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The absence of a mitochondrial genome in rho⁰ yeast cells extends lifespan independently of retrograde regulation

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Abstract

The absence of mtDNA in rho⁰ yeast cells affects both respiration and mitochondrial-nuclear communication (e.g., retrograde regulation, intergenomic signaling, or pleiotropic drug resistance). Previously, it has been reported that some rho⁰ strains have increased replicative lifespans, attributable to the lack of respiration and retrograde regulation. Here, we have been able to confirm that rho⁰ cells exhibit increased replicative lifespans but have found that this is not associated with the lack of respiration or reduced oxidative stress but instead, is related to the lack of mtDNA *per se* in rho⁰ cells. Also, we find no correlation between the strength of retrograde regulation and lifespan. Furthermore, we find that *pdr3* or *rtg2* mutations are not responsible for lifespan extension in rho⁰ cells, ruling out a specific role for *PDR3*-pleiotropic drug resistance or *RGT2*-retrograde regulation pathways in the extended lifespans of rho⁰ cells. Surprisingly, Rtg3p, which acts downstream of Rtg2p, is required for lifespan increase in rho⁰ cells. Together, these findings indicate that the loss of mtDNA *per se* and not the lack of respiration lead to extended longevity in rho⁰ cells. They also suggest that Rtg3p, acting independently of retrograde regulation, mediates this effect, possibly via intergenomic signaling.

Keywords

Aging; Respiration; mtDNA; Mitochondrial-nuclear communication; RTG3

1. Introduction

Point mutations and deletions in mtDNA accumulate and cause a progressive decline in mitochondrial function during aging (Wallace, 1999; Attardi, 2002; Hekimi and Guarente, 2003). These alterations in mtDNA have at least two fundamentally different effects; they change the activity of the mitochondrial respiratory chain complexes and they influence the expression of nuclear genes through crosstalk between the mitochondrion and the nucleus. Hence, mtDNA can affect longevity in at least two distinct ways; by supporting mitochondrial respiration or by modulating nuclear gene expression through mitochondrial-nuclear communication pathways. Many studies have examined the roles of mitochondrial respiration in longevity. These studies have led to some models that emphasize the role of the mitochondrial respiratory chain and oxidative phosphorylation (OXPHOS) in sustaining cell growth and longevity (Dillin et al., 2002) and other models that focus on mitochondrial respiration as a source of reactive oxygen species (ROS) which contribute to oxidative stress and aging phenotype (Laun et al., 2001; Balaban et al., 2005; Cypser et al., 2006; Martin and Grotewiel, 2006).

In contrast, current understanding of the role(s) of mitochondrial-nuclear communication in aging is incomplete. Multiple pathways are involved in the crosstalk from the mitochondrion to the nucleus. Some of these pathways are conserved in both simple and complex eukaryotes (Poyton, 1999; Dagsgaard et al., 2001; Poyton et al., 2003; Butow and Avadhani, 2004; Castello et al., 2006; Liu and Butow, 2006). The earliest of these pathways to be discovered is *retrograde regulation*, which functions to up-regulate some *aerobic* nuclear genes in response to the reduction of mitochondrial respiration (c.f., Butow and Avadhani, 2004; Liu and Butow, 2006). Components of this pathway in yeast cells include: a heterodimeric transcription factor (Rtg1p-Rtg3p), the Tup1-Cyc8 protein complex, which interacts with Rtg1p-Rtg3p (Conlan et al., 1999), and Rtg2p, a cytoplasmic protein that also functions in nitrogen sensing and catabolism (Butow and Avadhani, 2004). When retrograde regulation is induced Rtg3p is partially dephosphorylated and Rtg1p-Rtg3p moves from the cytosol to the nucleus (Sekito et al., 2000). A second well-studied type of mitochondrial-nuclear crosstalk is operational during *hypoxic signaling* and the induction of *hypoxic* genes (Chandel et al., 1998; Kwast et al., 1999; Poyton et al., 2003; Castello et al., 2006, 2008). Hypoxic signaling occurs in cells that experience reduced O₂ levels. It facilitates cellular adaptation to hypoxia and/or anoxia. When cells experience reduced O₂ levels mitochondria produce ROS, nitric oxide, and reactive nitrogen species like peroxynitrite. These can nitrate or nitrosate proteins (e.g., HIF-1 α , Li et al., 2007, involved in the induction of hypoxic genes (Chandel et al., 1998; Guzy et al., 2005; Mansfield et al., 2005; Castello et al., 2006). A third type of mitochondrial-nuclear crosstalk in yeast is involved in the regulation of genes for *pleiotropic drug resistance* proteins (Moye-Rowley, 2005). Pleiotropic drug resistance produces the multidrug-resistance phenotype in yeast cells by expressing ATP binding cassette (ABC) transporter proteins, such as Pdr5p. Loss of mtDNA, elimination of the mitochondrial inner membrane protein Oxa1p coded by the nuclear genome, or loss of normal functional levels of the *F₀* portion of the mitochondrial ATPase all induce pleiotropic drug resistance (Zhang and Moye-Rowley, 2001; Moye-Rowley, 2003, 2005). A fourth type of mitochondrial-nuclear crosstalk, *intergenomic signaling*, in yeast involves mtDNA *per se* and is independent of respiration or oxidative phosphorylation (Farrell et al., 1990; Poyton and McEwen, 1996; Dagsgaard et al., 2001; Woo et al., 2009). Unlike the retrograde regulation, hypoxic signaling, and pleiotropic drug resistance pathways, this pathway is functional in aerobic respiring cells. It affects the expression of 60 or so nuclear genes (Woo et al., 2009).

