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The *MEF2* gene is essential for yeast longevity, with a dual role in cell respiration and maintenance of mitochondrial membrane potential

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ABSTRACT

The *Saccharomyces cerevisiae MEF2* gene is a mitochondrial protein translation factor. Formerly believed to catalyze peptide elongation, evidence now suggests its involvement in ribosome recycling. This study confirms the role of the *MEF2* gene for cell respiration and further uncovers a slow growth phenotype and reduced chronological lifespan. Furthermore, in comparison with cytoplasmic ρ^0 strains, *mef2*Δ strains have a marked reduction of the inner mitochondrial membrane potential and mitochondria show a tendency to aggregate, suggesting an additional role for the *MEF2* gene in maintenance of mitochondrial health, a role that may also be shared by other mitochondrial protein synthesis factors.

1. Introduction

The process of mitochondrial protein translation takes place in four stages, initiation, elongation, termination and ribosome recycling, with each stage carefully coordinated by various nuclear encoded proteins known as mitochondrial translation factors [1,2]. The role of the mitochondrial protein translation factors in eukaryotes is poorly defined. Based on sequence similarity with bacterial elongation factors, the *Saccharomyces cerevisiae MEF2* gene and its human homolog, *EFG2*, were designated as mitochondrial elongation factors [3,4]. A recent study, however, predicts that these genes in fact encode a mitochondrial ribosome recycling factor [5].

The role of the yeast Mef2p protein is further confounded by the contradictory description of *mef2*Δ deletion mutants, with an original functional screen of the *S. cerevisiae* deletion library failing to detect a phenotype [6]. This has been subsequently cited in a number of papers, leading to a belief that the role of the yeast *MEF2* gene in mitochondrial translation is redundant [7–10]. More recent genome-wide screens have identified the *mef2*Δ mutant as a respiratory deficient yeast [11,12].

In this study, a detailed functional characterization of the *mef2*Δ deletion mutant is undertaken. Results confirm that the *MEF2* gene is required for respiratory growth and is essential for maintenance of the mitochondrial genome. Moreover, in comparison to cytoplasmic ρ^0 strains, which lack mitochondrial DNA (mtDNA), *mef2*Δ mutants exhibit a significant slow growth phenotype under normal growth conditions (in glucose) and a reduced chronological lifespan (CLS). Fluorescent staining results indicate that *mef2*Δ strains are compromised in their maintenance of an inner mitochondrial membrane potential (ΔΨ) and electron micrographs show a clustered mitochondrial morphology, raising the possibility that the *MEF2* gene has a dual function, for mitochondrial protein translation and for maintenance of ΔΨ and mitochondrial organization.

2. Materials and methods

2.1. Strains and growth conditions

The haploid wild-type *S. cerevisiae* strain used was BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). The *mef2*Δ/*MEF2* heterozygote deletion mutant was created by PCR amplifying the *KanMX* marker plus an additional flanking 500 bp from an existing *mef2*Δ deletion mutant (EUROSCARF) [6]. The forward primer used was 5′-TCGTCTGTTTCAACTCTGAAGG-3′ and reverse primer was 5′-TTGAAAAAGCAACGACCAGT-3′. The *KanMX* cassette was

Abbreviations: ΔΨ, inner mitochondrial membrane potential; mtDNA, mitochondrial DNA; DAPI, 4′,6-diamidino-2-phenylindol; OD, optical density; CLS, chronological lifespan; RFLP, restriction fragment length polymorphism

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transformed into the BY4743 wild-type diploid (*MAT*a/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 MET15/met15Δ0 ura3Δ0/ura3Δ0*) and the resulting *mef2*Δ/*MEF2* strain was sporulated to obtain the haploid *mef2*Δ deletion mutant used in this study (*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0; YJL102w.*:KanMX4). PCR was performed for deletion verification of all strains as previously described [6].

Cytoplasmic ρ^0 strains were generated by growing BY4742 cells in medium containing 10 μg/ml ethidium bromide for 48 h. Respiratory deficient clones were selected for their inability to utilize glycerol as a carbon source and absence of mtDNA was verified by 4′,6-diamidino-2-phenylindol (DAPI) staining.

