Viewpoint

New clues to old yeast

S. Michal Jazwinski *

*Department of Biochemistry and Molecular Biology,
Louisiana State University Health Sciences Center, 1901 Perdido St., Box P7-2, New Orleans,
LA 70112, USA*

Abstract

The yeast *Saccharomyces cerevisiae* has been used as an experimental model for the genetic and molecular dissecton of the aging process for the past decade. This period has seen the implication of some 30 genes in yeast aging. These genes encode a wide array of biochemical functions, suggesting the participation of multiple molecular mechanisms of aging. However, four principles appear to be at play: metabolism, stress resistance, gene dysregulation, and genetic instability. They unite the broad physiological aspects of yeast aging with those in other species. Genes and environment are not the only players; stochastic change also appears important in determining life span. This element of chance provides opportunities for an integrative approach, which is beginning to appear in yeast aging research. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Saccharomyces cerevisiae; Aging; Longevity genes; Caloric restriction; Signalling

1. Introduction

Yeast aging research has burgeoned in the last few years. The emphasis has been on the genetics, but this has resulted in the delineation of cellular and molecular mechanisms. Over 20 genes have now been implicated in yeast aging (Table 1). These genes depict a panorama of cellular biochemistry. None the less, the broad

---

* Tel./fax: + 1-504-5684725.
E-mail address: sjazwi@lsuhsc.edu (S.M. Jazwinski).

Table 1
Yeast longevity genes

Gene	Function	References
LAG1	Endoplasmic reticulum (ER) protein; GPI-anchored protein transport, sphingolipid metabolism	D'mello et al., 1994
LAC1	Homolog of LAG1; ER protein; GPI-anchored protein transport, sphingolipid metabolism	Jiang et al., 1998
RAS1	GTP-binding (G-) protein; signal transduction	Sun et al., 1994
RAS2	G-protein; signal transduction	Sun et al., 1994
CDC35	Adenylate cyclase	Sun et al., 1994; Lin et al., 2000
BCY1	Regulatory subunit of protein kinase A	Sun et al., 1994
PHB1	Mitochondrial protein; membrane-bound mitochondrial protein chaperone	Jazwinski, 1996; Coates et al., 1997; Berger and Yaffe, 1998
PHB2	Mitochondrial protein; homolog of PHB1; membrane-bound mitochondrial protein chaperone	Coates et al., 1997; Berger and Yaffe, 1998
CDC7	Protein kinase; cell cycle control	Jazwinski et al., 1989
BUD1	G-protein; cell polarity	Jazwinski et al., 1998
RTG2	Unknown; retrograde response	Kirchman et al., 1999
RTG3	Basic helix–loop–helix/leucine zipper transcription factor; retrograde response	Kirchman et al., 1999; Jiang et al., 2000
RPD3	Histone deacetylase; chromatin-dependent gene regulation	Kim et al., 1999
HDA1	Histone deacetylase; chromatin-dependent gene regulation	Kim et al., 1999
SIR2	ADP-ribosyltransferase; histone deacetylase; chromatin-dependent transcriptional silencing	Kim et al., 1999; Imai et al., 2000; Landry et al., 2000; Smith et al., 2000
SIR4	Transcriptional silencing	Kennedy et al., 1995
UTH4	Unknown	Kennedy et al., 1997
YGL023	Unknown; homolog of UTH4	Kennedy et al., 1997
SGS1	DNA helicase; DNA recombination	Sinclair et al., 1997; Heo et al., 1999
RAD52	DNA repair	Park et al., 1999
FOB1	Replication block	Defossez et al., 1999
CDC25	GDP-GTP exchange factor for RAS	Lin et al., 2000
TPK2	Protein kinase A catalytic subunit	Lin et al., 2000
GPR1	Glucose-binding protein	Lin et al., 2000
GPA2	G-protein	Lin et al., 2000
ZDS1	Transcriptional silencing	Roy and Runge, 2000
ZDS2	Transcriptional silencing	Roy and Runge, 2000
NMT1	*N*-myristoyltransferase	Ashrafi et al., 2000
SIP2	Unknown; *N*-myristoylprotein	Ashrafi et al., 2000
SNF1	AMP-activated protein kinase homolog	Ashrafi et al., 2000
SNF4	Regulatory subunit of Snf1complex	Ashrafi et al., 2000

principles underlying the aging process in yeast remain what they were 5 years ago, and include metabolism, resistance to stress, gene dysregulation, and genetic stability (Jazwinski, 1996). The first two of these are even more firmly than before entrenched as universal aspects of aging, while the latter two may soon approach this status.

This review focuses on the four principles enunciated above. In the process, it provides an analysis of the genetic and environmental factors that contribute to yeast aging. In addition, the role of chance in aging is discussed briefly. Yeast here refers to the budding yeast, *Saccharomyces cerevisiae*. Although it has recently been shown that fission yeast (*Schizosaccharomyces pombe*) display a similar aging process (Barker and Walmsley, 1999), little has as yet been done to dissect this further. The replicative life span is the subject of discourse. This is measured by the number of times individual cells divide, a finite quantity (Mortimer and Johnston, 1959; Müller et al., 1980). Chronological aging or, in other words, survival in stationary phase, has been studied much less intensely (Longo, 1999) and will not be discussed. However, it is interesting that events that occur in stationary phase affect replicative life span (Ashrafi et al., 1999 Jazwinski, 1999a).

2. Yeast age

The Gompertz equation readily describes yeast mortality (Pohley, 1987 Jazwinski et al., 1989). Interestingly, the mortality rate plateaus at later ages in yeast (Jazwinski et al., 1998), as it does in other species (Vaupel et al., 1998). Furthermore, yeasts display a wide range of age changes, some of which are decremental (Table 2). It is still too early to tell whether any of the experimental model systems are yielding information useful for the understanding of human aging. However, clues connecting yeast and human aging are beginning to appear (De Benedictis et al., 2000).

3. Metabolism and aging

It is almost a given that metabolism is an important factor in determining yeast longevity. After all, we measure the life span in this organism by counting the number of daughter cells an individual mother cell produces. This mechanism of aging received a firm footing with the discovery that an intracellular signaling pathway by which the mitochondrion communicates with the nucleus, called the retrograde response (Parikh et al., 1987), plays a role in determining life span (Kirchman et al., 1999).

