Is the Yeast a Relevant Model for Aging of Multicellular Organisms? An Insight from the Total Lifespan of *Saccharomyces cerevisiae*

Renata Zadrag, Grzegorz Bartosz and Tomasz Bilinski*

Department of Biochemistry and Cell Biology, University of Rzeszów, ul. Cegielniana 12, PL 35-595 Rzeszów, Poland

Abstract: The applicability of the free radical theory of aging to the yeast *S. cerevisiae* is a matter of debate. In order to get an insight into this question, we studied the reproductive potential (the number of buds produced), reproductive lifespan (the time during which a yeast cell is able to divide), postreproductive lifespan (duration of life of yeast cells which ceased to divide) and total lifespan (sum of reproductive lifespan and postreproductive lifespan) of three isogenic pairs of yeast strains. Each pair contained a parent strain and a disruptant of gene(s) coding for important antioxidant enzyme(s) (CuZn-superoxide dismutase, all five peroxiredoxins or glutaredoxin 5). Although the reproductive potential was decreased in all antioxidant enzyme-deficient mutants, the differences in the reproductive lifespan between the parent strains and the mutants were less pronounced while postreproductive lifespan and total lifespan were not diminished in the mutants. These results suggest that either the free-radical theory of aging is not applicable to *S. cerevisiae* or that this yeast is not a proper model organism for the study of aging of higher organisms. In our opinion the latter possibility is more apparent and the increase in cell volume (unavoidable for a cell propagating by budding) rather than accumulation of oxidative damage may be the main reason for the cessation of budding (and perhaps postreproductive death) in *S. cerevisiae*.

Keywords: Aging, yeast, *S. cerevisiae*, lifespan, postreproductive lifespan.

INTRODUCTION

The discovery that cells of the baker's yeast *Saccharomyces cerevisiae* are able to perform a limited number of buddings has become a basis of assumption that this organism can be a model for studies of aging of higher organisms including humans. Because of its short lifespan and the ease of culture and genetic manipulation, the aging of the budding yeast has been extensively studied in two respects [1-3]. The limitation in the number of buddings, referred to as replicative aging, is believed to model the aging of whole organisms or of their cells capable of replication [3-6]. The limited survival of yeast cells in a stationary culture is called chronological aging and is thought to model the aging of postmitotic cells of higher organisms [7-9]. Both assumptions may be questionable. In the stationary culture yeast cells are kept in a medium devoid of essential nutrients which have been used up and accumulating products of metabolism or in distilled water, a situation never experienced by postmitotic cells of the human body; therefore the chronological aging of the yeast may rather represent a test for their resistance to starving than anything else and thus not be relevant for studies of physiological aging. The limited number of yeast buddings is not homologous to the Hayflick limit of mammalian cells as the yeast has active telomerase and its telomeres do not shorten upon divisions. It has been pointed out that the "replicative lifespan" (nota bene expressed in divisions and not in time units) is a measure of fertility rather than lifespan [10]. The number of offspring would be a crude measure of mammalian lifespan, not including the postreproductive lifespan. However, a recent proposal of gathering information of total yeast lifespan (i.e. the sum of *reproductive* and *postreproductive lifespan*) [10], which could give further insight into the relevance of the yeast as a model organism in aging studies, to our surprise has not been addressed by researchers studying aging in the yeast.

In this paper we report results of comparison of *reproductive, postreproductive* and *total lifespan* of three pairs of isogenic strains containing mutants in important antioxidant proteins. The number of buds produced by these mutants is lowered; however, the total lifespan does not differ significantly within the pairs of strains. Our results demonstrate that the total lifespan measured does not differ significantly within the pairs of strains putting in doubt whether the lifespan of the yeast models that of the human and other metazoans.

MATERIAL AND METHODS

Yeast Strains

We analyzed three pairs of yeast strains, each pair consisting of a typical laboratory strain and its disruptant in an important antioxidant protein (Table **1**). The strains within each pairs showed a difference in both the average and maximal number of buddings.

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.