For the majority of assays, medium used was liquid YEPD medium (1% yeast extract, 2% peptone, 2% dextrose). Viability counts were performed on solid YEPD medium (liquid YEPD plus 2% agar) and YEPG medium (1% yeast extract, 2% peptone, 3% glycerol, 2% agar) was used to assess respiratory deficiency. Erythromycin treatment involved the addition of 2 mg/ml erythromycin (Sigma) to wild-type cells [13]. Prior to experimentation, yeast strains were cultured in YEPD medium for 24 h, subcultured and then grown to exponential phase for subsequent studies. Incubation temperature was 30 °C unless stated otherwise.

2.2. mtDNA restriction analysis

mtDNA was extracted as described elsewhere [14]. DNA was digested with *Dde*I according to the manufacturer's instructions (New England Biolabs) and DNA fragments were separated on a 0.8% agarose gel containing ethidium bromide. Electrophoresis was run at 80 V for 2 h and bands visualized using the Gel Doc EQ system (Bio-Rad laboratories).

2.3. DAPI staining

Vital cell staining of mtDNA and nuclear DNA was performed by adding DAPI as previously described [15].

2.4. Growth assay

Triplicate exponential phase cultures were diluted to an optical density (OD_{595nm}) of 0.2 and 50 μl of this culture was added to 150 μl of YEPD in wells of a 96-well plate. Cell growth was monitored by measuring OD at 595 nm every hour for 15 h in a Tecan Genios™ microplate reader [16]. Cell doubling time was calculated using the formula $\ln 2/k$ where k is the maximal slope of the curve when $\ln(OD_{595nm})$ is plotted against time.

2.5. CLS assay

CLS assay was performed in a 2 ml volume of water, essentially as previously described [17]. The assay was continued for a period of 13 days. Cell viability was monitored by plating various culture dilutions onto solid YEPD every 3–4 days and colony forming units were counted.

2.6. Visualization of mitochondrial membrane potential

MitoTracker Red CMXRos® (Molecular Probes®) was added directly to a 500 μl volume of exponential phase culture in YEPD to a final concentration of 250 nM (1×), 750 nM (3×) or 2500 nM (10×). Cells were incubated at 30 °C for 30 min, washed with fresh medium and resuspended in YEPD medium. Cells were mounted onto a glass slide and viewed immediately [18]. Fluorescent intensity was quantified by measuring the 'grey value' within the cell cytoplasm or mitochondria on the acquired digital photograph. Measurements were made using the Cell^F software (Olympus). Approximately 50 cells were used for each measurement.

2.7. Microscopy

Fluorescence images were viewed using an Olympus BX51 microscope equipped with a UV filter for DAPI and an intermediate green filter for MitoTracker Red® stained cells. Photographs were taken using Soft Imaging System's Colorview III camera (Olympus), controlled by the Cell^F software (Olympus).

2.8. Transmission electron microscopy (TEM)

Cells were harvested in early log phase, fixed and subjected to 0.15 mg/ml zymolyase (Seigaku) treatment to permeabilise the cell wall as previously described [19]. Further cell fixation was carried out in 2% OsO_4, followed by dehydration and resin infiltration as previously described [20]. Cells were then sectioned and viewed on a Philips CM100 transmission electron microscope.

2.9. Statistical analysis

Statistical tests were carried out using the GraphPad Prism® version 5.04 software for Windows (San Diego, CA).

3. Results

3.1. Deletion of the MEF2 gene results in a respiratory deficient ρ^0 strain

Deletion of the *MEF2* gene gives rise to small colonies on glucose and growth on media containing a non-fermentable carbon

