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Yeast replicative life span – the mitochondrial connection

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Abstract

Mitochondria have been associated with aging in many experimental systems through the damaging action of reactive oxygen species. There is more, however, to the connection between mitochondria and *Saccharomyces cerevisiae* longevity and aging. Induction of the retrograde response, a pathway signaling mitochondrial dysfunction, results in the extension of life span and postponement of the manifestations of aging, changing the metabolic and stress resistance status of the cell. A paradox associated with the retrograde response is the simultaneous triggering of extrachromosomal ribosomal DNA circle (ERC) production, because of the deleterious effect these circles have on yeast longevity. The retrograde response gene *RTG2* appears to play a pivotal role in ERC production, linking metabolism and genome stability. In addition to mother cell aging, mitochondria are important in establishment of age asymmetry between mother and daughter cells. The results more generally point to the existence of a mechanism to "filter" damaged components from daughter cells, a form of checkpoint control. Mitochondrial integrity is affected by the *PHB1* and *PHB2* genes, which encode inner mitochondrial membrane chaperones called prohibitins. The Phb1/2 proteins protect the cell from imbalances in the production of mitochondrial proteins. Such imbalances appear to cause a stochastic stratification of the yeast population with the appearance of short-lived cells. Ras2p impacts this process. Maintenance of mitochondrial membrane potential and the provision of Krebs cycle intermediates for biosyntheses appear to be crucial elements in yeast longevity. In sum, it is clear that mitochondria lie at the nexus of yeast longevity and aging.

Keywords: Retrograde response; Rtg2 protein; Ras2 protein; Prohibitin; Genome stability

1. Introduction

The yeast *Saccharomyces cerevisiae* has a finite replicative life span, which is measured by the number of times an individual cell divides rather than by chronological time [1,2]. With each division, the mother cell becomes older, but it produces daughter cells that, in principle, start from scratch, having the potential for a full life span. This latter characteristic, in which asymmetric cell division "erases" the experiences of the mother from the "memory" of the daughter, breaks down as the mothers get older [3], and the daughters of old mothers tend to have shorter life spans than those produced by young mother cells [4]. These aging phenotypes have been taken as evidence for the elaboration of a cytoplasmic senescence factor, which accumulates in old mother cells, increasing its probability of transmission to the daughters where it operates in a dominant fashion [3]. A senescence factor of this sort may accumulate in stationary phase. It has been shown that holding cells in stationary culture curtails their replicative life span when cell division resumes [5]. The loss of viability of stationary-phase cells has been termed chronological aging [6]. Thus, the same senescence factor may cause both replicative and chronological aging of yeast cells. This article focuses on the former process.

The fact that individual yeasts are mortal is not in itself evidence of the operation of an aging process. However, mother cells undergo a variety of changes as they progress through their replicative life spans, some of these represent functional decline [7]. Two of these age changes have been used frequently to assess the

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course of aging. They are the gradual increase in generation time [3] and the development of resistance to mating pheromone [8].

Studies of yeast aging have intensified over the past decade, and there are now over a dozen laboratories worldwide interested in this biological problem. It has been clear for some time now that both genetic and environmental factors contribute to the limited life span and aging of yeast [9]. However, the realization that chance has a predictable role in yeast aging has been a more recent development [10]. There are more than 30 genes with an effect on yeast longevity [9]. These genes describe a wide array of biochemical functions, but they can be grouped into four broad cellular processes: metabolism, stress resistance, chromatin-dependent gene regulation, and genome stability [11].

The accumulation of ERCs is viewed as the ultimate cause of yeast aging by some [12]. However, the prevention of circle accumulation does not result in immortality [13], and aging is not always associated with their accretion [14,15]. The presence of more than one mechanism or cause of aging in a genetically homogenous population of cells kept under constant and defined conditions seems to present a conceptual difficulty. However, the operation of chance in aging and the related epigenetic stratification of a yeast population remove any impediments to the acceptance of multiple, simultaneous causes of aging in a yeast population [9].

