Journal of Gerontology: BIOLOGICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci
2010 Vol. 65A, No. 1, 1–8
doi:10.1093/gerona/glp152

Advance Access published on October 29, 2009

Review

Fission Yeast and Other Yeasts as Emergent Models to Unravel Cellular Aging in Eukaryotes

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In the past years, simple organisms such as yeasts and worms have contributed a great deal to aging research. Studies pioneered in *Saccharomyces cerevisiae* were useful to elucidate a significant number of molecular mechanisms underlying cellular aging and to discover novel longevity genes. Importantly, these genes proved many times to be conserved in multicellular eukaryotes. Consequently, such discovery approaches are being extended to other yeast models, such as *Schizosaccharomyces pombe*, *Candida albicans*, *Kluyveromyces lactis*, and *Cryptococcus neoformans*. In fission yeast, researchers have found links between asymmetrical cell division and nutrient signaling pathways with aging. In this review, we discuss the state of knowledge on the mechanisms controlling both replicative and chronological aging in *S pombe* and the other emergent yeast models.

Key Words: Longevity—Yeast—*Schizosaccharomyces pombe*—*Candida albicans*—Replicative life span—Chronological life span.

In the past years, a considerable number of publications have significantly furthered our understanding on the mechanisms regulating aging (1,2). Despite this important advance, the molecular determinants of aging in eukaryotes remain ill-defined. Certainly, further research is needed to identify cellular factors and chart their interactions in order to elucidate the pathways regulating aging. For this endeavor, simple model organisms such as nematodes, flies, and yeast represent powerful tools for the study of aging because of their convenient short life span and their amenability to easy genetic manipulation. Several large-scale genetic screens carried out with worms or yeasts led to the identification of numerous genes involved in aging, which subsequently were tested in mammals (3). As a corollary, the extrapolation from one eukaryotic species to another is for the most part possible because altogether, these studies demonstrated that many functions known to control longevity are conserved within the eukaryotic kingdom (4).

In unicellular fungi, the mechanisms of aging are investigated by using two distinct kinds of studies. Replicative life span (RLS) refers to the number of daughters a single cell can produce before it dies, and so it is measured as a number of divisions or generations (5,6). Chronological life span (CLS) measures the time a population of cells survives in stationary phase (7). Some studies compared both types of aging in *Saccharomyces cerevisiae* and tried to reconcile them showing that replicative and chronological aging were dependent on each other (8,9). However, the effects of knocking out more than 500 genes on both kinds of life spans suggest that replicative and chronological aging are controlled independently (10).

For both replicative and chronological aging, budding yeast has been a highly effective organism for the discovery of novel genes involved in the regulation of aging. The relevance of the discoveries related to the study of aging in budding yeast to other eukaryotes has been shown numerous times. For example, dietary restriction increases RLS and CLS of *S cerevisiae* and extends longevity of metazoans as well (11,12). Importantly, the function and often the sequence of the proteins encoded by the genes discovered in yeast are frequently conserved in multicellular eukaryotes (3,13,14). Moreover, in yeast, nutrient signaling pathways are controlled by the serine threonine kinases Tor, Sch9, and PKA. These pathways are all known to have proaging effect, and their homologs in animals share conserved functions in aging. For instance, loss of Tor1 kinase extends both replicative and chronological aging of budding yeast (15–17) and has the same effect on flies and worms (18). Likewise, deletion of the adenylyl cyclase *CYR1*, which acts upstream of the PKA kinase, lengthens life span in budding yeast (19). The knockout of the adenylyl cyclase AC5-activating PKA increases mice's longevity as well (20). The Sch9 kinase, which controls replicative and chronological aging in yeast (17,19), was proposed to be the ortholog of the kinase S6K1, which is known to be related to the control of aging in mice, *Drosophila*, and *Caenorhabditis elegans* (14,21). The role of sirtuins in aging was first discovered in *S cerevisiae*, where an extra copy of the *SIR2* gene increases RLS (22). Later, these proteins have been found to belong to a highly conserved family of NAD^+-dependent enzymes acting as regulators of aging in other lower organisms (23). In yeast, invertebrates, and mammals, they act in functions related to longevity, such as genomic maintenance (24), regulation of stress resistance (25,26), metabolism, and glucose tolerance (27,28).

