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# Aging and Cell Death in the Other Yeasts, *Schizosaccharomyces pombe* and *Candida albicans*

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## Abstract

How do cells age and die? For the past twenty 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.

### Keywords

aging; cell death; *S. cerevisiae*; *Candida albicans*; *Schizosaccharomyces pombe*; apoptosis; autophagy; necrosis

## Introduction

Over the past fifty 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, Botstein & Fink, 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 individual yeast cell undergoes before senescence, i.e., its division potential (Mortimer & Johnston, 1959), whereas CLS measures the length of time cells remains viable at a non-dividing state, i.e., its post-mitotic survival (Fabrizio & Longo, 2003, Longo & Fabrizio, 2012). 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 since CLS can be readily determined by monitoring the viability of non-dividing stationary phase yeast cells over time (Fabrizio & Longo, 2003, Chen & Runge, 2009, Roux *et al.*, 2010, 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, Kaeberlein *et al.*, 2005, 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, Kaeberlein *et al.*, 2005). Both *tor1*• and *sch9*• mutants have been suggested to be genetic mimics of CR (Fabrizio *et al.*, 2001, Kaeberlein *et al.*, 2005).

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.*, 2011, 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 & Nystrom, 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, Murakami *et al.*, 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 the 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 pro-growth 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 metazoa 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 symmetrical 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.*, 2010).

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*, whose 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 approximately 9–16 divisions depending on the strain background (Barker & Walmsley, 1999, Erjavec *et al.*, 2008). Interestingly, older *S. pombe* cells show asymmetrical partitioning of damaged proteins (Erjavec *et al.*, 2008), a phenomenon that has also been reported in *S. cerevisiae* (Aguilaniu *et al.*, 2003). This asymmetrical 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 over-expressed (Zuin *et al.*, 2008, Roux *et al.*, 2010, 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.*, 2010, Azuma *et al.*, 2012, Ohtsuka *et al.*, 2013, Stephan *et al.*, 2013), ROS production/stress resistance (Mutoh & Kitajima, 2007, Zuin *et al.*, 2010, Ohtsuka *et al.*, 2011, Ohtsuka *et al.*, 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.*, 2010, Zuin *et al.*, 2010).

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 homologue 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 *pka1•* mutant showed increased stress resistance but the *sck2•* mutant did not (Roux *et al.*, 2006). Another AKT homolog *sck1*$^{+}$ appeared to play a minor role in CLS since *•sck1* only marginally extended CLS, however, the *sck1•sck2•* 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.*, 2010). A recent study confirmed that deletion of the Tor1 kinase indeed extended CLS (Ohtsuka *et al.*, 2013). Deletion of the Git3 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 *lcf1*$^{+}$ 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 $G_0$ by nitrogen starvation. Inactivation of the proteasome activity in $G_0$ results in accumulation of ROS, autophagy-mediated destruction of mitochondria and rapid loss of viability. This study suggests that proteasome function is essential for $G_0$ survival and that the degradation of mitochondria (the primary source of ROS) by autophagy during proteasome dysfunction is a defense mechanism of $G_0$ 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, Roux *et al.*, 2010, 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.*, 2010, Zuin *et al.*, 2010) 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 underling these two extreme yet very robust forms of CR remain unclear. Studies in glucose reduction-mediated CR have linked CR to the glucose sensing Git3 pathway (Roux *et al.*, 2009). Interestingly, CR appeared to activate respiration, ROS production and the Sty1 MAP kinase (Zuin *et al.*, 2010, Zuin *et al.*, 2010). 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.*, 2010). 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, Ohtsuka *et al.*, 2009, Ohtsuka *et al.*, 2011, Ohtsuka *et al.*, 2012, Ohtsuka *et al.*, 2013). The precise function of these genes in life span regulation remains unclear. A few of these genes have been further characterized. Overexpression of *ecl1*$^+$ (extender of chronological life span) appeared to function downstream or in parallel to the Sty1 MAP kinase pathway since *ecl1*$^+$ overexpression rescued the short CLS of the *sty1•* mutant. Ecl1 overexpression did not further extend the CLS induced by *pka1•* 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 *tor1•* mutant (such as Caffeine sensitivity and Canavanine resistance), suggesting 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 Gα subunit (Gpa2) of GPCR (Roux *et al.*, 2010). 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.*, 2010). It was suggested that these genes functioned in Pka1/Sck2/Tor pathways (Roux *et al.*, 2010). 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 anti-aging pathways and chemicals (Stephan *et al.*, 2013). Using a modified SD based CLS protocol (Roux *et al.*, 2010), 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 re-grow 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 phosphoinositite 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 $J_2$), inhibit Git3/PKA signaling (Prostaglandin $J_2$) and decrease intracellular GMP synthesis (mycophenolic acid and acivicin).

