Winter 12-2-1994

Daughter Cells of Saccharomyces cerevisiae from Old Mothers Display a Reduced Life Span

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Abstract. The yeast *Saccharomyces cerevisiae* typically divides asymmetrically to give a large mother cell and a smaller daughter cell. As mother cells become old, they enlarge and produce daughter cells that are larger than daughters derived from young mother cells. We found that occasional daughter cells were indistinguishable in size from their mothers, giving rise to a symmetric division. The frequency of symmetric divisions became greater as mother cells aged and reached a maximum occurrence of 30% in mothers undergoing their last cell division. Symmetric divisions occurred similarly in *rad9* and *ste12* mutants. Strikingly, daughters from old mothers, whether they arose from symmetric divisions or not, displayed reduced life spans relative to daughters from young mothers. Because daughters from old mothers were larger than daughters from young mothers, we investigated whether an increased size per se shortened life span and found that it did not. These findings are consistent with a model for aging that invokes a senescence substance which accumulates in old mother cells and is inherited by their daughters.

A hallmark of aging in an organism is that the probability of death increases exponentially with age (Gompertz, 1825). In *Saccharomyces cerevisiae*, cell division involves budding of daughter cells which are smaller than mother cells upon division (Hartwell and Unger, 1977). By micro-manipulating daughters away from mothers, the fate of mother cells can be followed during multiple rounds of cell division (Mortimer and Johnston, 1959). Through this kind of analysis, it was determined that mothers divide a relatively fixed number of times before stopping, and the probability of stopping increases exponentially as the number of prior divisions increases. These experiments therefore showed that yeast cells age and have a specific life span that varies around a given mean.

A number of phenotypes are manifest during the aging process in yeast. Cells enlarge as they age (Mortimer and Johnston, 1959). The increase in volume has been demonstrated to be linear with regard to the age of the cell (Egilmez et al., 1990). Aging cells divide more slowly: cell cycle time can increase as much as sixfold during the course of a cell's life span (Mortimer and Johnston, 1959). Finally, a decrease in fertility has been observed in old cells (Müller, 1985).

When old cells are mated to young cells, the resultant diploids live for a number of generations most similar to the remaining life span of the older cells, suggesting that aging in yeast may be a dominant characteristic (Müller, 1985). This dominance may be due to some substance that is synthesized or accumulates in old cells. Consistent with this possibility, Egilmez et al. (1989) have demonstrated that certain mRNAs are preferentially found in old cells.

Aging in yeast appears in many ways to be similar to senescence in mammalian fibroblasts. There appears to be an underlying genetic basis to both processes. Fibroblasts undergo an increase in cell size as they age (Sherwood, et al., 1988), and a correlation between cell size and senescence can be demonstrated by incubating young human diploid fibroblasts (HDFs)[1] in low serum medium. These cells arrest in the G1 phase of the cell cycle, enlarge and display a decreased division potential when returned to normal serum containing medium (Angello et al., 1989). Also, cell fusion studies between old and young HDFs indicate that senescence is dominant (Norwood et al., 1974). Injection of polyA$^+$ RNA from senescent HDFs into young cells was sufficient to inhibit DNA synthesis, further supporting the notion that senescence is dominant and suggesting that a gene or a number of genes encoding inhibitory proteins are expressed in senescent cells (Lumpkin et al., 1986). These apparent similarities between yeast and animal cells suggest yeast may be a fruitful model system in which to study aging. Indeed, the mechanisms of other cellular processes, such as that of transcription and cell cycle progression, appear to be conserved in these organisms.

Many theories have been put forth to explain yeast aging. The most prominent involves the accumulation of bud scars as the cell ages (Johnston, 1966). After each division, a mother cell accumulates a chitin-containing ring which

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1. *Abbreviations used in this paper*: ANOVA, analysis of variance; HDF, human diploid fibroblasts.

covers approximately 1% of the surface area of the cell (Bartholomew and Mittwer, 1953). Since the cell wall of the daughter is newly synthesized in the process of division, old scars are not transferred from mother to bud (Farkas, 1979; Johnson and Gibson, 1966). In *S. cerevisiae*, these bud scars have never been observed to overlap on the surface of a cell (Bartholomew and Mittwer, 1953). If metabolic processes are altered at the site of a bud scar, the mother may continually lose active surface area until it becomes unable to maintain division potential. Short-term exposure of *cdc24* ts mutant cells to the restrictive temperature, conditions under which it accumulates chitin in the cell wall, did not affect their life span (Egilmez and Jazwinski, 1989). Therefore, artificially induced deposition of a major component of bud scars is not life shortening.

The increase in size as a mother cell ages results in a decrease in the surface area-to-volume ratio. Researchers have speculated that a decrease in the ratio below a certain point may be detrimental to the cell (Mortimer and Johnston, 1959). Once the cell reaches a certain volume, transport of metabolites may no longer be sufficient to satisfy the growing need and thus the old cell may arrest division by a mechanism similar to starvation induced arrest.

In this report, we demonstrate that old mother cells are unable to produce daughters with a full life span potential, further indicating that bud scars are not the direct cause of aging. Instead these mothers produce larger than normal daughters with severely restricted life spans. Although these experiments show a direct correlation between size and life span of daughters cells, increasing the size of small daughters by a physiological regime did not shorten their life spans. Our findings are consistent with a model for aging in which a substance accumulates in old mother cells to cause senescence. When this substance is inherited by daughters, their life spans are shortened.

