

Cell volume as a factor limiting the replicative lifespan of the yeast *Saccharomyces cerevisiae*

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Abstract The number of cell divisions of the yeast *Saccharomyces cerevisiae* is limited, referred to as “replicative lifespan” of this organism and believed to be due to aging mechanisms similar to those of mammalian cells. We demonstrate, using three pairs of isogenic yeast strains (standard and a mutant deficient in an antioxidant defense protein) that although the lifespan differs significantly, the final volume attained after the last division is similar within each pair of strains. In a population, cells cease to bud after various number of cell cycles but attaining a similar final volume. These results indicate that the increase in the mother cell volume, intrinsic to the asymmetric cell division in *S. cerevisiae*, may be the main mechanism limiting the reproductive capacity of in this organism.

Keywords Cell volume · Lifespan · Replicative aging · *Saccharomyces cerevisiae* · Yeast

Introduction

The yeast *Saccharomyces cerevisiae* has been proposed and widely accepted as a simple eukaryotic model organism for the studies of aging, on the assumption that basic mechanisms of aging are conserved among eukaryotic organisms (Bitterman et al. 2003; Gershon and Gershon 2000). However, it has been pointed out that the public mechanisms of aging are accompanied by private mechanisms (Partridge and Gems 2002) and it is not clear what is the relation between the mechanisms underlying the limited replicative lifespan of yeast cells and of mammalian cells and the limited lifespan of the whole organisms. A yeast cell has a limited reproductive capacity which seems to be similar to the Hayflick limit of mammalian cells. However, both phenomena are analogous rather than homologous, one of obvious differences being the death of the whole population of cultured mammalian cells after reaching the Hayflick limit contrasting with the immortality of yeast population in which cells approaching their budding limit constitute a minute fraction. Moreover, the role of the limited division capacity of mammalian cells in the aging of the organism is doubtful since other model

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organisms employed in the studies of aging such as *Caenorhabditis elegans* or *Drosophila melanogaster* are in their mature forms composed of only postmitotic cells and in mammals the division limit of the cells does not seem to be reached even in old organisms.

Why the ability of yeast cells to produce buds become exhausted after several tens of divisions while the “counter of divisions” of their daughter cells is set at zero? An apparently obvious explanation could be the unequal distribution of a “senescence factor” which becomes accumulated in the mother cell and is not transmitted or negligibly transmitted to daughter cells. A cytoplasmic diffusible, degradable senescence factor has been suggested (Egilmez and Jazwinski 1989) but not identified. Accumulation of rDNA circles accumulating in old yeast mother cells has been proposed to limit the replicative lifespan of the yeast (Sinclair and Guarente 1997; Sinclair et al. 1998) although cases of replicative yeast aging not accompanied by accumulation of rDNA circles have been reported (Ashrafi et al. 1999; Park et al. 1999) and commonly occurring 2- μ m plasmids do not adversely affect the replicative life span of the yeast (Falcon et al. 2005). Theoretical considerations indicate that accumulation of rDNA circles cannot be a single factor underlying the limitation of cell divisions of yeast mother cells (Gillespie et al. 2004). Another factor which can fulfill the function of the senescence factor is the accumulation in the mother cell of oxidatively damaged proteins which are not transmitted to daughter cells (Aguilaniu et al. 2003). However, oxidized proteins have not been claimed to be responsible for the limited ability of yeast cells to reproduce. Moreover, we (Wawryn et al. 2002) and others (Koc et al. 2004) did not observe increased replicative lifespan of the yeast cultured under hypoxic and anoxic conditions when oxidative protein damage is attenuated.

Mitochondrial damage has also been taken into account as a potential course of yeast replicative aging (Jazwinski 2000; Piper et al. 2002) but divergent results of studies of rho^o mutants (Kaeberlein et al. 2005a, b) do not allow for drawing unequivocal conclusions concerning the role of mitochondria as a factor limiting the ability of yeast cells to reproduce.

