Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mechanisms of Ageing and Development 126 (2005) 243–248
mechanisms of ageing and development
www.elsevier.com/locate/mechagedev

Yeast longevity and aging—the mitochondrial connection

S. Michal Jazwinski

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

Available online 21 October 2004

Abstract

Studies of the yeast *Saccharomyces cerevisiae* reveal four processes determining life span: metabolism, stress resistance, chromatin-dependent gene regulation, and genome stability. The retrograde response, which signals mitochondrial dysfunction resulting in changes in nuclear gene expression, extends yeast life span and is induced during normal aging. This response involves extensive metabolic adaptations. The retrograde response links metabolism and genome stability during yeast aging. A reduction in the availability of nutrients also extends yeast life span. This metabolic mechanism operates by pathways distinct from the retrograde response, although it shares with the latter some longevity effectors. Life extension by calorie restriction entails re-modeling of mitochondrial function. The retrograde response appears to compensate for age changes, while calorie restriction may be a preventive mechanism. The maintenance of age asymmetry between the mother and daughter yeast cells also depends on mitochondrial function. Loss of this age asymmetry occurs during normal yeast aging and may be a paradigm for stem cell aging. The importance of mitochondrial integrity in yeast longevity is emphasized by the role of prohibition function in attenuating oxidative damage. Our studies point to the central role of mitochondria in yeast aging. They highlight the importance of the maintenance of mitochondrial membrane potential, which drives the transport of biosynthetic precursors derived from the Krebs cycle. Common threads weave their way through the studies of aging in yeast and in other model organisms. This suggests conserved features of aging across phyla.

Keywords: Retrograde response; Calorie restriction; Genome stability; Stress resistance; Mitochondrial membrane potential; Reactive oxygen species

1. Introduction

The yeast *S. cerevisiae* has become an accepted model in aging research (Guarente and Kenyon, 2000; Jazwinski, 2003a). The limits to yeast longevity have been examined in two different experimental paradigms: chronological aging is measured by the loss of cell viability during storage in stationary culture. The metric of replicative aging is the number of daughters produced by individual yeast cells. The latter is the subject of this discussion, although there appears to be a relationship between the two (Ashrafi et al., 1999).

Each time a yeast cell divides the probability that it will divide again decreases (Mortimer and Johnston, 1959), and this decline is exponential (Pohley, 1987; Jazwinski et al., 1989). Interestingly, the mortality rate plateaus for the last 10% or so of an aging yeast cohort (Jazwinski et al., 1998), indicating that the demise of the remaining yeasts is due to stochastic extrinsic events. A finite life span does not in itself demonstrate a biological aging process at work. It is apparent, however, that yeasts do age. This is readily concluded from the observation of the many changes that occur during the replicative life span, some of which clearly represent functional decline (Jazwinski, 2003b).

One of the strengths of the yeast model is the facility with which genetic analysis can be applied. Over the past 15 years, some 40 genes have been implicated in yeast aging. These genes encode proteins, which are involved in a wide array of biochemical processes. However, it has been clear for some time that these processes fall into just four categories: metabolism, stress resistance, chromatin-dependent gene regulation, and genome stability (Jazwinski, 1996). Importantly, this categorization is applicable to longevity genes identified in other species (Jazwinski, 1996; Martin et al., 1996; Guarente and Kenyon, 2000), including

E-mail address: sjazwi@lsuhsc.edu.

0047-6374/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2004.08.016

human (Geesaman et al., 2003; Barzilai et al., 2003). This article emphasizes the metabolic aspects of life span determination in yeast. These aspects appear to play a primary role in longevity with the other three categories listed above having a derivative function, as will be evident from the discussion.

2. Retrograde response

The measure of the replicative life span is the number of daughters produced by a yeast mother cell. This production requires significant metabolic activity. Thus, metabolism is in reality a surrogate measure of longevity. This argues that metabolism is important in determining life span. However, nothing beats an experimental demonstration of this truism. Such a demonstration first came with our direct implication of the retrograde response in determining yeast longevity (Kirchman et al., 1999).