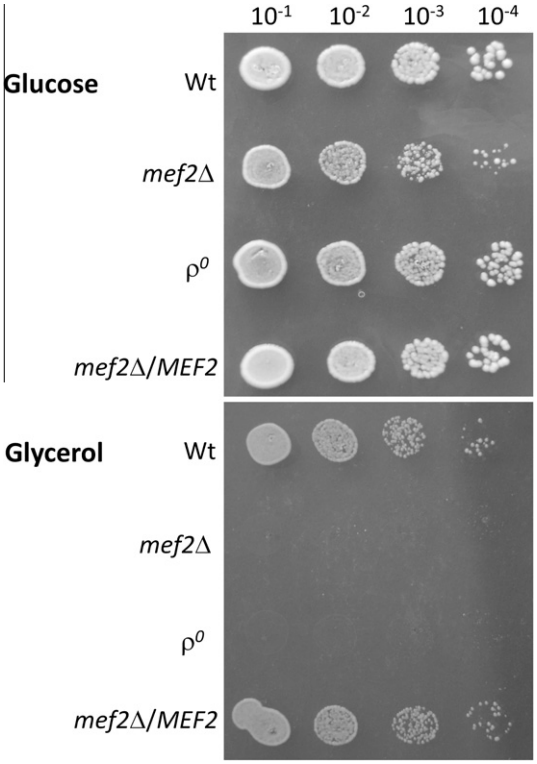


Fig. 1. Growth of the wild-type, *mef2*Δ mutant, ρ^0 strain and *mef2*Δ/*MEF2* heterozygous mutant after 48 h on solid medium containing glucose or the non-fermentable carbon source glycerol. Ten-fold serial dilutions ranging from 10^{-1} to 10^{-4} were spotted onto each plate.

source (glycerol) was absent, revealing a respiratory deficient phenotype (Fig. 1). Loss of respiratory ability in the *mef2*Δ deletion mutant occurred rapidly following sporulation of a *mef2*Δ/*MEF2* heterozygote. Respiratory deficiency does not extend to the *mef2*Δ/*MEF2* heterozygote, indicating that a single copy of the *MEF2* gene is sufficient to maintain respiratory function.

To test whether the absence of respiratory ability observed in the *mef2*Δ strain is a consequence of mtDNA damage, a restriction fragment length polymorphism (RFLP) was performed [14]. There are four distinct high molecular weight bands (greater than 6 kb) visible in DNA extracted from the wild-type yeast, however, no distinct bands are present in a cytoplasmic ρ^0 control strain, or the *mef2*Δ mutant (Fig. 2A). This signifies either the complete absence of mtDNA (ρ^0) in the *mef2*Δ mutant, or severe mtDNA rearrangements that result in a ρ^- strain, comprising of small repetitive fragments of mtDNA.

To decipher between a ρ^0 and a ρ^- genotype, cells were stained with DAPI. Microscopic observation showed that while mitochondrial nuclei were clearly visible in the wild-type strain as small round dots positioned around the cell periphery, these nuclei were absent from the *mef2*Δ mutant and the ρ^0 control (Fig. 2B). Further confirmation of the ρ^0 nature of the *mef2*Δ strain was obtained by performing genetic crosses with a wild-type ρ^+ strain. Whereas ρ^- mutants are frequently suppressive [21], producing respiratory deficient progeny, *mef2*Δ mating with the wild-type ρ^+ strain restored respiratory competence to the resulting crosses, verifying that the *mef2*Δ mutant is ρ^0 (results not shown).

3.2. The *mef2Δ* mutant has a slow growth phenotype and reduced chronological life span

It was noticed that colonies produced by the *mef2*Δ mutant are much smaller than those that arise from the wild-type and from the corresponding cytoplasmic ρ^0 strains (Fig. 1). A quantification of growth rate in glucose rich medium shows that *mef2*Δ strains have a considerably reduced growth rate, with a doubling time of 3.59 h, compared with 2.79 h for the ρ^0 strain and 2.43 h for the ρ^+ wild-type yeast (Fig. 3A). Several *mef2*Δ strains, each derived separately from sporulation of a *mef2*Δ/*MEF2* heterozygote, were investigated and each displayed the same growth defect. As a control, the growth rate of erythromycin treated wild-type cells, which are blocked in their ability to translate mitochondrial proteins, was also compared. Although these cells grew slower than the wild-type, both the ρ^0 strains and *mef2*Δ strains exhibited a significantly slower growth rate (data not shown).

CLS is a measure of cell survival in a population of non-dividing cells and has been shown to be closely linked with mitochondrial function [22–24]. To investigate the effect of deleting the *MEF2* gene on CLS, viability of cells grown to stationary phase was monitored for 13 days [17]. Results show that the *mef2*Δ deletion mutant has a reduced CLS compared with both wild-type and cytoplasmic ρ^0 strains (Fig. 3B). The ρ^0 strain took nine days for viability to fall below 50%, whereas viability of the wild-type remained above 50% until approximately day 11 (Fig. 3B). In contrast, the *mef2*Δ mutant took only 5 days to fall below 50% viability. So, whereas the reduction in CLS of the *mef2*Δ strain can be partially attributed to the absence of mtDNA [25], it does not solely account for the severely reduced CLS of strains devoid of *MEF2* gene function.