2. Retrograde response

The retrograde response is an intracellular signaling pathway, which for several years possessed the status of a curious phenomenon of gene regulation [16]. This pathway is triggered by dysfunctional mitochondria, and it results in the induction of numerous nuclear genes, which encode metabolic enzymes and stress response proteins destined for the cytoplasm, mitochondria, and peroxisomes [17]. The induction of the retrograde response causes a remodeling of cell metabolism. Importantly, it also results in an increase in yeast longevity and a postponement of the increase in cell cycle time characteristic of yeast aging [18]. Deletion of the retrograde response genes *RTG2* [18] or *RTG3* (J.C. Jiang, J. Wawryn, and S.M. Jazwinski, unpublished), which are signal transducers in the pathway, prevents the enhancement of life span. In some yeast strains, the induction of the retrograde response only becomes apparent on media possessing a non-repressive carbon source [18].

The retrograde response is not a simple on-off switch. Instead, it functions more like a rheostat. The greater the mitochondrial dysfunction is, the more extensive is the induction of the retrograde response and the larger the attendant enhancement of life span [19]. There is a gradual increase in mitochondrial dysfunction during the course of aging, as measured by the decline in mitochondrial membrane potential ($\Delta\Psi_m$) [20]. Concomitantly, there is a commensurate increase in the retrograde response [21]. Oxidative stress induces apoptotic death of yeast cells [22]. The age-dependent loss of mitochondrial function [20] occurs in conjunction with increased oxidative stress [23], marking the yeast cell for apoptotic demise at the end of its replicative life span [23]. Interestingly, apoptotic death punctuates the yeast chronological life span as well [24].

There are various ways in which genetic and environmental factors can be implicated experimentally in aging. The most straightforward occurs when such a factor fulfills four criteria: (1) its application extends life span; (2) it induces a postponement of the senescent phenotype; (3) the factor impacts or is associated with a normal aging process; and 4) the factor is induced during the course of normal aging. These are stringent criteria. Once they have been applied to identify an aging determinant, any additional factor that modulates the effect on longevity of such a determinant is readily implicated in aging. The retrograde response fulfills all four criteria for a determinant of longevity, and the retrograde response genes *RTG2* and *RTG3* can be considered longevity-determining genes.

The retrograde response pathway is elaborate. It is modulated by several other pathways that monitor the biosynthetic status of the cell, notably the target of rapamycin (TOR) [25,26] and RAS [18] pathways. In turn, the retrograde response pathway regulates the nitrogen catabolism pathway, which is induced to allow the utilization of low-quality nitrogen sources [27]. The key regulator is the Mks1p, which was originally identified as a downstream component of the RAS pathway [28]. Mks1p appears to be a target of the TOR pathway [29], and it displays a complicated relationship to Rtg2p, the proximal sensor of mitochondrial dysfunction in the retrograde response, with which it can form a complex [30]. In fact, the phosphorylated Mks1p, which blocks the retrograde response pathway, is complexed with the 14-3-3 proteins Bmh1 and Bmh2 that are also negative regulators of this pathway, rather than with Rtg2p which binds the dephosphorylated form and inhibits the negative effect of Mks1p on the retrograde response [31]. Glutamate, a rich nitrogen source, represses both the retrograde response and the nitrogen catabolism pathways and appears to be pivotal in regulation of cell metabolism [32].

The metabolic remodeling that the retrograde response portends encompasses a shift to the utilization of lipid/acetate as a carbon source. The acetate is utilized by the glyoxylate cycle, which is more economical than the Krebs cycle because it conserves the two carbons of acetate rather than releasing them in the form of carbon dioxide. The glyoxylate cycle is an anaplerotic process

that provides biosynthetic precursors in the face of declining Krebs cycle activity and the loss of $\Delta\Psi_m$ to power export of Krebs cycle intermediates from the mitochondrion for biosyntheses. Thus, the retrograde response is a compensatory anti-aging mechanism, whose induction during normal aging may explain why yeasts live as long as they do. Another metabolic anti-aging mechanism is calorie restriction, which remodels mitochondrial function [33,34] and may play a preventive role.

