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The links between hypertrophy, reproductive potential and longevity in the *Saccharomyces cerevisiae* yeast*

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The yeast *Saccharomyces cerevisiae* has long been used as a model organism for studying the basic mechanisms of aging. However, the main problem with the use of this unicellular fungus is the unit of "longevity". For all organisms, lifespan is expressed in units of time, while in the case of yeast it is defined by the number of daughter cells produced. Additionally, in yeast the phenotypic effects of mutations often show a clear dependence on the genetic background, suggesting the need for an analysis of strains representing different genetic backgrounds. Our results confirm the data presented in earlier papers that the reproductive potential is strongly associated with an increase in cell volume per generation. An excessive cell volume results in the loss of reproductive capacity. These data clearly support the hypertrophy hypothesis. The time of life of all analysed mutants, with the exception of *sch9*Δ, is the same as in the case of the wild-type strain. Interestingly, the 121% increase of the *fob1*Δ mutant's reproductive potential compared to the *sfp1*Δ mutant does not result in prolongation of the mutant's time of life (total lifespan).

Key words: yeast, lifespan, longevity, hypertrophy

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INTRODUCTION

Aging is defined as *"any age-specific decline in variables associated with individual fitness, specifically mortality, reproduction and physiological performance"* (Reznick *et al.*, 2004). Despite the complicated etiology of the process, we still lack certainty as to which factors lead to longevity. The choice of an appropriate model for research has been a challenge for numerous scientists. Model organisms used in many fields of science, such as medicine, biology or biotechnology, should help overcomes experimental constraints associated with ethical use of a host organism, including the human being. They are also useful in developing and optimising analytical methods. The important thing is that such organisms should be representative of a larger class of living forms. Unfortunately, the use of a specific model organism does not always ensure that the same results will be obtained in other organisms (Karathia *et al.*, 2011). From among the variety of types of such organisms, the *S. cerevisiae* yeast was proposed as a model organism for explanation of the general mechanisms of aging (Bitterman *et al.*, 2003; Jazwinski, 1999; Piper, 2006). The use of yeast in the studies of aging has its clear advantages, such as the small genome, short cell cycle, numerous protocols and broad knowledge of this unicellular eukaryotic species. Yeast is described in detail also at the molecular level. We can use successfully various tools to change and monitor cellular processes, such as translation and transcription. On the other hand, we should also take into consideration certain problems or limitations resulting from the use of this particular unicellular organism. An example of such consideration is homeostatic regulation of both intra- and extracellular environments by yeast and higher (multicellular) eukaryotes. Yeast regulates its intracellular processes in a way similar to other eukaryotes. However, as a unicellular organism yeast is more susceptible to environmental factors (changes of temperature, nutrients, metabolites and other) which can regulate its metabolism, as opposed to multicellular organisms where cells have a relatively constant external environment. Other controversies include absence of DNA silencing by methylation, lack of miRNA processing machinery or lack of telomere shortening during successive replication cycles (Gershon & Gershon, 2000). Furthermore, the amount of non-coding DNA and only marginal contribution of alternative splicing indicate that there are aspects where yeast biology is incomparable to the biology of higher eukaryotes. Apart from the differences associated with molecular, physiological or phylogenetic relations between yeast and higher eukaryotic organisms, there are important limitations concerning the way of expressing their lifespan. In the case of yeast, this parameter is defined as the number of daughter cells produced (Sinclair *et al.*, 1998), whereas for other organisms lifespan is expressed in units of time. Our recent studies with the use of the so called "longevity mutants" (organisms with increased reproductive potential) showed no significant differences in their time of life (total lifespan). In spite of significant differences in the reproductive potential, the total lifespan of the mutants remained almost identical to that of the wild-type strain (Molon *et al.*, 2015).

The phenomenon discovered by Mortimer and Johnston (Mortimer & Johnston, 1959) was termed replicative lifespan (RLS) and defined as the number of daughter cells produced by a mother cell (Kaeberlein, 2010), later named reproductive potential (Zadrag *et al.*, 2008). Mortimer and Johnston were the first to show that yeast cells can perform a limited number of cycles (Mortimer & Johnston, 1959). During that time, a lot of theories emerged describing various factors that might affect the numeric value of the replicative lifespan. One of the first candidates proposed for the role of the "senescence factor" was extrachromosomal rDNA circles (Sinclair

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Abbreviations: ERCs, extrachromosomal rDNA circles; RLS, replicative lifespan