3.3. The MEF2 gene has a role in maintenance of mitochondrial membrane potential

Although mtDNA is dispensable for cell viability in *S. cerevisiae*, the mitochondrial organelle is the location of several essential cellular processes. These processes are reliant upon the maintenance

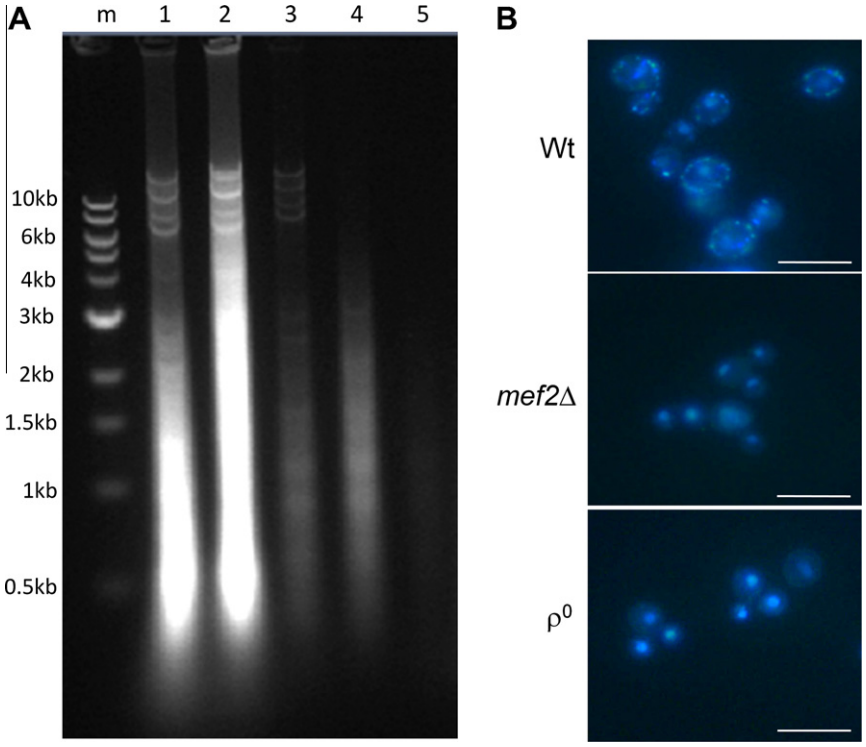
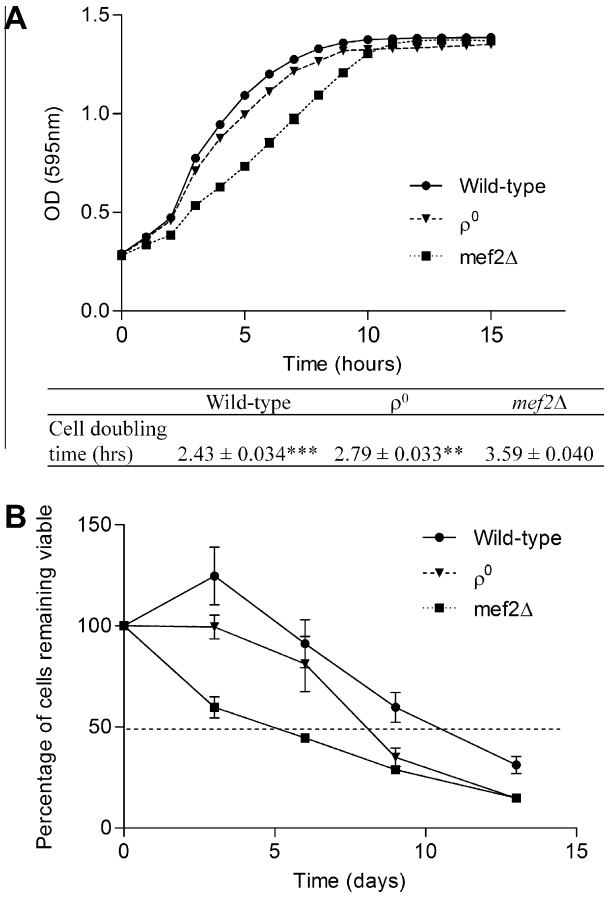


Fig. 2. mtDNA RFLP, cut using *Dde1*. Lane 1 contains the BY4742 wild-type strain (ρ^+), while lanes 2 and 3 show restriction digests from two other wild-type strains. Lanes 4 and 5 contain the *mef2*Δ deletion mutant and cytoplasmic ρ^0 strain, respectively (A). DAPI staining of wild-type (top panel), *mef2*Δ mutant (middle panel) and a ρ^0 control (lower panel). Mitochondrial nucleoids are visible in the wild-type strain as small bright spots, typically around the cell periphery (B). Cells are shown at ×1000 magnification. Scale bar = 10 μM.