There is a conundrum connected to this anti-aging mechanism. The retrograde response is associated with the production of ERCs [35], which have been shown to kill yeast cells [12]. How does life extension occur under these circumstances? The resolution of this dilemma comes with the discovery that the retrograde response protein Rtg2 plays a role in the production of these circles [21]. Rtg2p relays the signal from dysfunctional mitochondria to the Rtg1p–Rtg3p transcription factor [36]. It is indirectly responsible for the activation and translocation of the transcription factor from the cytoplasm to the nucleus [36]. It has been shown recently that Rtg2p is a component of the histone acetyltransferase complex SLIK and can be found at the promoters of retrograde responsive genes, where it serves as a co-activator by modifying chromatin [37]. Rtg2p, however, has an additional role. It suppresses the production of ERCs, but it can only do this when it is not engaged in transmitting the retrograde signal [21]. It appears that Rtg2p, either in the SLIK or some other complex, prevents the recombination events that result in ERC formation, or alternatively it is engaged in relaying the retrograde response signal.

The intensity of the signal provided by dysfunctional mitochondria is what limits the retrograde response and not limiting levels of Rtg2p [21]. With advancing age, mitochondrial dysfunction increases, and this is countered by the increase in the retrograde response, which enhances longevity. However, this engagement of Rtg2p in retrograde signaling reduces its availability to counter the production of ERCs, which accumulate with age and curtail life span. Fortunately, the retrograde response has two life-extending effects; one counters the negative effects of ERCs, while the other has an independent role in life extension [21]. Approximately one-half of the life-extending effect of the retrograde response is devoted to countering the negative effects of ERC production [21].

3. Age asymmetry

There are two main questions in yeast aging: Why do mother cells age? Why are daughters born young? The second of these questions can be restated in terms of the establishment of age asymmetry between the mother and the daughter. This is an important issue more generally for stem cell aging. It has been addressed by the isolation of mutants of age asymmetry in yeast. Conditional mutants that undergo clonal senescence were isolated and shown to produce daughters that possessed the same replicative age as their mothers at the time of birth [20]. The phenotype was subtle, took several cell generations to develop, and was reversible up to a point. The defect in the mutant had all of the features of the cytoplasmic senescence factor, described above.

One of the age asymmetry mutants isolated was shown to be the result of a point mutation in the *ATP2* gene, which encodes the β-subunit of mitochondrial ATP synthase [20]. The effect of the mutation on enzyme activity was subtle, because the cells could grow easily on a non-fermentable carbon source. However, it resulted in a gradual loss of $\Delta\Psi_m$, followed by the loss of mitochondrial mass under restrictive conditions. The mitochondria showed a change in morphology and distribution in the cell. Daughter cells often received less than their share of mitochondrial material. At the extreme, cells became totally devoid of mitochondria. The *atp2* mutant displayed a cell pathology. This pathology indicates the importance of the segregation of a complement of active mitochondria to maintain age asymmetry. It suggests more generally that certain "filters" might exist to guarantee that progeny receive a set of undamaged cell components. It has been shown that older mother cells have a tendency to segregate dysfunctional mitochondria to their daughters [20]. This indicates that a breakdown in age asymmetry is a feature of normal yeast aging.

How does a mutation in ATP synthase lead to the phenotypes described here? Normally, the mitochondrial electron transport chain generates the $\Delta\Psi_m$, which is needed for a variety of mitochondrial functions, including the transport of various biosynthetic precursors. Under fermentative conditions, this function is provided by the ATP synthase acting as an ATPase and pumping protons out of the mitochondrion. This requires cooperation with the ATP–ADP translocator, which brings the ATP generated during glycolysis into the mitochondrion [38]. In fact, only the F_1-ATPase portion of the ATP synthase is essential as a sink for ATP, because the translocator generates a $\Delta\Psi_m$ when it brings ATP into the mitochondrion in exchange for ADP. It is this $\Delta\Psi_m$ that appears to be important for age asymmetry, which is compromised during yeast aging. The loss of $\Delta\Psi_m$ with age affects the integrity of mitochondrial function during aging in at least two ways. It decreases the fidelity of segregation of active mitochondria to daughter cells, and it reduces the ability of mitochondria to provide the cell with precursors for biosyntheses.

