Mitochondrial Targeted *AFG3* Abolishment Triggers Higher Mitochondrial Membrane Potential (ΔΨm) in Young Yeast

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Abstract

Yeast *AFG3* gene is homologous to human *AFG3L2* and *SPG7* genes whose encoded proteins interact with each other on the mitochondrial inner membrane to form the m-AAA metalloproteinase complex. Mutations associated with the gene *SPG7* cause autosomal recessive disease spastic paraplegia and a type of ataxia in human but the mitochondrial activity in terms of mitochondrial membrane potential was not investigated previously. In our earlier study, we characterized *AFG3* gene deletion yeast mutant and found this mutant gained altered mitochondrial morphology and functions such as mitochondrial aggregation, absence of ROS, less ATP etc. In this current study, we further investigated the effect of *AFG3* deletion on mitochondrial health and activation in yeast models. To do so, the rate of oxygen consumption was measured and found that *afg3Δ* consumed less amount of oxygen compared to wild type (WT). In addition, mitochondrial membrane potential was measured and found that young *afg3Δ* gained significantly higher membrane potential (doubled) compared to WT. As Afg3 degrades unassembled or unfolded proteins, we also analyzed mitochondrial unfolded protein response (UPRmt) signal and found inactivated indicating mitochondrial proteostatic balance was any how managed and augmentation of ΔΨm may play role here. Physical interaction with AFG3 were sorted out and classified in order to find out how the interactive network may hamper due to abolishment of the Afg3 protein function. Thus this investigation in yeast (*Saccharomyces cerevisiae*) model may provide additional information in the study of human spastic paraplegia.

Significance | Molecular insight into the human genetic disease, spastic paraplegia through yeast model

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Abbreviations: ETC, Electron transport chain; ΔΨm, Mitochondrial membrane potential; UPRmt, mitochondrial unfolded protein response; YPD, Yeast extract peptone dextrose; YPG, Yeast extract peptone glycerol.

Introduction

Inside mitochondrial respiratory chain, electrons from reduced substrates are passed through electron transport chain (ETC) complexes (I, II, III and IV) to oxygen, forming water and causing protons to be pumped across the mitochondrial inner membrane (Hulbert et al., 2007). This activity of the ETC drives the synthesis of ATP and also generates the electrochemical potential or mitochondrial membrane potential (ΔΨm) that is utilized to power the transport of biosynthetic precursors out of the mitochondriaand into the cytoplasm (Jazwinski, 2004). Some of these precursors are the intermediates in the TCA cycle.

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When yeast cells (*Saccharomyces cerevisiae*) are fermenting glucose, the bulk of the ATP is generated at the substrate level through glycolysis. This generated ATP is used for maintaining ΔΨm through the exchange of mitochondrial ADP for ATP with the help of ADP–ATP translocator present in the inner mitochondrial membrane (Jazwinski, 2013). ΔΨm and the processes it supports may play a crucial role in mitochondrial functionality and affects mitochondrial integrity. However, the loss of ΔΨm leads to mitochondrial dysfunction (Jazwinski, 2013; Wright, 2004).

In fact yeast has been used as a single cell eukaryotic model organism for more than 50 years to study the molecular biology of the cell. This model is a rapid and powerful tool that has enabled a better understanding of human biology and many diseases hence it possesses 23% homologous genes to human (Liu et al., 2017). Yeast mitochondrial metabolism gene, *AFG3* (YER017C), is an ATPase family gene. Its protein product, Afg3, acts as a component of the m-AAA protease which is basically an ATP-dependent metalloprotease. Afg3 mediates the degradation of misfolded or unassembled inner membrane proteins of mitochondria (Arlt et al., 1996). This protein is necessary for the correct assembly of mitochondrial respiratory chain and ATPase complexes (Arlt et al., 1998; Di Bella et al., 2010). Thus *AFG3* is known to play an important role in regulating electron transport chain complexes (Nolden et al., 2005).