Logically, following the fruitful use of *S cerevisiae* to investigate the mechanisms of aging, in the past years, various research groups turned to other yeast models. In this review, we elaborate on the relevance of the use of different unicellular organisms to unravel the process of aging, particularly focusing on fission yeast. Data from other unconventional yeasts like *Candida albicans*, *Kluyveromyces lactis*, and others will be also discussed. The comparison of similar species having different life spans can help to identify universal molecular factors acting on aging.

What Makes Schizosaccharomyces pombe an Attractive Model for the Study of Aging?

For historical and cultural reasons, *S cerevisiae* is by far the most popular yeast used in the laboratory. The "other yeast" model, *Schizosaccharomyces pombe*, has been developed because it brings a distinct and complementary tool to study the biology of the eukaryotic cell. These two yeasts are separated from a common ancestor by 400 million years, leaving each other much time to evolve separately (29). A separate evolution took place during this long period of time; it has been speculated that budding yeast could have lost some functions that fission yeast and animals conserved from their common ancestor (30). Indeed, genome studies showed that most proteins for messenger RNA (mRNA) splicing are conserved between *S pombe* and metazoans but are not found in *S cerevisiae* (30). The same observation was done for other RNA-binding proteins, including those in the RNA interference machinery (31), nuclear structural proteins, chromatin- and centromere-binding proteins (30,32), and some glycoprotein-folding proteins in the endoplasmic reticulum (33). In addition, the Wis4/Wis1/Sty1 stress response pathway in fission yeast is mitogen-activated protein (MAP) kinases dependent, similar to mammals where the stress response activates the MAP kinases p38 and JNK (34,35).

Mitochondrion is a central organelle in the regulation of aging (36,37). As budding yeast, fission yeast is Crabtree positive, which refers to their ability to turn down respiration in the presence of glucose (38,39). However, mitochondrial activity proved to be differently regulated in these two yeasts. First, mutants without mitochondrial DNA (rho^0 or *petite*) are more difficult to isolate in *S pombe*. For a long time, *S pombe* was ranged as petite negative but such mutants were finally isolated in a recent study (40,41). The reason for such difficulty to obtain *S pombe* petite clones was probably linked to the very poor growth of this mutant (40). Furthermore, the basal level of respiration when this yeast is cultured in glucose is lower than in budding yeast (39). Besides, the mitochondrial inheritance in fission yeast is mediated through the microtubule network as in mammalian cells (42). Altogether, these observations suggest a tighter dependence of fission yeast on mitochondrial activity, as is the case in mammalian cells. Regarding the multiple conservation of *S pombe* functions with those of multicellular organisms, this yeast represents a complementary and very interesting model to study aging.

Table 1. RLS of *Schizosaccharomyces pombe*

Wild Type Background	Mean RLS*	Maximum RLS*,†	*N*	Reference
NCYC132	9.2	14	48	(45)
h$^-$ 972	15.9	21	75	(46)

Notes: RLS = replicative life span.
* Unit: number of divisions.
† Maximum RLS attained.

Replicative Aging in S pombe

RLS is measured by micromanipulating a single mother cell to count the number of daughters produced before death (43). In order to succeed in this analysis, the biologist has to separate the mother cell from its daughters, which implies that one needs to distinguish one from the other. This operation is easily carried out in *S cerevisiae*, in which the division is morphologically asymmetrical with the formation of a bud. It is not the case in *S pombe* because it divides by fission, which results in the synthesis of a septum in the middle of the cell. This renders the identification of mother and daughter cells more complicated. Due to this apparent symmetrical morphology of the two cells after division, *S pombe* siblings were considered as sisters (44). This particularity of binary fission makes this species a very interesting model to study RLS of higher eukaryotes because it represents a mechanism of division similar to that of mammalian cells.

Despite the difficulty to isolate the mother from its daughter, Barker and Walmsley (45) succeeded to measure RLS of *S pombe*. They observed that the first, the second, and sometimes the third divisions of a virgin mother cell are morphologically symmetrical. However, the fourth and the next divisions are not symmetrical. Taking advantage of the fact that the mother cell becomes rounder and bigger while it replicatively ages, they could recognize it from the forming daughter and measure its RLS.

