Uncovering new approaches for treatment of mitochondrial disease using budding yeast and fruit flies

by

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A Thesis Submitted to the
Graduate School of Sciences and Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Master of Science
in
Molecular Biology and Genetics

Koç University

July 2013

Koç University

Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a master's thesis by

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and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
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Date:

ABSTRACT

The mitochondrion is the compartment of the cell most involved in the conversion of food into energy. Mitochondria contain a small genome (mtDNA) that is highly involved in bioenergetics. Nearly 1 in 5000 people is afflicted with a heritable disease associated with mitochondrial dysfunction, and there are no useful treatments for most mitochondrial disease. Furthermore, as we age, we accumulate significant amount of damage to mtDNA, raising the possibility that mtDNA damage is a major factor in the process of aging.

I have performed studies in the yeast *Saccharomyces cerevisiae* and in the fly *Drosophila melanogaster* directed toward uncovering new approaches to the treatment of diseases caused by mtDNA damage. First, I explored how perturbation of two nutrient-sensing pathways can provide significant benefits to cells lacking a mitochondrial genome. Second, I have provided further evidence that a decrease in cytosolic protein synthesis increases the fitness of cells with catastrophic mtDNA damage. In my thesis, I will discuss my results and their potential application to human disease.

ÖZET

Mitokondri, hücrenin gıdayı enerjiye dönüştürmeden sorumlu tutulan organelidir. Mitokondri çoğu biyoenerji ile ilgili genleri barındıran bir genom (mtDNA) içermektedir. Yaklaşık her 5000 kişiden 1'i kalıtsal mitokondriyal işlev bozukluğu ile bağlantılı bir hastalığa maruz kalmaktadır ve mitokondriyal hastalıklar için faydalı tedaviler şu an bulunmamaktadır. Ayrıca, yaşlandıkça mtDNA'daki hasar önemli miktarda birikir ve bu nedenle mtDNA'daki hasarın yaşlanma sürecinde önemli bir faktör olduğu olasılığını arttırır.

Mitokondriyal DNA hasarının neden olduğu hastalıkların tedavisi için yeni yaklaşımlar ortaya çıkarılması doğrultusunda maya, *Saccharomyces cerevisiae*, ve meyve sineği, *Drosophila melanogaster*, üzerinde çalışmalar gerçekleştirdim. Birincisi, iki korunmuş besin algılama yollarının baskılanmasının mitokondriyal genomu eksik hücrelerine nasıl önemli yararlar sağlayabildiğini araştırdım. İkincisi, sitoplazmik protein sentezinde bir azalmanın mtDNA hasarlı hücrelerin formunu arttırdığı yönünde yeni kanıtlar sağladım. Tezimde sonuçlarımı ve insan hastalıklarını üzerine potansiyel uygulamayı tartışacağım.

This thesis is dedicated to my grandparents

ACKNOWLEDGEMENTS

I would like to thank all the people who have given their support during the writing process of this thesis.

I am grateful to my advisor, Dr. Cory Dunn, for his guidance and mentorship. It has been serendipitous that I have worked in his lab for 4 years. I will always be proud of being the first member of his lab and miss the lab environment he provided wherever I go.

I am also grateful to Prof. Howard T. Jacobs for his support and guidance for my internship in University of Tampere. I am indebted for the opportunity he provided and enthusiastic lab environment.

I would like to acknowledge to the Koç University, Koç Foundation and Koç Family for their financial support during my graduate and undergraduate education and for providing the best atmosphere for doing science.

I am indebted to my previous and current labmates at Koç Unviersity, Nebibe Mutlu, Emel Akdoğan, Gülkız Baytek and Tomris Şayan Örs for their help and friendship. I am also grateful to Esko Kemppainen and Jack George for their guidance and support during my work in University of Tampere. I also want to thank Troy Faithful for his friendship and support during my time in Tampere and Gülcan Semra Şahin for her support during the process of writing my thesis.

I would like to thank my family, my mother Gülşen Garipler, my father Şefik Garipler and my brother Tolga Garipler, for their endless patience, support and encouragement during my education and in the process of writing my thesis.

Special thanks to my dear friend Ugur Güler for his invaluable support whenever I face through tough times and his endless friendship for the past 10 years. I am lucky to have a friend like him.

Special thanks to two members of the three musketeers: Ece Özalp and Berk Emir Ildız. Their infinite support and friendship during my graduate studies and in the process of writing my thesis will never be forgotten. I am looking forward to having new adventures with them in New York.

Last, but not the least, I am grateful to Selena Kazancıoğlu for her endless support, infinite patience, undying positivity and eternal friendship. Without her support, this work would not have been possible.

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NOMENCLATURE

mtDNA	Mitochondrial DNA
ETC	Electron transport chain
NGS	Next-generation sequencing
tRNA	transfer RNA
rRNA	ribosomal RNA
WT	Wildtype
EtBr	Ethidium bromide
IM	Inner membrane
OM	Outer membrane
$\Delta\Psi^{mito}$	Mitochondrial electrochemical potential
ρ	petite
Δ	Deletion
YEPD	Yeast extract peptone dextrose
YEPGE	Yeast extract peptone glycerol ethanol
Ura	Uracil
His	Histidine
Leu	Leucine
Trp	Tryptophan
CHX	Cycloheximide
DOX	Doxycycline

Chapter 1

INTRODUCTION

Mitochondria contain their own genome as a relic of its prokaryotic ancestor. Mutation of mitochondrial DNA (mtDNA) affects the respiration machinery and energy production, which leads to many diseases in various organisms. The amount of accumulated mtDNA mutations correlate with age and it may be a partial cause of aging process [1]. Blocking the proofreading activity of mitochondrial DNA polymerase causes error prone DNA replication and yields mutations in the mtDNA [2]. Accumulation of these mutations leads to many deficiencies in mouse and gives a premature ageing phenotype [2]. Mutations in the mtDNA of budding yeast are also seen during the replicative lifespan in some yeast backgrounds [3]. Moreover, treatment of HIV-positive patients with certain anti-retroviral drugs can cause the accumulation of mtDNA damage [4]. Additional mutations in mtDNA can be obtained through reactive oxygen species created in electron transport chain, environmental factors and drugs that were used to treat virus infections and cancer.

Since many of the proteins functioning in electron transport chain (ETC) are generating $\Delta\Psi^{mito}$, mitochondrial DNA damage severely reduces the mitochondrial electrochemical potential ($\Delta\Psi^{mito}$) [5]. Also the mtDNA mutations impair the tricarboxylic acid cycle (TCA) [6] and drive the cell to express alternative pathways to produce intermediate metabolites that can be used in cellular processes [7]. Additionally cells lacking mtDNA demonstrates an active stress response with heat shock proteins

and iron-sulfur clusters [8]. mtDNA loss also results nuclear genome instability [3] and even apoptosis [9].

Saccharomyces cerevisiae offers a background which can proliferate in the absence of mitochondrial genome, and this sensitized background can be used to study mitochondrial biogenesis. Mitochondrial electrochemical potential and many of the processes in mitochondria are essential for cells, however ATP synthesis can be created by glycolysis on fermentation media which is sufficient for yeast to use in various pathways [5]. Mitochondrial DNA can be depleted by incubating cells in media containing ethidium bromide [10] converting wildtype (ρ^+) cells to mtDNA lacking (ρ^-) cells. Moreover, some of the nuclear encoded genes become essential after the mtDNA loss; these genes are called as petite-negative genes. Simultaneous deletion of these genes and mtDNA causes lethality. Finding suppressors that can rescue this petite-negative lethality can offer new interactions that can influence mitochondria biogenesis.

Mitochondrial biogenesis is regulated by various signaling pathways that are sensitive to the internal environment of the cell and to extracellular conditions. We have discovered an intriguing connection between conserved proteins functioning in Target of Rapamycin (TOR) and Protein Kinase A (PKA) pathways with mitochondrial biogenesis. We found that inhibition of phosphatase activity under TOR pathway and of upregulation of PKA pathway, which are conserved in mammals, were beneficial for budding yeast cells with damaged mtDNA. These mutations were benefiting cell proliferation, enhancing mitochondrial protein import and increasing the mitochondrial electrochemical potential ($\Delta\Psi^{mito}$). Interestingly, the yeast ortholog of the mammalian AMP-activated protein kinase is essential for the full benefits of phosphatase deletions in TOR pathway, however the involvement of SNF1 kinase in PKA pathway is unclear.

In addition, we also studied ribosomal biogenesis in two different models of mitochondrial disease. We showed that increasing ribosomal biogenesis and protein translation is harmful, and increasing the $\Delta\Psi^{mito}$ is beneficial for mtDNA damaged yeast cells. Moreover, preliminary data demonstrated that drugs inhibiting the protein synthesis may partially rescue the developmental defect of a mitochondrial disease

model in the fruit fly, *Drosophila melanogaster*, however reproducibility of this data was not possible which is discussed in the results section. Heat shock proteins (HSP) were found to be highly expressed both in yeast and in fly as a response to mitochondrial damage. Currently we are investigating the transcriptional factors and stress conditions affecting for such overexpression in mtDNA damaged yeast. Our findings may unveil new interactions between signaling pathways, proteostasis and mitochondrial biogenesis that can be used in therapeutic approaches.