Previous studies have concluded that lifespan extension in rho⁰ cells is initiated by the absence of respiration and mediated by retrograde regulation. This conclusion was based primarily on the observation that an *rtg2* mutation reversed the lifespan extension in rho⁰ cells (Kirchman et al., 1999). However, subsequent studies (Borghouts et al., 2004; Kaeberlein et al., 2005a) have reported that *RTG2* affects the lifespans of both rho⁺ and rho⁰ cells. Given that retrograde regulation is not induced in respiration-proficient rho⁺ cells (Butow and Avadhani, 2004), these findings cast doubt on the conclusion that retrograde regulation is responsible for the lifespan extension observed in rho⁰ cells. Moreover, it has reported by others that respiration-deficient cells experience shorter, not longer, lifespans (Coates et al., 1997; Piper et al., 2002; Kaeberlein et al., 2005a). Because the pleiotropic drug resistance and intergenomic signaling pathways were discovered after the early studies of Kirchman et al. (1999), it is not yet known whether these other types of mitochondrial-nuclear communication also contribute to longevity in yeast or other organisms. In this study, we have compared the lifespans of nuclear *pet* mutants, which lack respiration but retain a mitochondrial genome, with the lifespans of rho⁰ strains, which lack both respiration and a mitochondrial genome, in order to provide insight concerning the relative role of the mitochondrial genome *per se* and respiration in lifespan and, at the same time, ask if intergenomic signaling, retrograde regulation, or the pleiotropic drug resistance pathways are involved.

2. Materials and methods

2.1. Yeast strains

The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. As indicated, construction of strains JM43, JM43p⁰, JM43-GD6, JM43-GD100, JM6, JM48, JM55, D273-10B, and D273-10Bp⁰ has been published previously. Genetic complementation tests between JM43-GD6 or JM43-GD100 and JM6 were routinely performed to be sure that neither the JM43-GD6 nor JM43-GD100 cells used in this study was rho⁰. *RTG2*, *RTG3*, and *PDR3* deletion strains were constructed using homologous recombination of site-specific cassettes amplified by PCR (Petracek and Longtine, 2002). Deletion cassettes containing the *Escherichia coli* *kan^r* gene, which confers resistance to G418/geneticin (Jimenez and Davies, 1980; Hadfield et al., 1990), were used to transform JM43 and JM43p⁰ by the lithium acetate procedure (Ito et al., 1983). PCR primers used in deletion strain construction are listed in Table 2. Strains YDW2 and YDW6 were constructed by a *kar1*-mediated mtDNA transfer (Georgieva and Rothstein, 2002) to JM43p⁰. Briefly, Strain JM43p⁰ was mated with strain JM48 (or JM55) (Valencik et al., 1989) which contains a nuclear mutation in the *KAR1* gene (Conde and Fink, 1976), which is required for nuclear fusion. Cytoductants from a cross between JM43p⁰ and JM48 (or JM55) were picked and screened for the auxotrophic markers present in JM43p⁰ and JM48 (or JM55) to identify which nucleus they contain. Those cytoductants that contain the mitochondrial DNA from JM48 (or JM55) were identified by their ability to grow on glycerol, a nonfermentable carbon source. Strain YDW2 is a cytoductant with a JM43 nuclear background and mtDNA from JM48 and strain YDW6 is a cytoductant with a JM43 nuclear background and mtDNA from JM55.