Materials and Methods

Strains and Media

The haploid strain PSY142 (MATα *lys2-801, ura3-52, leu2-3, 112*) was used in most experiments reported. In the α-factor experiment, strain JFC17 (*MATa, his4, ura3-52, leu2-3, 2-112*) was used. All experiments were conducted on complete medium (YPD) prepared as described (Sherman et al., 1986). DBY747 and its Δ*rad9* derivative YJJ53 were a gift of L. Prakash (Schiestl et al., 1989). The STE12 disruption was constructed using plasmid pNC163 as described (Company et al., 1988).

Life Span Determinations

To determine the life span of a strain, cells were taken from logarithmically growing liquid cultures and plated at low density on complete medium. The cells were incubated at 30°C for approximately 3 h. At this time daughter cells were isolated as buds that had emerged from mother cells and moved with a Zeiss Micro-manipulator to uninhabited parts of the plate. All future buds produced by these daughter cells were then micro-manipulated away. The plates were grown at 30°C during working hours and shifted to 4°C overnight. The life spans generated in this manner were compared to life spans from cells incubated at 30°C continuously and the means were not found to be statistically different (unpublished data). The positions of mother cells relative to partially formed buds were carefully noted to distinguish mothers from daughters during symmetric division. The daughters were then picked with the needle and moved to a different location on the plate for analysis of life span. On very rare occasions, a cell was observed to lyse immediately after micro-manipulation and was excluded from the data set. We observed that it was important that the initial daughter cells are isolated from a logarithmically growing culture prior to being micromanipulated, otherwise they frequently do not begin to divide.

Photography

Photographs of cell divisions were taken with Nomarski Optics at 1,000× magnification using a Zeiss Axioskop microscope with an accompanying Zeiss MC100 camera attachment.

Statistical Methods

To determine if the strain PSY142 had an exponential increase in the rate of senescence, the rate of death per generation was calculated in the range where a sufficient amount of data was available and this rate was shown to increase exponentially by standard statistical methods.

The analysis of variance (ANOVA) was conducted according to standard statistical methods.

Results

The haploid strain PSY142 was used in all of the experiments presented. Fig. 1 depicts the mortality curve for this strain. The mean life span for this strain was 29.1 generations with a standard deviation of 10.9 generations. The aging characteristics of this strain follow a Gompertz distribution; the rate of death increases exponentially with age (see Materials and Methods). This is characteristic of many organisms including yeast (Pohley, 1987).

Symmetric Divisions in Old Mother Cells

During the life span determination of this strain, a number of observations were made concerning the size of the buds being removed. Mothers in the last third of their life span produced daughters that were significantly larger than normal and, in the extreme, were indistinguishable in size from the mother (a symmetric division). In no case was a daughter obviously larger than the mother at the time of division. Visual observation of divisions destined to be symmetric indicated that they occurred through the normal yeast budding process; the daughters were initially visible as small buds before enlarging to abnormal sizes. Also, the size of the mother

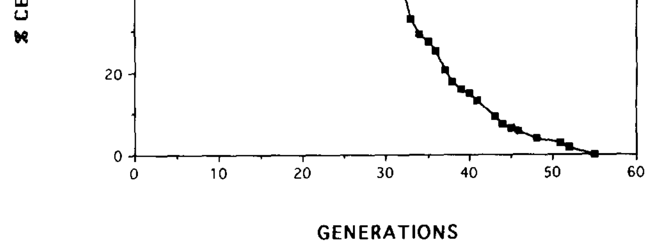

Figure 1. The mortality curve for strain PSY142. The method used to determine the life span of this strain is described in Materials and Methods.

