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Review: To Bud Until Death: The Genetics of Ageing in the Yeast, *Saccharomyces*

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Individual cells of the budding yeast, *Saccharomyces cerevisiae*, have a limited division capacity and undergo characteristic changes as they senesce, primarily increasing both their cell size and cell cycle time. The mortality curve for ageing yeast cells can be described by the Gompertz equation, the classical definition for an ageing population. Recent work from several laboratories has demonstrated that genes can determine the yeast lifespan. Studies with the *UTH* genes have implicated changes in transcriptional silencing during yeast ageing, but the roles of the *RAS2*, *LAG1* and *PHB1* genes in regulating yeast longevity are still unclear. What is becoming clearer, however, is that yeast ageing is more than just a bud scar phenomenon.

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INTRODUCTION

Species-specific lifespans and longevity differences among members of the same species have always been given as evidence that genetic factors influence ageing. The nature of the genetic elements involved, however, has remained controversial. One possibility, that of ageing genes which act solely to promote the deterioration and eventual death of the organism, has often been dismissed on evolutionary grounds. The primary argument is that there is no selection for genes which act during a period of life which most organisms in a natural population never reach because of predation or deleterious environmental conditions. In fact, natural selection would favour the selection of ageing gene loss-of-function mutants which do not age and therefore continue to produce immortal progeny.

A more non-adaptive view looks at ageing as the biological equivalent of wear and tear. The functional decline of the organism would arise from the accumulation of damage during its post-reproductive period of life which is not subject to the pressures of natural selection. The genetic elements which determine lifespan, according to this view, would not be ageing genes but longevity genes, which act to prevent damage and delay the organism's loss of viability.

A priori, loss of function mutations in ageing and longevity genes should lengthen and shorten an organism's lifespan respectively. In recent years, candidate longevity and ageing genes have been identified in different genetic model systems and the functional analysis of their gene products should answer some outstanding questions on the nature of the ageing process. On the one hand, mutants in *Caenorhabditis elegans* and *Drosophila melanogaster* are providing new insights into the ageing of postmitotic tissues.[16,18,27] On the other, *Saccharomyces cerevisiae* is emerging as one model for replicative senescence and cellular ageing.

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THE AGEING PROCESS IN BUDDING YEAST

Defining ageing in budding yeast is possible only because of the asymmetry of its cell cycle. The mother cell is usually larger than its newly budded daughter and can therefore be identified at the end of each cell division. This allowed Mortimer and Johnston[20] in 1959 to show for the first time that an immortal yeast colony was in fact made up of mortal yeast cells. Using a tetrad dissecting microscope, they were able to isolate virgin cells and determine that these cells only completed a certain number of cell cycles before arresting. They called this limited division potential the lifespan of the yeast cell. The colony, however, was immortal because each daughter cell reset its clock and had the potential for a full lifespan.[12]

In the past 35 years, a few laboratories have characterized the yeast ageing process and defined several biomarkers for this organism. Probably the most obvious feature of a senescent yeast cell is its large size. Several studies have shown that the volume of the yeast cell increases linearly with age and that the senescent cell can be four times as large as an exponentially growing cell.[9,20] Another parameter that changes with age is the duration of the cell cycle, which increases exponentially with increasing generations.[7,20] This lengthening in the period of the cell cycle results from an increase in the time spent in the unbudded portion of the cell cycle. Though it is not absolute, most old cells arrest during this G1 stage.[12] One thing that is clear is that ageing in yeast is not dependent upon chronological time. Extending the cell cycle time either by decreasing the temperature or by changing the carbon source does not change the mean number of generations that a population of cells can complete, even though it can double the calendar time required to age.[14,24] In fact, changing the carbon source from glucose to ethanol, which nearly doubles the cell cycle time, actually increases the lifespan.[24] Finally, ageing yeast cells undergo morphological changes while they age. They acquire wrinkles from a loss in turgor pressure[22] and accumulate refractile granules (Figure 1, lower panels) which stain with Sudan Black B, a lipid-specific dye (unpublished data). These lipid granules are reminiscent of the lipid pigment, lipofuscin, which is known to accumulate in aged cells of a diverse range of organisms.[13]

At the molecular level, RNA and protein content are increased several fold with age,[21] while specific transcripts have been shown to either increase or decrease with increasing generations.[8] In contrast, the protein synthetic rate measured by the incorporation of radioactive amino acids per unit amount of protein is reported to decrease linearly with age.[21] It has been speculated that the decrease in the synthesis of critical limiting proteins may be responsible for the prolongation of the unbudded phase in older cells.[21]

UNLIKELY EXPLANATIONS FOR YEAST AGEING

Several hypotheses have been proposed to explain the senescence of yeast cells. One of the earliest is that the yeast cell arrests when it has exceeded some critical cell size and a minimum surface-to-volume ratio.[12,20] Experiments that have increased cell size and volume either by varying the ploidy of the cell[22] or by enlarging arrested cells with mating pheromone,[14] however, demonstrate that these do not decrease lifespan.

