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Replicative Aging in Yeast: The Means to the End

K.A. Steinkraus,[1] M. Kaeberlein,[1,*] and B.K. Kennedy[2,*]

[1]Department of Pathology and [2]Department of Biochemistry, University of Washington, Seattle, Washington 98195; email: kaeber@u.washington.edu, bkenn@u.washington.edu

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*Corresponding authors.

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longevity, dietary restriction, SIR2, TOR, life span

Abstract

Progress in aging research is now rapid, and surprisingly, studies in a single-celled eukaryote are a driving force. The genetic modulators of replicative life span in yeast are being identified, the molecular events that accompany aging are being discovered, and the extent to which longevity pathways are conserved between yeast and multicellular eukaryotes is being tested. In this review, we provide a brief retrospective view on the development of yeast as a model for aging and then turn to recent discoveries that have pushed aging research into novel directions and also linked aging in yeast to well-developed hypotheses in mammals. Although the question of what causes aging still cannot be answered definitively, that day may be rapidly approaching.

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INTRODUCTION

Aging is paradoxical. On the one hand, it is universal in mammals, and therefore, unlike specific diseases, it seems to be a natural process. On the other hand, the deterioration in health and ultimate mortality associated with aging have caused humans for millennia to look for ways of impeding its seemingly inevitable progress.

Molecular and genetic studies of aging have gained pace over the last century to the extent that aging research is a thriving field of endeavor. Studies of aging in mammals are limited by the long life span of common model organisms. Mice and rats live 3–5 years and primates up to 40. Nevertheless, aging studies, particularly in rodents, have been highly informative, framing much of our understanding of the genetic factors and environmental conditions modulating longevity. One solution to speed up studies of aging has been to study primary human or mouse cells in culture. These cells (usually fibroblasts are chosen for study) have a limited proliferative capacity before they undergo terminal cell-cycle arrest (Hayflick 1965). Senescence in culture has been immensely informative, providing insights into cell-cycle control, differentiation, and cancer. However, its relevance to organismal aging remains a highly debated topic (Campisi & d'Adda di Fagagna 2007).

Insulin/IGF-1 signaling pathway: a hormonal signaling pathway conserved in higher eukaryotes

A second approach that has dramatically accelerated aging research is the use of invertebrate organisms, which age more rapidly and are readily amenable to genetic and environmental manipulation. Although a variety of organisms have been studied, a majority of studies have utilized fruit flies (*Drosophila melanogaster*) (Helfand & Rogina 2003), worms (*Caenorhabditis elegans*) (Houthoofd & Vanfleteren 2007, Olsen et al. 2006), or yeast (*Saccharomyces cerevisiae*) (Kaeberlein et al. 2007, Piper 2006). Worms live approximately 2–3 weeks and flies 2–3 months. Among the genetic interventions that result in life span extension in both organisms are mutations in the insulin/IGF-1 signaling pathway (Gami & Wolkow 2006). Because some mutations reducing insulin/IGF-1 signaling in mice also result in enhanced longevity (Bartke 2005), at least some lessons learned in worms and flies are likely to translate to mammal and, perhaps, humans.

As a single-celled eukaryote, *S. cerevisiae* seems an unlikely candidate for aging studies. Yet at least two aging assays have been developed and more than 100 studies have been published for this organism. In this review, we discuss genetic studies aimed at defining the pathways modulating yeast aging, and biochemical studies attempting to identify the root causes of one of the models: replicative aging. Readers are directed to other papers for a discussion of chronological aging in yeast (Fabrizio & Longo 2003, Laun et al. 2006). Furthermore, we address the likelihood that the aging process is conserved between yeast and multicellular eukaryotes.

REPLICATIVE AGING IN YEAST

S. cerevisiae divide by budding and therefore undergo asymmetrical cell division, with the mother cell retaining more volume than the

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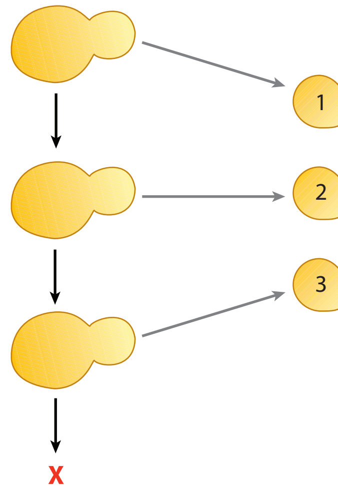
daughter (Hartwell & Unger 1977). Taking advantage of this asymmetry, Robert Mortimer & John Johnston (1959) were the first scientists to perform a yeast life span experiment. A pioneer in the field of yeast genetics and recently deceased, Mortimer was best known for developing techniques to monitor yeast meiosis and using them to generate invaluable genetic maps (**http://mcb.berkeley.edu/news-and-events/research-news/robert-mortimer/**). In this study, a small needle was used to microdissect daughter cells away from mothers, and the number of divisions a mother could undergo [later defined as replicative life span (RLS)] (**Figure 1**) was tabulated (Mortimer & Johnston 1959). However, this pioneering study made no attempt to link the limited proliferative potential of individual yeast cells to aging in multicellular organisms.

The result of each division of a mother cell can also be detected by the appearance of a circular bud scar composed of chitin on the cell surface (Bacon et al. 1966, Barton 1950, Seichertova et al. 1973). Because bud scars do not normally overlap, the Mortimer study addressed the hypothesis that available sites for the formation of new bud scars on the cell surface would become limiting as yeast mother cells continue to divide, a mechanism likely restricted to yeast. This model was discounted, however, by the observation that cell surface area expands dramatically as mother cells age, more than compensating for the loss of available surface area (Mortimer & Johnston 1959). Instead, Mortimer & Johnston speculated that reduced surface-to-volume ratios in old mother cells may limit metabolic processes. Although the bud scar and surface-to-volume hypotheses are now largely disfavored (Egilmez & Jazwinski 1989, Kennedy et al. 1994), this original study set the stage for exploration into yeast replicative aging.

After the initial foray by Mortimer & Johnston (1959), two decades passed before a second group restored interest in yeast aging. This latter group (Müller et al. 1980) addressed an important question that remained unresolved: Is the life span of a yeast cell limited by the number of mitotic cycles or calendar life span? A series of experiments led to the conclusion that the number of replicative cycles, and not the length of time since budding, was the primary determinant of life span (Müller et al. 1980). This is also supported by the observation by many groups that cells do not lyse immediately after reaching permanent cell-cycle arrest (Mortimer & Johnston 1959). Although lysis may be the final result, many cells can often remain metabolically active for days, similar to what is reported for senescent fibroblasts in cell culture. The chronological time a cell can remain viable in a postreplicative state [chronological life span (CLS)] has been established and extensively studied as a second model of aging in yeast (Fabrizio & Longo 2003, Longo et al. 1996). Which of these two assays

Replicative life span =
number of mitotic divisions



Chronological life span =
days viable in
postreplicative state

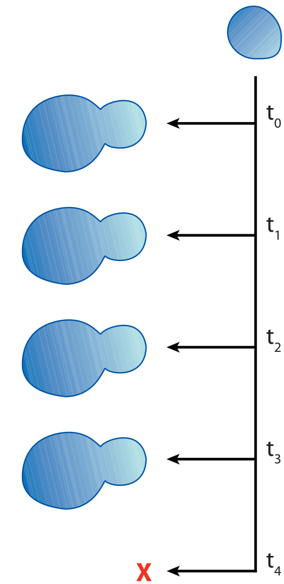


Figure 1
Replicative and chronological life span assays in yeast. (*Left*) In a replicative life span (RLS) assay, one mother cell is allowed to divide, and the smaller daughter cells are removed by microdissection. The number of daughter produced is tallied as the RLS. (*Right*) In a chronological life span (CLS) assay, cells are maintained in an undividing state. At given time (*t*) intervals, a subset of the population is placed onto media to allow cell division to resume. The life span is determined as the time point at which those cells are unable to reenter the cell cycle.

Replicative life span (RLS): the cumulative number of mitotic divisions a cell can undergo

Chronological life span (CLS): the amount of time a cell can remain viable while in a nondividing state

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better models aging in other eukaryotes remains unanswered. In fact, there may be an interesting interplay between the replicative and the chronological aging of yeast; passage of a cell through a postreplicative stage reduces its RLS once it reenters the cell cycle (Ashrafi et al. 1999). Therefore, postmitotic aging of a cell can delimit its RLS.

ASYMMETRY AND YEAST AGING

To survive in the wild, *S. cerevisiae* has adapted to undergo rapid mitotic expansion in nutrient-rich conditions. It must accomplish this feat while simultaneously maintaining the vitality of the population, and it accomplishes this in part through asymmetric division (**Figure 2**). Whereas the larger mother cell ages and ultimately loses replicative capacity, daughters produced by the aging mother generally are renewed in their capacity; this ensures that the colony survives even though aging mothers within the colony might perish. Asymmetric division may cause mother cells to retain an aging factor, thus sacrificing individual replicative potential while retaining full potential in resulting daughter cells (Egilmez & Jazwinski 1989, Kennedy et al. 1994).

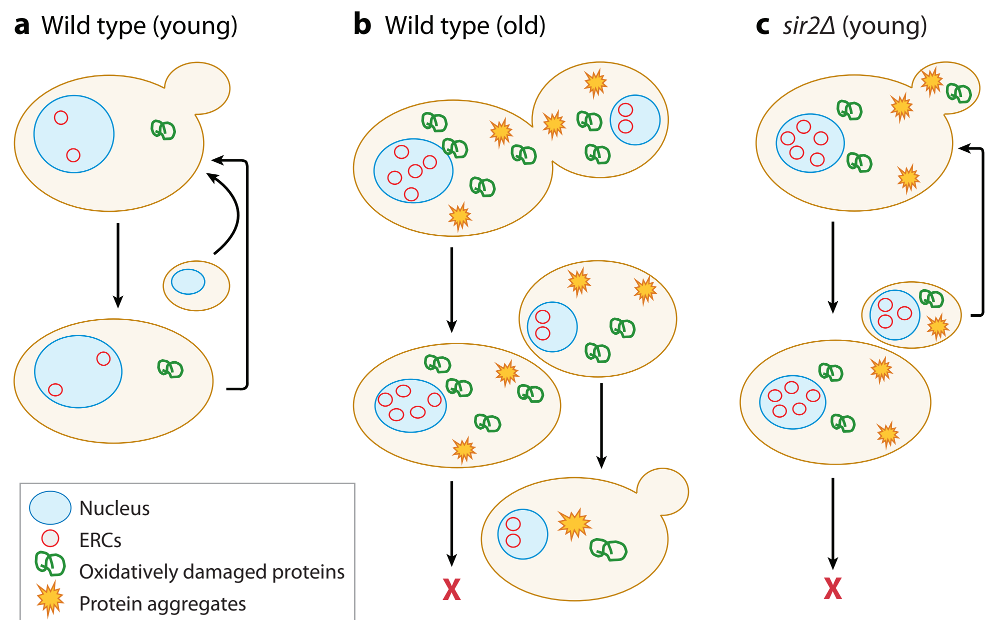

Figure 2

The asymmetry of division is lost in old mother cells and *sir2*Δ cells. The three aging factors diagrammed here are extrachromosomal ribosomal DNA circles (ERCs), oxidatively damaged proteins, and protein aggregates. (*a*) In young wild-type cells, levels of all three factors are low, and the factors are preferentially sequestered in the mother cell. Note the size differential between the mother cell and the budding daughter cell. Both mother and daughter will go on to further budding. (*b*) In old wild-type cells, the three factors accumulate to high levels. The budding daughter cell is similar in size to the mother cell and inherits all three of the aging factors. The old mother cell can no longer divide at the end of its replicative life span (RLS). The daughter cell, although the product of asymmetrical division, will proceed to normal asymmetric division. (*c*) *sir2*Δ cells accumulate abnormally high levels of ERCs and protein aggregates. Division is partially symmetric in that, although the daughter cell is much smaller than the mother, these aging factors are not preferentially sequestered away from the daughter. The precocious accumulation of aging factors limits the RLS of the mother, although the daughter can go on to divide.