Geometrical factors have been considered initially as a possible factor limiting the budding capacity of yeast cells (Mortimer and Johnston 1959) but

dismissed soon, at least in the form proposed originally i.e., as limitation of the division capacity due to accumulation of bud scars over the surface of mother cells (Egilmez and Jazwinski 1989). We have reconsidered this question recently suggesting that another geometrical factor may be critical for limitation of budding of yeast cells. The volume of yeast mother cell increases unavoidably upon each budding, due to the very nature of budding as a mechanism of reproduction in *S. cerevisiae* which can reach a size too big to allow for efficient completion of the cell cycle. We demonstrated that inhibition of budding but not of protein synthesis and cell volume growth by pheromone treatment decreases yeast replicative lifespan in a manner inversely related to the volume attained (Zadrag et al. 2005). Analysis of volume changes of two isogenic yeast strains of significantly different replicative lifespan during consecutive cell cycles demonstrated that both strains stop budding when reaching the same final volume (Zadrag et al. 2005, 2006). An obvious criticism to these results was that they are limited to one pair of strains and they should be confirmed on further cases of different origin. This paper confirms our previous findings on three pairs of isogenic strains consisting of different popular standard laboratory strains [except for D1CSP4-8C which is a cross between the SP-4 strain and its mutant (Zadrag et al. 2005)] and their disruptants in antioxidant enzymes, showing considerable differences in the replicative lifespan due to various defects of the antioxidant barrier (lack of CuZn, superoxide dismutase, glutaredoxin 5 and peroxiredoxins 1-5).

Materials and methods

Yeast strains

Yeast strains used are listed in Table 1.

Growth conditions

Yeast cells were grown in a standard liquid YPDextrose medium (1% Difco Yeast Extract, 1% Yeast Bacto-Peptone, 2% glucose) on a rotary shaker at 150 rpm or on a solid YPD medium containing 2% agar, at a temperature of 30°C.

Table 1 Yeast *S. cerevisiae* strains used in the study

Strain	Genotype	Reference
D1CSP4-8C	MATa leu1arg4 (WT)	Zadrag et al. (2005)
Δsod1 (DSCD1-1C)	MATa leu1arg4 Δsod1 (isogenic to D1CSP4-8C)	Bilinski et al. (1985)
BY4741	MATa his3Δ1 leu2Δmet15Δura3Δ (WT)	Wong et al. (2004)
Δprx (Δ5)	Isogenic to BY4741	Wong et al. (2004)
W303-1A	MATa ura3-1 ade2-1 leu2-3,112 trp1-1 his3-11,15 (WT)	Rodriguez-Manzaneque et al. (2002)
Δgrx5 (MML 100)	MATa grx5::kanMX4 (isogenic to W303-1A)	Rodriguez-Manzaneque et al. (2002)

Determination of replicative lifespan

Replicative life span of individual yeast cells was determined microscopically by a routine procedure (Kim et al. 1999; Wawryn et al. 1999) on cells placed on agar plates using a micromanipulator. The number of buds formed by each cell is referred to as its replicative life span. One plate with at least 40 cells was followed in each experiment and two replicates was carried out.

Estimation of cell volume

Cell volume was estimated by analysis of microscopic images recorded every each five cell divisions during a routine procedure of determination of replicative lifespan. Images were captured with a Nikon Eclipse E200 microscope with 20× objective, equipped with a Sony digital camera. Diameter of the cell was measured using a MicroImage 3.0 software. Cell diameter (d) was measured in four times in various planes for each cell and the mean value was used for calculations. It was assumed that each cell has a regular shape similar to a sphere and the cell volume was calculated as $4/3\pi(d/2)^3$.

Estimation of protein concentration

Protein concentration was estimated in by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Results and discussion

The three reference strains used differ in the mean and maximal replicative lifespan. The D1CSP4-8C strain is the most long-lived as judged by both these criteria, like its parent strain, SP-4 (Wawryn et al.