AFG3 is homologous to human *AFG3L2* and *SPG7*. In human they interact with each other on the mitochondrial inner membrane to form the m-AAA metalloproteinase complex (Arlt et al., 1996, 1998). The *SPG7* gene that is located on human chromosome 16, encodes a protein called paraplegin (Casari et al., 1998; De Michele et al., 1998) and point mutations associated with this gene cause autosomal recessive spastic paraplegia, a neurodegenerative disorder that is characterized by a slow, gradual, progressive weakness and spasticity of the lower limbs. *SPG7* mutations have also been associated with other undiagnosed ataxia (Pfeffer et al., 2015; Warnecke et al., 2010). Furthermore, *SPG7* deficiency with null mutation is associated with impaired respiratory activities and mitochondrial functions (Warnecke et al., 2010). So yeast *AFG3* gene and its deletion mutant *afg3Δ* has been used to study the molecular biology of spinocerebellar ataxia type 28 and hereditary spastic paraplegia in yeast model (Arlt et al., 1996, 1998). Delaney et al., 2013 also found that this mutant is unable to use non-fermentable carbon sources indicating an impaired mitochondrial function exists in this mutant.

Indeed, impaired mitochondrial function or, mitochondrial dysfunction is intimately connected to protein aggregation and protein folding disorders (Callegari and Dennerlein, 2018) and mitochondrial defects have recently been shown to lead to genome instability (Veatch et al., 2009) . In response to a mitochondrial perturbation there exists a stress response mechanism that is communicated to the nucleus to increase the expression of mitochondrial associated protein chaperones referred to as the mitochondria-specific unfolded protein response (UPRmt) (Benedetti et al., 2006). Thus mitochondrial unfolded protein response (UPRmt) is considered as mitochondrial proteostasis pathway (Jovaisaite et al., 2014). UPRmt is activated when protein balance in mitochondria is disturbed. This occurs, for instance, upon accumulation of misfolded or unfolded proteins (Haynes & Ron, 2010). This incident then triggers a mitochondria-to-nuclear stress-signal which induces the transcription of nDNA-encoded mitochondrial molecular chaperones, such as mt*HSP60* (Durieux et al., 2011).

Houtkooper et al., 2013 reported that mitochondrial ribosomal protein S5 (Mrps5) and other mitochondrial ribosomal proteins as metabolic regulators. Mrp knockdown triggers mitonuclear protein imbalance, reducing mitochondrial respiration and activating the mitochondrial unfolded protein response. UPRmt is also induced induced by mitochondrial stress, subsequently activating a nuclear transcriptional response, inducing the chaperone *HSP-60* to restore mitochondrial proteostasis (Haynes & Ron, 2010).

As human spastic paraplegia causing genes *AFG3L2* and *SPG7* are related to yeast *AFG3* that regulates ETC complex and mitochondrial proteostasis, we assumed that the null mutation of *AFG3* might affect mitochondrial health and function. So in the present investigation we aimed to test the effect of *AFG3* gene deletion on mitochondrial health and activity by analysing mitochondrial membrane potential and mitochondrial unfolded protein response.

Methods

Yeast strain and culture

Wild-type (WT) strain of yeast BY4743 (mat a/α his3Δ1 / his3Δ1 leu2Δ0 / leu2Δ0 LYS2 / lys2Δ0 met15Δ0 / MET15 ura3Δ0 / ura3Δ0) and the genetic background isogenic deletion mutant *afg3Δ* were obtained from EUROSCARF and used in the current study. Both WT and the mutant cells were grown in 30°C in solid and liquid YPD broth (1% yeast extract, 2% Peptone, 2% Dextrose and 2% Agar). The concentrations of the yeast cells according to the experimental conditions (optical density) at 600 nm wavelength (OD_{600}) were measured using a spectrophotometer.

Separation of young and old yeast cells

Logarithmically growing cells were considered as young cells since there is only a very small fraction of old cells present in an exponentially growing population. Thus, young yeast cells were obtained by growing them in fresh 2% YPD growth medium for 6 hours at 30°C while aged (20-generation old) cells were isolated by using an elutriation system (High speed centrifuge, Beckman Coulter Avanti J-26 XP). Overnight cultures of the cells were loaded into the separation chamber of that elutriation system with a flow rate of 40 ml/min at 2500 rpm. Then the centrifugation speed was reduced to 1000 rpm with a flow rate 30 ml/min to isolate cells larger than 15 µm (≤20 generation old cells). The resulting old cells were collected and stored at -80°C until they were analyzed.