The provision of biosynthetic precursors by mitochondria has an additional role to play in preventing the ravages of aging. Because the transport of these precursors out of the mitochondrion depends on $\Delta\Psi_m$, it

results in a slight reduction in its magnitude. This in turn would ameliorate the production of reactive oxygen species, which have a deleterious effect on cells and reduce life span. As discussed earlier, old yeast cells accumulate reactive oxygen species [23].

The notion of a mechanism that guarantees the transmission of undamaged components from mother to daughter cells, based on the unbiased screen for age asymmetry mutants, has gained additional support more recently. It has been shown that carbonylated proteins tend to be retained in mother cells and that this retention depends on *SIR2* [39]. These damaged proteins, in similarity to damaged mitochondria, could be representative of the cytoplasmic senescence factor. It has also been suggested that ERC constitutes this senescence factor [12], although no direct evidence for its involvement in age asymmetry or clonal senescence has been provided [20].

4. Mitochondrial biogenesis

The prohibitin genes, *PHB1* and *PHB2*, have been implicated in yeast longevity [9]. Although this has been clear for some time, the nature of this involvement has only become apparent recently. Deletion of either of these genes curtails life span. This is observed consistently in strains that lack a mitochondrial genome, and thus do not have fully functional mitochondria, in which the usual life extension due to induction of the retrograde response is not seen [40]. Surprisingly, the survival curves are bimodal, with a fraction of the population dying early, and the remainder showing a longer life span. This pattern is also observed when both genes are deleted, although the fraction dying early is larger. The expression of the prohibitin genes declines during the life span.

Phb1p and Phb2p are located in the inner mitochondrial membrane. They cooperate with the mitochondrial inner membrane mAAA-protease [41,42], which degrades supernumerary or improperly folded proteins of the inner membrane. An examination of cells lacking mitochondrial DNA and Phb1p shows a striking change in mitochondrial morphology and distribution. A fraction of the cells has few or no mitochondria [40]. This fraction corresponds closely to the portion of the yeast population that dies early in the life span. Interestingly, the deletion of *RAS2* in strains devoid of prohibitin suppresses the life span deficit and the altered mitochondrial morphology, distribution, and segregation. Deletion of *PHB1* in the absence of a mitochondrial genome generates a sub-population of cells that produce high amounts of reactive oxygen species in their mitochondria. The accumulation of these cells could readily explain in quantitative terms the decreased longevity of cells deficient for prohibitins. The production of oxidants in these cells is suppressed by deletion of *RAS2*.

Factors that attenuate life span are more difficult to consider than those that extend it. The induction of ''premature aging'' has been used on occasion for this purpose. The problem is the definition of premature aging, as opposed to the negative effects of abrogation of vital processes. If only one mechanism of aging exists in yeast, there is no difficulty in this definition, because one can choose any of the several manifestations of aging as a measure. If there is more than one mechanism, a very judicious choice that encompasses several truly independent measures is needed. Such an approach has been applied only on occasion [43]. There are other instances, however, when life-shortening factors can be informative. This occurs when they exacerbate and thus make more obvious events that occur during normal aging.

