

Effect of temperature on replicative aging of the budding yeast *Saccharomyces cerevisiae*

Mateusz Molon · Renata Zadrag-Tecza

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Abstract The use of the budding yeast *Saccharomyces cerevisiae* in gerontological studies was based on the assumption that the reproduction limit of a single cell (replicative aging) is a consequence of accumulation of a hypothetical universal “senescence factor” within the mother cell. However, some evidence suggests that molecules or structures proposed as the “aging factor”, such as rDNA circles, oxidatively damaged proteins (with carbonyl groups) or mitochondria, have little effect on replicative lifespan of yeast cells. Our results also suggest that protein aggregates associated with Hsp104, treated as a marker of yeast aging, do not seem to affect the numeric value of replicative lifespan of yeast. What these results indicate, however, is the need for finding a different way of expressing age and longevity of yeast cells instead of the commonly used number of daughters produced over units of time, as in the case of other organisms. In this paper, we show that the temperature has a stronger influence on the time of life (the total lifespan) than on the reproductive potential of yeast cells.

Keywords Yeast · *Saccharomyces cerevisiae* · Lifespan · Longevity · Hsp104p

Introduction

The budding yeast *Saccharomyces cerevisiae* is considered to be a useful model organism for research on aging. In aging studies conducted with the use of yeast, two main accepted types of aging have been used: replicative and chronological aging (Longo et al. 1996; Mortimer and Johnston 1959). Replicative aging (replicative lifespan, RLS) is defined as the number of daughter cells produced by a mother cell (Kaerberlein 2010) and represents replicative potential of a cell. This type of aging was proposed as a model in the studies of aging of mitotically active cells of higher eukaryotes (Longo et al. 2012). In turn, chronological aging (chronological lifespan, RLS) shows the survival time of cells in a non-dividing state and is a model for studies of aging of post-mitotic cells of higher eukaryotes (Fabrizio and Longo 2003). Numerous studies have shown that both these models are useful for analysing various aspects of aging, e.g. calorie restriction, rDNA stability, aneuploidy or bioactive substance influence (Georgieva et al. 2015; Lewinska et al. 2014; Rona et al. 2015).

In this paper we focus only on the replicative lifespan (RLS). The use of the budding yeast as a model organism in gerontology was based on several premises, the key assumption being that the limit of reproductive capacity of each individual yeast cell is a consequence of active accumulation of a hypothetical “senescence factor” within the mother cell (Egilmez

M. Molon (✉) · R. Zadrag-Tecza
Department of Biochemistry and Cell Biology, University
of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland
e-mail: mateuszmolon@univ.rzeszow.pl

and Jazwinski 1989). That factor was supposed to gradually accumulate within the mother cell because of the postulated mechanism of its asymmetrical distribution between the mother and daughter cells (Aguilaniu et al. 2003). Generally, for daughter cells the counter is reset to count the number of reproductive cycles that the cell undergoes during its life. However, rejuvenation of daughter cells of old mothers is much more problematic as the daughters inherit the age of their mothers, probably due to lower efficiency of the mechanism of retention of the aforementioned “senescence factor” (Kennedy et al. 1994). It was proposed that molecules which might potentially play the role of such the “senescence factor” should meet the following basic criteria: (i) they should accumulate with age; (ii) they should be distributed asymmetrically between the mother and the daughter cells; (iii) decreasing the level of the “senescence factor” should lead to an increase in replicative lifespan, whereas increase of its level should decrease the replicative lifespan (Henderson and Gottschling 2008). It was also suggested that the potential “senescence factor” should be soluble and partly degradable (Egilmez and Jazwinski 1989). However, despite numerous studies, the nature of the factor has not been clearly defined yet (Kwan et al. 2013).

Initially, three candidate molecules or structures were considered to be the causative factors of replicative aging of the budding yeast. One of them was rDNA circles (ERCs) accumulated in the mother cell with replicative age (Sinclair and Guarente 1997). It was postulated that ERCs accumulation has a causative role in replicative aging as high levels of ERCs in cells shorten their lifespan (Sinclair and Guarente 1997), whereas limitation of ERCs formation can extend cell lifespan (Defossez et al. 1999). However, recent data suggest that rather than accumulation of ERCs per se, it is factors such as rDNA instability, DNA repair-induced chromatin relocalisation, and replication stress that are responsible for a gradual loss of functions that underlies aging (Ganley and Kobayashi 2014). This explanation denies the direct role of rDNA circles as regulators of yeast replicative lifespan.

