ELSEVIER

Neurobiology of Aging 20 (1999) 471–478

NEUROBIOLOGY OF AGING

The *RAS* genes: a homeostatic device in *Saccharomyces cerevisiae* longevity☆

S. Michal Jazwinski*

Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112, USA

Received 3 August 1999; received in revised form 8 September 1999; accepted 17 September 1999

Abstract

The genetic analysis of the yeast replicative life span has revealed the importance of metabolic control and resistance to stress. It has also illuminated the pivotal role in determining longevity that the *RAS* genes play by the maintenance of homeostasis. This role appears to be performed by the coordination of a variety of cellular processes. Metabolic control seems to occupy a central position among these cellular processes that include stress resistance. Some of the features of metabolic control in yeast resemble the effects of the *daf* pathway for adult longevity in *Caenorhabditis elegans* and the metabolic consequences of selection for extended longevity in *Drosophila melanogaster*, as well as some of the features of caloric restriction in mammals. The distinction between dividing and nondividing cells is proposed to be less important for the aging process than generally believed because these cell types are part of a metabolic continuum in which the total metabolic capacity determines life span. As a consequence, the study of yeast aging may be helpful in understanding processes occurring in the aging brain.

Keywords: Aging; Longevity; Metabolic control; Stress resistance; Mitotic cells; Post-mitotic cells; *Saccharomyces cerevisiae*; *RAS1*; *RAS2*

1. Introduction

The metric of the yeast life span is the number of divisions (generations) that an individual cell completes or, in other words, the number of daughter cells produced [30,33]. We call this the replicative life span. More recently, aging has also been studied as the length of time yeast cells remain viable in stationary phase [25]. These two rather distinct ways of looking at longevity and aging in this organism may not be as disparate as it would seem. (This statement will be subjected to a critical evaluation later in this article.) Unless stated otherwise, life span will refer here to the replicative life span.

Yeasts undergo many morphological and physiological changes as they progress through their replicative life span [15]. Among the most universal age changes are the increase in generation time (the time between consecutive buddings) [8], increase in size [7], and progressive decline in mating ability [32,44]. It is difficult to use these changes as biomarkers of aging. The increase in generation time can be uncoupled from life span, resulting in an early appearance of this senescent phenotype [21]. This uncoupling has also been found for size increase [4]. The decline, with age, in the ability to respond to the mating pheromone α-factor has been used to define premature or accelerated senescence in yeast [42,44]. Mutations that give rise to such premature aging cause a redistribution of Sir-transcriptional silencing complexes [35,42] to either extrachromosomal ribosomal DNA [41] or double-stranded DNA breaks [29]. This redistribution results in a loss of transcriptional silencing at the silent mating-type loci that is the direct cause of the loss of response to the mating pheromone. Thus, it is difficult to use loss of response to the pheromone as a measure of the global aging process. Perhaps, the loss of mating ability in yeast is the equivalent of a unimodal progeroid syndrome [27].

This discussion regarding phenotypic changes points to the difficulty of defining biomarkers of aging in yeast, which mirrors the problem with biomarkers of aging in other species. The best predictor of mortality remains replicative age itself. Indeed, the mortality rate of yeast, defined as the probability that an individual yeast will fail to divide, rises exponentially as a function of age, which is the number

☆ The work in the author's laboratory was supported by grants from the National Institute on Aging of the National Institutes of Health.

* Corresponding author. Tel.: +1-504-568-4725; fax: +1-504-568-4725.

E-mail address: sjazwi@lsumc.edu (S.M. Jazwinski)

0197-4580/99/$ – see front matter
PII: S0197-4580(99)00095-0

of divisions already completed [16,37]. Thus, replicative life span is the tool most commonly used in yeast aging studies. Interestingly, the mortality rate plateaus late in the life span. A mathematical model based solely on change as the cause of aging predicts, counterintuitively, that such a plateau must occur, suggesting that the leveling of mortality rate at later ages may be an expression of age changes in individual yeast [17].

The genetics of aging in yeast is a well-developed discipline, with some 14 genes that influence longevity identified [15]. The first genes implicated in yeast longevity were *RAS1* and *RAS2*, the homologues of mammalian H-*RAS* [4]. The divergent roles of these two genes in determining yeast life span was later demonstrated [45]. The pleiotropic effects of yeast *RAS* are well known [46,47]. The role of *RAS* in metabolic control and in resistance to stress was instrumental in proposing the importance of these physiological mechanisms for aging and in their perceived interrelatedness, particularly given the emerging outlines of similar responses in other organisms [12]. Today, this proposal appears even better grounded [13,15].

2. Molecular and physiological mechanisms of aging

Because with each division the yeast cell must carry out the biosynthetic processes required to produce a daughter cell, any extension of replicative life span must entail an increase in the total metabolic effort, called metabolic capacity [12], if other things remain equal. Over expression of *RAS2* extends the life span and postpones the increase in generation time observed during yeast aging [45]. Thus, the yeasts complete more cell divisions and divide rapidly for an extended period. By definition, therefore, they display an enhanced metabolic capacity. Because this gene is important in coordinating cell growth and cell division *RAS2* is crucial [46]. Metabolic capacity is not as easy to assess in organisms in which life span is measured chronologically, because it is not directly related to the metric of longevity. In organisms other than yeast, this raises the question whether life extension has entailed more active life or simply more time.