The deletion of one or both of the prohibitin genes in cells lacking fully functional mitochondria creates a cellular pathology. This pathology informs on events that occur during normal aging of yeasts, creating a phenocopy of an old yeast cell. There is a decline in the expression of prohibitins during aging, as mentioned above. Furthermore, there is a loss of $\Delta\Psi_m$ with age. The combination of these two factors is equivalent to the deletion of the prohibitin genes in cells devoid of mitochondrial DNA. This would accelerate the production of reactive oxygen species, which are known to accumulate during yeast aging [23]. This implies that the loss of mitochondrial function may be the primary event, which is followed closely during aging by more extensive oxidative damage to mitochondria. This would represent a self-accelerating process. The probability that a given yeast cell would cross the threshold for life span limitation in this sequence of events would be finite, resulting in the type of epigenetic stratification of the population described earlier, with the amplification of oxidative damage and emergence of a sub-population suffering early demise [40].

This leaves the question of the role of Ras2p in this chain of events. It has been shown that Ras2p stimulates the expression of mitochondrial protein genes in the nucleus [44]. This would create an imbalance in the production of mitochondrial protein complexes in cells lacking a mitochondrial genome. This imbalance is rectified by the prohibitins, which ameliorate the presence of supernumerary mitochondrial proteins, likely by orchestrating their degradation by the mAAA-protease. In the absence of prohibitins, these excess proteins would accumulate. Under these circumstances, the deletion of *RAS2* would have a salutary effect. During the yeast life span, expression of prohibitins declines [40], but this may be balanced by the decline in expression of *RAS2* [45]. Nevertheless, the mitochondrial deficits that accrue during the life span would overwhelm the cells, due to the loss of Ras2p function. This is likely to be the

case, because it is known that deletion of *RAS2* makes cells more susceptible to chronic stress [46]. Furthermore, the loss of Ras2p activity impairs the induction of the life-extending retrograde response, which counters the metabolic deficits resulting from the loss of mitochondrial activity [18].

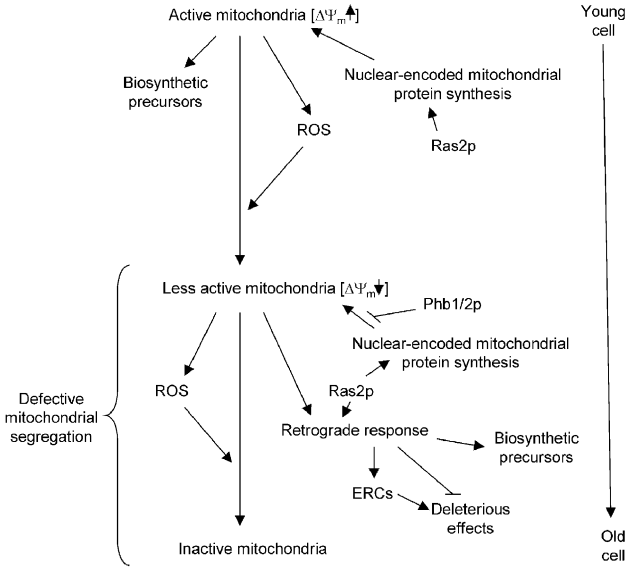

Fig. 1. Model of some of the age-related processes in yeast involving mitochondria. Active mitochondria in young cells have a high membrane potential ($\Delta\Psi_m$), which drives the export of biosynthetic precursors to the cytoplasm allowing the production of daughter cells. This transport process would dissipate somewhat the $\Delta\Psi_m$, reducing the production of damaging reactive oxygen species (ROS). Ras2p activity stimulates the expression of nuclear genes, encoding mitochondrial proteins. This, in conjunction with the expression of mitochondrial genes, facilitates the biogenesis of mitochondria. The production of ROS is believed to damage mitochondria, leading to the accumulation of less active organelles. The decline in Krebs cycle activity, along with loss of $\Delta\Psi_m$, markedly diminishes the capacity of mitochondria to provide biosynthetic precursors. The dysfunctional mitochondria, however, signal the induction of the retrograde response, which provides these precursors and thus would provide for production of daughter cells and the extension of life span that it measures. But the induction of the retrograde response leads to the production of ERCs, a phenomenon of genome instability. The deleterious effects of ERCs are countered by the retrograde response. Ras2p-stimulated mitochondrial biogenesis now appears to begin to have a sinister role, because the mounting damage in the organelle interferes with the proper assembly of membrane protein complexes. Fortunately, the Phb1/2p complex holds excess proteins and delivers them to the mAAA-protease in the mitochondrial membrane for degradation. This may only delay the inevitable accumulation of inactive mitochondria during aging, because there is a decrease in *PHB1/2* expression with age. That could be balanced for a time by the simultaneous reduction in *RAS2* expression. However, this latter process also reduces the induction of the retrograde response, diminishing progressively its salutary contribution to yeast longevity. As yeast cells age, they develop the tendency to segregate fewer functional mitochondria to daughter cells. The dysfunctional mitochondria possess the attributes of the cytoplasmic senescence factor. The significance of the retrograde response in determining yeast life span is bolstered by the observation of life extension in *Caenorhabditis elegans* as a result of lowering the expression of mitochondrial protein genes [47,48] and by the comparable metabolic remodeling observed in *daf-2* mutant worms that display extended longevity [49,50]. For further details and references, please see the text.