The two other postulated “senescence factors” have one feature in common: both carbonylated proteins (Aguilaniu et al. 2003) and damaged mitochondria (Klinger et al. 2010) are products of oxidative damage. This type of damages is strictly

connected with the presence of oxygen and production of reactive oxygen species. However, some studies on the role of oxygen and antioxidants in replicative aging suggest that the numeric value of RLS does not substantially depend on the presence of oxygen (Wawryn et al. 2002). The RLS value stays unchanged in a full range of oxygen concentrations from 0 through 3, 21 to 100 %. In addition, RLS of yeast *sod1Δ* mutant is not increased when the level of oxygen drops down or after treatment with ascorbate at low concentration (Krzepilko et al. 2004) to alleviate numerous negative consequences of the absence of CuZnSOD (Kozioł et al. 2005; Zyracka et al. 2005), one of the most important antioxidant enzymes (Bilinski et al. 1985). These data suggest that elimination of oxidative damage has a little influence on the RLS, which leads to a discussion on the role of the earlier mentioned products as causative factors of replicative aging.

Carbonylated proteins, which represent a subset of possible oxidative modifications to proteins, show progressive accumulation and their level increases with replicative age of a yeast mother cell. Thus, it has been proposed that the above accumulation is a consequence of asymmetrical distribution between the mother cell and its bud (Aguilaniu et al. 2003; Erjavec et al. 2007). Carbonylated proteins can form protein aggregates, thereby leading to perturbation of cellular function, but the aggregation can also occur for thermally damaged or misfolded proteins. It has been shown for a number of organisms that damaged and misfolded proteins accumulate with age causing loss of cell function and contribute to age-related diseases such as Parkinson’s or Alzheimer’s disease (Vilchez et al. 2014). It has also been proposed that in the budding yeast protein aggregates can play a role of a “senescence factor” and give rise to an age-associated phenotype (Hill et al. 2014). These aggregates, among others protein aggregates associated with Hsp104—a stress-tolerance chaperone—are accumulated during consecutive reproductive cycles due to their asymmetrical distribution between the mother and daughter cells (Erjavec et al. 2007; Liu et al. 2010). Accumulation of protein aggregates is considered to be one of those cellular changes that are age dependent. Moreover, protein aggregates associated with Hsp104 are also treated as a senescence marker in yeast (Unal and Amon 2011; Unal et al. 2011). These aggregates are not present in young cells

but found in replicatively old cells. An increase in protein aggregation can also be induced by exposure to oxidative or heat stress.

In this paper, we extend the term “senescence factor” to all molecules or structures of the cell that are vulnerable to thermal stress. Our goal was to verify the postulate that accumulation of protein aggregates can cause the existence of the reproduction limit in yeast cells. In this case, we used a model based on accumulation of protein aggregates associated with Hsp104. The protein aggregation was induced by growth under mild heat stress conditions (temp. 37 °C). It was assumed that an increase in temperature can lead to an increase in the amount of errors occurring during biosynthesis and folding of proteins, and consequently lead to accumulation of damaged molecules, in particular protein aggregates. The optimum temperature of growth of *Saccharomyces cerevisiae* was early established at 28 °C. We therefore assumed that reducing the temperature to 22 °C, which was the control value applied in studies of heat shock response, should measurably reduce the rate of protein aggregation. We also expected that the mild heat shock caused by increasing the temperature to 37 °C would strongly increase the protein aggregation rate.

Materials and methods

Chemicals

Components of culture media were obtained from BD Difco (Becton–Dickinson and Company, Spark, USA), except for glucose (POCH, Gliwice, Poland). Dihydroethidine (DHET) and FUN-1 were obtained from Molecular Probes (Eugene, OR, USA). Phloxine B came from Sigma-Aldrich (Poznan, Poland). Primary rabbit antibody specific to Hsp104 proteins (ab2924) and goat anti-rabbit secondary antibody conjugated with Chromeo™ 546 (ab60317) were obtained from Abcam (Cambridge, UK). All other reagents were purchased from Sigma-Aldrich (Poznan, Poland).

Yeast strains and growth conditions

Yeast *S. cerevisiae* wild-type strain BMA64-1A (*MATa*; *ura3-1*; *trp1Δ* 2; *leu2-3,112*; *his3-11,15*;

ade2-1; *can1-100*) was obtained from EUROSCARF (acc.no. 20000A). Yeast was grown on a standard liquid YPD medium (1 % yeast extract, 1 % bacto-peptone, 2 % glucose) on a rotary shaker at 150 r.p.m. at the temperature of 28 °C (control), 22 and 37 °C, respectively. Analysis of the reproductive potential was performed on a solid YPD medium containing 2 % agar. Total lifespan analysis was performed on a solid YPD medium with 2 % agar containing Phloxine B (10 µg/ml). All experiments were performed at 28 °C (control), 22 and 37 °C, respectively.