& Guarente, 1997). The role of the "senescence factor" was also attributed to oxidatively damaged proteins (Aguilaniu *et al.*, 2003) or thermal aggregates (Erjavec *et al.*, 2007). Replicative lifespan may also be influenced by various genes and environmental conditions (Steinkraus *et al.*, 2008). For many years, the flagship "longevity mutant" was *fob1*Δ. *FOB1* (*fork blocking less*) is a gene that has no homologues in other organisms. The *Fob1* protein occurs in the nucleolus (Defossez *et al.*, 1999). Blocking the replication forks by mutations in the *FOB1* gene proved to be an important factor in reducing the number of extrachromosomal rDNA circles (ERCs), which were regarded as one of the many "senescence factors". Studies have shown that ERCs accumulate in larger amounts in old cells; for cells this means that they have performed a lot of reproductive cycles. The increased number of ERCs was postulated to decrease the reproductive potential of yeast cells (Sinclair & Guarente, 1997). Another gene, the mutation of which leads to an increased reproductive potential, is *SCH9*. The yeast *SCH9* gene encodes a serine/threonine kinase involved in the glucose response and regulation of transcription by RNA polymerases I, II, and III. Sch9p also performs a function analogous to the mammalian S6K gene (Urban *et al.*, 2007). Mutation in this gene extends chronological lifespan and causes an increase in the reproductive potential. It also causes an increased resistance to oxidative and thermal stress (Fabrizio *et al.*, 2001; Kaeberlein & Kennedy, 2005). With yeast as a model organism used for studies on the aging process, much research has been devoted to the analysis of replicative lifespan, which refers only to the number of daughter cells produced. On that basis, numerous of "longevity mutants" were proposed. However, when in addition to the number of daughters we also consider the time of life, the only longevity yeast mutant (for longevity expressed in units of time) would be the one that has been recently described as *sfp1*Δ (Molon *et al.*, 2015). The product of the *SFP1* gene is a protein (split finger-zinc protein) that plays a key role in regulating the biosynthesis of ribosomal proteins. This protein can also have an impact on reproductive potential (Heeren *et al.*, 2009; Molon *et al.*, 2015).

An alternative postulate is that the limit of mitotic cycles is not a consequence of accumulation of a "senescence factor" but results from cell achieving its critical volume, which prevents further reproduction (Yang *et al.*, 2011; Zadrag-Tecza *et al.*, 2009). Studies concerning the impact of genes on RLS often face the problem of the impact of the genetic background on the phenotypic effect of deletion of selected genes, which makes it difficult to formulate clear conclusions. Therefore, it is important in this case to take into account other parameters, such as changes in cell size or duration of various phases in yeast cell life (reproductive and post-reproductive) because such parameters may facilitate interpretation of the differences observed in the studied yeast strains related to genetic backgrounds.

The aim of this work is to analyse the reproductive potential and time of life (expressed in time units) of yeast strains devoid of the genes known as "longevity genes", namely *FOB1*, *SCH9* and *SFP1*, and also to verify whether there is a relationship between the excessive volume of cells (hypertrophy) and reproductive potential. The role of cell size in the regulation of reproductive potential of yeast was presented in our team's previous papers. As the topic has recently spurred heated discussion, it is worth adding some further data. In addition, the new aspect of these studies was using the BMA64-1A genetic background with cells achieving larges sizes in both mean and maximum terms, in comparison with cells representing other genetic backgrounds.

MATERIALS AND METHODS

Chemicals. Components of culture media were from BD Difco (Becton Dickinson and Company, Spark), except for glucose (POCh, Gliwice, Poland).

Yeast strains. Strains used for the purpose of this paper are show in Table 1. The strains were constructed as described in Molon *et al.*, 2015.

Media and growth conditions. Yeast was grown in a standard liquid YPD medium (1% yeast extract, 1% yeast Bacto-peptone, 2% glucose) on a rotary shaker at 150 rpm at a temperature of 28°C.

Determination of reproductive potential. Reproductive potential of yeast (the number of daughter produced by a single cell during its life) was determined according to the previously described procedure (Wawryn *et al.*, 1999). Five microliter aliquots of culture from the exponential phase of growth (5×10^{-7} cells/ml density) was dropped on separate YPD plates. Forty single cells, randomly chosen, were used for each experiment. The analysis was performed by micromanipulation using the Nikon Eclipse E200 optical microscope with the attached micromanipulator. The number of buds formed by each cell signifies the reproductive potential of the cell. During the manipulation, the plates were kept at 28°C for 16 h and at 4°C during the night. The data represent the mean values from three separate experiments.

