Metabolic control and ageing

There appear to be multiple processes that are limiting for longevity and the associated mechanisms of ageing. Among these processes, metabolic control is coming to the forefront, because it has surfaced in studies in several model systems and because of its relevance to mammalian ageing. The genetic and molecular dissection of ageing in yeast points to mechanisms involving three aspects of metabolism. First, dysfunctional mitochondria signal many changes in nuclear gene expression that result in metabolic adjustments that extend life span. Second, manipulation of nutritional status can also increase longevity in a separate caloric-restriction pathway. Finally, protein synthesis is a third aspect, which depends on the transcriptional state of chromatin and the histone deacetylases that modulate it.
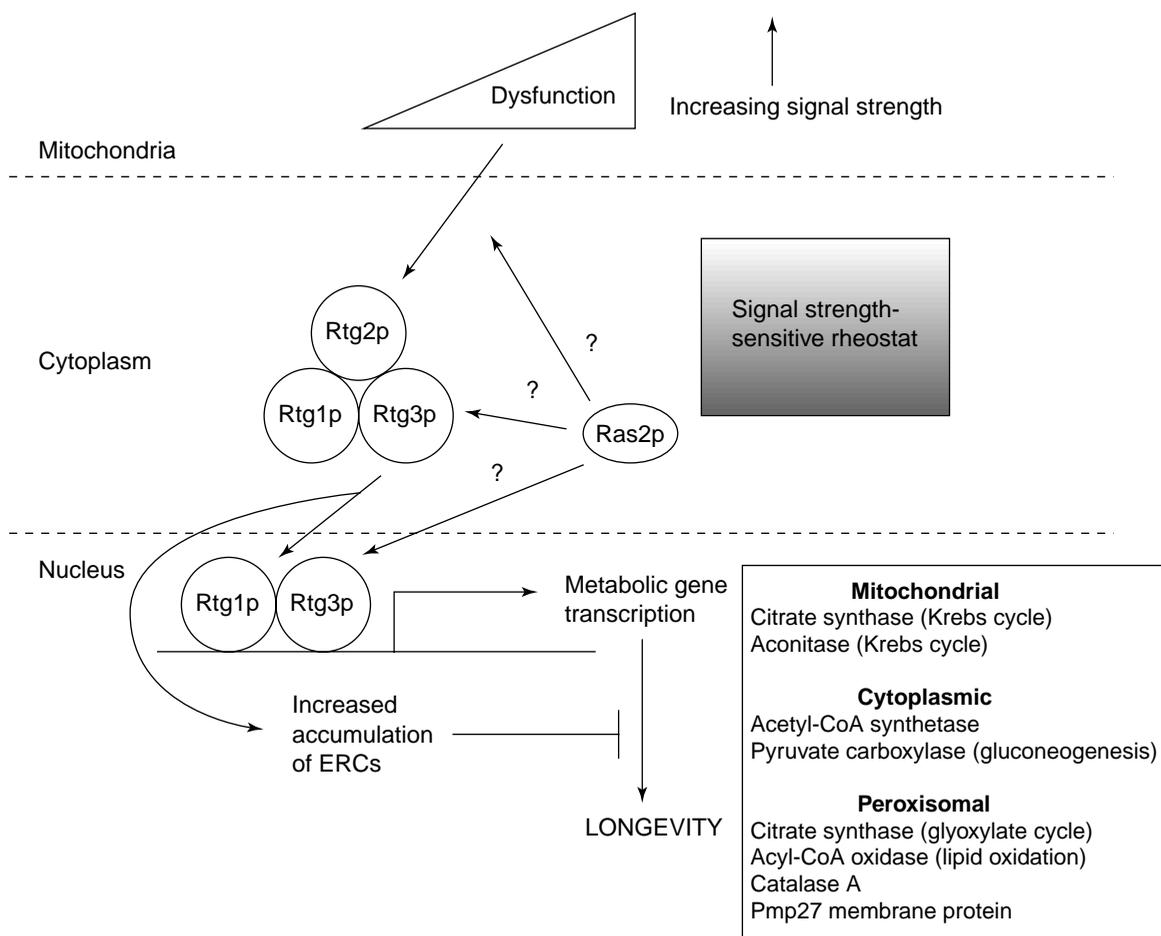
S. Michal Jazwinski
sjazwi@lsuhsc.edu