Determination of reproductive potential

The reproductive potential of individual mother yeast cells was defined as the number of mitotic cycles during cell life. The reproductive potential was determined microscopically by a routine procedure with the use of a micromanipulator (Wawryn et al. 1999). Yeast cultures were grown on a rich YPD medium (1 % bacto-peptone, 1 % yeast extract, 2 % glucose) to log phase. One-microliter aliquots of an overnight grown culture of yeast were collected and transferred onto YPD plates with a solid medium. Analysis was performed by micromanipulation using the Nikon Eclipse E200 optical microscope with an attached micromanipulator. The number of daughter cells (buds) formed by each mother cell signifies its reproductive potential. In each experiment, forty single cells were analysed. The data represent mean values from two separate experiments.

Determination of total lifespan

The total lifespan was defined as the length of life of a single mother cell expressed in units of time. The total lifespan was calculated as the sum of reproductive and post-reproductive lifespans (Zadrag et al. 2008). The reproductive lifespan was defined as the length of time between the first and the last budding, and post-reproductive lifespan as the length of time from the last budding until cell death. The lifespan of the *S. cerevisiae* yeast was determined as previously described (Minois et al. 2005; Zadrag et al. 2008). Yeast cultures were grown on a rich YPD medium (1 % bacto-peptone, 1 % yeast extract, 2 % glucose) to log phase. One-microliter aliquots of an overnight grown culture of yeast were transferred onto YPD plates with a solid medium containing Phloxine B

(10 µg/ml). In each experiment, forty single cells were analysed. Analysis was performed by micromanipulation using the Nikon Eclipse E200 optical microscope with an attached micromanipulator. During manipulation, the plates were kept, respectively, at 28, 22 and 37 °C for 16 h and at 4 °C during the night. The data represent mean values from two separate experiments.

Estimation of Hsp104 proteins

Yeast cells were cultured in an appropriate temperature (22, 28, 37 °C) to reach the late-exponential phase and were then centrifuged, washed with sterile water and suspended to the final density of 10^8 cells/ml in 100 mM phosphate buffer with pH 7.0, containing 0.1 % glucose and 1 mM EDTA. In situ detection of Hsp104 proteins was performed according to standard immunocytochemistry procedures. The anti-Hsp104 rabbit polyclonal antibody (ab2924) was used at a 1:200 dilution. The secondary goat anti-rabbit polyclonal antibody conjugated with Chromeo™ 546 (ab60317) was used at a 1:1000 dilution. Fluorescence pictures were taken with Olympus BX-51 microscope equipped with a DP-72 digital camera and cellSens Dimension software. For images acquisition the same parameters of exposure were used in each case.

Metabolic activity determination

Cells were suspended in 10 mM Na-HEPES with pH 7.2 containing 2 % glucose. The metabolic activity of cells was estimated with 0.5 µM FUN-1 (100 µM stock solution, dissolved in DMSO) according to the manufacturer's protocol (Molecular Probes) with modification described in our other document (Kwolek-Mirek and Zadrag-Tecza 2014). The metabolic activity of cells was expressed as a change in ratio of red ($\lambda = 575$ nm) to green ($\lambda = 535$ nm) fluorescence. The fluorescence of the cell suspension was measured using the TECAN Infinite 200 microplate reader at $\lambda_{\text{ex}} = 480$ nm, $\lambda_{\text{em}} = 500$ –650 nm.

Superoxide anion generation assay

Yeast cells were cultured in an appropriate temperature (22, 28, 37 °C). The cells were suspended to the density of 1×10^8 cell/ml in a 100 mM phosphate buffer with pH 7.0 containing 0.1 % glucose and

1 mM EDTA. Generation of superoxide anion was assessed with dihydroethidine (Benov et al. 1998) (DHET; 10.7 µM final concentration; stock solution in DMSO). The kinetics of fluorescence increase was measured for 30 min at 28 °C using a TECAN Infinite 200 microplate reader.

Statistical analysis

The results represent mean \pm SD values for all cells tested in two independent experiments (80 cells). Differences between the mutant strain compared to the wild-type strain were estimated using a one-way ANOVA and Dunnett's post hoc test. The values were considered significant if $p < 0.01$. Statistical analysis was performed using Statistica 10 software.