Oxygen consumption assay

WT and the mutant strain *afg3Δ* were grown in 5 ml YPD liquid culture media for overnight at 30°C and then 100 µl were transferred into 200 ml fresh YPD liquid media to obtain logarithmic phase cells. The culture was incubated until OD_{600} reached to 0.8. Cells were washed with distilled water (dH2O), suspended in glycerol media (YPG) and incubated at 30°C for 30 min. To make a control sample, the same amount of fresh YPG without cells was incubated at the same time. Hanna Instrument H12400 logging DO meter was used to measure the dissolved oxygen levels. The polarization and calibration procedure was performed using electrolyte fluid followed by the

instrumental protocol. The percent of dissolved oxygen read by the probe (H176407/2) for each case was subtracted from the control (only liquid YPG) value. Triplicate measurements were done for each of the three biological replicas.

Measurement of mitochondrial membrane potential

MitoProbe™ JC-1 Assay kit (Molecular Probes) was used for measuring mitochondrial membrane potential. Briefly, overnight YPD grown yeast strains were diluted and grown for 3 hours. OD_{600} values were adjusted to 0.7. Cells were pelleted and resuspended in 250µl of YPD containing JC-1 dye. Control cells were resuspended in YPD without the dye. Cells were incubated at 30°C for 30 min and were washed twice with PBS. After resuspension of the cells in PBS, red/green fluorescence ratio (535/595) of the cells was analyzed using a fluorescent spectrometer (Thermo Scientific Varioscan). Triplicate measurements were done for each of the three biological replicas. Statistical analysis was done by the Student's *t*-test.

Real-time PCR analysis of UPR^{mt} signal gene HSP60

The expression of *HSP60* gene was analyzed through a qPCR (Bio-Rad-iQ50) approach. The primers were designed as forward TAGTGGTCCAAAGGAAGCTATTC and reverse CAAACGCTCTTGCAGTTTCTC. Beta-actin gene was used for normalization. Total RNA was isolated and purified from 30 million cells for each strain by using an RNA isolation kit (Ambion Technology). Nano drop was used for quantification. DNAse 1 treatment was performed before the synthesis of cDNA. cDNA was made with the help of first strand cDNA sntehsis kit (Thermo scientific). 100 ng DNA in a 25-µL reaction was set using Maxima SYBR green /ROX qPCR master mix(2X) kit (Thermo Scientific) with 50 nmol forward and reverse primers. PCR was run as 95˚C for 10 min and 40 cycles of 60˚C for 15 sec, 60˚C for 30 sec and 72˚C for 30 sec. CT values were normalized and relative expression of that signal gene measured through 2-ΔΔCT method. Triplicate measurements were done for each of the three biological replicas.

Interaction analysis

The proteins that interact with Afg3 were identified with the help of Yeast genome data base and their molecular and biological functions were identified with MIPS (Munich Information Center for Protein Sequences) classification using the Fun-Spec analysis program.

Results and discussion

Level of oxygen consumption by *AFG3* deletion mutant, *afg3Δ*

We previously tested whether this mutant was defective in respiration by growing them on a glycerol-containing media. Proliferation rates of both of the cells in glucose were normal as they were assessed by a growth curve analysis in liquid YPD media but the mutant was not able to utilize glycerol which indicates that *afg3Δ* had respiration deficiency (Muid et al., 2019).

In this present investigation WT and *afg3Δ* were grown in glucose media (YPD) at first then transferred 20 million of each strain into YPG (glycerol) media in order to test the rate of oxygen consumption by them. Initially both WT and *afg3Δ* consumed a certain level of dissolved oxygen but the WT exhibited 3 times higher consumption than the mutant. As times goes, the mutant did not consumed oxygen more while WT showed a linear increase **(Fig. 1)** which also supports that the mutant has impaired respiration status.

The decreased level of oxygen abolishes the possible oxidation of reduced pyridine nucleotides in the respiratory chain coupled to oxidative phosphorylation indicating hampered of electron transport chain (Jazwinski & Kriete, 2012). The loss of the electron transport chain can prevent the utilization of the TCA cycle for production of biosynthetic intermediates. But cells can grow readily by generating ATP through glycolysis, however, they need a source of biosynthetic intermediates (Mullen et al., 2012).

S. cerevisiae, are well known by their capability of producing acetic acid under both aerobic and anaerobic or oxygen-limiting conditions. In *S. cerevisiae*, the further metabolism of acetic acid through acetyl–CoA synthetase (encoded by ACS1 and ACS2 genes) is the only source of cytosolic acetyl–CoA, an imperative building block of fatty acid biosynthesis (Flikweert et al., 1996; Van Den Berg & Steensma, 1995).

