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Review

Yeast aging research: recent advances and medical relevance

D. A. Sinclair

Department of Pathology, Harvard Medical School, 200 Longwood Avenue, Boston (Massachusetts 01215, USA), Fax + 1 617 432-3931, e-mail dsinclair@hms.harvard.edu

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Abstract. The molecular mechanisms of aging are most fully understood for the budding yeast *Saccharomyces cerevisiae*. Recent advances in our understanding of aging in this organism have enabled researchers to answer some fundamental questions about the aging process. Is aging due to a multitude of 'mechanisms' or can there be a key few? Can we design single-gene mutations that will prolong life? Can we prolong life whilst maintaining health and fecundity? The various contributing factors to yeast longevity, uncovered thus far, fall into three classes: DNA metabolism, heterochromatin, and metabolic activity. However, these separate classes may actually represent different aspects of the same aging mechanism based on genome stability. This review examines the recent advances in our understanding of yeast aging and discusses their relevance, if any, to the human condition.

Key words. Yeast; aging; senescence; helicase; silencing; nucleolus; metabolism; retrograde signaling.

In his philosophical treatise *On the Advancement of Learning*, Francis Bacon wrote that the "prolongation of life" is the most noble of all medical pursuits: "for if any such thing be found out [it] would be the greatest of earthly donations" [1]. Three hundred and fifty years later, are we truly on the verge of fulfilling Bacon's dream, as many researchers are now professing, or have we still a long way to go?

In the last few years, great advances have been made in our understanding of the molecular mechanisms of aging. This is particularly true for the yeast *Saccharomyces cerevisiae*. We now know that a main cause of yeast aging is the genetic instability of repeated, highly transcribed DNA. Multiple factors influence this instability, including DNA repair processes, replication, heterochromatic state and metabolic activity. As we investigate this (seemingly esoteric) mechanism of aging in more detail, the more likely it seems that broad aging principles will be learned by studying the mortality of unicellular organisms.

Mitotic versus non-replicative aging

Different parts of the human body are often considered to age by different mechanisms. Many researchers suspect that events leading to the deterioration of dividing 'mitotic' cells, such as those comprising skin and muscle, must be different from those that remain in a 'post-mitotic' quiescent state, such as neurons. The reason for this distinction is that aging in humans is often modeled using proliferating (i.e., dividing) cells in culture such as skin fibroblasts. The limited replicative capacity of these cells [2], due in part to telomere shortening [3], is considered by many to be a reflection of organismal aging [4]. Since this hypothesis would only operate in dividing cells, aging processes that are replication independent are often evoked to explain post-mitotic aging (e.g., oxidative damage and the accumulation of mutations).

In yeast, a distinction is also made between the aging of mitotic, actively growing cells and those in a quiescent state due to starvation. Mitotic aging in *S. cerevisiae*

was first identified by Barton [5] who found that individual 'mother' cells divide a finite number of times. This surprised many researchers who assumed unicellular organisms would be immortal. Similar to cellular senescence in metazoans, yeast mitotic life span is measured not in time but by the number of cell divisions. Yeast 'daughter' cells emerge from their mother as a bud, usually separating before the daughter reaches the mother's size [6].

By following the fate of individual mother cells using micromanipulation techniques, researchers have noted that aging yeast cells grow larger [5, 6], become sterile [7] and experience a slowing of the cell cycle [6]. Mother cells continue to produce small daughter cells throughout most of their life span. Approaching the final division, daughters tend to bud off when both cells are similar in size. These large daughter cells appear prematurely old and have a shorter than expected life span [8, 9]. However, this effect is not due to a permanent genetic alteration since descendants regain a normal life span.

Accurate markers of aging are essential to determine whether short-lived mutants are undergoing a normal aging process or are merely sick. This is true for both yeast and human aging. Unfortunately, none of the above yeast phenotypes, even in combination, has proved to be a definitive marker of aging. A good example is the *rad52* strain lacking recombinational DNA repair. *rad52* cells have a short life span and undergo rapid aging by all the above criteria, yet old *rad52* cells lack some crucial molecular aging markers [10] (discussed below). One solution to the current paucity of aging markers would be the generation of whole-genome transcriptional profiles during the life span using DNA microarrays.