Determination of reproductive lifespan, post-reproductive lifespan and total lifespan. Yeast lifespan was determined according to the previously described method (Minois *et al.*, 2005) with some modifications (Zadrag *et al.*, 2008). Five microliter aliquots of culture from the exponential phase of growth (5×10^{-7} cells/ml density) was dropped on separate YPD plates with solid medium containing Phloxine B (10 μg/ml). Forty single cells were used for each experiment. The analysis was performed by micromanipulation using the Nikon Eclipse E200 optical microscope with the attached micromanipulator. Phloxine B was used as the indicator of dead yeast cells during determination of the total lifespan of cells. The total lifespan was calculated as the sum of the reproductive and post-reproductive lifespans (each

Table 1. Strains used in this study

Strain	Genotype	Source
BMA64-1A (wild-type)	MAT a ura3-1 trp1Δ 2 leu2-3,112 his3-11,15 ade2-1 can1-100	Euroscarf
*fob1*Δ	MAT a ura3-1 trp1Δ 2 leu2-3,112 his3-11,15 ade2-1 can1-100 fob1::kanMX4	in this study
*sch9*Δ	MAT a ura3-1 trp1Δ 2 leu2-3,112 his3-11,15 ade2-1 can1-100 sch9::kanMX4	in this study
*sfp1*Δ	MAT a ura3-1 trp1Δ 2 leu2-3,112 his3-11,15 ade2-1 can1-100 sfp1::kanMX4	in this study