2.2. Measurement of replicative lifespan

Yeast replicative lifespan was measured as described (Park et al., 2002). Briefly, yeast cells were lightly patched to a YPGal plate (1% Bacto-yeast extract, 2% Bacto-peptone, 2% galactose, 2% Bacto-agar). After 3-4 h growth, individual cells were randomly picked under a microscope using a micromanipulator and aligned in isolated areas of the YPGal plate. Cells were allowed to undergo 1-2 divisions. Virgin cells (35-40 new buds) were then separated from their mother cells and subjected to analysis of their replicative lifespans, which was recorded as the total number of daughter cells produced and removed by micromanipulation. Plates were sealed with Parafilm, except during microdissection. Cells were grown at 30 °C during the day and stored at 4 °C at night. Cells were considered dead when budding ceased, and they lost refractility. Each experiment consisted of 35-40 mother cells and was performed at least twice, independently. Statistical significance was determined by the nonparametric Mann-Whitney test using SPSS software (SPSS, Chicago, IL). Strains are stated to have a significant difference in lifespan for *P*-values <0.05.

2.3. Isolation of mitochondria and quantitation of protein-carbonyl content by HPLC

For preparation of mitochondria, cells were grown to mid-exponential phase at 30 °C in YPGal. Cells were harvested and spheroplasted as described (Allen et al., 1995). Mitochondria were isolated from broken spheroplasts by differential centrifugation (McKee and Poyton, 1984). Mitochondrial protein concentrations were determined by the Lowry method using BSA as a standard (Lowry et al., 1951). Carbonyl content of mitochondrial protein fractions was determined after derivatization with 2,4-dinitrophenyl hydrazine (DNPH) (Levine et al., 1994). Derivatized protein samples were fractionated by HPLC on a Waters model 626 fitted with a Waters 600S controller and a Waters 996 Photodiode Array Detector, using Zorbax GF 450 and Zorbax GF 250 gel filtration columns run in series. The columns were calibrated and eluted (flow rate 1 ml/min) with 6 M guanidinium

hydrochloride, 0.5 M KPO₄, pH 2.5. Eluants were monitored for absorbance at 366 and 280 nm (Dirmeier et al., 2004).

2.4. Measurement of respiration

Mitochondrial respiration was measured in strains JM43, JM43p⁰, JM43-GD6, and JM43-GD100 as cyanide-sensitive oxygen consumption at 30 °C with a Strathkelvin Oxygen Meter and accompanying data analysis software. Whole cells (0.25 g wet weight per ml of 40 mM KPO₄, pH 7.4) were added to 3 ml of aerated, 40 mM NaPO₄, pH 7.4, plus 0.67% (w/v) glucose to start the reaction. The reaction was conducted at 30 °C with stirring for a minimum of 3 min. To determine the rate of cyanide-sensitive oxygen consumption, 1 mM KCN was added to the assay solution either at the end of the assay or before the addition of cells in separate assays.

3. Results

3.1. Rho⁰ cells with two different nuclear backgrounds have increased replicative lifespans

In comparing the replicative lifespans of four rho⁰ mutants and their rho⁺ counterparts Kirchman et al. (1999) found that only one rho⁰ strain, made in a YPK9 nuclear background, had an extended lifespan relative to the isochromosomal rho⁺ strain from which it was derived. Rho⁰ strains made in SP1-1 and A364A nuclear backgrounds have shortened lifespans while the lifespan of a rho⁰ strain made in a W303-1A nuclear background was identical to its rho⁺ parent. All of these strains are relatively short-lived. More recently, Kaerberlein et al. (2005a) reported that a rho⁰ strain made in the long-lived nuclear background S288C has a lifespan that was indistinguishable from its rho⁺ parent. Here, we have examined the replicative lifespans in two additional nuclear backgrounds, JM43 and D273-10B, strains with very high respiratory capacity (Sherman, 2002). In addition, replicative lifespan experiments in this study were performed on YPGal plates in which carbon source is galactose that exerts less repression on respiration than glucose, allowing more respiration in rho⁺ strains (Polakis et al., 1965). We have chosen to use galactose in this study because it is a fermentable but non-repressing carbon source which allows for optimal transcription of nuclear genes required for a fully functional respiratory chain and because non-repressing carbon sources (either galactose or raffinose) are used in studies on retrograde regulation (Epstein et al., 2001; Kirchman et al., 1999) and intergenomic signaling (Dagsgaard et al., 2001; Woo et al., 2009). The replicative lifespans in respiration-deficient JM43p⁰ cells show a 33% increase in their mean lifespans ($P < 0.001$) relative to those of the rho⁺ cells, JM43 from which they are derived (Fig. 1A). The replicative lifespans of D273-10Bp⁰ cells also exhibit an increase in lifespan, albeit slight (about 10%), relative to its parental rho⁺ strain, D273-10B ($P = 0.123$) (Fig. 1B). Together, these findings confirm those of Kirchman et al. (1999) that some rho⁰ strains have extended lifespans.