The retrograde response is induced by mitochondrial dysfunction. We do not know the nature of the signal that the mitochondrion elicits to activate this response. However, it is mediated by the products of the genes *RTG1*, *RTG2*, and *RTG3* (Liao and Butow, 1993; Jia et al., 1997). Rtg1p and Rtg3p are the subunits of a heterodimeric basic helix–loop–helix/leucine-zipper transcription factor.

Table 2
Changes in yeast cells during aging

Feature	Directon of change
Cell size	Increase
Cell shape	Altered
Granular appearance	Develops
Surface wrinkles	Develop
Loss of turgor	Develops
Cell fragility (prior to death)	None
Cell lysis	Occurs
Loss of refractility	Occurs
Bud scar number	Increase
Cell wall chitin	Increase
Vacuole size	Increase
Generation (cell cycle) time	Increase
Response to pheromones (haploids)	None/decrease
Mating ability (haploids)	Decrease
Sporulation ability (diploids)	Increase
Cell cycle arrest at G1/S boundary (putative)	Occurs
Senescence factor	Appears
Mutability of mitochondrial DNA	Decrease
UV resistance	Increase followed by decrease
Resistance to methylating agents	Decrease
Telomere length	None
Random budding	Increase
Specific gene expression	Altered
rRNA levels	Increase
rDNA circles	Increase
Cellular rRNA concentration	Decrease
Protein synthesis	Decrease
Ribosome activity, polysome recruitment	Decrease
Transcriptional silencing	Decrease
Nucleolar fragmentation	Appears
Migration of silencing complexes to nucleolus	Appears

Rtg2p associates with Rtg1p–Rtg3p (Rothermel et al., 1997), and it participates in the translocation from the cytoplasm to the nucleus and activation of this transcription factor (Sekito et al., 2000). This results in changes in the expression of many nuclear genes (Liao et al., 1991; Chelstowska and Butow, 1995; Small et al., 1995; Velót et al., 1996; Chelstowska et al., 1999). The gene expression changes portend a shift from the utilization of the Krebs cycle to the glyoxylate cycle, which allows the utilization of acetate as a source of biosynthetic intermediates normally provided by the Krebs cycle. This shift conserves carbon atoms and thus represents a more efficient utilization of resources. There is also an indication that gluconeogenesis is activated and that fatty acids are used as a source of energy.

A broader picture of the changes in gene expression has been presented recently in a microarray analysis of mRNAs in yeast cells with dysfunctional mitochondria (Traven et al., 2000). The metabolic changes suggested by this study are largely

consistent with previous analyses. In addition, they show that stress response genes are activated. This is consistent with the enhanced thermal resistance of these cells (Kirchman et al., 1999; Traven et al., 2000). However, there are some concerns with this study. The important control of deletion of one of the *RTG* genes to ensure that the mRNA profiles are in fact dependent on the retrograde response is missing. Furthermore, some of the genes known to be induced by the retrograde response, including the diagnostic *CIT2*, do not display increased mRNA levels. Thus, this study awaits further verification.

The induction of the retrograde response results in the extension of life span. This occurs in all four of the different yeast strains tested. Significantly, the details of the induction of the response differ, but an increase in longevity always pertains (Kirchman et al., 1999). Furthermore, the extension of life span depends upon the *RTG2* gene, showing that in fact it is the retrograde response pathway that is responsible (Kirchman et al., 1999). The *RAS2* longevity gene (Sun et al., 1994) is required to observe the increase in life span, and it has been found to modulate the retrograde response (Kirchman et al., 1999).

The retrograde response acts continuously, much like a rheostat, in compensating for mitochondrial dysfunction with an enhancement of longevity (Jazwinski, 2000). Mitochondrial damage and dysfunction is known to accumulate with age in many organisms (Shigenaga et al., 1994). The Rtg1p–Rtg3p transcription factor plays a special role in cell metabolic control. It takes over for the Hap (heme-dependent transcriptional activator) transcription complex to regulate a variety of mitochondrial activities under stressful conditions (Liu and Butow, 1999). Thus, it is appropriate to consider the conditions under which the retrograde response is induced to be a state of metabolic duress.

It has been known for some 70 years that a reduction in food intake in rodents results in an extension of mean and maximum life span and a retardation of the appearance of aging pathologies (Masoro, 1995). The effect observed is proportional to the extent to which food intake is reduced. It is not dependent on the specific nutrient, but rather it appears to involve a decrease in calories consumed. Thus, it is called caloric restriction. The phenotypic consequences of caloric restriction include the reversal or retardation of many age changes. Recently, this description has been taken to the molecular level in a global analysis of the patterns of gene expression during aging and the effect of caloric restriction in mouse skeletal muscle (Lee et al., 1999), brain (Lee et al., 2000), and liver (Han et al., 2000).

One of the earliest changes observed on the imposition of calorie restriction is a reduction in blood glucose levels (Cartee and Dean, 1994). This and other features of calorie restriction have led to the suggestion that it depends on a change in the way glucose is metabolized (Masoro, 1995). Glucose levels can be readily manipulated in the growth medium of yeasts, and this has been used to mimic the calorie restriction effect (Jiang et al., 2000). In this study, extension of both mean and maximum life span was seen, and it was proportional to the reduction in glucose in the medium. The effect was obtained both in a modified broth and in chemically defined medium, and there was a concomitant retardation of the appearance of an

age-related phenotype, the decline in the rate at which cells budded. Life extension was also obtained when amino acids concentrations were lowered, while glucose levels were maintained. Thus, the enhancement of longevity was not dependent on a specific nutrient, and it is reasonable to propose that this intervention closely resembles caloric restriction in rodents.

The obvious question is whether there is any overlap between the retrograde response and caloric restriction in yeast, both of which are metabolic mechanisms. At the level of expression of the diagnostic gene for the retrograde response *CIT2*, they appear separate (Jiang et al., 2000). Genetic analysis also indicates that these are two separate pathways. Caloric restriction does not operate via *RTG2* or *RTG3* mediators of the retrograde response. However, there may be some interaction between the longevity effectors under the control of the two pathways, as indicated by genetic evidence. It will be of interest to determine the actual extent of the interaction by examining the global patterns of gene expression changes induced by caloric restriction in yeast. These can also be compared to those seen in rodents.