Determination of Reproductive Potential, Reproductive Lifespan and Postreproductive Lifespan

Yeast lifespan was determined by a small modification of the method of [10]. Yeast cultures were grown in YPD liquid

*Address correspondence to this author at the Department of Biochemistry and Cell Biology, University of Rzeszów, Cegielniana 12, PL 35-595 Rzeszów, Poland; Tel: +48 17 872 1253; Fax: +48 17 872 1425;
E-mail: bilinski@univ.rzeszow.pl

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Table 1. Yeast Strains Used in this Study

Strain	Genotype	References
D1CSP4-8C	*MATa leu1arg4*	[26]
DSCD1-1C	*MATa leu1arg4 Δsod1* (disruptant in SOD-1, isogenic to D1CSP4-8C)	[31]
BY4741	*MATa his3Δ1 leu2Δ0met15Δ0ura3Δ0*	[32]
5Δ(prxΔ)	Disruptant in peroxiredoxins 1-5, isogenic to BY4741	[33]
W303-1A	*MATa ura3-1 ade2-1 leu2-3,112 trp1-1 his3-11,15*	[34]
Grx5 (MML 100)	*MATa grx5::kanMX4* (disruptant in glutaredoxin 5, isogenic to W303-1A)	[34]

medium overnight. One-microliter aliquots of each culture was dropped on separate YPD plates with solid medium containing Phloxine B at a concentration 10 µg/ml. Forty single cells were micromanipulated to an appointed area on the plates for each experiment. The first daughter were chosen and their successive buddings were followed to determine the total number of buds formed by each cell (which we prefer to call *reproductive potential* instead of the commonly used misnomer "replicative lifespan") and *reproductive lifespan expressed in time units* (defined as the length of time during which a cell is able to divide or the time from the first to the last division). After completion of buddings, the yeast cells were inspected in one-hour intervals to determine their *postreproductive lifespan* (the length of time from the last division to cell death). A cell was considered dead when it became red due to accumulation of the dye. *Total lifespan* was calculated as a sum of *reproductive lifespan* and *postreproductive lifespan*. The plates were kept at 30°C during the observations (16h) and at 4°C overnight (8 h). A total of 80 cells was analyzed for each strain in two separate experiments.

Because the dye Phloxine B has a photodynamic action generating superoxide [11], which has a discernible effect especially on the superoxide-dismutase deficient strain, a green filter absorbing the light in the range of the maximum absorption for this dye was applied to attenuate the incident light. This procedure resulted in a discernible increase in the *reproductive potential* and the *reproductive lifespan* of the mutants which became indistinguishable from that determined in the absence of Phloxine B even for the mutants.

Statistical Analysis of Data

The results represent mean ± SD from two independent experiments (each comprising 40 cells). As the replicates did not differ significantly, pooled data were analyzed. Normality of the distribution of lifespan was assessed by the Kolmogorov-Smirnov test. Differences between mean value reproductive potential and reproductive, postreproductive and total lifespan within each isogenic pair of strains were assessed with the Student's t-test. The correlation between reproductive potential and different types of lifespan were assessed by the Pearson's test. Statistical analysis of the data was performed using STATISTICA v 7.0.

RESULTS

We applied the methodological approach of Minois *et al.* [10] to compare the total lifespan of three pairs of isogenic strains consisting of a parent strain and of a strain of significant deficiency of the antioxidant defense (CuZn-superoxide dismutase, all five peroxiredoxins and glutaredoxin 5, respectively), as evidenced by the inability of the mutant strains to grow under the atmosphere of pure oxygen. We used deliberately three different pairs of strains to check whether the effects observed are independent of the genetic background. Various laboratory strains used commonly differ significantly in their physiology, including auxotrophies which are useful for their use but may affect their metabolism.