Chapter 2 provides a literature review about the origins of mitochondria and mtDNA mutations. Various diseases caused by mtDNA mutations and possible therapeutics are discussed in this chapter. Additionally, acquiring of petite phenotype and cellular mechanisms to improve the mitochondrial stress under mtDNA mutations are explained.

Chapter 3 elucidates a new approach for the rescue of petite-negative phenotype by deletion of conserved phosphatases functioning in the TOR pathway and also by deletion of PKA pathway activity, showing the importance of nutrient sensing pathways in mitochondrial biogenesis.

Chapter 4 discusses the effects of reduced or increased ribosomal biogenesis and protein translation in mtDNA depleted cells and also in mitoribosomal protein mutant fruit flies. Also this chapter aims to clarify why the heat shock proteins are used as a response to mitochondrial damage and identify the responsible elements activating these chaperones.

Chapter 2

LITERATURE REVIEW

2.1 Mitochondria and mitochondrial genome originate from bacteria

Mitochondria and chloroplasts have evolved as organelles as a result of an endosymbiotic event. One bacterium is hypothesized to engulf the secondary small bacterium and converted an organism into an organelle [11]. Chloroplasts and mitochondria are distinct organelles with the double membrane in eukaryotes. Aside from the membrane that they contained when they were an organism, they were also covered with the membrane of the host. Mitochondria-like organelles found in *Trichomonas vaginalis* such as hydrogenosome [12] or small and less functioning mitosomes found in *Encephalitozoon cuniculi* [13], *Mastigamoeba balamuthi* [14], *Trachipleistophora hominis* [15] also contain double membrane structure.

Aside from the fact that the engulfed organism's ability to hold onto its own membrane, the new organelle also kept its genome as a relic of endosymbiotic event. The amount of mitochondrial genome (mtDNA) is changing from organism to organism: *Plasmodium falciparum*, the parasite that causes malaria, has only 6-kb of mtDNA and encodes only three proteins. On the other hand plants contain very long mitochondrial genome over 11.000-kb genomes and the most protein rich mitochondrial DNA is carried by a protist *Reclinomonas americana* by expressing 27 proteins

[16][17][18]. Surprisingly it is very short in mammalian mitochondria, nearly 16-kb [19][20]. Most of the genes encoded in mitochondrial genome contribute to the energy generation from various food sources by expressing 13 different proteins that are functioning in the electron transport chain (ETC). For complex I (NADH dehydrogenase or NADH:ubiquinone oxidoreductase), the human DNA encodes for subunits *ND1* to *ND6* and *ND4L*, [21] mtDNA in other organisms also encode for *ND7* to *ND11* which are either evolutionarily lost or encoded by nuclear DNA [16]. mtDNA also encodes for cytochrome b used in Complex III and Cox1p, Cox2p and Cox3p in Complex IV. For Complex II, succinate dehydrogenase subunits *SDH2-SDH4* were expressed in nuclear DNA in yeast or in mammalians [21][16]. For the final complex of OXPHOS machinery, *ATP6* and *ATP8* subunits are encoded by mtDNA to be used in Complex V, ATP synthase [16]. Also mtDNA expresses ribosomal RNAs 12S SSU (small subunit rRNA) and 16S LSU (large subunit rRNA)[21]. Since mitochondria have a different triplet code for its codons and thus for the aminoacids, mitochondria encodes for 22 different transfer RNAs (tRNA) that are functioning in the translation of these genes [22]. The non-coded locuses of mtDNA contains replication and transcription initiation sites [21].

If the mitochondria is performing energy production or doing other essential processes; how does it function with few proteins and why does it contain so few genes? The answer is that nearly all of the proteins functioning in the mitochondria are encoded in the nuclear genome, translated in the cytoplasm into proteins and then imported into the mitochondria [23]. The mitochondrial genes were transferred into nuclear genome a process called endosymbiotic gene transfer [24]. Also some of the mitochondrial genes were lost and this lead to changes in the protein content of the mitochondria. Currently, 85% of the proteins in the mitochondria cannot be found in the bacterial ancestors [25]. Such change created high complexity and efficiency with new proteins evolved into new protein import and electron transport chain machinery [25]. Evolving of the import complexes was the first phase of transition from an

endosymbiont to an organelle since the proteins synthesized in cytoplasm can be imported into mitochondria [26].

2.2 Mitochondrial DNA mutations cause many diseases

Although most of the proteins functioning in mitochondria are generated outside of mitochondria, damage to the mitochondrial DNA causes many diseases in various organisms including humans. The first discovered mitochondrial diseases are Kearns-Sayre syndrome (KSS) [27] and Leber's hereditary octicus neuropathy (LOHN) [28]. For KSS, functional defects were found in Complex I and III which are highly linked to the maternal inheritance [27]. The biopsies of muscles of the patients showed large scale deletions to almost 7-kb in some tissues however mitochondria isolated from other muscles tissues were showing normal length mtDNA [27]. This is due to heteroplasmy and will be discussed in later chapters. Since respiration and ATP production is the main role of mitochondria, electron transport chain deficiencies also cause diseases like mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) [29] and Leigh's syndrome [30][31].

LOHN is also one of the first mitochondrial diseases to be identified in 1988. It is a R320H point mutation found in the *ND4* subunit of Complex I [28]. As in KSS, the disease is inherited in families with maternal inheritance and this showed the first link between neurological diseases and energy production machinery. Also KSS deletions may be more relevant to yeast mtDNA deletion work. Two years after the finding of KSS and LOHN, mycolinc epilepsy and ragged red fiber disease (MERRF) was discovered [32]. Interestingly the mutation that was identified is not in the energy production machinery. The tRNA that carries lysine is changed with another point mutation which converted conserved alanine residue to guanine. As LOHN, MERRF was also heteroplasmic however even a small percentage of wildtype mitochondrial DNA was sufficient to delay the effects of this disease to a late age [32]. Although

respiration deficiencies cause various diseases, other mutated genes in mtDNA encoding tRNAs also cause cardiomyopathy [33], and other research indicates extensive deletion of mtDNA also causes cardiomyopathy [34].

Although cancer cells shutdown the energy production in mitochondria to use these carbon products to build more cellular blocks, mtDNA mutations are also associating with cancers in many tissues with varying degrees [35][36][37]. However not all mitochondrial diseases were originating from mutations in mtDNA, there are also genomic DNA mutations evoking mitochondrial related defects. Mutations in genes encoding mitochondria GTPase mitofusin 2 causes Charcot-Marie-Tooth neuropathy [38]. Mutations in proteins required for iron-sulfer clusters and in mitochondrial fusion cause Friedreich's ataxia and autosomal dominant optic atrophy (ADOA) respectively [39][40].

2.3 There is no treatment found to cure mitochondria associated diseases

Unfortunately, there is no effective way found to cure for most of the diseases associated with mitochondria [41]. One approach for treating mitochondrial diseases is by Coenzyme Q10 treatments which showed a possible approach to alleviate the conditions of Friedriech's ataxia and Parkinson's disease [42][43][44]. Since the ETC mutations lead to higher concentration of free radicals, usage of antioxidants like coenzyme Q10 and N-acetylcycteine were improving OXPHOS conditions and reducing free radicals in a mutation that causes neuropathy, ataxia and retinitis pigmentosa (NARP) and maternally inherited Leigh's syndrome (MILS) [45]. Arginine is also used against MELAS as a preliminary method; but a controlled experiment is still needed for the clinical usage of arginine [46]. Besides the therapeutics, diagnostics of the mitochondrial disease in humans is also complex. Unlike genomic DNA which has two copies per cell, mitochondria have thousands copies per cell and the ratio of mutated mitochondrial genome versus wildtype (heteroplasmy) determines the fate of

the cell and the organism [47]. Mostly the COX (cytochorome c oxidase) activity is measured in order to detect the heteroplasmy level that can lead to a disease phenotype [21]. Most of the mutational genotypes do not have a clear phenotype and due to the heteroplasmy, the disease find in one tissue may not reflect the state of the whole body. Moreover, some of the mutations curtail the lifespan of the patients to early childhood, and many of the patients were deceased prior to diagnostics. Since muscle and neuronal tissue are excessively using ATP, a mutation in the respiration deficiency harshly affects the muscle and neuron tissues. Muscle biopsies were performed in order to visualize and experiment on the tissue containing disease phenotype. Increase in technology and usage of the next generation sequencing (NGS) is also shown as a direction in terms of diagnosing the mitochondrial diseases with exome sequencing [48][49]. However sequencing of the heteroplasmic mtDNA mutation containing tissue will not determine the ratio and also it might still give false positives and lead to false diagnosis.

Inheritance of mtDNA associated diseases is also important in therapeutics of a disease. Inheritance will be in Mendelian fashion if the mutation is encoded in genomic DNA, but if not, it will only be inherited from the mother since maternal mitochondria in the only mitochondria in the formation of zygote. So if the mother has a mitochondrial disease, it will be likely for the child to inherit the disease depending on the heteroplasmy [47]. The inheritance ratio of wildtype to mutant is based on chance and the progeny containing higher ratio of the mutant will likely have a more severe disease phenotype. Nuclear gene transfer techniques prevents involvement of maternal damaged mtDNA with transferring the pronucleus of the fertilized egg cells to another oocyte donated by another female which has wildtype mtDNA [21][50]. However there is still a chance for entry of the mutant mtDNA while transforming the nucleus into the third parent's egg cell. Also there are ethical concerns restricting a clinical trial for this approach.