	Wild-type	ρ^0	*mef2*Δ
Cell doubling time (hrs)	2.43 ± 0.034***	2.79 ± 0.033**	3.59 ± 0.040

Fig. 3. Growth curves and cell doubling times for wild-type, cytoplasmic ρ^0 and the *mef2*Δ mutant. Cells were grown in YEPD medium for 15 h and $OD_{(595nm)}$ was measured at hourly intervals. Cell doubling times are tabled as the means ± S.E.M. ($n = 3$). A Student's *t*-test was used to compare the mean cell doubling times for the wild-type and cytoplasmic ρ^0 strains to the *mef2*Δ deletion mutant (A). Chronological lifespan of the wild-type, cytoplasmic ρ^0 strain and the *mef2*Δ deletion mutant (B). Values plotted are the mean from three biological replicates and error bars represent S.E.M. $^{**}P < 0.01$ and $^{***}P < 0.001$.

of an electrochemical gradient across the inner mitochondrial membrane for the import of proteins into the mitochondria. Uptake of the MitoTracker Red CMXRos® dye into the mitochondrial matrix is dependent on ΔΨ, and so to analyze the integrity of ΔΨ in *mef2*Δ mutants, cells were stained with MitoTracker Red® [18]. In wild-type cells, where the ΔΨ is maintained through the activity of the electron transport chain, the ρ^+ mitochondria stain brightly and appear as a network of tubular structures within the cell (Fig. 4). In ρ^0 cells, the ΔΨ is maintained via a mechanism that involves the exchange of ATP and ADP across the inner mitochondrial membrane by the adenine nucleotide translocator and F_1 ATP synthase [26]. The ΔΨ generated by this alternative mechanism lacks in magnitude but, following a period of adaptation that may involve genomic alterations, the ΔΨ is sufficient to enable essential mitochondrial processes to function [27]. As a result of this adaptation process, the extent of ΔΨ in ρ^0 strains has been found to vary (Gottschling, personal communication; [27]) and indeed there was some variation in mitochondrial staining between several ρ^0 strains tested. Nevertheless, all ρ^0 strains, revealed punctate fluorescent structures in each cell, indicative of fragmented mitochondria and typical of cells that have lost their mtDNA (Fig. 4) [12]. Erythromycin treated wild-type cells, while showing an expected reduction in ΔΨ, still maintained a greater ΔΨ than ρ^0 cells (results not shown).

In contrast, mitochondria of the *mef2*Δ deletion mutants consistently stained poorly, with mitochondrial fluorescence, barely visible in a 1× concentration of MitoTracker® (Fig. 4). It was observed that cytoplasmic staining in the ρ^0 and *mef2*Δ cells was lower than that of wild-type cells. To overcome this reduction in intracellular MitoTracker® concentration and to improve visibility of mitochondrial morphology, ρ^0 and *mef2*Δ cells were stained with increasing concentrations of MitoTracker Red®. The results clearly show that even when cytoplasmic fluorescence of the *mef2*Δ mutant is greater than that of wild-type cells (cytoplasmic grey values for *mef2*Δ (10×) = 109; ρ^0 (3×) = 95 and wild-type (1×) = 88), the mitochondrial staining is notably reduced in *mef2*Δ cells. In some cells (approximately 30–40%), there is an increase in visibility of internal spherical structures, although these structures still do not stain as brightly as those in ρ^0 cells, appearing smaller and fewer (generally only one per cell) than those of ρ^0 cells. Furthermore, for the majority of *mef2*Δ cells (60–70%), an obvious mitochondrial structure is not visible (Fig. 4). It therefore appears that *mef2*Δ deletion mutants may have a decreased mitochondrial content, with fragmented mitochondria showing a tendency to aggregate, typically in a single cluster per cell.

3.4. The *mef2Δ* mutant has aggregated mitochondria

To further investigate the mitochondrial morphology of the *mef2*Δ strain, cell ultrastructure was visualized using TEM (Fig. 5). There were no obvious abnormalities in the mitochondrial content of *mef2*Δ cells, however, in several *mef2*Δ cells, mitochondria appeared clustered, supporting the observations made during fluorescence staining of the mitochondria. This phenotype was not observed in the ρ^0 cells where mitochondria were fragmented, but did not aggregate (Fig. 5). Based on these observations, the *MEF2* gene appears to have an essential role for mitochondrial health, impacting on maintenance of ΔΨ and altering mitochondrial organization.

