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Heat Stress-Induced Life Span Extension in Yeast

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The yeast *Saccharomyces cerevisiae* has a limited life span that can be measured by the number of times individual cells divide. Several genetic manipulations have been shown to prolong the yeast life span. However, environmental effects that extend longevity have been largely ignored. We have found that mild, nonlethal heat stress extended yeast life span when it was administered transiently early in life. The increased longevity was due to a reduction in the mortality rate that persisted over many cell divisions (generations) but was not permanent. The genes *RAS1* and *RAS2* were necessary to observe this effect of heat stress. The *RAS2* gene is consistently required for maintenance of life span when heat stress is chronic or in its extension when heat stress is transient or absent altogether. *RAS1,* on the other hand, appears to have a role in signaling life extension induced by transient, mild heat stress, which is distinct from its life-span-curtailing effect in the absence of stress and its lack of involvement in the response to chronic heat stress. This distinction between the *RAS* genes may be partially related to their different effects on growth-promoting genes and stress-responsive genes. The *ras2* mutation clearly hindered resumption of growth and recovery from stress, while the *ras1* mutation did not. The *HSP104* gene, which is largely responsible for induced thermotolerance in yeast, was necessary for life extension induced by transient heat stress. An interaction between mitochondrial petite mutations and heat stress was found, suggesting that mitochondria may be necessary for life extension by transient heat stress. The results raise the possibility that the *RAS* genes and mitochondria may play a role in the epigenetic inheritance of reduced mortality rate afforded by transient, mild heat stress.

Key Words: **aging; longevity; heat stress; *RAS* genes; *HSP104;* biodemography.**

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INTRODUCTION

Continuously changing environmental conditions are a source of stress for living organisms. Stress is also generated endogenously through metabolic activity. Thus, the ability to cope with stress may be a key factor that limits life span. Enhanced resistance to oxidative stress is found in mutants of *Caenorhabditis elegans* that display extended life span, and this is associated with elevated levels of catalase and Cu, Zn-superoxide dismutase [1, 2]. *Drosophila melanogaster* selected for postponed senescence are also more resistant to oxidative stress, and they have increased activity of several enzymes involved in protection from oxidative damage [3, 4]. Fruit flies displaying postponed senescence show enhanced resistance to starvation, dessication, heat, and ethanol [5–7]. Further selection for resistance to starvation and dessication yielded further gains in longevity [8]. Yeast selected for resistance to nitrogen starvation and cold stress show enhanced resistance to a variety of stresses, including heat shock, and have a longer life span [9]. Overexpression of Cu, Zn-superoxide dismutase and catalase extends the life span of *Drosophila* [10]. In fact, overexpression of the former in motorneurons is sufficient [11]. Dietary restriction is the only proven means by which the life span of mammals has been extended [12, 13]. Animals that are dietarily restricted maintain the levels of antioxidant enzymes late in life [14]. Furthermore, these animals are more resistant to heat stress, and this is correlated with a reversal of the age-dependent decline in *hsp70* expression [15].

The correlation between extended longevity and enhanced resistance to stress goes even further. Long-lived mutants of *C. elegans* display an increased intrinsic thermal tolerance [16]. Indeed, extended longevity appears to be associated with resistance to heat stress, oxidative stress, and ultraviolet radiation in the worm [17]. What is more, induction of thermal tolerance has been shown to extend longevity in both the worm and the fruit fly [18, 19]. In the case of the latter, this effect has been correlated with expression of the heat shock protein Hsp70 [20].

We have been using the yeast *Saccharomyces cerevisiae* as a model for the analysis of the molecular mech-

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anisms of aging [reviewed in Ref. 21]. Life span in yeast is most readily measured by the number of divisions an individual cell undergoes [22, 23]. The mean replicative life span of a strain, under given conditions, is a characteristic feature. The aging process in yeast is accompanied by morphological and physiological changes [reviewed in Ref. 24]. After a finite number of divisions yeast cells lyse, but their daughters have the potential for a full replicative life span, guaranteeing the immortality of the population. We have recently found that chronic, nonlethal thermal stress shortens yeast life span [25]. *RAS2*, which has a life extending effect [26] in the absence of stress, plays a role in efficient recovery from heat shock and maintaining replicative capacity under conditions of chronic stress [25].

Thermal stress in yeast induces a heat shock response that is characterized by a temporary arrest in the G_1 phase of the cell cycle at the regulatory step Start and a transient decline in the abundance of most cellular RNAs, while the expression of stress-responsive genes is induced [reviewed in Ref. 27]. A major stress-responsive gene is the one coding for the heat shock protein Hsp104, which has been implicated in resolubilization of proteins [28] and reactivation of mRNA splicing [29], after heat inactivation. Hsp104p is necessary for survival at high temperatures [30] and by itself is sufficient for the bulk of induced thermotolerance in yeast [31].