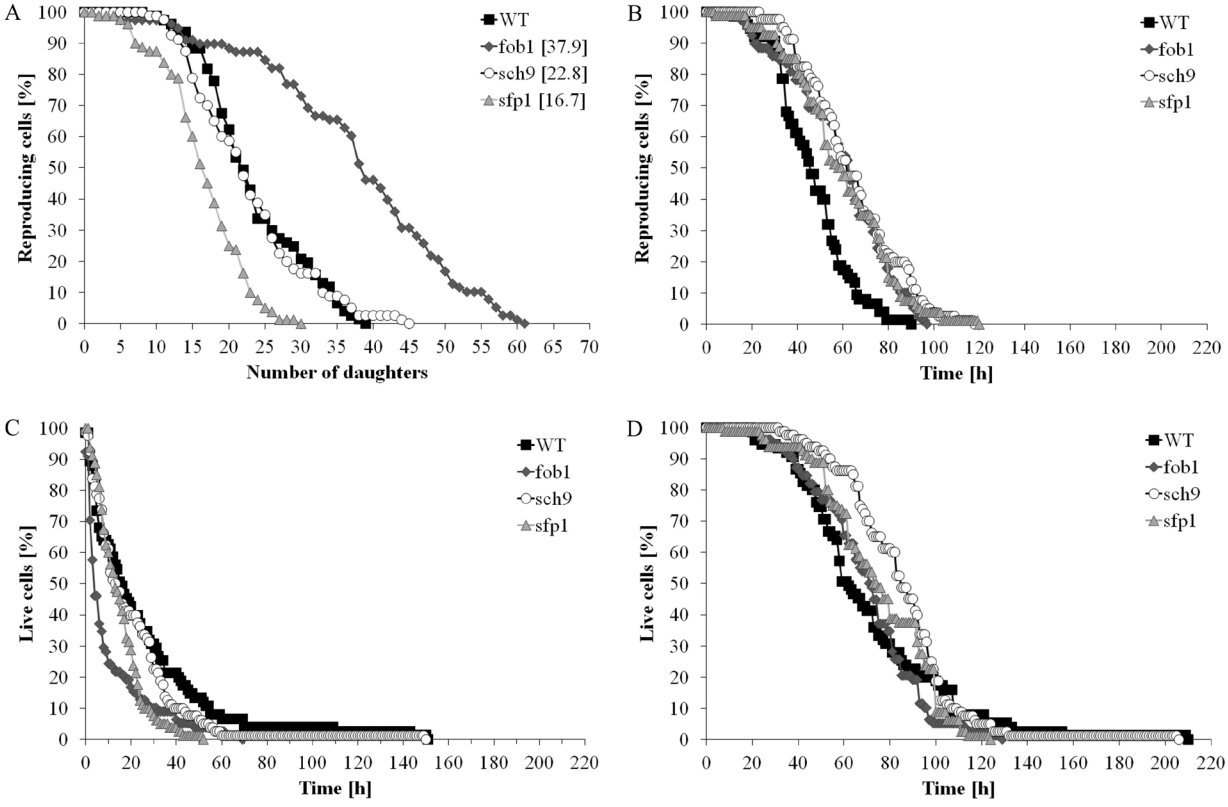

Figure 1. Comparison of the reproductive potential (A), reproductive lifespan (B), post-reproductive lifespan (C) and total lifespan (D) of the haploid wild-type yeast strain BMA64-1A and isogenic mutant strains *fob1Δ*, *sch9Δ* and *sfp1Δ*.
Values shown in parentheses are the mean values of the reproductive potential.

expressed in units of time). During the manipulation, the plates were kept at 28°C for 16 h and at 4°C during the night. The data represent the mean values from three separate experiments.

Estimation of cell volume. Cell volume was estimated by means of analysis of microscopic images recorded every fifth cell budding during the routine procedure of determining the reproductive potential. Images were captured with the Nikon Eclipse E200 microscope equipped with the Olympus DP26 digital camera. Cell diameter was measured using the Olympus cellSens Standard software. Cell diameter (d) was measured four times in various planes for each cell and the mean value was used for calculations. Assuming that each cell has a regular shape similar to the sphere, the cell volume was calculated as $4/3\pi\ (d/2)^3$.

Determination of mean doubling time. Mean doubling time was calculated individually for each of the analysed cells during routine determination of reproductive potential. During the calculations, the time of the two first reproductive cycles was not taken into account (in the case of virgin cells, the first as well as the second doubling time is longer than in older yeast cell); the time of the first reproductive cycle performed at the start of each day's experiment was also rejected (during the night the plates was kept at a low temperature of +4°C). The data represent the mean values from three independent experiments (with forty cells used in each experiment).

Statistics. Statistical analysis was performed with the use of StatSoft, Inc. (2011) (STATISTICA, data analysis software system, version 10. www.statsoft.com) using the *t*-test for independent samples in respect of the variable test and Dunnett's *post hoc* test. The results were presented as mean ± standard deviation. Statistically significant differences were taken at $P < 0.01$.

RESULTS AND DISCUSSION

The phenotypic effects of mutations often show a clear dependence on the genetic background, thus suggesting the need for an analysis of strains representing different genetic backgrounds. It is especially important in the analysis of reproductive potential where genetic background has an impact on the number of daughter cells produced (Dmello *et al.*, 1994; Kaeberlein *et al.*, 2005; Molon *et al.*, 2015). In this study, we used three yeast mutants — *fob1*Δ, sch9Δ and sfp1Δ — representing one standard genetic background, i.e. BMA64-1A, where the characteristic feature is the large size achieved by the cells in comparison with cells representing other genetic backgrounds. All the analysed genes belong to the "longevity" genes but represent different mechanisms of action. Furthermore, to assess the impact of the genetic background on the analysed lifespan parameters, we compared the obtained results with those presented in another paper (Molon *et al.*, 2015), where we used these mutants in the BY4741 genetic background.

To check the phenotypic effects of the analysed mutations, we determined the reproductive potential and lifespan expressed in units of time. First, we checked reproductive potential of the selected yeast strains, expressed as the number of daughters produced by a mother cell. Our results showed different values of reproductive po-

Table 2. Mean Reproductive potential (number of generations), reproductive lifespan, post-reproductive lifespan and total lifespan of the yeast strains studied (mean ± S.D., combined data from duplicate experiments). **$p < 0.01$ compared to the wild-type strain

Strain	Reproductive potential [number of generations]	Reproductive lifespan [h]	Post-reproductive lifespan [h]	Total lifespan [h]
BMA64-1A	23.6±7	46.4±18.9	24.1±25.6	70.5±32.8
*fob1*Δ	37±9.12**	58.7±22.4**	10.5±14.9**	69.2±23.0
*sch9*Δ	22.81±7.9	64.3±21.1**	18.5±15.3	82.9±21.4**
*sfp1*Δ	16.74±5.6**	59.4±22.3**	14.9±10.2**	74.4±24.3