3.5. Nuclear encoded genes with a dual role for respiratory function and cell growth

To determine how universal a *mef2*Δ-like growth defect is amongst other nuclear encoded genes that are essential for respiration, a literature analysis was conducted. A recent study by Merz and Westermann (2009) compiled a list of 163 deletion mutants that exhibit respiratory deficiency based on three published genome-wide screens for respiratory deficiency [11,12,28]. This dataset was correlated with that of another genome-wide screen in which homozygous *S. cerevisiae* knockout mutants were profiled for growth defects in YEPD medium [29]. To correct for fermentative slowing of cell growth normally observed in cytoplasmic ρ^0 strains, only strains with a fitness score <0.830 in the Deutschbauer et al. (2005) study were considered. This cut-off selects for strains with a fitness defect similar or less than that of *mef2*Δ (fitness score = 0.808). Of the 163 respiratory deficient deletion mutants reported by Merz and Westermann (2009), 48 (29%) have a growth defect that is likely to extend beyond that imposed simply by fermentative growth. An overwhelming proportion of the 48 genes (61%), have a role in mitochondrial protein synthesis (Fig. 6). This mitochondrial protein synthesis category constituted only 40% of the original 163 genes [11] and so there appears to be a considerable enrichment for mitochondrial protein synthesis genes among the subset of respiratory deficient, growth defective strains. It is not known to date whether these mitochondrial protein synthesis genes, of which the *MEF2* gene is a part, also have a reduced CLS or ΔΨ, but these results suggest that a number of nuclear encoded mitochondrial genes may have a dual function which, at least for the *MEF2* gene, is essential for ΔΨ maintenance. Also categorically

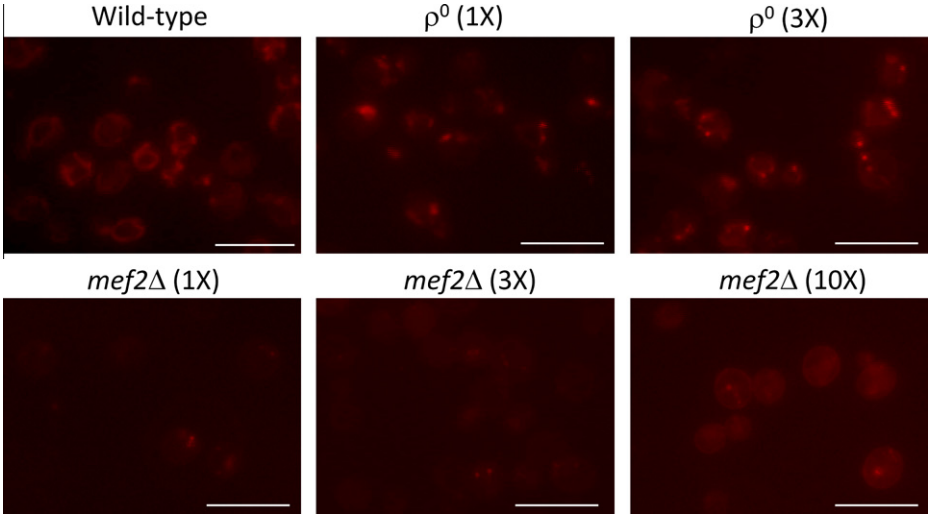


Fig. 4. MitoTracker Red CMXRos® staining of wild-type, ρ^0 and *mef2*Δ strains. To better resolve mitochondrial structure, ρ^0 and *mef2*Δ strains were stained with increasing concentrations of MitoTracker®. Scale bar = 10 μM.

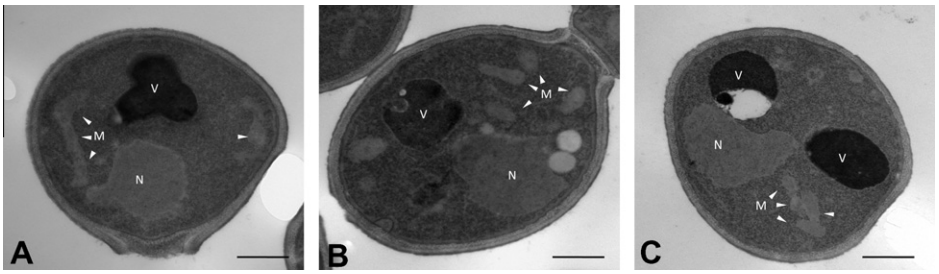


Fig. 5. TEM micrographs showing ultrastructure of the wild-type (A), ρ^0 (B) and *mef2*Δ (C) strains. Cell nucleus (N), vacuoles (V) and mitochondria (M) are labeled. Scale bar = 500 nm.