2.4 Yeast studies can elucidate new interactions influencing mitochondrial biogenesis

In order to find new possible cures for treating mitochondrial disease, *Saccharomyces cerevisiae* has been studied, due to its ease of genetic modification, reproducibility, conservation of genes and ease for using in biochemical assays. One advantage about using yeast is that deletion of its entire mitochondrial genome does not cause any lethal phenotype. Interestingly, it has been shown that mtDNA can be depleted in cultured human cells [51]. mtDNA is not essential for yeast to grow on fermentable carbon source and cells lacking their mitochondrial genome are called "cytoplasmic petite" and denoted with ρ^0 for complete mitochondrial loss or ρ^- for partial loss of mtDNA .

Petite cells were first found in 1949 during visualization of *Saccharomyces cerevisiae* cell size [52]. In a plate, 1-2% of the cells were giving small colony sizes and the rest were giving normal colony size. When the big colonies were re-streaked onto another plate, again 1-2% of the cells were giving small colony sizes. However when small colonies were streaked to another plate, 100% of the cells were giving small cellular size termed petites. Interestingly when wildtype big cells were treated with acriflavin, 100% of the cells were petite phenotype. Another interesting finding of Ephrussi was that petite colonies were not able to respire on non-fermentable carbon source, however big colonies had no problems growing on non-fermentable carbon source [52]. Also petites and anaerobic wildtype was growing at the same rate on fermentation media. Moreover, when the wildtype cells were crossed to petites, the progeny was not demonstrating Mendelian inheritance for colony sizes or respiration ability of spores.

By that time, they were not able to conclude that such difference is caused by the mitochondrial DNA mutations. Nevertheless the characterization experiments were performed for petites to better understand the genetics and biochemistry behind the formation of small size cells with respiration deficiencies. Aside from the knowledge

that petite cells were carrying a “cytoplasmic factor”, which is now known as mitochondrial DNA, for the phenotype to be inherited [53] and demonstration of a the growth defect by petite cells [54], other experiments were also shown that these cells were lacking OXPHOS compartments [55][5]. After 15 years, during mitochondrial purification studies, DNA has also been found to be co-purifying with mitochondria [56]. Also DNA was visualized with electron microscopy inside mitochondria [57]. With the finding of ethidium bromide’s ability to destroy entire mtDNA, a new notation is created for petites with ρ^0 which meant complete depletion of DNA [10][58].

2.5 Genes controlling the ATP distribution and electrochemical potential generation become essential after mtDNA depletion

Loss of mitochondrial genome causes the inhibition of expression in the subunits of ETC and this leads to inhibition of ATP synthesis and electrochemical potential generation [3]. Deletion of entire mitochondrial DNA impairs the electron transport machinery since it encodes for many subunits. NADH is generated in breakdown of acetyl-CoA in TCA cycle and beta-oxidation of fatty acids [59]. Electrons that are carried by NADH is then transferred to Complex I [59]. Deletion of NADH dehydrogenase compartments will lead to a deficiency in the electron transport chain initiation from NADH to Complex I. With the deletion of mtDNA, electron passage is also halted at the cytochorome c oxidase (COX) which accepts electrons from cytochorome c [59]. Stopping of energy production through acceptance/donations of electrons in the ETC also blocks the hydrogen ion, proton, passages to the IMS since that energy is crucial for the proton pumping [59]. As a conclusion a reduced inner membrane electrochemical potential is generated (Figure 2.1A and 2.1B).

Yeast requires some nuclear encoded genes in the absence of its mtDNA which are called petite-negative genes. Simultaneous mutation of petite-negative genes and deletion of mtDNA causes lethality phenotype, also called as petite-negative phenotype

[5]. None of the petite-negative genes is essential for wildtype yeast containing mtDNA. Deletion of ADP/ATP antiporter, α and β subunits of F1-ATPase, catalytic and regulator subunits of i-AAA protease, outer and inner membrane import proteins, prohibitins and many other genes have been found to cause a petite-negative phenotype [5][60][61][62][63]. Over the years many suppressors were identified for the rescue of lethality, however among them *aac2* mutations give the most severe petite-negative phenotype with no suppressors have been characterized so far. *AAC2* encodes for an ADP/ATP antiporter that is responsible for exchanging ATP that is generated inside of the mitochondria for ADP in cytoplasm thus supplying ATP to cytoplasm for cellular processes. However it also exchanges cytoplasmic ATP for mitochondrial ADP when there is no or reduced ATP synthesis in the mitochondria, like the conditions of petite yeast [5]. ATP generated by glycolysis inside the cytoplasm has to be imported into the mitochondria in mtDNA lacking yeast since many other essential ATP requiring reactions continue to proceed inside of the mitochondria. Hence removal of Aac2p or adding Aac2p inhibitor bongkrekic acid to mtDNA lacking yeast causes lethality [64][65] (Figure 2.1C).

Since mtDNA lacking cells have disrupted electron transport chain, the electrochemical potential ($\Delta\Psi^{mito}$) is decreased. The $\Delta\Psi^{mito}$ is crucial for mitochondria since many proteins and metabolites that are in the cytoplasm, imported into the mitochondria via potential dependent mechanisms. Even in the mtDNA lacking cell, there is still a reduced $\Delta\Psi^{mito}$ around -55 mV [66] compared to -180 mV in wildtype cells [67]. This makes mitochondria less efficient for import and proteins with N-terminal mitochondria localization sequence often localize to cytoplasm [3]. As in the case of ATP supplementation, Aac2p again takes the role in $\Delta\Psi^{mito}$ generation. While importing ATP into the mitochondria, Aac2p also increases the mitochondrial electrochemical potential since ATP is charged with -4 compared to ADP charged with -3. But how does the cell constantly supply ADP^{3-} to the Aac2p? ATP imported into the mitochondria is used by F_1ATPase to generate more ADP^{3-} [5]. In the presence of mitochondrial DNA, F_1F_O-ATP synthase normally generates ATP through passing the

protons inside of the complex machinery and initiating a motor force. α and β subunits of F_1-ATPase form hexamer structures and γ subunit rotate around the hexamer with the force supplied by passage of the proton by the help of F_O-ATPase [68]. When the mitochondrial DNA is depleted, F_O subunit of the ATP synthase is not expressed and the ATP synthase is nonfunctional [5]. However cells still need subunits of F_1-ATPase in order to proliferate in the absence of mitochondrial DNA [5]. Deletion of Also *ATP1*, *ATP2* and *ATP3*, α, β and γ subunits of the F_1-ATPase gene lead to petite-negative phenotype [5]. The reason cells need F_1-ATPase is the need for ADP^{3-} to be generated inside of the mitochondria to trade it for ATP^{4-}, generated in the cytoplasm, via ATP/ADP antiporter (Figure 2.1B and 2.1C) [5]. In budding yeast, mutation of δ subunit of the F_1-ATPase caused formation of petite colonies by loss of F_1-ATPase function [69]. These cells also showed a proliferation defect that is linked to the $\Delta\Psi^{mito}$ [69]. The blocking of F_1-ATPase via mutation of δ subunit disabled the hydrolyzation of ATP and could not supply ADP^{3-} to Aac2p [69]. *Trypanosoma brucei* mitochondrial DNA is kept in kinetoplast as kinetoplast DNA (kDNA) and mtDNA lacking ρ^- yeast has a parallel organism as dyskinetoplastic *T. brucei* [70]. The bloodstream, mitochondrial containing form of trypanosomes lacks the Complex III and Complex IV and it is impaired for ATP synthesis and electrochemical potential generation. However it compensates the loss of $\Delta\Psi^{mito}$ by pumping the protons via F_1F_O-ATP synthase [71][72]. Dyskinetoplastic *T. brucei* lacks the F_O-ATPase and the question asked was how these trypanosomes were alive without generating their potential via F_1F_O-ATPase [70]. The dominant γ subunit mutation in F_1-ATPase L262P and A273P were rescuing the proliferation defect of the kDNA lacking *T. brucei* [70]. In addition, a hyperactivated ATP/ADP antiporter is needed since bongkrekic acid fed trypanosomes could not get the full benefits of the γ subunit mutations [70]. Such finding in trypanosomes also fits with the model in the budding yeast. The interaction between F1 and AAC is not clear, however there are multiple data showing these two complexes forming super-complexes in both yeast [73] and *Leishmania* [74]. This super-complex formation may explain the connection between F1 and AAC to generate $\Delta\Psi^{mito}$ in the

absence of mtDNA. Such super-complex formation is also observed between the colocalization of Complex III and IV with inner membrane transport complexes, which uses the proton gradient generated by ETC complexes to perform protein import [75].