Interestingly, the average life span of the NCYC132 background of *S pombe* is only 9 divisions, and the maximum is attained after 14 divisions (Table 1) (45). The same experiment was repeated later in the wild-type background h$^-$ 972, which is commonly used by most laboratory today, and scored a mean RLS of young cell at 16.5 generations, with a maximum of only 21 generations (Table 1) (46). In this work, the protocol was improved; the authors took advantage of the presence of fission scar(s) on the mother to distinguish it from its daughter, which allowed separating them from the very first division.

The equivalent analysis in budding yeast resulted in an average life span of more than 20 divisions for most backgrounds (47). *Saccharomyces cerevisiae* can reach an average of 26 generations and a maximum RLS of more than 50 in the commonly used BY4742 strain, which makes this assay particularly long (47). The characteristic of fission yeast to have a shorter average and maximum RLS should greatly accelerate the experimentation with this species. Moreover, the fact that just a few different wild-type background exist

and that most laboratories use the h⁻ 972 background will avoid discrepancies associated to strain-specific effects on longevity, like it happened in budding yeast (48).

The forming bud and the following newborn daughter of *S cerevisiae* contain less carbonylated proteins (characteristic of old damaged proteins) than their corresponding mother (49). Consequently, the division is a rejuvenation event for the new cell. Despite the fact that mitosis in *S pombe* cells results in median fission, differences in stress resistance of the siblings were already known in this species. Later, the same asymmetrical distribution of carbonylated proteins was observed in fission yeast than in budding yeast (46). This partitioning mechanism is dependent on the histone deacetylase Sir2, like in *S cerevisiae*. Interestingly, conserved functions of heterochromatin assembly and DNA damage response have been reported for Sir2 and its homolog Hst4 in fission yeast (50,51). The partitioning during cell division relies also on Tea1, a protein known to act in polarized growth (52). Fission yeast cell polarity has been intensively studied, which will be of great advantage to explore the role of microtubule-mediated division in replicative aging. To date, few studies have looked for genes affecting RLS in fission yeast, and yet this model system looks very promising.

Chronological Aging in S pombe

Protocols and Conditions

Replicative aging in yeast is considered as a model for actively dividing cells like germ line cells, whereas chronological aging constitutes a model for differentiated somatic cells (53). CLS is measured on a population of billions of cells by following their survival during stationary phase, a low metabolic state following exponential growth (7). This protocol was validated recently in fission yeast with the characterization of the increased CLS in two knockouts of serine threonine kinases involved in nutrient sensing (54). Since then, several other studies of chronological aging have been carried out with this model.

The protocol to measure CLS is very similar to the one described previously for *S cerevisiae* (7). Basically, cells are grown in a given medium until saturation. When the maximum optical density is reached, the culture is left in the incubator for 1 or 2 days before the analysis in order to avoid late divisions. Then, samples from the liquid aging cultures are serially diluted and plated on rich solid medium to count colony-forming units. This method was verified by comparing results with those obtained with vital dyes like phloxine B and propidium iodide (54,55). Four different types of media were used in fission yeast aging assays: (a) synthetic medium (Edinburgh Minimal Medium, EMM, see (56)) completed with all amino acids, called synthetic dextrose completed or SDC (54); (b) synthetic dextrose (SD) medium (55,57); (c) rich medium based on yeast extract supplemented by auxotrophic compounds (55,58); and (d) minimal medium EMM alone (55). SD is a classical medium used in budding yeast. It is made from yeast nitrogen base, but today, it is less used in *S pombe* because it impairs normal growth. Indeed, in SD medium, fission yeast does not reach the optimal optical density like in yeast extract (YE) or in EMM, about $OD_{600\ nm}$ 2–3 in SD (57,59) and about $OD_{600\ nm}$ 8–10 in YE or EMM (54,60).