It has recently been shown that in yeast, the glyoxylate cycle can be induced. This allows acetyl-coenzyme A (acetyl-CoA) to be used for the synthesis of the TCA cycle metabolites citrate and malate (Muid et al., 2019). This, in turn, allows the first three reactions of the TCA cycle to proceed with the synthesis of α-ketoglutarate, which can be converted to glutamate, the ultimate source of nitrogen in biosynthesis. Thus taken together with these data, we think a metabolic remodeling of the biochemical pathways in *afg3Δ* took place. This metabolic remodeling may affect the electrochemical potential within the mitochondrial membrane.

Mitochondrial membrane potential (ΔΨm) in *afg3Δ*:

The mitochondrial membrane potential affects mitochondrial integrity during the life span, and it is important in driving the transport of biochemical precursors across the mitochondrial inner membrane (Hughes & Gottschling, 2012). To better evaluate the mitochondrial activation state of this *afg3Δ* cell, mitochondrial membrane potential was analyzed. It was found that ΔΨm values were about the same for young and old wild-type cells. However, the mutant cells had higher (about doubled) ΔΨm values (hyperpolarized) in the young stage (replicative stage) when compared to wild-type ($p<0.05$); interestingly when the mutant cells become replicatively old (senescent) it retains its ΔΨm parallel to wild type cells **(Fig.2).**

In addition to loss of respiration, the loss of mtDNA in mammalian (Jazayeri et al., 2003) or yeast cells (Dunn & Jensen, 2003) results in a reduction of ΔΨm. This potential is required for the transport of proteins through the mitochondrial matrix in all cells. In respiring cells the ΔΨ is normally generated through the reactions of electron transport and oxidative phosphorylation (Dunn & Jensen, 2003). Hughes & Gottschling, 2012 hypothesized that reduction in the ΔΨm contributed to the crisis after loss of mtDNA. In our previuos observation, we tested this idea by examining mitochondrial DNA copy number with the help of qPCR and found that young *afg3Δ* had less mtDNA while in senescent mutant cells had completely lost mtDNA (Muid et al., 2019).

Table 1| *AFG3* interaction and their molecular and biological functions were identified and grouped with MIPS (Munich Information Center for Protein Sequences) classification using the Fun-Spec web based analysis program. The p-values represent the probability that the intersection of a given list with any given functional category occurs by chance.

Category	*p*-value	In Category from Cluster	k	f
Protein/peptide degradation [14.13]	3.346e-08	AFG3 PHB1 YTA12 YME1	4	47
ATP binding [16.19.03]	0.0004468	AFG3 YTA12 YME1	3	191
Assembly of protein complexes [14.10]	0.0005043	AFG3 YTA12 YME1	3	199
Mitochondrial transport [20.09.04]	0.003537	AFG3 YTA12	2	104
Protein transport [20.01.10]	0.00642	AFG3 YTA12	2	141
Mitochondrion [42.16]	0.009234	MRPL32 YME1	2	170

k: number of genes from the input cluster in given category; f: number of genes total in given category.

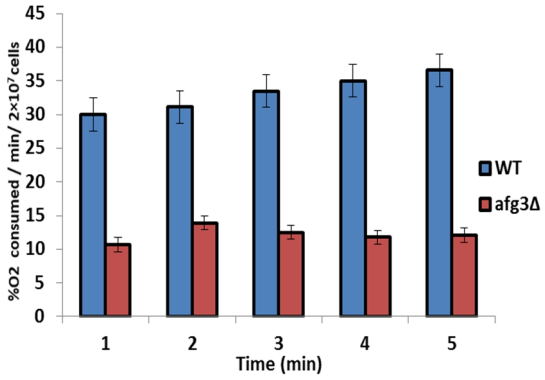

Figure 1| Oxygen consumption levels in WT and *afg3Δ*. The arrow indicates the linear increase of the oxygen consumption rate by the WT cells while the mutant *afg3Δ* rendered less amount of oxygen consumption ($p \leq 0.05$).