What is the significance of the multiple metabolic mechanisms of aging in yeast? The retrograde response is a compensatory pathway that adapts metabolism to mitochondrial dysfunction that can accumulate with age (Fig. 1). Caloric restriction appears to have a different role. It serves to prevent or retard the ravages of time by adjusting metabolism (Jiang et al., 2000).
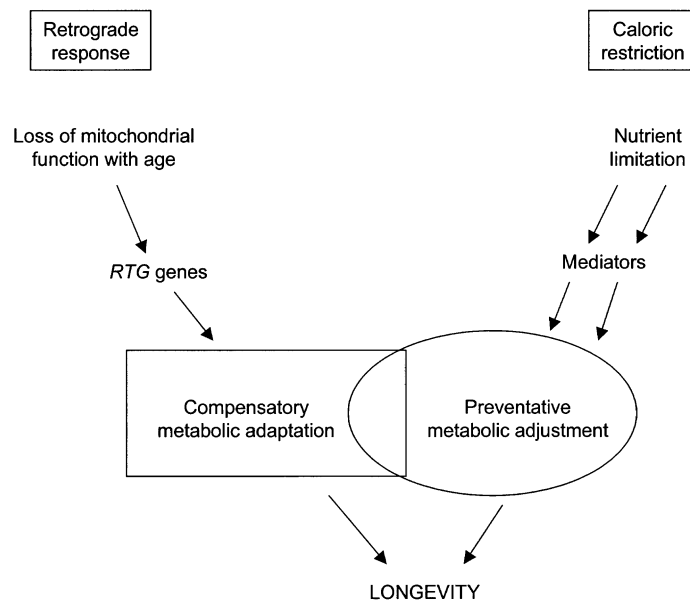

Fig. 1. Retrograde response and caloric restriction are separate pathways to extended longevity. See text for discussion. Genetic evidence points to some overlap between the longevity effectors of the retrograde response and caloric restriction.

It is noteworthy that nutrient deprivation in some yeast strains results in filamentous growth under appropriate conditions. Cells become elongated, and they bud in a unipolar fashion, providing for directional growth of filaments. This phenomenon has been considered a means by which yeasts forage for nutrients. It has been termed pseudohyphal growth in diploids (Gimeno et al., 1992) and invasive growth in haploids (Madhani and Fink, 1998). In haploid cells, filamentous growth is triggered by glucose deprivation (Cullen and Sprague, 2000), while in diploids, the growth is triggered by nitrogen source limitation (Madhani and Fink, 1998). Some of the similarities between filamentous growth and the yeast replicative life span were pointed out earlier, especially the role of *RAS2* in both phenomena (Jazwinski, 1993). In either case, it appears that the issue is the number of divisions before growth ceases. In the caloric restriction study (Jiang et al., 2000), no note of any frank filamentation was made.

In another study, it has been claimed that the NAD-dependent Sir2 histone deacetylase is required for caloric restriction to exert its life-extending effect in yeast (Lin et al., 2000). However, this study did not examine the effect of *SIR2* on life extension obtained through nutritional manipulation. Instead, it showed that deletion of *SIR2* suppresses the life extension obtained through disruption of the Ras-cAMP pathway, which was already known to curtail longevity (Sun et al., 1994). It is unlikely that the postulated depression of NAD levels by the Ras-cAMP pathway and the link this might provide between metabolism and aging through the Sir2 histone deacetylase (Lin et al., 2000) is a normal mechanism of aging, because NAD levels increase with age in yeast (Ashrafi et al., 2000). Furthermore, extension of life span by caloric restriction does not require *SIR2* (J.C. Jiang and S.M. Jazwinski, unpublished). The data summarized above are not contradictory; however, the conclusions are.

Recently, a further connection between nutrient utilization and aging has been adduced in yeast. Hyperactivation of the Snf1 protein kinase was shown to curtail life span (Ashrafi et al., 2000). Snf1p is necessary for the induction of glucose-repressed genes upon glucose deprivation. This protein is the homolog of the mammalian AMP-activated protein kinase, which functions as a fuel gauge. This result suggests to me that inappropriate activation of certain pathways involved in the response to glucose deprivation is harmful. It also prompts the notion that more than one pathway may be activated by caloric restriction, one of which could be the activation of Snf1p (Ashrafi et al., 2000) and another Gpr1p/Gpa2p signaling (Lin et al., 2000).

4. Resistance to stress

The secondary phenotype of resistance to cold and starvation was used to isolate mutants exhibiting an extended life span (Kennedy et al., 1995). These mutants were stated to possess resistance to a variety of other stressors. This would suggest that stress resistance goes hand in hand with a longer life span. However, no cause and effect relationship has been demonstrated to date. One of the mutants possesses

a semidominant mutation in the *SIR4* gene, which relieves transcriptional silencing at the heterochromatic mating-type locus and subtelomeric regions of the genome. The former are known to result in sterility, while the latter result in an increased resistance to stresses. The association of the stress resistance with longevity is confused by the fact that loss of silencing at subtelomeric regions is a consequence of normal aging in yeast (Kim et al., 1996). It is possible that either the relevant effects of *SIR4* go beyond action at the telomeres, or that the increased stress resistance is counterbalanced by other deleterious events dictated by the loss of silencing.

Although the case for enhanced stress resistance as a means for increasing life span is far from conclusive, it is clear that abrogation of stress responses has a negative role. Deficiencies in either the Cu, Zn- or the Mn-superoxide dismutases (*SOD1* and *SOD2*) decrease yeast life span (Barker et al., 1999; Wawryn et al., 1999). The resistance to ultraviolet radiation (UV) increases until mid-life and then declines precipitously (Kale and Jazwinski, 1996). This profile parallels the pattern of expression of *RAS2* (Sun et al., 1994; Kale and Jazwinski, 1996), which is required for the response to UV (Engelberg et al., 1994). This response is not mediated by DNA damage per se. Thus, the fact that the profile of resistance to other DNA damaging agents shows a monotonic decline with age is not surprising (Kale and Jazwinski, 1996). There may be some relationship between the response to oxidative stress and to UV, because the latter can give rise to oxidative damage.