DNA helicases and premature aging

Inherited human 'progeroid' diseases such as Werner syndrome (WS), Rothmund-Thompson syndrome (RTS) and Hutchinson-Guilford syndrome (HGS) are characterized by the appearance of symptoms that resemble premature aging [reviewed in ref. 11]. These diseases have attracted considerable attention because they may provide information about normal aging processes [12]. The genes responsible for WS [13] and a subset of RTS [14] have been cloned (designated *WRN* and *RECQL4*, respectively). In both cases, the genes encode members of the *Escherichia coli* RecQ DNA helicase family that convert double-stranded DNA into single-stranded DNAs [15] (fig. 1). While it is known that *E. coli* RecQ participates in recombination involving ssDNA gaps [16, 17] and the repair of UV-disrupted replication forks [18], the in vivo functions of the eukaryotic RecQ-like helicases are currently unknown. Other eukaryotic members of the RecQ family include the gene responsible for Bloom syndrome (*BLM*) [19], *Schizosaccharomyces pombe rqh1*$^+$ [20], *Drosophila melanogaster Dmblm* [21], a number of uncharacterized *Caenorhabditis elegans* genes, and *S. cerevisiae SGS1* [22, 23].

SGS1 was first identified in two separate studies: as a genetic and two-hybrid interactor with topoisomerase 3 (Top3p) [22], and as a two-hybrid interactor with Top2p [23]. Strains lacking *SGS1* have since been shown to exhibit elevated mitotic and meiotic chromosome missegregation [22–24], poor sporulation efficiency [25], and sensitivity to a variety of DNA-damaging agents [D. A. Sinclair and L. Guarente, unpublished results]. Mitotic recombination is also elevated in *sgs1* strains, as assessed by marker loss from the rDNA locus (*RDN1*) [22], subtelomeric Y′ elements and other loci [24]. In *sgs1* strains, telomere lengths and the stability of terminal telomeric sequences are the same as in wild type [24]. However, it is possible that Sgs1p is required for a secondary telomere maintenance pathway that would be revealed only in the absence of telomerase and the Rad52p-dependent recombinational pathway.

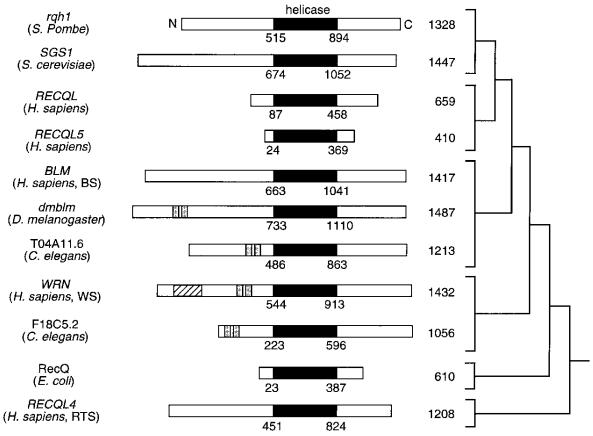

Figure 1. The RecQ family of DNA helicases. Schematic representation of RecQ family members aligned along the first residue of the helicase domain. *WRN*, *RECQL4* and *BLM* encode genes responsible for Werner syndrome (WS) and Rothmund-Thompson syndrome (RTS), and Bloom syndrome (BS), respectively. Patients with WS or RTS exhibit symptoms that resemble premature aging. BS is a disease of genetic instability characterized by elevated rates of cancer and sensitivity to sunlight. Total amino acid residues per polypeptide are shown to the right of the box. Repeated motifs are shown as gray boxes; the WRN domain homologous to RNaseD and DNA polymerase I is shown as a hatched box. Maximum-likelihood estimations of the five main RecQ family subgroups are shown far right [from top: yeast RecQ, RecQL, BLM, WRN, bacterial RecQ; adapted from ref. 21].

Sgs1p is a low-abundance nuclear protein concentrated in the nucleolus [26]. Human WRN also localizes predominately to the nucleoli of cultured fibroblasts, suggesting that WRN and Sgs1p have a similar function [27, 28]. The related BLM helicase is localized to discrete foci along synaptonemal complexes of bivalents in meiotic prophase [29]. The authors suggest that BLM may be required to expose ssDNA for homology checks prior to replication, or in the resolution of chromosome interlocks prior to anaphase I.