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A molecular confirmation of this hypothesis was provided by the identification of extrachromosomal ribosomal DNA circles (ERCs) as one cause of aging in yeast (Sinclair & Guarente 1997). The yeast ribosomal DNA (rDNA) consists of approximately 100 to 150 tandemly arrayed copies of a 9.1-kb repeat containing all the information necessary to code for the rRNA (Petes & Botstein 1977, Philippsen et al. 1978, Rustchenko & Sherman 1994). ERCs are formed by homologous recombination between rDNA repeats; are self-replicating, owing to the presence of an origin of replication within the rDNA repeat; and, because they lack a CEN element, display biased segregation to mother cells (Murray & Szostak 1983). This combination of asymmetric inheritance and self-replication leads to a mother cell–specific increase in ERC copy number with age. It is believed that, once a threshold level of ERCs accumulates in the mother cell, senescence occurs, perhaps owing to titration of essential cellular factors by the abundant rDNA sequence present as ERCs.

Interestingly, asymmetry breaks down in the oldest mothers, resulting in an increased frequency of symmetric divisions (Jazwinski et al. 1989, Johnston 1966, Kennedy et al. 1994). In these aberrant mitotic events, the mother cell and daughter cell are indistinguishable in size, and the daughter has a reduced life span that is comparable to the remaining life span of the mother cell. Progeny from symmetrical daughters recover full life span potential following asymmetric division (Kennedy et al. 1994), however, indicating that the damage that accumulates to limit mother cell life span is not permanent. For instance, if nuclear DNA damage to the mother cell accumulated during old age, it would be inherited in the daughter cells and would not be diluted out in the daughter lineage, and full life span would not be restored.

More recently, a screen has been performed to identify yeast mutants that fail to maintain age asymmetry with respect to divisions (Lai et al. 2002). These conditional mutants clonally senesce at the restrictive temperature. According to one proposal, normally functional mitochondria are specifically segregated to daughter cells, and this process is disrupted in symmetry mutants (Jazwinski 2005, Lai et al. 2002). More recently, Seo et al. (2007) reported that the abnormal segregation of mitochondria in one of these symmetry mutants (a point mutation in *ATP2*) could be suppressed by the overexpression of a peroxin protein (Pex6). They propose that Pex6, at least when overexpressed, can contribute to mitochondrial biogenesis in addition to its known peroxisomal roles. A second fruitful source of information regarding asymmetry may come from mathematical modeling, and studies have attempted to model yeast aging parameters in this fashion (Gillespie et al. 2004, Hirsch 1993, Jazwinski & Wawryn 2001).

At the molecular level, exciting data from Nyström and colleagues have shown that, after cytokinesis, levels of protein carbonyls and other forms of oxidatively damaged proteins are much higher in the mother cell than in the daughter (Aguilaniu et al. 2003, Erjavec & Nystrom 2007). Protein aggregates containing heavily carbonylated proteins are also retained in mother cells (Erjavec et al. 2007). Given that the accumulation of damage caused by reactive oxygen species (ROS) may be one cause of aging (discussed below), these findings suggest that minimizing the segregation of preexisting damage to the newly formed daughter cell is one method of ensuring a full life span (Nyström 2005).

The mechanism(s) by which asymmetry is achieved with respect to oxidized and aggregated proteins is beginning to be delineated. For instance, asymmetry must require a functional actin cytoskeleton because transient disruption of the cytoskeleton with Latrunculin-A disrupts the asymmetry of oxidized proteins and aggregates (Aguilaniu et al. 2003, Erjavec et al. 2007). The actin cytoskeleton has also been linked directly to life span in yeast; deletion of the actin bundling protein Scp1 results in increased actin dynamics, reduced ROS, and extended life span (Gourlay et al. 2004). However, protection of daughter cells during division seems to be accomplished not only by restricting access of damaged proteins

Extrachromosomal ribosomal DNA circles (ERCs): formed by recombination between rDNA repeats and preferentially sequestered in mother cells

Ribosomal DNA (rDNA): consists of 100 to 150 tandemly arrayed copies of the sequence coding for rRNA

Reactive oxygen species (ROS): highly reactive molecules due to the presence of unpaired valence shell electrons

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to daughters but also by increasing the ability of daughter cells to combat damage. This latter process occurs in part through a daughter-specific enhancement of catalase activity (Erjavec & Nystrom 2007). During aberrant symmetric divisions initiated by old mothers, one or more of these processes presumably break down, allowing both leakage of ERCs and higher levels of oxidized or aggregated proteins to migrate to daughters. Finally, the Sir2 histone deacetylase, a conserved longevity-promoting factor in yeast, worms, and flies, is also required for mother-daughter asymmetry with respect to oxidized aggregated proteins (Aguilaniu et al. 2003, Erjavec & Nystrom 2007, Erjavec et al. 2007). We discuss the role of Sir2 in this process in more detail in the subsection on sirtuins (below).

Other proteins (e.g., cell wall components) are segregated asymmetrically between mothers and daughters. However, old components are not always left in mother cells because the old spindle pole body always segregates to the daughter (Pereira et al. 2001). Studying the molecular asymmetry associated with mitotic division in yeast and how it relates to aging will no doubt continue to provide further novel insights into the aging process.

GENETIC MODULATION OF YEAST REPLICATIVE LIFE SPAN

Fundamentally, studies of the basic mechanisms of aging can be approached from two directions, and both have been exploited in yeast. Either one can study the genetic (or environmental) interventions that alter the rate of aging, or one can determine the phenotypic and molecular changes that are associated with aging (e.g., yeast cell volume increases with age). In this and the next section, we address both approaches in yeast replicative aging, beginning here with genetic approaches.

As with other model organisms, genetic changes can dramatically influence the longevity of yeast. This was first demonstrated by the finding that expression of an oncogenic retroviral derivative of human RAS, v-Ha-RAS, in yeast cells resulted in extension of RLS (Chen et al. 1990). Because expression of this oncogene was known to contribute to immortalization of mammalian fibroblasts, it was a reasonable starting point in yeast. Senescence in fibroblasts has long been studied in an attempt to gain an understanding of the mammalian aging process and has yielded extensive information about the control of cell proliferation and its links to cancer. The relationship between fibroblast senescence and organismal aging is still debated (Campisi & d'Adda di Fagagna 2007). Because yeast is a single-celled organism, it was also argued whether the RLS assay would be a better model for organismal aging or cellular senescence. Although this debate is not completely resolved, the genetic factors controlling yeast aging seem to align better with those controlling organismal aging in multicellular eukaryotes than with those controlling senescence (see below). However, many of the known regulators of mammalian cell senescence are not conserved in yeast, making a direct comparison difficult.

Life span extension by expression of v-Ha-RAS in yeast served as the motivation for testing the possibility that the two yeast RAS orthologs might function to modulate replicative aging. Paradoxically, deletion of *RAS1* resulted in life span extension, whereas deletion of *RAS2* shortened life span (Sun et al. 1994). The reasons for these divergent effects are not yet understood. Since these early studies, numerous yeast aging genes have been identified and are discussed in relevant places throughout this review. In this section, we highlight efforts to perform unbiased screens to identify yeast aging genes and the extent to which they have led to a better understanding of yeast replicative aging.

Although there are many benefits to studying aging in yeast, there are also limitations, in particular, the difficulty associated with procuring large populations of truly old cells. In a rapidly growing culture of yeast, ages of individual cells will distribute geometrically, with half of the population virgins, 1/4 one-division-old mothers, 1/8 two-division-old mothers, etc.

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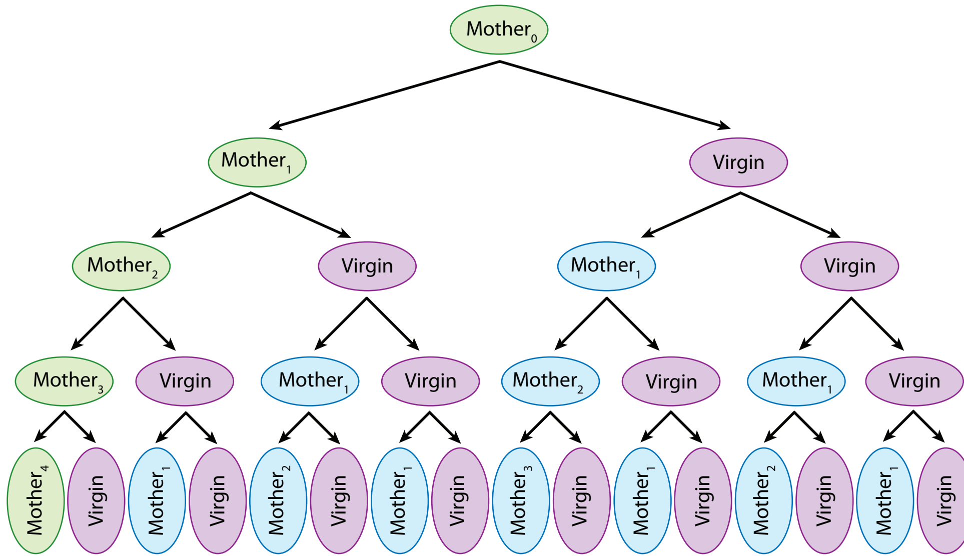

Figure 3

Old mother cells represent a fraction of an experimental population. The schematic shows the distribution of ages of cells in a dividing population. The number of divisions each mother cell has undergone is indicated in the subtext. Virgin daughters (*purple*) represent half of the population at each division. Among the remaining population are the younger mothers (*blue*) and the original mother (*green*).

(**Figure 3**). Therefore, the fraction of truly old cells (>20 generations) is negligible. This fact raises questions about whether the life span of individual yeast cells is relevant from the perspective of natural selection (discussed below), and also makes obtaining enough old cells for biochemical studies difficult. Several schemes have been employed to enrich for old cells, and populations of reasonably old cells can be obtained (Chen & Contreras 2007, Egilmez et al. 1989, Park et al. 2002, Smeal et al. 1996). Nevertheless, and in spite of attempts to automate life span analysis (Jarolim et al. 2004), the only truly accurate method for measuring RLS to date is micromanipulation of daughter cells away from mother cells while counting the number of daughters produced by each individual mother cell. The brute-force nature of this assay is not conducive to large-scale screens.

The first attempt around this problem by Jazwinski and colleagues involved purification of old (and young) cells, followed by RNA collection and differential hybridization to identify transcripts up- or downregulated with age (Egilmez et al. 1989). This led to the identification of a gene whose expression is dramatically downregulated with age and that later was named *LAG1* (D'Mello et al. 1994). *LAG1* encodes a ceramide synthase (Dickson et al. 2006, Guillas et al. 2001, Schorling et al. 2001, Spassieva et al. 2006). This is quite intriguing, given proposed roles for ceramides in stress response pathways, apoptosis, and cell senescence (Obeid & Hannun 2003). The relationship of *LAG1* to yeast life span is complex, as either deletion or mild overexpression results in life span extension (D'Mello et al. 1994). In contrast, deletion of another ceramide synthase gene (*LAC1*) does not alter RLS (Guillas et al. 2001, Jiang et al. 1998, Schorling et al. 2001).

In another attempt to study yeast aging, the Guarante lab's primary goal was to perform unbiased screens to identify aging genes. An initial screen was made possible by the finding that stress resistance and the maintenance of viability at 4°C correlated with longevity. Although

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this correlation later proved to be strain specific, it was grounds for screening stress-resistant mutants for enhanced longevity (Kennedy et al. 1995). The first mutant characterized was a complex mutation in the gene encoding Sir4, which, along with Sir2 and Sir3, forms a complex that mediates transcriptional repression at telomeres and silent mating-type loci *HML* and *HMR* (Ivy et al. 1986, Rine & Herskowitz 1987). *SIR2* encodes a histone/protein deacetylase, and this enzymatic activity is required for its silencing functions (Imai et al. 2000, Landry et al. 2000, Smith et al. 2000).