In this report, we show that a sublethal heat shock early in yeast life span results in an increase in longevity that is due to a marked decrease in the mortality rate. This effect is sustained over a period of many generations. However, it ultimately subsides. Both yeast *RAS* genes are required for this enhancement of life span, and it is associated with the induction of Hsp104p. *HSP104* is necessary for extension of longevity by transient, mild thermal stress, as are fully functional mitochondria. There is a clear distinction between the effect of sublethal heat stress when it is transient, as described here, and when it is repeated or chronic, as shown in the accompanying paper [25]. The former treatment prolongs life span, while the latter curtails it. The difference does not end there. Both phenomena involve *RAS2*, but *RAS1* is implicated only in life extension by transient heat stress. This interaction with thermal stress highlights further the divergent roles of the *RAS* genes in yeast longevity found in the absence of overt stress [26]. The results juxtapose the salutory role that exposure to transient stress plays with the deleterious effect of chronic stress.

MATERIALS AND METHODS

Strains, growth conditions, and transformations. *S. cerevisiae* YPK9 (*MAT***a**, *leu2-Δ1, ura3-52, trp1-Δ63, ade2-101*^{ochre}, *lys2-801*^{amber}, *his3-Δ200*) is a haploid derivative of YPH501 (supplied by P. Hieter, The Johns Hopkins University). The following strains were generated in the YPK9 background: A *RAS2*-null mutant (*ras2*) was generated using pRa530, and a *RAS1*-null mutant (*ras1*) was generated essentially as previously described [26], except that the *RAS1* sequences were cloned in pRS405 [32]. An *HSP104*-null mutant (*hsp104*) was generated by transformation with a 2.2-kb *Cla*I-*Hin*dIII *HSP104* fragment in which the *Apa*I-*Bgl*II fragment containing the coding region was replaced by the *URA3* gene in pYSU2 (S. Lindquist, University of Chicago). A respiratory-deficient petite strain (YSK365) that lacks fully functional mitochondria was generated by growing YPK9 cells in ethidium bromide-containing medium and selecting for inability to grow in YPG. Media were YPD (2% peptone, 1% yeast extract, 2% glucose), YPG (YPD containing 2% glycerol instead of glucose), and SC [0.67% yeast nitrogen base without amino acids and supplemented with uracil (0.012 mg/ml), adenine (0.041 mg/ml), leucine (0.051 mg/ml), lysine (0.029 mg/ml), histidine (0.0198 mg/ml), tryptophan (0.04 mg/ml), and 2% glucose]. For overexpression of *HSP104*, cells were transformed with the plasmid pYSGal104 (supplied by S. Lindquist, University of Chicago) [31], which carries the *HSP104* coding sequence behind the *GAL1* galactose-inducible promoter. A control plasmid, pYS104, was generated by deleting the 1-kb *HSP104*-containing fragment from the pYSGal104 plasmid. Overexpression was achieved by substituting the glucose in SC with 2% galactose. Prior to induction cells were grown in SC containing 2% raffinose in the place of glucose. Yeast cells were transformed with 1–2 μg DNA using the lithium acetate procedure [33] without carrier DNA. Transformants were selected on SC medium lacking the appropriate nutrients. Cells were cultured at 30°C. Heat shock treatment of batch cultures is described in the Fig. 3 legend.

Northern and Western blot analysis. Total yeast RNA and protein were prepared and analyzed as described in the accompanying paper [25].

Life span determination. A Nikon Labophot-2 microscope with a 20× long working-distance objective and a micromanipulator attachment was used [34]. A fresh colony of yeast cells was grown to logarithmic phase in the appropriate medium. One microliter of the culture was spotted onto a plate containing the same medium. Individual cells were pulled aside with the micromanipulator, and were allowed to grow until buds emerged. Thirty-five buds were removed, and were referred to as "virgins" (i.e., cells that have never budded). After they underwent their first cell division, buds were removed from these cells, and the virgin cells, now mothers, were recorded as one generation old. This process was continued until budding ceased and cells lysed. During the course of the experiment, cells were transferred to 12°C during the night to slow division and allow relief for the investigator. These cells resumed progress through the life span upon return to 30°C. This treatment does not alter the replicative life span [23]. Heat-shocked cells were exposed to 37°C for 2 h, after which they were returned to 30°C. Heat shocks were first applied to virgin cells and once more at the age of four generations. In the experiments with continuous induction of *HSP104*, cells transformed with the plasmid pYSGal104 were pregrown to mid-log phase in raffinose-containing media and spotted onto plates containing 2% galactose for life span determination. For transient expression of *HSP104*, pYSGal104-carrying cells were pregrown to mid-log phase in SC−uracil medium containing 2% raffinose as the carbon source. This culture was divided into two tubes. The culture in one tube was mixed with equal volume of SC−uracil + 4% galactose medium to achieve a final concentration of 2% galactose. The culture in the other tube was mixed with an equal volume of SC + 4% raffinose medium and used as the control. Both tubes were incubated at 30°C for a given length of time before one drop from each tube was spotted onto a YPD plate. Fifty cells from each strain were pulled out of the spots and their remaining life spans were determined using a micromanipulator. The nonparametric Mann–Whitney test was used

