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SHORT COMMUNICATION

Convergent lifespan reaction norms in the yeast cultures exposed to different environmental stresses

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Abstract

Lifespan extension under mild stress is frequently observed although difficult to quantify and generalize as previous studies differed substantially in specific experimental arrangements. We cultured the budding yeast in several environments defined by different temperature, source of energy, saline concentration or combinations of these factors. Cells obtained under different growth regimes were transferred to identical and generally nonstressful conditions except for an absence of organic carbon. Chronological lifespan (CL) of the starving cells showed an approximately common norm of reaction when plotted against the growth rate which served as a measure of stress intensity. CL increased roughly 50% in cultures raised at moderately slower pace, regardless of what particular single or multiple stress signals were present, and then decreased gradually with a deepening growth deceleration. We suggest that the strongly nonlinear relation between the metabolic rate and longevity can be a potent constraint controlling norms of phenotypic reaction in a variety of environmental gradients.

Introduction

Natural selection works to maximize fitness over the range of environments encountered by an organism. It takes a substantial work to determine how variable and successful are phenotypes in different conditions even though typically only a small fraction of potentially relevant environments is tested. Therefore, even if the goal is to identify only the chief factors determining the evolution of reaction norms, its realization will not be easy (Auld *et al.*, 2010; Chevin *et al.*, 2010). It may be thus encouraging that from the variety of observations made to date, some patterns are already emerging. One especially interesting result is that a moderate decline in the quality of environment may lead to a significant extension of lifespan. It can be dietary (caloric) restriction (Bishop & Guarente, 2007; Fontana *et al.*, 2010), mild external stress (Minois, 2000) or a small dose of a substance that is normally toxic at higher concentrations (Calabrese & Baldwin, 1998). The positive effect of a mild stress on the viability of starving cells was observed also in yeast (Fabrizio & Longo, 2003; Powers *et al.*, 2006). An increased resistance of cells to starvation is obviously only one aspect of longevity, but it likely applies to many organisms both uni- and multicellular.

A standard assay of the yeast's chronological lifespan (CL) has a phase of growth followed by a phase of starvation beginning after one of essential nutrients is depleted. Because different stresses ultimately slowdown growth, a simple question can be asked whether the extension of CL observed at the time of starvation relates directly to the rate of preceding growth. The answer is not easy because longevity can depend also on the metabolism of starving cells. Washing off cells and transferring them to water can extend CL; this suggests that the presence of 'left-over' resources is not necessarily helpful at the time of starvation (Fabrizio & Longo, 2003). More recent work confirmed that import, conversion and export of carbon compounds accumulating after conversion of an initial carbon source are damaging to the cell metabolism (Boer *et al.*, 2008; Wei *et al.*, 2008; Burtner *et al.*, 2009). We devised a simple experiment in which only the conditions of growth

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varied between cultures. We arranged three environmental gradients in which glucose was exchanged for other sugars of decreasing value, temperature was gradually lowered, or NaCl was added in increasing doses. In addition, we combined two or three of the listed stress factors. All cultures were then transferred to fresh medium lacking any utilizable organic carbon and maintained under standard conditions. This meant starvation for a source of energy in an environment that was otherwise nonstressful and the same for all cultures. The starving cells were periodically sampled and tested for viability. We also tested whether cells obtained under different growth regimes had different levels of trehalose or glycogen which are considered protectants and storage materials and thus, could influence longevity under starvation (Benaroudj *et al.*, 2001; Gray *et al.*, 2004).

Materials and methods

We used the BY 4743 *ura3 leu2 his3* diploid strain of *Saccharomyces cerevisiae* and synthetic complete (SC) medium with glucose and double levels of uracil, leucine and histidine. Growth was initiated by inoculating 1% of a stationary phase culture into fresh medium. Cultures were maintained at 30 °C in batches of 10 mL with 250 rpm shaking. These were the conditions of a control treatment. Other treatments were created by exchanging glucose for another sugar, lowering temperature, adding NaCl, or combining two or three of these factors of low intensity (all conditions listed in Results). Maximum growth rate was estimated by recurring measurements of optical density of an exponentially growing culture.

