appeared to activate respiration, ROS production, and the Sty1 MAP kinase (Zuin et al., 2010a, b). Activation of Sty1 by CR induces the expression of stress response genes essential for CLS extension. Sty1 activation is also required for the CLS extension mediated by deleting the two main nutrient sensing kinases Pka1 and Sck2. Deletion of the Sty1 phosphatase Pyp1, which resulted in constitutive active Sty1, partially rescued the short CLS of a mutant with hyperactive Pka1 (by deleting the Pka1 inhibitory subunit Cgs1; Zuin et al., 2010a, b). Overall these studies suggest that nutrient sensing, ROS homeostasis, and stress response play important roles in CR. More detailed studies are required to further delineate the complex interplay among these factors.

Genetic screens for novel longevity genes have also been carried out in S. pombe. Ohtsuka et al. have identified a number of genes that extend CLS when overexpressed (Ohtsuka et al., 2008, 2009, 2011, 2012, 2013). The precise function of these genes in life span regulation remains unclear. A few of these genes have been further characterized. Overexpression of cell1+ (extender of chronological life span) appeared to function downstream or in parallel to the Sty1 MAP kinase pathway as cell1+ overexpression rescued the short CLS of the sty1Δ mutant. Ecl1 overexpression did not further extend the CLS induced by pka1Δ or CR suggesting Ecl1 is associated with these pathways (Ohtsuka et al., 2008). Similar to CR, Ecl overexpression increased stress resistance (Ohtsuka et al., 2012), and required functional mitochondria (Azuma et al., 2009) for CLS extension. Overexpression of Hsf1 (heat shock factor) also extended CLS (Ohtsuka et al., 2011). Hsf1 appeared to bind to the upstream region of ecl2+ upon heat shock. Hsf1 overexpression required functional Ecl2 for CLS extension (Ohtsuka et al., 2011) and Ecl2 overexpression also extend CLS (Ohtsuka et al., 2012), however, the biological function of Ecl2 remains unclear. Cells overexpressing Oga1 exhibited phenotypes mimicking the tor1Δ mutant (such as Caffeine sensitivity and Canavanine resistance), suggesting that Oga1 may function in the Tor1 signaling pathway (Ohtsuka et al., 2013). Another group of genes were identified in a respiratory deficient mutant induced by expressing a constitutive active form of the Gz subunit (Gpa2) of GPCR (Roux et al., 2010a, b). Overexpression of these genes not only rescued the respiration defect of this Gpa2 mutant but could also extend CLS in the wild type background (Roux et al., 2010a, b). It was suggested that these genes functioned in Pka1/Sck2/Tor pathways (Roux et al., 2010a, b). Detailed mechanisms remain to be determined.