5. Conclusions

The studies reviewed here point to a central role for mitochondria in the yeast aging process. It appears that the impacts of stress and genome instability on yeast longevity are ultimately related to metabolism, especially mitochondrial metabolism (Fig. 1). Mitochondrial activity sows the seeds of mitochondrial decline during aging. However, this decline induces a compensatory reaction in the form of the retrograde response, which promotes longevity in the face of the mitochondrial deficits. Unfortunately, the retrograde response is in turn a Trojan horse creating genome instability, which it is able nevertheless to counter at least for a time. Even the elimination of this genome instability, however, only extends longevity up to a point, at which yet other life-limiting processes make their presence known. It is an open question whether this is an infinite series. Yeasts offer the opportunity to resolve this quandary.

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References

[1] Mortimer, R.K. and Johnston, J.R. (1959) Life span of individual yeast cells. Nature 183, 1751–1752.
[2] Müller, I., Zimmermann, M., Becker, D. and Flömer, M. (1980) Calendar life span versus budding life span of *Saccharomyces cerevisiae*. Mech. Ageing Dev. 12, 47–52.
[3] Egilmez, N.K. and Jazwinski, S.M. (1989) Evidence for the involvement of a cytoplasmic factor in the aging of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. 171, 37–42.
[4] Kennedy, B.K., Austriaco, N.R. and Guarente, L. (1994) Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life span. J. Cell Biol. 127, 1985–1993.
[5] Ashrafi, K., Sinclair, D., Gordon, J.I. and Guarente, L. (1999) Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96, 9100–9105.
[6] Fabrizio, P. and Longo, V.D. (2003) The chronological life span of *Saccharomyces cerevisiae*. Aging Cell 2, 73–81.
[7] Jazwinski, S.M. (2003) Yeast longevity and aging. In: Aging of Organisms (Osiewacz, H.D., Ed.), pp. 39–59. Kluwer Academic Publishers, Dordrecht, Netherlands.
[8] Smeal, T., Claus, J., Kennedy, B., Cole, F. and Guarente, L. (1996) Loss of transcriptional silencing causes sterility in old mother cells of *S. cerevisiae*. Cell 84, 633–642.