In theory, the differences in lifespan between rho⁰ strains and their rho⁺ parents observed both in earlier studies and in this study may result from different nuclear genomes or from different mitochondrial genomes. In considering yeast mitochondrial genotype it is important to note that these are at least two general types of mitochondrial genome: short and long (Morimoto and Rabinowitz, 1979). The short form is ~70 kb and the long form is ~76 kb. The short genome is identical to the long genome but lacks some introns and intergenic DNA. Strain JM43 has a long mitochondrial genome while strain D273-10B has a short mitochondrial genome. To ask if short and long yeast mitochondrial genomes have differential effects on lifespan we have introduced each one into JM43p⁰ by cytoduction. Strain YDW2 carries the short mitochondrial genome from D273-10B while strain YDW6 carries a long mitochondrial genome. Both short and long mitochondrial genomes added back to JM43p⁰ cells produce cells with essentially the same replicative lifespan as JM43 (P

= 0.089 for YDW2 and $P = 0.142$ for YDW6) (Fig. 1C), indicating that the presence of either mitochondrial genome *per se* reduces the lifespan extension enjoyed by JM43p⁰ cells. This finding supports the conclusion that the lifespan extension observed in rho⁰ cells is linked to the absence of a mitochondrial genome and that both long and short genomes serve to reduce longevity in rho⁺ cells. It also excludes a role for potential lifespan extending nuclear mutations arising during the creation of JM43p⁰ cells.

3.2. The increased replicative lifespans in JM43p⁰ cells are not associated with reduced levels of respiration or oxidative stress

Rho⁰ cells differ from rho⁺ cells in three important ways: they lack mtDNA, they are respiration-deficient, and they have reduced levels of oxidative stress. In order to determine if the lack of respiration or reduced levels of oxidative stress are responsible for the lifespan extension in JM43p⁰, we used two different respiration-deficient mutants, JM43-GD6 and JM43-GD100, which are isogenic with JM43 and JM43p⁰. Strain JM43-GD6 is a nuclear *pet* mutant deleted for *COX6* which encodes the cytochrome *c* oxidase subunit VI (Dagsgaard et al., 2001) and strain JM43-GD100 is a nuclear *pet* mutant deleted for *PET100* which encodes a cytochrome *c* oxidase assembly factor (Church et al., 1996). JM43p⁰, JM43-GD6, and JM43-GD100 each had no measurable cyanide-sensitive mitochondrial respiration (Fig. 2A). Cellular oxidative stress in these strains was assessed by quantifying protein carbonylation, a measure of oxidative protein damage (Dirmeier et al., 2004), using HPLC. When compared to the wild-type JM43, all three respiration-deficient mutants, JM43p⁰, JM43-GD6, and JM43-GD100, showed lower levels of protein carbonylation in their mitochondrial proteins (Fig. 2B). This finding indicates that all of these mutants experience lower oxidative stress relative to their wild-type respiration-proficient JM43. However, there is no correlation between the lack or respiration or level of protein carbonylation and replicative lifespan. As shown in Fig. 2C, JM43p⁰ has a longer replicative lifespan than JM43 whereas JM43-GD6 and JM43-GD100 do not. Surprisingly, JM43-GD6 which has a lower level of oxidative stress compared to JM43, has a shorter lifespan than JM43 (a 43% reduction in their mean lifespans, $P < 0.001$), indicating no direct correlation between lower oxidative stress and lifespan extension in JM43 strains. Based on these findings, it is unlikely that the extended lifespan in JM43p⁰ is attributable to a lack of respiration or reduced oxidative stress, suggesting that it is the loss of mtDNA *per se* and/or mitochondrial-nuclear communication pathways that are responsible.

3.3. Retrograde regulation is not responsible for the extended longevity of JM43p⁰ cells

It was concluded previously that retrograde regulation is responsible for the extended replicative lifespans in rho⁰ cells made in an YPK9 nuclear background (Kirchman et al., 1999). In order to examine a possible role for retrograde regulation in the JM43p⁰ lifespan extension, we have compared mRNA expression patterns for 19 retrograde regulation target genes in respiration-deficient cells with JM43 nuclear background (JM43p⁰ and JM43-GD6) with those in PSY142p⁰ with a strong retrograde response background using published genome-wide expression data (Epstein et al., 2001; Woo et al., 2009). As shown in Fig. 3A, many of the retrograde regulation target genes are not up-regulated in both JM43p⁰ and JM43-GD6 compared to their wild-type JM43 while all of them are induced robustly in PSY142p⁰. A subset of them is induced in JM43p⁰ and JM43-GD6 but not to same extent as in PSY142p⁰ which has a strong retrograde response. This indicates that both JM43p⁰ and JM43-GD6 have a weak retrograde response. Because JM43p⁰ and JM43-GD6 have markedly different lifespans and similarly weak retrograde responses it is clear that the strength of retrograde regulation is not correlated with replicative lifespan in these strains. Moreover, given that of the three respiration-deficient strains used here only JM43p⁰ shows a lifespan extension, it is apparent that the extended lifespan is not correlated with either the lack of respiration or the induction of retrograde regulation in respiration-deficient cells.