The long-lived *SIR4-42* allele was loss of function for telomere and *HM* silencing; however, it behaved as a dominant allele for longevity (Kennedy et al. 1995). This finding, although dampening interest in direct links between telomeres and yeast aging, proved highly valuable in the establishment of a model whereby the SIR4-42 mutation directed the SIR complex away from telomeres and *HM* loci to an undefined *AGE* locus (Kennedy et al. 1995), which was later proposed to be the rDNA (Kennedy et al. 1997). Consistently, mutations that reduce recruitment sites for the SIR complex at telomeres lead to life span extension (Austriaco & Guarente 1997). For a more detailed discussion of SIR2 and yeast aging, see the subsection on sirtuins, below.

Two other genes were linked to yeast aging in this initial screen. Recessive mutations in (or deletion of) *UTH1* were found to cause a modest increase in life span (Austriaco 1996, Kennedy et al. 1995). *UTH1* expression is induced by oxidative stress, and *uth1*Δ strains have altered sensitivities to different sources of oxidative stress (Bandara et al. 1998, Camougrand et al. 2004). Reported to be an outer mitochondrial membrane protein (Velours et al. 2002), *UTH1* is also required for induction of apoptosis in yeast by expression of mammalian Bax and for autophagy-mediated degradation of mitochondria (Camougrand et al. 2003, Kissova et al. 2004). Which, if any, of these activities are causally involved in the longevity-modulating role of Uth1 remains to be determined. The link to autophagy is particularly intriguing, however, given that reduced Tor signaling, a process known to induce autophagy (Martin & Hall 2005), leads to RLS extension (Kaeberlein et al. 2005c). In addition, autophagy has been implicated in life span–extending mutations in *C. elegans* (Melendez et al. 2003).

The final gene identified in this screen was *UTH4* (Kennedy et al. 1997), also referred to as *MPT5*. Identification of *MPT5* was fortuitous because the parental strain for this screen contained a C-terminal truncation allele, and the life span–extending *UTH4* mutation was a reversion that restored the wild-type open reading frame (ORF) and allowed production of the full-length protein. Overexpression of *MPT5* increases life span in two other strain backgrounds, and deletion of *MPT5* shortens life span (Kaeberlein & Guarente 2002, Kaeberlein et al. 2005b, Kennedy et al. 1997). It remains unclear how increased Mpt5 activity affects yeast aging. Mpt5 function leads to altered SIR-dependent silencing (Kennedy et al. 1997) but also regulates the integrity of the cell wall (Kaeberlein & Guarente 2002, Stewart et al. 2007) and a MAP kinase pathway linked to pseudohyphal growth (Prinz et al. 2007). Mpt5 also interacts genetically with Ssd1, a protein that promotes longevity via a Sir2-independent pathway (Kaeberlein & Guarente 2002, Kaeberlein et al. 2004). Structurally, Uth4 is one member of a family of yeast proteins that resemble *Drosophila* pumilio, an RNA binding protein that regulates the translation of specific messages (Wharton & Aggarwal 2006). Mpt5 controls the translation and/or degradation of a few mRNAs (Goldstrohm et al. 2006, 2007; Hook et al. 2007; Prinz et al. 2007; Stewart et al. 2007). Whether these transcripts (or as-yet-unidentified ones) are linked to longevity regulation by Mpt5 remains to be determined.

To date, only one unbiased screen for mutations that increase RLS has been reported; in this screen, longevity was used as the primary phenotype (Kaeberlein et al. 2005c). This work involved screening 564 single-gene deletion strains as part of an ongoing effort to

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quantify replicative aging properties across the haploid yeast ORF deletion collection (Kaeberlein et al. 2005c), a set of ∼4800 isogenic single-gene deletion strains (Brachmann et al. 1998, Winzeler et al. 1999). Generally, to get a reliable estimate of the mean RLS of a given strain, it is desirable to measure the life span of at least 40–50 mother cells in repeated assays. Given that the average life span of the deletion set parental strain (BY4742) is ∼26 generations, this would require microdissection of approximately 19.5 million daughter cells. Thus, it was necessary to take an alternative approach. Accordingly, Kaeberlein et al. (2005c) developed an iterative strategy that provides semiquantitative life span data for each deletion strain and allows for classification of each strain as potentially (or unlikely to be) long-lived. Potentially long-lived deletions are then subjected to rigorous validation in strains from both haploid mating types.

From the initial analysis of 564 gene deletions, 13 (2.3%) were verified to be long-lived (Kaeberlein et al. 2005c). Among the yeast aging genes identified was *TOR1*, providing the first link in yeast between Tor signaling and aging (see below subsection, Translational Regulation and Dietary Restriction). Also of note, approximately 20% of deletions were statistically short-lived. Although a very small number of cells were analyzed for most short-lived deletions, this high approximate frequency points to the challenge of mechanistically interpreting shortened life span. It is currently not possible to determine whether the normal aging process is accelerated in short-lived cells or whether cells are dying owing to defects unrelated to normal aging. We have now generated preliminary data on all nonessential yeast deletion strains, and the estimate of 2.3% for the percentage of long-lived deletions appears accurate, leading us to predict that, upon completion of verification, approximately 100–120 yeast aging genes will be identified. These numbers are in keeping with estimates from RNAi-based screens in *C. elegans* (Hamilton et al. 2005, Hansen et al. 2005, Lee et al. 2003).

MOLECULAR PHENOTYPES ASSOCIATED WITH REPLICATIVE AGING

Through micromanipulation, Mortimer & Johnston (1959) were the first to determine that aging mother cells take increasingly longer times to progress through the cell cycle and produce daughters. Accumulation of the proposed aging factor and/or cellular damage likely makes it progressively harder for aging mothers to continue mitotic division. Two such factors have been identified: ERCs (Sinclair & Guarente 1997) and oxidatively damaged proteins (Aguilaniu et al. 2003). Interestingly, both are regulated by the activity of the protein deacetylase Sir2 (discussed below) (Aguilaniu et al. 2003, Kaeberlein et al. 1999).

Several lines of evidence indicate that ERCs can cause the senescence of mother cells, at least under some conditions. The most elegant of these derives from a study by Sinclair & Guarente (1997), in which a plasmid containing an ERC- and *LoxP*-flanked ARS-CEN element was introduced into yeast cells. After Cre-mediated excision of the ARS-CEN, the plasmid accumulated in mother cells and dramatically shortened RLS. Interestingly, this toxicity does not seem to be related to the rDNA sequence present in the ERC because the presence of an asymmetrically inherited, self-replicating plasmid without an rDNA sequence is sufficient to cause a similar premature senescence phenotype (Sinclair & Guarente 1997). The rDNA locus may be the source of the ill-fated episome simply because of the increased likelihood of ERC formation by homologous recombination, owing to the highly repetitive nature of the rDNA locus. There remains no compelling evidence that ERCs or episomes of any kind are a contributing factor to aging in other eukaryotic organisms.

Unlike mammalian cells, which often have multiple nucleoli dispersed throughout the nucleus, yeast cells contain only one nucleolus, which is separated from the rest of the nucleoplasm. Dispersion of this nucleolus into

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USING YEAST TO STUDY AGE-RELATED DISEASES

S. cerevisiae is increasingly used to model age-related diseases. Two examples of diseases currently being modeled in yeast are cancer and neurodegenerative disorders.

Yeast and Cancer

One trait commonly seen during carcinogenesis is genomic instability. By studying inheritance patterns of genes from aging mother cells to daughter cells, investigators have demonstrated that yeast display age-associated genomic instability (MacMurray & Gottschling 2003). Work is currently under way to understand the mechanism of this instability.

Yeast and Neurodegeneration

Yeast models of two common neurodegenerative diseases, Huntington's and Parkinson's diseases, have been made. Huntington's disease is a polyglutamine disorder caused by an expansion of the polyglutamine tract in the huntingtin protein. Parkinson's disease can be caused by mutations in α-synuclein. In both cases, the mutant protein is associated with cellular toxicity. Both proteins aggregate into large inclusions in affected cells; however, whether these aggregates cause the cellular toxicity remains unclear. Work from the Lindquist lab has generated yeast strains expressing either mutant huntingtin or α-synuclein. These strains are being used to characterize the genetic factors leading to the aggregation and/or toxicity of the mutated proteins (Duennwald et al. 2006a,b; Ehrnhoefer et al. 2006; Willingham et al. 2003).

multiple fragments has been observed in aging mother cells (Sinclair et al. 1997). This is likely linked to the accumulation of ERCs; high-copy plasmids containing the rDNA locus cause similar fragmentation (Nierras et al. 1997). Fragmentation is unlikely to be required for yeast aging because ARS elements without rDNA repeats can also shorten life span (Sinclair & Guarente 1997). It is unlikely that these plasmids also cause nucleolar fragmentation, though this has not been formally tested.

Superoxide dismutases (SODs): protect against oxidative damage by catalyzing the dismutation of superoxide into hydrogen peroxide

Recessive mutations in the *WRN* locus, encoding a DNA helicase, cause Werner syndrome in humans (Yu et al. 1996). In this progeroid syndrome, affected individuals prematurely display many but not all of the phenotypes associated with normal aging (Epstein et al. 1966). The molecular basis of this disease remains unresolved, but increased DNA damage or defective repair in the absence of WRN remains a prominent candidate mechanism (Bohr 2005, Kudlow et al. 2007, Ozgenc & Loeb 2005). The yeast DNA helicase most similar to WRN is *SGS1* (Watt et al. 1996). Interestingly, *sgs1*Δ strains are short-lived owing to elevated rDNA recombination leading to ERC formation and elevated, age-independent arrest in mitosis that likely results from defective resolution of recombination intermediates (McVey et al. 2001, Sinclair & Guarente 1997).

ERCs are not the only factor that accumulates in aging mother cells; oxidative damage appears to do the same (Aguilaniu et al. 2003, Laun et al. 2001, Reverter-Branchat et al. 2004), as do protein aggregates (Erjavec et al. 2007). This observation is of particular interest, given the long-standing free-radical theory of aging, which posits that aging is caused by the accumulation of macromolecular damage caused by free radicals (Harman 1956). Whether ROS are a primary determining factor in aging in yeast or any other organism, however, remains to be conclusively established (Muller et al. 2007).

One indication that oxygen radicals may have an important role in yeast replicative aging comes from the observation that deletion of superoxide dismutases (SODs) can shorten life span (Kaeberlein et al. 2005b, Unlu & Koc 2007, Wawryn et al. 1999). Increased exposure to oxygen can also shorten life span (Nestelbacher et al. 2000). However, as discussed above, whether a short life span equates to accelerated aging is difficult to determine (Kaeberlein et al. 2005c).

Overexpressing SODs, in contrast, can confer a small extension of RLS. This extension is mild at best and sometimes results in a shorter life span, depending on the carbon source used for the aging assay (Harris et al. 2003, 2005). These overexpression assays were

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performed on media containing glycerol as a carbon source. Most life span assays have been performed on media containing 2% glucose. Yeast, being facultative anaerobes, generate energy primarily from fermentation when glucose is available and switch to a primarily respiratory growth state either when glucose is depleted or when they are grown on a nonfermentable carbon source such as glycerol (Gancedo & Serrano 1989). Kirchman & Botta (2007) have argued that yeast aging assays testing the importance of mitochondrial function should be performed on a glycerol medium because this pushes metabolic pathways generating energy into a mode more similar to that of mammalian cells. Under these conditions, copper supplementation is associated with life span extension, possibly through the upregulation of oxygen-radical defense mechanisms.

Mitochondria are one major source of ROS. Inefficient respiration and/or defective mitochondria may be major contributors to the enhanced generation of ROS found in aging organisms. However, the role of mitochondrial function in yeast replicative aging is enigmatic. For instance, yeast lacking mitochondrial DNA have varying RLSs depending on the strain background (Kaeberlein et al. 2005b, Kirchman et al. 1999). Whether life span extension by dietary restriction (DR) also requires enhanced respiration is also highly debated (discussed below) (Easlon et al. 2007, Kaeberlein et al. 2005a, Lin & Guarente 2006, Lin et al. 2002). Two groups have tested the effects of mitochondrial uncouplers on yeast replicative aging (**Figure 4**). Partial uncoupling by CCCP (carbonyl cyanide 3-chlorophenylhydrazone), a widely used uncoupler of oxidative phosphorylation, leads to enhanced ROS and shortened life span (Stockl et al. 2007), whereas dinitrophenol-mediated uncoupling results in reduced ROS and increased life span (Barros et al. 2004).