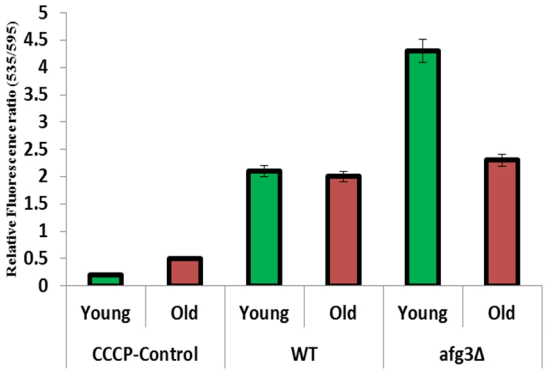

Figure 2| Mitochondrial membrane potential (ΔΨm) comparison: MitoProbe™ JC-1 Assay kit (Molecular Probes) was used to measure mitochondrial membrane potential both in young and old cells of WT and *afg3Δ*. Significant difference was observed between young WT and young *afg3Δ* ($^*p \leq 0.05$). When the mutant cell became senescent (replicatively old), the ΔΨm was decreased compared to its own young ($p \leq 0.05$) but the level was maintained parallel to WT ($p \geq 0.05$).

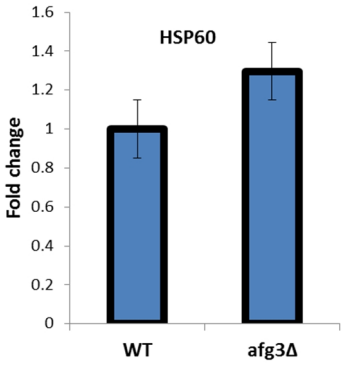

Figure 3| Analysis of *HSP60* gene expression: The expression of HSP60 gene was analyzed through a realtime PCR (Bio-Rad-iQ50). Significant difference was not observed between WT and *afg3Δ* ($p \geq 0.05$) thus UPRmt was thought not activated in this mutant.

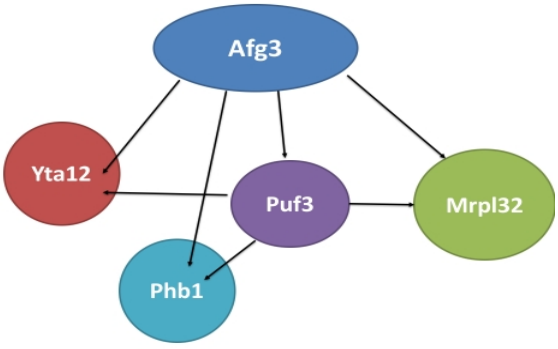

Figure 4| *AFG3* interaction network: The proteins that physically interact with Afg3 protein were analyzed with the help of Yeast genome data base. Yta12, Phb1, Puf3 and Mrpl32 were found to have network with Afg3.

Interestingly following mtDNA reduction could not hamper in maintaining the mitochondrial membrane potential in our studied *afg3Δ* mutant. The maintenance of the mitochondrial membrane potential by electron transport is critical to proper function of the organelle, and therefore, the cell. Maintenance of the essential ΔΨm in cells lacking sufficient mtDNA depends on electrogenic exchange of ATP4− generated by glycolysis for ADP3− produced by hydrolytic activity of the ATP synthase F1 sector in the mitochondrial matrix (Chen & Clark-Walker, 1999).

Deletion of the protein phosphatases raise ΔΨm and one potential mechanism of ΔΨm augmentation might be transcriptional up-regulation of a different ATP hydrolase residing in the mitochondrial matrix or a change in ion mobility and distribution across the mitochondrial inner membrane (Garipler et al., 2014). (Muid et al., 2019) published that *afg3Δ* has 40% increased replicative life span indicating better replicative performance. However, there was a gradual loss of ΔΨm as *afg3Δ* replicatively aged **(Fig. 2).**

Mitochondrial unfolded protein response (UPR_{mt}) in *afg3Δ*:

Houtkooper et al., 2013 showed that mitochondrial ribosomal protein MRP knockdown triggers mitonuclear protein imbalance, reducing mitochondrial respiration and activating the mitochondrial unfolded protein response in *C. elegans*. The authors also stated that UPRmt is induced by mitochondrial stress, subsequently activating a nuclear transcriptional response, inducing the chaperones *HSP-6* (*HSP-70* in mammals) and *HSP-60* (in Yeast) to restore mitochondrial proteostasis. Muid et al., 2019 proposed that retrograde signal may play role for the longevity of *afg3Δ* but how they maintain protein balance is still unclear.