Incubation in growth media lasted for 3 days (4 days in case of the least favourable environments). The resulting cultures were in stationary phase, but media still contained utilizable carbon (mostly ethanol and acetate). To begin starvation, cultures were washed three times with water and re-suspended in 10 mL of fresh SC dropped out for sugar or any other source of carbon. Starving cultures were kept at 30 °C, constantly shaken and loosely capped (water was periodically refilled to 10 mL). At the beginning of starvation and every next 2 days, a sample of 50 μL was taken, serially diluted and overlaid on YPD agar plates to count colony-forming units. Five replicate control cultures and three of every experimental cultures were used.

To measure the cellular content of trehalose and glycogen, three further replicates of every type of culture were grown and washed as described earlier. Water-washed pellets of cells were lysed to extract carbohydrates (Sillje *et al.*, 1997). The extracts were treated with trehalase (TREH, Megazyme) or amyloglucosidase (E-AMGDF, Megazyme) to digest trehalose or glycogen, respectively. Liberated glucose was determined enzymatically (D-GLUKHR, Megazyme).

Results

We used two measures of CL. Summed survival (SS) was obtained by adding the counts of viable cells at all time points, this was equivalent to calculating the area under survival curve. Late survival (LS) was defined as a time point when population reached 1% of its original density. LS was chosen over the maximum lifespan, that is, the day of the last appearance of alive cells, as less dependent on chance. The two measures, LS and the maximum lifespan, correlated with each other (r = 0.750, d.f. = 18, $P < 0.001$).

Figure 1a shows that SS and LS increased considerably in cultures in which growth rate was moderately reduced. Further slowdown of growth tended to lower CL. Because the results obtained for cultures exposed to different single stresses were generally similar, we pooled them and compared with cultures exposed to multiple stresses. Figure 1b shows that there was no significant difference in SS or LS between the single and multiple stress cultures. In both groups, the two measures of CL were enhanced to similar levels and within similar range of the growth rate.

We also tested whether CL depended on the amounts of trehalose and glycogen accumulated in the cells at the beginning of starvation. Figure 2 shows that there was no correlation between the cellular content of trehalose or glycogen and SS or LS.

Discussion

We found that extension of chronological lifespan is largely independent of what single or multiple stress signals are perceived by an organism. Neither the intracellular level of storage materials could explain the observed variation in CL (the last finding may look surprising but it is not in odds with earlier studies (Fabrizio & Longo, 2003; Gray *et al.*, 2004). The extension of lifespan was highest in cultures in which temperature was reduced to 25 °C, sucrose was used as a source of energy or NaCl at a relatively low level was present. All these conditions are nominally unfavourable because they do not permit growth at a rate as high as that obtainable with glucose, 30 °C and in absence of NaCl. However, it is questionable whether slight deteriorations of nutritional or physical conditions constitute real 'stresses'. They may be rather considered brakes to growth at rates harmful for future survival. A potential explanation could be that growth conditions as good as those created in the laboratory are unusual in the wild and therefore, yeast did not develop metabolic strategies securing both fast growth and good survival. But, many strains freshly isolated from different habitats and from human patients can grow even faster than the laboratory ones (Liti *et al.*, 2009). Thus, a seemingly 'excessive' growth rate is not an adaptation to laboratory conditions that developed recently at a cost of resistance to