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

Studies with the yeast *Saccharomyces cerevisiae* have suggested that metabolic control and its dysfunction is an important mechanism of ageing in this and other organisms. Yeast cells are mortal, as individual cells have a limited capacity for division[1]. The probability that a yeast cell will undergo a division decreases exponentially as a function of the number of divisions it has already completed; consequently, the mortality rate increases exponentially as a function of age[2,3] until old age, when a plateau occurs in the mortality rate[4,5]. The measure of the yeast life span is the number of divisions completed by a cell, not calendar time, because the chronological life span can be extended, for example by lowering the temperature, but the total number of cell divisions remains the same[6]. The difference between yeast and other organisms in the measure of longevity need not imply a difference in the mechanisms of the biological ageing process. In addition to reduced replicative capacity, there are a variety of age-related changes, morphological and physiological, suffered by this organism, some of which are clearly decremental[7], such as a decline in budding rate and loss of fertility. However, it is difficult to determine the final cause of yeast death. Its ultimate result is the loss of refractility and lysis.

The genetic dissection of the ageing process in yeast during the past dozen years has implicated 19 genes that determine life span (Table 1). These genes encode a variety of biochemical functions, suggesting the existence of multiple pathways and processes that impinge on ageing. Four broad physiological processes important for longevity have emerged: metabolic control, resistance to stress, gene dysregulation, and genetic stability. The possible significance of these processes in unifying the mechanisms of ageing across phyla was pointed out some time ago[8].

How is it that a genetically homogeneous yeast strain can display multiple ageing mechanisms? This question is related to the dilemma posed by the varied life spans of isogenic individuals kept under constant conditions, or, in other words, the fact that these individuals do not die all at once. This is a real problem because it indicates that more than one factor is limiting for longevity, simultaneously as it were. The resolution of this quandary lies in the realization that stochastic change leads to the epigenetic stratification of an ageing population, such that its individuals differ physiologically[4,5]. Thus, chance is an important ingredient in ageing, in addition to the genes and the environment. Several recent general reviews of yeast ageing are available[9,10]. This article focuses on metabolism and ageing.

TABLE 1. Longevity genes in yeast[a]

Gene	Function	Refs
LAG1	Endoplasmic reticulum (ER) protein; GPI-anchored protein transport	41
LAC1	Homolog of *LAG1*; ER protein; GPI-anchored protein transport	42
RAS1	GTP-binding (G-) protein; signal transduction	21
RAS2	G-protein; signal transduction	21
PHB1	Mitochondrial protein; regulates mitochondrial AAA protease	8,43,44
PHB2	Mitochondrial protein; homolog of *PHB1*; regulates mitochondrial AAA protease	43,44
CDC7	Protein kinase; cell cycle control	3
BUD1	G-protein; cell polarity	4
RTG2	Unknown; retrograde response	20
RTG3	Basic helix-loop-helix-leucine zipper transcription factor; retrograde response	20,37
RPD3	Histone deacetylase; chromatin-dependent gene regulation	28
HDA1	Histone deacetylase; chromatin-dependent gene regulation	28
SIR2	ADP-ribosyltransferase; histone deacetylase; chromatin-dependent transcriptional silencing	28,29
SIR4	Transcriptional silencing	45
UTH4	Unknown	33
YGL023	Unknown; homolog of *UTH4*	33
SGS1	DNA helicase; DNA recombination	46
RAD52	DNA repair	47
FOB1	Replication block	48

[a]The role of these genes in longevity was determined by studying the effects on life span of mutants and of overexpression.

Mitochondria and the retrograde response: a metabolic escape switch

Mitochondrial dysfunction in yeast is signaled to the nucleus via the products of three genes (*RTG1*, *RTG2*, *RTG3*), eliciting the retrograde response[11,12] and eventually

FIGURE 1. Activation of the retrograde response increases life span

The retrograde response constitutes a signal-intensity-sensitive rheostat that alters the expression of nuclear genes. Mitochondrial dysfunction generates the signal to which the rheostat responds: the greater the dysfunction, the stronger the signal. The exact site at which Ras2p modulates the retrograde response is not known. Extrachromosomal ribosomal DNA circle (ERC) production is a by-product of the retrograde response. The activation of the retrograde pathway in response to mitochondrial dysfunction is associated with an accumulation of ERCs, which can negatively affect longevity. The life-span-extending effect of the retrograde response predominates over this negative effect of the ERCs. The box contains the known gene products that are under the control of the retrograde response, and shows their subcellular localization. For further discussion, see the text.