Results and discussion

The mechanism of aging can be modulated by environmental and genetic factors (Fontana et al. 2010; Kenyon 2010). Temperature is one of the thermodynamic parameters that affect virtually all biochemical processes in the cell (Conti 2008). According to the binding procedures establishing the value of the reproductive potential of yeast cells, RLS allows for assessment of “replicative lifespan” or “longevity” of yeast cells. In this study, we use one of frequently used standard strains, namely BMA64-1A, a close relative of W303-1A. The experiments were performed at 37, 22 and 28 °C, respectively, with 28 °C as the control. For the *S. cerevisiae* yeast, the optimum growth is within the temperature range of 28–30 °C. Above 45 °C, yeast cells are severely stressed. After 5 min at 50 °C (lethal temperature), more than 99 % of growing non-adapted yeast cells die. In the range of 35–37 °C, yeast cells are “mildly stressed” but continue to grow and adapt to higher lethal heat exposures (Davidson and Schiestl 2001). The optimum temperature for heat shock induction is between 39 and 40 °C (Lindquist et al. 1982). These temperature conditions were used as a model for low (22 °C) and high (37 °C) level production of protein aggregates associated with Hsp104. Figure 1a presents the reproductive potential of yeast cells. This parameter has been considered by a number of research groups to be the measure of survival (Kaeberlein et al. 2005; Lindstrom and Gottschling 2009),

but in fact it only indicates the ability to produce daughter cells by the mother. There are no statistically significant differences between cells grown at different temperatures compared to the control (Table 1). The most surprising finding is the almost identical shape of curves obtained for cells grown at temperatures differing by as much as 15 °C. This suggests that the damages or protein aggregates that may occur under thermal stress have little influence on the reproductive potential of yeast cells. To make sure that we can draw such an unexpected conclusion, we used an alternative procedure developed several years before (Minois et al. 2005; Zadrag et al. 2008) for determining the time of life of yeast cells beside establishing RLS expressed in the number of daughters produced.

Figures 1b, c, d present the data obtained in the course of the analysis: reproductive lifespan, post-reproductive lifespan and total lifespan, respectively. The results concerning the reproductive period of life

(Fig. 1b) are presented exclusively to show that this parameter merely reflects the rate of reproduction, which apparently increases with temperature, up to the point when the damage starts to inhibit reproduction. The statistically significant differences observed in the length of the reproductive lifespan (Fig. 1b; Table 1), despite very similar values of the reproductive potential (number of daughter produced), may be explained by differences in the generation time, which are also statistically significant (Fig. 2). Cells grown at the temperature of 37 °C have the shortest reproductive lifespan, which may result from reduction of the generation time by ca. 21 % in comparison to the control cells grown at the optimum temperature of 28 °C. On the other hand, cells grown at 22 °C show the longest reproduction lifespan due to lengthening of the generation time by ca. 47 % in comparison to the control cells (Figs. 1b, 2). Differences in the generation time may reflect a “physiological fitness” of cells, such as protein biosynthesis abilities. However, the