The retrograde response is a pathway of interorganellar communication (Butow, 2002). This signaling pathway is triggered by mitochondrial dysfunction. It is most readily observed in petite yeast cells. These are cells that have dysfunctional mitochondria, because of mutations in or complete lack of mitochondrial DNA (Parikh et al., 1987) or because of mutations in nuclear genes that encode certain mitochondrial proteins (Kirchman et al., 1999). The key signaling proteins in the retrograde response are Rtg2p and the transcription factor Rtg1p–Rtg3p, which is activated by Rtg2p. However, this is an elaborate pathway, which cross talks with other pathways that sense cellular status (Jazwinski, 2003a). The retrograde response is potentiated by Ras2p (Kirchman et al., 1999). The net effect of activation of the retrograde pathway is the translocation of the Rtg1p–Rtg3p from the cytoplasm to the nucleus (Sekito et al., 2000), and the resulting induction of numerous nuclear genes (Epstein et al., 2001). These induced genes code for metabolic enzymes and stress proteins.

The retrograde response portends a remarkable metabolic adaptation to the metabolic duress resulting from mitochondrial dysfunction. Notably, there is an induction of glyoxylate cycle genes. It appears that the cell can utilize fatty acids and acetate, in general, as a carbon source. This is a more economical source of biosynthetic precursors than the Krebs cycle, because the two carbon atoms of acetate are conserved by the glyoxylate cycle, while the Krebs cycle releases them as carbon dioxide. This and other anaplerotic reactions bolster the flagging activity of the Krebs cycle in petite yeast cells. The retrograde response is induced in direct proportion to the extent of mitochondrial dysfunction (Kirchman et al., 1999). Furthermore, the extent of life extension is directly proportional to the activity of the retrograde response (Jazwinski, 2000). Thus, the retrograde response is not a simple on–off switch, but rather it responds in a continuous manner to the changing metabolic needs of the cell. The life extension observed in petites might be considered a curiosity that has nothing to do with normal aging. However, we have shown that mitochondrial dysfunction accumulates with age in yeast (Lai et al., 2002), as much as it does in human cells (Shigenaga et al., 1994). The dysfunction is likely linked to the increased production of oxidants by mitochondria in old cells (Laun et al., 2001). Significantly, this accumulating dysfunction coincides with an increasing induction of the retrograde response (Borghouts et al., 2004). Thus, the retrograde response has a role to play during the course of normal yeast aging. Perhaps its induction is the reason yeasts live as long as they do.

Evidence has been available that the glyoxylate cycle is active in long-lived *Caenorhabditis elegans* (Vanfleteren and De Vreese, 1995). This activity is part of a pattern of gene expression changes, similar to the retrograde response, which is characteristic of the long-lived dauer larval state in this nematode (Holt and Riddle, 2003). Interestingly, long-lived nematodes result from the attenuation of the expression of genes encoding mitochondrial proteins (Lee et al., 2003; Dillin et al., 2002). This finding was the result of a long-lived mutant hunt in one case (Lee et al., 2003). It is worth noting that mitochondrial encephalopathies take several years to develop pronounced symptoms. This may be partially due to metabolic adaptations, much like the retrograde response that compensate for the primary mitochondrial defect.

The activation of the retrograde response has a puzzling consequence in yeast. It results in a higher than usual steady state level of extrachromosomal ribosomal DNA circles (Conrad-Webb and Butow, 1995). These circles can cause cellular demise (Sinclair and Guarente, 1997). It is now known that the retrograde response can counteract the detrimental effects of these circles and at the same time potentiate life extension independently (Borghouts et al., 2004). The Rtg2p plays a critical role in the relationship between the production of these circles and the induction of the retrograde response. It suppresses the production of circles. However, it cannot do this when it is transmitting the retrograde signal (Borghouts et al., 2004). Rtg2p may function in several protein complexes in the cell, and its quantities appear to be limited, so that it cannot protect the cell from the circles and perform in other capacities at the same time. This protein is part of the SLIK histone acetyltransferase complex, which is a transcriptional co-activator (Pray-Grant et al., 2002). It is also involved in suppression of the events associated with trinucleotide repeat expansion by recombination (Bhattacharyya et al., 2002).