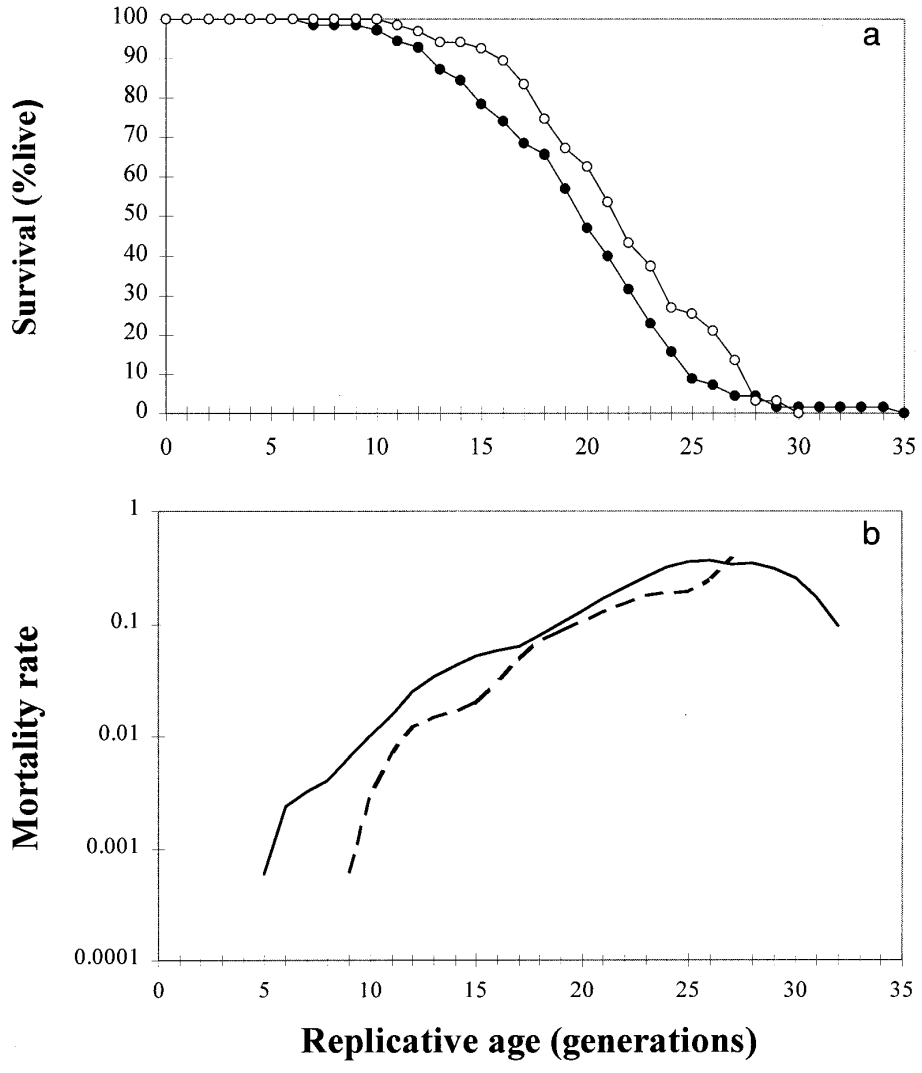

FIG. 1. Heat-induced life span extension in yeast cells. (a) Life span determination. Life span of parental YPK9 cells was determined on YPD plates as explained under Materials and Methods. Control cells were maintained at 30°C (closed circles). Heat-shocked cells (open circles) were transferred to 37°C for 2 h at zero (i.e., virgin cells) and 4 generations. The life span of the heat-shocked cells was significantly longer than that of the control cells ($P = 0.013$). The mean life spans were 21.3 and 19.4 for the heat-shocked and control cells, respectively. (b) Mortality rate. The mortality rate is the fraction of live cells that die during an interval [50]. Mortality rates were determined using the program MORTAL 1.0, designed and provided by J. W. Curtsinger (University of Minnesota) and smoothed over an eight-generation window by an algorithm contained in the program. Control group: solid line. Heat-shocked group: broken line.

to compare survival curves. Life spans were considered to be different if $P < 0.05$.

RESULTS

Sublethal Heat Stress Increases Longevity

Induction of thermotolerance early in life by subjecting roundworms [18] or fruit flies [19] to nonlethal heat stress extends their life spans. To determine if this is the case for yeast cells as well, they were subjected to two transient (2-h), sublethal heat shocks, at 37°C, early on in life, during the course of life span determination. The cells were newborn and 4 generations-old at the time of the two heat shocks, which were mild enough that they did not affect viability (colony-forming ability). This treatment resulted in a significant extension in mean life span (10%), compared to non-heat-shocked cells (Fig. 1a), similar to that found in fruit flies [19] and roundworms [18] in which thermotolerance was induced. This life extension was reproducible, as the average extension obtained in 10 independent experiments was 12%. The biodemographic cause of the increase in life expectancy was explored by analysis of the age-specific mortality rates of the con-

trol and heat-stressed yeasts. This revealed a reduction in the mortality rate following heat treatment, which persisted for several generations (Fig. 1b). Though long-term, the effect on mortality rate was not permanent, because at later ages the mortality rate was not discernibly different from that of the control.

The conditions required to observe an extension of life span were fairly stringent. When the duration of the two heat shocks at 37°C was less than 2 h, no effect was seen. Temperatures higher then 37°C caused a significant increase in mortality when applied twice for 2 h to young cells, abrogating any increase in life span (data not shown). Application of 1-h heat shocks on a daily basis resulted in shortening of life span [25]. Finally, heat shocks late in life did not extend life span, pointing to the importance of a process(es) taking place early on in life. The heat shock conditions that result in extended life span will be called the life span-extending protocol for easy reference.