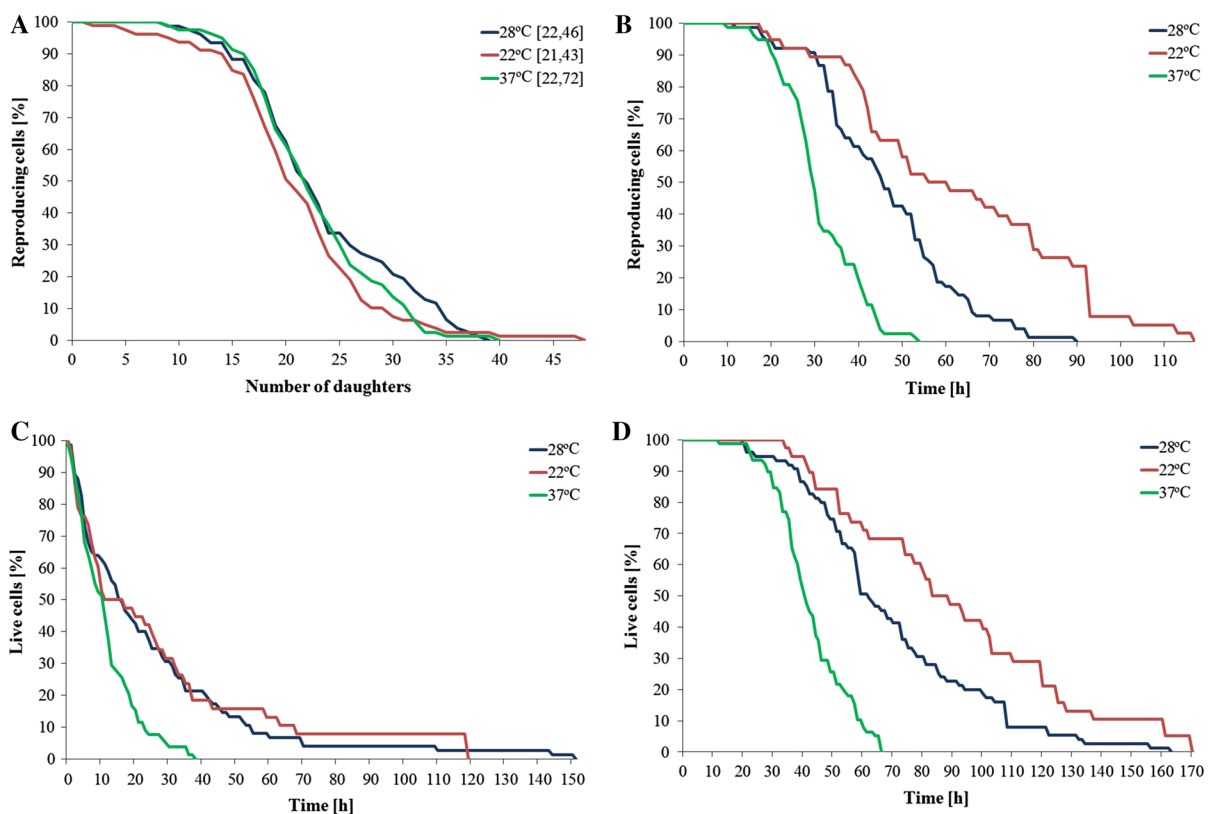


Fig. 1 Comparison of the reproductive potential (a), reproductive lifespan (b), post-reproductive lifespan (c) and total lifespan (d) of the haploid wild-type yeast strain BMA64-1A in different

growth temperatures (28 °C as the control, 22 and 37 °C, respectively). The mean value of the reproductive potential is shown in parentheses

Table 1 Mean reproductive potential (number of generations), reproductive lifespan, post-reproductive lifespan and total lifespan of the studied yeast strains

Strain/conditions	Reproductive potential (number of daughters)	Reproductive lifespan (h)	Post-reproductive lifespan (h)	Total lifespan (h)
BMA64-1A/28 °C	22.46 ± 7.05	46.42 ± 15.85	24.05 ± 25.57	70.47 ± 32.83
BMA64-1A/22 °C	21.43 ± 7.31	63.28 ± 26.97***	27.07 ± 32.38	90.35 ± 38.00**
BMA64-1A/37 °C	22.76 ± 5.98	31.21 ± 8.77***	10.76 ± 9.45***	41.97 ± 12.61***

The results represent the mean of ± SD 80 cells analysed in 2 independent experiments (40 cells in each experiment)

The statistical significance of differences in parameters between BMA64-1A/28 °C (control) and BMA64-1A/22 °C, BMA64-1A/37 °C was estimated using one-way analysis of variance (ANOVA)

* P < 0.05, ** P < 0.01, *** P < 0.001

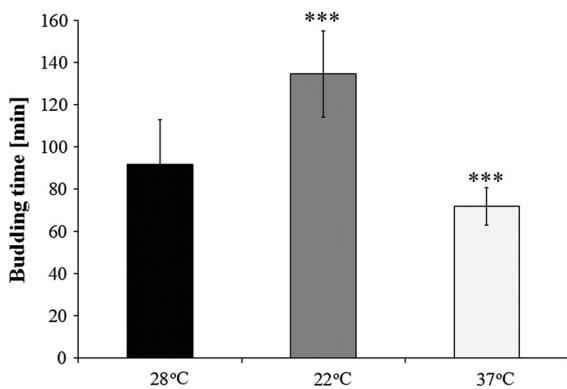


Fig. 2 Comparison of average generation time during reproduction. Data are presented as mean ± SD from 80 cells analysed in 2 independent experiments (40 cells in each experiment). Bars indicate SD; *P < 0.05, **P < 0.01 and ***P < 0.001 of significantly different values with respect to control growth conditions (temp. 28 °C) estimated ANOVA and Dunnet post hoc test

data presented in Fig. 1c provide the most interesting insights. Based on the comparison of the post-reproductive period of yeast cell life (PRLS), the results indicate that at the temperature of 37 °C cells that ceased reproduction die at a highly accelerated rate as opposed to cells grown at optimum conditions (28 °C). There are no substantial differences in the post-reproductive lifespan for cells grown in optimum conditions and at a lower temperature (Fig. 1c). A shortened generation time of cells growing at 37 °C and a large energy requirement associated with high rate of reproduction can result in a statistically significant shortening of the post-reproduction lifespan.