The role of thermotolerance in determining yeast life span is complicated. A bout of sublethal heat stress has no impact on longevity. However, chronic bouts of such thermal stress markedly curtail longevity (Shama et al., 1998a). Paradoxically, deletion of *RAS2*, but not *RAS1*, exacerbates this effect. The paradox derives from the fact that the Ras-cAMP pathway downregulates expression of genes under the control of the STRE (stress response regulatory element) promoter element, which activates transcription in response to a variety of stress conditions (Marchler et al., 1993). The Ras-cAMP pathway actually curtails longevity in the absence of overt stress (Sun et al., 1994), but this effect is apparently not mediated through the STRE (Lin et al., 2000). The responses to heat and other stresses are related (Wieser et al., 1991; Sanchez et al., 1992; Davidson et al., 1996).

The dilemma posed above is resolved on examination of the molecular effects of heat stress. Exposure to elevated temperatures results in a rapid induction of stress response genes and downregulation of genes involved in growth and cell division. These changes in gene expression persist for long periods, regardless of whether or not the cells remain at the elevated temperature in the absence of *RAS2* (Shama et al., 1998a). Thus, repeated bouts of stress result in a chronic state of stress response, which appears to be deleterious. Overexpression of *RAS2* rescues the yeasts from this condition. In fact, it results in an increase in longevity beyond what is seen in cells unexposed to the heat stress to begin with (Shama et al., 1998a). At least part of the effect of *RAS2* appears to lie in the reversal of the chronic state of stress through downregulation of stress genes and upregulation of growth and cell division genes. This follows from the fact that the $ras2^{ser42}$ (Kim et al., 1996) mutant, which is deficient in activation of adenylate cyclase, is not capable of this

effect and that the Ras-cAMP pathway is known to downregulate gene expression from the STRE. How does this fit with the result that the Ras-cAMP pathway curtails life span, presented earlier? This is addressed below.

The induction of thermal tolerance can have beneficial effects, even under conditions in which there is no overt stress (Shama et al., 1998b). Transient, sublethal heat shock delivered early in the life span increases longevity. This is due to a reduction in mortality rate that is persistent over several generations, but is not permanent. Not surprisingly, the life extension depends on *HSP104*, which is required for thermotolerance in yeast (Sanchez and Lindquist, 1990). Active mitochondria are necessary for this life extension, perhaps due to the requirement of mitochondrial function for resistance to oxidative stress (Grant et al., 1997), which is involved in heat-induced cell death (Davidson et al., 1996). The life extension also requires both *RAS1* and *RAS2*. The requirement for the latter comes as no surprise, given its role in downregulation of the stress response. However, the role of *RAS1* is puzzling. After all, *RAS1* is largely redundant to *RAS2* and performs less robustly in its place.

The key to the involvement of *RAS1*, which, under normal conditions, curtails longevity (Sun et al., 1994), may reside in the participation of *RAS1* in glucose-stimulated inositiol phospholipid turnover in yeast (Kaibuchi et al., 1986). Sphingolipids contain inositol in yeast, and they are required for resistance to a variety of stresses, including heat (Patton et al., 1992; Jenkins et al., 1997). The transient heat stress may cause a Ras1p-dependent, long-lived change in sphingolipid pools. Ceramide, which is a component of sphingolipids in eukaryotes, plays an important role in cell growth, differentiation, apoptosis, and senescence (Mathias et al., 1998).

In this context, it is interesting to note that *LAG1*, the first yeast longevity gene cloned (D'mello et al., 1994), appears to play a role in sphingolipid metabolism (Brandwagt et al., 2000). Yeast *LAG1* has homologs in higher eukaryotes, including humans (Jiang et al., 1998), and it has been implicated in the transport of glycosylinositolphospholipid-anchored proteins from the endoplasmic reticulum to the Golgi (Barz and Walter, 1999). This effect may be secondary to its involvement in sphingolipid metabolism (Brandwagt et al., 2000). Lag1p is most likely a component of ceramide synthase, although some seem to shy away from this conclusion (Brandwagt et al., 2000).

In the absence of overt stress, overexpression of *RAS2* extends life span, while its deletion shortens it (Sun et al., 1994). How do we reconcile this with its effects on stress responses? Yeast aging is a non-linear process (Jazwinski, 1999b). The organism is not the same throughout its life span. It is certainly not the same in the presence and absence of stress, even at the molecular level, as already discussed. Therefore, it should not be surprising that Ras2p has different effects on longevity depending on environmental conditions and on whether or not growth and cell division are feasible (Fig. 2). These choices represent trade-offs, and they establish *RAS2* as a homeostatic device in yeast longevity (Jazwinski, 1999a). In this capacity, Ras2p does not participate in pathways that determine life span, but instead, it modulates them. This allows it to affect many cellular processes, in effect channeling resources as and where they are needed.
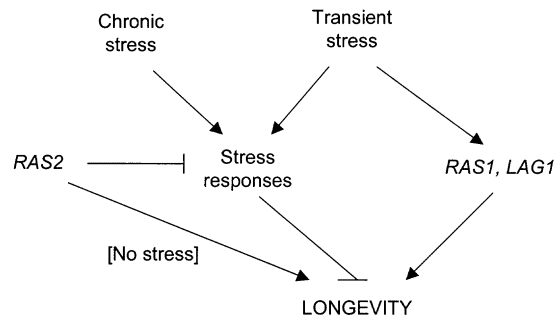
Fig. 2. *RAS* genes have differential effects on longevity depending on environmental conditions. The life span-shortening effect of *RAS1* in the absence of overt stress is not depicted. *LAG1* is proposed to affect longevity through its role in ceramide synthesis. For further details, see text.

5. Gene dysregulation

The epigenetic inheritance of different regulatory states of chromatin was recognized as a potential factor that determines a yeast cell's life span some time ago (Jazwinski, 1990). It was later shown that there is a loss of transcriptional silencing in subtelomeric chromatin (Kim et al., 1996) and at the silent mating-type loci (Smeal et al., 1996). These epigenetic changes provide the potential for involvement of unscheduled transcription and the gene dysregulation it pulls in tow as a contributor to the aging process. However, there are many genes whose expression is affected, making it difficult to identify factors that are proximal to life span determination. Indeed, it may not be important which individual genes are affected. Instead, any of a multitude of combinations may be culpable, providing a large number of routes to senescence. The changes in gene expression may feed back to cause a vast array of secondary effects, and this could give rise to the diversity of the aging phenotype from one individual to the next.