resulting in the extension of life span (Fig. 1), as discussed below. Rtg1p and Rtg3p constitute the subunits of a heterodimeric, basic helix-loop-helix/leucine zipper transcription factor that regulates the expression of genes possessing the retrograde-response promoter element[13]. Rtg2p, an accessory factor, is involved in the activation of the transcription factor[13] by transducing mitochondrial signals that affect the phosphorylation state and subcellular localization of Rtg3p[14]. Nine genes that are regulated by the Rtg pathway have been identified so far[15–17]. Judging by the presence of sequences related to the retrograde response element in the promoters of many other yeast genes, there are likely to be scores of others.

Yeasts in which the mitochondria are not fully functional, typically because of a disruption in the electron transport chain due to the partial or complete loss of mitochondrial DNA (mtDNA) are called rho^- or rho^0 petites, respectively. Such petites display novel patterns of gene expression[18]. It had been shown that both spontaneous as well as induced petites have the same life spans as their isogenic parents[19]. This seemed to rule out a role for mitochondria in yeast ageing. However, the effect of loss of mtDNA sequences on life span has recently been shown to be strain specific[20]. All three possible results were found: no effect, a shortening of the life span and a lengthening of the life span. It should be kept in mind that yeast can derive energy through fermentation and can forego respiration. The life extension observed in certain petites is dependent on loss of mitochondrial function, because the reintroduction of mitochondria by cytoduction reverses the life exension[20]. One proposed explanation for the life extension seen in petites is that the lack of mitochondria means that fewer free radicals are produced. This seems unlikely, as overexpression of superoxide dismutase and catalase (which should destroy free radicals) does not result in extended longevity of a parent rho^+ strain grown on glucose, suggesting that the life extension observed in the petite is not mediated by an effect on the production of reactive oxygen species[20]. The enhanced longevity in certain petites, however, was closely correlated with the

induction of the retrograde response, as measured by the activation of *CIT2* (peroxisomal citrate synthase) gene expression[20], which is diagnostic for the response (Fig. 1). Petites (*rho*[0]) of four different strains were tested for the expression of *CIT2* and for life extension[20]. In two cases, both were repressed by glucose, but growth on another sugar unveiled a concomitant response. In another strain, there was no glucose repression, and the response was seen on glucose. In a fourth strain, the expression of *CIT2* was induced even in the *rho*[+] background in the absence of glucose, and this activation of the retrograde response was associated with increased longevity. Thus, enhanced longevity always correlated with an active retrograde response. This analysis provides an explanation for the differential effects of a petite in different genetic backgrounds. The retrograde response is induced by mitochondrial dysfunction and not by the loss of mtDNA *per se*, because a petite resulting from a deletion of the nuclear gene *COX4*, which encodes a subunit of the mitochondrial cytochrome oxidase, displays an increase in *CIT2* expression and has an extended life span[20]. The life span extension is, in fact, proportional to the level of *CIT2* expression.

The causal connection between the petite (via the retrograde response) and longevity was established by the demonstration that the deletion of *RTG2* results in suppression of life extension in the petite[20]. This suppression occurs regardless of the conditions required to induce the retrograde response and elicit life-span extension. Thus, the retrograde response represents a molecular mechanism of ageing in yeast whose operation can be induced by both genetic and physiological means. The induction of the retrograde response not only extends the life span, but also postpones the development of the senescent phenotype[20].

To date, there are nine known target genes in the retrograde response. The obvious question is which (if any) of these are the effectors of the increased longevity. It would appear that *CIT2* is not involved, because its deletion does not affect the life span of either a petite or its parent *rho*[+] strain[20]. However, other genes, such as *CIT1* or a third homolog in the yeast genome, may bypass *CIT2* when it is absent. The interpretation is not simple, as enhanced expression of *CIT2*, either alone or in concert with other effectors, does increase life span. Given the number of potential longevity effectors, it is not easy experimentally to establish which ones (or which combinations) determine life-span extension.