1997, 2002). Maximal lifespan of the BY4741 strain is not much lower but that of the W303-1A strain does not reach 40 buds produced. The final volumes attained by cells of D1CSP4-8C and BY4741 strains do not differ significantly but that of the W303-1A strain is considerably higher (Table 2). We attribute this difference to the large vacuolar volume in the latter strain which is visible under the microscope in cells stained with 7-amino-4-chloromethylcoumarin L-arginine amide (Arg-CMAC) but difficult to quantify. However, an indirect measure of the large vacuolar volume in W303-1A is the mean cellular protein concentration which is much lower in this strain (24 pg/ μm^3) than in D1CSP4-8C and BY4741 strains (63, 54 pg/ μm^3), respectively.

Disruption of genes coding for vital antioxidant proteins lead to increased sensitivity to oxidative stress, including (in contrast to the standard strains) inability to grow in the atmosphere of pure oxygen (not shown). These defects lead also to a distinctly decreased replicative lifespan (1.7- to 4-fold decrease in mean replicative lifespan and about twofold decrease in the maximal replicative lifespan).

However, in spite of the dramatic differences in the replicative lifespan between the mutants and their

Table 2 Mean and maximum lifespan for each strain used in this study and cell volume which cell was obtained after last cell division

Strain	Replicative lifespan		Final cell volume (μm^3)
	Mean	Maximal	
D1CSP4-8C	40 ± 13	61	160 ± 23
Δsod1 (DSCD1-1C)	10 ± 5	26	166 ± 40
BY4741	24 ± 11	54	190 ± 43
Δprx (Δ5)	14 ± 5	27	189 ± 27
W303-1A	24 ± 8	39	520 ± 217
Δgrx5 (MML100)	8 ± 5	18	539 ± 202

Mean ± SD; data from 2 experiments of 40 cells each

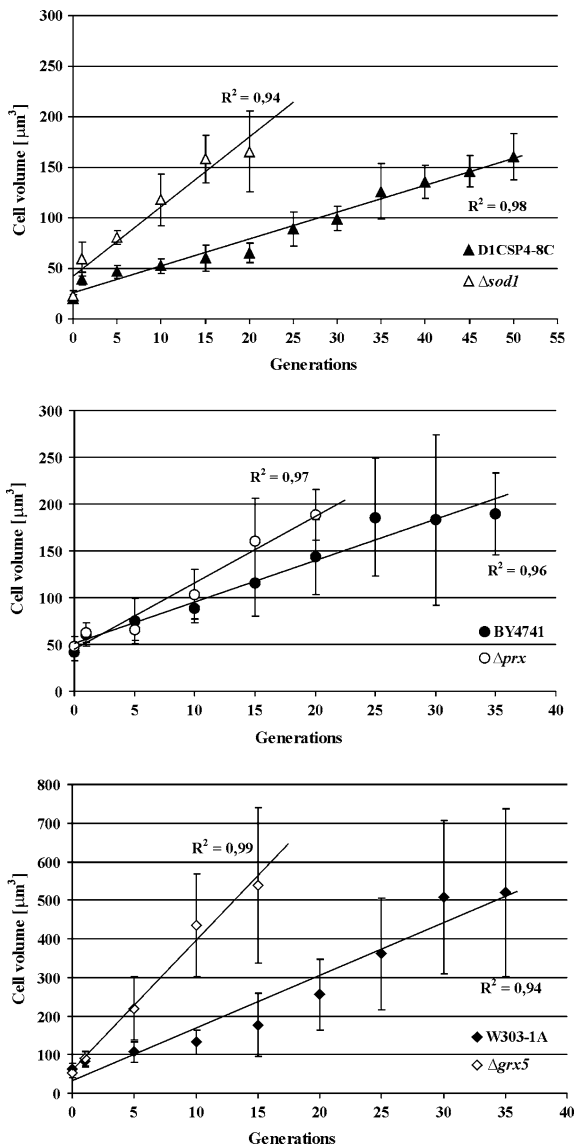


Fig. 1 Dependence of cell volume on the number of cell divisions accomplished by mother yeast cells for three pairs of isogenic yeast strains. The *bars* indicate SD

standard counterparts, the volumes attained by mother cells reaching their maximal replicative lifespan were identical in each pair of strains, within the limits of experimental error (Table 2; Fig. 1). This result corroborates our previous findings on one pair of strains (Zadrag et al. 2006) and indicates that reaching a critically high cell volume (different for strains of various genetic background) may be the main factor limiting the ability of mother yeast cells to accomplish a successful cell cycle. As mentioned

earlier, the progressive growth of the volume of yeast cells with each cycle is an unavoidable consequence of this particular mechanism of reproduction. The limited number of buddings may be due to this peculiarity rather than to aging (Bilinski and Bartosz 2006).