New methods and tools have recently been developed for CLS studies in S. pombe. A chemical genetic screen has identified novel antiaging pathways and chemicals (Stephan et al., 2013). Using a modified SD based CLS protocol (Roux et al., 2010a, b), which also allows CR to extend CLS (Chen & Runge, 2009), Stephan et al. developed a 96-well microtiter plate method to screen 522 natural products. In SD media, S. pombe cells show a uniform decline of viability until all cells in the culture are dead (Chen & Runge, 2012; Stephan et al., 2013) and hardly any cells survived beyond day 10 in SD supplemented with 3% glucose (non-CR condition). This assay represents an efficient and unique platform for screening genes or chemicals that can extend CLS in a high throughput manner. In S. cerevisiae, a small fraction of cells always regrow after most cells die in a CLS assay culture (Fabrizio et al., 2004). Therefore, long-lived S. cerevisiae cells identified using similar CLS assay may not indeed have longer CLS, and some of them may simply be better at scavenging nutrients. In addition, studies in S. cerevisiae showed that certain metabolite intermediates such as ethanol (Fabrizio et al., 2005) and acetic acid (Burtner et al., 2009), as well as the buffering capacity of both intracellular and extracellular environment may also determine CLS (Burtner et al., 2009; Longo et al., 2012; Wu et al., 2013). It is currently unknown whether these factors also have similar effects on S. pombe life span. Despite these caveat, Stephan et al. were able to identify nineteen compounds that extended CLS. Among these, one was wortmannin, a known inhibitor of phosphoinositide 3-kinases and TOR kinases (Stephan et al., 2013). The TOR kinase inhibitors have been shown to extend life span in several organisms (Fontana et al., 2010). This study also identified compounds that increase vacuolar acidification (monensin and nigericin), inhibit mitochondrial fission (Prostaglandin...
J2, inhibit Git3/PKA signaling (Prostaglandin J2), and decrease intracellular GMP synthesis (mycophenolic acid and acicin). In S. cerevisiae, proper vacuolar function has been shown to be essential for maintaining normal CLS (Fabrizio et al., 2010) and RLS (Hughes & Gottschling, 2012). Schizosaccharomyces pombe cells treated with monensin or nigerin showed increased vacuolar acidification and CLS, and that both effects were abolished by deleting the vacuolar v-ATPase Vma1 or Vma3. Overexpressing Vma1 was sufficient to extend CLS and increase vacuolar acidity. Chemical treatments did not further enhance the phenotypes induced by Vma1 overexpression, suggesting that these chemicals are likely to extend CLS by maintaining vacuolar pH homeostasis (Stephan et al., 2013). This result is in line with a recent study in S. cerevisiae that Vma1 overexpression can extend CLS of budding yeast (Hughes & Gottschling, 2012). In this study, replicatively aged S. cerevisiae mother cells showed increased vacuolar pH and mitochondrial dysfunction. It was suggested that proper vacuolar pH is required to maintain mitochondrial membrane potential. One possibility is that is when vacuolar pH is high (old cells), proton-facilitated amino acid (and other metabolites) transport into the vacuole is impaired leading to accumulation of intracellular amino acid (and other metabolites) transport into the vacuole is impaired leading to accumulation of intracellular amino acid (and other metabolites), which may depolarize mitochondrial membrane via an unknown mechanism (Hughes & Gottschling, 2012). Another study in S. pombe also supports the importance of intracellular pH maintenance in cellular life span (Ito et al., 2010). Deleting a P-type proton ATPase Pma1 decreases the export of H+ and extends CLS. It would be interesting to determine whether Pma1 deletion extends CLS by maintaining vacuolar acidity and mitochondrial function in S. pombe.

Decreased mitochondrial fusion (by deleting the Dnm1) has been shown to extend both RLS and CLS in S. cerevisiae (Palermo et al., 2007; Scheckhuber et al., 2007). Impaired mitochondrial fusion shortens life span in both S. pombe (by deleting Msp1) and S. cerevisiae (by deleting Mgm1; Scheckhuber et al., 2011; Stephan et al., 2013). However, unlike studies in budding yeast (Palermo et al., 2007; Scheckhuber et al., 2007), deleting Dnm1 was not sufficient to extend CLS in S. pombe, suggesting additional factors may play a more important role in regulating mitochondrial dynamics or CLS. Although the antiaging compound prostaglandin J2 was able to increase mitochondrial fusion in old S. pombe, its life span extension effect did not require functional Dnm1 or Msp1. It was suggested that prostaglandin J2 extended life span also by inhibiting the Git3/PKA pathway (Stephan et al., 2013). Given the connection between vacuolar acidity, mitochondrial function/dynamics, and life span discussed above, it would be very interesting to determine whether the compounds that affect vacuolar acidity would also affect mitochondrial activity and fission/fusion and vice versa. Finally, a recent study discussed the construction of a bar-coded DNA insertion library in S. pombe. This library will allow the isolation of partially inactivated essential genes and gain-of-function mutants, which are not possible using the currently available deletion collection. Overall, aging studies in S. pombe are promising and have helped pinpoint the key conserved longevity factors/pathways and define the proper conditions for CR studies.

Aging studies in Candida albicans

Candida albicans is a polymorphic fungus, which has recently been established as a Crabtree negative microbial model for aging. Candida albicans is a prevalent opportunistic fungal pathogen in humans, and can grow in both budding (single-celled blastospore) form or filamentous (hyphal) form. This organism is amenable to RLS analysis, and both the single-celled and filamentous forms have similar RLS of about twenty generations (Fu et al., 2008). At 30 °C, the yeast form of C. albicans proliferates by budding whereas at 37–40 °C in response to serum, the yeast form is induced to become filamentous multicellular hyphae. The switch to filamentous form is irreversible but the hyphal cells can still give rise to smaller yeast form daughters when they are grown at 30 °C. Taking advantage of these morphological changes, cells of different age (in hyphal form) can be sorted by changing the growth conditions and repeated cycles of separation of smaller young yeast form daughters and the old hyphae mothers by centrifugation on a sucrose gradient (Fu et al., 2008). Similarly to S. cerevisiae, old C. albicans cells appear to accumulate glycogens and damaged proteins. SIR2 also regulate C. albicans RLS in a dose-dependent manner: deletion of SIR2 decreases RLS whereas one extra copy of SIR2 extends RLS. Although SIR2 deletion does not cause accumulation of extra chromosomal rDNA circles (a cause of replicative aging in S. cerevisiae), it helps the retention of oxidatively damaged proteins in mother cells (Fu et al., 2008). The efficient isolation of old C. albicans cells allows large-scale biochemical, genomic, and proteomic studies and perhaps genetic screens for long-lived mutants.