The physiological effect of the retrograde response is a profound adjustment to metabolism. The target genes of this response encode metabolic enzymes that are located in the mitochondrion, cytoplasm and peroxisome[15–17] (Fig. 1). The most obvious effect is a shift towards the utilization of the glyoxylate cycle. This allows the generation of biosynthetic Krebs-cycle intermediates from acetate, and it conserves carbon atoms. Another apparent effect is the maintenance of gluconeogenesis and anaplerotic reactions (conversion of pyruvate to Krebs-cycle intermediates). There are also effects on lipid metabolism that suggest its utilization as fuel. Without the three *RTG* genes, yeast cannot utilize acetate as a carbon source[15]. Thus, the retrograde response pathway may signal a switch from a time of plenty, which growth on glucose would define, to a time of relative scarcity, defined by growth on acetate (as acetate has a lower caloric content than glucose). This may seem paradoxical, in that respiratory-deficient yeast cannot grow on acetate. However, the respiratory defect that is used experimentally to induce and measure the retrograde response may simply be a drastic example of the milder insufficiencies that might be encountered by the cell under normal circumstances. The retrograde response may be a metabolic escape switch that allows yeast to surmount such insufficiencies. In essence, it is a compensatory response.

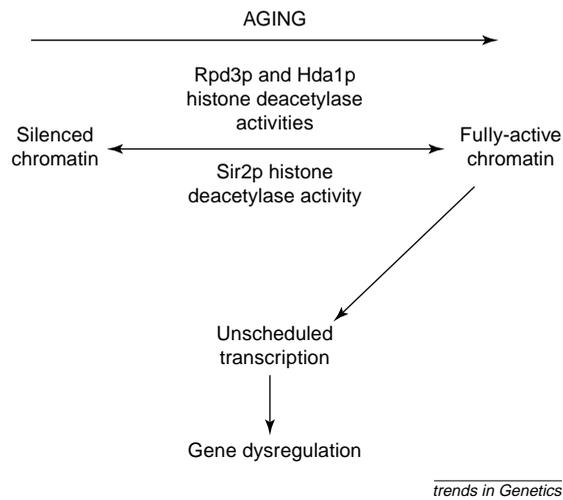## Other genes affect the retrograde response and longevity

The proper function of the *RAS2* gene determines yeast longevity[21]. Deletion of this gene curtails the life span, whereas overexpression extends it. It modulates the response of the cell to the nutritional state, thus impinging on metabolism. The deletion of *RAS2* completely suppresses the life extension seen in petites[20], suggesting that Ras2p (or a function under its control) lies in the pathway to extended longevity. This pathway is likely to be the retrograde response, because *RAS2* is required to produce the full induction of *CIT2* in a petite[20]. However, these results do not indicate that this is the sole mechanism by which *RAS2* operates as a homeostatic device in yeast longevity[22].

The retrograde-response pathway for extended longevity in yeast bears many similarities to the pathways and processes of life extension in animals[23]. The resemblance to the *daf-2* pathway in *Caenorhabditis elegans* is particularly close. DAF-2 is an insulin-like receptor that initiates a signal transduction pathway which senses crowding and starvation and results in a developmental detour to an alternative larval state called the dauer. The dauer larva can persist in a lethargic state without food for long periods. If the *daf-2* pathway is induced in the adult nematode, an extension of the life span occurs. Concomitantly, metabolic changes take place, including changes in lipid metabolism and a shift to the glyoxylate cycle[24], which resemble the retrograde response. Interestingly, sporulation in yeast requires the glyoxylate cycle and it, too, results in the generation of a dispersal form, the spore, under starvation conditions. Perhaps the retrograde response possesses features of the dauer/sporulation pathway that, under trying metabolic conditions, allow the yeast to survive. The retrograde response may be a phylogenetically primitive device for ensuring survival under metabolic duress, short of generating a dormant dispersal form.

Chromatin modification and yeast ageing

Extrachromosomal ribosomal DNA circles (ERCs) are found at very high levels in old yeast cells[25]. It was demonstrated some time ago that petites display a remarkable increase in ERC content compared to that of their isogenic parent strains[26]. Despite the deleterious effect on life span of the artificial accumulation of ERCs, their natural accumulation in the petite[26] does not prevent extension of life span[20]. Thus, ERCs are not a cause of ageing under these conditions and are an epiphenomenon, because it is the retrograde response that elevates ERC production (A. Benguria and S.M. Jazwinski, unpublished), indicating that it is predominant in life-span extension (Fig. 1). This interpretation stands in contrast to that of others[25].