Dietary Restriction

Different nutritional manipulations allowed an extension of life span in yeast. In *S cerevisiae*, the most common intervention is to grow cells in a low concentration of glucose during exponential phase and follow survival in stationary phase. It can also be achieved by replacing the growth media by water or by changing the amino acids composition (7,14,61). In fission yeast, some of these conditions extended CLS. First, this was obtained by lowering the glucose in the medium from the classical 20 g/L concentration by a factor of 4–40 (0.5%–0.05% final). This intervention does not work in all conditions as only two types of media enabled such regulation of life span by glucose: yeast extract–based medium (58) and SD medium (55,57). Interestingly, glucose restriction in synthetic minimum media like EMM or EMM completed with amino acids (SDC) failed to increase CLS (55,58). One proposed explanation was that growth in synthetic media is already a dietary restriction that is dominant over the effects of glucose on longevity (58). This hypothesis is reinforced by the observation that, in this minimal medium, the respiration rate is upregulated even in high concentration of glucose, similar to what happens in rich medium with low glucose (62). Chen and Runge (55) even showed that overnutrition of glucose in EMM could slightly enhance life span. In this case, a larger availability of energy appears to be helpful and can favor the maintenance functions to increase CLS because dietary restriction is attained independently of glucose. Alternatively, the glucose signaling pathway may be altered in EMM medium, consistent with our results that mutants of this pathway live longer despite growing in high levels of glucose.

In budding yeast, synthetic media supplemented with selected nutrients also lengthen CLS compared with rich media based on yeast extract (7). The use of different media per se increases life span in fission yeast as well, through a mechanism probably linked to dietary restriction. In fact, growth in EMM minimum medium precedes a very long survival in stationary phase compared with that in rich YE medium or in SD, which both induce short life span (55,57,58). Interestingly, completing the EMM synthetic minimum medium with all amino acids in a medium called SDC decreased CLS without changing the concentration of glucose (54,57). This result suggests that exogenous amino acids induce proaging signals, like noticed in *S cerevisiae* with the Tor-dependent signal turned on after amino acids

addition (15). Another possibility to explain the divergences in survival of a strain grown in different media comes from recent observations made in budding yeast showing that the metabolic intermediates ethanol and acetic acid determine chronological longevity (12,63). We can speculate that differences in metabolic state of fission yeast dictated by the nutritional environment could influence the production of these metabolites and consequently the CLS. To date, no study has reported an effect of ethanol and acetic acid on life span of *S pombe*.

Another protocol used in budding yeast to study dietary restriction is "extreme calorie restriction," and it consists in isolating cells that have entered stationary phase from their medium, wash them and let them age in sterile water, in the absence of any nutrient (7,12). This condition has been reproduced in *S pombe* in the SD medium and also increased strongly CLS (64). Finally, growing fission yeast in glycerol 3% as sole carbon source in SDC medium increased considerably CLS, up to sevenfold the life span of the same wild-type strain in glucose 2% (58). To date, this condition is reportedly the most efficient to extend longevity in this organism. This strong life-span extension with glycerol could be the consequence of a compilation of different effects: lack of glucose signaling and increased respiration (58), increased osmolarity (65,66), and chemical chaperone protection (67).

Nutrient Signalings

Nutrient signaling via the kinases Sch9/Tor/PKA has strong proaging effects in *S cerevisiae*, and downregulation of these pathways is partially responsible for dietary restriction–dependent CLS increase (14,16,68). Our laboratory first focused on similar pathways in fission yeast. We found that two homologs of these kinases display similar proaging activity in *S pombe*, Pka1 and Sck2 (54). Other studies confirmed our results thereafter (55,57). The first gene, *pka1*$^+$, codes for the active subunit of the PKA complex, which is regulated by the Cgs1 subunit, as represented in Figure 1 (69,70). Contrary to its budding yeast counterpart, the activity of Pka1 is carried out by a single protein and is not essential. The effect of the PKA active subunits on longevity has not been reported in budding yeast because the triple knockout of the three orthologs with redundant PKA activity (*TPK1-3*) is lethal. Indirect involvement of PKA activity in aging was determined by deletion of the regulatory subunit *BCY1*, of the adenylyl cyclase *CYR1* or using G protein *RAS2* mutants, and led to contradictory results (71). In *S pombe*, we took advantage that the knockout of Pka1 is viable to show the direct link between aging and this serine threonine kinase (54). Upstream from PKA, the membrane glucose receptor Git3 induces proaging effects through the Gα protein Gpa2, which signals the presence of glucose to Pka1 via cyclic adenosine monophosphate (cAMP) produced by the adenylyl cyclase Git2 (Figure 1)