It is currently unknown whether CR extends RLS in C. albicans. On the other hand, a recent study showed that the CLS of C. albicans could be extended by reducing glucose concentrations from 2% to 0.5% in SC (synthetic glucose) media (Chen et al., 2012). Interestingly, CR was able to extend the CLS of a respiration deficient goa1Δ mutant. It has been shown that Goa1 is required...
for mitochondrial function. In cells lacking Goa1, reduced activities of mitochondrial respiration, membrane potential, electron transport chain complex I, and ATP production were observed. These factors were suggested to contribute to the short CLS of the goa1Δ mutant (Li et al., 2011a, b). This result seemed to contradict CR studies in budding and fission yeasts, in which an optimal level of mitochondrial respiration activity is essential for CR-induced CLS extension (Azuma et al., 2009; Li et al., 2011a, b; Ocampo et al., 2012). It is noteworthy that C. albicans (and other fungi) have three respiratory pathways: the classical respiratory pathway, an alternative oxidase (AOX), and parallel respiratory pathways (PAR; Li et al., 2011a, b). CR appeared to induce the expression of AOX2 (an alternative oxidase) and a number of stress response genes in the goa3Δ mutant (deficient in the classical respiratory activity), which may explain why CR can extend CLS in this background (Chen et al., 2012). CR also induces alternative carbon metabolism by β-oxidation leading to more ROS production in the goa3Δ mutant. In addition, this organism appears to have an atypical response to glucose concentration: higher concentrations of glucose increases resistance to certain stresses (Rodaki et al., 2009), whereas restriction of glucose increases stress resistance in S. cerevisiae (Bonawitz et al., 2007; Wang et al., 2009; Li et al., 2011a, b; Ocampo et al., 2012) and S. pombe (Roux et al., 2009; Zuin et al., 2010a, b). Unlike Crabtree positive S. cerevisiae and S. pombe, which prefer to utilize glucose and use glucose to repress aerobic respiration, C. albicans is a Crabtree negative fungus and prefers respiration to fermentation even in the presence of glucose. The Crabtree effect (Crabtree, 1929) refers to inhibition of aerobic metabolism when the preferred carbon source, glucose, is available. This inhibition occurs in the presence or absence of oxygen, and the term is not specific to yeasts: many mammalian tumor cells also display a Crabtree effect (De Deken, 1966; Golshani-Hebronzi & Bessman, 1997). Given the differences in carbon source utilization preferences and multiple choices of respiratory pathways, C. albicans is thus a good model for providing complimentary comparisons to aging and CR studies in S. cerevisiae and S. pombe.

Background: cell death studies in Saccharomyces cerevisiae

Defined as any cell death that results from the activation of a genetic program, programmed cell death (PCD) has recently been classified by the Nomenclature Committee on Cell Death (NCCD) into twelve different functional categories based on measurable biochemical features (Kroemer et al., 2009; Galluzzi et al., 2012). Though PCD was first described in multicellular organisms, there is growing evidence that it is also found in unicellular organisms including yeast and bacteria (Engelberg-Kulka et al., 2006; Bayles, 2007). Here, we begin by summarizing the pioneering work done to interrogate cell death in the budding yeast, Saccharomyces cerevisiae, by focusing on the three primary NCCD categories of cell death found in this yeast, called intrinsic apoptosis, autophagic cell death, and regulated necrosis.