Kirchman et al. (1999) reported that the extended lifespan in ρ^0 cells is reversed upon the deletion of the *RTG2* gene. We have been able to confirm that *rtg2* ρ^0 double mutant cells (YDW9) have shorter lifespans than *RTG2* ρ^0 cells (JM43 ρ^0) ($P < 0.001$) (Fig. 3B). However, we also find that *rtg2* ρ^+ cells (YDW7) have shorter lifespans than *RTG2* ρ^+ cells (JM43) ($P < 0.001$). These findings support the conclusion that *RTG2* plays a role in the longevity of both respiration-proficient ρ^+ cells and respiration-deficient ρ^0 cells. Because retrograde regulation is activated by mitochondrial dysfunction and respiration deficiency (Liao and Butow, 1993; Butow and Avadhani, 2004), these findings suggest that the role of *RTG2* in longevity of JM43 strains is unrelated to retrograde regulation.

3.4. Pleiotropic drug resistance is not involved in the lifespan extension in JM43 ρ^0

A second pathway for mitochondrial-nuclear crosstalk is involved in pleiotropic drug resistance. To determine if this pathway functions in the lifespan extension in JM43 ρ^0 cells we made use of strains carrying a deletion in *PDR3*, which encodes a transcription factor Pdr3p that activates transcription of nuclear genes under the control of pleiotropic drug resistance (Moye-Rowley, 2005). We removed the entire coding sequence of *PDR3* from JM43 and JM43 ρ^0 genomes and measured the replicative lifespans in the resulting *pdr3Δ* mutants. As indicated in Fig. 4, no significant changes in replicative lifespans were observed between JM43 and YDW17 (JM43 *pdr3Δ*) ($P = 0.671$) as well as between JM43 ρ^0 and YDW19 (JM43 ρ^0 *pdr3Δ*) ($P = 0.705$). These findings clearly demonstrate that pleiotropic drug resistance is not implicated in the prolonged lifespan in JM43 ρ^0 .

3.5. The *RTG3* gene is required for the lifespan extension in JM43 ρ^0

Recent studies have reported that the Tor pathway (Inoki et al., 2005) can regulate the activity of Rtg3p and bypass Rtg2p (Komeili et al., 2000; Diloova et al., 2002; Butow and Avadhani, 2004; Diloova et al., 2004). Given the key roles of the Tor pathway in aging (Kaeberlein et al., 2005b), it was of interest to ask if *RTG3* is required for the lifespan extension in JM43 ρ^0 , independently of its role in retrograde regulation. As shown in Fig. 5 the *rtg3Δ* has no effect on the lifespan in JM43 ($P = 0.627$) but it reverses the extended lifespan in JM43 ρ^0 back to that of JM43 ($P = 0.018$). In addition, no significant changes in replicative lifespans were observed between JM43 and YDW14 (JM43 *rtg3Δ*) ($P = 0.922$). From the finding that *rtg3Δ* abolishes the increased lifespan in JM43 ρ^0 we conclude that *RTG3* function is necessary for the lifespan extension in JM43 ρ^0 .

4. Discussion

4.1. Mitochondrial DNA per se is responsible for lifespan extension in yeast ρ^0 cells

In this study, we found that ρ^0 mutants made in two different nuclear backgrounds have increased replicative lifespans. Using respiration-deficient *pet* mutants in one of these nuclear backgrounds, JM43, we have been able to effectively rule out both the absence of respiration and reduced levels of oxidative stress as causes of the increased replicative lifespan in JM43 ρ^0 cells. Given that ρ^0 cells differ from ρ^+ cells genotypically in lacking mitochondrial DNA and phenotypically in lacking respiration and having reduced levels of oxidative stress, it appears likely that the extended lifespan in JM43 ρ^0 cells is the result of the lack of mtDNA and not the phenotypic differences between ρ^+ and ρ^0 cells. This conclusion is supported by our finding that cytoductant JM43 strains with either short or long mitochondrial genomes produce cells with essentially the same replicative lifespan as JM43, indicating that it is the presence of a mitochondrial genome *per se* that affects lifespan and that the presence of a mitochondrial genome in JM43, D273-10B and in some other ρ^+ strains (Kirchman et al., 1999) effectively reduces lifespan.