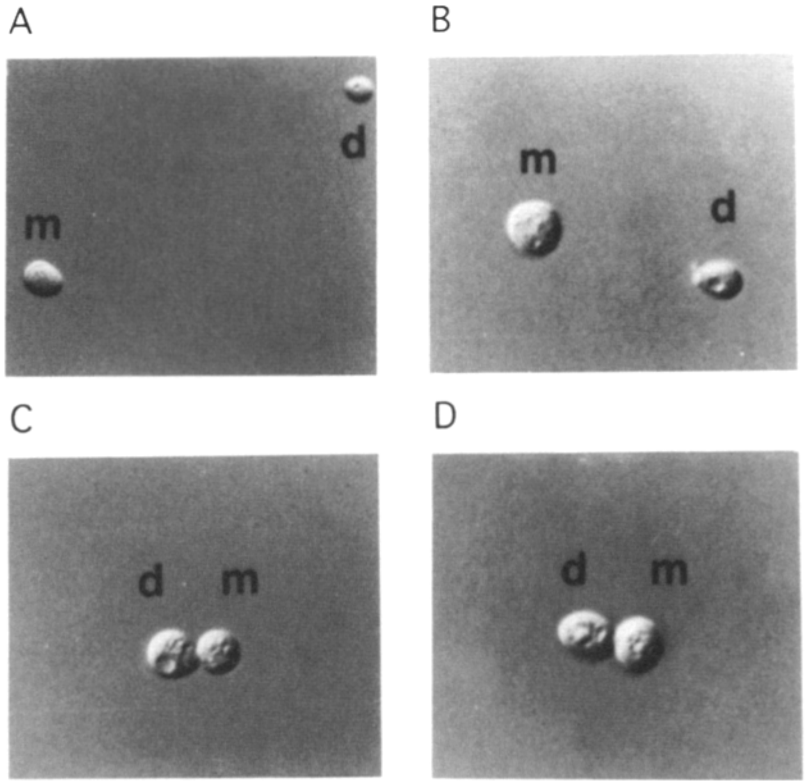

Figure 2. Symmetric and asymmetric cell divisions. All photographs were taken at 1,000× magnification using Nomarski optics. (*A*) A mother having completed one generation with her daughter. Mother cells are labeled with the letter "m" and daughters with the letter "d." (*B*) A mother cell and her 42nd daughter to the right. While this is clearly an asymmetric division, the daughter is noticeably larger than the daughter cell in *A*. (*C*) A symmetric division. The mother has undergone 26 divisions prior to this one. (*D*) Another symmetric division. The mother has undergone 30 divisions prior to this one.

cell did not decrease noticeably upon symmetric division. Instead, the daughter grew aberrantly large. These observations have also been made with a number of other unrelated strains in our laboratory (data not shown).

Larger and/or symmetric divisions occurred later in a mother's life span. Fig. 2 *a* shows a young mother undergoing a normal asymmetric division, while Fig. 2 *b* depicts an old mother producing a bud larger than usual, yet asymmetric. Symmetric divisions are shown in Fig. 2 (*c* and *d*). In all non-symmetric cases the buds were micro-manipulated away from the mother prior to photography to demonstrate that the budding cycle was completed.

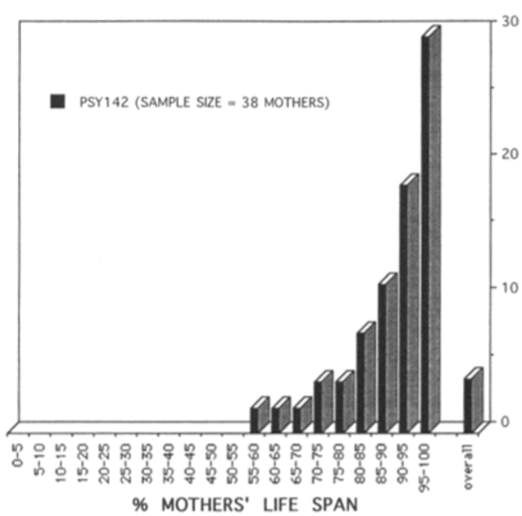

Figure 3. The exponential rate of increase of symmetric division with increasing age of the mother cell. The age of the mothers are given in terms of the percent of a mother's life span that had been completed when a symmetric bud occurred. The life spans of 38 mothers were followed in this experiment. The column labeled "overall" provides the percent likelihood per cell division that a mother would produce a symmetric bud regardless of its age. Symmetric buddings occur at a rate of 4.0% for the strain PSY142.

We sought to quantitate the frequency of appearance of symmetric cell divisions as a function of the relative age of the mother cell (Fig. 3). Since the life spans of the mother cells varied substantially, the best way to align the mothers for analysis was to consider not how many buds a mother had produced prior to a symmetric division, but what percentage of that mother's own life span had been completed. No symmetric buds were observed in mothers which had completed less than 50% of their life span. However, as the mothers aged further, symmetric budding began at a low frequency and increased exponentially to a maximum of 30% during the last 5% of the mothers' life span.

The daughters arising from symmetric divisions were not aberrant in that they gave rise to normal asymmetric divisions (8/11 divisions scored) at a frequency similar to daughters arising asymmetricly from old mothers (7/11 scored in the same experiment).

Symmetric Divisions Do Not Require Integrity of the RAD9 Checkpoint or the Pseudohyphal Growth Pathway

We considered the possibility that symmetric divisions arose in old mother cells as a consequence of a pause in the cell cycle due to the accumulation of genetic damage. This pause could cause the bud to grow abnormally large. The one well-characterized regulatory step that halts the cell cycle in re-