2.1. The retrograde response

A clear example of a molecular mechanism of yeast aging comes from the analysis of the role of mitochondria in longevity. A signaling pathway from the mitochondrion to the nucleus that affects the expression of nuclear genes encoding mitochondrial, cytoplasmic, and peroxisomal proteins [34] also regulates yeast longevity [23]. Typically, this pathway, called the retrograde response, is induced in yeast lacking part or all of their mitochondrial DNA (rho^0), resulting in mitochondria that are not fully functional. The retrograde response has been shown to be induced by both genetic and environmental means, with a concomitant extension of life span in four different yeast strains. Although the details of the conditions that elicit the retrograde response differ among these strains, life span is always extended when the retrograde response is induced. Increased longevity requires the activity of *RTG2*, a downstream effector of the retrograde response. The nature of the physiological signal that the mitochondrion sends to the nucleus is not clear at present, nor is it known whether it can be elicited by means other than the disruption of mitochondrial function.

The retrograde response underscores the fundamental role that metabolic control plays in yeast longevity. Interestingly, this pathway and its effect on the yeast life span are modulated by *RAS2* [23]. The induction of the retrograde response postpones senescence, as defined by a prolonged period of rapid cell division, and it enhances metabolic capacity [23]. The effect of this intracellular signaling pathway on yeast life span can be likened to a rheostat, rather than a simple on-off switch, in that the greater its induction the larger the effect on life span.

The retrograde response results in the increase in activity of several metabolic enzymes [3,43,49]. The net effect is a shift from the Krebs cycle to the glyoxylate cycle, a switch from the use of glucose to the use of acetate, and an increase in gluconeogenic activity. This is to some extent reminiscent of the mobilization of fat stores. The metabolic and enzymatic changes that occur in *Caenorhabditis elegans* whose life has been extended by the manipulation of the *daf-2*/*daf-16* pathway suggest similar biochemical adjustments. There are also similarities to the metabolic changes that are found in *Drosophila* that have been selected for an extended life span, including the storage of fat. The activation of gluconeogenic enzymes is also found during caloric restriction (without malnutrition), which results in the extension of life span in mammals. Rather than simply a caloric reduction, the retrograde response represents a shift from an energy source of high caloric content (glucose) to one of lower caloric content (acetate). All of these facts support the crucial role of metabolic control in determining life span across several species. These connections are more thoroughly discussed in Jazwinski [13,15].

2.2. Stress resistance

The potential significance of stress resistance in yeast aging is inherent in the action of *RAS2* in determining life span [45] because this gene modulates a variety of stress responses [26]. However, the fact that the resistance of yeast to ultraviolet radiation (UV) changes with age provided further grounding for this proposal [19]. UV resistance increases with age through midlife and then plummets. This biphasic profile parallels the *RAS2* expression during the yeast life span. *RAS2* is required to protect yeast from UV [9].

The viability of *Saccharomyces cerevisiae* in the stationary phase or after lethal heat shock is enhanced when the

RAS2 gene is deleted [46]. However, the shift from the stationary phase to logarithmic growth involves *RAS2* and glucose signaling [46,47]. Interestingly, rho^0 yeast, in which the retrograde response is induced, are more resistant to lethal heat stress [23], linking metabolic control to stress resistance.

When yeast are subjected to chronic bouts of sublethal heat stress, their replicative life span declines. This decline is ameliorated by the *RAS2* gene, but not by *RAS1* [39]. This role in resistance to heat stress is the opposite of the effect of this gene on survival following a lethal dose of heat. Yeast are more likely to encounter sublethal heat stress, as opposed to a lethal heat shock, during their life span. Thus, the activity of *RAS2* represents a balance. The yeast pay a price to be able to withstand chronic, sublethal heat stress during their replicative life span. *RAS2* allows the cells to recover rapidly from the sublethal heat stress, as evidenced by the expression of stress genes and growth-promoting genes [39]. Thus, this gene facilitates a rapid response to changing conditions in which the yeast cell finds itself, especially in the shift from stressful to relaxed conditions. Yeast have, therefore, two responses to stress. In the first, they "stay and fight" by inducing stress responses, as happens in the stationary phase. The induction of gene expression that this entails is downregulated by *RAS2* [26]. In the second, they "run away" by resuming growth and division, thus overcoming the stress by shear numbers. This appears to be the preferred response during the replicative life span. One effect of the decline in *RAS2* expression late in life [45] would be to reduce this capability. Aging yeast cells experience difficulty in maintaining a rapidly dividing state, which can be remedied by stimulating downstream elements of the *RAS2* pathway [31].

