

Replicative aging of the yeast does not require DNA replication

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Abstract

Mating pheromone treatment resulting in shmoo formation is a physiologically relevant model for separation of cell growth and division processes in the yeast *Saccharomyces cerevisiae*. Using this attitude we demonstrate that yeast loses its capacity for division at a faster rate when engaged in intensive growth and metabolism without cell divisions (in the shmoo state) than during normal reproductive growth. These results suggest that limitation of the division potential in the yeast is not due to a counter of cell divisions but is of growth/metabolic nature, perhaps involving attaining a limitation of cell volume.

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The yeast *Saccharomyces cerevisiae* has become a model organism in the studies of aging [1–4], due to its limited capacity for cell divisions. The number of divisions a yeast cell can accomplish (corresponding to the number of buds it forms) is referred to as its replicative lifespan. The phenomenon of limited ability of yeast cells to divide is sometimes compared to the Hayflick limit of mammalian cells. However, there are reasons to think that both these phenomena are rather analogous than homologous. One of such reasons is that the Hayflick limit is a consequence of telomere shortening after each round of DNA replication, a phenomenon which does not occur in yeast cells. A fundamental question appears: what is the mechanism limiting the number of cell divisions in yeast cells? The most broadly accepted view links this effect to the accumulation of extrachromosomal rDNA circles [4–6]. However, in some cases shortening of replicative lifespan is not accompanied by an accumulation of ERCs, e.g., in *rad52* mutants defective in DNA repair through homologous recombination, [7] or during aging in the stationary phase [8].

Theoretical considerations show that accumulation of rDNA circles alone is insufficient to account for the limited number of buddings [9]. Moreover, an intriguing phenomenon of asymmetrical distribution of oxidized proteins between mother and daughter cells [10] may lead to the accumulation of damage in the mother cells and may contribute to its limited replicative lifespan. Accumulation of extrachromosomal rDNA circles takes place during recombination while oxidative modification of proteins is usually correlated with the rate of metabolic processes. The main energetic cost of the yeast growth is the production of biomass, mainly proteins, rather than processes related to DNA replications or cell divisions. Further insight into the mechanisms limiting the number of yeast cell divisions can be gained by separation of processes of cell growth from DNA replication and mitotic events.

In order to answer the question as to which factor determines the limited division capacity of yeast cells, it is important to find which stage of the cell cycle plays a dominant role in this phenomenon. The typical cell cycle consists of two growth phases (G1 and G2) separated by a DNA replication stage (S) and cell division (M). However, there are examples of omission of the growth

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phases (early stages of blastulation) or arresting cells at the G1 phase without inhibition of growth processes (various cases of differentiation). In the case of the yeast, an example of such process is differentiation of haploid cells under the action of mating pheromones into shmoo forms, corresponding functionally to gametes of higher organisms. Such cells are arrested in the G1 phase and grow in the direction of a partner of an opposite mating type in order to reach it and form a zygote. During this process, cell volume increases significantly.

In this study, we have made use of this unique possibility of separation of cell growth and division processes available for yeast cells. Such an attitude has been employed for the first time by Kennedy et al. [11] to examine the effect of cell size on the replicative lifespan of the yeast. The aim of the present study was to address the question whether the processes of extensive growth, including biosynthesis of protein, affect the replicative lifespan of the yeast; in other words, which of the two processes: cell growth or other processes taking place during cell divisions plays a key role in the determination of the replicative lifespan of the yeast. We have used two a-type yeast strains of significantly different number of divisions.

Materials and methods

Yeast strains and culture. Yeast of two isogenic strains: D1CSP4-8C (MATa leu1 arg4) being a cross of Sp4 [12] and DSCD1-1C [13], and DSCD1-1C (MATa leu1 arg4 sod1) were used. Yeast was grown in a standard liquid YPDextrose medium (1% Difco yeast extract, 1% yeast Bacto-peptone, and 2% glucose) on a rotary shaker at 150 rpm or on a solid YPD medium containing 2% agar, at a temperature of 30°C.