Mortimer and Johnston[20] also suggested that bud scars may limit the availability of surface area for budding or for nutrient exchange with the environment. Recent studies of daughters of older mothers have obtained data inconsistent with this hypothesis.[14] As mentioned before, during each asymmetric division, the smaller bud has a full life expectancy. At a low probability, however, an ageing cell produces a bud of similar size which has a lifespan identical to the mother's remaining lifespan. These symmetric divisions argue against a causal role for bud scars in determining longevity because mother and daughter cells do not have the same number of scars. In addition, increasing the deposition of chitin, the major component of bud scars, using a conditional *cdc24* mutant has little effect on longevity.[7]

Yeast senescence cannot be easily explained by theories which posit the accumulation of mutations or genetic errors, because most daughters of old cells which inherit a copy of the old genome are young[12,14] and because haploid and diploid cells have identical lifespans.[14,15] In addition, no increase in the numbers of auxotrophies or petite mutations have been observed in old cells.[22] Finally, senescence has been shown to be dominant in the rare zygotes generated between a young and an old haploid.[25] Thus, the young genome cannot replace any functions that may have been lost in the old cell.

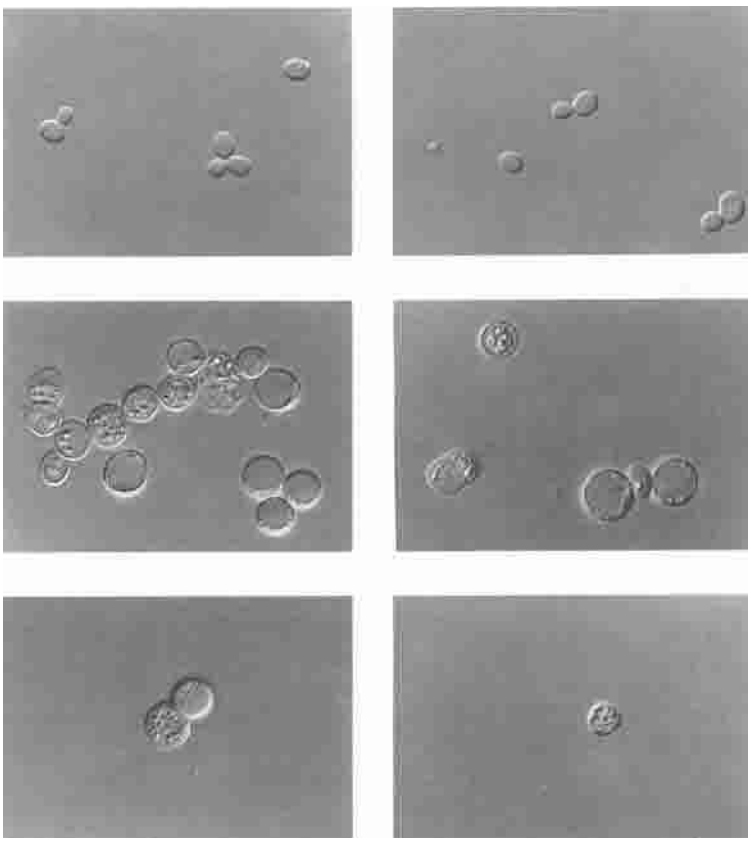


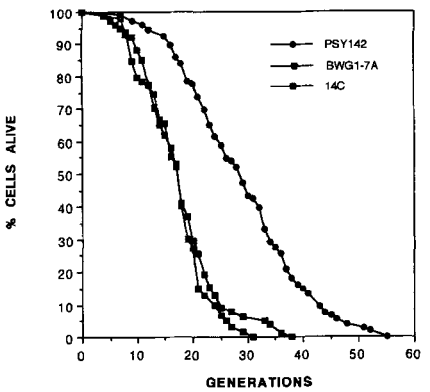
Figure 1. Similarity between ageing and starving cells. Exponentially growing haploid yeast cells in rich media (upper panels). Haploid cells growing in sporulation media (middle panels) increase in volume and accumulate granular particles. These starving cells resemble cells of the same strain which have been micromanipulated until they senesced (lower panels). All photographs were taken at 1000 x magnification using Nomarski optics.