In all three pairs of isogenic yeast strains used, the mutants deficient in the antioxidant defense had a considerably lowered *reproductive potential*. Plotting the number of divisions accomplished by a yeast cell *as a function of time* makes the differences between the mutants and their isogenic parent strain smaller since the generation times, i. e. the time intervals between successive buddings are longer for the mutants. The *reproductive potential* and *reproductive lifespan* of the mutant lacking superoxide dismutase 1 were lower with respect to the isogenic parent strain but the *postreproductive lifespan* was longer for the mutant. As a result, while the mean total lifespan of the mutant was shorter for the *Δsod1* mutant than for its parent counterpart, the maximal total lifespans were indistinguishable for both strains (Fig. **1**, Table **2**). The disruptant in all five peroxiredoxins had a considerably lower *reproductive potential* than its parent counterpart but its mean and maximal *reproductive lifespans* were only slightly shorter with respect to the parent strain. The *postreproductive lifespan* and *total lifespan* did not differ significantly between the parent strain and the disruptant (Fig. **2**, Table **2**). The *reproductive potential* of disruptant in glutaredoxin 5 was much lower than that of its parent counterpart; this difference became much reduced when comparing *reproductive lifespan*. The *postreproductive lifespan* did not differ between the mutant and the parent strain. As a result, mean *total lifespan* was slightly lower for the mutant than for the parent strain but the *maximal total lifespan* was the same for both strains (Fig. **3**, Table **2**). In fact, all three pairs of strains, in spite of differences in genetic background between the pairs and vast differences in *reproductive potential*, have very similar *maximal total lifespan*, of about 200 hours.

Smaller differences in the reproductive lifespan between the strains in each pair, as compared with the differences in the reproductive potential are due to longer generation times of the mutants (Table **3**).

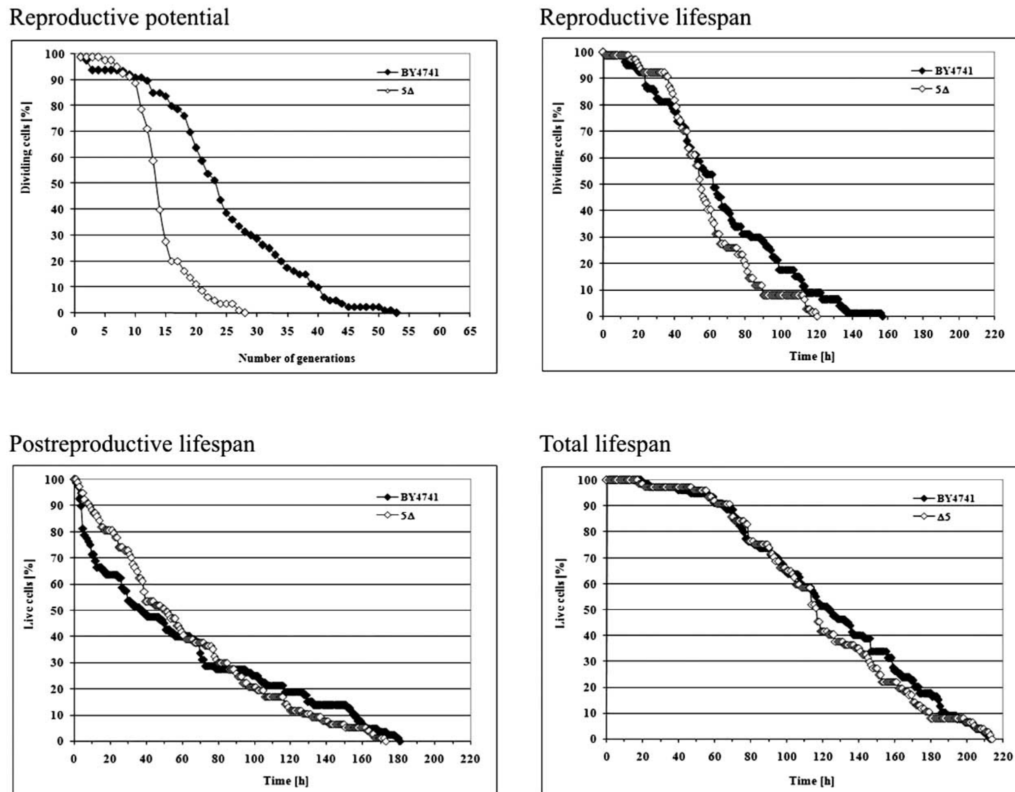

Fig. (1). Reproductive potential, reproductive lifespan, postreproductive lifespan and total lifespan of a mutant lacking CuZn-SOD (DSCD1-1C) and its isogenic parent strain (D1CSP4-8C).