2.6 Petite-negativity phenotype can be used as a sensitized strain to find new suppressors

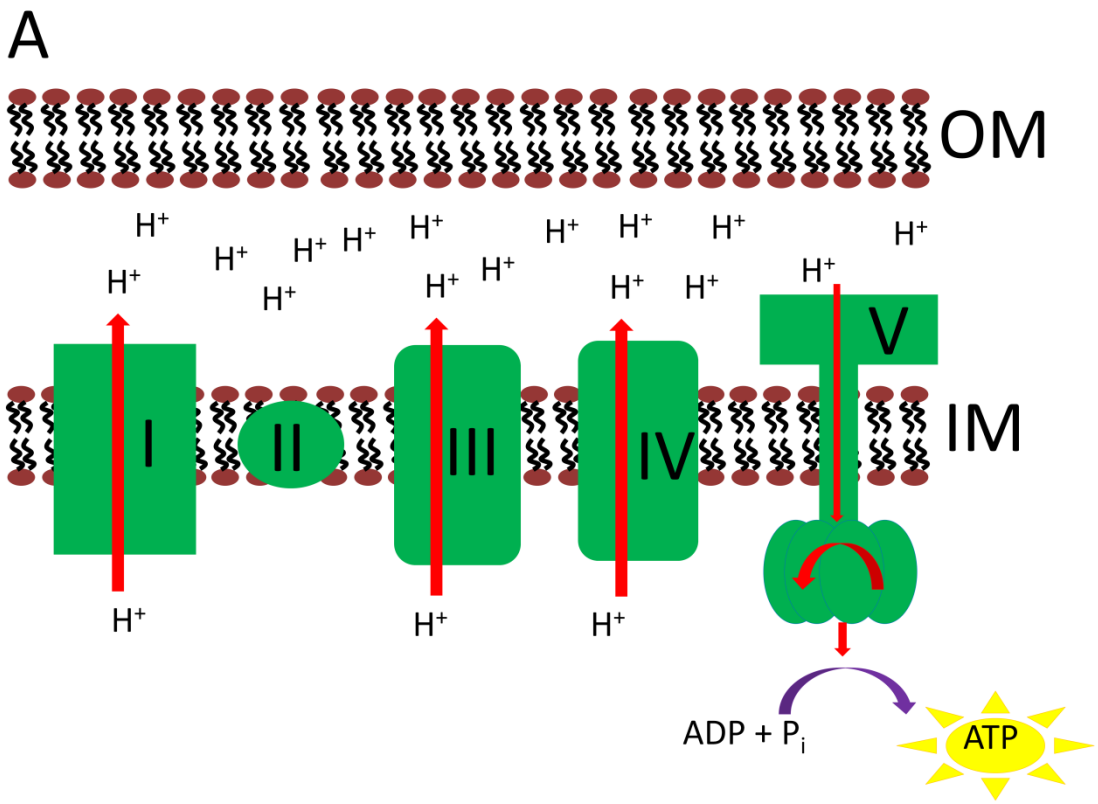
Petite-negative phenotype can be used as a sensitized yeast background in order to uncover novel interactions influencing mitochondrial biogenesis. Random mutagenesis or library plasmid overexpression screens can be used in order to find new pathways influencing mitochondrial biogenesis. Petite-negative gene deletion followed by mtDNA depletion leads to a lethal phenotype but insertion of random mutation or library overexpression plasmid may lead to living colonies. Suppressors were previously identified by complementation assays however nowadays with the improvements in next-generation sequencing, finding the random mutagenesis is much easier. For the suppressors in overexpression plasmids, it is also easy with sequencing the plasmid to unveil the gene(s) that are benefiting mitochondria.

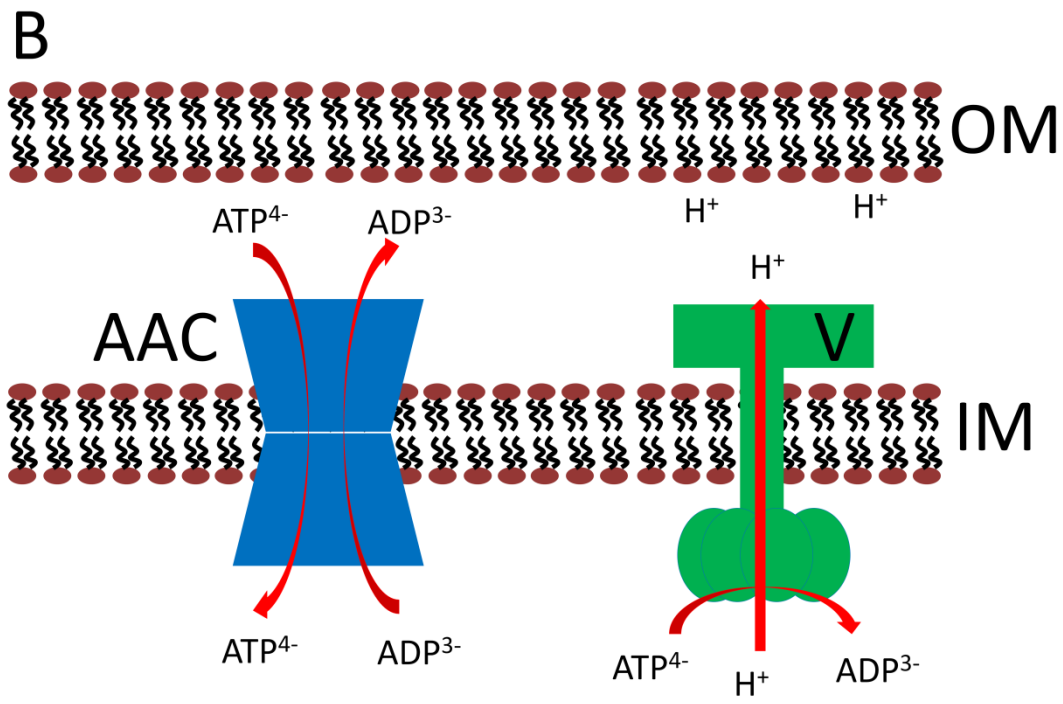
A hypothesized reason for the cause of lethality of petite-negative phenotype is reduced mitochondrial potential [5]. Mutations in specific residues of γ subunit of F_1-ATPase were shown to rescue the lethality of *yme1*Δ ρ^-, i-AAA protein functioning for the protein quality control and turnover, also a petite-negative gene [76]. Mutating similar residues in a different organism, *Kluyveromyces lactis*, also rescued the lethal phenotype [77]. Besides, our lab previously identified that increasing vacuolar pH was rescuing the petite-negative phenotype of *mgr1*Δ, subunit of i-AAA and *tom70*Δ, outer membrane import protein, cells lacking mtDNA [78]. Although the mechanism behind the rescue was unknown, increasing the pH or decreasing the acidity of vacuole was also shown to increase the $\Delta\Psi^{mito}$[78].

2.7 Other mitochondrial disease models in higher eukaryotes can also be used to find new suppressors and test the findings in yeast in more complex organisms

There are two different approaches that can be used to convert a wildtype yeast cell into a petite cell: depleting mtDNA by incubating the yeast in EtBr containing media or deleting the most genes encoding mitochondrial ribosomal proteins. Unfortunately a higher eukaryote, it is not possible to deleted entire mtDNA of fruit fly, *Drosophila melanogaster*. However a similar mutation at its mitochondrial translational machinery can be used as a disease model [79]. tko^{25t} mutation causes reduced mitochondrial ribosomal translation and flies show prolonged average eclosion day and also go under paralysis after brief mechanical vibration due to impaired mechanoreceptor neurons [80][81]. tko^{25t} flies are also can be used as petite-negative yeast to search for pathways that can rescue its mitochondria associating defects [79].