Our findings that neither the respiration deficiency nor reduced levels of oxidative stress in rho^0 cells are responsible for increased lifespan in rho^0 cells are consistent with the observations of Kirchman et al. (1999). However, the finding that rho^0 cells have extended lifespans is in apparent conflict with the recent findings from Guarente and colleagues, who have reported that mitochondrial respiration is both necessary and sufficient to extend replicative lifespan (Lin et al., 2002). The findings from this latter study have been used to propose that a shift from fermentative to respiratory metabolism activates Sir2p (an NAD-dependent histone deacetylase involved in yeast longevity) by increasing the NAD/NADH ratio. In this study, we have found that there is no correlation between the lack of respiration and lifespan in three different strains; JM43p⁰, JM43-GD6, and JM43-GD100. This suggests that it is not simply respiration that influences lifespan. One way to reconcile our findings with those of Kirchman et al. (1999) and Lin et al. (2002) is to propose that *both* mitochondrial genotype and respiration influence lifespan but do so independently of one another. It is also possible that the use of galactose instead of glucose in the present study contributes to the observed differences because, unlike glucose, galactose is a non-repressing carbon source.

4.2. Involvement of the mitochondrial genome in aging

Evidence for a role of mtDNA *per se* in aging is also accumulating from studies with several organisms other than *S. cerevisiae*. Four examples are especially cogent. First, studies with the filamentous fungus, *Podospora anserina*, senescence is linked to the accumulation of circular DNA molecules, called senDNA, which originate from different parts of the mtDNA (Griffiths, 1992). One of them, senDNA α , which comes from the first intron (intron α) of *COX1*, is present in all senescent cultures of wild-type strain. The finding that mitochondrial mutants carrying a deletion of 'the intron α ' escape senescence demonstrates a role of the intron α in accelerating senescence (Begel et al., 1999), indicating that a sequence of mtDNA can be a longevity factor. Second, studies with mtDNA-mutator mice carrying a proof-reading-deficient version of mtDNA polymerase showed that increased levels of mtDNA mutations induced in these mice are associated with premature aging and reduced lifespan (Trifunovic et al., 2004; Kujoth et al., 2005) even though there is no direct correlation between the increased levels of mtDNA mutations and elevated oxidative damages in these mice (Kujoth et al., 2005; Trifunovic et al., 2005). Third, a recent study with human cybrid cell lines showed that rho^0 mutations are distinct from mtDNA point mutations in their effects on nuclear gene expression (Jahangir Tafrechi et al., 2005).

A fourth example comes from human haplogroups. It has been reported that there is an increased incidence of a specific human mtDNA haplogroup (e.g., haplogroup J) in long-lived individuals (Niemi et al., 2003; Ross et al., 2001; De Benedictis et al., 1999; Tanaka et al., 1998; Ivanova et al., 1998), indicating that mtDNA genotype *per se* may predispose resistance to adult-onset diseases and be one of the genetic factors for longevity. One way of explaining these findings is to propose that mtDNA sequence variation within the protein or RNA coding regions on mtDNA may cause slight differences in the level or function of the respiratory chain. While there is some evidence that certain haplogroups may exacerbate or mitigate harmful mutations linked to certain OXPHOS diseases (c.f., Salvioli et al., 2001) there is currently little evidence to support the hypothesis that respiratory chain or OXPHOS functions are altered in those individuals that possess the specific haplogroups that are enriched in long-lived individuals. Thus, it is possible that one or more of the polymorphisms on this haplogroup is silent *vis-à-vis* mitochondrial function but not with respect to longevity.

4.3. Mitochondrial-nuclear communication and lifespan in ρ^0 cells

Although it has been concluded previously that lifespan extension in ρ^0 yeast cells involves retrograde regulation (Kirchman et al., 1999), findings reported earlier (Kaeberlein et al., 2005a) and in this study are inconsistent with that conclusion. These studies find that the loss of respiration, which activates retrograde regulation, by itself does not account for an extended lifespan. Indeed, our findings with JM43 ρ^0 , JM43-GD6, and JM43-GD100, Kirchman's findings (1999) with antimycin A inhibition and a respiration-deficient *cox4* mutant, and studies with mitochondrial prohibitin mutants (Coates et al., 1997; Piper et al., 2002; Kaeberlein et al., 2005a) all support the conclusion that the loss of respiration can have variable effects on lifespan. Moreover, we find here that there is no correlation between the strength of retrograde regulation initiated by respiratory deficiency and lifespan. Although it has been reported earlier that an *rtg2* mutation shortens lifespan in ρ^0 cells, subsequent studies (Borhouts et al., 2004; Kaeberlein et al., 2005a) as well as data reported here reveal that an *rtg2* mutation also shortens lifespan of ρ^+ cells in which retrograde regulation is not activated. Together all of these findings argue against a role for retrograde regulation in the lifespan extension observed in ρ^0 cells. Also, our finding that YDW19 (JM43 ρ^0 *pdr3* Δ) has a similar replicative lifespan to JM43 ρ^0 argues against a role for the pleiotropic drug resistance pathway in the lifespan extension observed in ρ^0 cells.