One popular hypothesis for the senescence of cell populations, both yeast and mammalian, is that division capacity is limited by the length of telomeres—cells die when their telomeres get too short.[1,36] This can be directly tested in yeast by taking advantage of mutations which modulate telomere length. For example, a deletion of *SIR4* shortens telomeres while a *rap1s* mutation lengthens them.[28,35] Lifespan analyses of strains bearing these mutations show that there is no correlation between telomere length and longevity (unpublished data). Furthermore, populations of senescent yeast do not show any evidence of telomere shortening, suggesting that telomeres play no part in longevity control.[4,32]

Given these unlikely explanations, why do yeast cells age? One possibility, supported by several observations, is that yeast contain a senescence factor that accumulates in old mother cells and is inherited by their daughters.[7,14] A primary goal for yeast ageing research has become the isolation and characterization of genes which may encode this senescence factor or other regulatory elements of longevity.

THE SEARCH FOR YEAST AGEING AND LONGEVITY GENES

Ageing in yeast can be described by the Gompertz equation, the classical definition for ageing populations, which states that the mortality rate, the probability of dying in the next time interval or generation, increases exponentially with age.[31] This is evident in the sigmoidal shape of the yeast



	LIFESPAN mean	maximum	SAMPLE SIZE
14C	16	31	94
PSY142	28	55	105
BWG1-7A	17	37	87

Figure 2. Mortality curves for different wild-type yeast strains. Populations of haploid yeast strains of different genotypes were micromanipulated until they ceased further division. The difference in means between strain PSY142 and strains BWG1-7A and 14C was deemed statistically significant by the Wilcoxon Signed Rank Test.[15]

mortality curve, which depicts the percentage of a cell population able to complete a given number of cell cycles (Figure 2). Furthermore, different wild-type yeast strains have different mean and maximum lifespans, suggesting that ageing in yeast is not a purely stochastic process and that the genetic background can influence the longevity of the strain (Figure 2).[14,15,20] Tetrad analysis has shown that longevity in yeast is a polygenic trait that is not cytoplasmically inherited.[15]

Mutations in many yeast genes have no effect on longevity. Auxotrophic mutations fall into this category, as do mutations in the mating pheromone response pathway.[14,15] Not surprisingly, loss-of-function changes in genes involved in the maintenance of crucial growth functions shorten lifespan. Included in this class are UV-sensitive mutants,[23] ρ minus mutants, and mutants in non-essential components of the transcriptional apparatus (unpublished data). Most of these, however, also have a slow growth phenotype. It is therefore difficult to interpret the role of these genes in the control of the ageing process because the shortened lifespan could simply be a consequence of a decrease in the overall fitness of the mutant strain. Ideally, a candidate longevity gene should be defined by loss-of-function mutations which shorten lifespan without changing any cell cycle parameters or growth characteristics of the cell. In addition, overexpression of the gene might be expected to extend lifespan. The *RAD9* gene involved in cell cycle checkpoint regulation is one gene which satisfies the first criterion.[14] The second criterion for *RAD9* has not yet been tested.

Yeast homologs of genes involved in proliferation control of higher eukaryotic cells are prime candidates for genes which determine longevity in yeast. Jazwinski and colleagues have reported that a deletion in *RAS2*, one of the yeast homologs of the mammalian RAS oncogene, shortens lifespan while overexpression leads to a 30% increase in longevity.[34] The precise role of the *RAS2* gene in longevity control is still unclear, however, because deletions of this gene have also been reported to lengthen lifespan.[30] Finally, deletion of the yeast gene *PHB1*, the yeast prohibitin homolog, is reported to lengthen lifespan.[10] Prohibitin is a candidate tumour suppressor gene which has been implicated in the biogenesis of breast cancer.[19] Because mammalian prohibitin is a mitochondrially localized protein (K. McClung, personal communication),[11] it will be interesting to see how the mitochondria influences cell cycle and proliferation control.

Two approaches, one biochemical and one genetic, have been taken to identify novel genes which regulate longevity. Jazwinski and coworkers used a differential hybridization screen to isolate cDNAs that are preferentially expressed either in young or in old cells.[8] One gene called *LAG1* (longevity assurance gene) encodes a putative transmembrane protein.[5] Transcript levels decreased with replicative age and deletion of the gene reportedly increased the yeast lifespan by 50%. Five more transcripts remain uncharacterized.