tential for each of the analysed yeast strains, even though all of them were treated as "longevous": as such, they should have produced more daughter cells compared to the wild-type strain (Fig. 1A). The reproductive potential of the *fob1*Δ mutant is higher, while that of *sfp1*Δ is statistically significantly lower in comparison with the wild-type strain. In turn, the *SCH9* gene deletion has no effect on the reproductive potential in that genetic background (Table 2). The increased reproductive potential of the *fob1*Δ mutant was initially associated with low levels of ERCs (Defossez *et al.*, 1999). However, the results obtained by Takehiko Kobayashi showed that despite the significant reduction in the amount of ERCs in one of the strains (rARS Δ-3), the reproduction potential dropped. This was postulated to be the consequence of the rDNA instability; such instability occurs more frequently in mother cells rather than daughters (Ganley *et al.*, 2009; Lindstrom *et al.*, 2011). These results confirm a hypothesis which assumes that stability of the rDNA regulates the stability of the whole genome, and thus is an important factor in regulating the "longevity" of yeast (Kobayashi, 2008). The decrease of the reproductive potential in the case of *sfp1*Δ is consistent with the data obtained by Heeren and coworkers (Heeren *et al.*, 2009) but differs significantly from the data obtained by Molon and coworkers (Molon *et al.*, 2015) in the studies where the BY4741 background was used. Given that all of the analysed genes are defined as "longevity genes", these results tend to be somewhat surprising and suggest that the phenotypic effect of the mutations is strongly dependent on the genetic background.

For an explanation of the differences in the reproductive potential observed in the analysed BMA64-1A genetic background, we have studied changes in cell size during the entire reproductive period. That experimental approach was associated with the hypertrophy hypothesis, which explains the relationship between the reproductive potential and the cell volume growth rate per generation (Bilinski, 2012, Bilinski *et al.*, 2012). The hypertrophy hypothesis assumes that limited reproductive potential is related to cell achieving its maximum volume (hypertrophy). Analysis of the kinetics of cell volume changes during successive cycles in the case of the wild-type yeast strain and selected mutants indicates an important role of cell volume in limiting the reproductive potential. The maximum volume of the *fob1*Δ mutant cell remains at the same level as in the wild-type strain. However, its doubling time is shorter, hence the increase in size during single generation is lower, and therefore cells of that mutant produce more daughters. In the case of the *sch9*Δ mutant, cells were larger compared to the wild-type and the *fob1*Δ mutant cells. On the other hand, in the case of *sfp1*Δ the observed volume of the cells was the biggest (Fig. 2). Cells of that strain attain a huge volume, which consequently leads to their breakdown and destruction (not shown). A significant increase in the volume of *sch9*Δ and *sfp1*Δ mutant cells may be associated with statistically significant prolongation of the doubling time (Fig. 3). This, in turn, may be explained by the fact that knock-out of the *SFP1* and *SCH9* genes slows down the growth rate of the yeast cells (Blumberg & Silver, 1991, Toda *et al.*, 1988). Both Sch9p and Sfp1p participate in regulation of ribosome biogenesis and RiBi genes transcription, which are essential for the cell (Jorgensen *et al.*, 2004). Among the factors involved in ribosome biogenesis, the protein Sfp1 is the strongest START repressor because it regulates expression of numerous genes affecting the cell volume (Jorgensen *et al.*, 2002). Furthermore, we suspect that hypertrophy of *sfp1*Δ and partially of *sch9*Δ may be a result

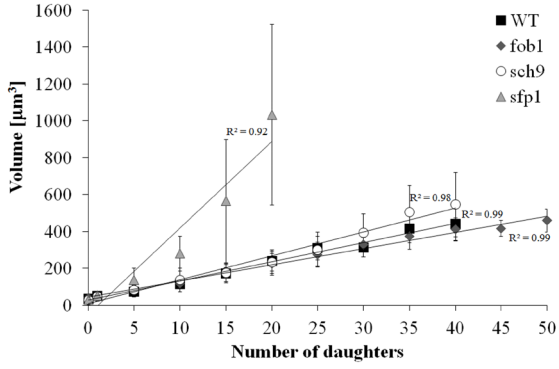

Figure 2. Dependence of cell volume on the number of daughters accomplished by mother yeast cells. The bars indicate S.D.