Nutrient limitation can also extend yeast longevity (Jiang et al., 2000; Lin et al., 2000), and in many ways it resembles the calorie restriction paradigm described in rodents (Masoro, 1995). This mode of life extension operates in a pathway clearly separate from the retrograde response (Jiang et al., 2000). However, some of the downstream longevity effectors may be common to both, as indicated by the genetic analysis. Combining nutrient limitation with mutations in histone deacetylase genes has delimited the range of gene expression changes associated with life extension by calorie restriction in

yeast (Jiang et al., 2002). The results point to carbohydrate/energy metabolism and reflect a re-modeling of mitochondrial function. Similar conclusions have been reached by others (Lin et al., 2002), although the details likely differ, given the inconsistencies in the role that the Sir2p appears to play. It is not likely that enhanced respiration increases life span, as has been claimed (Lin et al., 2002). When yeasts are grown on a non-fermentable carbon source that results in respiratory growth, there is no increase in life span (Egilmez et al., 1990). Calorie restriction may be a preventive mechanism that extends life span, while the retrograde response is obviously compensatory.

3. Age asymmetry between mother and daughter cells

A full understanding of yeast aging requires an elucidation of the mechanism underlying the aging of the mother cell. As importantly, it is essential to grasp the processes that determine that daughter cells are born young and have before them the potential for a full life span. This capacity is not absolute, however. Daughters born of old mothers have this capacity diminished (Högel and Müller cited in Jazwinski, 1993; Kennedy et al., 1994). Indeed, the senescent phenotype is restricted to mother cells early in the life span, but it progressively becomes part of the inheritance of the daughters (Egilmez and Jazwinski, 1989). Daughters receive a cytoplasmic 'senescence factor' from their mothers, whose effects they can gradually overcome. It appears that the quantity they receive from old mother cells is, however, overwhelming.

We have instituted a hunt for mutants in this senescence factor by screening for conditional mutants for clonal senescence (Lai et al., 2002). This yielded mutants in which the age asymmetry between mother and daughter is abrogated. These mutants showed neither abnormal accumulation of extrachromosomal ribosomal DNA circles nor telomere shortening. One of the mutants was shown to be the result of a single-base change in the nuclear *ATP2* gene, which encodes the -βsubunit of mitochondrial ATP synthase. This mutation caused a subtle and progressive loss of mitochondrial membrane potential ($\Delta\psi_m$) over time. This was followed by the loss of mitochondrial mass from the cells. It was the young cells in the population, which showed this loss of mitochondria preferentially. Microscopic examination demonstrated a change in mitochondrial morphology and distribution in the cell. There was a defect in the segregation of mitochondria from mothers to daughters during cell division. The net result was the generation of a population of cells that were totally devoid of mitochondria.

The *atp2* mutant demonstrates the significance of active mitochondria in the maintenance of age asymmetry between mother and daughter. The phenotype of this mutant is, however, an induced pathology, and the question arises as to the role of this phenomenon in normal aging. We have demonstrated that older mother cells have a tendency to segregate dysfunctional mitochondria to their daughters (Lai et al., 2002). This indicates that what we observe to be accentuated in the *atp2* mutant is part and parcel of normal yeast aging. Our results also support the notion that dysfunctional mitochondria are the senescence factor whose operation we had adduced some time ago.

The studies of age asymmetry in yeast have implications for stem cell aging. The renewal of many tissues requires the function of stem cells. This renewal is critical for maintenance of function as the organism ages. However, it is essential that the cells that the stem cells produce are pristine and possess full functional ability. Our studies show that such stem cells may age, if the analogy with the yeast mother cell is appropriate, and that this leads to the production of cells with reduced functional ability. At least one of the factors important in maintenance of this functional ability is active mitochondria. We have proposed, in general, that there must be 'filters' operative that guarantee that daughter cells receive fully functional components (Lai et al., 2002). The decay of these filters may be a cause of aging of cells and tissues, and ultimately organisms.