Pheromone treatment. Virgin cells (buds) were isolated by centrifugation in a sucrose (10–30%) density gradient. The bud suspension in YPD medium (10^6 cells/ml) was spun down, resuspended in fresh medium, and added with the α -pheromone (BioVectra, DCL, Canada) to the final concentration of 5 μ M. The cell suspensions were incubated on the shaker (150 rpm, 30 °C) for various time intervals. In order to counteract the effect of pheromone degradation, a fresh portion of the pheromone (8.5 μ g/ml) was added every 8–9 h. After appropriate time intervals, the cells were centrifuged and transferred onto fresh solid YPD medium.

Determination of replicative lifespan. Lifespan of individual yeast cells was determined by a routine procedure [14,15] of cells placed on agar plates using a micromanipulator. The number of buds formed by each cell is referred to as its replicative lifespan. At least 40 cells were taken for each experimental point.

Results and discussion

We found that incubation of cells arrested in G1 phase of the cell cycle by the action of α -pheromone resulted in a progressive decrease in the number of divisions they were able to accomplish after release from the action of the pheromone by transfer to a new medium. This phenomenon was observed for both yeast

strains used (Figs. 1 and 2). It should be stressed that in our strains we did not observe any significant mortality in the shmoo state, i.e., almost all cells were able to resume divisions after release of the pheromone action (Zadrag et al., submitted). Such a behavior of our strains contrasts with that reported by Severin and Hyman [16] who reported a considerable mortality of yeast treated with the pheromone (albeit at a much higher concentration).

An attitude similar to that presented here has been previously employed by Kennedy et al. [11] (nota bene, also these authors did not report any significant mortality after pheromone treatment). They came to a different conclusion, suggesting that arresting division and allowing for cell growth do not affect the number of yeast cell division capacity. However, their experiment had dealt a short time of pheromone action (4 h) which

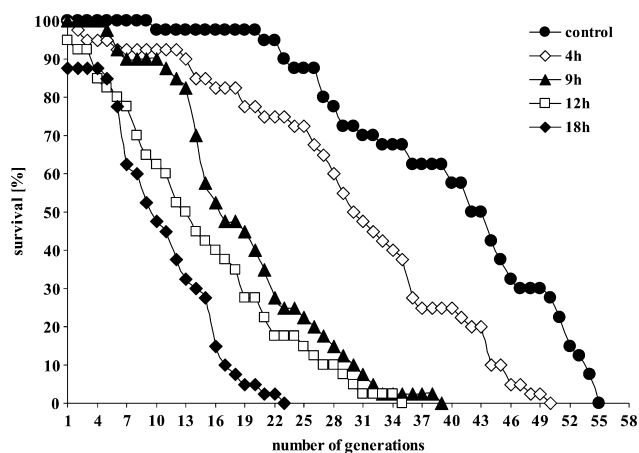


Fig. 1. Survival curves of wild-type yeast cells (strain D1CSP4-8C) treated with α -pheromone for indicated time periods. Yeast cells (5×10^6 cells/ml) were incubated with the pheromone (5 μ M initial, then supplemented as described in Materials and methods).

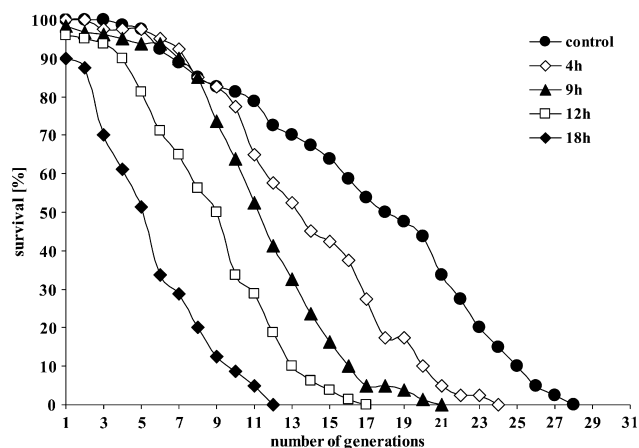


Fig. 2. Survival curves of Δ sod1 yeast cells (strain DSCD1-1C) treated with α -pheromone for indicated time periods. Conditions as in legend to Fig. 1.

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