In vitro, Sgs1p unwinds linear dsDNA but has a preference for both forked and tetrahelical structures of guanine-rich sequences (G-DNA) [30]. Both *BLM* and *WRN* also resolve G-DNA in vitro [31, 32], although their affinity for certain structures differs significantly [32]. This has led to the attractive idea that these helicases resolve potentially deleterious DNA secondary structures that form spontaneously during replication, transcription or recombination [30, 32]. Such structures are more likely to form in GC-rich regions such as rDNA, telomeres and GC-containing trinucleotide repeats [32–36]. The latter postulate has recently been examined by Fry and Loeb [32], who showed that, at least in vitro, *WRN* efficiently unwinds bimolecular tetraplex structures of the Fragile X syndrome sequence, $d(CGG)_n$. Telomeres in $WRN^{-/-}$ fibroblasts are relatively unstable [37, 38], so perhaps the reduced replicative potential of $WRN^{-/-}$ cells is due to increased G-DNA formation and more rapid telomere erosion. G-DNA structures have not been identified in vivo, but this of course does not belie the theory. The development of novel G-DNA-specific antibodies, dyes or biochemical assays may finally permit a direct test of this theory.

Yeast mutants lacking Sgs1p function have life spans only 40% that of congenic wild-type strains and undergo what appears to be a rapid aging process [26]. Initially, young *sgs1* cells are indistinguishable from wild type by external phenotypes [26] and whole-genome transcript assay [D. A. Sinclair and L. Guarente, unpublished results]. After about five divisions, the *sgs1* cell cycle begins to slow and cells eventually become sterile due to a loss of silencing at the mating-type loci. As *sgs1* cells age, their nucleoli enlarge and eventually fragment into discrete foci that tend to array around the nuclear periphery. Nucleolar fragmentation has been proposed as an embodiment of damage occurring at that site, perhaps to the ribosomal DNA (rDNA) within nucleoli. This seemed plausible given the greatly increased level of rDNA instability in *sgs1* strains [22]. Although the exact function of Sgs1p is unknown, clues have recently been gained by studying the processes that lead to rDNA instability (see below).

Heterochromatin and mobile silencing factors

One theory of aging states that changes in the expression of specific genes may directly contribute to the aging process [39]. Changes in gene expression during aging have been well documented in many organisms, from yeast to human [40, 41]. Jazwinski and colleagues have characterized two yeast genes, *LAG1* [42] and *LAG2* [43] that are down-regulated in old cells and affect yeast longevity. Both encode proteins with putative transmembrane domains, but beyond this, little is known.

One way to control the expression of genes in a more global manner is through the establishment of silent heterochromatin. Heterochromatic changes have been proposed as a key determinant of the aging process [41]. In light of new findings, the heterochromatin model of aging could be expanded to include the fact that silencing factors also participate in cell cycle control [44–46], DNA repair [47–49] and the suppression of recombination at repetitive DNA [44, 50, 51]. In recent years it has become clear that the organization of heterochromatin within the yeast nucleus is central to longevity.

The yeast heterotrimeric Sir protein complex, composed of Sir2p, Sir3p, and Sir4p, is crucial for the establishment of 'silent' heterochromatin at telomeres and mating-type (*HM*) loci [52] (fig. 2). Deletion of any one of the *SIR* genes leads to telomere instability and shortening [51] but not senescence [52]. Sir2p is distinct from Sir3p and Sir4p because it localizes predominately to the nucleolus [53] where it contributes to rDNA stability [50], cell cycle control [45] and the silencing of Pol II-transcribed genes placed within the rDNA array [54, 55].

It is currently thought that silencing is mediated by the distribution of limited nuclear pools of Sir proteins to the various silent loci during replication [56, reviewed in ref. 57]. Evidence for this comes mostly from genetic analyses. Overexpression of Sir2p increases the strength of silencing at both telomeres and rDNA [58], implying that Sir2p is a limiting component of the silencing apparatus. Deletion of *SIR4* abolishes telomeric and *HM* silencing but increases rDNA silencing by presumably allowing the relocalization of Sir2p and Sir3p to the nucleolus [58].

The first link between yeast life span and heterochromatin was uncovered in a search for mutations that increased starvation resistance and life span [59]. Since there was no simple way to screen for long-lived individual yeast cells other than by microscopic examination, Kennedy and colleagues capitalized on the well-established correlation between stress resistance and longevity [reviewed in ref. 60]. Four mutations were isolated, named *uth1–4*, that conferred starvation resistance and a longer life span [59]. To date, the function