Guarente and colleagues have taken advantage of a correlation between stress resistance and longevity to isolate long-lived yeast mutants.[15] As shown in Figure 1, ageing cells morphologically resemble haploid cells starving in sporulation media, suggesting that starvation and ageing may be similar processes regulated by the same genes. This was confirmed when we identified mutations in the *UTH* (youth) genes which increased both stress resistance and longevity.

UTH2 was the first gene to be cloned by complementation of its sterile phenotype.[15] Sequencing

showed that it is allelic to *SIR4*, a gene involved both in the repression of the silent mating type loci and in the regulation of telomeric silencing and structure.[28] *Uth2-42* is a gain-of-function mutation of the *SIR4* gene; a deletion of *SIR4* has little effect on the yeast lifespan. The extension of lifespan, however, is dependent upon an intact *SIR3/4* complex, suggesting that the silencing complex somehow regulates the expression of an ageing gene(s), the putative *AGE* locus. Strikingly, recent work with populations of old cells isolated with a purification protocol involving sorting with magnetic beads demonstrated that derepression of the silent mating type information occurs with age.[32] Furthermore, experiments with single cells deleted of the HML a information have demonstrated that derepression of the silent mating type information occurs in individual senescent cells.[32]

Cloning of *UTH1* was done by complementation of the paraquat sensitivity of the mutant (unpublished data). It is a novel gene located on chromosome XI. Disruption of the open reading frame extends longevity, suggesting that *UTH1* is a yeast ageing gene. The gene is a founding member of the *SUN* (*SIM1*, *UTH1*, *NCA3*) family of yeast genes which share a 215 amino acid domain (the SUN domain) at their C-termini. *SIM1* (START independent of mitosis) was isolated in a screen for cell cycle mutants which undergo two rounds of DNA synthesis without an intervening mitosis.[3] *NCA3* (nuclear control of ATPase) has been implicated in the regulation of the expression of the mitochondrial ATP synthetase.[29] Another yeast gene in this family, tentatively called *SUN4*, has been identified by the yeast sequencing effort. Lifespan analysis of cells carrying disruptions in the other members of the *SUN* family revealed that *SIM1*, *NCA3* and *SUN4* have characteristics of yeast longevity genes.

Finally, the dominant mutation in *UTH4* is a change in the previously isolated gene *HTR1*[17]/*MPT5*[2] which was identified in screens for genes required either for high temperature growth and recovery from pheromone arrest or for suppression of a mutation in *POP2*, a gene involved in glucose repression (B. Kennedy and L. Guarente, unpublished data). It is still unclear how these phenotypes are related to the yeast ageing process.

Our preliminary experiments have placed the different *UTH* genes in a genetic pathway that may regulate the yeast ageing process. Like the original *uth2-42* mutant, mutations in *UTH1* and *UTH4* which extend lifespan require the presence of an intact *SIR4* gene. This places these *UTH* genes upstream of the SIRs, suggesting that they may affect the localization or activity of the silencing complex at the putative AGE locus. Attempts are underway to determine the molecular basis for *UTH* gene action and to identify downstream targets. A paradigm that is emerging is that ageing in yeast may result from inappropriate gene expression late in the lifespan causing death, in this case from loss of gene silencing.

FUTURE DIRECTIONS

What is next? A priority in the field should be to tighten the genetic and ideally the mechanistic relationships between the different genes which are thought to determine yeast longevity. In *C. elegans*, for example, genes which affect lifespan isolated in different ways by different laboratories now seem to all belong to the dauer pathway.[6,16,18] It will therefore be interesting to see if links between the *UTH* genes and *LAG1* or *PHB1* exist.

One reason put forward earlier for believing that there were longevity-determining genes in yeast is that different strains have different lifespans. How does one explain this? Laboratory strains of yeast are under no selection for longevity and there is no reason to expect all longevity mechanisms to be preserved in divergent strains. Hypothetically, long-lived strains could already have lost a subset of genes which limit longevity. Given the redundancy and non-essential nature of the *SUN* genes, for example, it is not difficult to imagine that different combinations of alleles could exist in different strains accounting for the variation in lifespan. The contrasting effects of *RAS2* deletions on lifespan mentioned earlier may be one manifestation of polymorphism. On the other hand, there is no evidence that different strains age in the same way. All yeast ageing may result from inappropriate gene expression but different genes may become deregulated in different strains. Only a study of the effects of the same combinations of mutations in different strains will resolve this issue.