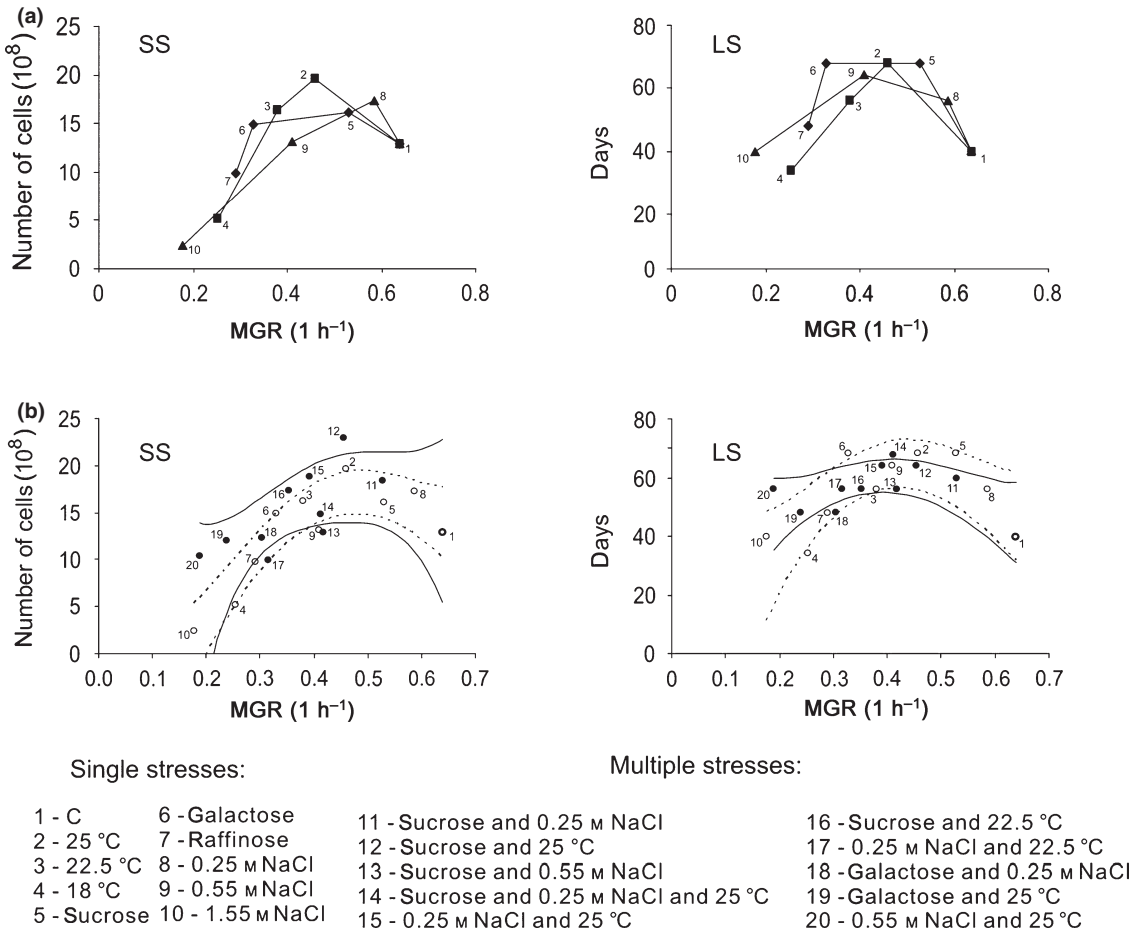

Fig. 1 Summed survival (SS) and late survival in relation to maximum growth rate (MGR). (a) Gradients of single stress factors: low temperature (squares), inferior source of energy (diamonds), addition of NaCl (triangles). (b) Comparison between single and multiple stress factors. Quadratic regressions were calculated for single-factor (open circles) and multiple-factor cultures (filled circles). Ninety-five per cent of confidence limits of these predictions are shown for the single-factor (dashed lines) and multiple-factor (solid lines).

starvation. Fast growth is probably a natural way of competition for resources and apparently cannot be accomplished without negative consequences for future survival.