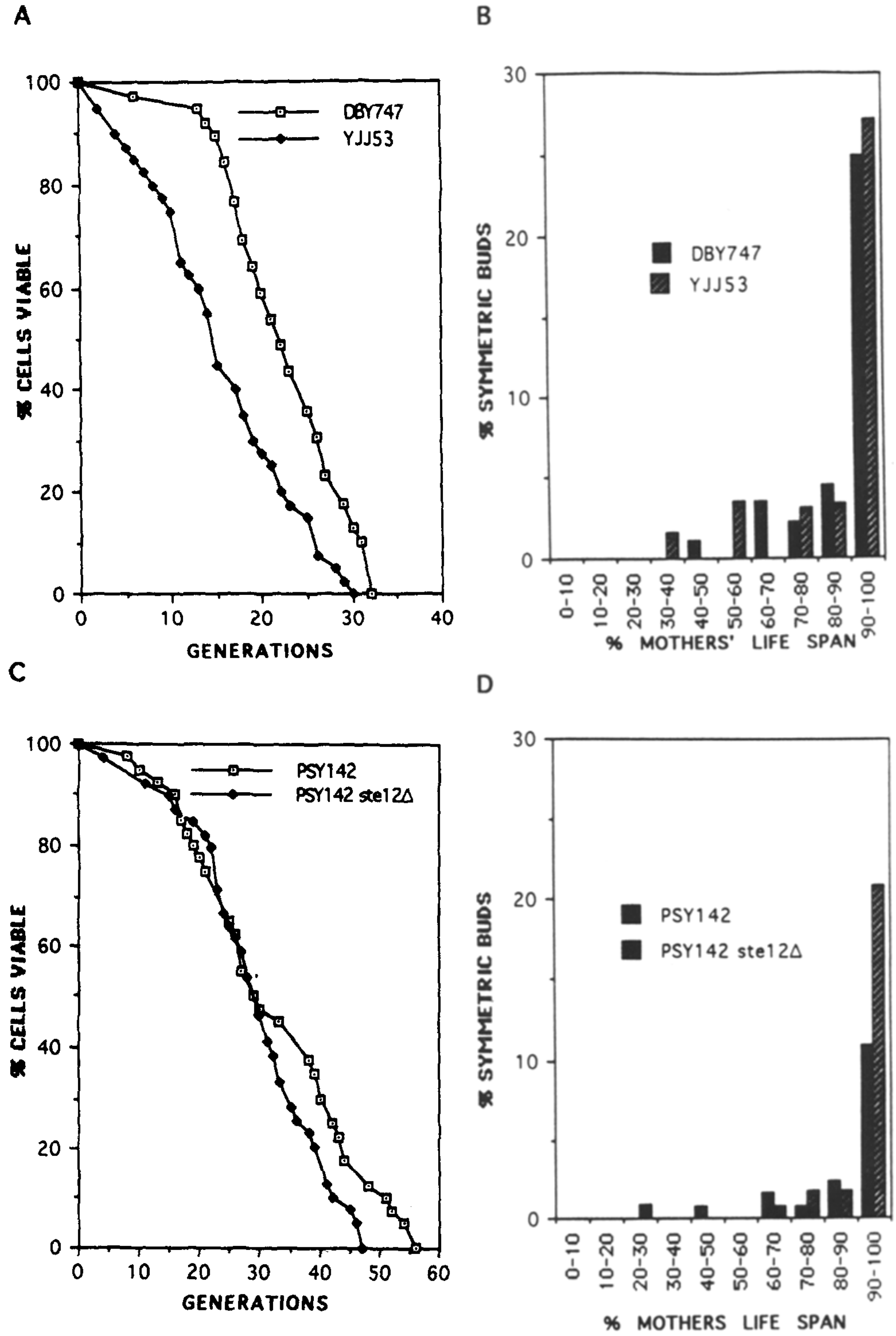

Figure 4. Life spans and symmetric bud formation in Δ*rad9* and Δ*ste12* strains. *A* and *B* depict the wild type DBY747 and its isogenic *rad9* derivative YJJ53 examined for life span (*A*) and symmetric bud formation (*B*). *C* and *D* show analogous experiments for PSY142 and its isogenic Δ*ste12* derivative. In both experiments the sample consists of 40 cells of the wild type and 40 cells of the mutant, which were followed microscopically for life span and symmetric divisions.

sponse to DNA damage is the *RAD9* checkpoint (Weinert and Hartwell, 1988). Thus, we examined whether symmetric divisions occurred in Δ*rad9* cells and their isogenic *RAD9* parent at comparable frequencies. Initially, we found that the Δ*rad9* mutant YJJ53 had a life span that was reduced about 30% compared to the isogenic *RAD9* parent DBY747 (Fig. 4 *A*). Nonetheless, symmetric divisions were observed, and, when plotted as a function of the life span of the *RAD9* strain, these divisions occurred at a frequency indistinguishable from the *RAD9* parent (Fig. 4 *B*).

We also considered whether other cases in which normal budding is altered might be relevant to symmetric divisions. One such case is pseudohyphal formation, which results in symmetric divisions and requires the integrity of the pheromone-response pathway (Liu et al., 1993). We determined whether this pathway was also required for symmetric divi-

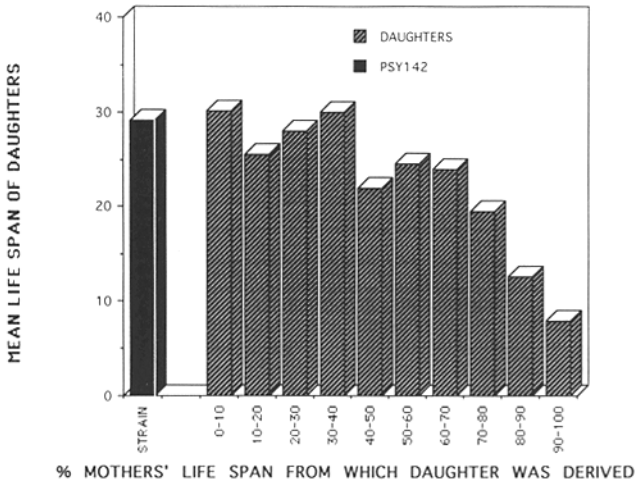

Figure 5. The progressive decrease in the life span of daughters as a function of the mother's age at the time of division. The strain column reflects the mean life span for the strain. The sample sizes for each column are as follows: strain (106 cells), 0–10 (16), 10–20 (12), 20–30 (10), 30–40 (7), 40–50 (10), 50–60 (11), 60–70 (8), 70–80 (10), 80–90 (9), 90–100 (8).

sions by constructing a deletion of *STE12* in PSY142 using plasmid pNC163 (Company et al., 1988), and analyzing these cells microscopically. The deletion of *STE12* did not shorten the life span of PSY142 (Fig. 4 *C*). Moreover, there was no substantial change in the frequency of symmetric divisions in the Δ*ste12* strain (Fig. 4 *D*).