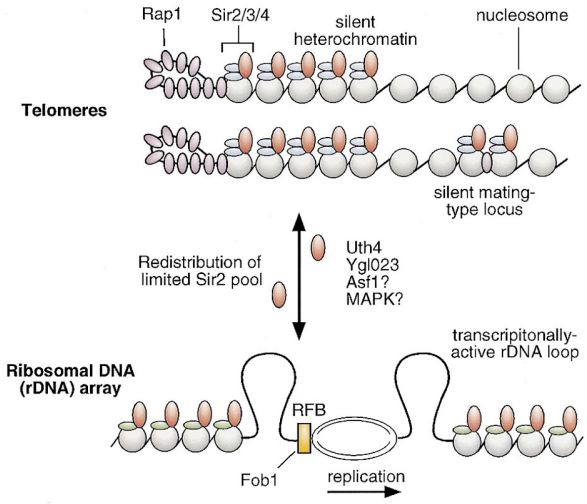

Figure 2. Yeast life span is regulated by the distribution of limiting heterochromatic factors. The Sir complex, composed of Sir2p, Sir3p, and Sir4p, is essential for the formation of 'silent' heterochromatin at telomeres and the two mating type loci, *HML* and *HMR*. Sir2p is distinct from Sir3p and Sir4p because it localizes predominately to the nucleolus where it contributes to rDNA stability, cell cycle control and the silencing of Pol II-transcribed genes placed within the rDNA array. Redistribution of the limited pools of Sir2 to the various silent loci is thought to occur in S phase. An increase in the abundance of Sir2p at the rDNA may reduce extrachromosomal circle (ERC) formation and extend life span. Some genes that affect yeast life span (e.g. *UTH4* and *YGL023*) may regulate the redistribution of silencing factors between the telomeres, HM loci and the rDNA locus. Fob1p sets up a unidirectional replication fork block (RFB) at the rDNA that is required for expansion, contraction, and homogenization of rDNA repeats.

of *UTH1* has not been ascertained and *UTH3* has not been cloned.

UTH4 encodes a homologue of the *Drosophila* Pumillio involved in RNA translational regulation and development [61, 62]. Both Uth4p and its yeast homologue, Ygl023p, appear to regulate the distribution of the Sir complex within the nucleus [53, 63]. Deletion of *UTH4* strengthens silencing at telomeres and weakens rDNA silencing, whereas *YGL023* has the opposite effect [63]. Overexpression of *UTH4* extends life span by 20%, whereas *YGL023* shortens it. The biochemical function of Uth4p and Ygl023p may be to regulate the translation of factors involved in the establishment of heterochromatin.

The semi-dominant *uth2* mutation (renamed *SIR4-42*), which extended life span by 30%, introduced a stop codon in the *SIR4* gene. This removed 121 amino acids from the C terminus of the peptide that is required for anchoring the Sir complex to telomeres. Although the precise mechanism by which *SIR4-42* extends life span is unknown, we do have some clues. The life-span-extending properties of the mutation were thought to be due to the liberation of the Sir complex from telomeres, which then bound to the putative 'age locus' [59]. This idea gained credence from immunofluorescence data, which showed that Sir4-42p was not at telomeres, but in the nucleolus [63]. Consistent with their roles in Sir relocalization, mutation of both *UTH4* and *YGL023* abrogated the localization of Sir4-42p to the nucleolus. Currently, the most plausible idea is that Sir4-42p reduces the chance of deleterious events at the nucleolus, or more specifically, the rDNA.

In addition to their role at telomeres and rDNA, Sir proteins have recently been shown to directly participate in dsDNA break (DSB) repair throughout the genome [48, 49]. Following the detection of a DSB, a DNA damage signal results in relocalization of the Sir complex from telomeres to DSBs, perhaps to modify chromatin at the site. It is not known if this activity is related to the life-span-extending properties of *SIR4-42*, nor if this activity is crucial for life span.

In addition to the elusive biochemical function of Sir4-42p, numerous questions raised by this research remain unanswered. For example, does Sir2p have a role in aging distinct from Sir3p or Sir4p? What is the life span effect of increasing the level of one, or all three, of these proteins? What effect do the yeast homologs of Sir2p, the Hst proteins [44], have on aging? Are the mammalian orthologs of Sir2p also important for the aging process, and if so, can we extend life span?

A molecular cause of yeast mitotic aging

As described above, two independent yeast aging studies identified the nucleolus as a putative 'Achilles heel' of the organism. The yeast nucleolus is a compact nuclear structure containing 100–200 tandem copies of a 9-kb rDNA unit on chromosome XII and other components required for ribosome assembly [reviewed in ref. 64]. The non-transcribed spacer (NTS) of each repeat contains three adjacent autonomously replicating sequences (*ARS*s) [65] that can support the replication of recombinant plasmids [66]. However, less than one-third of the repeats are used as origins of replication each S phase [67, 68]. In strains with low topoisomerase activity, up to 50% of rDNA exists as extrachromosomal circles known as ERCs [69].