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). *S. pombe* cells treated with monensin or nigericin 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 these chemicals likely to extend CLS by maintaining vacuolar pH homeostasis (Stephan *et al.*, 2013). This result is in line with a recent study in *S. cerevisae* that Vma1 overexpression can extend the RLS 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 that 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), 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 fission (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. cerevisie* (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 anti-aging compound prostaglandin $J_2$ 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 $J_2$ 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 insertional 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. *C. 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°C–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 *goa1*• 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 *goa1*• mutant (Li *et al.*, 2011). 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.*, 2011, 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.*, 2011). CR appeared to induce the expression of *AOX2* (an alternative oxidase) and a number of stress response genes in the *goa3*• 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 *goa3*• 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.*, 2011, Ocampo *et al.*, 2012) and *S. pombe* (Roux *et al.*, 2009, Zuin *et al.*, 2010). 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-Hebroni & Bessman, 1997). Given the differences in carbon source utilization preferences and multiple choices of respiration 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, Madeo *et al.*, 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 $H_2O_2$ 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.*, 1997, Ludovico *et al.*, 2002, Pereira *et al.*, 2010)

Mechanistically, it is clear that caspase-dependent intrinsic apoptosis exists in *S. cerevisiae* (Liang *et al.*, 2008, Mazzoni & Falcone, 2008). This yeast has one ortholog of the mammalian capsases, 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.*, 2000, 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 pro-apoptotic protease, Kex1p, that is essential for hypochlorite-induced apoptosis (Carmona-Gutierrez *et al.*, 2013).

Caspase-independent intrinsic apoptosis can also occur in this organism (Liang *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), *BXI1* (Buttner *et al.*, 2011, Cebulski *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/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 *atg8*• 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 $H_2O_2$ 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.*, 2011). 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 (Pss) 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 metazoa. For instance, the yeast homolog of the lysosomal endoprotease cathepsin D, called Pep4p in *S. cerevisiae*, has both anti-apoptotic and anti-necrotic functions where prolonged overexpression of the protein extended the chronological lifespan of yeast, specifically by inhibiting necrosis (Carmona-Gutierrez *et al.*, 2011). 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 pro-apoptotic 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, and 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 pro-apoptotic 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, Guerin *et al.*, 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 since deletion of the gene did not protect cells from $H_2O_2$, 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 anti-apoptotic 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*.

Calnexin is an ER transmembrane chaperone that is involved in protein translocation, protein folding, and the quality control of newly synthesized polypeptides (Rutkevich & 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 anti-apoptotic 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, Guerin *et al.*, 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, Guerin *et al.*, 2009).

Finally, Rad9 is a component of the Rad9-Hus1-Rad1 complex that functions as a sensor of DNA damage (Parrilla-Castellar *et al.*, 2004). It belongs to the pro-survival and pro-apoptotic 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 anti-apoptotic protein, BCL-2 (Komatsu *et al.*, 2000, Komatsu *et al.*, 2000). Nonetheless, in fission yeast, transcriptional up-regulation 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, phosphatdylserine 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, $H_2O_2$, 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, i.e., 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. Austriaco, 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 $H_2O_2$ while 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 defensing RsAFP2 and the echinocandin, caspofungin, in *Candida* does not require this metacaspase (Aerts *et al.*, 2009). Moreover, Lu et al. have discovered that $H_2O_2$-induced apoptosis in *Candida* is mediated by a rise in intracellular $Ca^{2+}$ 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 $H_2O_2$-induced apoptosis partially by downregulating the calcineurin-caspase pathway (Dai *et al.*, 2012). This pathway is reminiscent of the calcineurincaspase 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 while 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 $H_2O_2$-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 gluthathione content in *C. albicans* (Dai *et al.*, 2009, Dai *et al.*, 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, pro-growth 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 from programmed cell death more generally. 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, Guarente, 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. cervisiae*, *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.

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