Decreased Life Span in Daughters from Old Mother Cells

The life spans of daughters derived from mothers of varying ages were determined by taking at random daughters produced during different points in mothers' life spans. In Fig. 5, the mean life span of daughters is plotted against the percent of the mothers' life span that had been completed at the time of the division. Daughters from mothers in the first 40% of their life span generally enjoyed full life spans themselves, while daughters from older mothers exhibited reduced life spans, the percent reduction increasing progressively as the mothers increased in age. At the extreme, daughters from mothers in the last 10% of their life span lived only 25% as long as the mothers from which they were derived.

This decrease in life span in daughters of old mothers was not heritable. We analyzed the life spans of daughters of the daughters of mothers of varying ages (grand-daughters). Fig. 6 shows that the reduced life spans of daughters of old mothers was restored back toward normal in granddaughters. Further, great granddaughters, great great granddaughters, and great great great granddaughters all displayed a similar normal life span, regardless of the age of the mother cells from which they descended.

To determine if the increased relative size of daughters from old mothers was correlated to the decreased life span, the life spans of symmetric buds were analyzed and compared to both the mean life span of the strain and the remaining life spans of the mothers after they produced the symmetric bud (Fig. 7). An ANOVA was performed to compare the variance of each set of two data points relative to the variance of all points combined. The results demonstrate that a symmetric bud's life span was significantly more similar to the post-symmetric division life span of its mother than to the life spans of the other cells in the data set ($P < 0.05$). In fact, the remaining life spans of the daughters and mothers in a symmetric division are not obviously different. The data in Fig. 7 compares the life spans of symmetrically arising daughters with the life spans of cells randomly distributed with regard to age. However, their life span is also much shorter than asymmetrical daughters from older mothers

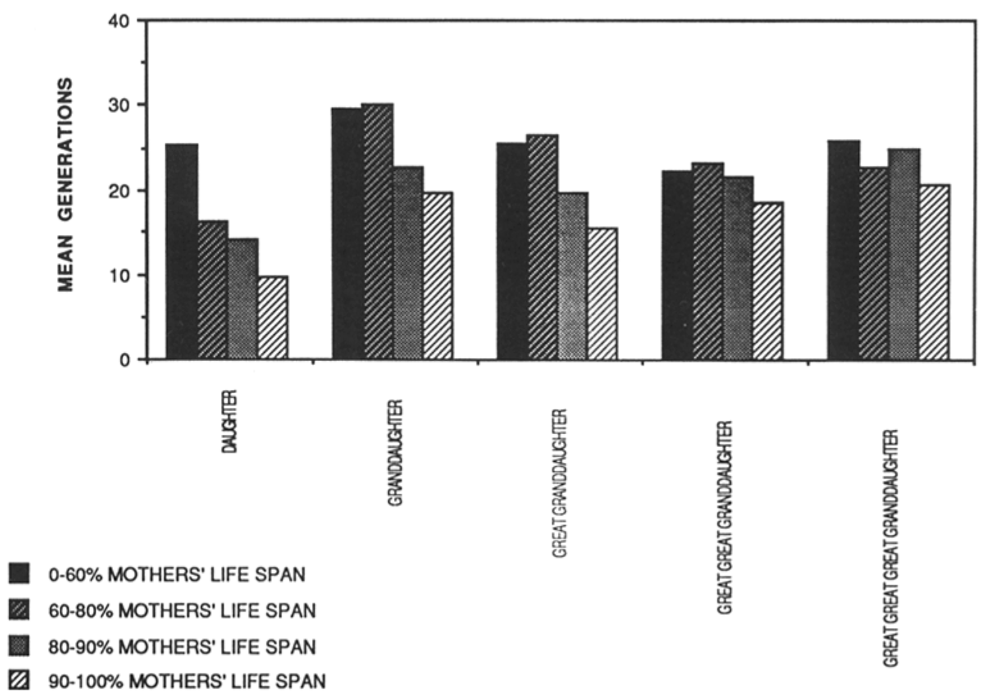

Figure 6. The shortening in life span in daughters from old mothers declines in their descendents. Life spans of daughters from mothers of varying ages were determined microscopically. Daughters were from mothers that had passed through the first 60% of their life span (solid black, 11 cells), 60–80% of their life span (dark hatched, 6 cells), 80–90% of their life span (solid grey, 5 cells), or 90–100% of their life spans (light hatched, 21 cells). The life spans of the first daughters of each of these cells (granddaughters) were also determined, and so on for great granddaughters, etc. The differences in life spans of daughters depending on the age of their mothers is evident in the first generation, and is reduced or eliminated in subsequent generations.