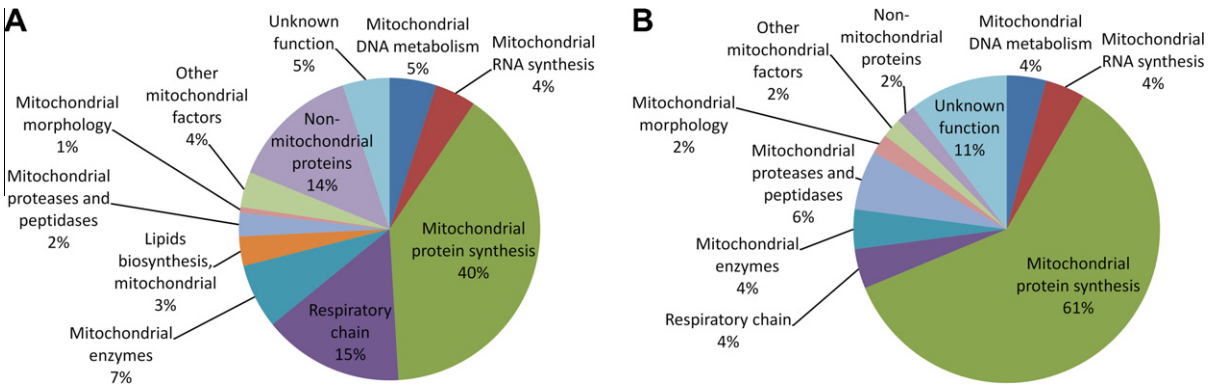


Fig. 6. Functional distribution of genes essential for cell respiration as determined in the Merz and Westermann (2009) study based on overlapping data from three published genome-wide screens for respiratory deficiency [11,12,28]. The 13 questionable ORFs included in the Merz and Westermann data-set were omitted from this analysis (A). Functional distribution of genes with a dual role for respiratory function and cell growth as determined in this study, based on the dataset compiled by Merz and Westermann [11] and results from the fitness profiling study for growth defects by Deutschbauer et al. (2005) [29] (B). Functional annotations are those used in the Merz and Westermann (2009) study [11].

enriched are genes encoding mitochondrial proteases and peptidases (6%, compared with 2% in the original study [11]).

4. Discussion

The *MEF2* gene encodes a mitochondrial translation factor, with a predicted involvement in mitochondrial ribosome recycling [5]. Yeast cells lacking components of the mitochondrial translation apparatus generally become ρ^0, likely due to the inhibition of translation of mitochondrial proteins necessary for mtDNA replication and repair [30]. However, as there have been some discrepancies in the literature [6,11,12], a number of reports continue to maintain that the role of the *MEF2* gene in mitochondrial translation is redundant [7–10]. This study presents the first detailed functional characterization of *mef2*Δ and findings confirm that the Mef2p protein is indispensable for the maintenance of mtDNA. Deletion of the *MEF2* gene leads to rapid respiratory deficiency and a detailed analysis of the mtDNA content of the *mef2*Δ deletion

mutant shows that there is a complete loss of mtDNA. This demonstrates that the Mef2p protein plays an essential role in mitochondrial protein translation.

In addition to respiratory deficiency, deletion of the *MEF2* gene confers a significant growth defect and a reduction in CLS. A slow growth phenotype for ρ^0 cells is expected as cells are forced to produce energy via the less efficient fermentative pathways [31]. The shortened CLS of the *mef2*Δ deletion mutant can also, in part, be attributed to lack of respiratory function, with cytoplasmic ρ^0 cells having previously been shown to have a reduced CLS, although the mechanism for this is not clear [25]. However, both the growth and lifespan defects of the *mef2*Δ deletion strain extend beyond what is observed for a cytoplasmic ρ^0 strain. These observations raise the possibility that the *MEF2* gene also has a non-respiratory related function; at least in cells that have no mtDNA, and that this function is necessary for normal growth and viability.