As it was mentioned in the introduction, a nonlinear relation between the intensity of stress and longevity is commonly observed. This suggests that also in other organisms, the dependence between stress and the longevity reaction norms can be mediated by the growth rate. The idea that a moderately lower rate of metabolism permits to survive longer is an old one (Pearl, 1928). It was proposed that high metabolism is destructive because it results in high levels of reactive oxygen species (ROS) in the cell (Harman, 1956, 2009; Balaban *et al.*, 2005). The role of ROS in shortening yeast's lifespan was explored many times, the results were often mixed and difficult to interpret (Fabrizio *et al.*, 2001; Passos *et al.*, 2006; Bonawitz & Shadel, 2007; Unlu & Koc, 2007). There is also growing uncertainty about the role of ROS in ageing of other organisms (Kenyon, 2010). However, senescence of cells can involve many processes but still be generally similar in different species (Goldberg *et al.*, 2009). Indeed, recent studies showed that as much as a quarter of all yeast genes are expressed in a way that is closely correlated, either negatively or positively, with the rate of growth rather than any specific environmental factors (Regenberg *et al.*, 2006; Castrillo *et al.*, 2007; Brauer *et al.*, 2008). This likely applies to other organisms because the main pathways of remodelling the growth metabolism are well conserved in distant taxa (Fontana *et al.*, 2010). Thus, not only standard evolutionary theory but also new data on gene expression support the view that organisms evolve under the necessity of making trade-offs between fast synthesis of biomass and its good preservation (Lopez-Maury *et al.*, 2008).

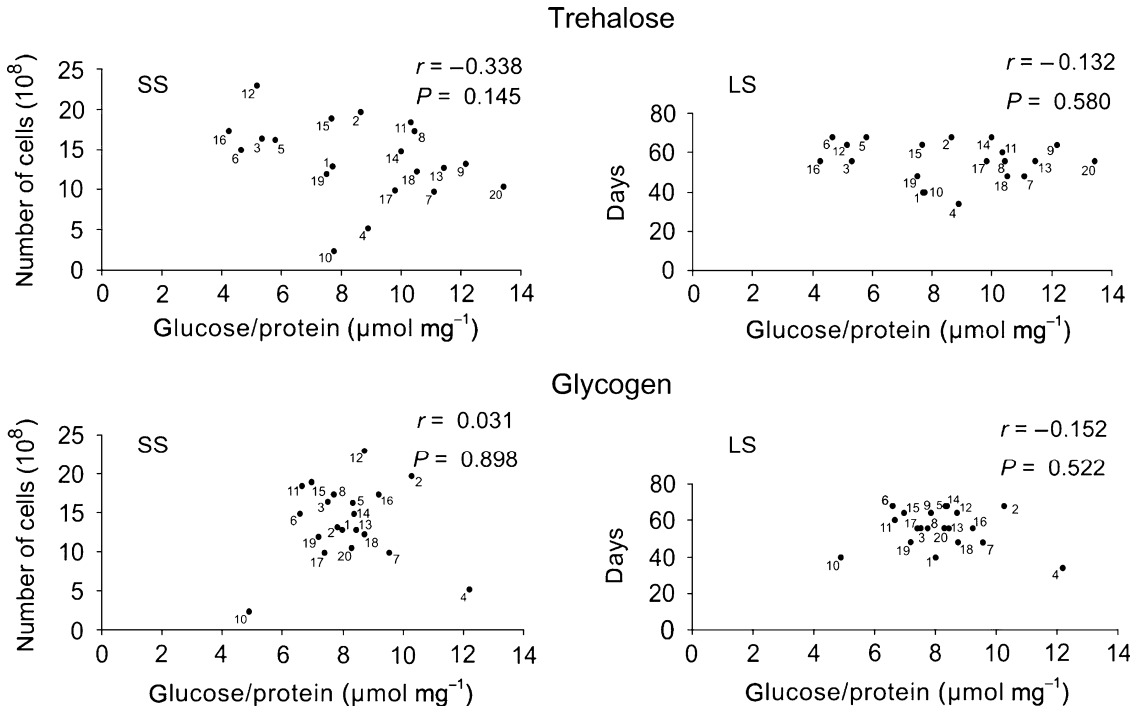

Fig. 2 Summed survival (SS) and late survival (LS) in relation to trehalose and glycogen content at the beginning of starvation. Contents of carbohydrates are scaled against contents of proteins to account for differences in cultures' density. Pearson's correlation coefficients and associated with them *P*-values are shown in the graphs.

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