One can argue that we will never really understand the yeast ageing process until we can completely manipulate it at will. What are the prospects for an immortal yeast cell? Three possibilities should be explored. The first two depend on the number and nature of the pathways which regulate lifespan and the third is simply a matter of symmetry.

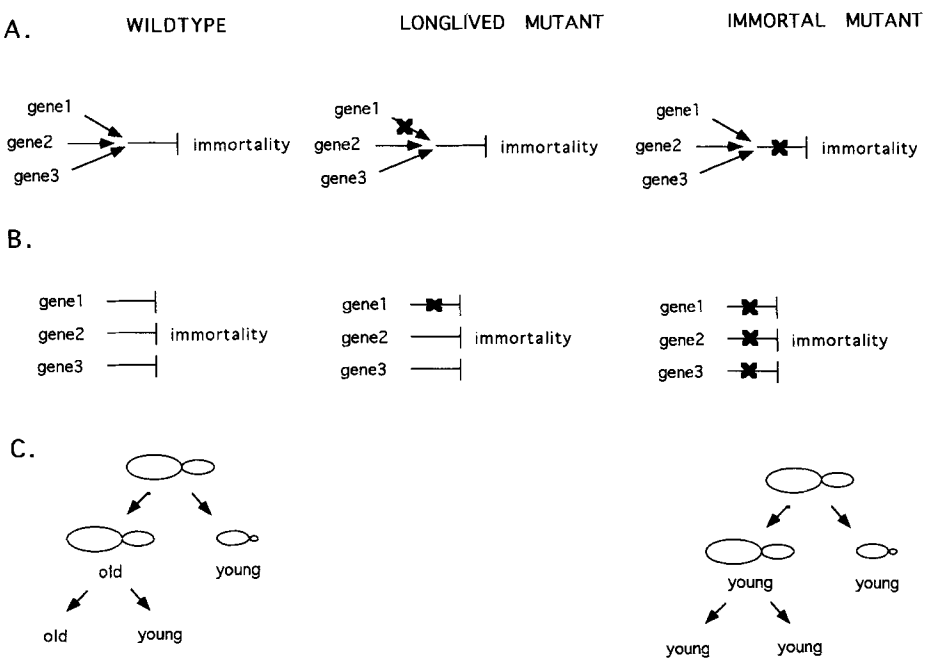


Figure 3. Three scenarios for yeast immortality. (A) If one genetic pathway regulates longevity, then a single gene mutation can result in immortality. (B) Several independent genetic mechanisms preventing immortality would mean that multiple genetic events would be required for perpetual youth. (C) A mutation which breaks the asymmetry of the clock-resetting mechanism would make the mother cell immortal without affecting the basic yeast ageing mechanism.

Simplistically, one may postulate that there is a single pathway-regulating ageing as there is only one biosynthetic pathway in the cell for methionine production. The mutations in the known longevity-determining genes lie upstream of a common pathway. A mutation downstream of the junction point like the elimination of the putative senescence factor would remove ageing and the cell would be immortal (Figure 3A). At the other extreme, one can envision a situation where there are several parallel and independent blocks to immortality. To achieve perpetual youth, the cell must acquire the correct combination of mutations, which maximizes longevity and minimizes ageing. This might involve deletions of all ageing genes with concurrent high overexpression of several longevity gene products (Figure 3B). This second scenario predicts that no single gene mutation can ever result in immortality.

The third route to the fountain of youth for yeast may be easier to achieve. The key to immortality may already exist given that the yeast colony is already immortal. This immortality results from the asymmetric resetting of the clock which determines the reproductive potential of the cell. Only one other instance exists where the mother/daughter asymmetry is clear and that is in the asymmetric regulation of *HO* expression; mothers switch, daughters do not.[26] Even so, in some instances, daughters have been found to switch at a low frequency.[33] Is this break in asymmetry analogous to the symmetric buds seen in old cells where the daughter fails to be rejuvenated? If asymmetry is the key to immortality for the colony, then a mutant which partitions the clock-resetting mechanism to increase the chances that the mother's clock is reset at some frequency, making her 'young', would be immortal (Figure 3C). One must keep in mind, however, that a symmetry mutant could also result in clonal senescence and a mortal colony. Nevertheless, the question of immortality is reduced then to a question of symmetry.

CONCLUSION

The identification of single gene mutations which modulate longevity has opened up the field of ageing to the power of yeast genetics. It remains to be seen, however, if any of these regulatory

pathways is functionally conserved in models of replicative senescence in higher cells. What is clear is that yeast ageing has become more than just a bud scar phenomenon. It is an area of research which is still young.

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