ERCs are thought to arise through homologous recombination at the *RDN1* chromosomal locus, which is composed of a tandem array of between 100 and 200 copies of the 9.4-kb rDNA repeat. Mutants in *SIR2* result in the loss of

FIGURE 2. Ageing is associated with gene dysregulation
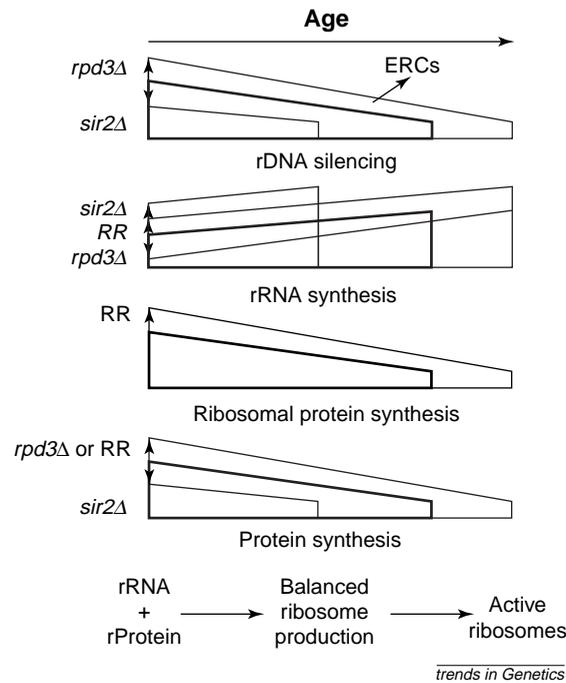

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The equilibrium between silenced and fully active chromatin is modulated by the histone deacetylases Rpd3p, Hda1p and Sir2p. During ageing, the balance shifts towards fully active chromatin, resulting in unscheduled transcription. The ensuing gene dysregulation leads to a loss of homeostasis.

FIGURE 3. Mechanisms of ageing associated with protein synthesis
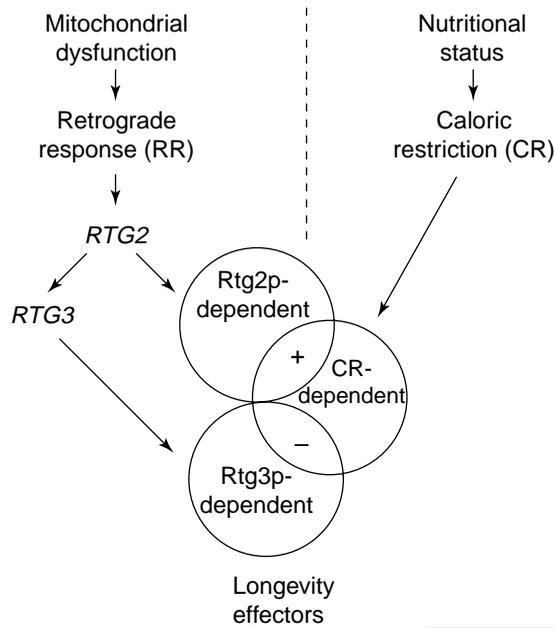

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Maintenance of protein synthesis requires an active translation machinery. A decline in protein synthesis occurs during ageing, because of an abundance of rRNA relative to ribosomal protein production. Factors that rectify this imbalance extend life span. The histone deacetylases, Rpd3p, Hda1p and Sir2p, affect the balance through their role in rDNA silencing, which impinges on rRNA synthesis. The production of ERCs is an epiphenomenon associated with events at the rDNA locus. The retrograde response (RR) affects both rRNA and ribosomal protein synthesis. The heavy lines designate the normal situation, and the light lines depict the changes associated with the various treatments. For further discussion, see the text.

transcriptional silencing and promote mitotic recombination at the *RDN1* locus[27]. Thus, if ERCs cause ageing, deletion of *SIR2* should curtail the life span. A *sir2*-Δ strain does, indeed, have a shortened life span, but this is not associated with the formation of ERCs[28]. This suggests that the role that this gene plays in determining life span is mediated by a different mechanism. This mechanism appears to be the silencing of rDNA[28], and Sir2p has very recently been shown to be a histone deacetylase[29], indicating the enzymatic mechanism responsible for its role in silencing. The histone deacetylase genes *RPD3* and *HDA1*, which encode homologs of human histone deacetylases HDAC1 and HDAC2, had already been shown to affect the yeast life span, and this effect, too, is most closely associated with transcriptional silencing of the *RDN1* locus[28].