The end of the road for replicatively aging mother cells may be apoptosis, as initially suggested by findings that old mother cells are often TUNEL and annexin V positive (Laun et al. 2001). Apoptosis seems paradoxical for a single-celled organism but is induced under a variety of conditions (Buttner et al. 2006), including both chronological and replicative aging (Fabrizio et al. 2004a, Herker et al. 2004). Under these conditions, it is possible that a subset of cells dies for the benefit of the others, although this has not been formally demonstrated. Replicatively aged cells are likely to be a relatively small fraction of any growing colony of cells, so it is difficult to imagine apoptosis providing a benefit to other young cells. More likely, the cellular defects that accompany aging may tap into an apoptotic program that is beneficial in other settings where similar defects occur.

An early aging study by Müller et al. (1985) noted that aging yeast cells have a reduced

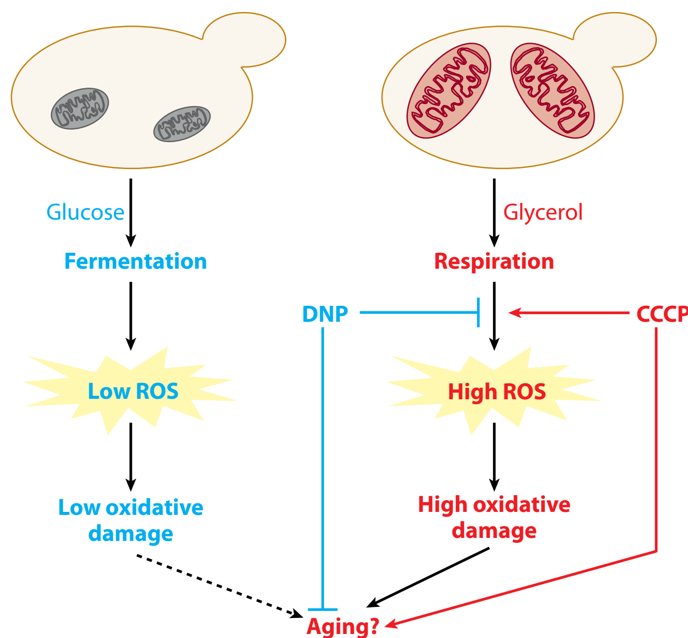

Figure 4

Mitochondria, reactive oxygen species (ROS) production, oxidative damage, and aging. Yeast cells produce energy via different pathways, depending on the carbon source. Pathways that promote aging are diagrammed in red; pathways that do not promote aging are diagrammed in blue. When in the presence of glucose, fermentation results in low levels of ROS production. However, in the presence of glycerol, respiration results in higher levels of ROS production. Elevated levels of ROS may lead to increased oxidative damage, which in turn may promote aging. The effects of two different mitochondrial uncouplers are also diagrammed: Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) leads to increased ROS production and a shortened life span, whereas dinitrophenol (DNP) treatment results in decreased levels of ROS and enhanced life span.

Dietary restriction (DR): the limitation of dietary intake short of nutrient deprivation

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mating capacity. This phenotype is now well understood and involves an age-dependent relocalization of the SIR complex (Sir2, -3, and -4) (Kennedy et al. 1997, Smeal et al. 1996). In young cells, this protein complex transcriptionally represses two silent mating-type (*HM*) loci that serve as templates for mating-type switching in homothallic yeast strains (Hicks & Herskowitz 1977, Ivy et al. 1986, Rine & Herskowitz 1987). Unlike *HM* loci, the mating-specific genes at the *MAT* locus are expressed and dictate whether heterothallic haploid yeast strains are *MAT*α or *MAT***a**. In cells that have lost silencing at the *HM* loci, genes for both mating types are expressed, and cells enter a pseudodiploid state accompanied by sterility. Loss of SIR-dependent *HM* silencing occurs during replicative aging (Smeal et al. 1996), in addition to loss of telomere silencing at some chromosome ends (Kennedy et al. 1997, Kim et al. 1996). Expression of the *HM* loci in a haploid throughout the life span (e.g., in *sir3*Δ or *sir4*Δ strains) results in a slight decrease in mean RLS independently of ERCs (Kaeberlein et al. 1999). That expression of *HM* loci limits the life span of wild-type strains seems unlikely, given that increased expression would only occur approximately midway through the life span of a mother cell and that deletion fails to increase life span significantly (Kaeberlein et al. 1999).

Not all things change with aging. For instance, telomere length remains relatively constant throughout replicative aging, seemingly eliminating telomere shortening as a determinant of yeast aging (D'Mello & Jazwinski 1991). Nevertheless, it is clear that many more molecular changes occur and that a subset of these likely contributes to limiting the life span of mother cells.

A handful of attempts have been made to characterize the cellular changes that accompany aging in yeast by the use of microarrays to examine the global gene expression profiles of young and old cells. In one study, microarray analysis was carried out on "young" (0–1-generation-old) or "old" (7–8-generation-old) wild-type cells, which were obtained by magnetic sorting (Lin et al. 2001). From this analysis, it was concluded that gluconeogenesis and glucose storage increase as cells age, suggesting a metabolic shift away from glycolysis and toward gluconeogenesis. In a second study of this type, elutriation was used to obtain an aged population in which 75% of the cells were at least 15 generations old and 90% of the cells were more than 8 generations old (Lesur & Campbell 2004). Microarray analysis of aged cells relative to young cells suggested an increase in expression of enzymes associated with glucose storage and gluconeogenesis, consistent with the previous study (Lin et al. 2001). In addition, certain stress- and damage-responsive genes were also elevated in aged cells (Lesur & Campbell 2004).

Several hurdles associated with this type of approach have limited the utility of microarray studies of yeast aging. First, all these studies have faced the same technical difficulty—how to obtain sufficiently large numbers of relatively pure populations of aging cells (see section, Genetic Modulation of Yeast Replicative Life Span, above). Although enrichment of aged cells is feasible, there is always a detectable percentage of daughter cells present in any "old" population, and the resulting contamination in gene expression profile is hard to gauge. In addition, all the aging-related microarray experiments reported to date have been performed in liquid growth media—an environment quite different from that experienced by cells in the standard RLS assay. Finally, the "aged" populations used in studies published to date have generally been relatively young: between 8 and 15 generations. Because the median life span of most laboratory strains is between 21 and 25 generations, the "old" cells in these studies are not even middle-aged. In one case, an attempt was made to examine the gene expression profile of senescent cells obtained by elutriation (Laun et al. 2005). Approximately 30% of the resulting population were senescent and showed gene expression profiles consistent with cells undergoing an apoptosis-like response (Madeo et al. 1997).

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DIETARY RESTRICTION AND YEAST REPLICATIVE AGING

Much of recent research on yeast replicative aging has focused on trying to determine the mechanism by which DR (also called calorie restriction) enhances longevity. The reasons for this obsession are obvious: DR is the only intervention that extends life span in all common model organisms for aging research. The mechanisms proposed to explain the effects of DR in yeast remain highly controversial. In this section, we discuss what is known about DR in yeast, including the generally agreed upon immediate signal transduction pathways downstream of DR and two nonexclusive possible targets of these pathways (**Figure 5**).

Dietary Restriction and Signal Transduction

In yeast, DR is generally invoked by reducing levels of glucose in the media during the life span experiment (Lin et al. 2000). A reduction of amino acid levels has also been reported to extend life span, but this form of DR has not been extensively studied (Jiang et al. 2000). Normal yeast growth media contains 2% glucose. This is well in excess of what is needed for rapid growth and has generally been employed to ensure that yeast reach high densities in liquid media for biochemical experimentation. Different labs favor different levels of glucose reduction for DR experiments, with glucose concentrations ranging from 0.5% to 0.005% (Kaeberlein et al. 2004, Lin et al. 2000). Ideally, a yeast strain should be analyzed at a series of reduced glucose concentrations to determine where the maximum increase in life span can be achieved. In fact, one commonly used strain (W303) may be totally unresponsive to DR, although this is controversial (Kaeberlein et al. 2006, Lamming et al. 2005). For the yeast ORF deletion strain background, maximum life span extension is achieved at 0.05% glucose (Kaeberlein et al. 2004). Although this seems to represent a drastic reduction of glucose levels (40-fold), yeast cells do not divide at dramatically lower rates under these conditions as long as glucose levels are held constant, which is the case for individual mother cells growing on solid media in the absense of neighboring cells during life span analysis (in contrast to liquid cultures, in which glucose is rapidly depleted by an increasing number of dividing cells, resulting in a drop in cell division rate upon glucose depletion) (V.L. MacKay, K. Steffen, M. Kaeberlein & B.K. Kennedy, unpublished). The reduction of growth rate that

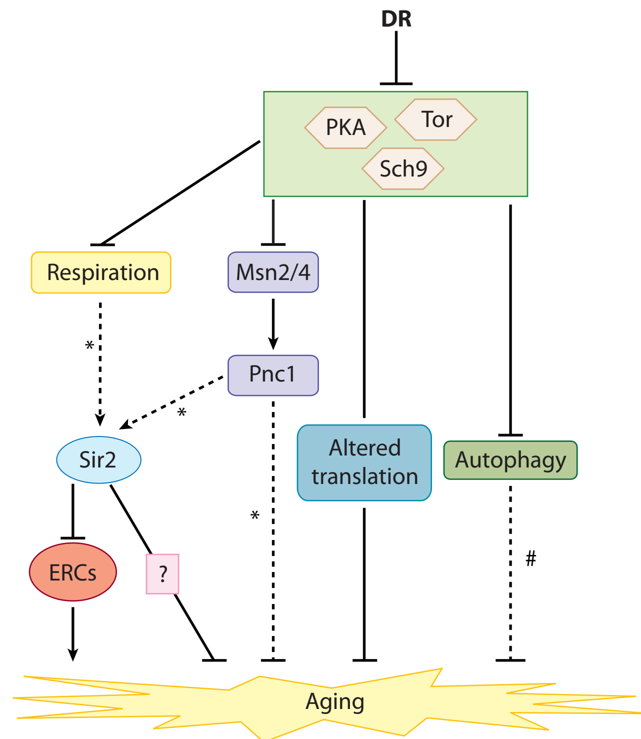

Figure 5

Model for the mechanism of dietary restriction (DR). In this proposed model, DR inhibits the three protein kinases Tor, protein kinase A (PKA), and Sch9. This inhibition results in increased levels of respiration and activation of the stress response proteins Msn2/4. Inhibition of Msn2/4 results in loss of activity of Pnc1, a nicotinamide deaminase. Both events lead to the activation of Sir2, which then affects aging by inhibiting ERC formation and by an unknown pathway. Inhibition of the three kinases may also result in altered translation levels as well as higher levels of autophagy. The cumulative effect of all events is the extension of life span. The asterisks indicate that the relevance of these branches of the pathway is in question among different research groups. The pound sign indicates where a role for autophagy in regulating yeast replicative life span is possible but not formally demonstrated. ERCs, extrachromosomal ribosomal DNA circles.

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does exist is largely attributable to the slow first cell cycle of daughter cells; they must achieve a certain size before DNA replication ensues and the rate of cell growth is reduced (Hartwell & Unger 1977).

Several genetic mimetics of DR also lead to RLS extension. For instance, deletion of *HXK2*, which encodes the glycolytic enzyme hexokinase II (Hxk2) (Walsh et al. 1983), leads to life span extension (Lin et al. 2000). When glucose is abundant, Hxk2 is the major kinase that converts glucose to glucose-6-phosphate, leading to the entry of glucose into the glycolytic pathway. Two other glucose kinase genes, *HXK1* and *GLK1*, are not expressed at high levels in these conditions; however, they are induced in strains lacking *HXK2*, and it remains unclear to what extent glucose phosphorylation is reduced in this background (Rodriguez et al. 2001, Walsh et al. 1991). Hxk2 also acts in a signaling pathway important for maintaining glucose repression, and this activity may also be linked to longevity control (Ahuatzi et al. 2004).