2.8 Literature Review Figures

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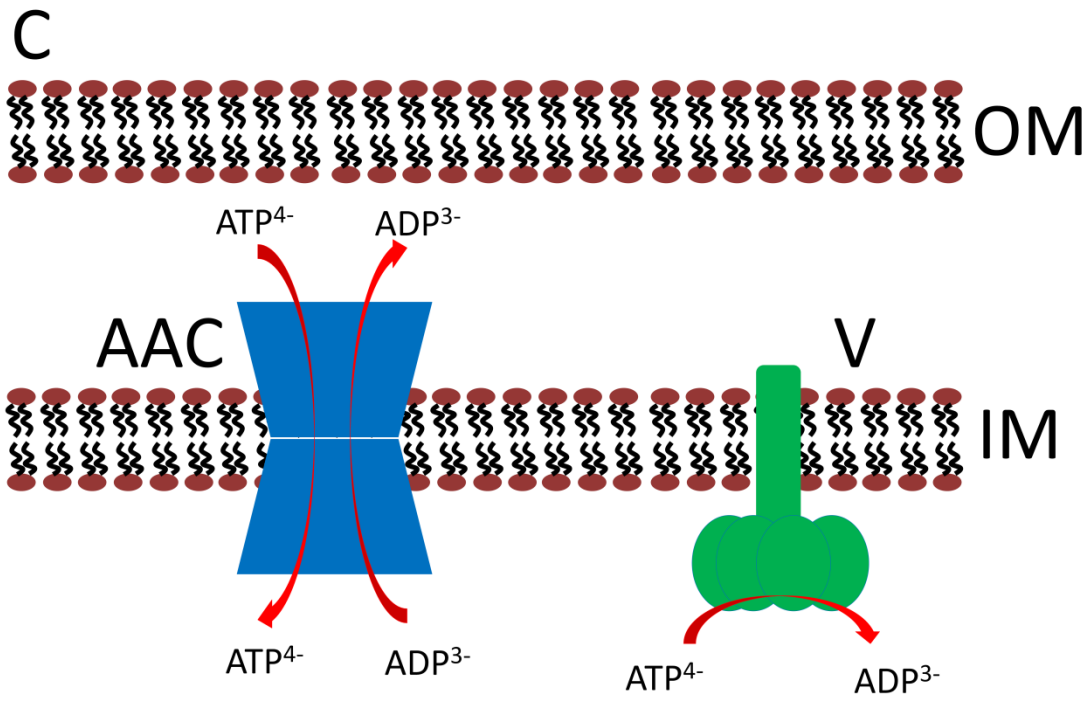
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Figure 2.1 Mitochondrial electrochemical potential generation in (A) wildtype cells, (B) cells with impaired ETC and (C) cells lacking mitochondrial DNA. (A) Mitochondrial electron transport chain complexes NADH dehydrogenase (I), Cytochrome bc_1 complex (III) and Cytochrome c oxidase (IV) transfer protons carried by NADH and $FADH_2$ from inner membrane (IM) to inter membrane space between IM and outer membrane (OM). These protons are then used by ATP synthase (V) to generate ATP. (B) In cells having impaired electron transport chain (ETC) function, ATP cannot be generated inside of the mitochondria and imported by ATP/ADP antiporter AAC complex. This introduces a negative charge into the matrix since ATP is charged by -4 compared to -3 by ADP. This ATP^{4-} is then hydrolyzed by F_1F_O-ATPase to pump protons to IMS to generate further mitochondrial potential. (C) Cells with depleted mitochondrial DNA still have same AAC function as in (B). However since they cannot encode for F_O portion of the F_1F_O-ATPase they cannot pump protons to the IMS. However they still hydrolyze ATP^{4-} to supplement ADP^{3-} to AAC for further exchange process.

Chapter 3

SUPPRESSION OF DEFECTS FOLLOWING MITOCHONDRIAL DNA LOSS BY MODULATION OF NUTRIENT SENSING PATHWAYS

3.1 Introduction

Cells respond to different nutrient conditions and arrange their metabolism through various nutrient sensing signals. Cells decide when to grow or halt proliferation, when to generate more organelles or recycle organelles to generate energy depending on the nutrient sensing machineries [82]. Target of Rapamycin (TOR) pathway is core machinery for helping the cell with such decision making. Since TOR is the centerpiece of proliferation and homeostatic balance, mutations in TOR pathway leads to severe malfunctions in the cellular state and even lead to cancers [82]. TOR responds to nutrients, growth factors, stress and energy. TOR Complex I (TORC1) is activated when there are nutrients in the environment and drives the anabolism, energy generation and consumption, on the other hand, it is in activated in starvation conditions which drives macroautophagy [83].

One of the upstream signal that regulate TOR is the Rheb GTPase which interacts with TOR to increase its activity [84]. GTPase activating protein TSC2 interacts with TSC1 and inhibits Rheb activity to downregulate TOR stimulation [84]. In mammalians, TSC1 and TSC2 also plays important role in mammalian since many

signaling pathways like AMP-activated protein kinase (AMPK) pathway and Akt/Protein Kinase B pathway interact and either activate or inactivate TOR pathway through their regulation of TSC1 and TSC2 [85]. GTPase activity of Rheb is also inhibited by BH3 proteins during hypoxia conditions which generally inhibits TOR pathway as a stress response [86]. External nutrient signaling can still stimulate TOR activity in the absence of TSC1 and TSC2 [87]; moreover, deletion of Akt pathway phosphorylation residues in TSC1 and TSC2 still does not change the growth factor signaling and the general growth of *Drosophila melanogaster* [88]. These findings state that there are still upstream regulators of TOR pathway remain to be found. Another GTPase family that acts on TOR is Rag family with Gtr1p and Gtr2p activating TOR upon amino acid signaling [89]. Upon GTPase activation with amino acid sensing, RAPTOR or Kog1p in yeast is activated by Gtr and Rag family proteins [90] These two proteins also function as subunits of EGO complex, also enable to halt the growth arrest furnished by rapamycin [89].

mTOR (mammalian TOR) is the catalytic subunit of the TORC1 however there are additional subunits of this complex in mammalians: regulatory-associated protein of mTOR (RAPTOR) and Rapamycin-insensitive companion of mTOR (RICTOR) [91][92]. Depletion of regulatory subunit RAPTOR in muscle tissue causes mitochondrial dysfunction, reduced activity in OXPHOS machinery and muscle dystrophy [93]. Apart from TSC, APMK and Akt also converge upon TOR pathway through RAPTOR [84]. AMPK was found to phosphorylate RAPTOR and drives the phosphorylation dependent interaction with 14-3-3 inhibitor protein [94]. Akt also phosphorylates RAPTOR binding protein PRAS40 and drives the phosphorylation dependent recruitment of 14-3-3 inhibitor protein [95]. RAPTOR is also phosphorylated at two clusters of serine residues and particularly phosphorylated at S863, when stimulated by nutrients and growth factors to activate mTOR in a rapamycin dependent manner [96].

Inactivation of TOR pathway drives mitophagy which is the destruction of mitochondria to produce immediate energy [97]. Inactivation of TOR due to lack of

nutrients or with rapamycin also drives autophagy and generate energy from already generated organelles of the cell rather than external sources [84]. Activation of TORC1 induces mitochondrial biogenesis and activates the OXPHOS (oxidative phosphorylation) machinery [98]. TORC1 forms complex with transcriptional factor PGC1α and YY1 and this complex induces the activity of mitochondria [98]. This is not the only mechanism by which TOR affects mitochondria. One of the substrates of the TORC1 is eIF4E binding protein (4E-BP1) and it is activated in starvation conditions where TORC1 is inactivated [99]. Activation of 4E-BP1 leads to selective translation of specific mRNAs coding for important mitochondrial oxidative phosphorylation machinery subunits [100].

TOR pathway is also effected by other nutrient signaling pathways. AMP activated protein kinase (AMPK) is activated in high AMP/low ATP conditions and activation of AMPK leads to the inactivation of TORC1 [101]. Macroautophagy and mitophagy are occurring when TORC1 is deactivated to generate rapid ATP and balance the AMP to ATP ratio [94]. However other data also suggests that beta-guanidinopropionic acid (GPA) led to an increase in AMP to ATP ratio and lead to increase in the AMPK activity in muscle tissue and thus stimulation of the biogenesis of mitochondria [102]. SNF1 kinase is the yeast ortholog of mammalian AMPK and it is also activated in stress conditions such as oxidative stress, alkaline pH and sodium ion stress [103]. Also in hypoxia conditions where OXPHOS is not possible and ATP concentration is low, AMPK is activated which then leads to the inactivation of the TORC1 [104]. AMPK together with calcium/calmodulin dependent protein kinase-kinase (CAMKK) also downregulates TOR activity and initiates autophagy upon increase in the cytoplasmic Ca^{2+} levels [105].

SNF1 kinase is regulated by upstream kinases and phosphatases. Sak1p, Tos3p and Elm1p phosphorylate SNF1 kinase on Thr210 [106]. Three different kinases impinge on SNF1 kinase under different carbon source and metabolic conditions and deletion of all three kinases deplete the SNF1 activity in yeast and in mammalians [107][106][108]. SAK1 kinase localizes to vacuole as Sip1p [109] and forms complexes

with Snf1p through amino terminal sequence [110][111]. Tos3p is important for Snf1p functions since it is the only kinase that is sufficient to activate SNF1 kinase by itself under different carbon sources [112]. SNF1 activity is also downregulated by phosphatase Glc7p which is under regulation of Reg1p [113]. Glc7p cleaves the phosphate off of SNF1 Thr210 in response to glucose deprivation to activate AMPK [114]. However AMPK shows resistance against low glucose and ATP levels in the absence of Reg1p activity [108].

SNF1 protein kinase plays a crucial role while switching media conditions [115]. Yeast prefers to live on glucose medium to perform fermentation, and transition to another carbon source stimulates expression of different genes including genes expressing OXPHOS machinery [116]. SNF1 protein kinase is targeted to downstream processes through its interaction with its beta subunits Sip1p, Sip2p and Gal83p [117] and each of the beta subunits have distinct N terminal localization signal with driving Snf1p to different locations to activate various processes [117]. In high glucose conditions in which SNF1 protein kinase is inactive, all three beta subunits localize to cytoplasm [118]. However in low glucose conditions, Sip1p localizes to vacuolar membrane, Sip2p localizes to cytoplasm and Gal84p localizes to nucleus and drive the Snf1p to perform phosphorylations to activate various processes [118].