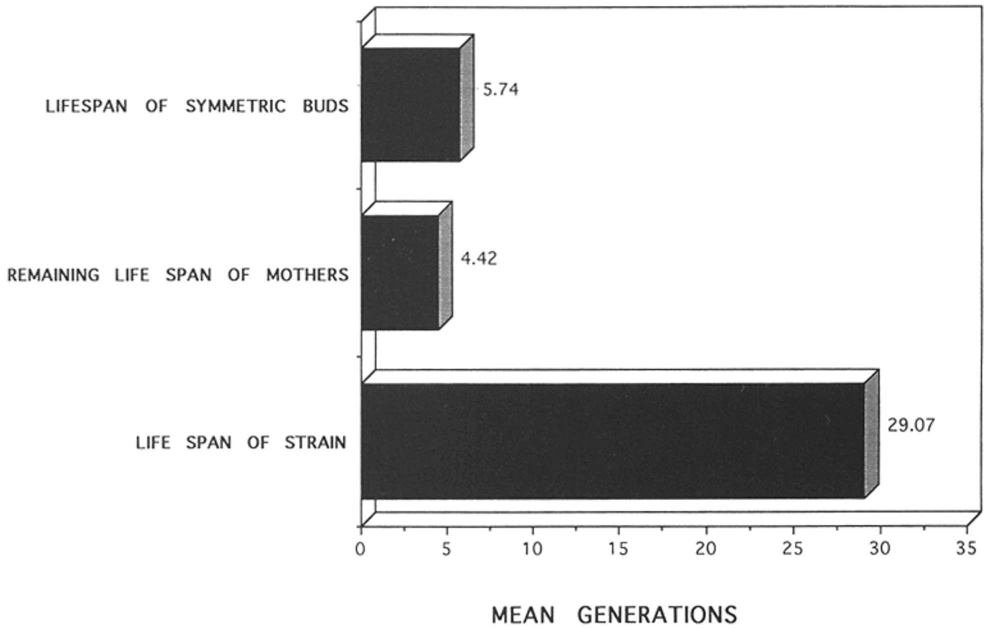

Figure 7. The life spans of symmetric buds compared to the post symmetric bud life spans of their mothers and the life span of the strain. The sample size is 19 sets of symmetric buds and their mothers. The strain sample size was 106 cells.

(i.e., mothers of a similar age to those giving symmetrical daughters, not shown).

The substantial reduction in the mean life span of daughters derived from old mothers indicates that bud scars are not a necessary agent in yeast aging. The similarity in the remaining life spans of daughters and mothers from symmetric buddings is a further confirmation of this conclusion. The only way to resurrect the bud scar hypothesis is to assume that many of the bud scars are somehow transferred to the bud in a symmetric division (an event which has been shown not to occur in typical asymmetric divisions). If many of the bud scars were being transferred to the daughter in instances of symmetric divisions, not only would the daughter's life span be diminished, but the mother's life span would be substantially lengthened. To determine the effect on the mother cell, the life spans of 56 cells were compared to the number of symmetric buds they generated. Table I depicts both the number of cells having 0, 1, 2, etc., symmetric buds and the mean life spans of those cells. Mothers which produced more symmetric buds had at most a slight increase in mean life span, though not the mean life span increase that would be expected if many of the bud scars had been transferred to the daughters. The slight observed increase in life span can be explained simply by the fact that mothers who live longer will have more chances to produce symmetric buds. It is thus likely that the production of symmetric buds does not affect the mother's life span.

Increased Size Does Not Shorten Life Span

Because old mother cells are larger than young mother cells, their daughters are larger than daughters from young mothers. A correlation between the size of mother cells and their age was noted previously (Mortimer and Johnston, 1959), and it was proposed that an increase in size might cause senescence. We thus wished to test whether the shortened life span in daughters from old mothers was due to their large size. Our approach was to cause the size of young cells to increase in the absence of cell division, and to measure whether this shortened their life span. We used α-factor to arrest cells from a random culture at start. After 4 h of arrest, these treated cells were much larger than cells of the same culture that were not treated and showed the characteristic morphology of pheromone-arrested cells (Fig. 8 *A*). The size of the treated cells remained large throughout their life spans. The arrows in the untreated control cells indicate cells that are in G1 and can be directly compared to the arrested cells. The α-factor was removed and mortality curves derived for the treated and control cells. Although we could not determine the age of cells at the start of the experiment, in a random population 1/2 are virgins, 1/4 are mothers that have divided once, 1/8 are mothers that have divided twice, etc. Thus these life spans will closely approximate those starting with only virgin daughters. Strikingly, there was no difference in the life spans of these two samples (Fig. 8 *B*), indicating that an increase in cell size does not necessarily lead to a shortening in life span.

Discussion

In this report we have microscopically followed mother cells in the budding yeast, *S. cerevisiae*, through many cell divisions to senescence. As previously noted, the number of cell divisions that mother cells undergo to give rise to daughters is finite and fixed around a distribution characteristic of ag-

Table I. Life Span of Mothers with Differing Numbers of Symmetric Buds

Number of symmetric buds	Number of mothers	Mean life span
0	21	27
1	17	27
2	10	29
3	6	33
4	2	32
>4	0	–

The mean life spans of mothers producing differing numbers of symmetric buds. No mothers from this strain were observed to produce more than four symmetric buds. In other strains, as many as six symmetric buds have been recorded (data not shown).