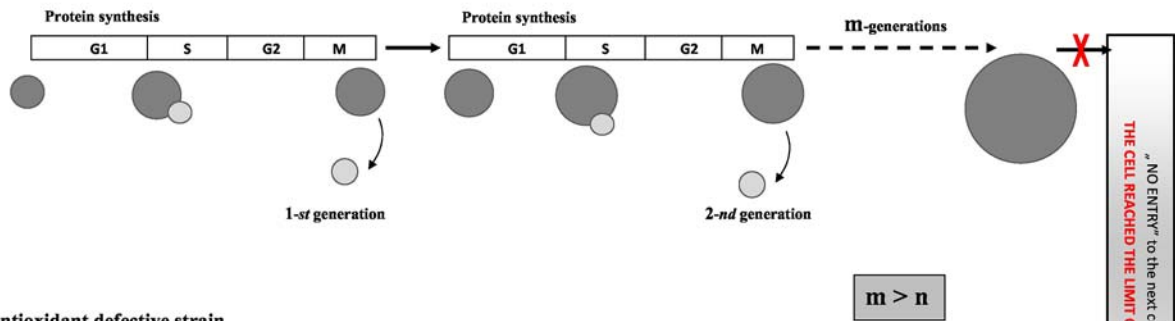
Plotting the cell volume of yeast mother cells as a function of the number of buddings shows that all the mutants deficient in antioxidant proteins tested reach the limiting volume within a smaller number of cell cycles completed (Fig. 1). Apparently, oxidative stress leading to enhanced DNA damage stops the divisions of the cells of the mutants at cell cycle checkpoints without hampering protein synthesis and cell volume growth (Fig. 2). A similar situation occurs when cell entry into the next cycle is transiently inhibited by treatment with alpha pheromone; also in this case the increase of the volume of the cells drives them to reach faster the limiting cell volume (Zadrag et al. 2005).

Another argument pointing to the cell volume as the main parameter determining the reproductive capacity of yeast cells comes from measurements of the final volumes of the yeast mother cells which ceased to divide after accomplishing various number of buddings. Except for the cells which lost their reproductive capacity very soon, other subpopulations of the cells had similar volumes at the moment of reaching their division limit, irrespective of the number of buds formed (not shown due to the low number of data, precluding statistical analysis). Therefore, cell volume attained rather than the number of buds formed is the factor limiting further reproduction.

A similar inverse relationship between the cell size and number of cell divisions has been noted previously for human fibroblasts in culture (Angello et al. 1987, 1989) and, more recently, for normal and neoplastic cells under conditions of prolonged unbalanced growth induced by suppression of cell divisions (Sumikawa et al. 2005).

Due to various reasons, scaling up of a single cell is possible only within a limited range of size. One of the problems accompanying the increase in cell volume is the limited size of the nuclear surface necessary for the exchange of signaling proteins with the cytosol; even if the volume of the nucleus grows proportionally to the growth in cells size, the ratio of the surface to volume of the nucleus decreases.