How does the mutation in *ATP2* lead to the profound changes in cell physiology described here? This mutation has no effect on energy production and growth (Lai et al., 2002). The activity of the mitochondrial electron transport chain drives the synthesis of ATP. However, it also generates the $\Delta\psi_m$ that is utilized to power the transport of biosynthetic precursors out of the mitochondrion. Some of these precursors are the intermediates in the Krebs cycle. When yeasts are fermenting glucose, the bulk of the ATP is generated at the substrate level in glycolysis. The ATP is used among others in the maintenance of $\Delta\psi_m$. This occurs through the exchange of mitochondrial ADP for ATP by the action of the ADP–ATP translocator in the inner mitochondrial membrane. For this reaction to proceed, the ATP synthase must act in reverse to hydrolyze ATP within the mitochondrion (Dupont et al., 1985). The *atp2* mutant exhibits a subtle defect in this activity, which gives rise to a reduction of $\Delta\psi_m$ over time. The age asymmetry phenotype of the *atp2* mutant is observed when the cells are grown on glucose, but not on glycerol, when the $\Delta\psi_m$ is generated by the electron transport chain (Lai et al., 2002). As mentioned earlier, the $\Delta\psi_m$ declines with age. This indicates that $\Delta\psi_m$ and the processes it supports may play a crucial role in determining yeast longevity, a conclusion, which is supported by our studies of age asymmetry. Among the crucial processes, the provision of biochemical precursors from the Krebs cycle seems to be of paramount importance, as the studies of the retrograde response and calorie restriction suggest.

4. Prohibitins, mitochondrial sentinels

Some time ago, we reported that yeast prohibitin plays a role in determining life span (Jazwinski, 1996). We

indicated that this was observed in petite yeast and that there was an interaction with the *RAS2* gene. Several studies have since been published detailing the biochemistry and cell biology of the yeast prohibitins, Phb1p and Phb2p, and their mammalian homologs (Nijtmans et al., 2002). It is clear that these proteins are located at the inner mitochondrial membrane and that they play a role as chaperones of mitochondrial membrane protein subunits. However, many of the published findings have been inconsistent and incomplete. Therefore, we have recently detailed and expanded our initial studies (Kirchman et al., 2003).

Deletion of either or both of the prohibitins has no effect on the life span of yeasts with fully functional mitochondria. However, there is a marked reduction in longevity when *PHB1* and/or *PHB2* are deleted in petites. This reduction in life span is due to a large extent to a very pronounced increase in the mortality of cells early in the life span. In fact, the mortality curves are clearly bimodal, with a portion of the population suffering early demise, and the remainder leading a near normal life span. Interestingly, deletion of the *RAS2* gene suppresses this decreased life span. The expression of the prohibitins declines during yeast aging. This, in conjunction with the decline in mitochondrial function noted earlier, could result in old yeast cells in the equivalent of the prohibitin deletion in the petite.

We next examined the effect of deletion of the prohibitin genes on the appearance of the mitochondria (Kirchman et al., 2003). The mitochondria were visualized by immunostaining the outer mitochondrial membrane porin. Although no effect was observed in cells with fully functional mitochondria, the deletion of *PHB1* in petites resulted in a dramatic change in mitochondrial morphology and distribution in the cell. There was also a defect in mitochondrial segregation. Quantitatively, this defect could readily account for the early demise of cells that had been observed. A fraction of the cells showed a very pronounced production of reactive oxygen species, which was localized to the mitochondria. Although the steady state level of these oxidatively stressed cells was low, their number could accumulate to account for the high initial mortality of the prohibitin mutant in the petite background. The deletion of *RAS2* reversed the morphological changes in the mitochondria caused by prohibitin deletion, and it also suppressed the appearance of cells producing large amounts of reactive oxygen species.

One interpretation is that the oxidative damage to the mitochondria is stochastic. However, the production of oxidants would accelerate once a threshold in damage had been sustained, resulting in the stratification of the cell population and the emergence of cohort suffering early death. Such an epigenetic stratification of aging populations has been proposed earlier on theoretical grounds (Jazwinski et al., 1998). In any case, it would be damaged mitochondria that would limit life span, regardless of any overt morphological changes that would be secondary.

The deletion of prohibitins in petite yeasts produces an induced pathology. However, this pathology is informative of the events that occur during normal yeast aging. Mitochondrial dysfunction, as measured by loss of $\Delta\psi_m$, accumulates with age (Lai et al., 2002), as does the appearance of oxidative damage (Laun et al., 2001). Expression of the prohibitin genes declines during yeast aging (Kirchman et al., 2003). This would accelerate the deleterious mitochondrial changes, resulting in further compromise of mitochondrial function and the improper segregation of the organelle. These deleterious changes may be balanced for a time by the normal reduction in *RAS2* expression that occurs with age (Sun et al., 1994). However, this reduction in Ras2p activity also decreases the compensating retrograde response (Kirchman et al., 1999), and it further accelerates the decline in $\Delta\psi_m$ because of the role of *RAS2* in potentiating the expression of ATP synthase (Mabuchi et al., 2000). The deletion of prohibitins in petite yeasts results in a phenocopy of an old yeast cell with compromised mitochondria that produce large amounts of reactive oxygen species (Kirchman et al., 2003).