Interestingly, mutation of three catalytic subunits of protein kinase A (PKA) pathway localizes Sip1p to vacuole even in the presence of high glucose [119]. This demonstrates another crosstalk between nutrient sensing pathways with PKA negatively regulating the localization of beta subunits of AMPK while there is glucose in the environment. Targeting of phosphorylated, active PKA to mitochondrial outer membrane by A-kinase anchor protein 121 (AKAP121) shows various phosphorylation in outer membrane proteins [120]. Blocking of the targeting by mutation in AKAP121 leads to apoptosis [120]. PKA also demonstrate an inner membrane (IM) role on mitochondrial respiration with localization to IM in matrix of bovine heart mitochondria [121]. Such localization also causes phosphorylations of proteins inside the mitochondrial matrix and specifically Complex I in OXPHOS machinery. Nuclear

encoded NDUFS4 gene is phosphorylated by protein kinase A at its C terminus which enhances its activity [121]. Activation of PKA is not always work in favor of mitochondria. Constitutively activated PKA in a *ras2* mutant, shortens the life-span of yeast cells and lead to the production of reactive oxygen species and oxidative protein damage [122]. However overexpression of Pde2p, a negative regulator of PKA reverts this phenotype in the background of the *ras2* mutant [122]. Together TOR, AMPK and PKA effects the mitochondrial biogenesis and studying these pathways can unveil new interaction influencing mitochondrial diseases.

The budding yeast has two branches downstream of the TOR pathway. *SCH9* encodes an AGC family protein kinase and Sch9p is the yeast ortholog of mammalian S6K [123]. Sch9p is phosphorylated by TORC1 to activate ribosomal biogenesis, ribosomal protein synthesis and protein translation [123]. Sch9 kinase or S6K activity is downregulated by dephosphorylation by rapamycin treatment or PI3K inhibitors [124][125]. However rapamycin and PI3K regulate S6K separately since deletion of amino-terminal sequence of S6K gains resistance against rapamycin however the sensitivity against PI3K continues [126]. Mutation of *SCH9* causes parallel phenotype as rapamycin treated cells which causes a decrease in the expression of genes regulating ribosomal translation mainly through Rim15p stimulation [124][127]. The beneficial effect of calorie restriction by increased lifespan is also seen in *TOR1* or *SCH9* mutations. However the longevity of these cells were not increased in the calorie restriction [128].

Another branch downstream of TOR pathway is through Tap42p [129]. Tap42p regulates the stability and activation of Pph21p and Pph22p, yeast ortholog of PP2A, and Sit4p, yeast ortholog of PP6 [130]. Deletion of TAP42 is lethal for wildtype cells and temperature sensitive version of *TAP42*, *tap42-11* demonstrates a drastic reduction in protein translation [130]. Tap42p is phosphorylated by TORC1 and thus regulated for its interaction to the downstream phosphatases with competition to bind to the catalytic subunits of PP6 and PP2A [131][132]. Tor2p is also shown for its ability to phosphorylate Tap42p [132]. This interaction breaks apart in the presence of rapamycin

or absence of nutrients with dephosphorylation of Tap42p [130]. Tap42p itself is not affected by rapamycin with *tor1* mutants showing resistance to rapamycin still show Tap42p phosphorylation [132]. So Tap42p has an inhibitory function against PP2A and PP6 [133]. The mammalian version of Tap42p, α4 also binds to the catalytic subunits of PP2A and PP6 [134][135]. Again, as Tap42p-Sit4p, the interaction between α4 and PP2A is inhibited upon treatment with rapamycin [134].

There is also contradictory evidence showing that downregulation of Tap42p activity by using temperature sensitive allele at non-permissive temperature shows similar transcriptional regulation as rapamycin fed cells [131]. Stress response genes are activated when cells were treated with rapamycin since rapamycin blocks the nuclear export signal for these genes [136]. Nuclear import of Msn2p is not TOR dependent [136] and it is hypothesized that there is a Tap42p-PP2A or Tap42p-PP6 complex that exports the Msn2p from nucleus to inhibit the expression of stress response genes [131]. Also for nitrogen deprivation pathway (NDP) and retrograde signaling pathway (RTG) genes are induced in the presence of rapamycin, however disruption of Tap42p activity blocked the expression of these pathways [131]. Transcription factors regulating the expression of NDP genes mainly located in the cytoplasm and phosphorylated versions of Gln3p and Ure2p localize to nucleus to activate gene expression [137][138]. Sit4 dephosphorylates Gln3p and Ure2p and when rapamycin is fed and Tap42p activity is blocked, it is expected to see dephosphorylated versions of Gln3p and Ure2p [137]. However this is not the case. Tap42p, in this case, is not inactivating Sit4p to dephosphorylate downstream targets since impaired Tap42p also inactivate Sit4p [131]. Interestingly loss of Tap42p and Sit4p or Pph21p/Pph22p generates the same response with rapamycin treatment which highlights the concordance of activities of Tap42p and PP6 or PP2A [131].

Another protein that is influencing the Tap42p activity is Tip41p (Tap42p interacting protein) which binds and inactivates the Tap42p [139]. Tip41p activation increases the PP6 and PP2A activity and Sit4p cleaves the phosphate group on Tip41p to tighten the interaction between Tap42p-Tip41 as a feedback loop [139]. Ser/Thr

protein kinase NPR1 is activated when TOR pathway is active [133] however rapamycin treatment results activation of Sit4p and dephosphorylation of Tip41p to influence the dephosphorylation of NPR1 kinase [139]. Tip41p activity increases the Sit4p interaction with SAPs (Sit4p associating proteins) to target to downstream pathways and deletion of *TIP41* lead to reduced Sit4p activity and hyperphosphorylation of NPR1 kinase which is also seen when cells are deleted of *SAP155* [139]. Mammalian TIP41 also was shown to inhibit the TOR activity [140].

TAP42 forms complexes with PP2A and PP6 to regulate downstream targets [130][141]. The budding yeast encodes for orthologs of mammalian PP2A and PP6 as redundant activity of Pph21p/Pph22p and Sit4p activity respectively [142] [143]. Deletion of one of the redundant Pph21p or Pph22p does not severely affect the cell growth however simultaneous deletion causes almost complete suppression of complex activity [142]. Both Pph21p and Pph22p perform same functions by participating in a heterotrimeric complex also consisting of α and β subunits [144]. The scaffolding and stability of the complex is maintained through α subunit which is expressed by *TPD3* in budding yeast [145]. The beta subunits are expressed by *CDC55* and *RTS1* which perform the catalytic activity for the complex [146][147]. Mammalian PP6 activity is furnished by *SIT4* in yeast [143]. Like Pph21p/Pph22p, Sit4p associates with SAPs (Sap4p, Sap155p, Sap185p and Sap190p) to form complexes to regulate G1 cyclin production and initiates the process of G1 in cell cycle [148][149][143].

The complex formation between TAP42 and PP6 or PP2A does not depend on the associating subunits of PP6 or scaffolding α subunit of PP2A [150][130]. TOR pathway activation phosphorylates Tap42p to enhance the interaction between Tap42p and PP2A and PP6; such interaction inhibits PP2A and PP6 function [132][130]. Amino terminus of Sit4p and Pph21p are highly similar and this region is used to bind to Tap42p [141]. Mammalian two hybrid assay also showed that 50 aminoacid residues located at the N terminal of PP6 is interacting with α4, mammalian ortholog of TAP42 [135]. This interaction is halted when cells treated in the presence of rapamycin or in the absence of wildtype TOR function [130]. Such abolishment of interaction results the

increasing phosphatase activity of PP6 and PP2A which results to dephosphorylation of downstream targets NPR1 kinase [133] and transcriptional activator GLN3 [151]. Tap42p is also known to block the complex formation between PP2A and its α and β subunits through competition for PP2A binding sites [132] and maintains its role as a phosphatase inhibitor [139].

As discussed in Chapter 2: Literature Review, deletion of mitochondrial genome via EtBr treatment in budding yeast leads to (i) ATP synthesis deficiency, (ii) reduced inner membrane electrochemical potential generation and (iii) reduced mitochondrial protein import [5][3][152]. F1β subunit of ATP synthase, *ATP2,* becomes essential in the absence of mitochondrial DNA since it hydrolyze the ATP^{4-} generated in and imported from cytoplasm to ADP^{3-} which is then used by ATP/ADP antiporter to import more ATP^{4-} to generate more potential [5]. Deletion of *ATP2* leads to a petite-negative phenotype in mtDNA lacking cells. Previous data showed that overexpression of Tap42p inhibitor TIP41 gene in high-copy plasmid rescued the proliferation defect of mtDNA lacking cells deleted of Atp2 protein [63]. As described above, Tip41p interacts and blocks the activity of Tap42p which regulates the activity of PP2A and PP6 [139]. We found that temperature-sensitive allele for Tap42p and deletion of phosphatases functioning downstream of Tap42p rescued the proliferation defect of the *atp2*Δ ρ^- cells. Aside from the rescue we also found that these phosphatase deletions are beneficial for the proliferation delay, electrochemical potential generation, mitochondrial protein import and wildtype transcriptome level maintenance in mtDNA lacking cells. Interestingly we also demonstrated that SNF1 kinase is essential for the rescue of petite-negative lethality and also contributes to the beneficial effects of phosphatase deletions on mtDNA lacking cells.