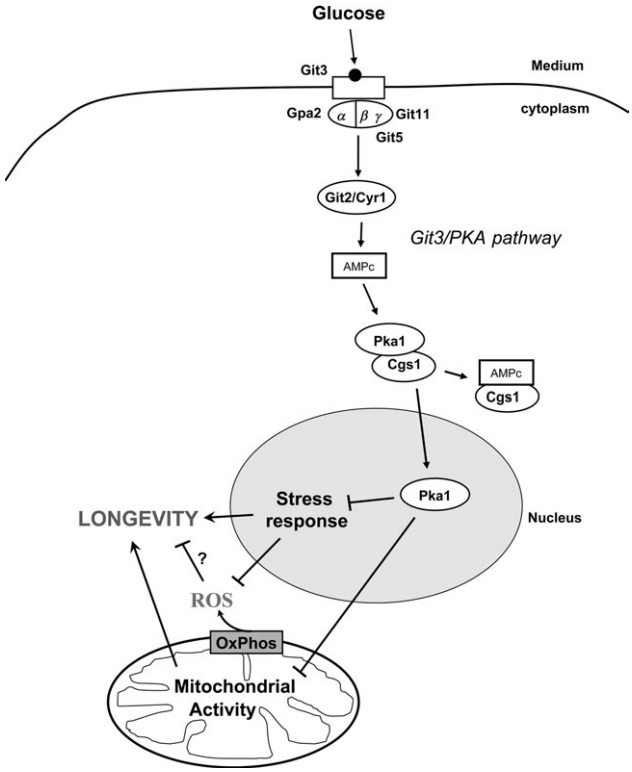

Figure 1. The Git3/Pka1 pathway in *Schizosaccharomyces pombe* shortens life span. The Git3 membrane receptor is activated by glucose and transduces a signal to the Gpa2 Gα protein, which in turn activates the adenylyl cyclase Git2/Cyr1. The production of cAMP induces the release of the Pka1 kinase from the Cgs1 regulatory subunit and its translocation to the nucleus. This correlates with decreased stress resistance, mitochondrial respiration, and shortened chronological life span. The Sck2 kinase has a similar effect on aging but the underlying mechanism is unknown. ROS = reactive oxygen species.

(58,72). The Git3/PKA pathway is responsible for the downregulation of stress resistance and mitochondrial activities, possibly causing premature aging by promoting the accumulation of reactive oxygen species (ROS; Figure 1) (54,58). The genes responsible for longevity regulation in fission yeast are summarized in Table 2.

The Sch9 serine threonine kinase is the gene that has the strongest proaging effect in *S cerevisiae*. Two homologs exist in fission yeast: Sck1 and Sck2, which stand for *S*uppressor of loss of *c*AMP-dependent protein *k*inase (74,75). The deletion

Table 2. Genes Involved in Chronological Longevity Extension in Fission Yeast

Gene	Function	Manipulation*	Medium Used	Reference
pka1	Ser/Thr kinase	Δ	SDC, SD	(54,57)
git3	Ser/Thr kinase	Δ	YEC	(58)
sck2	Ser/Thr kinase	Δ	SDC, SD	(54,55,57)
icl2	Fatty acyl-CoA synthetase	Δ	SD	(73)
ecl1/2/3	Unknown	oe	SD/EMM/H_2O	(57,64)

Notes: EMM = Edinburgh Minimal Medium; SD = synthetic dextrose; SDC = synthetic dextrose completed; YEC = yeast extract completed.

*Δ refers to the deletion of the corresponding gene; oe refers to its overexpression on plasmid.