The epigenetic inheritance of different regulatory states of chromatin was proposed as a determinant of life span a decade ago[30] and a molecular mechanism of ageing was ascribed to progressive gene dysregulation resulting from the loss of silencing during ageing[8]. Indeed, loss of transcriptional silencing during ageing has been demonstrated in yeast[31,32]. Histone deacetylases may maintain an equilibrium between transcriptionally active and silent regions of chromatin (Fig. 2). With age, a shift occurs towards the active state. This may be due to the loss of expression of proteins that maintain heterochromatin[28] and to migration of these proteins to other chromosomal regions[33]. The result is the transcription of genes that are silent in young cells. As this process intensifies, a state of gene dysregulation ensues, which could result in the loss of homeostasis. There has been speculation very recently that histone deacetylase activity may couple age-related inappropriate gene expression to metabolism[29].

Protein metabolism and yeast ageing

The retrograde response and histone deacetylases, acting at the *RDN1* locus, were proposed to impinge on yeast life span through their effects on protein metabolism[28].

Protein synthetic activity declines with age in yeast[34]; this could be due to an imbalance in the synthesis of rRNA and ribosomal proteins, resulting in the defective assembly of active ribosomes (Fig. 3). In fact, there is an increase in the rRNA content of old yeast cells[9,34]. This increase may not be balanced by an increase in other components of the translation machinery. Deletion of *RPD3* (or *HDA1* in conjunction with a *sir3*-Δ, which unmasks the effect of the *HDA1* deletion) enhances the silencing of rDNA[28], which could then restore the balance between rRNA and the rest of the protein biosynthetic apparatus. Because of its differential effects on silencing, perhaps by virtue of Sir2p specificity for different lysine residues, the deletion of *SIR2* has just the opposite effect, exacerbating the problem. The retrograde response actually increases rRNA production[26]. However, it is proposed that it also increases the expression of genes that encode other components of the translation machinery, creating a new balance that results in a marked extension of life span. The maintenance of protein metabolism depicted here is a molecular mechanism of ageing that may be broadly applicable[35].

Caloric restriction does not work through the retrograde response

One method for extending the life span of mammals has a long history. It involves the reduction of food intake and

FIGURE 4. Interaction of the retrograde response and caloric restriction

The retrograde response and caloric restriction are two non-overlapping pathways. They interact at the level of the longevity effectors. The interaction can be either positive or negative. The extent of the overlap between the effectors of both pathways is not known at present. For more details, see the text.

is variously called food restriction, dietary restriction or caloric restriction[36]. The basic observation is that a decrease in food intake leads to an increase in both life expectancy and maximum life span. Furthermore, there is a delay in the appearance of the functional decline and diseases associated with ageing. This effect is due to an overall reduction in calorie intake and not to a particular component of the diet, hence the name caloric restriction. Caloric restriction is not malnutrition, because all of the essential components of the diet are provided.

The implication of a mechanism involving metabolic control in yeast ageing has prompted an attempt to manipulate the yeast life span by using caloric restriction[37]. In the case of yeast, it is possible to manipulate the available glucose levels directly. A decrease in the glucose concentration in the growth medium causes an increase in the life span, as measured by the number of divisions of individual cells. This effect can be achieved both in a modified broth as well as in a chemically defined medium. There is, however, a limit beyond which decreases in glucose levels are no longer effective in extending life span. Obviously, glucose becomes limiting for growth in yeast, just as there is a limit to the extent to which a reduction in the calories of an animal's diet has a beneficial effect on longevity. The life extension observed in yeast correlates with a postponement in the appearance of a senescent phenotype (Ref. 37 online at www.fasebj.org), that is, a decrease in the budding rate.

Does the reduction in glucose that leads to an increase in yeast life span operate through the retrograde-response pathway? This does not appear to be the case[37], as glucose concentrations that result in life extension do not induce

the CIT2 gene. On the contrary, they result in a reduction in CIT2 mRNA levels[37]. This suggests that the life extension either requires an attenuation of some of the functions controlled by the retrograde response or works via an independent mechanism.