Similar to the *TIP41* rescue, previous work also showed that a high-copy plasmid carrying *PDE2* gene has been found rescue the petite-negative phenotype of *tim18*Δ ρ^0 cells [153]. As *TIP41,* it also benefits to the proliferation of wildtype (*WT*) ρ^0 cells. Pde2p is a cyclic AMP phosphodiesterase and is a negative regulator of the PKA pathway [154]. Since it is a component of the PKA pathway, we investigated the

deletion of this gene and the downstream genes participating in the PKA pathway in the background of ρ^- cells. The beneficial effects seen on ρ^- cells by downregulation of these two highly conserved pathways can be converging on the same protein/pathway or separately benefiting the cells with mtDNA damage. Unfortunately there is no therapeutic approach that has been found yet to cure mitochondrial disease [155]. However recent findings about how nutrient sensing pathways, such as TORC1 [156] or PKA [157], influence the mitochondrial biogenesis and disease may be a direction towards curing mitochondrial disease.

3.2 Results

3.2.1 Petite-negativity phenotype of *atp2*Δ cells lacking mtDNA rescued by removal of type 2A and type2A-like phosphatase activity

We already know that Tip41p overexpression in cells with petite-negative phenotype was rescuing both *tim18*Δ ρ^- cells [63], *atp2*Δ ρ^- cells [158] and *pde2*Δ ρ^- cells [153]. Tip41 protein interacts with Tap42p to regulate the stability and activation of the type 2A and type 2A-related phosphatases downstream of the TORC1 [139]. Since *TAP42* is an essential gene, we used a temperature sensitive allele *tap42-17* which has wildtype Tap42p activity at 24^{o}C, impaired activity at 30^{o}C and is depleted at 37^{o}C. We demonstrated that the mutant, at semi-permissive temperature at 30^{o}C, rescued the petite-negative lethality of *atp2*Δ ρ^- cells (Figure 3.1). The rescue states that Tip41p and Tap42p were performing opposite functions with Tap42p mutation or higher levels of Tip41 lead to beneficial effects on the proliferation of petite-negative phenotype.

Tap42p is controlling the assembly and folding of downstream phosphatases in mammalians such as PP2A, PP4 and PP6 [150]. In *S. cerevisiae*, PP2A activity is generated by Pph21p and Pph22p, PP4 activity is delivered by Pph3p, PP6 activity is supplied with Sit4p and PP2A-like phosphatases activity is furnished by Ppg1p. We have found that depletion of PP2A and PP6 activity with simultaneous deletion of

Pph21p and Pph22p or of Sit4p rescues the petite-negative lethality of *atp2*Δ ρ^- cells (Figure 3.2). However blocking of PP4 and PP2A-like phosphatase activity by deleting Pph3p and Ppg1p could not suppress the growth defects of *atp2*Δ ρ^- cells. This study only focuses on the effects of the rescue of Pph21p/Pph22p and Sit4p; since Pph3p and Ppg1p were not influencing the phenotype of *atp2*Δ ρ^- cells, they were not studied further. We also had to be sure that these cells were ρ^- cells. If the deletion of Pph21p/Pph22p or of Sit4p blocking the loss of mitochondria, it was no surprise to see the proliferation of *atp2*Δ ρ^+ cells. We demonstrated that such deletions were not inhibiting EtBr activity and were able to deplete mitochondrial DNA [158].

Since we already know that two different petite-negative genes can be rescued by the overexpression of *TIP41*, we wanted to know whether other petite-negative genes can be rescued by deletion of Sit4p. Interestingly *sit4*Δ enabled the proliferation of ρ^- cells lacking Tom70p, a mitochondrial outer membrane receptor functioning in mitochondrial import, or lacking Yme1p, mitochondrial i-AAA protease [158]. Previously found mutation raising vacuolar pH was able to suppress Tom70p deletion but was not able to suppress the Yme1p deletion [78]. This showed that petite-negativity phenotypes vary in terms of their severity [78] and Sit4p was able to rescue all of petite-negative genes except for Aac2p removal. Deletion of Sit4p was not able to rescue the loss of ADP/ATP antiporter loss after mtDNA depletion [158]. Mitochondrial ATP/ADP antiporter, Aac2p, becomes essential after mtDNA deletion because of its role in supplying ATP into the cell and generating mitochondrial electrochemical potential [5]. Deletion of *SIT4*, *PPH21* and *PPH22* in the same background was impossible since triple mutant lead to synthetic lethality [145][Dunn, unpublished data]. Such lethality disabled us from investigating the consequences of removing both the PP6 and PP2A activity in *atp2*Δ ρ^- cells.

Sit4p is directed toward downstream substrates by four SAPs (Sit4p-Assocaited-Proteins): Sap4p, Sap155p, Sap185p and Sap190p [148]. We deleted each SAP by itself in the background of *atp2*Δ ρ^- cells however single deletions were not able to suppress the petite-negative phenotype (Figure 3.3). Single colonies that are visible in cells

deleted of SAPs are also seen in *atp2*Δ ρ^- cells which can be a random mutagenesis that can rescue the lethality phenotype. Double deletions of specific pairs of SAPs suppressed the proliferation defect of *atp2*Δ ρ^- cells (Figure 3.4). Plate assay is a semi-quantitative approach and quantification can only be done with liquid proliferation assay however from the growth that is visualized on plate we can say that *atp2*Δ *sap155*Δ *sap190*Δ ρ^- cells had similar proliferation rate as *atp2*Δ *sit4*Δ ρ^- cells (Figure 3.4). Moreover, *atp2*Δ *sap185*Δ *sap190*Δ ρ^- cells also showed a mild proliferation compared to high proliferation of *atp2*Δ *sap155*Δ *sap190*Δ ρ^- cells (Figure 3.4). As a conclusion, we can say that blocking of Sit4p activity leads to the rescue of *atp2*Δ ρ^- cells is maintained through specific SAP pairs that are influencing on mitochondrial function. Similar to Sit4p, Pph21p and Pph22p are also directed toward downstream of the pathway via regulatory subunits Cdc55p and Rts1p [159]. Yet again similar to Sit4p, single deletions of the regulatory subunits could not suppress the petite-negativity phenotype of *atp2*Δ ρ^- cells (Figure 3.5) but when deleted simultaneously, loss of regulatory subunits of Pph21p and Pph22p lead to the proliferation of *atp2*Δ ρ^- cells at a similar rate as *atp2*Δ *pph21*Δ *pph22*Δ ρ^- cells (Figure 3.5). Beneficial effects seen in rescuing of petite-negative phenotype is controlled by the downstream associating and regulatory subunits of conserved phosphatases Sit4p and Pph21p/Pph22p.

3.2.2 SNF1 kinase activity is required for the rescue of petite-negative gene deletion followed by mtDNA loss

We used *sit4*Δ as a suppression of the petite-negative phenotype in order to further analyze the effects seen by Tip41p hyperactivity/Tap42p dysfunction. Previous work states that Sit4p is cleaving the phosphorylation of T210 on Snf1p and converts it into an inactive state [160]. Snf1 is the catalytic subunit of the SNF1 kinase complex which is the yeast ortholog of AMPK complex and deletion of Sit4p would lead to constant Snf1p phosphorylation thus the activation. The dephosphorylation ability of Sit4p phosphatase could be the reason for the rescue of *atp2*Δ ρ^- cells. Interestingly, we

found that *atp2*Δ *sit4*Δ *snf1*Δ cells showed a significant decrease in proliferation rate however still showed a viable phenotype (Figure 3.6). This indicated that Snf1p plays a crucial role for the rescue of *atp2*Δ ρ^- petite-negative lethality phenotype after Sit4p removal however the mild proliferation rate observed means that Sit4p removal also benefits petite-negativity phenotype in an alternative pathway. Mig1p is a transcriptional repressor in its inactive state and is a well-characterized target of Snf1p which phosphorylates Mig1p [161]. Phosphorylation of Mig1p activates the transcriptional repressor to be released from the DNA and this leads to transcriptional activation of repressed genes controlled by Mig1p. Among the genes whose expression is blocked by Mig1p, there are many genes involved in the metabolism of various carbon sources except glucose [161]. In order to find whether phosphorylation of Mig1p by Snf1p is the key for the petite-negative rescue, we deleted *MIG1*, and found that the *atp2*Δ *sit4*Δ *snf1*Δ *mig1*Δ ρ^- cells can proliferate at the similar rate as *atp2*Δ *sit4*Δ ρ^- cells (Figure 3.6). In order for cells to be proliferating in *atp2*Δ *sit4*Δ, the Mig1p must be phosphorylated by Snf1p to activate the expression of one or more genes. However *mig1*Δ could not rescue the proliferation defects of *atp2*Δ ρ^- cells by itself (Figure 3.10). This means that impaired PP6 activity is needed to visualize beneficial effects on the *atp2*Δ ρ^- cells.

Also there is no evidence suggesting the hyperactivation of SNF1 kinase is the sole reason for the rescue of *atp2*Δ ρ^- cells. Snf1p is also directed toward downstream targets by its associated β subunits Sip1p, Sip2p and Gal83p [117]. However, single deletions of the associating β subunits did not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells (Figure 3.7). There are also kinases, Sak1p and Tos3p functioning in the upstream of the SNF1 kinase in order to activate it [117]. However deletion of the Sak1p or Tos3p did not block the rescue of the petite-negative phenotype by *sit4*Δ (Figure 3.8). Deletion of a third kinase, Elm1p, which was functioning in the upstream of Snf1p, caused synthetic lethality with *sit4*Δ which restricted us from studying this gene in our studies. Deletion of a phosphatase, Reg1p, which inhibits the Snf1p activation, could not rescue *atp2*Δ ρ^- cells (Figure 3.11).