Replication of the yeast rDNA is halted in a unidirectional manner by a replication fork block (RFB) that prevents the replication machinery from meeting 35S transcription head-on [68, 70]. RFBs have also been identified in *Xenopus* [71] suggesting that this is not an isolated phenomenon. Establishment of the yeast RFB requires Fob1p [72], a nucleolar protein [73] whose

biochemical activity is unknown. The RFB is central to the process that homogenizes the rDNA array, perhaps by gene conversion [72, 74]. In a *fob1* strain, the rate of marker gene loss from the rDNA is reduced about tenfold [73] and the copy number of rDNA repeats remains fixed [72].

The ERC model of yeast aging [75] was formulated to explain the exponential increase in aging phenotypes seen with age and the tendency for cells arising from old mothers to inherit a shorter life span. It is in agreement with the idea originally proposed by Jazwinski [40] that yeast aging is due to a triggering event that then leads to the exponential accumulation of an inheritable senescence factor. The main aspects of the ERC model are shown in figure 3. At a stochastically determined point in a yeast cell life span, a single ERC excises from the rDNA array. From this single molecule, ERCs then accumulate exponentially in the mother cell because ERCs have replicative potential (less than once per cell cycle is required) and remain predominately within the mother cell. When mother cells become old with abundant ERCs, they will inevitably leak into daughters aging them prematurely. In successive generations, ERCs are diluted out so that the daughters of the daughters do not inherit an ERC, and thus experience a normal life span. ERCs are thought to titrate essential cellular components, causing a slowing of the cell cycle and senescence.

What is the evidence for the ERC model? Old yeast cells contain between 500 and 1000 ERCs, both monomers and multimers of the 9-kb rDNA repeat [75]. These circles can replicate and segregate to the mother cell in a biased fashion. When mother cells become old, ERCs do tend to leak across into daughter cells, presumably making them prematurely old. Importantly, when a single ERC is introduced into young cells, it shortens their life span by 40% and accelerates the onset of age-associated phenotypes including sterility [75]. Thus, ERCs can cause yeast aging. Further validation of the model came from Defossez et al. [73] who showed that *fob1* strains have a reduced rate of ERC formation and live up to twice as long as their wild-type counterparts. Old *sgs1* cells also contain abundant ERCs, although it is not known if they cause premature aging [75]. A *fob1* mutation does not suppress the short life span, or rDNA instability of *sgs1* strains, suggesting that ERC formation in *sgs1* strains is independent of the RFB.

The precise events leading to ERC formation are not known, but recent results have helped formulate a plausible theory. It is known from work in *E. coli*, that a stalled replication fork is an inherently unstable structure that is converted into a DSB by physical or chemical insult [76]. The RFB in yeast may also result in a DSB before the opposing replication fork arrives (fig. 3). The free DNA end may then recombine with homologous sequences of adjacent rDNA repeats, resulting in an ERC [73].

Consistent with the ERC/RFB model of yeast aging is the finding that only certain types of DNA repair are required for a normal life span. Strains lacking DNA repair by single-stranded annealing (*rad1*), nucleotide excision (*rad7*), and transcription-coupled repair (*rad26*) have life spans equivalent to wild-type strains, whereas those lacking DSB-induced homologous recombination have compromised life spans [10]. Deletion of *rad52* abolishes homologous recombination and prevents ERCs from forming, but reduces life span by 60%. In this strain, it is thought that lethal DNA damage occurs at such a high frequency throughout the genome that the predicted life span extension is not observed. It is worth noting that in *rad52* strains, dying cells exhibit a relocalization of the Sir complex to the nucleolus and a loss of silencing at *HM* loci [10], implying that these markers of aging are not definitive. The finding that the Sir complex is mobilized to genome-wide DSBs [48, 49] also suggests that this age-related phenomenon may be part of a more general DNA damage response.

Metabolism, stress, and retrograde regulation

There is substantial evidence in support of the theory that reactive oxygen species (ROS) generated by aerobic metabolism cause aging by damaging a variety of cellular components [77]. Abundant molecular and genetic evidence for the theory comes from studies of the nematode *C. elegans*. The long-lived *age-1 C. elegans* mutants have increased levels of superoxide dismutase (SOD) and catalase [78, 79] and are more resistant to oxidative stresses. Similarly, overexpression of ROS defense enzymes can extend the life span of both *C. elegans* and *D. melanogaster* [80, 81]. Exposure of worms, flies, and yeast to heat stress also extends life span [82, 83]. The correlation between metabolism and longevity is also observed for mammals. For example, calorie-restricted rodents have a lower body temperature, reduced metabolic rate, and enjoy a relatively disease-free life that is 40% longer than mice fed ad libitum [reviewed in ref. 84].