RAS Genes Are Involved in Life Extension by Thermal Stress

The *RAS* genes play a role in determining yeast longevity [26]. To ascertain whether the *RAS* genes are required for heat stress-induced increase in longevity, we applied the life span-extending protocol to *ras* mutant strains. In sharp contrast to the wild-type strain, the same protocol not only did not extend but in fact shortened the mean life spans of both *ras1* and *ras2* strains by 15 and 20%, respectively (Fig. 2). Thus, both genes are required for life span to be extended by the transient heat stress. This requirement could explain the ineffectiveness of heat stress late in life in extending life span, because the expression of these genes declines with age [26].

Role of RAS Genes in Life Extension by Heat Stress

We attempted to look for a possible molecular explanation for the reciprocal effects of transient heat stress on life span in wild-type and *ras* mutants. Cultures of the wild-type and *ras* mutant strains were heat shocked for 2 h at 37°C, after which they were allowed to recover and their growth and gene expression profiles were monitored. Upon temperature shift-up, the wild-type and *ras1* strains were only slightly affected, while the *ras2* strain completely arrested its growth (Fig. 3a). The *ras2* strain resumed growth within 3 h and reached stationary phase (not shown). The expression of *RPL16A*, a representative growth-promoting gene, was correlated with the growth rates. *RPL16A* mRNA slowly accumulated in wild-type and *ras1* strains, even while they were being heat shocked. This expression was accelerated as they were allowed to recover at 30°C. The *ras2* strain, on the other hand, did not show any increase until 1 h after the cells had been recovering (Fig. 3b). Thus despite the similar impact of *ras* mutations on heat-stress-induced extension of life span, the effects of the two *RAS* genes on cell growth were very different.

We wondered whether our treatment actually induced a stress response. We therefore analyzed the expression of the heat shock protein gene, *HSP104*. *HSP104* mRNA was transiently induced in all three strains (Fig. 3c). However, its down-regulation was delayed in the *ras2* strain (Fig. 3c). These differences were also evident at the protein level. Hsp104p was maintained at a higher level in the *ras2* strain at a time when its level in the wild-type and *ras1* strains decreased to the basal level (Fig. 3d).

Hsp104p Is Necessary for Life Extension by Heat Stress

Since Hsp104p has been reported to be both necessary and sufficient for induction of thermotolerance [30, 31], we asked whether it is required for the heat-induced extension of life span. Applying the heat shocks in the life span-extending protocol to an *hsp104* mutant was not effective in extending life span (Fig. 4). This treatment of the mutant may actually have a somewhat deleterious effect, because it resulted in a decrease in mean life span (14%) which was not, however, statistically significant. Thus, lack of Hsp104p abrogates the heat-induced life extension, and this protein appears to be necessary for the life span-extending effect of transient, mild heat stress.

To determine whether expression of Hsp104p is sufficient to mimic the life span-prolonging effects of mild, transient heat shocks, we overexpressed *HSP104* from the *GAL1* promoter in the presence of galactose. We attempted to express this gene transiently for different lengths of time and found no increase in life span. Figure 5 shows the results of one of these attempts. It should be emphasized that it is difficult to equate this overexpression experiment with the one in which heat stress was used to extend life span (Fig. 1a). The cells were all induced twice at the same, precisely defined ages in the latter. The conditions are sufficiently different that one would be hard-pressed to say whether the extent and duration of *HSP104* induction was equivalent and its physiological impact comparable. In contrast to transient expression of various durations, overexpression of *HSP104* throughout the life span actually caused a marked decrease in longevity, which amounted to 29% of the mean life span.

Presence of Petites Is Not the Cause of Life Extension Induced by Heat Stress

It has been recently found that respiratory-deficient (petite) yeast cells have a longer life span than their parent grande cells (P. A. Kirchman, S. Kim, C.-Y. Lai,