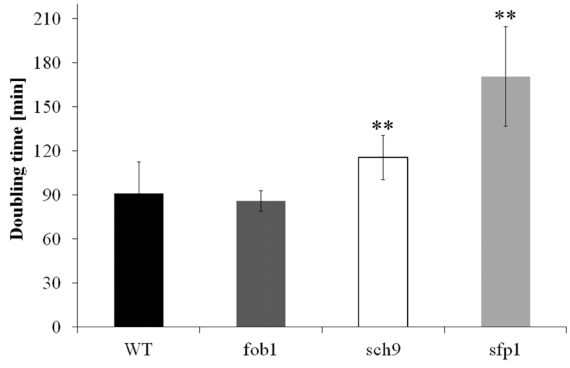

Figure 3. Comparison of average doubling time during reproduction of the haploid wild-type yeast strain BMA64-1A and isogenic mutant strains *fob1Δ*, *sch9Δ* and *sfp1Δ*.
**$p < 0.01$ compared to the wild-type strain.

of certain problems with coordination of two processes, namely cell volume growth and budding. In this genetic background (BMA64-1A), a problem with the correct cell cycle process, caused e.g. by the mutation, will lead to an increased volume — a phenomenon that was not observed in other genetic backgrounds. The cracking of oversized cells explains significant reduction in the reproduction potential of the *sfp1*Δ mutant compared to the previous data obtained for the other genetic background, i.e. BY4741 (Molon *et al.*, 2015).

As showed by the literature data and the results obtained in this study, reproductive potential is regulated by the rate of cell volume increase during successive reproducing cycles (Yang *et al.*, 2011; Zadrag-Tecza *et al.*, 2009). Therefore, the numerical dimension of the reproductive potential does not reflect the actual length of cell life. Yeast lifespan encompasses not only the time when the yeast cell reproduces (reproductive lifespan). After the end of the last cycle, the cell does not die but continues to live for a certain period of time, the length of which depends on the doubling time and the number of daughters produced by the mother cell (Zadrag *et al.*, 2008). Disregarding that stage of yeast cell life may lead to some problems with interpretation of the results, especially when these results are strain-dependent and vary between genetic backgrounds. An analysis of the duration of different stages of yeast cell life, i.e. reproductive and post-reproductive phases, may provide useful information for identification of the role of genes in relation to reproductive potential and total lifespan.

The reproductive lifespan was statistically significantly extended in the case of all the analysed strains (Fig. 1b). Cells of the *fob1*Δ mutant demonstrated a significant increase in their reproductive potentials; therefore, their reproductive lifespans also increased in comparison with the wild-type strain. The *sfp1*Δ mutant had a significantly decreased reproductive potential, while its reproductive lifespan was much longer compared to the wild-type strain. This extension of the reproductive phases of the *sch9* and *sfp1* mutants, with lack of differences in reproductive potential (*sch9*), or even lack of reduction in reproductive potential (*sfp1*) (Fig. 1A), may be a consequence of the extension of the doubling time, clearly seen in the case of the *sfp1* mutant (Fig. 3). We then examined the lifetime after reproduction (post-reproductive lifespan). A significant shortening of that phase in the case of *fob1*Δ and *sfp1*Δ compared to the wild-type strain is associated with two different factors. The *fob1*Δ mutant demonstrates an increase in reproductive potential; therefore, its post-reproductive lifespan is inversely proportional to the value of reproductive potential, as discussed previously (Zadrag-Tecza *et al.*, 2013). In turn, *sfp1*Δ has a decreased reproductive potential and a very short post-reproductive lifespan, which might be explained by a significant increase in the cell volume during successive cycles and disintegration of cells. We did not observe changes of that parameter in the *sch9*Δ mutant (Fig. 1C). The sum of the reproductive and post-reproductive lifespans indicates the total lifespan of cells. In our study, only the *sch9*Δ strain showed a statistically significant increase in the total lifespan. This was associated with a significant extension of the reproductive lifespan of that mutant, and only a slightly shortened post-reproductive phase. Interestingly, the *fob1*Δ mutant performed 37 generations on average and lived as long as the *sfp1*Δ strain which performed only 16 generations on average (Fig. 1D; Table 2).

The obtained data confirm our earlier observations that the phenotypic effect of the mutation is in many cases strain-dependent. In such a situation, it is difficult to draw any general conclusions without analysing the effect of a particular mutation in different genetic backgrounds. Even in the case of conserved pathways, such as Sch9p, the effect of the mutation may depend on the phenotypic properties of a wild-type strain. Further analysis should answer the question why we have seen such large variations in reproductive potential with the same mutants in different genetic backgrounds. It seems that some mutations may lead to dysregulation of the coordination between cell growth and budding. This may lead to an increase in the doubling time and to faster achievement of the state of hypertrophy. The results confirm earlier observations that reproductive potential (fertility) is strongly associated with cell volume growth per generation (Zadrag-Tecza *et al.*, 2009; Bilinski *et al.*, 2012). High hypertrophy can effectively reduce reproductive potential in yeast. Expressing lifespan of yeast not only in the number of daughters but also in units of time could provide a better explanation for the obtained results.