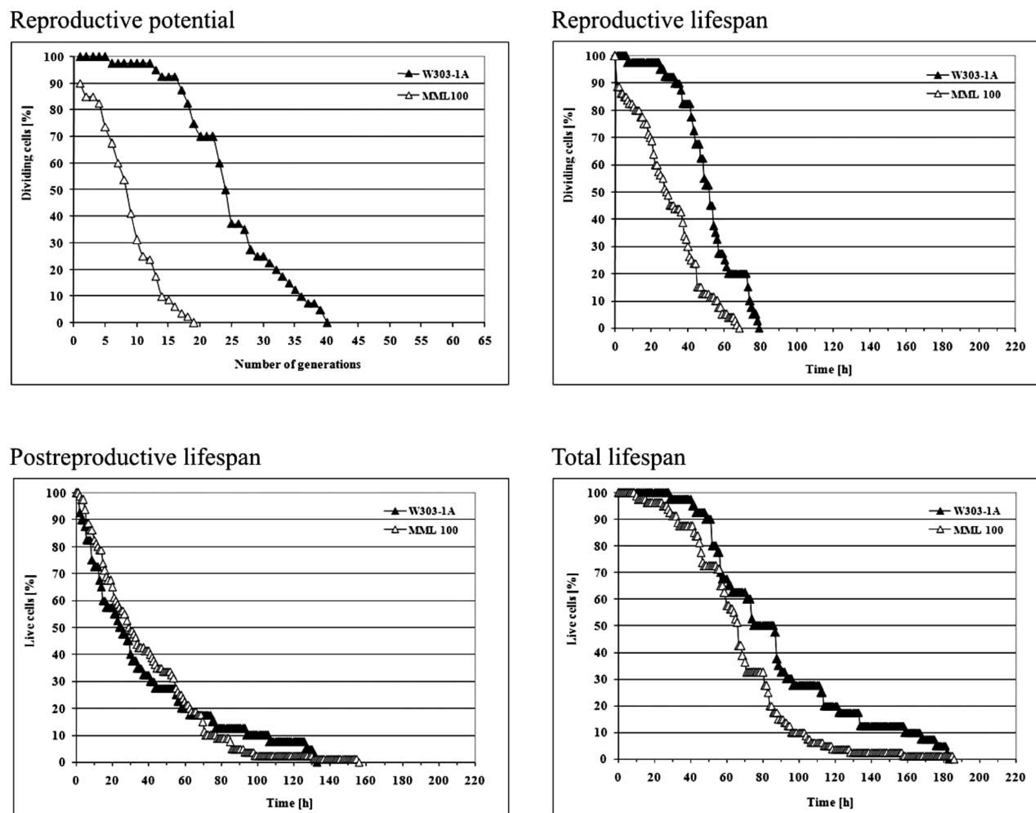

Fig. (2). Reproductive potential, reproductive lifespan and total lifespan of a mutant lacking all five peroxiredoxins (5Δ) and its isogenic parent strain (BY4741).