In the present study, we predicted the similar UPRmt hence the deletion of mitochondrial metabolism gene AFG3 were shown to interact with mitochondrial translation system and electron transport system **(Fig. 4)** that harbor impaired respiration by means of oxygen depletion (**Fig. 1**). Thus the cells may fall under a kind of stress condition leading to mitochondrial homeostatic imbalance. To test this hypothesis, we analyzed the expression of the key gene *HSP60* of UPR_{mt} pathway in a realtime PCR. Significant difference was not observed between WT and *afg3Δ* **(Fig. 3)**, suggesting that UPRmt is not activated in this deletion strain. Further investigation is highly required to find out how proteostasis is maintained in *AFG3* deletion mutant.

Interaction with *AFG3*

AFG3 was found to interact with *YTA12, PHB1, PUF3 and MRPL32* physically **(Fig. 4).** Protein protein interaction and their molecular and biological functions were identified with MIPS (Munich Information Center for Protein Sequences) classification using the Fun-Spec software **(Table 1).** Gerdes et al. 2012 stated that m- AAA proteases play an important role in the maintenance of mitochondrial proteostasis. He also mentioned that in human, mutations in AFG3L2 have distinct and more severe phenotypes as they affect all m-AAA protease isoenzymes and lead to a drastic reduction of the overall m-AAA protease activity. However, in our studied yeast, m-AAA proteases are the hetero-oligomers of two subunits, Yta10 (Afg3) and Yta12. Whereas Yta11 (Yme1) is a catalytic subunit of i-AAA protease complex that also involves in protein import, folding and maturation (Schreiner et al., 2012). From our bioinformaticanalysis we found that Afg3 interacts with Puf3 and both have interactive networks with Yta12, Phb1 and Mrpl32 (Fig. 4). Lapointe et al., 2018 reported that Puf3 directly linked to mitochondrial movement and promotes degradation of mRNAs for selecting mitochondria targeted nuclear proteins. Mrpl32 that interacts with Afg3, is the mitochondrial ribosomal protein of the large subunit, mediates translation in the mitochondria (Amunts et al., 2014). Another found interactive partner and regulator is Phb1 which is a subunit of prohibitin complex, an inner mitochondrial membrane chaperone. Schlei et al., 2013 published that Phb1 stabilizes newly synthesized proteins but deficiency or disturbance induces UPRmt. However we did not find UPRmt due to Afg3 deficiency (Fig. 3).

Conclusion

In the present investigation we analyzed the mitochondrial targeted *AFG3* gene deletion mutant in order to observe the effect of Afg3 abolishment on mitochondrial health and activity in yeast model. Due to absence of Afg3 protein function, oxygen consumption was reduced but the mutant cell were able to promote higher membrane potential at the young stage. We hypothesized that the augmentation of ΔΨm may facilitate protein balance in mitochondria. Hence Yta12 and afg3 double mutation is linked to human spastic paraplegia and ataxia, further study in mammalian model is highly required.

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Author Contribution

AMK did the experiments and analyzed it. AK guided and provided experimental supports.

Competing Interests

The authors declare that they have no competing interests.

References

Arlt, H., Steglich, G., Perryman, R., Guiard, B., Neupert, W., & Langer, T. (1998). The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease. *EMBO Journal*, *17*, 4837-4847. https://doi.org/10.1093/emboj/17.16.4837

Arlt, H., Tauer, R., Feldmann, H., Neupert, W., & Langer, T. (1996). The YTA10-12 complex, an AAA protease with chaperone-like activity in the inner membrane of mitochondria. *Cell*, *85* (6), 875-885. https://doi.org/10.1016/S0092-8674(00)81271-4

Benedetti, C., Haynes, C. M., Yang, Y., Harding, H. P., & Ron, D. (2006). Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. *Genetics*, *174* (1), 229-239. https://doi.org/10.1534/genetics.106.061580

Callegari, S., & Dennerlein, S. (2018). Sensing the stress: A role for the UPRmt and UPRam in the quality control of mitochondria. In *Frontiers in Cell and Developmental Biology*, *6*, 31. https://doi.org/10.3389/fcell.2018.00031

Casari, G., De Fusco, M., Ciarmatori, S., Zeviani, M., Mora, M., Fernandez, P., De Michele, G., Filla, A., Cocozza, S., Marconi, R., Dürr, A., Fontaine, B., & Ballabio, A. (1998). Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell*, *93* (6), 973-983.