The analytical difficulties described above have prompted a top-down approach to the problem, starting with genes high up in the regulatory hierarchy. The histone deacetylases Rpd3 and Hda1 play a role in the maintenance of equilibrium between silent and transcriptionally active chromatin. Both of these genes have been shown to play a role in determining life span (Kim et al., 1999). The activity of these deacetylases regulates silencing at all three of the known heterochromatic regions of the yeast genome, the silent mating-type loci, subtelomeric genes, and the ribosomal DNA cluster (rDNA) (Rundlett et al., 1996; Kim et al., 1999; Smith et al., 1999). The life span-extending effects of deletions in these genes correspond most closely to their effects on the rDNA locus (Kim et al., 1999). Sir2p has opposing effects to Rpd3p and Hda1p, suggesting that all three genes act in tandem to maintain equilibrium between active and silent chromatin (Kim et al., 1999) (Fig. 3). The mechanism of action of Sir2p as a NAD-dependent histone deacetylase clarifies its role further (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). The conclusion that the effects of Rpd3p and Hda1p on silencing are not due to an effect on the transcription of the Sir proteins, but rather, the result of the histone

acetylation pattern (Kim et al., 1999) has received experimental support recently (Bernstein et al., 2000).

Three other genes are known to affect transcriptional silencing and longevity in yeast. *RAS2* is required to maintain silencing at subtelomeric loci (Jazwinski et al., 1998), another example of its role as a homeostatic device in yeast longevity. Deletion of *ZDS1* results in an increase in silencing at rDNA and silent mating-type loci at the expense of telomeres with a concomitant increase in life span (Roy and Runge, 2000). Deletion of *ZDS2*, in contrast, decreases rDNA silencing and life span. The molecular mechanism underlying these effects is not known.

It has been proposed that the retrograde response and histone deacetylases act upon the rDNA genes, influencing yeast life span through their effects on protein metabolism (Kim et al., 1999). Protein synthesis declines with age in yeast (Motizuki and Tsurugi, 1992). This decline occurs at the same time as an increase in cellular rRNA content. It is likely that this may not be coupled to a coordinate increase in the proteins of the translation apparatus, resulting in an imbalance in the assembly of active ribosomes. Deletion of *RPD3* or *HDA1* increases silencing of rDNA, which could restore the balance. Deletion of *SIR2* has the opposite effect. The retrograde response increases synthesis of rRNA (Conrad-Webb and Butow, 1995). It is proposed that it also augments the levels of ribosomal protein and translation factors. Unfortunately, it is not clear at this time whether or not this is the case, given the discrepancies in the microarray analysis of the retrograde response noted earlier. In any case, protein metabolism dysfunction may constitute yet another of the multiple metabolic mechanisms of aging.

6. Genetic instability

The argument has been forcefully made that mutation is not the cause of aging and death of a yeast mother cell (Mortimer and Johnston, 1959). In fact, there is
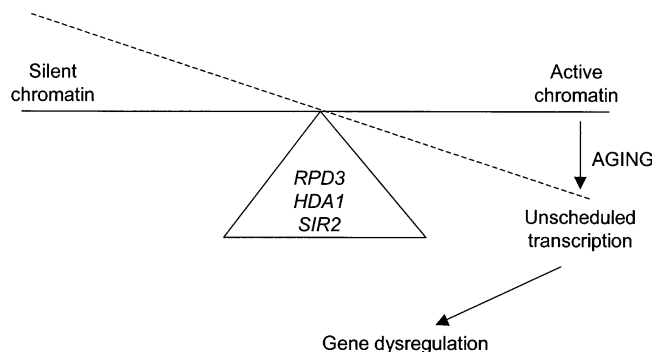

Fig. 3. Histone deacetylases maintain equilibrium between silent and transcriptionally active chromatin. Disruption of this balance leads to gene dysregulation, which may be a cause of aging. *RPD3*, *HDA1*, and *SIR2* play a role in determining yeast life span. See text for more information.

no accumulation of mutations in the mother cell during aging (Müller and Wolf, 1978). In some strains, petite mutants (missing part or all of the mitochondrial genome) accrue during the life span, but this induces the retrograde response and extends life span (Kirchman et al., 1999).

However, there is evidence that nuclear DNA is subject to rearrangements that impact longevity. Tandem rDNA repeats in yeast, which are present as an array of 100–200 copies, are known to exist in equilibrium with extrachromosomal circular species of various sizes. These extrachromosomal rDNA circles (ERCs) are maintained at various copy numbers thanks to the presence of an origin of replication. It has been shown that ERCs are present at much higher levels in old cells (Sinclair and Guarente, 1998). Their accumulation in such high numbers kills the cells. Deletion of the *FOB1* gene prevents this (Defossez et al., 1999), arguing for a role of genetic instability in yeast aging. This argument depends on the assumption that *FOB1* affects ERC production alone. Unfortunately, this is very difficult to prove, a conundrum that frequently underlies evidence for causality in aging research. Nevertheless, the case for the role of genetic instability in aging is tempting.

The finding of ERC increase during yeast aging has important implications for mechanisms of human aging. The *WRN* gene, which, when mutated, results in a human premature aging syndrome, is a DNA helicase homologous to the yeast *SGS1* gene (Yu et al., 1996). Deletion of *SGS1* curtails yeast life span, and this has been called premature aging in yeast, due to the appearance of sterility and nucleolar fragmentation (Sinclair et al., 1997). Contrary to expectation (Sinclair and Guarente, 1998), deletion of *SGS1* does not cause ERC production (Heo et al., 1999). The deficits associated with this deletion, including life-span shortening, are not rescued by *WRN*, but they are by *BLM*, a different human DNA helicase gene (Heo et al., 1999). This raises questions regarding the nature of premature aging in yeast. All of the postulated phenotypes associated with it, including sterility, nucleolar fragmentation, and accumulation of ERCs, coincide with the migration of silencing complexes to the nucleolus (Kennedy et al., 1997). It is difficult to extend this direct, unitary phenotype as a marker for the global and varied changes associated with the aging process.