When longevity of yeast is expressed in number of daughters produced, no differences are observed between cells growing under different temperature conditions. However, if the same parameter is expressed in units of time, it becomes clear that the time of life of yeast (total lifespan) strongly depends on the temperature (Fig. 1d). Despite nearly the same value of the reproductive potential of cells, the time of life of cells is significantly different. Compared to cells growing in optimum conditions, for yeast cells growing at the temperature of 37 °C the mean value of the total lifespan decreases by approximately 40 %, whereas it increases by ca. 28 % in the case of yeast cells growing at 22 °C. It is worth noting that expressing the lifespan both in units of time and a number of daughter cells produced seems to be more informative and gives a possibility of a better evaluation of the effect of the aforementioned factors on longevity of yeast. Moreover, for the first time the results show the influence of temperature not only on the reproductive potential but also on the total lifespan of yeast cells. These results are consistent with those obtained from studies on other organisms. Reduction of the temperature of the environment has an impact on an increase in longevity and delays aging of various species of invertebrate, e.g. the fruit fly *Drosophila melanogaster* (Lamb 1968) or the roundworm *Caenorhabditis elegans* (Hosono et al. 1982; Xiao et al. 2013), as well as vertebrate, e.g. the *Cynolebias adloffii* fish (Liu and Walford 1966). Furthermore, lowering the core body temperature of homeothermic animals such as mice also leads to increased lifespan (Conti et al. 2006). On the other hand, *D. melanogaster* and *C. elegans* have shorter lifespans at a high temperature than at a low temperature (Klass 1977;

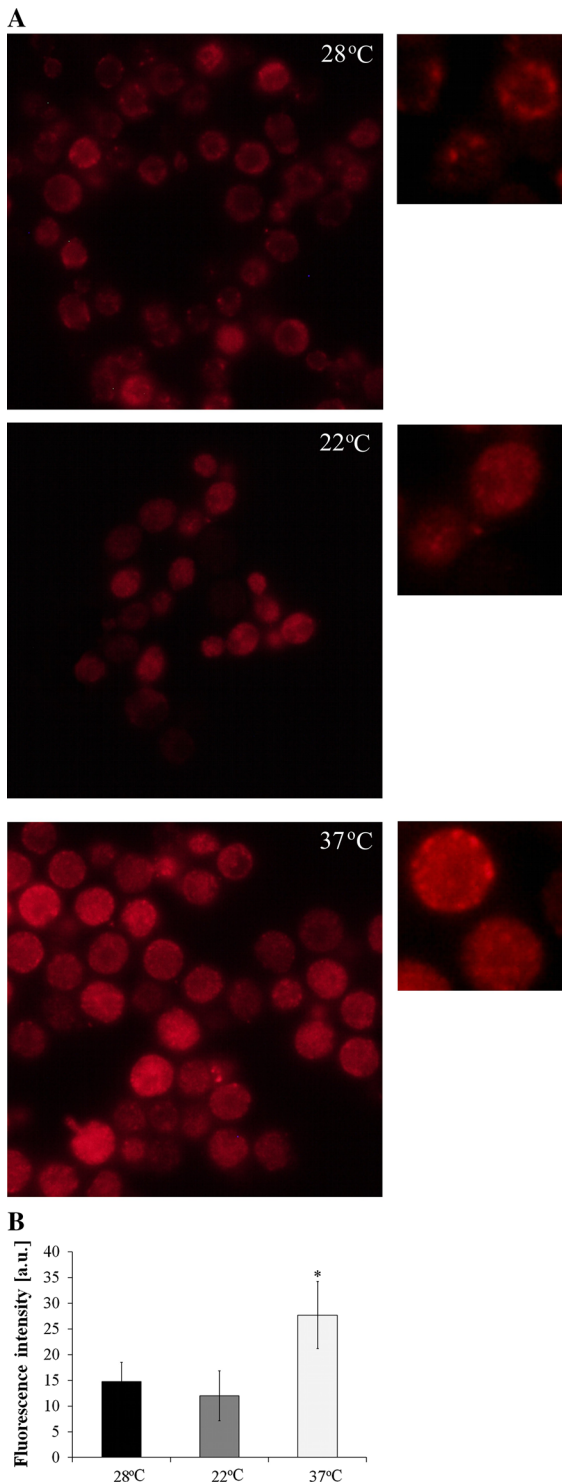
Miquel et al. 1976). In the case of yeast cells, it was shown that a heat shock of short duration (37 °C, 30 min) can prolong replicative lifespan (Shama et al. 1998; Swicilo et al. 2000) but these results are different in comparison to results received in the case of yeast cells, which were grown permanent on temperature 37 °C. As in the case of the fruit fly and roundworm, the results of our studies on the *S. cerevisiae* yeast confirm that changes in the lifespan are generally attributed to the effect of temperature on metabolic rates (Pearl 1924). For an explanation of these results, the hypothesis developed by Lloyd Demetrius seems quite important. Demetrius suggests that the most important factor affecting the duration of life is not the metabolic rate or oxidative stress but metabolic stability defined as the ability of cells to resist fluctuations in the steady state concentration of metabolites within the cell. Moreover, longevity is positively correlated with stability of the steady state concentrations of reactive oxygen species, whereas senescence-related loss of function is due to the dysregulation of the steady-state values of redox couples and the concomitant impairment of homeostasis (Demetrius 2004).