Wild type strain



Antioxidant defective strain

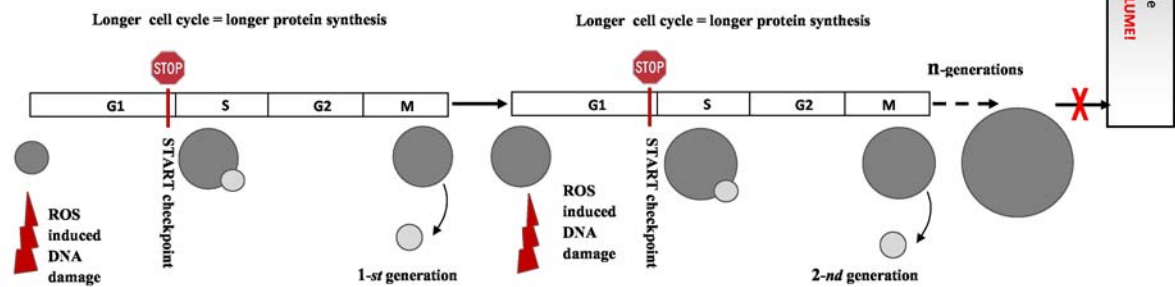


Fig. 2 Probable mechanism of shortening the replicative lifespan of yeast mutants defective in antioxidant barrier. The increased DNA damage due to oxidative stress in the mutants delays cell divisions why not inhibiting protein synthesis and

therefore growth in mother cell volume. As a results, the mutants reach the limiting cell volume after a lower number of cell divisions

There are also reasons to link the value of limiting cell volume to the capacity of protein synthesis (especially to the rate of synthesis of proteins critical for the progression through the cell cycle) which in turn is dependent on of the rate of protein synthesis and in turn on the efficiency of the translational apparatus. It is believed that a measure of the cell ability to reproduce is the concentration of cyclin Cln3, which complexes with the cyclin-dependent kinase Cdk1 to activate Start by phosphorylating and thereby inactivating the Start repressor Whi5 (de Bruin et al. 2004; Jorgensen et al. 2002). Cln3 is a highly unstable protein so its absolute abundance is expected to be highly sensitive to its rate of synthesis and thereby to reflect the current translation rate (Cross and Blake 1993; Jorgensen et al. 2004). Strong effects of ribosomal proteins Rpl10 and Rps6 on the replicative lifespan of the yeast (Chiocchetti et al. 2007) further illustrates the importance of the translation efficiency in the control of yeast cell doubling potential.

Our hypothesis answers the first of the two questions posed in the “Introduction”, concerning

the mechanism of limited reproductive potential of a single yeast cell. The second question, related to the mechanism of resetting the “counter of cell cycles” in the daughter cells can be addressed on the basis of measurements of changes in the volume of mother and daughter cells in consecutive cell cycles of the mother cell (Fig. 3). The ratio of volumes of the bud and the mother cell in the wild-type strains studied was 0.50–0.70 in the first budding decreasing to about 0.28 in the last buddings. It means, taking into account the progressive volume increase of the mother cell, that the absolute volume of the buds grows with successive buddings although at a lower rate with respect to that of the mother cell.

This big difference in size between the bud and the mother cell is a means of preventing increase in cell size of a yeast population. On the other hand, a decrease of all size in the population is prevented by the mechanism of inhibition of their cell cycle at START till reaching a minimal permissible volume (Hartwell et al. 1974; Johnston et al. 1977; Rupes 2002) prevents a decrease in cell size in the population. Therefore resetting of the “cell cycle