[9] Jazwinski, S.M. (2001) New clues to old yeast. Mech. Ageing Dev. 122, 865–882.
[10] Jazwinski, S.M. and Wawryn, J. (2001) Profiles of random change during aging contain hidden information about longevity and the aging process. J. Theor. Biol. 213, 599–608.
[11] Jazwinski, S.M. (1996) Longevity, genes, and aging. Science 273, 54–59.
[12] Sinclair, D.A. and Guarente, L. (1997) Extrachromosomal rDNA circles – a cause of aging in yeast. Cell 91, 1033–1042.
[13] Defossez, P.A., Prusty, R., Kaeberlein, M., Lin, S.J., Ferrigno, P., Silver, P.A., Keil, R.L. and Guarente, L. (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell 3, 447–455.
[14] Kim, S., Benguria, A., Lai, C.-Y. and Jazwinski, S.M. (1999) Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell 10, 3125–3136.
[15] Heo, S.J., Tatebayashi, K., Ohsugi, I., Shimamoto, A., Furuichi, Y. and Ikeda, H. (1999) Bloom's syndrome gene suppresses premature ageing caused by Sgs1 deficiency in yeast. Genes Cells 4, 619–625.
[16] Parikh, V.S., Morgan, M.M., Scott, R., Clements, L.S. and Butow, R.A. (1987) The mitochondrial genotype can influence nuclear gene expression in yeast. Science 235, 576–580.
[17] Epstein, C.B., Waddle, J.A., Hale, W., Dave, V., Thornton, J., Macatee, T.L., Garner, H.R. and Butow, R.A. (2001) Genome-wide responses to mitochondrial dysfunction. Mol. Biol. Cell 12, 297–308.
[18] Kirchman, P.A., Kim, S., Lai, C.-Y. and Jazwinski, S.M. (1999) Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. Genetics 152, 179–190.
[19] Jazwinski, S.M. (2000) Metabolic control and gene dysregulation in yeast aging. Ann. N. Y. Acad. Sci. 908, 21–30.
[20] Lai, C.-Y., Jaruga, E., Borghouts, C. and Jazwinski, S.M. (2002) A mutation in the *ATP2* gene abrogates the age asymmetry between mother and daughter cells of the yeast *Saccharomyces cerevisiae*. Genetics 162, 73–87.
[21] Borghouts, C., Benguria, A., Wawryn, J. and Jazwinski, S.M. (2004) Rtg2 protein links metabolism and genome stability in yeast longevity. Genetics 166, 765–777.
[22] Madeo, F., Engelhardt, S., Herker, E., Lehmann, N., Maldener, C., Proksch, A., Wissing, S. and Fröhlich, K.U. (2002) Apoptosis in yeast: a new model system with applications in cell biology and medicine. Curr. Genet. 41, 208–216.
[23] Laun, P., Pichova, A., Madeo, F., Fuchs, J., Ellinger, A., Kohlwein, S., Dawes, I., Fröhlich, K.U. and Breitenbach, M. (2001) Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. Mol. Microbiol. 39, 1166–1173.
[24] Herker, E., Jungwirth, H., Lehmann, K.A., Maldener, C., Fröhlich, K.U., Wissing, S., Buttner, S., Fehr, M., Sigrist, S. and Madeo, F. (2004) Chronological aging leads to apoptosis in yeast. J. Cell Biol. 164, 501–507.
[25] Komeili, A., Wedaman, K.P., O'Shea, E.K. and Powers, T. (2000) Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. J. Cell Biol. 151, 863–878.
[26] Tate, J.J. and Cooper, T.G. (2003) Tor1/2 regulation of retrograde gene expression in *Saccharomyces cerevisiae* derives indirectly as a consequence of alterations in ammonia metabolism. J. Biol. Chem. 278, 36924–36933.
[27] Pierce, M.M., Maddelein, M.L., Roberts, B.T. and Wickner, R.B. (2001) A novel Rtg2p activity regulates nitrogen catabolism in yeast. Proc. Natl. Acad. Sci. USA 98, 13213–13218.
[28] Matsuura, A. and Anraku, Y. (1993) Characterization of the *MKS1* gene, a new negative regulator of the Ras-cyclic AMP pathway in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 238, 6–16.
[29] Dilova, I., Chen, C.Y. and Powers, T. (2002) Mks1 in concert with TOR signaling negatively regulates RTG target gene expression in *S. cerevisiae*. Curr. Biol. 12, 389–395.
[30] Sekito, T., Liu, Z., Thornton, J. and Butow, R.A. (2002) RTG-dependent mitochondria-to-nucleus signaling is regulated by *MKS1* and is linked to formation of yeast prion [*URE3*]. Mol. Biol. Cell 13, 795–804.