One of the earliest types of cell death described, apoptosis is a form of regulated cell death that is characterized by distinctive morphological and biochemical changes, including the production of reactive oxygen species (ROS), the degradation of DNA, and the condensation and fragmentation of the nucleus (Kerr et al., 1972; Gerschenson & Rotello, 1992). Intrinsic apoptosis is a cell death process that is mediated by the permeabilization of the mitochondrial outer membrane and the release of, and often, the relocalization of proteins normally found in the intermembrane space (Galluzzi et al., 2012). Intrinsic apoptosis is further differentiated into caspase-dependent and caspase-independent intrinsic apoptosis based on the extent of cytoprotection conferred by the pharmacological or genetic inhibition of the caspases, the cysteine proteases that effect cell death in most eukaryotic cells (Galluzzi et al., 2012). In mammalian cells, activation of the intrinsic apoptotic program leads to the destruction of proteins by a caspase-dependent proteolytic cascade and to the fragmentation of genomic DNA by a mechanism requiring apoptosis-inducing factor (AIF) and endonuclease G (Ekert & Vaux, 2005).

In yeast, an apoptotic-like phenotype, characterized by the condensation and fragmentation of DNA, the generation of ROS, and the exposure of phosphatidyl serine, was first observed in S. cerevisiae (Madeo et al., 1997, 1999). Since then, numerous external triggers have been shown to induce apoptosis in budding yeast including hydrogen peroxide, acetic acid, ethanol, high salt, osmotic stress, lipids, UV irradiation, heat stress, and numerous heavy metal ions (Carmona-Gutierrez et al., 2010). Internal signals including ammonia, nitric oxide, and reactive oxygen species, among others, also lead to apoptotic cell death in this organism (Vachova & Palkova, 2005; Almeida et al., 2007; Perrone et al., 2008). Notably, the heterologous expression of the human proapoptotic protein BAX leads to apoptotic cell death, one which has been linked to endoplasmic reticulum function (Austriaco, 2012; Cakir, 2012), while conversely, the heterologous expression of the human antiapoptotic proteins BCL-2, BCL-XL, or BI-1 prevents BAX-induced lethality and increases the viability of budding yeast cells cultured in H2O2 or acetic acid (Eisenberg et al., 2007; Khoury & Greenwood, 2008; Sano et al., 2012). Both replicative life...
span (RLS) and chronological life span (CLS) have also been associated with an apoptotic-like cell death mechanism accompanied by ROS overproduction, phosphatidylserine externalization, and DNA fragmentation, suggesting that aging and cell death are linked in *S. cerevisiae* (Herker et al., 2004; Fabrizio & Longo, 2008; Laun et al., 2008; Rockenfeller & Madeo, 2008). Finally, like their metazoan counterparts, yeast mitochondria undergo dramatic organelle fragmentation during programmed cell death, which is accompanied by the concomitant release of cytochrome-c (Abdelwahid et al., 2011). However, a causal role linking cytochrome-c release to cell death has not been firmly established in this model system, though there is a report that the conserved mitochondrial protein AAC/ANT is required, not only for cytochrome-c release, but also for the cell death induced by acetic acid (Manon et al., 2004; Fabrizio & Longo, 2008; Pereira et al., 2010).

Mechanistically, it is clear that caspase-dependent intrinsic apoptosis exists in *S. cerevisiae* (Li et al., 2008; Mazzoni & Falcone, 2008). This yeast has one ortholog of the mammalian caspases, the metacaspase, Yca1p/Mca1p, which has been linked to numerous cell death scenarios including, for example, the cell death triggered by oxygen stress, osmotic stress, viral killer toxins, chronological aging, or sphingolipid dysregulation (Uren et al., 2006; Madeo et al., 2002; Mazzoni et al., 2005; Vachova & Palkova, 2007; Wilkinson & Ramsdale, 2011; Kajiwara et al., 2012; Wong et al., 2012; Shrestha et al., 2013). In one report, Yca1p was placed in the sphingolipid-induced apoptotic pathway downstream of both the calcineruin-dependent calcium signaling pathway and the mitochondrial apoptotic pathway mediated by cytochrome-c (Kajiwara et al., 2012). Significantly, caspase-3-, 6-, and 8-like activities that do not appear to depend on Yca1p have also been detected in dying yeast cells, implicating other proteases with caspase-like activity in the cell death response of *S. cerevisiae* (Wilkinson & Ramsdale, 2011). One example is the proapoptotic protease, Kex1p, that is essential for hypochlorite-induced apoptosis (Carloma-Gutierrez et al., 2013).