The question is what is the underlying mechanism of the effects described above? Petites lacking mitochondrial DNA cannot make intact complexes of the electron transport chain. However, they can still make the nuclear encoded subunits of these complexes. These supernumerary subunits are held in the inner mitochondrial membrane by the prohibitins acting as chaperones (Nijtmans et al., 2000), and they are degraded there by the mAAA-protease (Steglich et al., 1999). We have proposed that the prohibitins deliver such supernumerary subunits to the mAAA-protease for disposal (Kirchman et al., 2003). The absence of the prohibitins results in an accumulation of these subunits, which is likely to result in the enhanced production of reactive oxygen species by the defective complexes of the electron transport chain with all of the sequela discussed above. The reason that deletion of *RAS2* suppresses the phenotypes of the prohibitin deletion in petites is that it reduces the production of the nuclear encoded components of the electron transport chain (Dejean et al., 2002).

5. From yeast to human

The genetic analysis of aging in the yeast model system points to the role of mitochondria in the etiology of aging. The maintenance of $\Delta\psi_m$ appears to have a predominant role in determining yeast longevity. The $\Delta\psi_m$ affects mitochondrial integrity during the life span, and it also is important in driving the transport of biochemical precursors out of the mitochondria and into the cytoplasm where they are available for the synthesis of new daughter cells. Thus, mitochondria determine life span in a rather direct way in yeast. The fundamental nature of the role of providing these biochemical precursors for yeast longevity is underscored by the compensatory retrograde response, which substitutes the

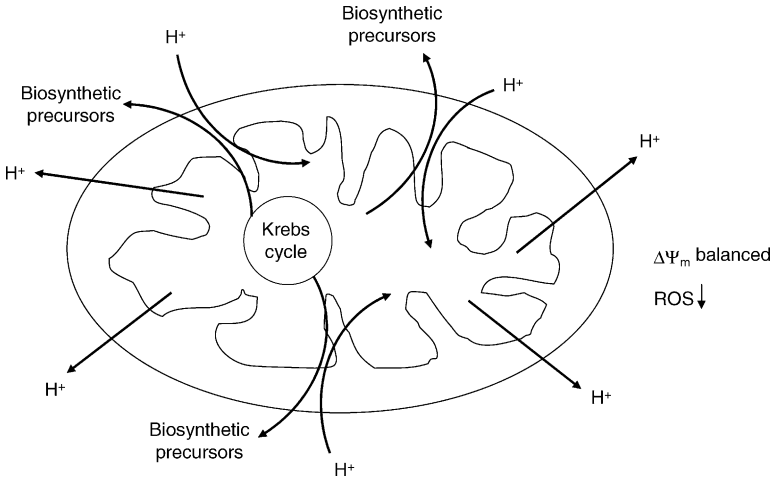

Fig. 1. Role of the mitochondrion in yeast longevity. The Krebs cycle and other biochemical pathways that are localized within the mitochondrion provide biosynthetic precursors for the production of daughter cells, which are the measure of the yeast life span, making metabolism the de facto metric. These precursors must be transported out of the mitochondrion. This transport is driven by the mitochondrial membrane potential ($\Delta\psi_m$). Thus, mitochondrial metabolism, which drives yeast cells through their replicative life span, coincidentally dissipates the $\Delta\psi_m$. An attenuation of $\Delta\psi_m$ lowers the production of reactive oxygen species (ROS), which reduces oxidative stress and damage to the mitochondrion and to the cell. This has its own salutary effect on yeast longevity. A disruption of the balance in the generation and dissipation of $\Delta\psi_m$ results in aging.

glyoxylate cycle for the Krebs cycle for this purpose. Mitochondrial integrity denotes the proper morphology, distribution in the cell, and segregation of this organelle. This integrity has substantial implications not only for the aging of yeast mother cells but also for the production of daughter cells that have full functional capabilities. In this respect, the yeast model can be informative in the study of stem cell aging in mammals.