It seems that further studies are needed to strengthen this statement; however, the data presented in this work as well as in the previous one (Molon *et al.*, 2015; Zadrag-Tecza *et al.*, 2013) support it strongly.

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SREFERENCES

Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* **299**: 1751–1753, http://dx.doi.org/10.1126/science.1080418.

Bilinski T (2012) Hypertrophy, replicative ageing and the ageing process. *FEMS Yeast Res* **12**: 739–740, http://dx.doi.org/10.1111/j.1567-1364.2012.00843.x.

Bilinski T, Zadrag-Tecza R, Bartosz G (2012) Hypertrophy hypothesis as an alternative explanation of the phenomenon of replicative aging of yeast. *FEMS Yeast Res* **12**: 97–101, http://dx.doi.org/10.1111/j.1567-1364.2011.00759.x.

Bitterman KJ, Medvedik O, Sinclair DA (2003) Longevity regulation in Saccharomyces cerevisiae: linking metabolism, genome stability, and heterochromatin. *Microbiology and molecular biology reviews: Microbiol Mol Biol Rev* **67**: 376–399, http://dx.doi.org/10.1128%2FMMBR.67.3.376-399.2003.

Blumberg H, Silver P (1991) A split zinc-finger protein is required for normal yeast growth. *Gene* **107**: 101–110.

Defossez PA, Prusty R, Kaeberlein M, Lin SJ, Ferrigno P, Silver PA, Keil RL, Guarente L (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. *Mol Cell* **3**: 447–455.

Dmello NP, Childress AM, Franklin DS, Kale SP, Pinswasdi C, Jazwinski SM (1994) Cloning and characterization of *LAG1*, a longevity-assurance gene in yeast. *J Biol Chemi* **269**: 15451–15459.

Erjavec N, Larsson L, Grantham J, Nystrom T (2007) Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes & Develop* **21**: 2410–2421. http:/ /dx.doi.org/10.1101/gad.439307.

Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD (2001) Regulation of longevity and stress resistance by Sch9 in yeast. *Science* **292**: 288–290. http:/ /dx.doi.org/10.1126/science.1059497.

Ganley ARD, Ide S, Saka K, Kobayashi T (2009) The effect of replication initiation on gene amplification in the rDNA and its relationship to aging. *Mol Cell* **35**: 683–693. http:/ /dx.doi.org/10.1016/j.molcel.2009.07.012

Gershon H, Gershon D (2000) The budding yeast, *Saccharomyces cerevisiae*, as a model for aging research: a critical review. *Mech Ageing Develop* **120**: 1–22. http:/ /dx.doi.org/10.1016/s0047-6374(00)00182-2.

Heeren G, Rinnerthaler M, Laun P, Von Seyerl P, Koessler S, Klinger H, Hager M, Bogengruber E, Jarolim S, Simon-Nobbe B, Schueller

C, Carmona-Gutierrez D, Breitenbach-Koller L, Mueck C, Jansen-Duerr P, Criollo A, Kroemer G, Madeo F, Breitenbach M (2009) The mitochondrial ribosomal protein of the large subunit, Afo1p, determines cellular longevity through mitochondrial back-signaling *via* TOR1. *Aging-Us* **1**: 622–636.

Jazwinski SM (1999) Longevity, genes, and aging: a view provided by a genetic model system. *Exp Gerontol* **34**: 1–6.

Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M (2002) Systematic identification of pathways that couple cell growth and division in yeast. *Science* **297**: 395–400. http:/ /dx.doi.org/10.1126/science.1070850.

Jorgensen P, Rupes I, Sharom JR, Schneper L, Broach JR, Tyers M (2004) A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes & Develop* **18**: 2491–2505. http:/ /dx.doi.org/10.1101/gad.1228804.

Kaeberlein M (2010) Lessons on longevity from budding yeast. *Nature* **464**: 513–519. http:/ /dx.doi.org/10.1038/nature08981.

Kaeberlein M, Kennedy BK (2005) Large-scale identification in yeast of conserved ageing genes. *Mechanisms of ageing and development,* **126**: 17–21. http:/ /dx.doi.org/10.1016/j.mad.2004.09.013.