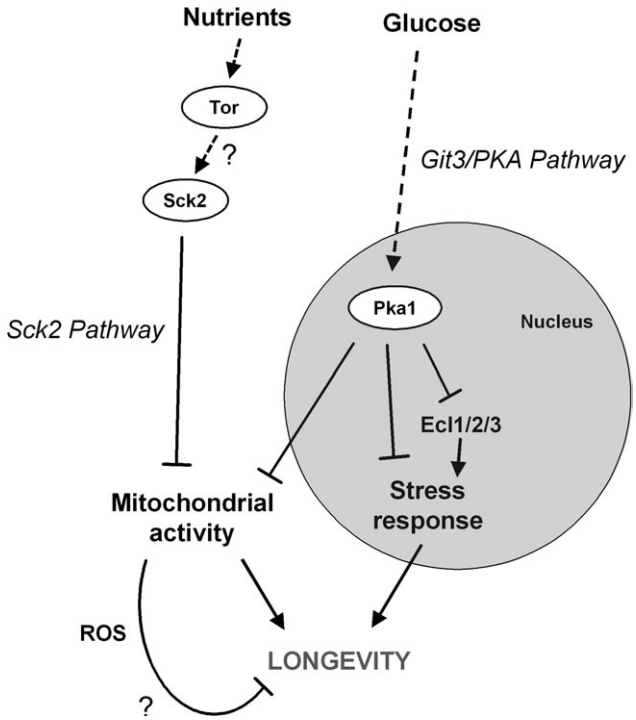

Figure 2. Model for the mechanisms responsible for aging regulation downstream of kinases Pka1/Sck2/Tor.

of *sck2*$^+$, but not *sck1*$^+$, was shown to increase significantly CLS of *S pombe*. Interestingly, this life-span extension is not followed by a gain of stress resistance in stationary phase like in Δ*pka1*, which may explain why the effects of these two deletions are additive (54). In *S cerevisiae*, Sch9, the Sck2 homolog, is a major target of Tor1, and both these kinases are important regulators of longevity (14,21). Moreover, Tor1 kinase inhibits respiration and mitochondrial translation, including the synthesis of numerous proteins involved in respiratory chain complexes (16). Interestingly, deletion of *TOR1* enhances life span independently of mitochondrial ROS-detoxifying enzyme Sod2. However, this is accompanied by decreased ROS production. The authors proposed that sustained renewal of oxidative phosphorylation chain components could ameliorate electron transfer during mitochondrial respiration, thus lowering ROS production. In a microarray study, we found that knocking out *sck2*$^+$ gene increased the quantity of mitochondrial-encoded mRNAs in stationary phase compared with wild type (unpublished data, 2009). This suggests that Sck2, like Sch9 in budding yeast, is regulated by TOR complex and could act on mitochondria instead of decreasing stress resistance (Figure 2) (54).

Sck1, the other homolog of Sch9, was first described not to be responsible for aging effects (54). However, another study revealed a late and slight proaging effect after 12 days in stationary phase; at earlier time points, the wild-type and the Δ*sck1* curves are indistinguishable (55). At this point, only 0.01%–0.001% of the cells are still alive, so Sck1 has a minor effect on longevity compared with its homolog Sck2.

Stress Response Pathway

The stress-activated protein kinase (SAPK) pathway is governed by the Wis4/Wis1/Sty1 MAP kinase cascade activated through many external stimuli, like nutrients, osmotic stress, thermal stress, and oxidative stress (35,76). Interestingly, the downstream transcription factor Atf1 is required for long-term survival as well as the Sty1 MAP kinase (57,77). Sty1 is responsible for the expression of many stress response proteins, including ROS scavengers, heat shock proteins, and osmotic response proteins (35). Interestingly, the rapid death following the loss of Sty1 is rescued by overexpression of *ecl1*$^+$, although this overexpression failed to further increase life span of Δ*pka1* (57). Moreover, a link between the Pka1 serine threonine kinase and Wis1/Sty1/Atf1 has already been proposed (54,78). Similarly, the Tor1 serine threonine activity has been shown to regulate Sty1 phosphorylation through Pyp2 phosphatase (79). Altogether, these results suggest that downregulation of the SAPK pathway could be involved in the proaging function of the Git3/PKA pathway.

Other Genes

A family of very small proteins called Ecl (*e*xtender of *c*hronological *l*ife span) has been described as positive regulators of chronological longevity (64,80). Combined to Pka1 deletion, the overexpression of *ecl1*$^+$ does not increase further the CLS, although in a wild-type background, it activates the expression of *ste11*$^+$, a target negatively regulated by Pka1 (64). These findings argue for a negative regulation of Ecl1 by Pka1 (Figure 2). Finally, the loss of the acyl-CoA synthetase Icf2 lengthens CLS in cells grown in SD medium, whereas the loss of its homolog Icf1 induces the rapid death in stationary phase (73,81). Interestingly, the deletion of *icf2*$^+$ did not increase further the life-span extension obtained by glucose reduction, suggesting a role for long-chain fatty acyl synthesis in calorie restriction (73). The essential role of lipid metabolism in longevity has been depicted in other eukaryotic organisms and was particularly analyzed in budding yeast and invertebrates (82).

