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# LONGEVITY, GENES, AND AGING: A VIEW PROVIDED BY A GENETIC MODEL SYSTEM[1]

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**Abstract—**The genetic analysis of aging in the yeast *Saccharomyces cerevisiae* has revealed the importance of metabolic capacity, resistance to stress, integrity of gene regulation, and genetic stability for longevity. A balance between these life maintenance processes is sustained by the *RAS2* gene, which channels cellular resources among them. This gene cooperates with mitochondria and *PHB1* in metabolic adjustments important for longevity. It also modulates stress responses. Transcriptional silencing of heterochromatic regions of the genome is lost during aging, suggesting that gene dysregulation accompanies the aging process. There is evidence that this age change plays a causal role. Aging possesses features of a nonlinear process, and it is likely that application of nonlinear system methodology to aging will be productive. 

**Key words: metabolism, stress responses, gene dysregulation, nonlinear system**

## INTRODUCTION

IF WE WERE to design an organism, we would endow it with sufficient lifetime metabolic capacity, stress resistance, integrity of gene regulation, and genetic stability. Given a limit to the resources available, we would need to strike a balance between these processes and reproduction. Genetic analysis of the aging process has confirmed the soundness of these engineering principles (Jazwinski, 1996). It is natural selection that actually dictates the distribution of these resources, and thus, indirectly, sets limitations on life expectancy (Kirkwood and Rose, 1991).

The genetics of aging, in our hands, is really the genetics of longevity. We identify genes that play a role in determining life span, because the best predictor of mortality is life span itself. Although our interest is in human aging, a facile application of genetic methodology requires a suitable model system. For us, this has been the yeast *Saccharomyces cerevisiae*. This unicel-

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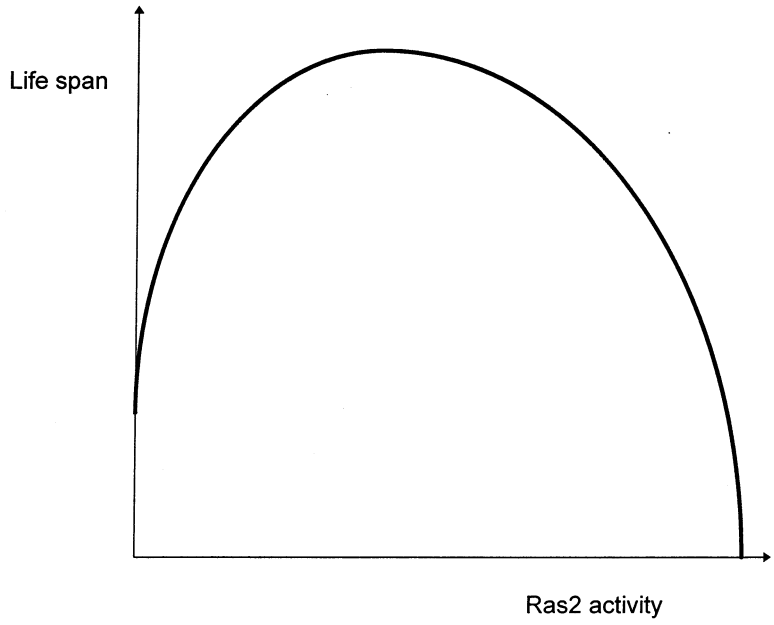


FIG. 1. Nonlinear relationship between Ras2 activity and life span. A model explaining the effects of *RAS2* overexpression on yeast longevity.

lular eukaryote displays a limited life span, which is measured by the number of divisions or daughter cells produced (Mortimer and Johnston, 1959; Muller *et al*., 1980). Time is not as useful a measure. We express age in generations. Although the individual yeast cell is mortal, the population is immortal, because the daughters essentially begin the process all over and display full life-span potential. As yeasts proceed through their replicative life span, they undergo many changes (Jazwinski, 1993). The probability that a yeast will continue dividing decreases exponentially as a function of the number of divisions (generations) completed (Pohley, 1987; Jazwinski et al., 1989). This conforms nicely to the Gompertz equation.