Mitochondrial dysfunction can trigger the retrograde response causing an increase in life span, as discussed above. It also results in the appearance of large numbers of ERCs in the cell (Conrad-Webb and Butow, 1995). The appearance and exponential accumulation of these ERCs during aging are dependent on the retrograde response (A. Benguria and S.M. Jazwinski, unpublished). Thus, the retrograde response predominates, causing increased longevity in the face of ERC production. ERCs are therefore not sufficient to cause aging. They are also not necessary (Heo et al., 1999; Kim et al., 1999). It is possible that the effects of ERCs on the cell are only manifest under particular conditions. Another possibility is that they are not the cause of aging per se, but only the symptom or the proximal cause of demise.

7. Individual change and aging

The discussion thus far has dealt with genetic and environmental factors that affect yeast aging. Could there be anything more? In a comparatively simple situation, the molecules of gas in a container possess a kinetic energy, which, averaged over their entire collection, can be read out as the temperature. However, individual molecules can vary in kinetic energy. Furthermore, their distribution throughout the container can vary over time, although, on average, it is uniform. Thus, the molecules are subject to random fluctuations or changes, even though the properties of the gas are deterministic. This description goes beyond the inherent characteristics of the gas and the forces to which it is subjected. It speaks to the element of chance. In the same sense, we must add a stochastic element to the genetic and environmental factors that contribute to aging.

Yeasts display a broad array of changes as they proceed through their replicative life spans (Table 2). No two cells are identical in the way they present. Here, we have a stochastic element. It has been postulated that stochastic change is the cause of aging and not simply an effect (Jazwinski et al., 1998). This single assumption has been used to construct a mathematical model, which has the form of an exponential difference equation (Jazwinski et al., 1998). This model describes how an aging population becomes stratified epigenetically, due to random change at the level of the individual. (The model is applicable to all levels of biological organization, including the molecular, cellular, organismal, and population; therefore, the change that enters into it can occur at all of these levels.) Several predictions of this model at the molecular, cellular, and organismal level have been tested.

The epigenetic stratification of the aging population explains why individuals die at different ages, even when they are genetically identical and they are maintained under the same conditions. It also explains why, under the same circumstances, there are multiple and independent limiting factors for longevity. It is interesting to note that the variance in life span of isogenic individuals in a constant environment is itself under genetic control (de Haan et al., 1998), which is also consistent with the model. The difference equation, which constitutes the model, describes a non-linear dynamic system. Several lines of evidence indicate that yeast aging is such a system (Jazwinski, 1999b). This is not surprising because biological systems tend to be non-linear, because of multiple interactions and feedback loops (Fig. 4). This suggests that the aging system as a whole will have properties that constitute more than a simple sum of the parts.

It will be essential to develop methodologies that take into account the complexity of the aging process. The global analysis of the patterns of gene expression during aging presents an opportunity in this direction. Perturbations of the aging system can be analyzed in the context of all of the mutual interactions that the system supports, providing an understanding of the trajectories involved in the aging process. This is particularly important, because individual or isolated patterns may not be causal. It should not be construed, however, that the reductionist approach is to be discarded, because it identifies mechanistic details that can form the bases for interventions.
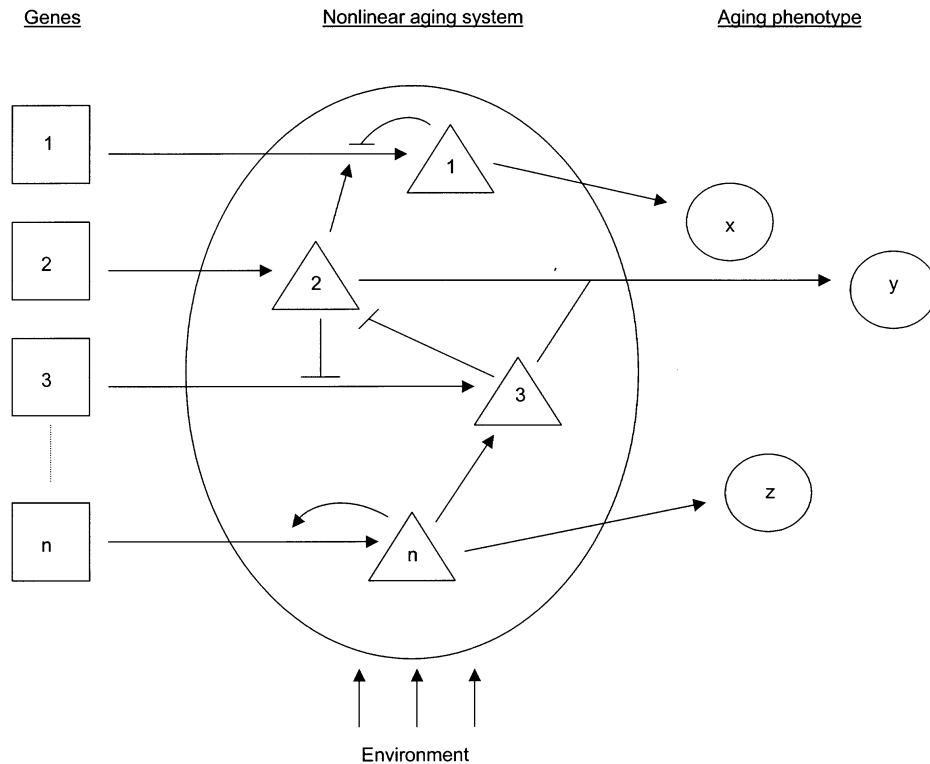
Fig. 4. Aging occurs in biological systems that display non-linear dynamics. Multiple interactions and feedback loops are indicated schematically. The system displayed here is oversimplified for clarity. Time is an additional dimension.

Can an integrative approach to aging work? A mathematical model was generated of the profiles of random change in budding pattern of individual yeast cells during aging (Jazwinski and Wawryn, submitted for publication). This model accurately categorizes the longevity of individual cells. More importantly, it makes counterintuitive predictions regarding mechanisms of aging, which have been verified. This analysis points to specific molecular mechanisms that can be explored further. The general methodology described (Jazwinski and Wawryn, submitted for publication) can be applied to other systems to extract information from profiles of random change during aging. This example prompts the optimism that integrative approaches can be coupled to genetics to help elucidate, and intervene in, the aging process.