[31] Liu, Z., Sekito, T., Spirek, M., Thornton, J. and Butow, R.A. (2003) Retrograde signaling is regulated by the dynamic interaction of Rtg2p and Mks1p. Mol. Cell 12, 401–411.
[32] Butow, R.A. (2002) Cellular responses to mitochondrial dysfunction: it's not always downhill. Cell Death Differ. 9, 1043–1045.
[33] Jiang, J.C., Wawryn, J., Shantha Kumara, H.M.C. and Jazwinski, S.M. (2002) Distinct roles of processes modulated by histone deacetylases Rpd3p, Hda1p, and Sir2p in life extension by caloric restriction in yeast. Exp. Gerontol. 37, 1023–1030.
[34] Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G.R. and Guarente, L. (2002) Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature 418, 344–348.
[35] Conrad-Webb, H. and Butow, R.A. (1995) A polymerase switch in the synthesis of rRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15, 2420–2428.
[36] Sekito, T., Thornton, J. and Butow, R.A. (2000) Mitochondria-to-nuclear signaling is regulated by the subcellular localization of the transcription factors Rtg1p and Rtg3p. Mol. Biol. Cell 11, 2103–2115.
[37] Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R. and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. Mol. Cell. Biol. 22, 8774–8786.
[38] Dupont, C.-H., Mazat, J.P. and Guérin, B. (1985) The role of adenine nucleotide translocation in the energization of the inner membrane of mitochondria isolated from ρ^+ and ρ^0 strains of *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 132, 1116–1123.
[39] Aguilaniu, H., Gustafsson, L., Rigoulet, M. and Nyström, T. (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science 299, 1751–1753.
[40] Kirchman, P.A., Miceli, M.V., West, R.L., Jiang, J.C., Kim, S. and Jazwinski, S.M. (2003) Prohibitins and Ras2 protein cooperate in the maintenance of mitochondrial function during yeast aging. Acta Biochim. Polon. 50, 1039–1056.
[41] Steglich, G., Neupert, W. and Langer, T. (1999) Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol. Cell. Biol. 19, 3435–3442.
[42] Nijtmans, L.G.J., de Jong, L., Artal Sanz, M., Coates, P.J., Berden, J.A., Back, J.W., Muijsers, A.O., van der Spek, H. and Grivell, L.A. (2000) Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J. 19, 2444–2451.
[43] Hoopes, L.L., Budd, M., Choe, W., Weitao, T. and Campbell, J.L. (2002) Mutations in DNA replication genes reduce yeast life span. Mol. Cell. Biol. 22, 4136–4146.
[44] Dejean, L., Beauvoit, B., Bunoust, O., Guérin, B. and Rigoulet, M. (2002) Activation of Ras cascade increases the mitochondrial enzyme content of respiratory competent yeast. Biochem. Biophys. Res. Commun. 293, 1383–1388.
[45] Sun, J., Kale, S.P., Childress, A.M., Pinswasdi, C. and Jazwinski, S.M. (1994) Divergent roles of *RAS1* and *RAS2* in yeast longevity. J. Biol. Chem. 269, 18638–18645.
[46] Shama, S., Kirchman, P.A., Jiang, J.C. and Jazwinski, S.M. (1998) Role of *RAS2* in recovery from chronic stress: effect on yeast life span. Exp. Cell Res. 245, 368–378.

[47] Lee, S.S., Lee, R.Y.N., Fraser, A.G., Kamath, R.S., Ahringer, J. and Ruvkun, G. (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat. Genet. 33, 40–48.
[48] Dillin, A., Hsu, A.-L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J. and Kenyon, C. (2002) Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.
[49] Vanfleteren, J.R. and De Vreese, A. (1995) The gerontogenes *age-1* and *daf-2* determine metabolic rate potential in aging *Caenorhabditis elegans*. FASEB J. 9, 1355–1361.
[50] Holt, S.J. and Riddle, D.L. (2003) SAGE surveys *C. elegans* carbohydrate metabolism: evidence for an anaerobic shift in the long-lived dauer larva. Mech. Ageing Dev. 124, 779–800.