https://doi.org/10.1016/S0092-8674(00)81203-9

Chen, X. J., & Clark-Walker, G. D. (1999). The petite mutation in yeasts: 50 years on. In *International Review of Cytology*, *194,* 197-238. https://doi.org/10.1016/s0074-7696(08)62397-9

Copeland, J. M., Cho, J., Lo, T., Hur, J. H., Bahadorani, S., Arabyan, T., Rabie, J., Soh, J., & Walker, D. W. (2009). Extension of Drosophila Life Span by RNAi of the Mitochondrial Respiratory Chain. *Current Biology*, *19* (19), 1591-1598. https://doi.org/10.1016/j.cub.2009.08.016

De Michele, G., De Fusco, M., Cavalcanti, F., Filla, A., Marconi, R., Volpe, G., Monticelli, A., Ballabio, A., Casari, G., & Cocozza, S. (1998). A new locus for autosomal recessive hereditary spastic paraplegia maps to chromosome 16q24.3. *American Journal of Human Genetics*, *63* (1), 135-139. https://doi.org/10.1086/301930

Delaney, J. R., Ahmed, U., Chou, A., Sim, S., Carr, D., Murakami, C. J., Schleit, J., Sutphin, G. L., An, E. H., Castanza, A., Fletcher, M., Higgins, S., Jelic, M., Klum, S., Muller, B., Peng, Z. J., Rai, D., Ros, V., Singh, M., … Kaeberlein, M. (2013). Stress profiling of longevity mutants identifies Afg3 as a mitochondrial determinant of cytoplasmic mRNA translation and aging. *Aging Cell*, *12* (1), 156-166. https://doi.org/10.1111/acel.12032

Delaney, J. R., Murakami, C. J., Olsen, B., Kennedy, B. K., & Kaeberlein, M. (2011). Quantitative evidence for early life fitness defects from 32 longevity-associated alleles in yeast. *Cell Cycle*, *10* (1), 156-165. https://doi.org/10.4161/cc.10.1.14457

Di Bella, D., Lazzaro, F., Brusco, A., Plumari, M., Battaglia, G., Pastore, A., Finardi, A., Cagnoli, C., Tempia, F., Frontali, M., Veneziano, L., Sacco, T., Boda, E., Brussino, A., Bonn, F., Castellotti, B., Baratta, S., Mariotti, C., Gellera, C., … Taroni, F. (2010). Mutations in the mitochondrial protease gene AFG3L2 cause dominant hereditary ataxia SCA28. *Nature Genetics*, *42,* 313-321. https://doi.org/10.1038/ng.544

Dunn, C. D., & Jensen, R. E. (2003). Suppression of a defect in mitochondrial protein import identifies cytosolic proteins required for viability of yeast cells lacking mitochondrial DNA. *Genetics*, *165* (1), 35-45.

Durieux, J., Wolff, S., & Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell*, *144* (1), 77-91. https://doi.org/10.1016/j.cell.2010.12.016

Flikweert, M. T., Van Der Zanden, L., Janssen, W. M. T. M., Steensma, H. Y., Van Dijken, J. P., & Pronk, J. T. (1996). Pyruvate decarboxylase: An indispensable enzyme for growth of Saccharomyces cerevisiae on glucose. *Yeast*, *12* (3), 247-257. https://doi.org/10.1002/(SICI)1097-0061(19960315)12:3<247::AID-YEA911>3.0.CO;2-I

Garipler, G., Mutlu, N., Lack, N. A., & Dunn, C. D. (2014). Deletion of conserved protein phosphatases reverses defects associated with mitochondrial DNA damage in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences of the United States of America*, *111* (4), 1473-1478. https://doi.org/10.1073/pnas.1312399111

Haynes, C. M., & Ron, D. (2010). The mitochondrial UPR - Protecting organelle protein homeostasis. In *Journal of Cell Science*, *123* (22), 3849-3855. https://doi.org/10.1242/jcs.075119

Houtkooper, R. H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R. W., & Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature*, *497,* 451-457. https://doi.org/10.1038/nature12188

Hughes, A. L., & Gottschling, D. E. (2012). An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature*, *492,* 261-265. https://doi.org/10.1038/nature11654

Hulbert, A. J., Pamplona, R., Buffenstein, R., & Buttemer, W. A. (2007). Life and death: Metabolic rate, membrane composition, and life span of animals. In *Physiological Reviews*, *87,* 1175-1213. https://doi.org/10.1152/physrev.00047.2006