Sublethal heat stress can have a different effect on yeast longevity when it is transient rather than chronic. This can be likened to a conditioning effect that results in an extension of life span through a persistent, but not permanent, reduction in mortality rate [40]. This effect requires both the *RAS1* and *RAS2* genes, as well as the major heat shock gene that is largely responsible for induced thermal tolerance, *HSP104*, and active mitochondria [40]. The effects of *RAS2* on yeast longevity involve cyclic adenosine 5′-monotriphosphate-dependent and -independent pathways, depending on the presence and absence of stress [39,45]. After transient stress, *RAS2* appears to be involved in the rapid recovery from the stressful conditions, as discussed above for chronic heat stress. *RAS1*, on the other hand, may function in the long-term effects of the transient stress, perhaps operating through a pathway of inositol phosholipid turnover [40].

2.3. Genetic stability

Genetic stability plays an important role in aging of *S. cerevisiae*. This phenomenon manifests itself in two, closely related ways. The first concerns the release of ribosomal DNA circles from the array of 100–200 repeats of the basic 9.1 kb unit that encodes the 35S precursor of ribosomal RNA and the 5S RNA [41]. The second involves the formation of double-stranded DNA breaks in the rDNA that are the likely precursor of the rDNA circles [6], although the causal relationship between breaks and circle formation during aging has not been demonstrated directly.

Double-strand breaks, as such, have also been purported to be a cause of aging in yeast [35]. This conclusion rests on the life-shortening effects of a mutation in the *RAD52* gene, which is involved in the recombinational repair of double-stranded breaks in DNA. This curtailment of life span by *rad52* is accompanied by a decline in the response of the cells to α-factor, which has been construed to indicate premature senescence. However, double-strand DNA breaks attract the Sir silencing complex, which causes the loss of silencing at the silent mating-type loci and loss of response to the α pheromone [29]. Thus, the conclusion that the life-shortening effect of the *rad52* mutation causes premature aging is difficult to sustain. Although this mutation is not lethal, the abrogation of the fundamental function that it specifies may require several cell divisions to be expressed. More persuasive would be the demonstration that the effect of this mutation is limited to the mother cell or that life span is extended by the over expression of *RAD52* in conjunction with a demonstration that double-stranded DNA breaks accumulate with age.

The appearance of rDNA circles and their accumulation with age may cause senescence or outright death because of the sequestration of DNA replicative complexes or transcription factors by these circles [41]. There may be other ways in which these molecules could clutter up the yeast cell, leading to its demise. However, these rDNA circles are not always detrimental to *S. cerevisiae*. Induction of the retrograde response that results in a substantial extension of life span [23] also causes a several-fold increase in the rDNA circle content [5]. Thus, an increase in life span occurs in spite of accumulation of circles, indicating that they are not a cause of aging under these conditions. Perhaps some accumulation of these circles is beneficial and contributes to extended longevity.

There is also a possibility that gene dysregulation in the form of loss of chromatin-dependent transcriptional silencing may be a cause of aging in yeast. Loss of silencing at subtelomeric loci [41] and at the silent mating-type loci [44] has been demonstrated. Mutants in *SIR4* [20] and in *CDC7* [8] affect silencing and yeast longevity, providing additional support for the significance of gene dysregulation in *S. cerevisiae* aging. Any conclusions are, however, plagued by the pleiotropic effects of these genes. It should be noted that *RAS2* functions in setting the basal silencing state of subtelomeric heterochromatin [17].

3. *RAS* coordinates cellular activities important for longevity

The role of *RAS2* in longevity through its involvement in metabolic control, and thus in cell growth, is demonstrated

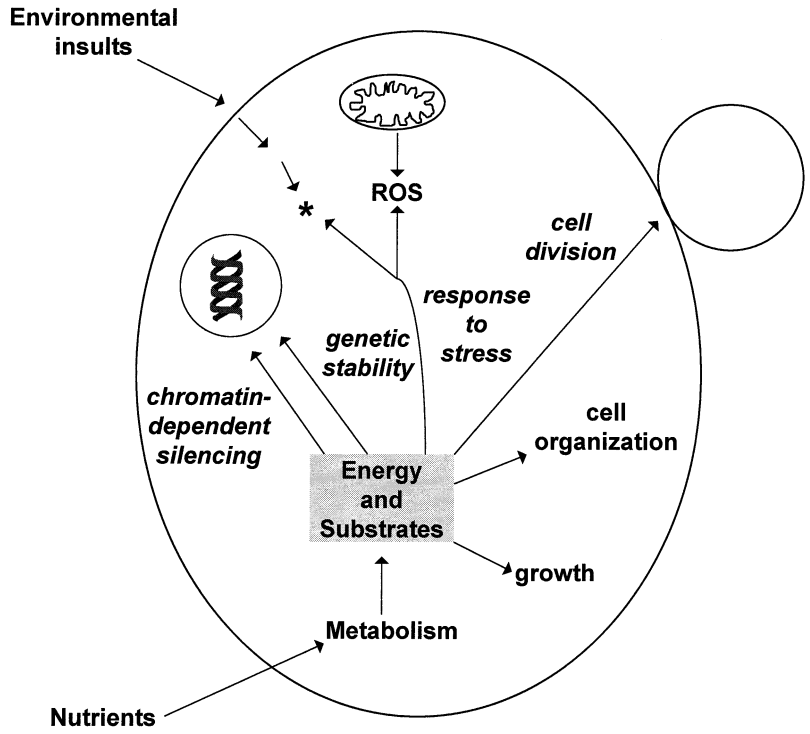

Fig. 1. Cellular processes important in yeast longevity. In principle, the processes indicated in the figure must be important for yeast longevity. There is evidence that each of these processes undergoes changes during aging. Nutrients are taken up by the cell and in metabolism are converted to energy and substrates that are used in the indicated processes.