Mutations leading to reduced activity of partially redundant nutrient-responsive signal transduction pathways [TOR and protein kinase A (PKA)] also lead to RLS extension (Fabrizio et al. 2004b, Kaeberlein et al. 2005c, Lin et al. 2000). The life spans of these mutants are not further extended by reducing the glucose levels in the media, consistent with a model in which these pathways directly mediate the longevity effects of DR. Yeast contain three different PKA catalytic subunits, which together are essential for cell viability. Two sensing pathways upstream of PKA mediate the induction of cAMP in yeast cells exposed to glucose, although the mechanisms by which these sensors are activated are unknown (Santangelo 2006). One sensing pathway leads to RAS activation, and the other is a G protein–coupled receptor system consisting of *GPA2* and *GPR1*. Deletion of either *GPA2* or *GPR1* leads to life span extension, and these mutants are often used to reduce PKA activity in life span experiments (Lin et al. 2000).

TOR signaling is regulated in an unknown fashion by glutamine levels, which in turn are determined by nitrogen availability (Carvalho & Zheng 2003, Crespo et al. 2002). Two TOR proteins, Tor1 and Tor2, exist in two Torc complexes (Dann & Thomas 2006). Torc2 is rapamycin insensitive and dynamically controls the actin cytoskeleton. Tor2 preferentially exists in Torc2 and is essential for viability, whereas Torc1 can contain either Tor1 or Tor2. Reduced TOR signaling, either in a *tor1*Δ strain or in the presence of the Tor inhibitors rapamycin or methionine sulfoximide, leads to life span extension in yeast (Kaeberlein et al. 2005c). DR of the *tor1*Δ mutant does not further increase life span, consistent with TOR signaling acting through a similar pathway as DR. Reduced TOR activity leads to life span extension in worms and flies as well (Jia et al. 2004, Kapahi et al. 2004, Vellai et al. 2003), indicating that links between this pathway and longevity are likely conserved in divergent eukaryotic species (see below).

Sch9 is a third nutrient-responsive kinase linked to yeast RLS. Deletion of *SCH9* leads to robust extension of both mean and maximum life span (Fabrizio et al. 2004b, Kaeberlein et al. 2005c). By sequence homology, *SCH9* is most similar to Akt, a central component in insulin signaling pathways (Burgering & Coffer 1995, Paradis & Ruvkun 1998). A formal insulin signaling pathway does not exist in yeast, however, and recent evidence suggests that Sch9 may be the functional ortholog of S6 kinase (Powers 2007, Urban et al. 2007), a substrate of TOR in multicellular eukaryotes and a central regulator of translation (Hay & Sonenberg 2004, Jorgensen et al. 2004). Whether yeast Sch9 plays roles carried out by both Akt and S6 kinase in multicellular eukaryotes remains to be determined. Interestingly, deletion of either Akt homologs or S6 kinase is sufficient to increase life span in worms (Hansen et al. 2007, Pan et al. 2007, Paradis & Ruvkun 1998).

There is general agreement that PKA, TOR, and Sch9 are responsive to nutrient deprivation, modulate longevity in evolutionarily divergent organisms, and mediate at least some of the health and longevity benefits associated with DR (Kennedy et al. 2007). An important

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unresolved question, however, is which downstream targets of these kinases are most important for the regulation of life span in yeast and other organisms. This question is not easily answered because these kinases have overlapping roles in regulating a number of cellular responses to nutrient exposure. In nutrient-replete conditions, high activity of these kinases drives ribosome biogenesis and cell growth while limiting stress response pathways and autophagy. As nutrients levels become limiting, reduced activity of these kinases leads to a reversal of these phenotypes, with emphasis placed on stress response pathways, including free-radical-scavenging enzymes and autophagy. Any of these cellular events could modulate longevity.

Two nonexclusive models have been proposed for how reduced TOR signaling could increase RLS in response to DR: activation of sirtuins and altered translational regulation (Kaeberlein et al. 2005c, Medvedik et al. 2007, Steffen et al. 2008). Both of these pathways are linked to longevity regulation in worms and flies, and therefore they may represent conserved pathways mediating DR. In the following subsections, we examine the evidence for and against both models, noting in advance that all evidence is not yet in and that further clarifying experiments are in high demand.

Sirtuins

In addition to Sir2, yeast have four other related class III deacetylases, Hst1–4. The distinguishing feature of class III deacetylases is their substrate requirement for NAD, which is metabolized during the deacetylation reaction to nicotinamide and *O*-acetyl-ADP-ribose (Imai et al. 2000, Landry et al. 2000, Smith et al. 2000). Recent reviews discuss the complex mechanisms by which this reaction occurs. Some Hsts have overlapping roles with Sir2 that both have and have not been reported to be relevant for RLS, and we discuss these Hsts in that context. The primary substrate of Sir2 in yeast appears to be acetyllysine residues in the N-terminal tails of histones H3 and H4 (Imai et al. 2000, Tanny & Moazed 2001, Xu et al. 2007), although the targets of Sir2 orthologs in worms, flies, and mammals are largely reported to be other transcription factors.

Sir2, when in complex with Sir3 and Sir4, is required for the establishment of silenced regions of chromatin in regions near telomeres and silent mating-type loci (Aparicio et al. 1991, Rine & Herskowitz 1987), and its deacetylase activity is required for silencing (Rusche et al. 2003). In addition, Sir2 can function independently of Sir3 and Sir4; it both silences PolII genes experimentally inserted in rDNA and suppresses rDNA recombination, thus inhibiting the formation of ERCs (Bryk et al. 1997, Defossez et al. 1999, Gottlieb & Esposito 1989, Smith & Boeke 1997). The Sir2/3/4 complex also localizes to rDNA in replicatively aged cells, reflective of a potential role for the complex at this locus as well (Kennedy et al. 1997). One possible mechanism for the effect of Sir2 on replicative aging is its repression of rDNA recombination (Sinclair & Guarente 1997); evidence for this comes from multiple experimental approaches, including the aforementioned decreased longevity that results from the generation of ERCs in young cells. Additional support for this model comes from analysis of *fob1*Δ strains. Fob1 is a replication fork barrier protein with activity specific for rDNA repeats, which leads to elevated recombination at that locus. Mutants lacking Fob1 have reduced rDNA recombination and thus low ERC levels; moreover, they are long-lived (Defossez et al. 1999). Fob1 also participates in a protein complex that recruits Sir2 to rDNA loci (Huang & Moazed 2003), raising the possibility that one effect of *fob1*Δ on longevity is caused by the relocalization of Sir2 to other loci. However, this has not been formally demonstrated.

There are likely other aging-specific functions of Sir2 that remain to be discovered. For example, *fob1*Δ mutants and *fob1*Δ *sir2*Δ double mutants behave differently for life span and ERC levels (Kaeberlein et al. 1999). The *sir2*Δ strain is short-lived and has elevated ERC levels, whereas the *fob1*Δ strain is long-lived

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and has reduced ERC levels. Loss of *FOB1* is epistatic to *SIR2* for rDNA recombination because the *fob1*Δ and *fob1*Δ *sir2*Δ strains both have very low ERC levels. However, the life span of the *fob1*Δ *sir2*Δ strain is shorter than that of the *fob1*Δ strain, a finding incongruous with ERC production being the sole downstream effect of Sir2 activity relevant to aging. One interpretation of this is that Sir2 has a second function important for mediating longevity that is not compensated for by loss of *FOB1*.

Sequestration of oxidized and aggregated proteins to mother cells may also relate to the life span functions of Sir2. However, the mechanism(s) by which Sir2 performs this function remains unknown. Moreover, this function may have contrasting effects on life span. With Sir2 allowing for the retention of damaged proteins in mother cells, the daughter cells are young and have full life span potential. However, this may simultaneously cause mother cells to accumulate damage more rapidly with age. The process is linked to RLS by the recent striking finding that overexpression of *HSP104*, a stress tolerance factor that acts along with chaperones on aggregated proteins, suppresses the short life span of *sir2*Δ (Erjavec et al. 2007). Increased Hsp104 activity also partially restores asymmetric distribution of damaged and aggregated proteins to mother cells in the *sir2*Δ mutant. This finding suggests that *sir2*Δ strains are not short-lived solely because of ERC accumulation, but possibly also because of missegregation of damaged proteins to daughters. An alternate possibility, that increased Hsp104 levels mitigate ERC toxicity, has not been examined.

Opinions over the importance of Sir2 and other sirtuins in the DR from many of the protagonists (including M.K. and B.K.K.) are already abundant in the literature, and the reader is referred to several papers (Chen & Guarente 2007, Kennedy et al. 2005, Longo & Kennedy 2006, Sinclair 2005). The goal in this section is to address the strengths and weaknesses of this model, with particular attention to recent findings. One issue still not fully resolved is whether DR enhances the activation of Sir2 in yeast. Paradoxically, this is more clearly established in mice, for which several groups have reported that DR activates the *SIR2* ortholog, Sirt1 (Cohen et al. 2004, Nemoto et al. 2004, Picard et al. 2004). Whether Sirt1 is required for life span extension by DR in mice remains to be determined, a question complicated by the severe phenotype of *Sirt1*$^{-/-}$ mice. In yeast, however, DR enhances rDNA silencing in a Sir2-dependent manner and lowers rates of rDNA recombination (Lamming et al. 2005), although we have not observed any effect on telomere silencing (Kaeberlein et al. 2005a).

The mechanism by which DR may activate Sir2 in yeast is also a matter of debate. Two nonexclusive models are possible. First, DR in yeast may lead to enhanced respiration, which may increase NAD levels and lead to Sir2 activation (Lin et al. 2002). Consistent with this model, overexpression of *HAP4* leads to increased transcription of respiratory genes and extends RLS (Lin et al. 2004). However, we have found that DR extends life span in respiratory-deficient Rho0 strains, which lack mitochondrial DNA (Kaeberlein et al. 2005a). A recent study from Lin and colleagues has carefully studied respiration and replicative aging, finding that deletion of *LAT1*, a component of mitochondrial pyruvate dehydrogenase, blocks life span extension by DR and that overexpression of this gene extends life span (Easlon et al. 2007). Interestingly, life span extension by *LAT1* overexpression is independent of yeast sirtuins.

A second model by which DR may activate Sir2 is through increased Msn2/4-dependent transcription of *PNC1*, which encodes an enzyme that deaminates nicotinamide, a product of sirtuin catalysis that, if not catabolized, inhibits sirtuins. Deletion of *PNC1* either blocks (Anderson et al. 2003) or partially blocks life span extension by DR (Lin et al. 2004); furthermore, overexpression of *PNC1* extends life span (Anderson et al. 2003). Although we have found no evidence that sirtuins mediate life span extension by DR, our studies indicate that increased nicotinamide can block much of the life span extension by DR without inhibiting sirtuin activity, indicating that this metabolite

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may have other, undefined roles in the regulation of RLS (Tsuchiya et al. 2006). Msn2 and -4 are maintained in the cytoplasm during optimal growth conditions but translocate to the nucleus when either glucose levels or Tor signaling are reduced (Beck & Hall 1999, Gorner et al. 1998, Mayordomo et al. 2002). A recent study indicates that the life span extension conferred by rapamycin requires nuclear localization of Msn2/4 and increased transcription of *PNC1* (Medvedik et al. 2007). However, life span extension in the *tor1*Δ strain is independent of *SIR2* (Kaeberlein et al. 2005c). Whether Hst2 can also compensate for loss of *SIR2* under these conditions remains to be determined (see below).

It is agreed that genetic mimetics of DR do not extend life span in a *sir2*Δ strain (Kaeberlein et al. 2004, Lin et al. 2000) and that DR does extend life span in the *sir2*Δ *fob1*Δ strains (Kaeberlein et al. 2004, Lamming et al. 2005). These findings have led to two different interpretations, which hinge on an experiment in which different labs have reached discordant results. It has been suggested that DR works in the *sir2*Δ *fob1*Δ strain because other sirtuins (primarily Hst2) become activated by DR and inhibit rDNA recombination (Lamming et al. 2005, 2006). This finding has not been successfully repeated (Kaeberlein et al. 2004, Tsuchiya et al. 2006). Why Hst2 is apparently unable to compensate for the loss of *SIR2* in strains with functional Fob1 has also not been experimentally addressed.