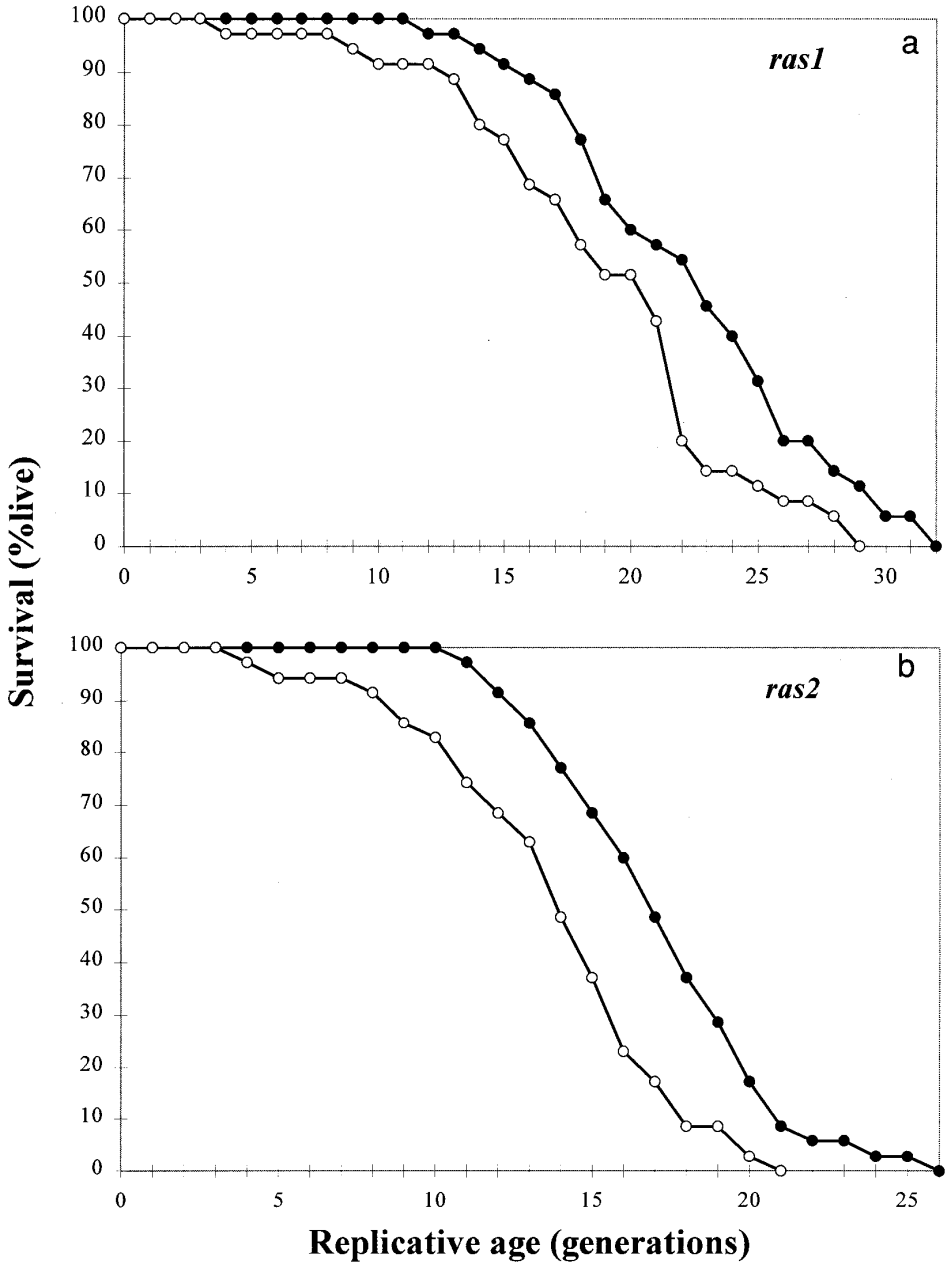

FIG. 2. The effect of mild heat stress on life span in *ras* mutants. Heat shock during life span determination of *ras1* (a) and *ras2* (b) mutants was performed as described in the legend to Fig. 1. The life span of *ras1* ($P = 0.007$) and *ras2* ($P = 0.0003$) heat-shocked cells was significantly shorter than non-heat-shocked cells. The mean life spans (in generations) of control (closed circles) versus heat-shocked (open circles) cells were: (a) 22.6 vs 19.2; (b) 17.4 vs 13.9.

and S. M. Jazwinski, unpublished results). Data in the literature suggest that mild heat shock may give rise to petites [35]. We, however, did not observe any increase in the number of petites during our life span determination experiments when the cells were subjected to heat stress (data not shown), indicating that extension of life span under these conditions operates through a different mechanism. We examined the possibility that the two modes of extension may be additive. Petites have a longer life span compared to the grande strain. However, our results clearly show that application of the life span-extension protocol to petites actually shortened their life span (Fig. 6). This is a synthetic phenotype that suggests an interaction between the two mechanisms for life span extension. This result clearly indicates that the presence of petites in the population or their possible generation due to heat shock is not the cause of life span extension by mild