The majority of ROS species are generated in mitochondria during aerobic metabolism. It has been proposed that damage to mitochondrial genomes is a direct cause of aging [reviewed in ref. 85]. The appearance of deletions in the mitochondrial genome and a decline in the activity electron transport chain correlate with age [86]. However, there is still considerable debate about their causative role in aging [reviewed in ref. 12].

A loss of respiratory function in yeast is readily discernable because cells fail to grow on non-fermentable

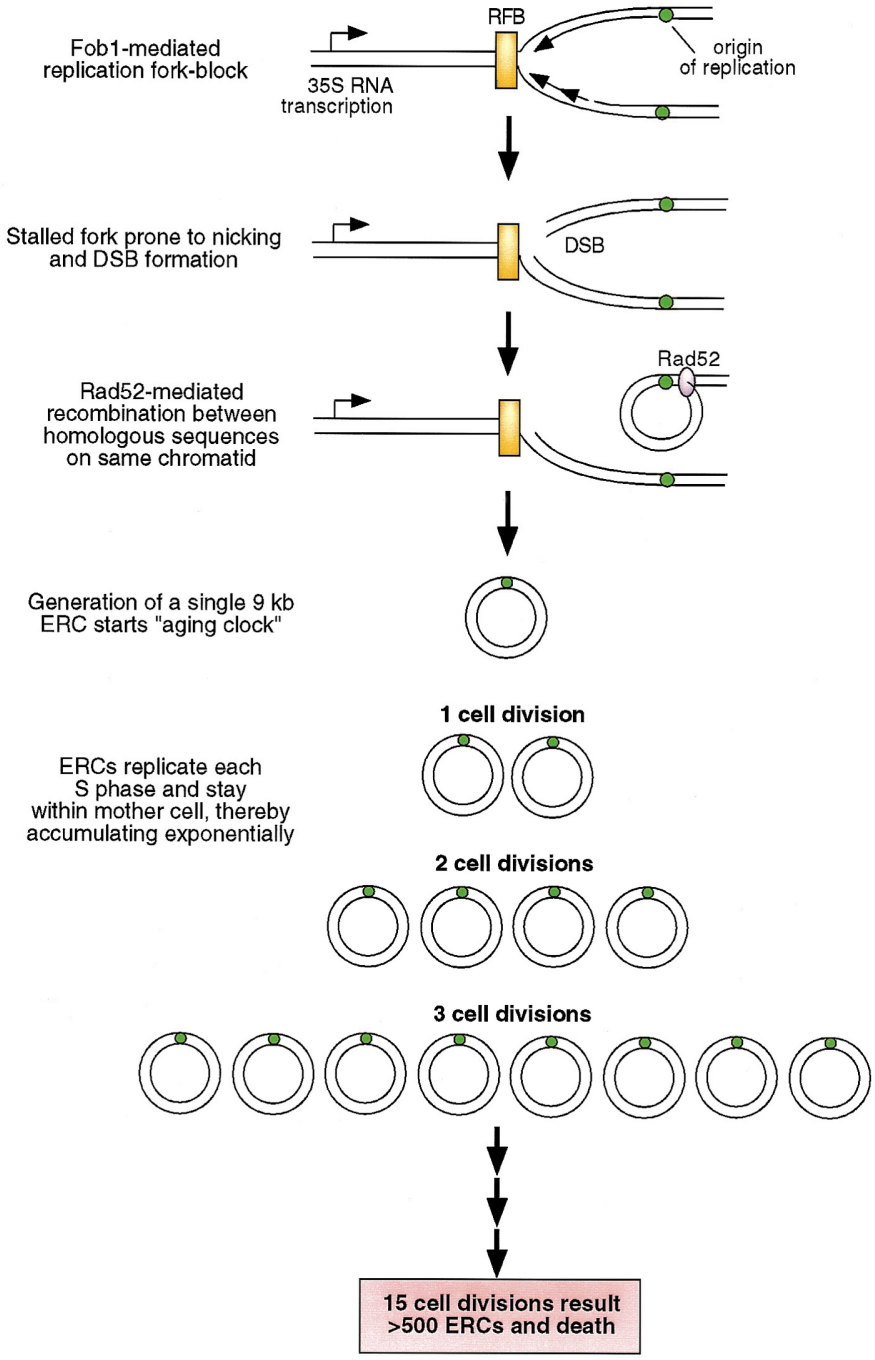

Figure 3. The rate of ERC formation governs yeast life span. At the rDNA, a Fob1-mediated replication fork block is an inherently unstable structure. Nicking of the ssDNA region leads to collapse of the replication fork, generating a gapped region and a double-strand DNA break (DSB). The free DNA end then recombines with an adjacent repeat unit on the same chromatid via Rad52, resulting in an extrachromosomal rDNA circle (ERC) composed of one or more 9-kb rDNA repeats. Because each rDNA repeat has three potential origins of replication, ERCs replicate during subsequent S phases. The majority of ERCs are held within the mother cell, causing them to accumulate exponentially. Old cells typically contain between 500 and 1000 ERCs that titrate essential transcription or replication factors. When mother cells become old, ERCs tend to leak into daughter cells, explaining why daughters of old cells are prematurely old.

carbon sources such as glycerol and ethanol. If mitochondrial mutations were a cause of yeast aging, we might expect cells to become increasingly respiratory incompetent as they age. A recent study by Kirchman et al. [87] has shown that this is not the case. However, in the same study, an intriguing correlation between the respiratory incompetence of 'petite' strains and life span extension was established. For some strains, petites have life spans up to 35% longer than the wild-type strain, whereas others are shorter than wild type. The life span extension of petites cannot be due simply to the loss of respiratory capacity since treament of respiratory-competent cells with antimycin A does not extend life span. Interestingly, the longer life span of petites requires *RTG2*, a gene involved in the retrograde signaling pathway that regulates the expression of nuclear genes when mitochondrial function is compromised. Deletion of the nuclear gene *COX4* (encoding cytochrome oxidase subunit IV) also extends life span, demonstrating that an aspect of the mitochondrial dysfunction per se, and not a specific mitochondrial mutation, is responsible for the effect.