Caspase-independent intrinsic apoptosis can also occur in this organism (Li et al., 2008). Examples include the cell death that occurs during long-term development of yeast multicellular colonies, and the cell death that results when mutations disrupt the N-glycosylation that occurs in the endoplasmic reticulum (Vachova & Palkova, 2005; Hauptmann et al., 2006). Moreover, the cell death that is triggered by the yeast homologs of mammalian apoptosis inducing factor (AIF) and endonuclease-G (EndoG), called Aif1p and Nuc1p in budding yeast respectively, can occur even in the absence of YCA1 (Wissing et al., 2004; Buttner et al., 2007). Intriguingly, like their mammalian counterparts, both Aif1p and Nuc1p are translocated from the mitochondria to the nucleus during the dying process (Wissing et al., 2004; Buttner et al., 2007).

Finally, other homologs of mammalian genes that have been implicated in metazoan apoptosis have also been identified in budding yeast, including, among others, BIR1 (Walter et al., 2006), BXXI (Buttner et al., 2011; Cebulska et al., 2011), ESP1 (Yang et al., 2008), NMA111 (Belanger et al., 2009), NDI1 (Cui et al., 2012), and SIR2 (Yang et al., 2008). A functional network linking these genes into interacting molecular pathways that regulate cell death has begun to emerge (Kazemzadeh et al., 2012) and is being curated by the yeast apoptosis database, yApoptosis (http://www.sysbio.se/ycellddeath/yapoptosis/).

Next, autophagic cell death is cell death that is accompanied by massive cytoplasmic vacuolization usually indicating an increased autophagic flux (Galluzzi et al., 2012). Autophagy is a self-degradative process that is involved in removing misfolded proteins, clearing damaged organelles, and eliminating intracellular pathogens (Glick et al., 2010). It appears to be responsible for the physiological cell death that occurs during the developmental program of Drosophila (Denton et al., 2009) and for the death of cancer cells that lack key apoptotic proteins including BAX, BAK, and the caspases (Fazi et al., 2008). By definition, autophagic cell death can be suppressed by the inhibition of the autophagic pathway by pharmacological or genetic means (Galluzzi et al., 2012).

In yeast, there are reports that suggest that the loss of the autophagy ATG genes can enhance the viability of *S. cerevisiae* cultured in media containing cell death triggers, pointing to the existence of autophagic cell death in this organism. For example, cells treated with high concentrations of Zn^{2+} died unless any one of seven autophagy genes was inactivated (Dziedzic & Caplan, 2011). A similar phenotype was observed in autophagy-deficient *atg8Δ* cells undergoing leucine- but not nitrogen starvation (Dziedzic & Caplan, 2012). The cell death associated with the heterologous expression of mammalian BAX in budding yeast has also been linked to the appearance of autophagic features, though the inactivation of autophagy did not prevent this BAX-induced cell demise (Kissova et al., 2006).

Finally, necrosis is a form of cell death that until recently had been linked to an accidental and uncontrolled cell death lacking any of the morphological traits of apoptosis (Walker et al., 1988). However, it is now clear that regulated necrosis can be triggered by a wide range of both external and internal stimuli in mammalian cells, especially in those cells whose caspases are inhibited either by pharmacological or genetic means (Zong & Thompson, 2006; Golstein & Kroemer, 2007; Galluzzi et al., 2012). Mechanistically, execution of regulated necrosis in these dying mammalian cells is often
associated with the activity of the serine/threonine kinases RIP1 and RIP3 (Galluzzi et al., 2009). Notably, regulated necrosis in metazoan cells has also been linked to lysosomal rupture (Artal-Sanz et al., 2006; Qin et al., 2008; Messner et al., 2012; Lima et al., 2013).

In yeast, there are data suggesting that regulated necrosis exists in S. cerevisiae (Eisenberg et al., 2010). Extrinsic triggers include well-known cell death inducers like H$_2$O$_2$ or acetic acid, which elicit apoptosis at low concentrations but necrosis at higher concentrations, and less-known molecules like copper and manganese (Madeo et al., 1999; Ludovico et al., 2001; Liang & Zhou, 2007). Fatty acids and ceramide too stimulate cells to undergo regulated necrosis (Rockenfeller et al., 2010; Carmona-Gutierrez et al., 2011a, b). Finally, the heterologous expression of human immunodeficiency virus (HIV-1) protease (Blanco et al., 2003), human α-synuclein, a trigger of neurodegeneration in Parkinson’s Disease (Buttner et al., 2008), and the proteinaceous elicitor harpin (Ps) from Pseudomonas syringae (Sripriya et al., 2009), also led to regulated necrosis in budding yeast.