Given the marked difference between the conditions under which the retrograde response is induced (mitochondrial dysfunction) and the nutritional manipulations that result in life-span extension, it is possible that CIT2 is not an accurate indicator of the retrograde response under the latter conditions. The deletion of the RTG2 gene, a mediator of the retrograde response, however, results in no decrease in life extension on reduced glucose[37], indicating the lack of a requirement for the retrograde response. In fact, deletion of RTG2 causes some reduction in life span at high glucose levels, which is not seen on low glucose. It is possible, however, that at reduced glucose concentrations the requirement for RTG2 is bypassed but that the Rtg1p–Rtg3p transcription factor is still required. Deletion of RTG3 did not suppress life extension upon glucose reduction[37]. Thus, the retrograde response and glucose reduction seem to represent two distinct metabolic mechanisms of life extension. Furthermore, the expression of some or all of the genes under the control of the Rtg1p–Rtg3p transcription factor in the absence of a retrograde-response signal from the mitochondria apparently suppresses longevity and prevents the full life-span benefit from being obtained from growth on reduced glucose, as suggested by the life-span extension caused by the RTG3 deletion and its additive effect with respect to reduced glucose levels[37]. The opposite effects of Rtg2p and Rtg3p suggest that there is some separation of the functions of these downstream mediators of the retrograde response.

Life-span extension can be effected not only by a reduction in the glucose levels of the growth medium, but also by reductions in amino acid concentrations; this effect, too, is independent of the retrograde response[37]. Thus, it is not the nature of the nutrient that dictates the effect. This resembles the situation found with the caloric restriction of rodents, in which it is the reduction in calories and not a specific nutrient that is crucial. Therefore, it is reasonable to give the nutritional response found in yeast the provisional label 'caloric restriction'.

Multiple metabolic mechanisms of ageing

Studies in yeast indicate that there are multiple metabolic mechanisms of ageing. To date, three of these mechanisms have been delineated: the retrograde response, caloric restriction, and protein synthesis. The relationship between the retrograde response and protein synthesis involves transcriptional silencing and a putative induction of ribosomal protein genes. Histone deacetylases have a modulatory effect on the protein metabolic mechanism of ageing. In fact, it has recently been speculated that the Sir2 histone deacetylase provides a link between metabolism and ageing through its dependence on NAD[29]. The increase in NAD levels in ageing yeast would mitigate against this[38], and extension of life span by caloric restriction does not require SIR2 (J.C. Jiang and S.M. Jazwinski, unpublished).

Whether or not there is any interaction between the retrograde response and caloric restriction is open to some speculation (Fig. 4). Clearly, these are separate signaling pathways. However, both positive and negative interactions appear to exist, in view of the effects on life

span of caloric restriction and deletion of *RTG2* and *RTG3*, detailed above. Even though certain features of the retrograde response appear to have a negative effect on the extension of life span afforded by caloric restriction, this does not rule out some overlap between the longevity effectors induced by these pathways. It was demonstrated recently that there is a switch in the requirement for transcription factors that regulate the expression of certain metabolic enzyme genes, depending on the functional state of the mitochondria[39]. This switch shifts the regulation of these genes from the Hap (heme-dependent transcriptional activator) to the Rtg transcription complex, such that some genes respond to both whilst others respond to one or the other. To determine the extent of the overlap, it will be important to define the cellular targets that are the downstream effectors of both the retrograde response and the caloric-restriction effects on life span.

What are the relative roles of the retrograde response and caloric restriction pathways in yeast physiology? Although both represent metabolic mechanisms of ageing, their roles appear to differ. The retrograde response seems to compensate for the dysfunction that develops in mitochondria during ageing[40]. It functions like a rheostat,

adjusting the activity of nuclear genes and longevity to the intensity of a signal provided by dysfunctional mitochondria. Presumably, the greater the accumulated dysfunction, the greater the compensatory retrograde response required to preserve cellular function. Caloric restriction, on the other hand, seems to increase the efficiency with which energy is extracted from the nutrients received by the organism. Thus, the retrograde pathway is an adaptation to the ravages of time, whereas caloric restriction tends to prevent the deficits associated with ageing. It is important to determine whether a retrograde response of the sort found in yeast is operational in humans. The development of yeast as a model for the study of caloric restriction should facilitate the dissection of this phenomenon at the molecular level. The most fundamental question is whether nutritional manipulation triggers responses that enhance survival, or whether a reduction in excess nutrients eliminates a negative effect on life span.

Acknowledgements

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