The temperature increase leads to a number of cellular changes, in particular concerning the kinetics of enzymatic reactions, thereby leading to an increase in the metabolic rate; this also increases frequency of errors and defects and can lead to higher protein aggregation levels. Aggregation of proteins, on the other hand, can lead to perturbation of cellular function and aging. To analyse the relationship between the reproductive potential of yeast cells and the level of protein aggregates, which can potentially play the role of a “senescence factor”, we used proteins aggregates associated with Hsp104 as a protein aggregation marker. Hsp104p belongs to a group of heat shock proteins (HSP) which are present in cells under normal conditions, but are expressed at high levels when exposed to thermal or other stress, such as oxidative stress. Hsp104p remains diffused in cytoplasm in the absence of aggregates or accumulates as foci in their presence. The sizes of such foci depend on the type and time of exposure to stress. A mild stress leads to the formation of small aggregates, while larger aggregates develop under prolonged exposure to stress. In the case of unstressed cells, there are no visible aggregates that accumulate during replicative aging, which somehow resembles accumulation of

carbonyl proteins (Erjavec et al. 2007). Hsp104p is a general anti-stress chaperone of the *HSP100* gene family (Bosl et al. 2006). Most chaperones prevent aggregation of proteins; however, Hsp104p in cooperation with the chaperone and co-chaperone Ydj1p and Ssa1p helps to disassemble protein aggregates that are formed under stress conditions (Glover and Lindquist 1998; Parsell et al. 1994). During vegetative growth Hsp104 protein aggregates are distributed asymmetrically between the mother and daughter cells. This protein is used as a marker for studies of protein aggregation in situ (Erjavec et al. 2007; Liu et al. 2010; Unal et al. 2011). It is important to note that the Hsp104p function has been evolutionarily conserved in numerous organisms, such as bacteria, fungi or plants (Parsell et al. 1991; Schirmer et al. 1994; Zenthon et al. 2006).

The data presented in Fig. 3 show a direct relationship between thermal conditions of cell growth and the level of Hsp104-associated protein aggregates. On the basis of fluorescence intensity and the number of Hsp104p foci, it can be concluded that yeast cells grown at the temperature of 37 °C accumulate the highest number of protein aggregates. In contrast to them, yeast cells grown at 22 °C accumulate the lowest number of protein aggregates (Fig. 3).

These results indicate that accumulation of protein aggregates has little effect on the reproductive potential of the *S. cerevisiae* yeast. The Hsp104-associated aggregates are formed even under optimum conditions; therefore Hsp104p is essential to maintain normal life. This is confirmed by the results where Hsp104p overexpression has no effect on the lifespan (Erjavec et al. 2007). Moreover, some results indicate that *hsp104Δ* deletion causes a significant decrease in RLS (Erjavec et al. 2007; Shama et al. 1998), although other results for the same mutation show a slight increase in RLS (Kaeberlein et al. 2005). In such circumstances, it was important to check other parameters that could help explain the lack of differences in the reproductive potential and significant differences in the total lifespan. We analysed the metabolic activity of yeast cells and the superoxide anion level. The metabolic activity was determined using the FUN-1 dye. The cells display the highest level of metabolic activity in optimum growth conditions (28 °C), and slightly lower at 22 and 37 °C, but these values are not statistically significant (Fig. 4). This is associated with cell adaptation to the prevailing



conditions. The studies concerning the effect of temperature on yeast cells also showed that heat shock can induce accumulation of superoxide anion

◀ **Fig. 3** Effect of temperature conditions on the Hsp104p aggregation in the yeast cells. Hsp104p aggregates were estimated by immunocytochemistry using polyclonal antibody to Hsp104p and a Chromo 546-conjugated, secondary antibody. *Data* are presented as representative images from three independent experiments. Magnification $\times 1000$. The quantitative results of fluorescence intensity are presented as mean \pm SD from three independent experiments (**b**). *Bars* indicate SD; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ of significantly different values with respect to control growth conditions (temp. 28 °C) estimated ANOVA and Dunnet post hoc test

(Davidson et al. 1996; Moraitis and Curran 2004; Sugiyama et al. 2000). Moreover, the high temperature induces expression of numerous genes, such as *CTT1*, *TPx*, *GSH1* or *GSH2* (Lee and Park 1998; Sugiyama et al. 2000; Wieser et al. 1991). In our studies, the analysis of the superoxide anion level in yeast cells confirmed the results of previous research. Cells grown at the temperature of 37 °C show an elevated superoxide anion level compared to the control cells (Fig. 5). On the other hand, decreasing the growth rate of cells by lowering the temperature during their cultivation has no effect on the superoxide anion level. This result is also consistent with the observed increase in the level of protein aggregates associated with Hsp104 in cells grown at 37 °C (Fig. 3), as well as with the previous conclusion that thermal or