If retrograde regulation and the pleiotropic drug resistance pathways are not involved in lifespan extension in ρ^0 cells what pathway for mitochondrial-nuclear communication is involved? The finding that the lifespan extension is correlated with the lack of mtDNA and not the loss of respiration or oxidative stress suggests intergenomic signaling as a likely candidate. We have shown previously that the mitochondrial genome can affect the expression of several yeast nuclear genes independently of respiration (Farrell et al., 1990; Dagsgaard et al., 2001; Woo et al., 2009), via intergenomic signaling (Poyton and McEwen, 1996). These genes which are down-regulated in ρ^0 cells but not in respiration-deficient nuclear *pet* mutants may, in turn, affect lifespan. And the finding that an *rtg3* mutation shortens lifespan in a JM43 ρ^0 cells but not in JM43 cells suggests that *RTG3* may mediate the effect of mtDNA on lifespan, independently of its role in retrograde regulation. Interestingly, *RTG3* has been implicated in the Tor pathway which plays an important role in aging in many model organisms including yeast, worm, and fly (Vellai et al., 2003; Kapahi et al., 2004; Kaeberlein et al., 2005b). The Tor pathway modulates the activity of Rtg1p-Rtg3p transcription factor by bypassing Rtg2p through Mks1p and Lst8p (Komeili et al., 2000; Dilova et al., 2002, 2004; Butow and Avadhani, 2004). Thus, it is possible that the extended longevity in JM43 ρ^0 is achieved by the interplay between the mitochondrial genome, intergenomic signaling, the Tor pathway, and Rtg3p. Future studies are aimed at addressing this possibility.

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Fig. 1.

Effect of a ρ^0 mutation, which results in complete loss of mtDNA, on replicative aging in JM43. (A) The lifespan of JM43 ρ^0 (n (number cells examined) = 172) was significantly longer compared to its parent wild-type JM43 (n = 100) (P < 0.001). Mean lifespans are shown in parentheses. (B) The lifespan of D273-10B (n = 27) was slightly longer relative to D273-10B ρ^0 (n = 29). (C) The lifespan of the cytoductants (YDW2 (n = 37) and YDW6 (n = 37)) does not differ from JM43. Re-introduction of mtDNA in JM43 ρ^0 returns the lifespan of JM43 ρ^0 to its wild-type JM43.

Fig. 2.

Effects of respiration deficiency and oxidative stress on lifespan in JM43. (A) Respiration was measured as oxygen consumption at 30 °C by using a Strathkelvin Oxygen Meter. For each measurement, the rate of O₂ consumption measured in the presence of 1 mM potassium cyanide was subtracted from the rate of O₂ consumption. Values are the average of three measurements for each strain grown in YPGal. The respiration rates for strains JM43p⁰, JM43-GD6, and JM43-GD100 are normalized to that of their wild-type parent JM43. The values corresponding to 100% in JM43 was 9.1 ± 1.7 nmol of O₂ consumed per min per mg of cell wet weight. (B) Carbonyl contents for mitochondrial proteins in various respiration-deficient mutants with the JM43 nuclear background are shown. Bars represent mean values $\pm$ standard deviations for three independent measurements. (C) Lifespans in respiration-deficient mutants (JM43p⁰ ($n = 40$), JM43-GD6 ($n = 39$), and JM43-GD100 ($n = 37$)) were compared to their respiration-proficient wild-type JM43 ($n = 40$). Lifespan of JM43-GD6 was significantly shorter than JM43 ($P < 0.001$) while lifespan of JM43-GD100 was not different from JM43 ($P = 0.259$). Mean lifespans are shown in parentheses.

Fig. 3.