Acknowledgements

References

Ashrafi, K., et al., 1999. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96, 9100–9105.

Ashrafi, K., et al., 2000. Sip2p and its partner Snf1p kinase affect aging in *S. cerevisiae*. Genes Dev. 14, 1872–1885.

Barker, M.G., et al., 1999. Effect of Cu,Zn superoxide dismutase disruption mutation on replicative senescence in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 177, 199–204.

Barker, M.G., Walmsley, R.M., 1999. Replicative ageing in the fission yeast *Schizosaccharomyces pombe*. Yeast 15, 1511–1518.

Barz, W.P., Walter, P., 1999. Two endoplasmic reticulum (ER) membrane proteins that facilitate ER-to-Golgi transport of glycosylphosphatidylinositol-anchored proteins. Mol. Biol. Cell 10, 1043–1059.

Berger, K.H., Yaffe, M.P., 1998. Prohibitin family members interact genetically with mitochondrial inheritance components in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18, 4043–4052.

Bernstein, B.E., et al., 2000. Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA 97, 13708–13713.

Brandwagt, B.F., et al., 2000. A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *Lycopersici* toxins and fuminosin B1. Proc. Natl. Acad. Sci. USA 97, 4961–4966.

Cartee, G.D., Dean, D.J., 1994. Glucose transport with brief dietary restriction: heterogeneous response in muscles. Am. J. Physiol. 266, E946–E952.

Chelstowska, A., Butow, R.A., 1995. 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. 270, 18141–18146.

Chelstowska, A., et al., 1999. Signalling between mitochondria and the nucleus regulates the expression of a new D-lactate dehydrogenase activity in yeast. Yeast 15, 1377–1391.

Coates, P.J., et al., 1997. The prohibitin family of mitochondrial proteins regulate replicative lifespan. Curr. Biol. 7, 607–610.

Conrad-Webb, H., Butow, R.A., 1995. A polymerase switch in the synthesis of rRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15, 2420–2428.

Cullen, P.J., Sprague, G.F., 2000. Glucose depletion causes haploid invasive growth in yeast. Proc. Natl. Acad. Sci. USA 97, 13619–13624.

D'mello, N.P., et al., 1994. Cloning and characterization of *LAG1*, a longevity-assurance gene in yeast. J. Biol. Chem. 269, 15451–15459.

Davidson, J.F., et al., 1996. Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 93, 5116–5121.

De Benedictis, G., et al., 2000. Does a retrograde response in human aging and longevity exist? Exp. Gerontol. 35, 795–801.

Defossez, P.A., et al., 1999. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell. 3, 447–455.

de Haan, G., et al., 1998. A putative gene causes variability in lifespan among genotypically identical mice. Nat. Genet. 19, 114–116.

Engelberg, D., et al., 1994. The UV response involving the Ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. Cell 77, 381–390.

Gimeno, C.J., et al., 1992. Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell 68, 1077–1090.

Grant, C.M., et al., 1997. Mitochondrial function is required for resistance to oxidative stress in the yeast *Saccharomyces cerevisiae*. FEBS Lett. 410, 219–222.

Han, E.-S., et al., 2000. cDNA expression arrays reveal incomplete reversal of age-related changes in gene expression by calorie restriction. Mech. Ageing Dev. 115–174.

Heo, S.J., et al., 1999. Bloom's syndrome gene suppresses premature ageing caused by Sgs1 deficiency in yeast. Genes Cells 4, 619–625.

Imai, S., et al., 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795–800.

Jazwinski, S.M., et al., 1989. Replication control and cellular life span. Exp. Gerontol. 24, 423–436.

Jazwinski, S.M., et al., 1998. Epigenetic stratification: the role of individual change in the biological aging process. Exp. Gerontol. 33, 571–580.

Jazwinski, S.M. and Wawryn, J., submitted for publication. Profiles of random change during aging contain hidden information about longevity and the aging process.

Jazwinski, S.M., 1990. Aging and senescence of the budding yeast *Saccharomyces cerevisiae*. Mol. Microbiol. 4, 337–343.

Jazwinski, S.M., 1993. The genetics of aging in the yeast *Saccharomyces cerevisiae*. Genetica 91, 35–51.

Jazwinski, S.M., 1996. Longevity, genes, and aging. Science 273, 54–59.

Jazwinski, S.M., 1999a. The RAS genes: a homeostatic device in *Saccharomyces cerevisiae* longevity. Neurobiol. Aging 20, 471–478.

Jazwinski, S.M., 1999b. Nonlinearity of the aging process revealed in studies with yeast. In: Bohr, V.A., Clark, B.F.C., Stevnsner, T. (Eds.), Molecular Biology of Aging. Munksgaard, Copenhagen, pp. 35–44.

Jazwinski, S.M., 2000. Metabolic control and ageing. Trends Genet. 16, 506–511.

Jenkins, G.M., et al., 1997. Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*. J. Biol. Chem. 272, 32566–32572.

Jia, Y., et al., 1997. A basic helix–loop–helix–leucine zipper transcription complex in yeast functions in a signaling pathway from mitochondria to the nucleus. Mol. Cell. Biol. 17, 1110–1117.

Jiang, J.C. et al., 2000. An intervention resembling caloric restriction prolongs life span and retards aging in yeast, *FASEB J*. 10.1096/fj.00-242fje

Jiang, J.C., et al., 1998. Homologs of the yeast longevity gene *LAG1* in *C. elegans* and human. Genome Res. 8, 1259–1272.

Kaibuchi, K., et al., 1986. Possible involvement of RAS-encoded proteins in glucose-induced inositolphospholipid turnover in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 83, 8172–8176.

Kale, S.P., Jazwinski, S.M., 1996. Differential response to UV stress and DNA damage during the yeast replicative life span. Dev. Genet. 18, 154–160.

Kennedy, B.K., et al., 1995. Mutation in the silencing gene *SIR4* can delay aging in *S. cerevisiae*. Cell 80, 485–496.

Kennedy, B.K., et al., 1997. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. Cell 89, 381–391.