by its involvement in the retrograde response [23]. The significance of *RAS1* and *RAS2* in the effects of heat stress on longevity has been documented by the analysis of the response to transient and to chronic heat treatment [39,40]. *RAS2* regulates recovery from the stress of nutritional deprivation, such as can occur in the stationary phase [46,47, 50]. Clearly, these genes play a role in determining the number of divisions available to the cell [45], perhaps through an effect on cyclin activity [1,48]. Cellular spatial organization, measured as an effect on budding pattern, and chromatin-dependent transcriptional silencing at subtelomeric loci are modulated by *RAS2* to maintain a youthful pattern, and this effect of *RAS2* on these cellular processes coincides with its positive effect on longevity [17]. For both of these processes, a causal effect on yeast aging remains to be determined. *RAS* may have a modulatory effect on genetic stability that is important for aging.

It is possible to propose on fundamental grounds that certain cellular processes must be maintained within appropriate bounds to prevent early aging. These processes guarantee the function of the cell under normal conditions and when the yeast cell is under stress. These processes include cell growth, cell division, response to stress (both environmental and intrinsically generated), cellular spatial organization or polarity, chromatin-dependent transcriptional silencing, and genetic stability (Fig. 1). The yeast cell takes up nutrients that become a source of energy and substrates through metabolic activity. Because energy and substrates are the resources the cell needs to perform the processes just listed, it is reasonable to propose that metabolic activity is the primary process that is involved in life maintenance and life span determination.

RAS regulates the flow of energy and substrate resources to the competing demands represented by the cellular processes involved in life maintenance, as proposed in the model in Fig. 2. In this way, *RAS2* (and *RAS1*) perform their longevity-determining functions. The ras proteins do not lie directly in any of the pathways that constitute or regulate these cellular processes; instead, they modulate these pathways. This allows coordination of these cellular processes by *RAS*. Alternatively, *RAS* may only be involved in metabolic control, responding to feedback from the critical cellular processes. In this case, *RAS* would help discern the quality and quantity of the energy and substrate resources available, which, in turn, would dictate their relative use by the competing cellular processes. The only known signal to which *RAS* responds is nutritional status.

Two possible ways in which *RAS* affects the aging process immediately spring to mind (Fig. 3). It may function as an adaptation gene, in which it responds to the change associated with aging, in essence compensating for a decline in homeostasis. On the other hand, it may function as a regulatory gene. As such, it would coordinate life maintenance processes, preserving homeostasis based on regulatory feedback. The latter alternative is more probable because changes in *RAS2* expression alter the life span and affect a wide array of processes. Furthermore, there is an optimal level of *RAS* activity that results in the maximal life

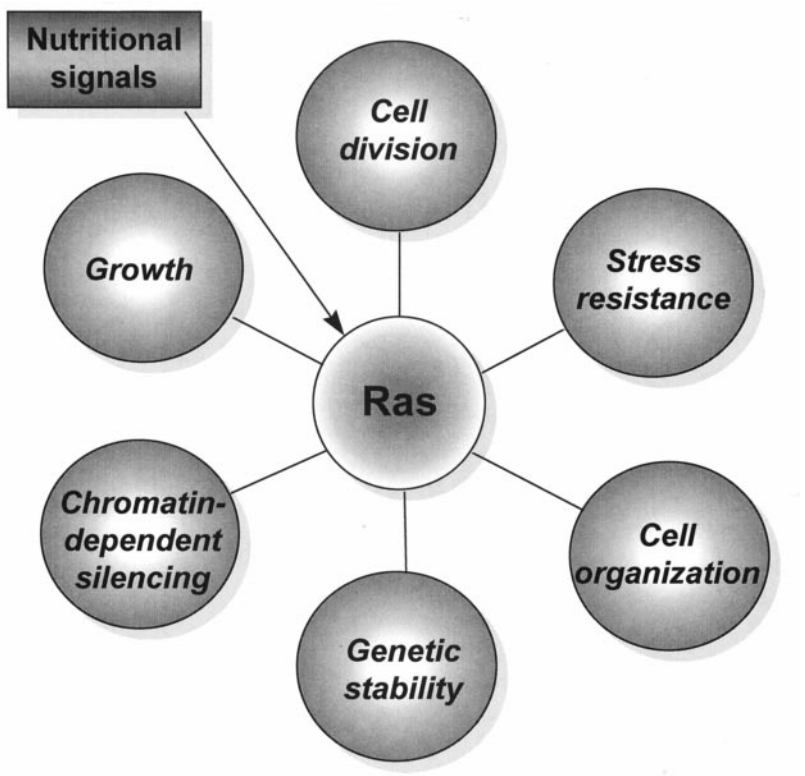

Fig. 2. The role of the *RAS* genes in yeast longevity. *RAS2* has been shown to modulate each of the indicated processes, except for genetic stability. (It is proposed that this gene also modulates this process.) By modulating these processes, *RAS2* "channels" cellular resources (energy and nutrients) to the competing demands that they represent. In this way, it maintains homeostasis. *RAS2* is involved in nutritional assessment.