I will focus here on a few of the several longevity genes we have identified in our studies of yeast aging. The yeast *RAS* genes play a pivotal role in our analyses. These genes function as central integrators of cell growth and cell division by operating as part of a nutrient sensor (Tatchell, 1993). They exert their effects through several signal transduction pathways. Originally, it was thought that the only role they had to play was to stimulate adenylate cyclase, a role reserved for heterotrimeric G proteins in mammals. Now, it is clear that *RAS1* is involved in stimulating inositol phospholipid turnover, and that *RAS2* participates in a MAP kinase pathway (Roberts *et al*., 1997), just like mammalian *ras*. The two genes play opposing roles in determining life span (Sun *et al*., 1994). *RAS1* tends to limit it, while *RAS2* extends it. This can be readily observed by either deletion or overexpression of these genes.

Overexpression of *RAS2* increases life span. However, too much of a good thing is not so good. Too much Ras activity abrogates the life extension (Chen *et al*., 1990). The picture that emerges from this (Fig. 1) is that there must be an optimal level of Ras2 activity for the maximal life span to be observed. Depending on the position on the curve in Fig. 1, overexpression could increase or even decrease life span. The effects of Ras2 activity are proposed to be determined

by the genetic background and by the epigenetic and environmental exigencies to which the organism is subjected. *RAS2*, acting through the cAMP pathway, regulates passage through Start in the $G_1$ phase of the cell cycle, which is dependent on the metabolic activity necessary for growth of the cell to a critical size (Pringle and Hartwell, 1981). One means by which this control is achieved is through repression of cyclin expression by cAMP-dependent protein kinase (Baroni *et al*., 1994). Another is through cAMP stimulation of growth (Pringle and Hartwell, 1981). The cAMP pathway also modulates transcription of a variety of stress-responsive genes (Marchler *et al*., 1993). Thus, *RAS2* channels resources between cell growth (metabolic activity), cell division (reproduction), and stress resistance. It would be too dangerous to put all the eggs in one basket.

### *Metabolic capacity*

Overexpression of *RAS2* not only increases life span, but it also postpones the age-dependent increase in generation time that is a hallmark of yeast aging (Sun *et al*., 1994). Thus, the yeasts display postponed senescence. It is useful to consider what happens in this instance. The yeasts generate and consume more energy, and they synthesize more biomass. All of this is necessary to produce the additional daughter cells, which are, in fact, produced at a fast rate over a longer period. Thus, metabolic capacity and efficiency are expanded. This requires some coordination, which may fall apart with age. Recently, additional evidence in support of the role of metabolic capacity in determining life span has been obtained. This involves our studies on the *PHB1* gene.

*PHB1* is a homologue of the human prohibitin gene (McClung *et al*., 1995). We have identified a genetic interaction between this gene and the *RAS2* gene in determining life span (PA Kirchman and SM Jazwinski, unpublished). This interaction is seen only in petite yeasts, whose mitochondria are not fully functional. Our studies suggest that mitochondria, *PHB1*, and *RAS2* participate in metabolic adjustments that are important for longevity. Our working model is that these genes cooperate to signal a decreasing requirement for mitochondrial activity (Fig. 2). We suggest a physical interaction between Phb1 and Ras2, because Phb1 possesses domains that are found in Ras-GAPs (Ras GTPase-activating proteins) (Sato *et al*., 1992).

### *Stress resistance*

Yeasts can either run away from danger, or they can stay and fight. The first of these alternatives does not really involve motility. Instead, it involves exponential cell division, such that the number of individuals is so large that at least a few are bound to survive. The second alternative involves the induction of stress responses. One stressor that yeasts encounter naturally is ultraviolet radiation (UV), as they sun themselves on grape leaves. We have shown that resistance to UV is biphasic as a function of age, first increasing through midlife and then dropping precipitously (Kale and Jazwinski, 1996). The expression of *RAS2* parallels this pattern. This, in fact, may be the cause for the biphasic profile observed, because it is known that *RAS2* is necessary for UV resistance (Engelberg *et al*., 1994). However, this requirement is not at the level of repair of DNA damage (Engelberg *et al*., 1994). It would appear that *RAS2* is essential to "organize" the response to the UV damage by affecting the kinetics of replication checkpoint arrest in the cell cycle. *ras2* mutant cells take longer than wild type to reach the checkpoint (S Kim and SM Jazwinski, unpublished).