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2005) Genes determining yeast replicative life span in a long-lived genetic background. *Mecha Ageing Develop* **126**: 491–504. http:/ /dx.doi.org/10.1016/j.mad.2004.10.007.

Karathia H, Vilaprinyo E, Sorribas A, Alves R (2011) Saccharomyces cerevisiae as a model organism: a comparative study. *PloS One* **6**: e16015. http:/ /dx.doi.org/10.1371/journal.pone.0016015.

Kobayashi T (2008) A new role of the rDNA and nucleolus in the nucleus-rDNA instability maintains genome integrity. *Bioessays* **30**: 267–272. http:/ /dx.doi.org/10.1002/bies.20723.

Lindstrom DL, Leverich CK, Henderson KA, Gottschling DE (2011) Replicative age induces mitotic recombination in the ribosomal RNA gene cluster of Saccharomyces cerevisiae. *PLoS Genetics* **7**: e1002015. http:/ /dx.doi.org/10.1371/journal.pgen.1002015.

Molon M, Zadrag-Tecza R, Bilinski T (2015) The longevity in the yeast *Saccharomyces cerevisiae*: A comparison of two approaches for assessment the lifespan. *Biochemi Biophys Res Commun* **460**: 651–656. http:/ /dx.doi.org/10.1016/j.bbrc.2015.03.085.

Mortimer RK, Johnston JR (1959) Life span of individual yeast cells. *Nature* **183**: 1751–1752. http:/ /dx.doi.org/10.1038/1831751a0.

Piper PW (2006) Long-lived yeast as a model for ageing research. *Yeast* **23**: 215–226. http:/ /dx.doi.org/10.1002/yea.1354.

Reznick DN, Bryant MJ, Roff D, Ghalambor CK, Ghalambor DE (2004) Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature* **431**: 1095–1099. http:/ /dx.doi.org/10.1038/nature02936.

Sinclair D, Mills K, Guarente L (1998) Aging in *Saccharomyces cerevisiae*. *Ann Rev Microbiol* **52**: 533–560. http:/ /dx.doi.org/10.1146/annurev.micro.52.1.533.

Sinclair DA, Guarente L (1997) Extrachromosomal rDNA circles — a cause of aging in yeast. *Cell* **91**: 1033–1042. http:/ /dx.doi.org/10.1016/s0092-8674(00)80493-6.

Steinkraus KA, Kaeberlein M, Kennedy BK (2008) Replicative aging in yeast: the means to the end. *Ann Rev Cell Develop Biol* **24**: 29–54. http:/ /dx.doi.org/10.1146/annurev.cellbio.23.090506.123509.

Toda T, Cameron S, Sass P, Wigler M (1988) SCH9, a gene of *Saccharomyces cerevisiae* that encodes a protein distinct from, but functionally and structurally related to, cAMP-dependent protein kinase catalytic subunits. *Genes & Develop* **2**: 517–527.

Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, Deloche O, Wanke V, Anrather D, Ammerer G, Riezman H, Broach JR, De Virgilio C, Hall MN, Loewith R (2007) Sch9 is a major target of TORC1 in Saccharomyces cerevisiae. *Mol Cell* **26**: 663–674. http:/ /dx.doi.org/10.1016/j.molcel.2007.04.020.

Yang J, Dungrawala H, Hua H, Manukyan A, Abraham L, Lane W, Mead H, Wright J, Schneider BL (2011) Cell size and growth rate are major determinants of replicative lifespan. *Cell Cycle* **10**: 144–155. http:/ /dx.doi.org/10.4161/cc.10.1.14455.

Zadrag-Tecza R, Kwolek-Mirek M, Bartosz G, Bilinski T (2009) Cell volume as a factor limiting the replicative lifespan of the yeast *Saccharomyces cerevisiae*. *Biogerontology* **10**: 481–488. http:/ /dx.doi.org/10.1007/s10522-008-9192-0.

Zadrag-Tecza R, Molon M, Mamczur J, Bilinski T (2013) Dependence of the yeast *Saccharomyces cerevisiae* post-reproductive lifespan on the reproductive potential. *Acta Biochim Pol* **60**: 111–115.

Zadrag R, Bartosz G, Bilinski T (2008) Is the yeast a relevant model for aging of multicellular organisms? An insight from the total lifespan of *Saccharomyces cerevisiae*. *Curr Aging Sci* **1**: 159–165.