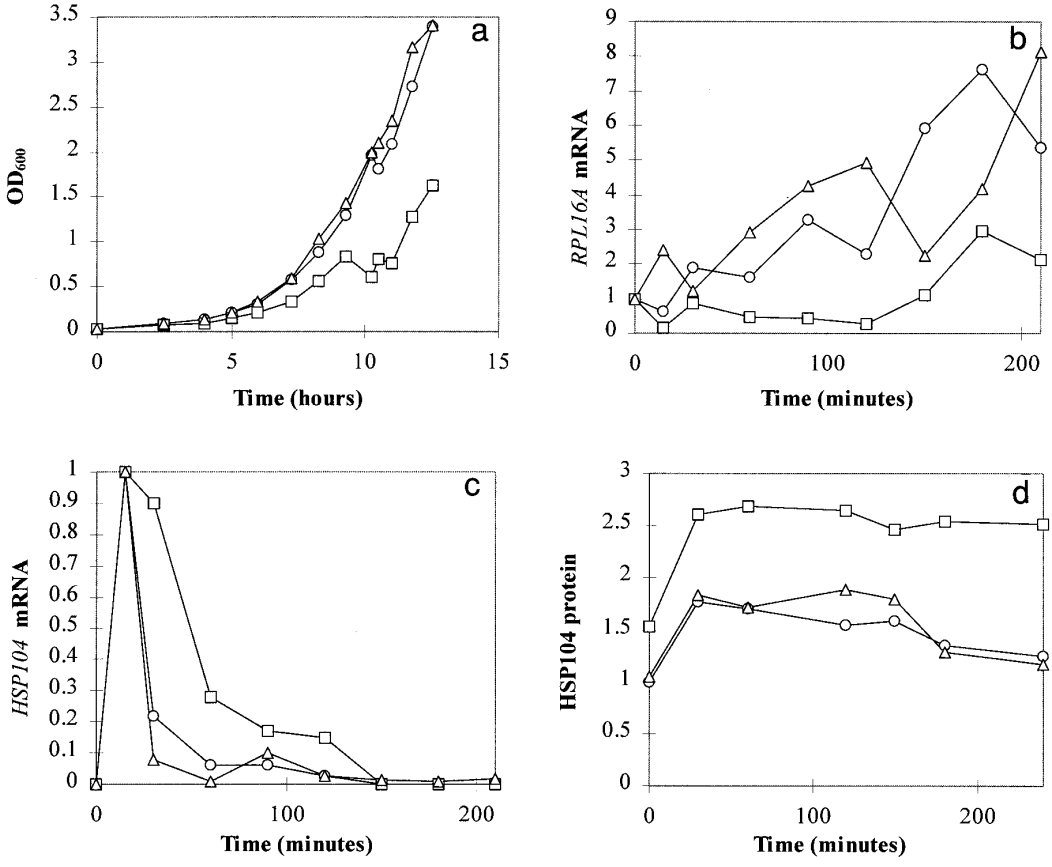

FIG. 3. The effect of heat shock on growth and gene expression. (a) Growth curves. Exponentially growing cells (wild-type, circles; *ras1*, triangles; *ras2*, squares) were transferred to 37°C at the 7.25-h time point for 2 h and then returned to 30°C. Growth was monitored by measuring optical density at 600 nm at the indicated times. (b) Analysis of *RPL16A* mRNA abundance. Ten micrograms of RNA isolated from the cells in (a) were analyzed by Northern blot hybridization with an *RPL16A*-specific probe and quantitated. Values for each strain were related to its zero time point of heat shock, which corresponds to the 7.25-h time point in (a). (c) Analysis of *HSP104* mRNA abundance. This was carried out as in (b). (d) Analysis of Hsp104 protein levels. Ten micrograms of protein were extracted at the indicated time points of heat shock and recovery and were subjected to Western blot analysis with Hsp104p-specific antiserum and quantitated. Changes in HSP104p abundance in all strains were related to its level in YPK9 cells at zero time point of heat shock. YPK9, circles; *ras1*, triangles; *ras2*, squares.

heat stress. Our results suggest that mitochondrial function may be required for the extended longevity induced by heat stress.

DISCUSSION

We have shown that brief exposure of yeasts to a transient, mild heat stress early in life provides a persistent, though not permanent, reduction in mortality rate that results in an increase in life expectancy (Fig. 1). The heat-induced extension of yeast life by 12% is in agreement with the extensions found in other organisms [18, 19]. This salutory effect on longevity is dependent on both *RAS1* and *RAS2* (Fig. 2). The role these two genes play in this response cannot be readily associated with their effects following heat treatment on the expression of a growth-promoting gene (*RPL16A*) and on growth rate itself (Fig. 3). *RAS1* signaling appears to have a role to play in heat stress-induced life extension that is distinct from that of *RAS2*. Mutations in *RAS1* and *RAS2* have quite different effects on the expression profile of the *HSP104* gene (Fig. 3). *HSP104* is largely responsible for induced thermotolerance in yeast [30, 31]. The distinct effects of *ras1* and *ras2* mutations on expression of this gene suggest that there is more to life extension by heat stress than simply the induction of Hsp104p expression and associated thermotolerance. However, we find that Hsp104 protein is necessary for the life extension (Fig. 4). Although we could not demonstrate that the expression of Hsp104 protein is sufficient for this response (Fig. 5), the interpretation of this negative result is subject to caveats.

A difference between the *RAS* genes is evident in their different effects on longevity under various environmental conditions. The two genes have reciprocal