Other Emergent Yeast Models to Study Aging

Apart from *S pombe*, other yeast models for eukaryotic aging have been developed in the past few years. Fu and colleagues focused on the replicative aging of *C albicans*. They took advantage of the fact that this species has two distinct morphological states: a yeast-like form called blastopore and a filamentous form called hyphae. The passage from one to the other depends on nutrient composition, pH, or temperature. They showed that both forms have similar RLS (about 20 generations) (83). They took advantage of the fact that the hyphae mother cell gives rise to smaller blastopore daughters that can be sorted out by centrifugation on a sucrose gradient. This way, they easily isolated replicatively old mother cells. Finally, they showed that the RLS was not determined by extra chromosomal

circles in this organism, like in budding yeast, but was nevertheless dependent on *SIR2* gene dosage. This study proposes a new model for aging with unique features greatly facilitating the large-scale screens for genes involved in replicative aging. This represents a significant advance as this protocol is hardly achievable in budding and fission yeasts, which require micromanipulation to isolate mother from daughter cells.

Kluyveromyces lactis is a Crabtree-negative yeast for which glucose limitation does not promote an enhancement of the respiratory capacity (84). Recently, a study compared the effect of dietary restriction by glucose reduction in *S cerevisiae* and *K lactis* (80). Surprisingly, the CLS of *K lactis* is not sensitive to glucose concentration like in *S cerevisiae* and *S pombe* (55,57,58,80). This feature coincides with the lack of respiration regulation by glucose, unchanging cytochrome content, and NADH-cytochrome c reductase activity, functions that are enhanced in low concentration of glucose in *S cerevisiae* (80). These results suggest that calorie restriction–dependent increase in longevity may be due to mitochondrial control and more particularly the regulation of oxidative phosphorylation activity.

Cryptococcus neoformans is an encapsulated yeast causing fungal meningoencephalitis in patients with advanced HIV infection and in some cases in immunocompetent hosts (85). In a recent study, Jain and colleagues (86) analyzed replicatively old *C neoformans* that they referred as "senescent" cells. Older cells lose replicative capacity but unexpectedly displayed better survival to the antifungal agents and no significant differences in virulence in mice. The authors showed that this yeast can undergo at least 31 generations before it dies and maybe more generations during infection. *Cryptococcus neoformans* is a promising model in part due to the data already accumulated on the signaling pathways in this species (87). For instance, the homologs of the Sch9 and Pka1 kinases have been studied to investigate their effect on the virulence of this pathogenic yeast in mice (87,88). The Sch9 homolog controls resistance to thermal stress. It would be interesting to examine if Sch9 and Pka1 homologs are also involved in replicative and chronological aging like in other yeasts.

Today, comparative studies using different mammalian models like the mole rat versus mice are in progress and promise novel insights into the mechanisms of aging (89,90). However, many yeast species are routinely grown in laboratories. The few already characterized for their longevity displayed differences in longevity as, for example, *S pombe* has shorter CLS and RLS than *S cerevisiae*. New yeast models could be quickly used for such comparative approaches that might bring new insights into the biology of aging. Furthermore, life span can be rapidly scored in these organisms, but it takes decades with long-lived mammals. For example, using yeast in comparative studies of aging, it would be easy to test the disposable theory of aging in comparing the relation between the rate of growth and longevity (91). Similarly, it would not require much effort to test the oxidative stress theory of aging on different yeast models or to study protein damage to obtain insights into the "proteotoxicity" hypothesis of aging (92).

Funding

This work was funded by Canadian Institutes for Health Research Grants IAP-79713 and GI MOP-89702 to L.A.R.

Acknowledgments

We wish to thank the members of the Rokeach laboratory for helpful discussions. P.C. and G.F. are senior scholars from the Fonds de la Recherche en Santé du Québec.

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Received July 24, 2009
Accepted September 17, 2009
Decision Editor: Huber R. Warner, PhD