Effects of retrograde regulation on lifespan in JM43. (A) Retrograde regulation is weak in JM43 strains. Expression of a range of retrograde regulation targets was extracted from published microarray data. Majority of retrograde regulation targets are not induced in respiration-deficient JM43 strains but robustly induced in a ρ^0 strain with PSY142 nuclear background (Epstein et al., 2001). (B) Deletion of RTG2 shortens lifespans in both JM43 ($P < 0.001$) and JM43p⁰ ($P < 0.001$). Mean lifespans are shown in parentheses. The number of cells examined was: 37 for JM43, 38 for JM43p⁰, 36 for YDW7, and 37 for YDW9.

Fig. 4.

Effects of *PDR3* on lifespan in JM43. Deletion of *PDR3* has no effect on lifespans in both JM43 ($P = 0.671$) and JM43 p^0 ($P = 0.705$), demonstrating that pleiotropic drug resistance is not important for the lifespan extension in JM43 p^0 . Mean lifespans are shown in parentheses. The number of cells examined was: 31 for JM43, 35 for JM43 p^0 , 35 for YDW17, and 39 for YDW19.

Fig. 5.

Roles for *RTG3* in lifespan determination in JM43. Deletion of *RTG3* has no effect on JM43 lifespan ($P = 0.627$) (A) but abolishes the lifespan extension in JM43^{p0} ($P = 0.018$) (B). Mean lifespans are shown in parentheses. The number of cells was: 32 for JM43, 34 for YDW11, 37 for JM43^{p0}, and 40 for YDW14.

Yeast strains used in this study

Table 1

Strain	Genotype	Reference
D273-10B	<i>MATa</i> [ρ^+]	Cumsky et al. (1985)
D273-10B ρ^0	<i>MATa</i> [ρ^0]	McEwen et al. (1986)
JM43	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52</i> [ρ^+]	McEwen et al. (1986)
JM43 ρ^0	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52</i> [ρ^0]	Waterland et al. (1991)
JM43-GD6	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 cox6::URA3</i> [ρ^+]	Dagsgaard et al. (2001)
JM43-GD100	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 pet100::LEU2</i> [ρ^+]	Church et al. (1996)
JM6	<i>MATa his4-580</i> [ρ^0]	Wright et al. (1986)
JM48	<i>MATa leu canf kar1</i> [ρ^+ 'short']	Valencik et al. (1989)
JM55	<i>MATa leu canf kar1</i> [ρ^+ 'long']	Valencik et al. (1989)
YDW2	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52</i> [ρ^+ 'short']	This study
YDW6	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52</i> [ρ^+ 'long']	This study
YDW7	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 rig2 Δ::KAN</i> [ρ^+]	This study
YDW9	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 rig2 Δ::KAN</i> [ρ^0]	This study
YDW11	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 rig3 Δ::KAN</i> [ρ^+]	This study
YDW14	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 rig3 Δ::KAN</i> [ρ^0]	This study
YDW17	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 pdr3 Δ::KAN</i> [ρ^+]	This study
YDW19	<i>MATa his4-580 trp1-289 leu2-3, 112 ura3-52 pdr3 Δ::KAN</i> [ρ^0]	This study

Table 2**PCR primers used in deletion strain construction**

Primers for RTG2 deletion

5'-AACGAAAAGTGTAGGCGTGCCACAAATGTCAACACTTAGCGATAGTGATACCGGATCCCCGGGTTAATTAA-3'

5'-TTATATAAGGATTTCGTATTTATTGTTCAAGTATTTAAAGACTAGATGTCTGAATTCGAGCTCGTTTAAAC-3'

Primers for checking RTG2 deletion

5'-CGTGCCACAAATGTCAACAC-3' and 5'-TCACGGGATTTATTCGTGAC-3'

5'-CGTGCCACAAATGTCAACAC-3' and 5'-GCCTGAGCGAGACGAAATAC-3'

Primers for RTG3 deletion

5'-AACCTACTTCTTAAATAAGTGAAGAATGATGAACAATAACGAAAGTGAGGCCGGATCCCCGGGTTAATTAA-3'

5'-GTCTACCTGACCTTTTTCAAATTTAATTTTTCCCGCTAATAAGACCATAAAGAATTCGAGCTCGTTTAAAC-3'

Primers for checking RTG3 deletion

5'-CTCGGTCCGGACAGTAGGAT-3' and 5'-GCCTGAGCGAGACGAAATAC-3'

Primers for PDR3 deletion

5'-ATTGCGTGACCGCAATGAAAGTGAAGAAATCAACTAGATCCGGATCCCCGGGTTAATTAA-3'

5'-TACTATGTTATGCTCTGCTTCCCTATTTCTTTTGCCTTTGAATTCGAGCTCGTTTAAAC-3'

Primers for checking PDR3 deletion

5'-CCTCTATGCCCTTGTCGGTA-3' and 5'-GCCTGAGCGAGACGAAATAC-3'