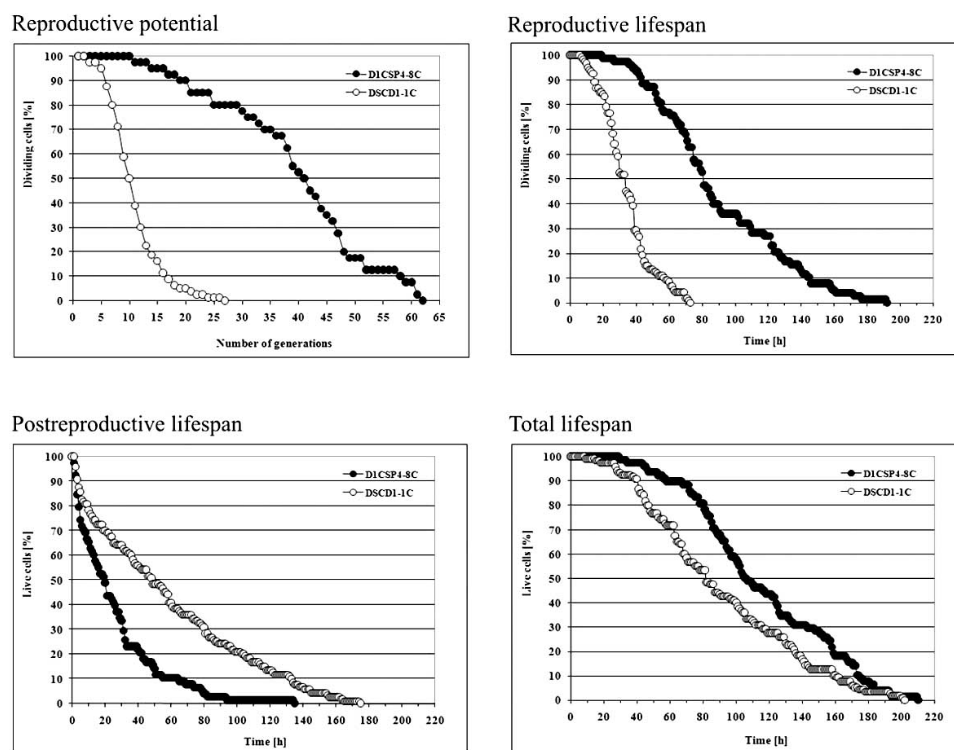

Fig. (3). Reproductive potential, reproductive lifespan and total lifespan of a mutant lacking glutaredoxin 5 (MML100) and its isogenic parent strain (W303-1A).

Table 2. Mean Reproductive Potential (*Number of Generations*), Mean Reproductive Lifespan, Postreproductive Lifespan and Total lifespan of the Yeast Strains Studied (Mean ± SEM, Combined Data from Duplicate Experiments; Maximal Values Given in Parentheses)

Yeast Strain	Reproductive Potential [number of buddings]	Reproductive Lifespan [h]	Postreproductive Lifespan [h]	Total Lifespan [h]
D1CSP4-8C	*N* = 78	*N* = 78	*N* = 78	*N* = 78
	40.1±1.5 (62)	89.8±4.4 (191.9)	25.35±2.9 (93.5)	115.1±4.9 (209.5)
Δsod1	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	10.3±0.4 (26)	33.8±1.4 (72.1)	57.0±4.3 (174.6)	90.7±4.2 (201.1)
BY4741	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	23.7±1.3 (54)	66.3±3.9 (156.2)	58.4±6.3 (200.9)	124.7±5.4 (212.2)
5Δ	*N* = 78	*N* = 78	*N* = 78	*N* = 78
	13.5±0.5 (27)	58.7±2.8 (119.6)	61.1±5.2 (172.3)	119.8±5.1 (213.2)
W303-1A	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	26.3±1.0 (39)	57.2±2.1 (96.4)	29.2±3.5 (132.5)	86.4±3.9 (182.2)
MML 100	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	7.7±0.5 (18)	28.9±2.1 (67.3)	38.2±3.4 (155.3)	67.1±3.3 (185.0)

Statistical analysis showed a strong, positive correlation between the reproductive potential (*number of generations*) and length of time spent in reproductive phase. This correlation is statistically significant for each pair of yeast strain and two replicates ($P > 0.05$ for all strains; Table **4**). No significant correlation was found between the reproductive potential and postreproductive lifespan or total lifespan.