span [4,45]. Expression of *RAS2* below or above this level results in a shorter life span. Thus, the dependence of life span on *RAS* is biphasic [14]. Where a particular yeast cell lies on this biphasic curve depends on its genetic background and on the environmental and epigenetic exigencies to which this cell is subjected. The source of this biphasic response to *RAS* activity is likely to reside in the coordination by *RAS* of cellular processes that exert competing demands on limited cellular resources. The actual dependence of life maintenance on any individual cellular process may vary with the circumstances. The yeast cell must be able to dynamically balance the requirements of competing cellular processes as its needs and dependence upon them change. It cannot put all of its "eggs in one basket." For example, the cell effects a trade-off between survival in the face of heat stress and growth and cell division, as evidenced by the transient arrest of growth and division following heat shock [39].

RAS constitutes a homeostatic device in yeast longevity, as implied by its modulation of several cellular processes important for life maintenance. The loss of homeostasis has been proposed to be the cause of aging [17]. The decline with age in the levels of both of the *RAS* gene products [45] could contribute to the loss of homeostasis with age. The resulting imbalance could be the cause of death in yeast. The imbalance between cell growth and division in many *cdc* mutants arrested at nonpermissive temperature results in cell lysis [38], which is the usual mode of death in yeast at the end of their replicative life span.

4. Aging of mitotic and postmitotic cells

Mitotic and postmitotic cells are frequently set apart in discussions of aging. It is often stressed that the mechanisms of aging must be quite distinct in these two broad cell types. Mitotic cells have the capacity for renewal in the process of cell division that is lacking in postmitotic cells. This capability of dividing cells generally brings in tow telomere shortening, which is absent from nondividing cells, and ultimately leads to cessation of division and potential changes in the expression of subtelomeric genes. However, both mitotic and postmitotic cells perform many of the same functions, although many processes are peculiar to each. Certainly, both are metabolically active. Indeed, some postmitotic cells, such as neurons, are more active than typical mitotic cells, and differences in metabolism are not greater than can be found among various dividing or nondividing cell types.

S. cerevisiae provides a cogent example of the metabolic continuum to which all dividing and nondividing cells belong (Fig. 4). The long-term viability of yeast cells in stationary culture has been proposed as a model for the study of aging of nondividing cells [25]. However, the

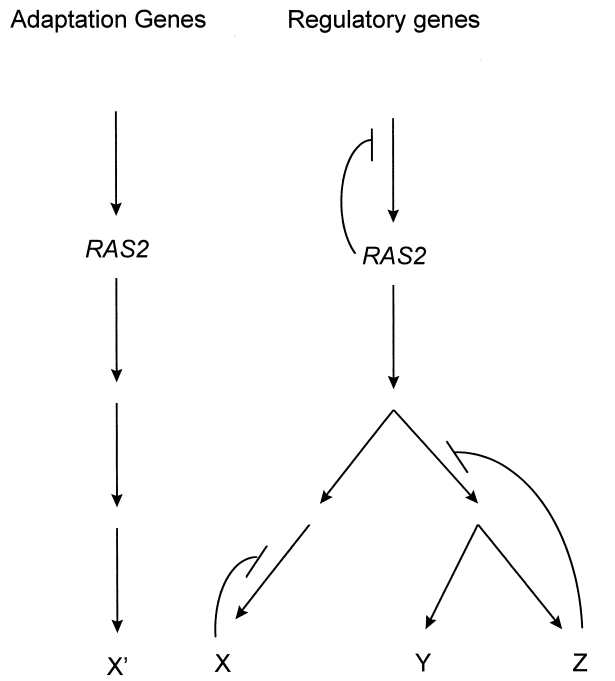

Fig. 3. Two alternative modes of action for *RAS2* in determining life span. *RAS2* may be an adaptation gene. In this model, this gene would be part of a pathway that responds to an age change(s) to compensate for it. *RAS2* may function as a regulatory gene. In this case, this gene would be part of a genetic hierarchy whose action plays a role in determining life span through its regulation of normal cellular processes. This model does not invoke a genetic program [11]. The two models are not mutually exclusive. The arrows indicate steps in pathways in which *RAS2* participates. The letters indicate the end products or outcomes of these pathways.

distinction between the replicative life span of yeast and their survival in stationary culture may not be as stark as perceived. The greatest difference is the markedly reduced metabolic activity in the stationary phase compared to exponential growth [50]. Depending on the conditions, the mix of respiratory versus fermentative metabolism may vary. Clearly, yeast in the stationary phase are stressed, as indicated earlier, and the relative importance of stress resistance is, therefore, greater. It takes nondividing yeast considerably more time to achieve the lifetime metabolic capacity of dividing yeast. Thus, the metabolic mechanisms of aging should be accentuated during the replicative life span, although nuances may be detected through the analysis of nondividing cells.