Lest the impression is left that all stress is bad, I rush to note that some stress may induce pathways that rally or organize the yeast, improving its fitness. We have found that induction of thermal tolerance early in the life span can reduce the mortality rate, resulting in an increase

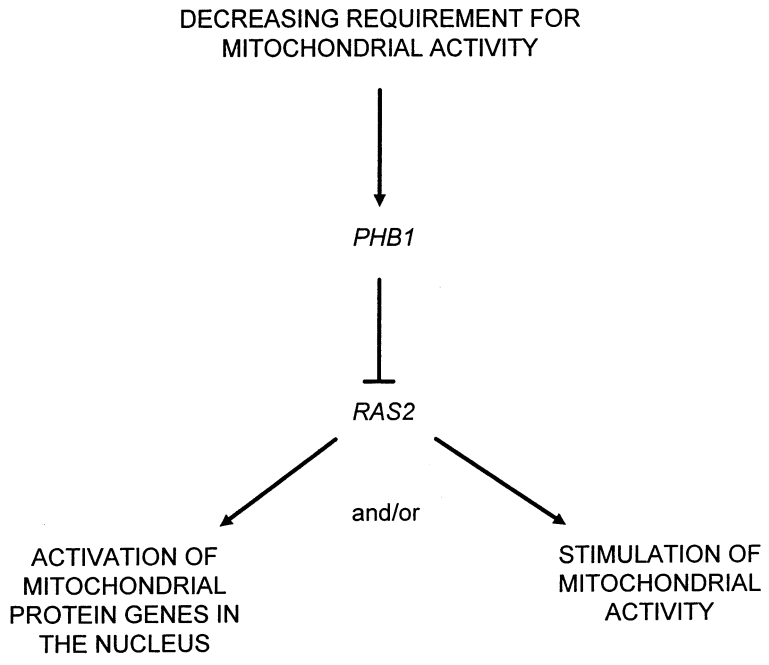


FIG. 2. Working model for a pathway signaling metabolic adjustments that determine longevity. (→) activation, (⊣) inhibition.

in life span (Shama *et al*., 1998a). This beneficial effect is transient, and ultimately the mortality rate becomes identical to that of yeasts in which thermal tolerance was not induced. This salutory effect on longevity requires both of the yeast *RAS* genes. In contrast to the benefits of induced thermal tolerance, chronically repeated heat stress has a clear life span shortening effect that is more profound in the absence of *RAS2* (Shama *et al*., 1998b). *RAS1* does not appear to play a role here. The effect of absence of *RAS2* may reside in the fact that rapid downregulation of stress response genes is no longer intact, as we have found. In sum, it appears that *RAS2* is important for switching between different cellular states, growth vs. response to stress.

*Gene dysregulation*

Some years ago, we proposed that yeast aging may be determined by the epigenetic inheritance of different regulatory states of chromatin (Jazwinski, 1990). It was discovered that genes in the vicinity of yeast telomeres are transcriptionally silenced (Gottschling *et al*., 1990), which provided a foundation for our hypothesis. Shortly thereafter, it was shown that telomeres shorten in normal human cells as they complete successive population doublings (Harley *et al*., 1990). This led to various proposals that this telomere shortening could alter the subtelomeric heterochromatin and the expression of genes that are buried in it (Wright and Shay, 1992). We determined that in yeast telomere shortening does not occur as a function of replicative age (D'mello and Jazwinski, 1991). We reasoned, however, that this did not preclude other changes in the telomeric heterochromatin that could affect the expression of neighboring genes during aging.

We have shown that there is a substantial loss of silencing of subtelomeric genes during yeast aging, at least at one telomere (Kim *et al*., 1996). Because no telomere shortening occurs, our result may be applicable to other cells in which telomere shortening does not occur, such as postmitotic cells. Indeed, the observation may be extended to other heterochromatic regions of the genome, resulting in gene dysregulation. The fact that these effects may be important in determining longevity comes from several observations. One observation from our laboratory shows that the transient inactivation of the Cdc7 protein leads to a reduction in the life span (Egilmez and Jazwinski, 1989). The *CDC7* gene plays a role in silencing (Axelrod and Rine, 1991), and we have found that *RAS2* modulates it (Jazwinski *et al*., 1998).

### *Nonlinear processes*

In our studies, we have found frequent examples of apparently nonlinear processes. These include the effect of *RAS* overexpression on longevity and the UV resistance profile of yeasts with age. This has suggested to us that aging is a nonlinear, dynamic process. We have generated a mathematical model of aging for such a process (Jazwinski *et al*., 1998). The model takes change as a cause rather than an effect of aging, and it places loss of homeostasis as its central feature. The model makes specific predictions at the levels of population mortality, cellular spatial organization, and gene dysregulation that have been tested. Further tests of this model are ongoing.

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