DISCUSSION

The aging of *S. cerevisiae* has been originally postulated to model the aging of higher organisms, including man. It should be mentioned that majority of yeast studies (including the present one) are made on haploid cells. Haploids are in allowing for immediate observation of effects of recessive

Table 3. Mean Generation Times of the Strains Studied (Mean ± SEM)

Yeast Strain	Mean Generation Time [min]	P
D1CSP4-8C	N = 1520 95.9 ± 0.8	< 0.05
Δsod1	N = 702 137.9 ± 1.8	
BY4741	N = 855 115.5 ± 2.5	< 0.05
5Δ	N = 512 165.1 ± 3.2	
W303-1A	N = 800 100.3 ± 2.1	< 0.05
MML 100	N = 252 167.3 ± 6.3	

Table 4. Pearson's Correlation Coefficient for each Strain Between the Reproductive Potential vs Reproductive, Postreproductive and Total Lifespan for each Strain Tested

Yeast Strain	Reproductive Potential / Reproductive Lifespan	Reproductive Potential / Postreproductive Lifespan	Reproductive Potential / Total Lifespan	Reproductive Lifespan / Postreproductive Lifespan
D1CSP4-8C	*N* = 78	*N* = 78	*N* = 78	*N* = 78
	r = 0.700*	r = -0.144	r = 0.546	r = -0.146
	p < 0.0001	p = 0.207	p < 0.0001	p = 0.199
Δsod1	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	r = 0.930*	r = -0.206	r = 0.08	r = -0.230
	p < 0.0001	p = 0.023	p = 0.335	p = 0.011
BY4741	*N* = 78	*N* = 78	*N* = 78	*N* = 78
	r = 0.890*	r = -0.465	r = 0.100	r = -0.527
	p < 0.0001	p < 0.0001	p = 0.376	p < 0.0001
5Δ	*N* = 77	*N* = 77	*N* = 77	*N* = 77
	r = 0.810*	r = -0.320	r = 0.115	r = -0.294
	p < 0.0001	p = 0.004	p = 0.315	p = 0.009
W303-1A	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	r = 0.880*	r = -0.129	r = 0.364	r = -0.102
	p < 0.0001	p = 0.250	p = 0.0008	p = 0.366
MML 100	*N* = 80	*N* = 80	*N* = 80	*N* = 80
	r = 0.910*	r = -0.257	r = 0.171	r = -0.366
	p < 0.0001	p = 0.021	p = 0.127	p = 0.0008

*P < 0.05.

mutations. Moreover, the possibility of sporulation of diploid strains may complicate studies of their reproductive potential and lifespan.

The plots of *reproductive potential* (plots of fraction of cells able of further divisions vs the number of divisions accomplished, commonly referred to as "survival plots") of the haploid yeast cells and human survival curves have similar shape [12] which has been used as an argument for the homology of aging between the yeast and mammals, including human. However, if these processes are similar, the *total lifespan* of the yeast should be used for comparison, so also for the yeast the fraction of surviving cells should be plotted versus the time (and not versus a parameter describing *reproductive potential*). There is no justification for applying the term "longevity" to a quantity which is a number of cell divisions, not expressed in the units of time, and for a simultaneous treatment of the yeast as an organism (describing its "longevity" and as a cell, measuring the number of its divisions).

Our data (Figs. **1-3**) support the finding of Minois *et al*. [10] that the *postreproductive lifespan* represents a significant fraction of the *total lifespan* of the yeast and cannot be neglected in the analysis. These data demonstrate also that mutations have different effect on yeast *reproductive potential* and *total lifespan*, mutations which significantly affect the *reproductive potential* having much less or no effect on the *total lifespan* of *S. cerevisiae*. Similar conclusion can be drawn from analysis of data presented by Minois *et al*. [10].

The finding that disruption of genes coding for key antioxidant proteins does not affect significantly the *total lifespan* of the yeast is intriguing and may indicate that either the free radical theory of aging does not apply to *S. cerevisiae*. Our other findings would be in line with such an explanation. Since more than twenty years we have been interested in physiologic effects of defects of the antioxidant defense in the yeast, including the effects on their *reproductive potential* (budding capacity). Our initial results concerning the effects of mutations in the genes coding for antioxidant enzymes were ambiguous. We found that disruption of genes coding for the cytosolic and mitochondrial superoxide dismutases (SOD1 and SOD2, respectively), though not for catalases T or A, lead to a diminution of the *reproductive potential* [13, 14].