What is the relationship between retrograde signaling and the ERC mode of yeast aging? We do not know, but it is possible that an *rtg2* mutation down-regulates the rate of rDNA transcription, thereby increasing the stability of the rDNA, reducing the rate of ERC formation. It has been reported that a petite strain has higher level of ERCs [88], exactly opposite to what we would expect by this model. However, given that petite mutations can cause either a substantial extension or an extreme shortening of life span depending on the strain used, no conclusions can be drawn at this stage about this finding, or the relationship between ERCs and retrograde signaling.

Disruption of the yeast *RAS2* gene, encoding a G-protein involved in the yeast nutrient response, has previously been shown to increase life span [89]. A clue to this function has come from the finding that a *ras2* deletion completely abrogates the life span extension of an *rtg2* mutation, although the retrograde response appears intact [87]. The authors favor the idea that Ras2p modulates the part of the retrograde signal required for life span extension.

Yeast cells enter stationary phase when deprived of nutrients, where they remain viable for months. It is hoped that the study of stationary-phase-dependent aging will be useful for understanding aging in other non-dividing cells. Stationary phase survival of yeast requires the products of Cu/Zn SOD, implying that ROS are damaging during this period [90]. Cells that are released from stationary phase have a reduced mitotic life span, proportional to the length of time they are held in stationary phase [91]. Importantly, daughter cells from starved mothers experience a normal life span, demonstrating that the effect is not due to irreversible genetic damage. Of the cells that re-enter the cell cycle, many of the aging phenotypes are observed, proportional to the shortened life span. ERCs do not accumulate to high levels, implying that another process causes these cells to senesce.

Do yeast cells really age?

Individual yeast cells do experience aging. They go through a predictable series of biochemical changes as their cellular functions break down exponentially with age, leading to senescence. This is the hallmark of any aging process [92]. But definitions aside, is there a reason to believe that yeast aging has any resemblance to human aging? Without sufficient experimental data to answer this question, we can at least take an evolutionary approach. Like all other life forms, yeast cells are subject to the laws of natural selection. All organisms have evolved to invest sufficient energy into their development and survival to produce competitive offspring. This is not to say that all organisms age in exactly the same way, but that the selective forces (or more precisely, the lack of forces) that shaped their mortality are similar.