The yeast spore is virtually dormant metabolically; it is a true dispersal form. The dauer larva of *C. elegans* performs a similar function, but its metabolism is not as stringently shut down. In terms of energy expenditure or metabolic rate, nondividing *S. cerevisiae* cells in the stationary phase [24] show greater activity than do spores [28], whereas dividing cells are most active. It is proposed that the adult dauer that displays extended longevity lies somewhere between the stationary yeast and the dividing yeast on the metabolic continuum. Measurement of life span in time (calendar life span) is relevant for *C. elegans*, the yeast spore, and the stationary yeast. It can also be used for dividing yeast cells; however, determination of the replicative life span is more facile when individual cells are concerned. The issue is really to assess the metabolic capacity in each case.

The existence of a metabolic continuum that circumscribes both the calendar and replicative life spans suggests

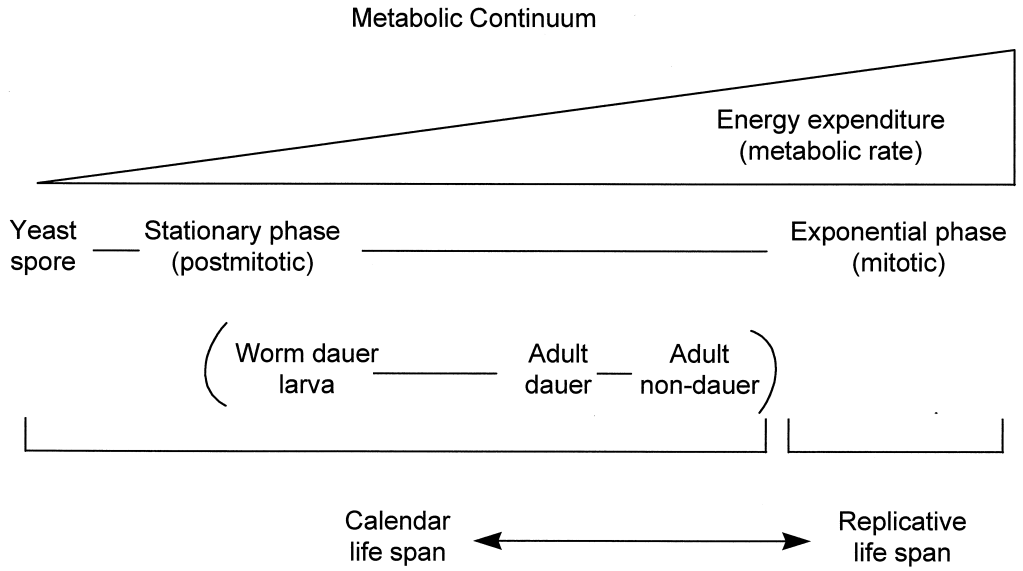

Fig. 4. Proposed role of metabolic capacity in determining life span. Dispersal forms, such as the yeast spore and the worm dauer larva, lie at one end of a metabolic continuum. At the other end lie metabolically very active cells and organisms. In the case of yeast, these are dividing cells. Nondividing yeast cells can be found somewhere between spores and dividing cells. Time is a reasonable measure of life span (calendar life span) at the lower end of the metabolic continuum. At its upper end, replicative life span is more useful for yeast. There is no discontinuity between calendar and replicative life span. The true measure of longevity is metabolic capacity. Certain aging processes and molecular mechanisms of aging operate throughout the metabolic continuum, whereas others may apply to discrete regions. For further discussion, see the text.

that metabolic capacity is being accrued whether or not cells are dividing. This has indeed been proposed to be the case in *S. cerevisiae* [10,11]. In fact, the loss of transcriptional silencing that occurs in subtelomeric chromatin in *S. cerevisiae* during the replicative life span, without telomere attrition, has led to the proposal that the distinction between dividing and nondividing cells may not be as great as might be thought [22]. If metabolic capacity is accrued in nondividing yeast cells, then this may contribute to the shortening of their replicative life span. Anecdotal observations indicate that individual cells from stationary cultures of *S. cerevisiae* have shorter life spans than cells from exponential cultures (J.B. Chen, N.P. D'mello, S.M. Jazwinski, unpublished observations).

Whether yeast are dividing or not, some processes of damage and stress are active throughout the metabolic continuum, albeit at different intensities. Thus, dividing and nondividing cells will display some unique, but also some common, specific aspects of aging. Among the unique properties of the nondividing cells is enhanced resistance to stress. However, this is not true for all types of stress. Although stationary phase *S. cerevisiae* are more resistant to heat and to oxidative stress [26], they are more sensitive to UV [36]. Many aging processes will be similar in both dividing and nondividing cells, and these processes will involve metabolic control, resistance to stress, and maintenance of homeostasis in general. As a consequence, molecular mechanisms of aging will be shared between these cell types. The extension of life span and the postponement of aging will require the expansion of metabolic capacity.