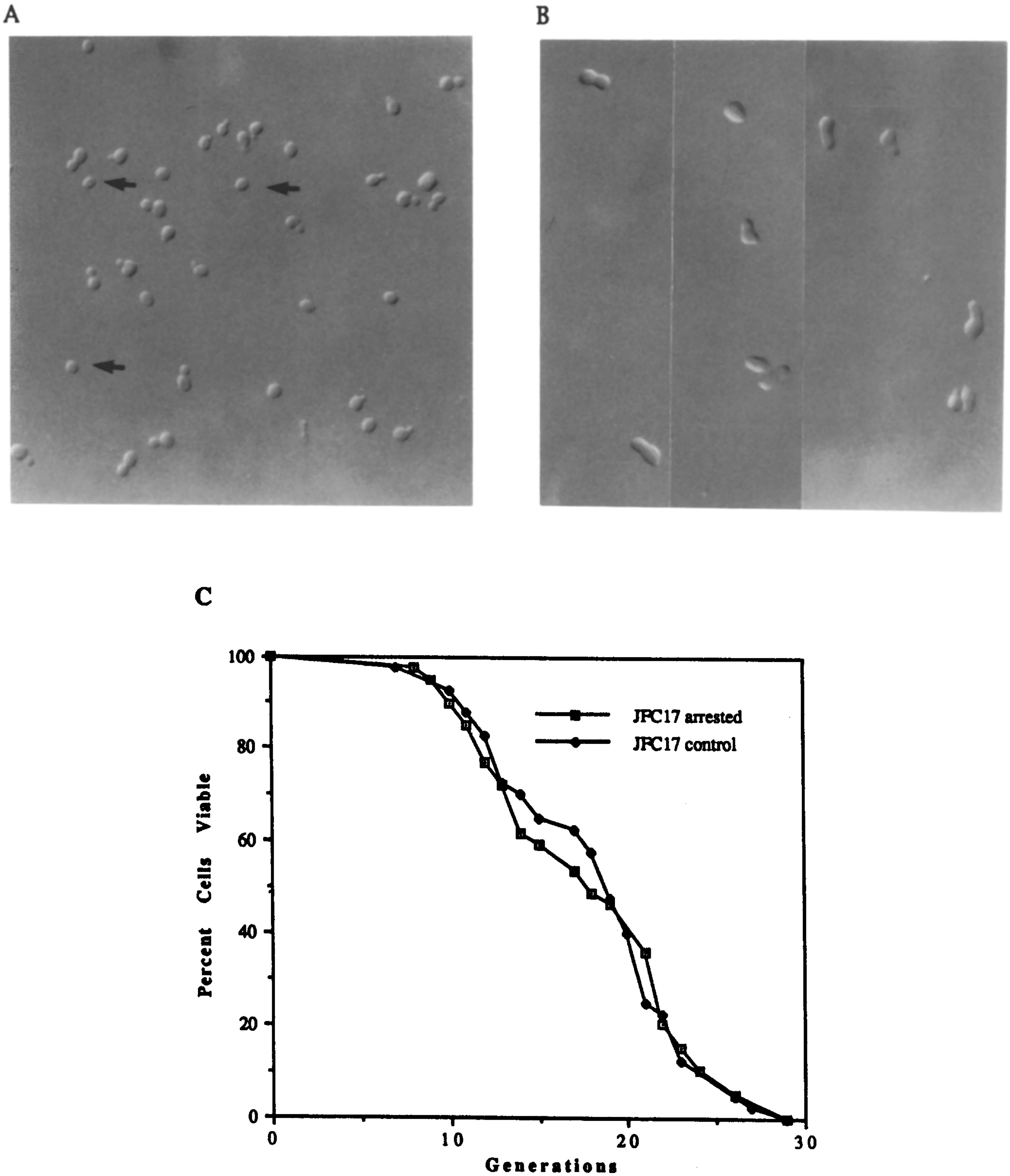

Figure 8. Enlargement of young cells does not shorten their life span. Cells from a growing culture of JFC17 (at a density of 10^7 cells/ml) were treated with 20 mg/ml α-factor for 4 h. After washing away the α-factor, cells were spread on plates for life span analysis. *A* shows untreated cells and *B* shows treated cells. The arrows point to cells in G1, the sizes of which can be compared to the G1-arrested cells in *B*. *C* shows that the life spans of the arrested and control cells are very similar.

ing in many organisms (Mortimer and Johnston, 1959; Pohley, 1987; Sacher, 1978). In this Gompertz distribution, the probability of cessation of life increases exponentially with age. Further, the size of mother cells, as well as their daughters, increases with age. We made three observations, discussed in greater detail below, that provide insight into the aging process. First, the fidelity with which cells divide asymmetricly to give a small daughter cell and a large mother cell, the normal mode of cell division in budding yeasts, decreases with age. Older cells can display symmetric divisions, in which the bud grows to the same size as the mother at division. The oldest mother cells, which are in the last 10% of their life span, feature up to 30% symmetric divisions. Second, the daughter cells of the oldest mother cells have much shorter life spans than daughters of younger cells. This finding goes against the conventional view that a full life span is regenerated in all daughter cells (Johnston, 1966). Third, an increase in cell size does not shorten life span.

Symmetric Divisions

Why do symmetric divisions occur in old mother cells? One possible explanation is that an active mechanism is required to give rise to the normal asymmetric divisions in budding yeasts and this mechanism breaks down in older cells. Whether such a breakdown may be related to the events that cause senescence is not evident. It is clear that symmetric divisions are not related to aging in any obligatory way. A significant fraction of aging cells reach senescence without ever giving rise to a symmetric division. Intriguingly, after a symmetric division, the life spans of both the mother and daughter cells are short and approximate the remaining number of divisions that a mother cell of that age would be expected to possess. Thus, the mother cell apparently does not gain any division potential by giving rise to a daughter of equivalent size.