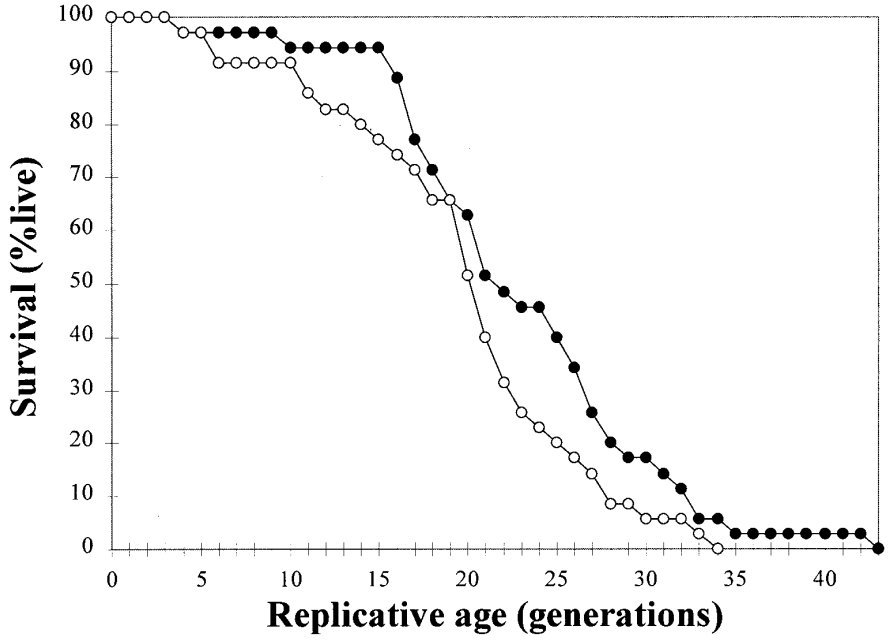

FIG. 4. The effect of heat stress on life span in *hsp104* mutants. Life span of the *hsp104* strain was determined as described in the legend to Fig. 1. There was no significant difference between heat-shocked and control (non-heat-shocked) cells ($P = 0.134$). The mean life spans (in generations) of control (closed circles) and heat-shocked (open circles) cells were 22.6 and 19.9, respectively.

effects on yeast longevity in the absence of overt stress [26]. The key difference actually seems to involve the *RAS1* gene. Deletion of *RAS1* extends life span in the absence of stress, and it has no effect under chronic stress conditions [25]. However, this mutation eliminates the salutory effect that life span-extending, transient heat stress has on longevity (Fig. 2). In contrast, *ras2* mutations have identical effects under all three conditions (Fig. 2) [25, 26]; they curtail life span. The effect of the *ras2* mutation is consistent with the role of

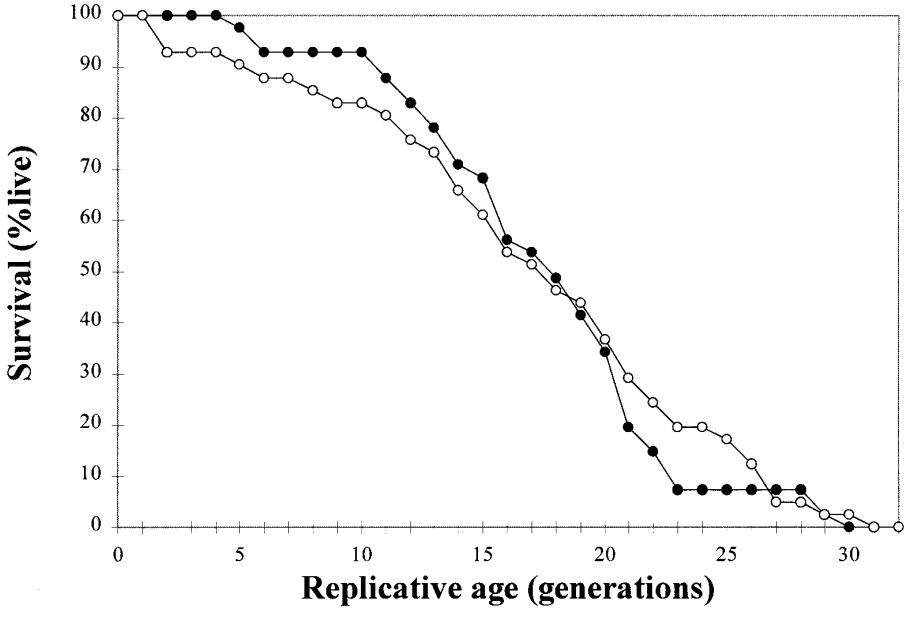

FIG. 5. The effect of *HSP104* overexpression on life span. Survival curves of YPK9 cells transiently induced with 2% galactose for 30 min. There was no significant difference between the life span of cells carrying the empty vector and that of cells with the vector containing *HSP104* ($P = 0.351$). The mean life span of control (closed circles) and *HSP104*-expressing (open circles) cells were 17.2 and 17.6, respectively.