Physiological defects other than decrease in the reproductive potential, observed in this mutant, like methionine and lysine auxotrophy and sensitivity to oxidative stress, have been effectively prevented by antioxidants [15-17]. The effects of antioxidants and of hypoxia were additive [15, 16]. These experiments demonstrate that the adverse effects of the lack of CuZnSOD can be alleviated in two ways: by hypoxia and/or by exogenous antioxidants. Consequently, one can expect that if the reduction of *reproductive potential* of *Δsod1* cells is due to free radical reactions, the number of cell divisions of these cells should be increased and brought closer to that of the parent strain in hypoxia/anoxia. However, hypoxic or anoxic atmosphere did not cure the decreased budding capacity of *Δsod1* yeast [14], contrary to what could be expected by the free radical theory of aging. A similar result has been reported for yeast disruptants in methionine sulfoxide reductase [18]. Ascorbate brought about some increase of the reproductive capacity of the *Δsod1* mutant but only at very high concentrations [19].

Another explanation of the finding of previous researchers [10] and ours is also possible and, in our view, more plausible. It has been postulated that the reason for the cessation of budding by an aging yeast mother cell is the accumulation in the mother yeast cell of a "senescence factor" which is not transmitted or negligibly transmitted to daughter cells. The existence of a cytoplasmic diffusible, degradable senescence factor has been suggested [20] but the factor has never been identified. Accumulation of rDNA circles accumulating in old yeast mother cells has been proposed to limit the replicative lifespan of the yeast [21, 22] although cases of replicative yeast aging not accompanied by accumulation of rDNA circles have been reported [23, 24]. 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 [25]. However, reproductive lifespan of the yeast cultured under hypoxic and anoxic conditions (i. e. when oxidative protein damage is attenuated) is not increased, as mentioned already [14, 18]. We have demonstrated that two isogenic yeast strains bud until they reach the same final volume although their differ significantly in the *reproductive potential.* These data suggest that the inevitable increase in cell volume with each division of a yeast mother cell is the factor limiting the number of buddings it can accomplish [26-28]. We have then measured the increase in volume of the three pairs of strain presented in this paper and found that within each pair of strains, cells of both a standard laboratory strain and of the mutant cease to divide after attaining the same volume. Within a given strain, cells attaining higher volume at a higher rate stop budding after production of a smaller number of divisions (Zadrag *et al*., submitted).

The increase in the mother cell volume on each division is inherent to the budding as a mode of cell division. We suggest that it may lead to reaching a cell volume incompatible with efficient functioning of cell division machinery and make impossible first further cell divisions and then effective cell functioning thus leading ultimately to cell death. It should be recalled that, unlike metazoan cells, a yeast cell is programmed for permanent growth and division if only nutrients are present in the medium. If cell division is delayed by proofreading mechanisms in the mutants which accumulate more DNA damage due to defective antioxidant defense, or due to disturbed mechanisms of metabolic regulation, their volume grows faster due to running protein synthesis and reach the limiting cell divisions with a lower number of buddings. If so, what we are measuring in "aging" studies of *S. cerevisiae* are the effects of attaining limiting cell volume (inevitable due to budding as a mode of cell divisions) rather than aging. From this point of view, the budding yeast may be not a valid model for human cell aging. This conclusion does not totally preclude the usefulness of the approach aimed at identification of genes increasing yeast *reproductive potential* and searching for their homologs in other organisms which could affect their *lifespan* but indicates that we are dealing with processes which are analogous and not homologous.

Finally, it can be mentioned that the Hayflick phenomenon of limited number of population doublings of culture cells *in vitro* may be not relevant to aging of the whole organism. It has been demonstrated that there is no correlation between the age of the donor and the number of population doublings of fibroblasts derived from the donor [29] indicating that the Hayflick limit is not a major cause of the limited lifespan of multicellular organisms. Instead, it may represent an artifact of tissue culture conditions under which the cells are deprived of interactions with other cell types and cell matrix, forced to grow in a monolayer and exposed to higher oxygen tension [30]. Therefore, the studies of reproductive potential of the yeast may concern a phenomenon which is analogous to an effect not critical for the aging of a multicellular organisms as a whole.

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