Several possible explanations for symmetric divisions have been eliminated. One is that old cells accumulate DNA damage which invokes the *RAD9* checkpoint (Weinert and Hartwell, 1988) to slow the cell cycle in emerging buds. We report that deletion of *RAD9* does not reduce the frequency of symmetric divisions. We can not rule out the possibility, however, that other controls on the cell cycle slow progression in old cells and give rise to symmetric divisions. While our observations suggest that symmetric cycles occur over a longer period of time than asymmetric cycles, our attempts to quantitate these measurements were confounded by a highly variable time required for cytokinesis and daughter cell detachment in old cells.

While deletion of *RAD9* does not eliminate symmetric divisions, it does result in a significant shortening of the life span (by ~30%). This could indicate that *RAD9* serves a function in non-irradiated cells, perhaps to allow repair of DNA that is damaged in the absence of irradiation. When *RAD9* is deleted, the accumulation of genetic damage may impose an artificial limit on the life span of cells.

A second explanation for symmetric divisions is that an altered program of budding is accessed in old cells, such as that used in pseudohyphal growth, which involves symmetric divisions and requires the pheromone-response pathway (Liu et al., 1993). Again, since deletion of *STE12* does not alter symmetric divisions in old cells, we conclude that there must be some other underlying basis.

It would be of interest to inquire whether regulatory processes based upon the distinction between mother and daughter cells break down after a symmetric division. One such process is regulation of the HO endonuclease that initiates mating type switching (Strathern and Herskowitz, 1979). The HO gene is transcribed only in mother cells (Nasmyth, 1983). The basis for this regulation is not known but may relate to the increased time in G1 spent by daughter cells to increase their size (Nasmyth et al., 1990). After a symmetric division, because the mother and daughter are the same size, they appear to transit G1 with equivalent kinetics. If HO regulation were retained after symmetric divisions, then some difference between mother and daughter cells that is not related to their difference in size would be implicated in the control.

Decreased Life Span in Daughters of Old Mothers

The decrease in the life spans in daughters of old mothers is substantial, 7.9 divisions in daughters from mothers in the last 10% of their life spans, as compared to 26.5 divisions for the daughters of mothers in the first 70% of their life span. Daughters from mothers in the last 70–80% and the last 80–90% of their life spans show reductions intermediate between daughters from young mothers and daughters of the oldest mothers. The reduction in life span applies to daughters arising from asymmetric and symmetric divisions alike.

What does this reduction in life span imply about the mechanism of senescence? We considered two models consistent with these findings. First, the increased size of daughters from old mothers per se shortens their life span. To address this possibility, we used α-factor to increase the sizes of G1-arrested cells in a random population. Mortality curves of the treated cells and untreated controls were indistinguishable. Since the treated cells were much larger than the control cells throughout their life spans, this experiment shows that an increase in cell size does not necessarily cause a shortening in life span.

A second model consistent with our findings is that the daughters of old mothers inherit a substance that shortens their division potential. Assuming the premature aging of these daughters is related to the normal senescence of mother cells, the substance would be the agent that accumulates in old mother cells to cause senescence. That substance may occur in the form of macromolecular damage that can not be repaired rapidly enough to prevent accumulation. The levels of this substance may be so high in old cells that daughters have a high probability to inherit a portion. Since this shortening of life span in daughters does not occur until mothers are very advanced in their aging program, it is likely that the substance does not accumulate until cells are fairly old. According to this model, the substance could kill cells directly or prevent growth by arresting the cell cycle.

Can the damage that accumulates in old cells be genetic damage? Several observations render this explanation unlikely. First, the daughters of the daughters of old mother cells displayed a life span that was corrected back toward normal. This finding is consistent with a senescence substance that is inherited in daughters of old mothers and is diluted in subsequent generations. It is not consistent with a theory of aging invoking damage to the DNA. Second, the cell cycling time of daughters from old mothers is increased in their first cell cycle (Egilmez and Jazwinski, 1989). This slowing of the cell cycle is alleviated in subsequent divisions. Third, yeast chromosomes are not inherited in a manner that is biased to confine the old DNA strand to the mother cell (Neff and Burke, 1991). However, none of these findings exclude the possibility that genetic damage might occur in a minority of old cells.

The reduction in life span in daughters of old mothers argues against bud scars as the immediate causative agent in yeast senescence. Bud growth in old cells is visually identical to growth in young cells. Thus, it is very likely that the physical parameters of bud growth, including confinement of the bud scars to the mother cells, does not change in old cells. Since the cell wall and membrane of the bud are derived from new synthesis, the substance inherited by the daughters of old mothers is probably an intracellular constituent. It is still possible, however, to retain the notion that bud scars cause senescence, but only if their effect is indirectly mediated by an intracellular component.

In summary, we have described several properties of aging in *S. cerevisiae* that help delimit possible mechanisms of senescence in that organism. Our findings argue against two proposals for aging in this organism: an accumulation of bud scars, or an enlarged cell size. Rather, we suggest that an intracellular substance accumulates in old mother cells that is generated by a failure to repair macromolecular damage. The identity of this substance and insights into its generation would shed light on the aging process in yeast cells, and perhaps other eukaryotic cells.

We would like to thank Chris Kaiser for use of his Zeiss microscope and camera, L. Prakash for the *RAD9* strains, and B. Errede for permission to use the Δ*ste12* vector.

This work was supported by grants to L. Guarente from the National Institutes of Health (AG11119 and GM30454). N. R. Austriaco is a predoctoral fellow of the Howard Hughes Medical Institute.

Received for publication 27 December 1993 and in revised form 7 October 1994.

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