In summary, the model proposed by Sinclair and colleagues (Sinclair 2005) posits that life span extension by DR results mainly from reduced rDNA recombination and ERC generation through the activation of sirtuins. One weakness of this model is that ERC-dependent aging appears to be specific to yeast. Thus, conserved longevity regulators (DR and/or increased *SIR2* expression) regulate yeast aging through a nonconserved mechanism. One possible resolution of this puzzle is the proposal that mammalian sirtuins extend life span through chromatin regulation, reducing DNA damage at other, more vulnerable sites in the mammalian genome. This would be consistent with the model discussed above.

On the basis of our data (Kaeberlein et al. 2004, 2006; Tsuchiya et al. 2006), we favor a model in which life span extension by increased *SIR2* expression is through a non-DR pathway. In this model, there are at least two pathways that enhance replicative aging in wild-type yeast. DR would fail to extend the short-lived *sir2*Δ because ERC levels are elevated; thus, all mother cells would die at an early age through an ERC-dependent mechanism. In the *sir2*Δ *fob1*Δ double mutant, however, ERC levels are low, and DR confers robust life span extension through the second pathway, which is more prominent when the influence of ERCs is muted. Data from our nonbiased genome screen have led us to favor a model whereby life span extension by DR is conferred through mechanisms leading to altered translational regulation in reduced nutrient environments.

Translational Regulation and Dietary Restriction

In addition to several deletions that are predicted to result in reduced TOR signaling, screening of the ORF deletion collection identified two *rpl*Δ strains (*rpl31a*Δ and *rpl6b*Δ) as long-lived (Kaeberlein et al. 2005c). Mutations of four other *RP* genes (three in haploid cells, one in heterozygous diploid cells) have since been reported to have enhanced longevity (Chiocchetti et al. 2007), although not every *RP* deletion has this effect (Kaeberlein et al. 2005c). Although most ribosomal subunits are required for viability, yeast differs from other eukaryotes in that its ribosomal protein genes have been duplicated. This means that yeast strains lacking a gene encoding a ribosomal subunit are generally viable owing to the presence of the duplicated highly similar gene. This permits replicative analysis of most *rp*Δ strains, which result in reduction but not elimination of their respective subunit. Recently, we have reported the existence of 14 more *rp*Δs that confer long life span, and surprisingly all the genes

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identified encode components of the ribosomal large subunit (Steffen et al. 2008). This specificity occurs at least in part because reduced 60S (and not 40S) subunit biogenesis leads paradoxically to enhanced translation of *GCN4* (Foiani et al. 1991, Steffen et al. 2008), a transcription factor induced in nutrient-deprived conditions (Yang et al. 2000). The transcriptional targets of Gcn4 that are important for longevity remain to be identified.

There is a well-established connection between the protein kinase Tor and ribosome biogenesis; decreased Tor signaling results in decreased levels of ribosomes (Jorgensen et al. 2004, Powers et al. 2004). Thus, altered translation may lie directly downstream of TOR and DR in longevity modulation.

Interestingly, this effect of loss of ribosomal proteins on life span does not seem to be restricted to yeast. RNAi screens for longevity genes in *C. elegans* have revealed both *rpl* and *rps* genes to be long-lived as well (Chen & Contreras 2007, Curran & Ruvkun 2007, Hansen et al. 2007, Pan et al. 2007). Unlike yeast, worm *RP* genes are not duplicated, and RNAi initiated in the embryo results in developmental lethality. Accordingly, in these screens, worms were not exposed to the RNAi bacteria until early adulthood. As such, it is not clear as to how much expression of the *RP* genes is knocked down, or why reduced expression of worm *rp* genes in adulthood allows for enhanced longevity. However, these results do suggest that life span extension by reduced ribosome biogenesis or function may be conserved in the eukaryotic lineage.

The mechanism for the effect of translation on extended life span is not yet known. One possibility is that reduced translation rates result in decreased accumulation of damaged proteins. Altered protein homeostasis may be one causal factor in aging and age-related disease, a theory supported by the finding that aggregated proteins accumulate aging yeast cells and in several mammalian neurodegenerative disorders. Reducing ribosomal protein expression in worms and yeast leads to decreased levels of translation, which may reduce the rate of accumulation of damaged proteins. This may allow better degradation or repair of existing damage with age. Alternatively (or in addition), we have recently shown that *rpl*Δ strains may lead to changes in the translation of specific messages linked to life span modulation. Whether this latter mechanism is conserved is unknown. Future research will be necessary to determine why reduced ribosome biogenesis can equate to long life.

CONCLUSIONS

Aging is not thought to be a programmed event but, rather, the inevitable consequence of an age-dependent decline in the forces of selection (Austad 2004, Kirkwood 2005). If this is the case, why should longevity pathways be conserved among disparate eukaryotic species with dramatically different reproductive strategies, life histories, and life spans? Now that genome-wide genetic approaches to longevity are feasible in more than one aging model organism, it has become possible to determine the levels of conservation that exist and to identify the specific genes and pathways with conserved effects. We have recently performed a genome-wide comparison between the genetic determinants of longevity in yeast and worms, finding that yeast orthologs of known worm aging genes are significantly more likely to modulate yeast aging than are genes chosen at random (Smith et al. 2008). In addition, these studies indicate that translational regulatory pathways are highly conserved for their effects on life span. It is our view that these pathways should be given specific attention in invertebrate studies because they have a higher likelihood of teaching us about processes that affect human life span and that the mammalian orthologs of conserved worm-yeast ortholog pairs may be of particular interest with regard to aging.

The state of yeast aging research is strong. In the past 15 years, the number of researchers in the field has grown exponentially, leading to unexpected discoveries and exciting new avenues of research. Although there is substantial controversy regarding key questions that

need to be resolved, this is more a reflection of rapid progress than a cause for serious concern. Despite the identification in yeast and other organisms of numerous genetic modulators of aging and several molecular phenotypes accompanying aging, the key question remains unanswered: What are the fundamental causes of aging? Given the rate of recent progress, it does not seem overly optimistic to forecast that the puzzle pieces will fit together at some point in the near future. With this knowledge, opportunities to intervene pharmacologically in the aging process as a therapeutic avenue to age-related diseases will increase dramatically.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T. 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299:1751–53

Protein carbonyls and other oxidatively damaged proteins accumulate preferentially in mother cells in an actin-dependent manner.

Ahuatzi D, Herrero P, De la Cera T, Moreno F. 2004. The glucose-regulated nuclear localization of hexokinase 2 in *Saccharomyces cerevisiae* is Mig1-dependent. *J. Biol. Chem.* 279:14440–46

Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. 2003. Nicotinamide and *PNC1* govern lifespan extension by caloric restriction in *Saccharomyces cerevisiae*. *Nature* 423:181–85

Aparicio OM, Billington BL, Gottschling DE. 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* 66:1279–87

Ashrafi K, Sinclair D, Gordon JI, Guarente L. 1999. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 96:9100–5

Austad SN. 2004. Is aging programmed? *Aging Cell* 3:249–51

Austriaco NR Jr. 1996. *UTH1 and the genetic control of aging in the yeast*, Saccharomyces cerevisiae. PhD thesis. Mass. Inst. Technol., Cambridge, MA

Austriaco NR Jr, Guarente LP. 1997. Changes of telomere length cause reciprocal changes in the lifespan of mother cells in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 94:9768–72

Bacon JS, Davidson ED, Jones D, Taylor IF. 1966. The location of chitin in the yeast cell wall. *Biochem. J.* 101:36C–38C

Bandara PD, Flattery-O'Brien JA, Grant CM, Dawes IW. 1998. Involvement of the *Saccharomyces cerevisiae UTH1* gene in the oxidative-stress response. *Curr. Genet.* 34:259–68

Barros MH, Bandy B, Tahara EB, Kowaltowski AJ. 2004. Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:49883–88

Bartke A. 2005. Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging. *Endocrinology* 146:3718–23

Barton AA. 1950. Some aspects of cell division in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* 4:84–86

Beck T, Hall MN. 1999. The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402:689–92

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org by UNIVERSITY OF WASHINGTON - HEALTH SCIENCES LIBRARIES on 08/18/08. For personal use only.

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org
by UNIVERSITY OF WASHINGTON - HEALTH SCIENCES LIBRARIES on 08/18/08. For personal use only.

Bohr VA. 2005. Deficient DNA repair in the human progeroid disorder, Werner syndrome. *Mutat. Res.* 577:252–59

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, et al. 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–32

Bryk M, Banerjee M, Murphy M, Knudsen KE, Garfinkel DJ, Curcio MJ. 1997. Transcriptional silencing of Ty1 elements in the *RDN1* locus of yeast. *Genes Dev.* 11:255–69

Burgering BM, Coffer PJ. 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 376:599–602

Buttner S, Eisenberg T, Herker E, Carmona-Gutierrez D, Kroemer G, Madeo F. 2006. Why yeast cells can undergo apoptosis: death in times of peace, love, and war. *J. Cell Biol.* 175:521–25

Camougrand N, Grelaud-Coq A, Marza E, Priault M, Bessoule JJ, Manon S. 2003. The product of the UTH1 gene, required for Bax-induced cell death in yeast, is involved in the response to rapamycin. *Mol. Microbiol.* 47:495–506

Camougrand N, Kiššova I, Velours G, Manon S. 2004. Uth1p: a yeast mitochondrial protein at the crossroads of stress, degradation and cell death. *FEMS Yeast Res.* 5:133–40

Campisi J, d'Adda di Fagagna F. 2007. Cellular senescence: when bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* 8:729–40

Carvalho J, Zheng XF. 2003. Domains of Gln3p interacting with karyopherins, Ure2p, and the target of rapamycin protein. *J. Biol. Chem.* 278:16878–86

Chen C, Contreras R. 2007. Identifying genes that extend life span using a high-throughput screening system. *Methods Mol. Biol.* 371:237–48

Chen D, Guarente L. 2007. SIR2: a potential target for calorie restriction mimetics. *Trends Mol. Med.* 13:64–71

Chen JB, Sun J, Jazwinski SM. 1990. Prolongation of the yeast life span by the v-Ha-*RAS* oncogene. *Mol. Microbiol.* 4:2081–86

The first demonstration that genetic modification of yeast can alter its life span.

Chiocchetti A, Zhou J, Zhu H, Karl T, Haubenreisser O, et al. 2007. Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span. *Exp. Gerontol.* 42:275–86

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, et al. 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305:390–92

Crespo JL, Powers T, Fowler B, Hall MN. 2002. The TOR-controlled transcription activators GLN3, RTG1 and RTG3 are regulated in response to cellular levels of glutamine. *Proc. Natl. Acad. Sci. USA* 99:6784–89

Curran SP, Ruvkun G. 2007. Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet.* 3:e56

D'Mello NP, Childress AM, Franklin DS, Kale SP, Pinswasdi C, Jazwinski SM. 1994. Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *J. Biol. Chem.* 269:15451–59

D'Mello NP, Jazwinski SM. 1991. Telomere length constancy during aging of *Saccharomyces cerevisiae*. *J. Bacteriol.* 173:6709–13

Dann SG, Thomas G. 2006. The amino acid sensitive TOR pathway from yeast to mammals. *FEBS Lett.* 580:2821–29

Defossez PA, Prusty R, Kaeberlein M, Lin SJ, Ferrigno P, et al. 1999. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. *Mol. Cell* 3:447–55

Dickson RC, Sumanasekera C, Lester RL. 2006. Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. *Prog. Lipid Res.* 45:447–65

Duennwald ML, Jagadish S, Giorgini F, Muchowski PJ, Lindquist S. 2006a. A network of protein interactions determines polyglutamine toxicity. *Proc. Natl. Acad. Sci. USA* 103:11051–56

Duennwald ML, Jagadish S, Muchowski PJ, Lindquist S. 2006b. Flanking sequences profoundly alter polyglutamine toxicity in yeast. *Proc. Natl. Acad. Sci. USA* 103:11045–50

Easlon E, Tsang F, Dilova I, Wang C, Lu SP, et al. 2007. The dihydrolipoamide acetyltransferase is a novel metabolic longevity factor and is required for calorie restriction-mediated life span extension. *J. Biol. Chem.* 282:6161–71

Egilmez NK, Chen JB, Jazwinski SM. 1989. Specific alterations in transcript prevalence during the yeast life span. *J. Biol. Chem.* 264:14312–17

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org
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Egilmez NK, Jazwinski SM. 1989. Evidence for the involvement of a cytoplasmic factor in the aging of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 171:37–42

Ehrnhoefer DE, Duennwald M, Markovic P, Wacker JL, Engemann S, et al. 2006. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum. Mol. Genet.* 15:2743–51

Epstein CJ, Martin GM, Schultz AL, Motulsky AG. 1966. Werner's syndrome: a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine* 45:177–222

Erjavec N, Larsson L, Grantham J, Nystrom T. 2007. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Dev.* 21:2410–21

Accelerated aging of *sir2* mutants is suppressed by overexpressing Hsp104, indicating that Sir2's aging function may not be restricted to extrachromosomal rDNA circles.