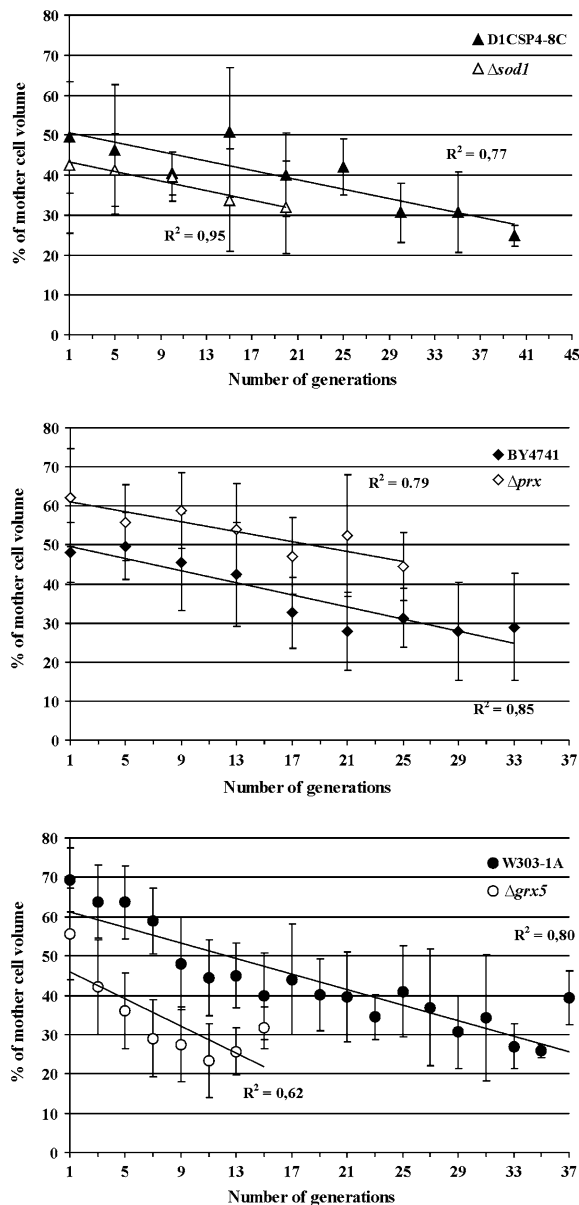


Fig. 3 Relative volume of buds in successive buddings of yeast of various strains. The volume of buds is presented as percent of the size of the mother cell. The data are mean values from 8 to 20 measurements. The variable number of measurement is due to the gradual decrease of the number of cells able to divide. The bars indicate SD

counter” is a consequence of the mechanism controlling the ratio between the daughter and the mother cells.

It should be emphasized that changes in the volume of yeast mother cells undergoing successive cell cycles have been studied since decades (Gershon

and Gershon 2000; Mortimer and Johnston 1959; Muller 1971) but treated as a result of aging and not the cause of the limitation of cell divisions. We are aware that our hypothesis putting this relationship upside down may be treated as a reasonable alternative only if it does not contradict the results of numerous studies. Our analysis of the literature on the replicative aging of the yeast did not point to such discrepancies but to a simple explanation of the observed phenomena without the necessity of invoking a mysterious “senescence factor” as exemplified by the following examples.

The volume of the last buds of a mother cell is usually higher in some strains than the minimal permissive value which may be the simplest explanation of the reason for the lower number of divisions these cells can accomplish (Jazwinski et al. 1989).

At the end of reproductive period some cells divide symmetrically producing giant buds of size similar to their own size which have a replicative lifespan identical to the remaining lifespan of the mother cells. This phenomenon speaks against a causal role for bud scars in determining replicative lifespan because mother and daughter cells do not have the same number of scars (Austriaco, 1996). However, it can be easily explained by our idea that attaining excessive cell volume is the factor limiting further divisions of yeast cells, without invoking a failure of the mechanism retaining the putative “senescence factor” in the mother cell.

The “calorie restriction” procedure increasing the number of cell cycles, leads to decreased cell size (unpublished). These changes are mediated by cell signaling pathways including Ras, PKA and TOR (Bonawitz et al. 2007; Hlavatá et al. 2008; Medvedik et al. 2007; Sun et al. 1994).

Data on the volume of yeast strains capable of producing increased number of buds (longevity mutants) are lacking in the literature which does not allow to decide whether this feature is due to decreased initial size, decreased rate of volume growth or increase in the maximal limiting volume. It would be of great interest to compare these parameters in yeast “longevity” mutants.

In summary, the results of this study point out that reaching of limiting cell volume may be the main factor determining the ability of cells of the budding yeast to reproduce. The replicative lifespan seems to be a resultant of two main factors: the value of the

limiting cell volume, determined genetically, and the rate of increase of cell volume growth determining the rate of reaching this volume limit. It would be of great interest to compare these parameters in yeast longevity mutants.

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