The use of fluorescent staining as an indicator of $\Delta\Psi$ has revealed a severely diminished $\Delta\Psi$ in *mef2*Δ cells. It was also observed that *mef2*Δ cells have a reduced cellular uptake of the MitoTracker® stain when compared with the ρ^0 strain. This possibly indicates a hyperactive retrograde response, which increases the activity of multidrug resistance pumps [32]. Further investigations of the retrograde response in the *mef2*Δ strain are required to establish this. Nevertheless, using increased concentrations of MitoTracker®, it was possible to standardize intracellular MitoTracker® concentrations with that of ρ^0 cells and still mitochondrial incorporation of the probe remained low, signifying a reduction in $\Delta\Psi$ of *mef2*Δ mutants. The role of $\Delta\Psi$ for cell function is critical. In addition to ATP production, the mitochondria are home to a number of important conserved cellular processes including iron metabolism, apoptosis, heme synthesis and fatty acid and steroid synthesis [33–35]. The majority of proteins involved in these processes are encoded in the nuclear genome and must be imported into the mitochondria, an action that is reliant on the existence of an electrochemical potential across the inner mitochondrial membrane [36]. Arguably the most significant mitochondrial process for cell viability is that of iron metabolism, which is responsible for the synthesis of iron–sulfur clusters. Iron–sulfur clusters can bestow catalytic or structural properties on a protein, or can serve as electron carriers or regulatory sensors. A wide variety of cell proteins rely on iron–sulfur clusters, from those that are required for nuclear DNA synthesis and repair, as well as proteins involved in cytoplasmic protein translation [33]. The inhibition of the synthesis of iron–sulfur clusters is believed to be the underlying mechanism for why lack of $\Delta\Psi$ is lethal to cells [33].

The $\Delta\Psi$ also enables the import and post-translational processing of nuclear encoded proteins that are responsible for mitochondrial fusion and the tubular mitochondrial morphology observed in wild-type cells [37]. Dissipation of $\Delta\Psi$ causes mitochondrial fragmentation as seen in ρ^0 cells [37], but further defects in mitochondrial import through mutation of mitochondrial import proteins, such as Hsp70p has been shown to cause mitochondrial aggregation [38]. TEM ultrastructure analysis shows numerous cells with aggregated mitochondria. It is possible that the abnormally low $\Delta\Psi$ in *mef2*Δ mutants compromises mitochondrial protein import and this may be a factor in the aberrant mitochondrial morphology of the *mef2*Δ mutant. However, additional studies are required to establish a link between the dissipated $\Delta\Psi$ and mitochondrial clustering phenotype of *mef2*Δ cells.

Phenotypes similar to that of *mef2*Δ, which have been linked to loss of $\Delta\Psi$, have also been observed in other nuclear encoded mitochondrial proteins. Perturbation of a yeast transposon, *HsTnII*, gives rise to respiratory deficient cells that have a reduced CLS. These cells also have fragmented mitochondria and a reduced $\Delta\Psi$ [23]. Additionally, mutations that impinge on the mitochondrial F_1–F_0 ATP synthase have been found to confer a ρ^0 phenotype with a significant growth defect [39]. The F_1–F_0 ATP synthase complex catalyzes ATP synthesis, but in the absence of mtDNA, the F_1 portion is believed to contribute to the maintenance of the $\Delta\Psi$. These results point to a very strong link between $\Delta\Psi$ and cell growth rate and viability.

This study has revealed the importance of the Mef2p protein for mtDNA maintenance and, in addition, results have further uncovered an unexpected bi-functional nature of the Mef2p protein, with a likely role in the maintenance of the $\Delta\Psi$ and mitochondrial organization. Further investigations are needed to establish the precise role of the *MEF2* gene in maintenance of the $\Delta\Psi$, but an analysis of existing literature has identified many genes essential for cell respiration that also confer a significant growth defect when deleted. A great proportion of these genes are involved in mitochondrial protein synthesis. It is expected that the role of these genes in cell growth is separate from their function for mitochondrial protein synthesis as the majority of deletion mutants for these genes are devoid of mtDNA. It is not known whether $\Delta\Psi$ is a factor in the growth phenotype of these genes, but considering the close association of the mitochondrial translation apparatus with the inner mitochondrial membrane [40], it is plausible to speculate that perhaps some aspect of the mitochondrial protein synthesis system, of which the Mef2p protein is a part, could play a role in maintenance of $\Delta\Psi$ in ρ^0 cells.

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