Often the question is asked: if producing more offspring per individual is advantageous, why are yeast cells (or humans for that matter) not immortal? The answer lies in the idea that fitness is costly [93, 94]. For an organism, it may be a better allocation of resources to produce offspring rapidly without building a soma that is long-lasting [95]. This is most true for species living in extremely hazardous environments where there is little advantage conferred by investing in reproduction much beyond an age where it is likely to have been killed [95]. This explains why small animals and insects at the bottom of the food chain tend to breed and age rapidly, while it is advantageous for animals at the top of the food chain to build bodies that last.

What is the relevance of yeast aging to humans?

The conservation of metabolic and signaling pathways between yeast and humans is strikingly high, leading to the expectation that aging mechanisms will also be common to both organisms. This is not necessarily the case. While there are only a few highly efficient ways of metabolizing energy and responding to stimuli, there are thousands of ways for organisms to die. However, as discussed earlier, there are reasons to believe that in both organisms, similar cellular systems will fail earlier than others. Certainly, many of the genes that extend yeast life span have human counterparts (table 1).

Table 1. Homologues of *S. cerevisiae* genes involved in aging.

S. cerevisiae	*C. elegans*	*H. sapiens*	Function in *S. cerevisiae*
FOB1	?	?	replication fork block
LAG1, *LAC1*	C09G4.1, K02G10.6	*PGDF1*	unknown
LAG2	?	?	unknown
RAS2	*RRP-1*, *LET-60*	*Ras2*, *R-RAS2*, *RAP1-B*, *RAP1-A*	GTP-binding protein, signaling
RTG2	?	Hsp70-type ATP-binding-domain proteins (eg. Grp78)	signaling from mitochondria to nucleus
SGS1	EA3A3.2, T04A11.6, K02F3.1	*WRN*, *BLM*, *RECQL*, *RECQL4*, *RECQL5*	DNA helicases
SIR2	R11A8.4, F46G10.3, F46G10.7	*SIR2α*, *SIR2β*, *SIR2γ*	silencing/NHEJ/rDNA stability
SIR3	?	?	silencing/NHEJ
SIR4	?	?	silencing/NHEJ

? = no clear homologues in database (11 June 1999); NHES = non-homologous end-going of DNA breaks.

Given that the causes of human aging are not known, it is difficult to assess the relevance of yeast aging research at this stage. Nonetheless, it is worth addressing. Arguably the greatest contributions from yeast aging research thus far are the answers to some fundamental questions about aging. For example, we now know that:
1) Aging can be surprisingly simple. This is not to say that human aging will be simple, but that aging need not be overwhelmingly complex.
2) As an extension of this, not all components of an organism necessarily break down simultaneously as the organism ages. The corollary is that not all biological systems are equally prone to failure.
3) Single-gene mutations can be *designed* that dramatically extend an organism's life span. This ability is perhaps the best test of any aging hypothesis.
The strict analogy between mitotic aging in yeast and cultured mammalian cells is not a good one. First, individual human cells are not subject to natural selection as is a yeast cell. A human mitotic cell in an adult is an expendable, transient component of the organism. A yeast cell is the organism. Secondly, telomeres do not shorten during the life time of a yeast cell because of intact telomere maintenance mechanisms. But all is not lost. Yeast lacking telomerase activity will continue to serve as an excellent model of cellular senescence and, if the telomere hypothesis is correct, will be crucial aids to the development of drugs that stem the aging process.
If there is one unifying theme emerging from recent aging studies, it is that the genome is a particularly difficult cellular system to maintain over long periods. It suffers great onslaughts from DNA-damaging agents and normal cellular processes, and to make matters worse, minor defects can be lethal. In mitotic yeast cells, longevity is critically dependent on genome maintenance mechanisms especially at the repeated rDNA locus. The rDNA is the 'Achilles heel' of the organism not only because it is a difficult system to maintain but also because the deleterious effects of ERC formation are delayed until an age where additional longevity does not confer an advantage.
What is the relevance of yeast ERCs to humans? Extrachromosomal DNAs have been found in all mammalian tissues examined thus far [96]. However, no study has shown a clear correlation between the accumulation of circular DNAs and aging. Will they be found to accumulate in a subset of cells, such as stem cells? In light of the evolutionary arguments above: not necessarily. There are certain to be other sequences in the human genome more likely to encounter instability in the absence of maintenance. Telomeric sequences and repetitive GC-rich sequences are good candidates. Even if the specific unstable DNA sequences differ between species, the proteins that help maintain the genome are likely to be conserved. Elucidation of the roles of these proteins in yeast and human aging will be a great step forward in our quest to understand the molecular causes of aging.

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