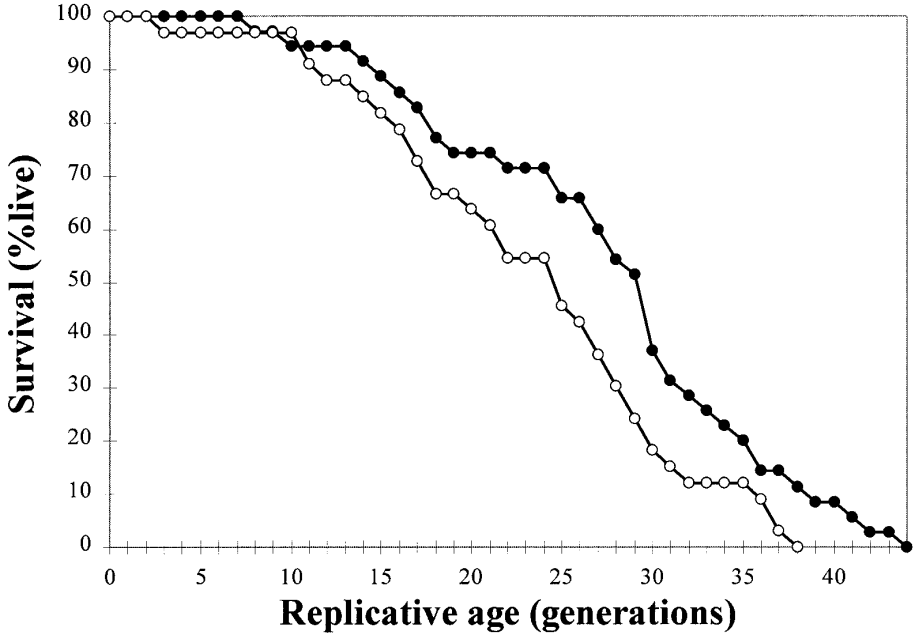

FIG. 6. The effect of heat stress on life span in respiratory-deficient (petite) cells. Life span of the respiratory deficient strain, YSK365, was determined as described in the legend to Fig. 1. The life span of the petite was significantly shorter when it was heat shocked ($P = 0.026$). The mean life spans (in generations) of control (closed circles) and heat-shocked (open circles) cells were 27.7 and 23.6, respectively.

RAS2 in maintaining life span under a variety of environmental conditions. This role appears to be associated with the ability to recover from stress, as measured by *HSP104* gene expression and resumption of growth when stress is present whether transient (Fig. 3) or chronic [25]. Indeed, the life span-shortening effect of continuous *HSP104* expression, described here, is consistent with this interpretation. Overexpression of *RAS2* extends life span in the absence of overt stress [26] and even in the face of chronic stress [25]. It is important to note that the level of stress discussed here is nonlethal. In fact, *ras2* mutants are more resistant than the wild-type to lethal heat stress [36]. Thus, there is a trade-off between the ability to maintain life span in the face of sublethal stress or to harness this response to extend life span, on the one hand, and the capacity to withstand a lethal stress, on the other.

The induction of the heat shock response is temporary (Fig. 3). Expression of *HSP104* is down-regulated within two generations. Even in the *ras2* mutant, this is evident at the mRNA level, though it may be substantially delayed at the protein level. This lack of rapid down-regulation could be the reason why the *ras2* mutant is defective in transient, heat stress-induced life extension, just as it is in the response to chronic heat stress [25]. However, this does not explain the defect in the *ras1* mutant. *RAS2* is known to modulate expression of stress response genes at the level of transcription through the STRE (stress response element) [36]. *RAS1* appears to have little impact on this expression. The results we have presented suggest that *RAS1*-dependent signaling pathways have a distinct role to play in the resistance to stress, as revealed over the course of a life span. These pathways could include inositolphospholipid turnover [37]. *RAS2* appears to function in a rapid switch between the stressed and nonstressed state. We suggest that *RAS1*, on the other hand, is involved in longer term propagation of the response to transient stress that results in life extension. Transient and chronic stress may share *RAS2*-dependent functions, but they are distinguished by the *RAS1*-dependent ones.

The interaction that we have observed between heat stress and mitochondrial function (Fig. 6) may provide an avenue for the exploration of possible additional functions that are essential for life extension by mild heat stress. The result we obtained may not be surprising as it has been shown that mitochondrial function is required for resistance to oxidative stress [38], which plays a role in heat-induced cell death [39]. The interaction we found is only visible when replicative life span is determined. Petites are actually more resistant to lethal heat shock at 50°C in terms of viability (colony-forming ability) than their grande counterparts (C.-Y. Lai and S. M. Jazwinski, unpublished results). This fact lends further support to the notion that mitochondria may play a distinct role in the extension of longevity by thermal stress.

The effect of heat stress acting early in life on longevity occurs in the absence of any overt stress acting

later in life. It is important to note that stress may not be easily identified especially in the case of yeasts. In addition to environmental insults, stress is also generated endogenously through normal metabolic activities. The expression of *hsp70*, the gene responsible for induced thermotolerance in *Drosophila*, increases with age and in response to oxidative stress [40]. This suggests the presence of covert stress or the presence of gene dysregulation [21]. The expression of *hsp70* may not be sufficient to counteract the levels of prevailing stress later in life. On the other hand, other factors may also come into play that determine how the organism responds to stress. We have shown that the life span-extending protocol is not effective after mid-life. This is when expression of *RAS1* and *RAS2* decline substantially [26]. This also coincides with a decrease in resistance to UV stress [41].

It is interesting that the effect of heat stress early in life is so persistent. This is a stably inherited epigenetic change, at least during its duration. This implies the operation of "molecular memory" [42]. The consequences of the activity of certain genes can encompass several cell generations [43]. Many of the *CDC* gene products are synthesized in amounts sufficient to sustain more than one cell cycle [44, 45]. Control of *HO* gene transcription is an example of a molecular mechanism that coordinates gene expression with events in prior cell cycles [46]. The epigenetic inheritance of different regulatory states of chromatin is another example [47]. Heat stress could reset the silencing status of chromatin, which would have consequences for many generations beyond the initial heat treatment. Changes in transcriptional silencing occur with age in yeast [48, 49]. The effect of the *SIR4-42* mutation [9] and the *cdc7* mutation [50, 51] on longevity suggest that such changes may determine life span.

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