Kim, S., et al., 1996. Effect of replicative age on transcriptional silencing near telomeres in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 219, 370–376.

Kim, S., et al., 1999. Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell 10, 3125–3136.

Kirchman, P.A., et al., 1999. Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. Genetics 152, 179–190.

Landry, J., et al., 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA 97, 5807–5811.

Lee, C.-K., et al., 1999. Gene expression profile of aging and its retardation by caloric restriction. Science 285, 1390–1393.

Lee, C.-K., et al., 2000. Gene-expression profile of the ageing brain in mice. Nat. Genet. 25, 294–297.

Liao, X., Butow, R.A., 1993. RTG1 and RTG2: two yeast genes required for a novel path of communication from mitochondria to the nucleus. Cell 72, 61–71.

Liao, X., et al., 1991. Intramitochondrial functions regulate nonmitochondrial citrate synthase (CIT2) expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 11, 38–46.

Lin, S.J., et al., 2000. Requirement of NAD and *SIR2* for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science 289, 2126–2128.

Liu, Z., Butow, R.A., 1999. A transcriptional switch in the expression of yeast tricarboxylic acid cycle genes in response to a reduction or loss of respiratory function. Mol. Cell. Biol. 19, 6720–6728.

Longo, V.D., 1999. Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. Neurobiol. Aging 20, 479–486.

Madhani, H.D., Fink, G.R., 1998. The control of filamentous differentiation and virulence in fungi. Trends Cell Biol. 8, 348–353.

Marchler, G., et al., 1993. A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions. EMBO J. 12, 1997–2003.

Masoro, E.J., 1995. Dietary restriction. Exp. Gerontol. 30, 291–298.

Mathias, S., et al., 1998. Signal transduction of stress via ceramide. Biochem. J. 335, 465–480.

Mortimer, R.K., Johnston, J.R., 1959. Life span of individual yeast cells. Nature 183, 1751–1752.

Motizuki, M., Tsurugi, K., 1992. The effect of aging on protein synthesis in the yeast *Saccharomyces cerevisiae*. Mech. Ageing Dev. 64, 235–245.

Müller, I., et al., 1980. Calendar life span versus budding life span of *Saccharomyces cerevisiae*. Mech. Ageing Dev. 12, 47–52.

Müller, I., Wolf, F., 1978. A correlation between shortened life span and UV-sensitivity in some strains of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 160, 231–234.

Parikh, V.S., et al., 1987. The mitochondrial genotype can influence nuclear gene expression in yeast. Science 235, 576–580.

Park, P.U., et al., 1999. Effects of mutations in DNA repair genes on formation of ribosomal DNA circles and life span in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 3848–3856.

Patton, J.L., et al., 1992. Phenotypes of sphingolipid-dependent strains of *Saccharomyces cerevisiae*. J. Bacteriol. 174, 7180–7184.

Pohley, H.-J., 1987. A formal mortality analysis for populations of unicellular organisms (*Saccharomyces cerevisiae*). Mech. Ageing Dev. 38, 231–243.

Rothermel, B., et al., 1997. Rtg3p, a basic helix–loop–helix/leucine zipper protein that functions in mitochondrial-induced changes in gene expression, contains independent activation domains. J. Biol. Chem. 272, 19801–19807.

Roy, N., Runge, K.W., 2000. Two paralogs involved in transcriptional silencing that antagonistically control yeast life span. Curr. Biol. 10, 111–114.

Rundlett, S.E., et al., 1996. HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. Proc. Natl. Acad. Sci. USA 93, 14503–14508.

Sanchez, Y., et al., 1992. HSP104 is required for tolerance to many forms of stress. EMBO J. 11, 2357–2364.

Sanchez, Y., Lindquist, S.L., 1990. HSP104 required for induced thermotolerance. Science 248, 1112–1115.

Sekito, T., et al., 2000. Mitochondria-to-nuclear signaling is regulated by the subcellular localization of the transcription factors Rtg1p and Rtg3p. Mol. Biol. Cell 11, 2103–2115.

Shama, S., et al., 1998a. Role of *RAS2* in recovery from chronic stress: effect on yeast life span. Exp. Cell Res. 245, 368–378.

Shama, S., et al., 1998b. Heat stress-induced life span extension in yeast. Exp. Cell Res. 245, 379–388.

Shigenaga, M.K., et al., 1994. Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. USA 91, 10771–10778.

Sinclair, D.A., et al., 1997. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. Science 277, 1313–1316.

Sinclair, D.A., Guarente, L., 1998. Molecular mechanisms of aging. Trends Biochem. Sci. 23, 131–134.

Small, W.C., et al., 1995. Enzymatic and metabolic studies on retrograde regulation mutants of yeast. Biochemistry 34, 5569–5576.

Smeal, T., et al., 1996. Loss of transcriptional silencing causes sterility in old mother cells of *S. cerevisiae*. Cell 84, 633–642.

Smith, J.S., et al., 1999. A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. Mol. Cell. Biol. 19, 3184–3197.

Smith, J.S., et al., 2000. A phylogenetically conserved NAD$^+$-dependent protein deacetylase activity in the Sir2 protein family. Proc. Natl. Acad. Sci. USA 97, 6658–6663.

Sun, J., et al., 1994. Divergent roles of RAS1 and RAS2 in yeast longevity. J. Biol. Chem. 269, 18638–18645.

Traven, A. et al. (2000) Inter-organellar communication: altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. J. Biol. Chem. 276, 4020–4027.

Vaupel, J.W., et al., 1998. Biodemographic trajectories of longevity. Science 280, 855–860.

Velót, C., et al., 1996. The *Saccharomyces cerevisiae RTG2* gene is a regulator of aconitase expression under catabolite repression conditions. Genetics 144, 893–903.

Wawryn, J., et al., 1999. Deficiency in superoxide dismutases shortens life span of yeast cells. Acta Biochim. Polon. 46, 249–253.

Wieser, R., et al., 1991. Heat shock factor-independent heat control of transcription of the *CTT1* gene encoding the cytosolic catalase T of *Saccharomyces cerevisiae*. J. Biol. Chem. 266, 12406–12411.

Yu, C.E., et al., 1996. Positional cloning of the Werner's syndrome gene. Science 272, 258–262.