Mechanistically, there is no evidence that homologs of the mammalian necrotic effectors, RIP1 or RIP3, exist in budding yeast. However, it appears that regulated necrosis in budding yeast involves the lysosome — called the vacuole in yeast — like it does in the metazoan. For instance, the yeast homolog of the lysosomal endoprotease cathepsin D, called Pep4p in S. cerevisiae, has both antiapoptotic and antinecrotic functions where prolonged overexpression of the protein extended the chronological lifespan of yeast, specifically by inhibiting necrosis (Carmona-Gutierrez et al., 2011a, b). More strikingly, Kim et al. have demonstrated that ER stress in yeast can lead to a necrotic cell death mediated by the permeabilization of the vacuolar membrane, a process that can be blocked by the action of calcineurin, a Ca$^{2+}$-dependent serine/threonine protein phosphatase (Dudgeon et al., 2008; Kim et al., 2012). Intriguingly, this vacuolar membrane permeabilization (VMP) mechanism for necrotic cell death — which is reminiscent of the lysosomal rupture observed in the necrosis-like cell death seen in degenerating neurons — may have evolved in the context of meiosis and spore development: Undomesticated budding yeast strains execute this mode of cell death during gametogenesis within the context of a maturing colony to the apparent benefit of sibling cells (Eastwood et al., 2012). This is one clear instance where programmed cell death in a unicellular organism is clearly adaptive.

**Cell death studies in Schizosaccharomyces pombe**

There are fewer studies of PCD of the fission yeast, S. pombe, than either of S. cerevisiae or of C. albicans. To date, of the three primary NCCD categories of cell death found in budding yeast, only two, intrinsic apoptosis and autophagic cell death — and there is only one published example of this in the literature — have been described in fission yeast.

The earliest studies of intrinsic apoptosis in fission yeast focused on the heterologous expression of metazoan proapoptotic effectors in this organism. Overproduction of BAX and BAK led to cell death with some of the classic hallmarks of intrinsic apoptosis including chromatin condensation and fragmentation, nuclear blebbing and fragmentation (Ink et al., 1997; Jurgensmeier et al., 1997; Torgler et al., 1997). A similar apoptotic phenotype with the additional apoptotic hallmarks of ROS production and an altered mitochondrial physiology and membrane potential was observed in fission yeast overexpressing the proapoptotic human immunodeficiency virus type 1 (HIV-1) Vpr protein (Huard et al., 2008). Inositol starvation, replication stress, and inappropriate mitosis too lead to cell death with apoptotic features including ROS production, metacaspase activation, DNA breakage, and/or nuclear fragmentation (Marchetti et al., 2006; Guerin et al., 2008, 2009). Lipid-associated apoptosis triggered by the abnormal metabolism of intracellular lipids has also been described as the onset of DNA fragmentation and phosphatidylserine externalization in fission yeast (Zhang et al., 2003; Low et al., 2008). Finally, the chronological aging of fission yeast is accompanied by the production of ROS suggesting that this dying process, like it does in S. cerevisiae, may involve an apoptotic-like death mechanism (Roux et al., 2006).

Mechanistically, the genome of S. pombe encodes a single metacaspase named Pca1, which when overexpressed, appears to stimulate and not inhibit growth in fission yeast (Lim et al., 2007). Moreover, its precise function in fission yeast apoptosis remains unclear because deletion of the gene did not protect cells from H$_2$O$_2$, acetic acid, valproic acid, or chronological aging, though it was able to protect cells from lipid-induced cell death in minimal media but not in rich media (Low et al., 2008; Mutoh et al., 2011). Indeed, overexpression of pca1+ protects cells from the toxicity associated with cadmium suggesting that it can also act as an antiapoptotic regulator (Lim et al., 2007). Nonetheless, the data does suggest that caspase-independent intrinsic apoptosis is the form of apoptosis that predominates in S. pombe.