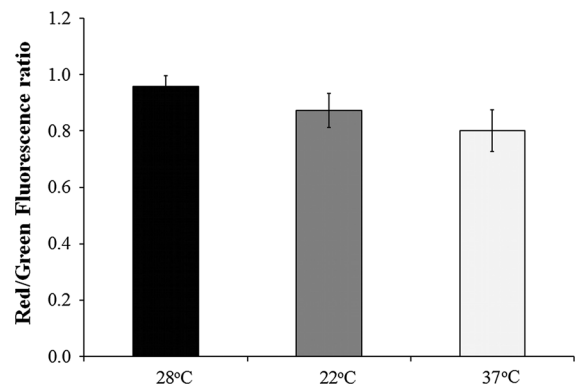


Fig. 4 Effect of temperature conditions on the metabolic activity of yeast cells. Metabolic activity of the cells in different growth temperatures was estimated with FUN-1 stain. *Data* are expressed as ratio of red ($\lambda = 575$ nm) to green ($\lambda = 535$ nm) fluorescence and presented as mean \pm SD from three independent experiments. *Bars* indicate SD; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ of significantly different values with respect to control growth conditions (temp. 28 °C) estimated ANOVA and Dunnet post hoc test

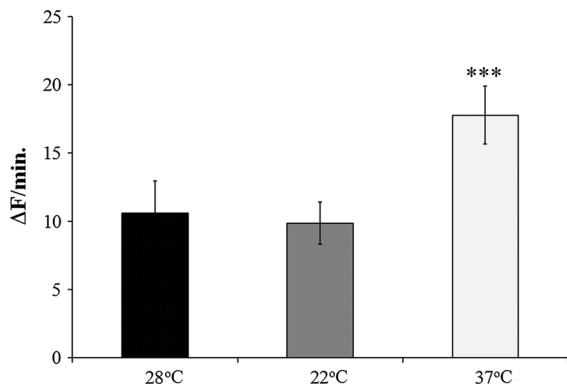


Fig. 5 Effect of temperature conditions on superoxide anion generation by yeast cells. ROS production in the cells was estimated with dihydroethidine. Data are presented as mean \pm SD from three independent experiments. Bars indicate SD; * indicates $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ of significantly different values with respect to control growth conditions (temp. 28 °C) estimated ANOVA and Dunnet post hoc test

oxidative stress induce protein aggregation (Erjavec et al. 2007). The short-term exposure of cells to a mild heat stress can lead to increased tolerance to other stress; however, permanent exposure of cells to such conditions can accelerate accumulation of defects, both thermal and oxidative, which are formed as a result of an increased production of superoxide anion. This may explain the significantly shorter total lifespan of the yeast cells. A short-term exposure to mild stress is a well-known phenomenon of hormesis. Hormesis is a dose response phenomenon, with a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect (Calabrese et al. 2007). They are well known examples of conserved hormetic pathways involving response to heat stress (Arumugam et al. 2006). High doses may inhibit growth, decrease fecundity and reduce longevity. When the same factor is in low dose, it may evoke stress response (Calabrese 2005). In the case of our results, the dose of the tested factor is low but the time of exposure is extended; therefore, we observe a reduction rather than extension of the length of life.

The results of the experiments show that accumulation of protein aggregates and other defects developing under increased temperature conditions seem to have no significant effect on the reproductive potential of yeast cells. A similar conclusion was drawn by Kaya et al. in respect of mutation accumulation (Kaya

et al. 2015). The use of the budding yeast as a model organism of gerontology was based on assumptions that the reproductive potential is limited by accumulation of a “senescence factor”. However, the majority of molecules proposed as “senescence factors” seem to have a little or no causative role in limiting the reproductive potential of yeast cells. Therefore, it would be important to take into account also the analysis of the total lifespan. This dual approach would allow for a comprehensive analysis of the impact of numerous factors on the lifespan of yeast cells. The need for such a change was postulated along with suggestions to introduce certain modifications to the already used methods (Minois et al. 2005; Zadrag et al. 2008). Using the number of daughters produced by the mother cell to express age and longevity of yeast cells is controversial because numerous genes or factors can change the reproductive potential without effect on the total lifespan; conversely, such genes or factors can change the total lifespan without effect on the reproductive potential. Therefore, as in the case of other organisms, it would be useful if age and longevity of the budding yeast were also expressed in units of time.

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Conflict of interest The authors declare that they have no conflict of interest.

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