It has already been suggested that metabolism plays a primary role in determining longevity. The evolutionary conservation of metabolic pathways and the essential similarity of metabolic activity in mitotic and postmitotic cells suggest that the molecular mechanisms underlying the metabolic control processes that play a role in determining yeast life span should provide important insights into aging in other species, including mammals.

5. Perspectives on brain aging

Are the studies on *S. cerevisiae* aging relevant for our understanding of the aging brain? One may only speculate. The brain consists of both mitotic glial cells and postmitotic neurons. It might seem that the studies on yeast have little importance for the understanding of the aging of the latter. However, this judgment may be too rapid considering the discussion of dividing and nondividing yeast cells.

The brain is the most metabolically active tissue in the body. Thus, metabolic control in this organ may be particularly prone to the aging process and constitute a major cause of aging. The brain is also subjected to considerable stress of various sorts during the life span, often mediated by the glucocorticoids. Therefore, stress resistance is especially important in the aging brain.

The study of *S. cerevisiae* aging, and of the *RAS* genes in particular, may help to illuminate some of the features of brain aging related to metabolic control and stress resistance. However, the utility of these studies does not stop with the *RAS* genes. The human homolog of the yeast longevity gene, *LAG1*, is expressed in the brain [18]. The *LAG1* protein affects glycosylphosphatidylinositol-anchored protein transport [2]. Glycosylphosphatidylinositol-anchored proteins include plasma membrane proteins, such as N-CAM, that are important in the development and function of the nervous system.

References

[1] Baroni MD, Monti P, Alberghina L. Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast. Nature 1994; 371:339–42.

[2] Barz WP, Walter P. Two endoplasmic reticulum (ER) membrane proteins that facilitate ER-to-Golgi transport of glycosylphosphatidylinositol-anchored protein. Mol Biol Cell 1999;10:1043–59.

[3] Chelstowska A, Butow RA. RTG genes in yeast that function in communication between mitochondria and the nucleus are also required for expression of genes encoding peroxisomal proteins. J Biol Chem 1995;270:18141–6.

[4] Chen JB, Sun J, Jazwinski SM. Prolongation of the yeast life span by the v-Ha-*RAS* oncogene. Mol Microbiol 1990;4:2081–6.

[5] Conrad-Webb H, Butow RA. A polymerase switch in the synthesis of rRNA in *Saccharomyces cerevisiae*. Mol Cell Biol 1995;15:2420–8.

[6] Defossez P-A, Prusty R, Kaeberlein M, Lin S-J, Ferrigno P, Silver PA, Keil RL, Guarente L. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol Cell 1999;3: 447–55.

[7] Egilmez N K, Chen JB, Jazwinski SM. Preparation and partial characterization of old yeast cells. J Gerontol Biol Sci 1990;45:B9–17.

[8] Egilmez NK, Jazwinski SM. Evidence for the involvement of a cytoplasmic factor in the aging of the yeast *Saccharomyces cerevisiae*. J Bacteriol 1989;171:37–42.

[9] Engelberg D, Klein C, Martinetto H, Struhl K, Karin M. The UV response involving the Ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. Cell 1994;77:381–90.

[10] Jazwinski SM. An experimental system for the molecular analysis of the aging process: the budding yeast *Saccharomyces cerevisiae*. J Gerontol 1990;45:B68–74.

[11] Jazwinski SM. Aging and senescence of the budding yeast *Saccharomyces cerevisiae*. Mol Microbiol 1990;4:337–43.

[12] Jazwinski SM. Longevity, genes, and aging. Science 1996;273:54–9.

[13] Jazwinski SM. Coordination of metabolic activity and stress resistance in yeast longevity. In: Hekimi S, editor. Molecular biology of aging. Heidelberg: Springer, in press.

[14] Jazwinski SM. Longevity, genes, and aging: A view provided by a genetic model system. Exp Gerontol 1999;34:1–6.

[15] Jazwinski SM. Molecular mechanisms of yeast longevity. Trends Microbiol 1999;7:247–52.

[16] Jazwinski SM, Egilmez NK, Chen JB. Replication control and cellular life span. Exp Gerontol 1989;24:423–36.

[17] Jazwinski SM, Kim S, Lai C-Y, Benguria A. Epigenetic stratification: the role of individual change in the biological aging process. Exp Gerontol 1998;33:571–80.

[18] Jiang JC, Kirchman PA, Zagulski M, Hunt J, Jazwinski SM. Homologs of the yeast longevity gene *LAG1* in *Caenorhabditis elegans* and human. Genome Res 1998;8:1259–72.