Metabolism, and especially mitochondrial metabolism, plays an important role in yeast aging. This role is primary, while the role of stress resistance, chromatin-dependent gene regulation and genome stability are derivative. The relationship of the latter two processes to metabolism should be transparent from what has been described already. The Rtg2p links metabolism with genome stability in yeast longevity, as discussed above. The histone deacetylases have a permissive role in the calorie restriction response that extends yeast life span, as alluded to earlier. The link between metabolism and stress, especially oxidative stress, seems intuitively obvious. The central position of $\Delta\psi_m$ in connecting yeast longevity and stress resistance may be less so. As stated earlier, the significance of the mitochondrion in yeast longevity is to provide biochemical precursors, first and foremost. This function has a secondary salutary effect, because the transport of these precursors is driven by the $\Delta\psi_m$ (Fig. 1). This transport results in an attenuation of the $\Delta\psi_m$, which in turn would have the effect of reducing the production of reactive oxygen species (Nicholls, 2004).

The yeast-aging model is a distant mirror, in which we can view human aging. We do not expect a one on one relationship between yeast and human aging. However, the yeast model focuses our attention on metabolism, and especially energy metabolism, as a key factor in human aging. We have instituted the Louisiana Healthy Aging Study to test the hypothesis that the characteristics of an individual's energy metabolism determine longevity with the retention of physical and cognitive ability, which in turn is the source of the feeling of well being associated with healthy aging. This study will provide a critical test of the utility of model systems for the analysis of human aging.

Acknowledgements

This research was supported by grants from the National Institute on Aging of the National Institutes of Health (U.S.P.H.S.) and by the Louisiana Board of Regents Millennium Trust Health Excellence Fund Grant [HEF (2001-06)-02].

References

Ashrafi, K., Sinclair, D., Gordon, J.I., Guarente, L., 1999. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96, 9100–9105.

Barzilai, N., Atzmon, G., Schechter, C., Schaefer, E.J., Cupples, A.L., Lipton, R., Cheng, S., Shuldiner, A.R., 2003. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA 290, 2030–2040.

Bhattacharyya, S., Rolfsmeier, M.L., Dixon, M.J., Wagoner, K., Lahue, R.S., 2002. Identification of *RTG2* as a modifier gene for CTG*CAG repeat instability in *Saccharomyces cerevisiae*. Genetics 162, 579–589.

Borghouts, C., Benguria, A., Wawryn, J., Jazwinski, S.M., 2004. Rtg2 protein links metabolism and genome stability in yeast longevity. Genetics 166, 765–777.

Butow, R.A., 2002. Cellular responses to mitochondrial dysfunction: it's not always downhill. Cell Death Differ. 9, 1043–1045.

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

Dejean, L., Beauvoit, B., Bunoust, O., Gu.rin, B., Rigoulet, M., 2002. Activation of Ras cascade increases the mitochondrial enzyme content of respiratory competent yeast. Biochem. Biophys. Res. Commun. 293, 1383–1388.

Dillin, A., Hsu, A.-L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., Kenyon, C., 2002. Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.

Dupont, C.H., Mazat, J.P., Guerin, 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.

Egilmez, N.K., Jazwinski, S.M., 1989. Evidence for the involvement of a cytoplasmic senescence factor in the aging of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. 171, 37–42.

Egilmez, N.K., Chen, J.B., Jazwinski, S.M., 1990. Preparation and partial characterization of old yeast cells. J. Gerontol. 45, B9–B17.

Epstein, C.B., Waddle, J.A., Hale, W.T., Dave, V., Thornton, J., Macatee, T.L., Garner, H.R., Butow, R.A., 2001. Genome-wide responses to mitochondrial dysfunction. Mol. Biol. Cell 12, 297–308.

Geesaman, B.J., Benson, E., Brewster, S.J., Kunkel, L.M., Blanche, H., Thomas, G., Perls, T.T., Daly, M.J., Puca, A.A., 2003. Haplotype-based identification of a microsomal transfer protein marker with the human lifespan. Proc. Natl. Acad. Sci. USA 100, 14115–14120.

Guarente, L., Kenyon, C., 2000. Genetic pathways that regulate ageing in model organisms. Nature 408, 255–262.