Cln3exin is an ER transmembrane chaperone that is involved in protein translocation, protein folding, and the quality control of newly synthesized polypeptides (Rutkевич & Williams, 2011). In mammalian cells, calnexin-deficient cells are more resistant to ER-stress associated cell death. Similarly, it appears that calnexin in fission yeast, Cnx1, is linked to apoptosis. First, it is required for...
the cell death mediated by the heterologous expression of mammalian BAK (Torgler et al., 1997). Overexpression of Cnx1 alone causes apoptosis, which is counteracted by the S. pombe homolog of the human antiapoptotic protein, HMGB1 (Guerin et al., 2008). Next, the protein is involved in ER stress associated cell death (ERSAD): Apoptosis induced by both ER stress and inositol starvation is also dependent on calnexin. (Guerin et al., 2008, 2009). The apoptotic cell death linked to inositol starvation, but not the cell death induced by the overexpression of Cnx1, is dependent on the metacaspase, Pca1 (Guerin et al., 2008, 2009).

Finally, Rad9 is a component of the Rad9-Hus1-Rad1 complex that functions as a sensor of DNA damage (Parilla-Castellar et al., 2004). It belongs to the prosurvival and proapoptotic BH3-only branch of Bcl-2 family of proteins that also include BAD, BID, and BIM (Giam et al., 2008). Paradoxically, however, overproduction of SpRad9, which is the fission yeast homolog of human Rad9, can induce apoptosis in human cells that could be blocked by co-overexpression of the human antiapoptotic protein, BCL-2 (Komatsu et al., 2000a, b). Nonetheless, in fission yeast, transcriptional upregulation of sprad9+ is correlated with improved viability under nitrogen stress conditions (Kang et al., 2007). Moreover, it has been implicated in the regulation of lipid-induced cell death in minimal media (Low et al., 2008).

Finally, as for autophagic cell death, fission yeast cells dying from the overexpression of mammalian BAX and BAK also display massive cytoplasmic vacuolization that is a feature of this category of cell death (Ink et al., 1997; Jurgensmeier et al., 1997). However, a genetic and molecular analysis of the underlying mechanism for this phenomenon has not yet been done.

Cell death studies in Candida albicans

Given its significance as a major opportunistic human pathogen, it is not surprising that the majority of studies investigating programmed cell death in Candida albicans have focused on the mechanism of action of present and potential antifungal agents (Ramsdale, 2008; De Brucker et al., 2011). They revealed that intrinsic apoptosis exists in C. albicans. However, examples of autophagic cell death and regulated necrosis – where specific genes demonstratively regulate the necrosis process – have not yet been confirmed in this yeast.

Like apoptosis in S. cerevisiae, apoptosis in C. albicans is accompanied by the classical hallmarks of metazoan apoptosis including ROS production, phosphatidylserine externalization, DNA and nuclear fragmentation, mitochondrial dysfunction, cytochrome c release, and metacaspase activation (Phillips et al., 2003; Ramsdale, 2008; Hao et al., 2013). In addition to a wide range of antifungals including, among others, amphotericin B (Phillips et al., 2003; Al-Dhaheri & Douglas, 2010; Yang et al., 2010), caspofungin (Hao et al., 2013), coprisin (Lee et al., 2012), medioresinol (Hwang et al., 2012), baicalein (Dai et al., 2009), miconazole (Vandenbosch et al., 2010), and curcumin (Sharma et al., 2010), other apoptotic triggers in this yeast include acetic acid, H2O2, plant defensins, polyunsaturated fatty acids (PUFAs), human lactoferrin, and the quorum sensing molecule, farnesol (Phillips et al., 2003; Andres et al., 2008; Aerts et al., 2009; Shirtliff et al., 2009; Aerts et al., 2011; Zhu et al., 2011; Thibane et al., 2012).

Intrinsic apoptosis can occur in both the blastospore, that is, yeast, and the hyphal forms of this dimorphic yeast, though the hyphal form may be relatively more resistant to apoptosis than the yeast form (D. Laprade and N. Aus triaco, manuscript in revision). Finally, apoptosis also occurs even when Candida forms biofilms (Al-Dhaheri & Douglas, 2010; Thibane et al., 2012).