[19] Kale SP, Jazwinski SM. Differential response to UV stress and DNA damage during the yeast replicative life span. Dev Genet 1996;18: 154–60.
[20] Kennedy BK, Austriaco NR, Zhang J, Guarente L. Mutation in the silencing gene *SIR4* can delay aging in *S. cerevisiae*. Cell 1995;80: 485–96.
[21] Kim S, Benguria A, Lai C-Y, Jazwinski SM. Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol Biol Cell 1999;3125–36.
[22] Kim S, Villeponteau B, Jazwinski SM. Effect of replicative age on transcriptional silencing near telomeres in *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 1996;219:370–6.
[23] Kirchman PA, Kim S, Lai C-Y, Jazwinski SM. Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. Genetics 1999;152:179–90.
[24] Lagunas R, Ruiz E. Balance of production and consumption of ATP in ammonium-starved *Saccharomyces cerevisiae*. J Gen Microbiol 1988;134:2507–11.
[25] Longo VD, Gralla EB, Valentine JS. Superoxide dismutase activity is essential for stationary phase survival in *Saccharomyces cerevisiae*. Mitochondrial production of toxic oxygen species in vivo. J Biol Chem 1996;271:12275–80.
[26] Marchler G, Schuller C, Adam G, Ruis H. A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions. EMBO J 1993;12: 1997–2003.
[27] Martin GM. Genetics and aging: the Werner syndrome as a segmental progeroid syndrome. Adv Exp Med Biol 1985;190:161–70.
[28] Miller JJ. Sporulation in *Saccharomyces cerevisiae*. In: Rose, AH, Harrison, JS, editors. The yeasts. San Diego, CA: Academic Press, 1989. p. 489–550.
[29] Mills KD, Sinclair DA, Guarente L. *MEC1*-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. Cell 1999;97:609–20.
[30] Mortimer RK, Johnston JR. Life span of individual yeast cells. Nature 1959;183:1751–2.
[31] Motizuki M, Tsurugi K. Effect of 17β-estradiol on the generation time of old cells of the yeast *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 1992;183:1191–6.
[32] Muller I. Parental age and the life-span of zygotes of *Saccharomyces cerevisiae*. Antonie Van Leeuwenhoek 1985;51:1–10.
[33] Muller I, Zimmermann M, Becker D, Flomer M. Calendar life span versus budding life span of *Saccharomyces cerevisiae*. Mech Ageing Dev 1980;12:47–52.
[34] Parikh VS, Morgan MM, Scott R, Clements LS, Butow RA. The mitochondrial genotype can influence gene expression in yeast. Science 1987;235:576–80.
[35] Park PU, Defossez P-A, Guarente L. Effects of mutations in DNA repair genes on formation of ribosomal DNA circles and life span in *Saccharomyces cerevisiae*. Mol Cell Biol 1999;19:3848–56.
[36] Parry JM, Davies J, Evans WE. The effects of "cell age" upon the lethal effects of physical and chemical mutagens in the yeast, *Saccharomyces cerevisiae*. Mol Gen Genet 1976;146:27–35.
[37] Pohley H-J. A formal mortality analysis for populations of unicellular organisms (*Saccharomyces cerevisiae*). Mech Ageing Dev 1987;38: 231–43.
[38] Pringle JR, Hartwell LH. The *Saccharomyces cerevisiae* cell cycle. In: Strathern JN, Jones EW, Broach JR, editors. The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Plainview, NY: Cold Spring Harbor Laboratory Press, 1981. p. 97–142.
[39] Shama S, Kirchman PA, Jiang JC, Jazwinski SM. Role of *RAS2* in recovery from chronic stress: effect on yeast life span. Exp Cell Res 1998;245:368–78.
[40] Shama S, Lai C-Y, Antoniazzi JM, Jiang JC, Jazwinski SM. Heat stress-induced life span extension in yeast. Exp Cell Res 1998;245: 379–88.
[41] Sinclair DA, Guarente L. Extrachromosomal rDNA circles: a cause of aging in yeast. Cell 1997;91:1033–42.
[42] Sinclair DA, Mills K, Guarente L. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. Science 1997;277:1313–16.
[43] Small WC, Brodeur RD, Sandor A, Fedorova N, Li G, Butow RA, Srere PA. Enzymatic and metabolic studies on retrograde regulation mutants of yeast. Biochemistry 1995;34:5569–76.
[44] Smeal T, Claus J, Kennedy B, Cole F, Guarente L. Loss of transcriptional silencing causes sterility in old mother cells of *S. cerevisiae.* Cell 1996;84:633–42.
[45] Sun J, Kale SP, Childress AM, Pinswasdi C, Jazwinski SM. Divergent roles of *RAS1* and *RAS2* in yeast longevity. J Biol Chem 1994; 269:18638–45.
[46] Tatchell K. *RAS* genes in the budding yeast *Saccharomyces cerevisiae*. In: Kurjan T, editor. Signal transduction: prokaryotic and simple eukaryotic systems. San Diego, CA: Academic Press, 1993. p. 147–88.
[47] Thevelein JM. Signal transduction in yeast. Yeast 1994;10:1753–90.
[48] Tokiwa G, Tyers M, Volpe T, Futcher B. Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. Nature 1994;371:342–5.
[49] Vélot C, Haviernik P, Lauquin GJ-M. The *Saccharomyces cerevisiae RTG2* gene is a regulator of aconitase expression under catabolite repression conditions. Genetics 1996;144:893–903.
[50] Werner-Washburne M, Braun E, Johnston GC, Singer, RA. Stationary phase in the yeast *Saccharomyces cerevisiae*. Microbiol Rev 1993;57:383–401.