Holt, S.J., 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.

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., 2000. Metabolic control and gene dysregulation in yeast aging. Ann. N. Y. Acad. Sci. 908, 21–30.

Jazwinski, S.M., 2003a. Mitochondria, metabolism, and aging in yeast. In: Nystrm, T., Osiewacz, H.D. (Eds.), Model Systems in Ageing. Springer, Berlin, Germany, pp. 39–59.

Jazwinski, S.M., 2003b. Yeast longevity and aging. In: Osiewacz, H.D. (Ed.), Aging of Organisms. Kluwer, Dordrecht, Netherlands, pp. 1–30.

Jazwinski, S.M., Egilmez, N.K., Chen, J.B., 1989. Replication control and cellular life span. Exp. Gerontol. 24, 423–436.

Jazwinski, S.M., Kim, S., Lai, C.-Y., Benguria, A., 1998. Epigenetic stratification: the role of individual change in the biological aging process. Exp. Gerontol. 33, 571–580.

Jiang, J.C., Jaruga, E., Repnevskaya, M.V., Jazwinski, S.M., 2000. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. FASEB J. 14, 2135–2137.

Jiang, J.C., Wawryn, J., Shantha Kumara, H.M., 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.

Kennedy, B.K., Austriaco, N.R., Guarente, L., 1994. Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life span. J. Cell Biol. 127, 1985–1993.

Kirchman, P.A., Kim, S., Lai, C.-Y., Jazwinski, S.M., 1999. Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. Genetics 152, 179–190.

Kirchman, P.A., Miceli, M.V., West, R.L., Jiang, J.C., Kim, S., Jazwinski, S.M., 2003. Prohibitins and Ras2 protein cooperate in the maintenance of mitochondrial function during yeast aging. Acta Biochim. Pol. 50, 1039–1056.

Lai, C.-Y., Jaruga, E., Borghouts, C., 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.

Laun, P., Pichova, A., Madeo, F., Fuchs, J., Ellinger, A., Kohlwein, S., Dawes, I., Frohlich, K.U., Breitenbach, M., 2001. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. Mol. Microbiol. 39, 1166–1173.

Lee, S.S., Lee, R.Y.N., Fraser, A.G., Kamath, R.S., Ahringer, J., Ruvkun, G., 2003. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat. Genet. 33, 40–48.

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

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G.R., Guarente, L., 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature 418, 344–348.

Mabuchi, T., Ichimura, Y., Takeda, M., Douglas, M.G., 2000. *ASC1/RAS2* suppresses the growth defect on glycerol caused by the *atp1-2* mutation in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 275, 10492–10497.

Martin, G.M., Austad, S.N., Johnson, T.E., 1996. Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nat. Genet. 13, 25–34.

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

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

Nicholls, D.G., 2004. Mitochondrial membrane potential and aging. Aging Cell 3, 35–40.

Nijtmans, L.G., Artal, S.M., Grivell, L.A., Coates, P.J., 2002. The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing, and degenerative disease. Cell. Mol. Life Sci. 59, 143–155.

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., Grivell, L.A., 2000. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J. 19, 2444–2451.

Parikh, V.S., Morgan, M.M., Scott, R., Clements, L.S., Butow, R.A., 1987. The mitochondrial genotype can influence nuclear gene expression in yeast. Science 235, 576–580.

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

Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R., Grant, P.A., 2002. The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. Mol. Cell. Biol. 22, 8774–8786.

Sekito, T., Thornton, J., 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.

Shigenaga, M.K., Hagen, T.M., Ames, B.N., 1994. Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. USA 91, 10771–10778.

Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. Cell 91, 1033–1042.

Steglich, G., Neupert, W., Langer, T., 1999. Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol. Cell. Biol. 19, 3435–3442.

Sun, J., Kale, S.P., Childress, A.M., Pinswasdi, C., Jazwinski, S.M., 1994. Divergent roles of *RAS1* and *RAS2* in yeast longevity. J. Biol. Chem. 269, 18638–18645.

Vanfleteren, J.R., De Vreese, A., 1995. The gerontogenes *age-1* and *daf-2* determine metabolic rate potential in aging *Caenorhabditis elegans*. FASEB J. 9, 1355–1361.