Erjavec N, Nystrom T. 2007. Sir2p-dependent protein segregation gives rise to a superior reactive oxygen species management in the progeny of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 104:10877–81

Fabrizio P, Battistella L, Vardavas R, Gattazzo C, Liou LL, et al. 2004a. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J. Cell Biol.* 166:1055–67

Fabrizio P, Longo VD. 2003. The chronological life span of *Saccharomyces cerevisiae*. *Aging Cell* 2:73–81

Fabrizio P, Pletcher SD, Minois N, Vaupel JW, Longo VD. 2004b. Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in *Saccharomyces cerevisiae*. *FEBS Lett.* 557:136–42

Foiani M, Cigan AM, Paddon CJ, Harashima S, Hinnebusch AG. 1991. GCD2, a translational repressor of the *GCN4* gene, has a general function in the initiation of protein synthesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 11:3203–16

Gami MS, Wolkow CA. 2006. Studies of *Caenorhabditis elegans* DAF-2/insulin signaling reveal targets for pharmacological manipulation of lifespan. *Aging Cell* 5:31–37

Gancedo C, Serrano R. 1989. Energy-yielding metabolism. In *The Yeasts: Metabolism and Physiology of Yeasts*, ed. AH Rose, JS Harrison, pp. 205–59. London: Academic

Gillespie CS, Proctor CJ, Boys RJ, Shanley DP, Wilkinson DJ, Kirkwood TBL. 2004. A mathematical model of ageing in yeast. *J. Theor. Biol.* 229:189–96

Goldstrohm AC, Hook BA, Seay DJ, Wickens M. 2006. PUF proteins bind Pop2p to regulate messenger RNAs. *Nat. Struct. Mol. Biol.* 13:533–39

Goldstrohm AC, Seay DJ, Hook BA, Wickens M. 2007. PUF protein-mediated deadenylation is catalyzed by Ccr4p. *J. Biol. Chem.* 282:109–14

Gorner W, Durchschlag E, Martinez-Pastor MT, Estruch F, Ammerer G, et al. 1998. Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev.* 12:586–97

Gottlieb S, Esposito RE. 1989. A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. *Cell* 56:771–76

Gourlay CW, Carpp LN, Timpson P, Winder SJ, Ayscough KR. 2004. A role for the actin cytoskeleton in cell death and aging in yeast. *J. Cell Biol.* 164:803–9

Guillas I, Kirchman PA, Chuard R, Pfefferli M, Jiang JC, et al. 2001. C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lag1p and Lac1p. *EMBO J.* 20:2655–65

Hamilton B, Dong Y, Shindo M, Liu W, Odell I, et al. 2005. A systematic RNAi screen for longevity genes in *C. elegans*. *Genes Dev.* 19:1544–55

Hansen M, Hsu AL, Dillin A, Kenyon C. 2005. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genet.* 1:119–28

Hansen M, Taubert S, Crawford D, Libina N, Lee SJ, Kenyon C. 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6:95–110

Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11:298–300

Harris N, Bachler M, Costa V, Mollapour M, Moradas-Ferreira P, Piper PW. 2005. Overexpressed Sod1p acts either to reduce or to increase the lifespans and stress resistance of yeast, depending on whether it is Cu^{2+}-deficient or an active Cu,Zn-superoxide dismutase. *Aging Cell* 4:41–52

Harris N, Costa V, MacLean M, Mollapour M, Moradas-Ferreira P, Piper PW. 2003. Mnsod overexpression extends the yeast chronological (G_0) life span but acts independently of Sir2p histone deacetylase to shorten the replicative life span of dividing cells. *Free Radic. Biol. Med.* 34:1599–606

REVIEWS IN ADVANCE

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org
by UNIVERSITY OF WASHINGTON - HEALTH SCIENCES LIBRARIES on 08/18/08. For personal use only.

Hartwell LH, Unger MW. 1977. Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. *J. Cell Biol.* 75:422–35

Hay N, Sonenberg N. 2004. Upstream and downstream of mTOR. *Genes Dev* 18:1926–45

Hayflick L. 1965. The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* 37:614–36

Helfand SL, Rogina B. 2003. Genetics of aging in the fruit fly, *Drosophila melanogaster*. *Annu. Rev. Genet.* 37:329–48

Herker E, Jungwirth H, Lehmann KA, Maldener C, Frohlich KU, et al. 2004. Chronological aging leads to apoptosis in yeast. *J. Cell Biol.* 164:501–7

Hicks JB, Herskowitz I. 1977. Interconversion of yeast mating types. II. Restoration of mating ability to sterile mutants in homothallic and heterothallic strains. *Genetics* 85:373–93

Hirsch HR. 1993. Accumulation of a senescence factor in yeast cells. *Exp. Gerontol.* 28:195–204

Hook BA, Goldstrohm AC, Seay DJ, Wickens M. 2007. Two yeast PUF proteins negatively regulate a single mRNA. *J. Biol. Chem.* 282:15430–38

Houthoofd K, Vanfleteren JR. 2007. Public and private mechanisms of life extension in *Caenorhabditis elegans*. *Mol. Genet. Genomics* 277:601–17

Huang J, Moazed D. 2003. Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes Dev.* 17:2162–76

Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403:795–800

Ivy JM, Klar AJ, Hicks JB. 1986. Cloning and characterization of four SIR genes of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 6:688–702

Jarolim S, Millen J, Heeren G, Laun P, Goldfarb DS, Breitenbach M. 2004. A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 5:169–77

Jazwinski SM. 2005. Yeast longevity and aging: the mitochondrial connection. *Mech. Ageing Dev.* 126:243–48

Jazwinski SM, Egilmez NK, Chen JB. 1989. Replication control and cellular life span. *Exp. Gerontol.* 24:423–36

Jazwinski SM, Wawryn J. 2001. Profiles of random change during aging contain hidden information about longevity and the aging process. *J. Theor. Biol.* 213:599–608

Jia K, Chen D, Riddle DL. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. *Development* 131:3897–906

Jiang JC, Jaruga E, Repnevskaya MV, Jazwinski SM. 2000. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. *FASEB J.* 14:2135–37

Jiang JC, Kirchman PA, Zagulski M, Hunt J, Jazwinski SM. 1998. Homologs of the yeast longevity gene LAG1 in *Caenorhabditis elegans* and human. *Genome. Res.* 8:1259–72

Johnston JR. 1966. Reproductive capacity and mode of death of yeast cells. *Antonie van Leeuwenhoek* 32:94–98

Jorgensen P, Rupes I, Sharom JR, Schneper L, Broach JR, Tyers M. 2004. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes Dev.* 18:2491–505

Kaeberlein M, Burtner CR, Kennedy BK. 2007. Recent developments in yeast aging. *PLoS Genet.* 3:655–60

Kaeberlein M, Guarente L. 2002. *Saccharomyces cerevisiae* MPT5 and SSD1 function in parallel pathways to promote cell wall integrity. *Genetics* 160:83–95

Kaeberlein M, Hu D, Kerr EO, Tsuchiya M, Westman EA, et al. 2005a. Increased life span due to calorie restriction in respiratory deficient yeast. *PLoS Genet.* 1:614–21

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK. 2004. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* 2:1381–87

Dietary restriction does not extend the life span of yeast *sir2*Δ single mutants, although it does extend the life span of *sir2*Δ *fob1*Δ double mutants.

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK. 2005b. Genes determining yeast replicative life span in a long-lived genetic background. *Mech. Ageing Dev.* 126:491–504

Kaeberlein M, McVey M, Guarente L. 1999. The *SIR2/3/4* complex and *SIR2* alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 13:2570–80

Kaeberlein M, Powers RW III, Steffen KK, Westman EA, Hu D, et al. 2005c. Regulation of yeast replicative life-span by TOR and Sch9 in response to nutrients. *Science* 310:1193–96

The only reported unbiased screen for mutants that extend replicative life span; 13 of 564 gene deletions resulted in extended life span, including a strain lacking *TOR1*.

Kaeberlein M, Steffen KK, Hu D, Dang N, Kerr EO, et al. 2006. Comment on "*HST2* mediates *SIR2*-independent life-span extension by calorie restriction". *Science* 312:1312b

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org
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Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. 2004. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr. Biol.* 14:885–90

Kennedy BK, Austriaco NR Jr, Guarente L. 1994. Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life span. *J. Cell Biol.* 127:1985–93

Kennedy BK, Austriaco NR, Zhang J, Guarente L. 1995. Mutation in the silencing gene SIR4 can delay aging in *S. cerevisiae*. *Cell* 80:485–96

A screen for stress-resistant, long-lived mutants identifies the SIR complex as a key regulator of yeast aging.

Kennedy BK, Gotta M, Sinclair DA, Mills K, McNabb DS, et al. 1997. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. *Cell* 89:381–91

Kennedy BK, Smith ED, Kaeberlein M. 2005. The enigmatic role of Sir2 in aging. *Cell* 123:548–50

Kennedy BK, Steffen KK, Kaeberlein M. 2007. Ruminations on dietary restriction and aging. *Cell Mol. Life Sci.* 64:1323–28

Kim S, Villeponteau B, Jazwinski SM. 1996. Effect of replicative age on transcriptional silencing near telomeres in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 219:370–76

Kirchman PA, Botta G. 2007. Copper supplementation increases yeast life span under conditions requiring respiratory metabolism. *Mech. Ageing Dev.* 128:187–95

Kirchman PA, Kim S, Lai CY, Jazwinski SM. 1999. Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. *Genetics* 152:179–90

Kirkwood TB. 2005. Understanding the odd science of aging. *Cell* 120:437–47

Kissova I, Deffieu M, Manon S, Camougrand N. 2004. Uth1p is involved in the autophagic degradation of mitochondria. *J. Biol. Chem.* 279:39068–74

Kudlow BA, Kennedy BK, Monnat RJ Jr. 2007. Werner syndrome and Hutchinson-Gilford progeria: mechanistic basis of human progeroid syndromes. *Nat. Rev. Mol. Cell Biol.* 8:394–404

Lai CY, Jaruga E, Borghouts C, Jazwinski SM. 2002. A mutation in the *ATP2* gene abrogates the age asymmetry between mother and daughter cells of the yeast *Saccharomyces cerevisiae*. *Genetics* 162:73–87

Lamming DW, Latorre-Esteves M, Medvedik O, Wong SN, Tsang FA, et al. 2005. *HST2* mediates *SIR2*-independent life-span extension by calorie restriction. *Science* 309:1861–64

Lamming DW, Latorre-Esteves M, Medvedik O, Wong SN, Tsang FA, et al. 2006. Response to comment on "*HST2* mediates *SIR2*-independent life-span extension by calorie restriction." *Science* 312:1312c

Landry J, Slama JT, Sternglanz R. 2000. Role of NAD^+ in the deacetylase activity of the SIR2-like proteins. *Biochem. Biophys. Res. Commun.* 278:685–90

Laun P, Pichova A, Madeo F, Fuchs J, Ellinger A, et al. 2001. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. *Mol. Microbiol.* 39:1166–73

Laun P, Ramachandran L, Jarolim S, Herker E, Liang P, et al. 2005. A comparison of the aging and apoptotic transcriptome of *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 5:1261–72

Laun P, Rinnerthaler M, Bogengruber E, Heeren G, Breitenbach M. 2006. Yeast as a model for chronological and reproductive aging: a comparison. *Exp. Gerontol.* 41:1208–12

Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. 2003. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* 33:40–48

Lesur I, Campbell JL. 2004. The transcriptome of prematurely aging yeast cells is similar to that of telomerase deficient cells. *Mol. Biol. Cell* 15:1297–312

Lin SJ, Defossez PA, Guarente L. 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289:2126–28

Established both glucose limitation and genetic methods as means of achieving dietary restriction in yeast.

Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. 2004. Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev.* 18:12–16

Lin SJ, Guarente L. 2006. Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet.* 2:e33

Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, et al. 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418:344–48

Lin SS, Manchester JK, Gordon JI. 2001. Enhanced gluconeogenesis and increased energy storage as hallmarks of aging in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 276:36000–7

Longo VD, Gralla EB, Valentine JS. 1996. Superoxide dismutase activity is essential for stationary phase survival in *Saccharomyces cerevisiae*. Mitochondrial production of toxic oxygen species in vivo. *J. Biol. Chem.* 271:12275–80

REVIEWS IN ADVANCE

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Longo VD, Kennedy BK. 2006. Sirtuins in aging and age-related disease. *Cell* 126:257–68

MacMurray MA, Gottschling DE. 2003. An age-induced switch to a hyper-recombinational state. *Science* 301:1908–11

Madeo F, Frohlich E, Frohlich KU. 1997. A yeast mutant showing diagnostic markers of early and late apoptosis. *J. Cell Biol.* 139:729–34

Martin DE, Hall MN. 2005. The expanding TOR signaling network. *Curr. Opin. Cell Biol.* 17:158–66

Mayordomo I, Estruch F, Sanz P. 2002. Convergence of the target of rapamycin and the Snf1 protein kinase pathways in the regulation of the subcellular localization of Msn2, a transcriptional activator of STRE (stress response element)-regulated genes. *J. Biol. Chem.* 277:35650–56

McVey M, Kaeberlein M, Tissenbaum HA, Guarente L. 2001. The short life span of *Saccharomyces cerevisiae sgs1* and *srs2* mutants is a composite of normal aging processes and mitotic arrest due to defective recombination. *Genetics* 157:1531–42

Medvedik O, Lamming DW, Kim KD, Sinclair DA. 2007. *MSN2* and *MSN4* link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PLoS Biol.* 5:230–41

Inhibition of Tor activity extends life span through a similar pathway as does dietary restriction.

Melendez A, Talloczy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. 2003. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* 301:1387–91

Mortimer RK, Johnston JR. 1959. Life span of individual yeast cells. *Nature* 183:1751–52

This pioneering paper in yeast aging was the first to measure the replicative aging properties of yeast cells.

Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. 2007. Trends in oxidative aging theories. *Free Radic. Biol. Med.* 43:477–503

Müller I. 1985. Parental age and the life-span of zygotes of *Saccharomyces cerevisiae*. *Antonie van Leeuwenhoek* 51:1–10

Müller I, Zimmermann M, Becker D, Flomer M. 1980. Calendar life span versus budding life span of *Saccharomyces cerevisiae*. *Mech. Ageing Dev.* 12:47–52

Murray AW, Szostak JW. 1983. Pedigree analysis of plasmid segregation in yeast. *Cell* 34:961–70

Nemoto S, Fergusson MM, Finkel T. 2004. Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. *Science* 306:2105–8

Nestelbacher R, Laun P, Vondrakova D, Pichova A, Schuller C, Breitenbach M. 2000. The influence of oxygen toxicity on yeast mother cell-specific aging. *Exp. Gerontol.* 35:63–70

Nierras CR, Liebman SW, Warner JR. 1997. Does *Saccharomyces* need an organized nucleolus? *Chromosoma* 108:444–51

Nyström T. 2005. Role of oxidative carbonylation in protein quality control and senescence. *EMBO J.* 24:1311–17

Obeid LM, Hannun YA. 2003. Ceramide, stress, and a "LAG" in aging. *Sci. Aging Knowl. Environ.* 2003:PE27

Olsen A, Vantipalli MC, Lithgow GJ. 2006. Using *Caenorhabditis elegans* as a model for aging and age-related diseases. *Ann. N.Y. Acad. Sci.* 1067:120–28

Ozgenc A, Loeb LA. 2005. Current advances in unraveling the function of the Werner syndrome protein. *Mutat. Res.* 577:237–51

Pan KZ, Palter JE, Rogers AN, Olsen A, Chen D, et al. 2007. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 6:111–19

Paradis S, Ruvkun G. 1998. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev.* 12:2488–98

Park PU, McVey M, Guarente L. 2002. Separation of mother and daughter cells. *Methods Enzymol.* 351:468–77

Pereira G, Tanaka TU, Nasmyth K, Schiebel E. 2001. Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. *EMBO J.* 20:6359–70

Petes TD, Botstein D. 1977. Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. *Proc. Natl. Acad. Sci. USA* 74:5091–95

Philippsen P, Thomas M, Kramer RA, Davis RW. 1978. Unique arrangement of coding sequences for 5 S, 5.8 S, 18 S and 25 S ribosomal RNA in *Saccharomyces cerevisiae* as determined by R-loop and hybridization analysis. *J. Mol. Biol.* 123:387–404

Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, et al. 2004. SirT1 promotes fat mobilization in white adipocytes by repressing PPARγ. *Nature* 429:771–76

Piper PW. 2006. Long-lived yeast as a model for ageing research. *Yeast* 23:215–26

Annu. Rev. Cell Dev. Biol. 2008.24. Downloaded from arjournals.annualreviews.org
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Powers T. 2007. TOR signaling and S6 kinase 1: Yeast catches up. *Cell Metab.* 6:1–2

Powers T, Dilova I, Chen CY, Wedaman K. 2004. Yeast TOR signaling: a mechanism for metabolic regulation. *Curr. Top. Microbiol. Immunol.* 279:39–51

Prinz S, Aldridge C, Ramsey SA, Taylor RJ, Galitski T. 2007. Control of signaling in a MAP-kinase pathway by an RNA-binding protein. *PLoS ONE* 2:e249

Reverter-Branchat G, Cabiscol E, Tamarit J, Ros J. 2004. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction. *J. Biol. Chem.* 279:31983–89

Rine J, Herskowitz I. 1987. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* 116:9–22

Rodriguez A, De la Cera T, Herrero P, Moreno F. 2001. The hexokinase 2 protein regulates the expression of the *GLK1*, *HXK1* and *HXK2* genes of *Saccharomyces cerevisiae*. *Biochem. J.* 355:625–31

Rusche LN, Kirchmaier AL, Rine J. 2003. The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu. Rev. Biochem.* 72:481–516

Rustchenko EP, Sherman F. 1994. Physical constitution of ribosomal genes in common strains of *Saccharomyces cerevisiae*. *Yeast* 10:1157–71

Santangelo GM. 2006. Glucose signaling in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 70:253–82

Schorling S, Vallee B, Barz WP, Riezman H, Oesterhelt D. 2001. Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisae*. *Mol. Biol. Cell* 12:3417–27

Seichertova O, Beran K, Holan Z, Pokorny V. 1973. The chitin-glucan complex of *Saccharomyces cerevisiae*. II. Location of the complex in the encircling region of the bud sear. *Folia Microbiol.* 18:207–11

Seo JG, Lai CY, Miceli MV, Jazwinski SM. 2007. A novel role of peroxin PEX6: suppression of aging defects in mitochondria. *Aging Cell* 6:405–13

Sinclair DA. 2005. Toward a unified theory of caloric restriction and longevity regulation. *Mech. Ageing Dev.* 126:987–1002

Sinclair DA, Guarente L. 1997. Extrachromosomal rDNA circles: a cause of aging in yeast. *Cell* 91:1033–42

Identified extrachromosomal rDNA circles as an aging factor in yeast.

Sinclair DA, Mills K, Guarente L. 1997. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. *Science* 277:1313–16

Smeal T, Claus J, Kennedy B, Cole F, Guarente L. 1996. Loss of transcriptional silencing causes sterility in old mother cells of *S. cerevisiae*. *Cell* 84:633–42

Smith ED, Tsuchiya M, Fox LA, Dang N, Hu D, et al. 2008. Quantitative evidence for conserved longevity pathways between divergent eukaryotic species. *Genome Res.* 18:564–70

Smith JS, Boeke JD. 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev.* 11:241–54

Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, et al. 2000. A phylogenetically conserved NAD^+-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl. Acad. Sci. USA* 97:6658–63

Spassieva S, Seo J-G, Jiang JC, Bielawski J, Alvarez-Vasquez F, et al. 2006. Necessary role for the Lag1 motif in (dihydro)ceramide synthase activity. *J. Biol. Chem.* 281:33931–38

Steffen KK, MacKay VL, Kerr EO, Tsuchiya M, Hu D, et al. 2008. Yeast lifespan extension by depletion of 60S ribosomal subunits is mediated by Gcn4. *Cell*. In press

Stewart MS, Krause SA, McGhie J, Gray JV. 2007. Mpt5p, a stress tolerance- and lifespan-promoting PUF protein in *Saccharomyces cerevisiae*, acts upstream of the cell wall integrity pathway. *Eukaryot. Cell* 6:262–70

Stockl P, Zankl C, Hutter E, Unterluggauer H, Laun P, et al. 2007. Partial uncoupling of oxidative phosphorylation induces premature senescence in human fibroblasts and yeast mother cells. *Free Radic. Biol. Med.* 43:947–58

Sun J, Kale SP, Childress AM, Pinswasdi C, Jazwinski SM. 1994. Divergent roles for *RAS1* and *RAS2* in yeast longevity. *J. Biol. Chem.* 269:18638–45

Tanny JC, Moazed D. 2001. NAD breakdown by the yeast silencing protein Sir2: evidence for acetyl transfer from substrate to an NAD breakdown product. *Proc. Natl. Acad. Sci. USA* 98:415–20

Tsuchiya M, Dang N, Kerr EO, Hu D, Steffen KK, et al. 2006. Sirtuin-independent effects of nicotinamide on lifespan extension from calorie restriction in yeast. *Aging Cell* 5:505–14

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Unlu ES, Koc A. 2007. Effects of deleting mitochondrial antioxidant genes on life span. *Ann. N.Y. Acad. Sci.* 1100:505–9

Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, et al. 2007. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell* 26:663–74

Provides evidence that Sch9 may be the functional ortholog of S6 kinase, a target of Tor, in yeast.

Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. 2003. Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* 426:620

Velours G, Boucheron C, Manon S, Camougrand N. 2002. Dual cell wall/mitochondria localization of the 'SUN' family proteins. *FEMS Microbiol. Lett.* 207:165–72

Walsh RB, Clifton D, Horak J, Fraenkel DG. 1991. *Saccharomyces cerevisiae* null mutants in glucose phosphorylation: metabolism and invertase expression. *Genetics* 128:521–27

Walsh RB, Kawasaki G, Fraenkel DG. 1983. Cloning of genes that complement yeast hexokinase and glucokinase mutants. *J. Bacteriol.* 154:1002–4

Watt PM, Hickson ID, Borts RH, Louis EJ. 1996. *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics* 144:935–45

Wawryn J, Krzepilko A, Myszka A, Bilinski T. 1999. Deficiency in superoxide dismutase shortens life span of yeast cells. *Acta Biochim. Pol.* 46:249–53

Wharton RP, Aggarwal AK. 2006. mRNA regulation by Puf domain proteins. *Sci. STKE* 2006:pe37

Willingham S, Outeiro TF, DeVit MJ, Lindquist SL, Muchowski PJ. 2003. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science* 302:1769–72

Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, et al. 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285:901–6

Xu F, Zhang Q, Zhang K, Xie W, Grunstein M. 2007. Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. *Mol. Cell* 27:890–900

Yang R, Wek SA, Wek RC. 2000. Glucose limitation induces *GCN4* translation by activation of Gcn2 protein kinase. *Mol. Cell. Biol.* 20:2706–17

Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, et al. 1996. Positional cloning of the Werner's syndrome gene. *Science* 272:258–62

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