Jazayeri, M., Andreyev, A., Will, Y., Ward, M., Anderson, C. M., & Clevenger, W. (2003). Inducible expression of a dominant negative DNA polymerase-γ depletes mitochondrial DNA and produces a ρ0 phenotype. *Journal of Biological Chemistry*, *278* (11), 9823-9830. https://doi.org/10.1074/jbc.M211730200

Jazwinski, S. M. (2004). Yeast replicative life span - The mitochondrial connection. In *FEMS Yeast Research*, *5* (2), 119-125. https://doi.org/10.1016/j.femsyr.2004.04.005

Jazwinski, S. M. (2013). The retrograde response: When mitochondrial quality control is not enough. In *Biochimica et Biophysica Acta - Molecular Cell Research*, *1833* (2), 400-409. https://doi.org/10.1016/j.bbamcr.2012.02.010

Jazwinski, S. M., & Kriete, A. (2012). The yeast retrograde response as a model of intracellular signaling of mitochondrial dysfunction. In *Frontiers in Physiology*, *3,* 139. https://doi.org/10.3389/fphys.2012.00139

Jovaisaite, V., Mouchiroud, L., & Auwerx, J. (2014). The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. In *Journal of Experimental Biology*, *217,* 137-143. https://doi.org/10.1242/jeb.090738

Liu, W., Li, L., Ye, H., Chen, H., Shen, W., Zhong, Y., Tian, T., & He, H. (2017). From Saccharomyces cerevisiae to human: The important gene co-expression modules. *Biomedical Reports*, *7,* 153-158. https://doi.org/10.3892/br.2017.941

Miwa, S., Jow, H., Baty, K., Johnson, A., Czapiewski, R., Saretzki, G., Treumann, A., & Von Zglinicki, T. (2014). Low abundance of the matrix arm of complex I in mitochondria predicts longevity in mice. *Nature Communications*, *5,* 3837. https://doi.org/10.1038/ncomms4837

Muid, K. A., Kimyon, Ö., Reza, S. H., Karakaya, H. C., & Koc, A. (2019). Characterization of long living yeast deletion mutants that lack mitochondrial metabolism genes DSS1, PPA2 and AFG3. *Gene*, *706,* 172-180. https://doi.org/10.1016/j.gene.2019.05.001

Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P. H., Sullivan, L. B., Cheng, T., Yang, Y., Linehan, W. M., Chandel, N. S., & Deberardinis, R. J. (2012). Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature*, *481* (7381), 385-388. https://doi.org/10.1038/nature10642

Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E. I., & Langer, T. (2005). The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. *Cell*, *123* (2), 277-289. https://doi.org/10.1016/j.cell.2005.08.003

Pfeffer, G., Pyle, A., Griffin, H., Miller, J., Wilson, V., Turnbull, L., Fawcett, K., Sims, D., Eglon, G., Hadjivassiliou, M., Horvath, R., Németh, A., & Chinnery, P. F. (2015). SPG7 mutations are a common cause of undiagnosed ataxia. *Neurology*, *84* (11), 1174-1176. https://doi.org/10.1212/WNL.0000000000001369

Van Den Berg, M. A., & Steensma, H. Y. (1995). ACS2, a Saccharomyces Cerevisiae Gene Encoding Acetyl-Coenzyme A Synthetase, Essential for Growth on Glucose. *European Journal of Biochemistry*, *231* (3), 704-713. https://doi.org/10.1111/j.1432-1033.1995.0704d.x

Veatch, J. R., McMurray, M. A., Nelson, Z. W., & Gottschling, D. E. (2009). Mitochondrial Dysfunction Leads to Nuclear Genome Instability via an Iron-Sulfur Cluster Defect. *Cell*, *137* (7), 1247-1258. https://doi.org/10.1016/j.cell.2009.04.014

Warnecke, T., Duning, T., Schirmacher, A., Mohammadi, S., Schwindt, W., Lohmann, H., Dziewas, R., Deppe, M., Ringelstein, E. B., & Young, P. (2010). A novel splice site mutation in the SPG7 gene causing widespread fiber damage in homozygous and heterozygous subjects. *Movement Disorders*, *25* (4), 413-420. https://doi.org/10.1002/mds.22949

Wright, S. H. (2004). Generation of resting membrane potential. *American Journal of Physiology - Advances in Physiology Education*, *28* (4), 139-142. https://doi.org/10.1152/advan.00029.2004