Mechanistically, C. albicans has a single gene encoding a metacaspase, CaMCA1, that is orthologous to the S. cerevisiae metacaspase, YCA1 (Cao et al., 2009). Deletion of CaMCA1 increased the viability of cells cultured in H2O2 whereas simultaneously increasing intracellular concentrations of trehalose, a disaccharide of glucose that plays a protective role against oxidative stress, suggesting that CaMca1 regulates cell death (Cao et al., 2009). In contrast, the apoptosis that is induced by the antifungal plant defensin, RaSFP2 and the echinocandin, caspofungin, in Candida does not require this metacaspase (Aerts et al., 2009). Moreover, Lu et al. have discovered that H2O2-induced apoptosis in Candida is mediated by a rise in intracellular Ca2+ levels that triggers the calcineurin-dependent calcium signaling pathway to activate CaMca1 (Lu et al., 2011). The crucial molecular chaperone, Hsp90, which had been linked to the calcineurin pathway, also regulates H2O2-induced apoptosis partially by downregulating the calcineurin-caspase pathway (Dai et al., 2012). This pathway is reminiscent of the calcineurin-caspase pathway described above that has been linked to sphingolipid-induced apoptosis in S. cerevisiae (Kajiwara et al., 2012). An independent pathway anchored by the Ras1 signal transduction GTPase has also been implicated in Candida apoptosis (Phillips et al., 2006). Mutations that block this pathway suppressed or delayed cell demise whereas mutations that stimulated the pathway accelerated the rate of entry of cells into apoptosis. It is not clear if this Ras1-dependent pathway is linked to the calcineurin-caspase pathway associated with H2O2-induced cell death. Finally, two studies have implicated the bZip transcription factor Cap1 in baicalein-induced apoptosis, possibly by regulating the expression of the glutathione reductase gene (GLR1) and glutathione content in C. albicans (Dai et al., 2009, 2013).
In sum, the study of Candida programmed cell death is still in its infancy. Given the apparent similarities between the intrinsic apoptosis of S. cerevisiae and that of C. albicans that are emerging, it will be important to determine if the other C. albicans homologs of metazoan cell death genes have similar functions in this yeast. Moreover, in light of this yeast’s ability to stochastically switch between two developmental states, white and opaque (Lin et al., 2013; Si et al., 2013), it would be interesting to see if opaque and white cells have similar or different cell death phenotypes, especially in the context of a biofilm. It could reveal additional adaptive explanations for the existence of programmed cell death in unicellular organisms.

Conclusion

Although metazoans are much more complicated than single-celled yeasts, many conserved intracellular processes in yeast are very similar to that in metazoans at the molecular and/or cellular level. Many longevity and death-associated factors/pathways are highly conserved from yeast to mammals, including mitochondrial respiration and dysregulation, Sir2 family proteins (sirtuins) and caspases, progrowth TOR and PKA signaling pathways and prodeath calcium networks, and metabolic pathways such as NAD⁺ biosynthesis. The significance of some of these longevity and death factors was first recognized in simple model organisms, which was later found parallel in higher eukaryotes. To date, the detailed mechanisms for how these longevity and death factors/pathways either extend life span and respond to CR or mediate cell death in response to both external and internal triggers have remained unclear. It is not even clear if overlapping molecular mechanisms regulate both longevity and death. For instance, it would be intriguing to determine if calorie restriction (CR) protects yeast not only from aging but more generally from programmed cell death. Contradictions also persist as to whether these factors indeed affect longevity by similar mechanisms in mammals. For example, whether increased or decreased mitochondrial activity is beneficial to lifespan and whether mitochondrial respiration and the Sir2 family proteins are major mediators of CR are still highly debatable (Guarente, 2007, 2008; Imai & Guarente, 2010; Someya et al., 2010; Kanfi et al., 2012; Longo & Fabrizio, 2012; Longo et al., 2012). Much clarification has yet to be done on the effects of these longevity and death factors on life span, CR, and PCD. Microbial models such as S. cerevisiae, S. pombe, and C. albicans not only provide a powerful genetic tool for the identification of critical components in these life and death processes but also serve as a platform for studying these longevity and death factors at the biochemical/molecular level.

Acknowledgements

We are grateful to the scientists whose work provided the basis for this review, and apologize to our colleagues whose relevant work was not cited or discussed because of length constraints. The work in our laboratories is supported by grants from the National Institutes of Health/NIH/NIGMS (R01-GM102297 to S. Lin and R15-GM094712 to N. Austriaco), the National Science Foundation (MRI-R2 0959354 to N. Austriaco), and the NIH/Rhode Island INBRE Program (R20 GM103430-12 to N. Austriaco for undergraduate student training).

References