Since Snf1p is crucial for the rescue of *atp2*Δ ρ^- cells by deletion of *sit4*Δ, we asked whether Snf1p mutant is petite-negative. In fact Snf1p deletion by itself demonstrated petite-negative phenotype after loss of mitochondria genome (Figure 3.9). Interestingly, the petite-negative phenotype of *snf1*Δ ρ^- cells could not be rescued by deletion of Mig1p [158] however it can be rescued by the expression of the *ATP3-5* allele (Figure 3.9) which raises the mitochondrial electrochemical potential in ρ^- cells [158]. These results indicate that electrochemical potential loss in the *snf1*Δ cells can be the reason for the lethality in the absence of mitochondrial DNA. Rescue of *snf1*Δ ρ^- cells by Sit4p deletion also supports this idea since deletion of Sit4p also increases the $\Delta\Psi^{mito}$ even in the absence of the SNF1 kinase.

3.2.3 Other possibilities were also investigated to determine the beneficial effects of ρ^- cells by Sit4p removal

Transcriptional activator Rtg1p and its subcellular localization regulator Rtg2p are controlling the retrograde (RTG) signaling and TOR pathway [162]. We wanted to block the retrograde signaling pathway since lack of Sit4p and Pph21p/Pph22p prevents the full activation of retrograde signaling pathway by rapamycin treatment [131]. However deletion of Rtg1p or of Rtg2p does not suppress the lethality of *atp2Δ* ρ^- cells (Figure 3.12).

Npr1p protein kinase is negatively regulated with phosphorylation by TORC1 [133]. Npr1p is excessively phosphorylated in *tap42* mutant in the absence of rapamycin which leads to its inactivation or in *sit4*Δ in the presence of rapamycin. However deletion of Npr1p does not enable the proliferation of *atp2Δ* ρ^- cells (Figure 3.13). Npr1p has a paralog Prr2p that arose from whole genome duplication [163] which is also a protein kinase. However as Npr1p, deletion of Prr2p does not enable the proliferation of *atp2Δ* ρ^- cells (Figure 3.21).

Increased TOR activity were shown to decrease the autophagy and macroautophagy [83]. Also deletion of Sit4p decreases the enhanced autophagy by rapamycin treatment [164]. However deletion of Atg1p neither suppresses the petite-negative phenotype of *atp2*Δ ρ^- cells nor blocks the proliferation of *atp2*Δ *sit4*Δ ρ^- cells (Figure 3.14).

Furthermore, we wanted to inhibit the response to nitrogen starvation signaling since lack of Sit4p and Pph21p/Pph22p prevents the full activation of nitrogen discrimination pathway by rapamycin treatment [131]. Deletion of transcriptional activators of nitrogen catabolite repression genes Gat1p and Gln3p were used to block the response to nitrogen limitations [165]. However simultaneous deletion of Gat1p and Gln3p does not suppress the petite-negative phenotype of *atp2*Δ ρ^- cells (Figure 3.15). We also inhibited G1 cyclins which have role in the cell cycle progression. Sit4p is required for full accumulation of G1 cyclins during G1 stage of cell cycle [149]. However deletion of Bck2p and Swi4p does not enable the proliferation of *atp2*Δ ρ^- cells (Figure 3.16). Sit4p also activates multidrug resistance in yeast and deletion of Sit4p decreases the levels of Pdr1p and Pdr3p which makes the yeast cells less resistant to drugs like cycloheximide and azoles [166]. However loss of pleotropic drug resistance pathway by simultaneous deletion of Pdr1p and Pdr3p does not enable the proliferation of *atp2*Δ ρ^- cells (Figure 3.17).

Cory D. Dunn demonstrated via RNAseq experiments that many genes were upregulated/downregulated in the absence of Sit4p deletion in the background of Mrpl16p deletion, in which mtDNA cannot be translated and give a similar phenotype as ρ^- cells [158]. Such hyper or hypoactivation of specific proteins may unveil the specific interactions in the absence of a protein. Sno1p and Snz1p which are expressed during the stationary phase and respond to nutrient limitations and growth arrest [167]. Also they are involved in pyridoxine synthesis [168]. However deletions of Sno1p or Snz1p do not suppress the lethality of *atp2Δ* ρ^- cells (Figure 3.18). Rim15p is a protein kinase that responds to nutrients to regulate cell proliferation and activates genes in the stationary phase [169]. Although TOR pathway controls Rim15p in a Sit4p independent

fashion, downregulations of stationary phase genes SNO1 and SNZ1 brings the question whether Rim15 is essential for the beneficial effects of Sit4p removal [170][127]. However deletion of Rim15p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.28).

Ornithine carbamoyltransferase, Arg3p is involved in biosynthesis of aminoacid arginine by catalyzing the biosynthesis of its precursor citruline [171]. Arg3p is also shown to be downregulated in the absence of Sit4p, however deletion of Arg3p does not suppress the lethality of *atp2Δ* ρ^- cells (Figure 3.19). Rtc2p, a vacuolar aminoacid transporter, exports cationic amino acids from the vacuole and lysine was shown to be highly regulated by Rtc2p similar to its mammalian ortholog PQLC2 [172]. RTC2 expression is also shown to be downregulated in *sit4Δ mrpl16Δ*, however deletion of Rtc2p does not suppress the lethality of *atp2Δ* ρ^- cells (Figure 3.20).

Moreover, *TMT1* expression is downregulated in *sit4Δ mrpl16Δ* cells. Tmt1p is a trans-aconitate methylltransferase to inhibit citric acid cycle and functions in the leucine biosynthesis pathway. Deletion of Tmt1p is not found to suppress the lethality of *atp2Δ* ρ^- cells (Figure 3.22). ρ^- cells show iron starvation phenotype with constantly activating genes regulating iron uptake into mitochondria which is tightly linked to mitochondrial electrochemical potential [3]. However deletion of Sit4p or of Pph21p/Pph22p converts the expression of iron regulon genes back to a similar state as wildtype [158]. Blocking of the response to iron starvation by deletion of Aft1p and Aft2p did not suppress the proliferation defects of *atp2Δ* ρ^- cells (Figure 3.23). Gene encoding general aminoacid permease, *GAP1* is also found to be downregulated in *sit4Δ mrpl16Δ* cells. Gap1p is regulated by TOR pathway in its amino acid passage activity, however it also as an crucial role for PKA pathway with its aminoacid sensing ability which activates PKA upon stimulation [173]. Gap1p is stabilized to remain at the plasma membrane by activation through Npr1p [174]. Similar to deletion of Npr1p (Figure 3.13), deletion of Gap1p also cannot suppress the petite-negative phenotype of *atp2Δ* ρ^- cells (Figure 3.24).

Degradation of Tec1p induced by rapamycin treatment is blocked by deletion of Sit4p [175]. We demonstrated that deletion of transcriptional factor Tec1p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.25). One of the substrates of the TORC1 is eIF4E binding protein (4E-BP1) [99] and deletion of Sit4p results in the hyperphosphorylation and negative regulation of eIF2α [176]. However inhibition of translation by hyperphosphorylation of eIF2α by deletion of Gcn2p or mutation of serine 51 of eIF2α to alanine does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.26). Hyperphosphorylation of eIF2α also leads to activation of Gcn4p which is a transcriptional factor that activates genes involve in aminoacid biosynthesis [176][177]. However deletion of Gcn4p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.27). Shifting a temperature-sensitive allele of TAP42 to the restrictive temperature is reported to cause glycogen accumulation [130]. *sit4* mutation also causes glycogen accumulation [178]. We simultaneously deleted *GSY1* and *GSY2* in *atp2Δ sit4Δ* ρ^- cells to halt the glycogen synthesis, which does not block the proliferation of the petite-negative rescue.

PHO5 encodes for secreted acid phosphatase which is induced in the absence of phosphate [179]. Although it is also shown to be upregulated in *sit4Δ mrpl16Δ* cells, deletion of Pho5p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.30). *PHM6* encodes for a protein regulated by phosphate levels [180]. PHM6 is also found to be upregulated in *sit4Δ mrpl16Δ* cells, however deletion of Phm6p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.31). Another upregulated gene in *sit4Δ mrpl16Δ* cells is *HXK1* that encodes for a cytosolic protein that catalyzes the first irreversible step in the glucose metabolism [181]. Deletion of Hxk1p does not block the proliferation of *atp2Δ sit4Δ* ρ^- cells (Figure 3.32).

3.2.4 Removal of Pde2p generates a petite-negative phenotype however lethality can be rescued by increasing mitochondrial electrochemical potential

We already know that overexpression of *PDE2* by a high copy plasmid was beneficial for ρ^- cells and rescuing the petite-negativity of the *tim18*Δ ρ^- cells [153]. Also deletion of Pde2p has been shown to be harmful for ρ^- cells [153]. We wanted to determine whether increasing the mitochondrial potential would benefit to the proliferation of the *pde2*Δ ρ^- cells. *ATP3-5* allele was, as expected, benefiting the wildtype ρ^- cells, but surprisingly we have found that increasing the $\Delta\Psi^{mito}$ by the *ATP3-5* allele was also rescuing the petite-negative phenotype of Pde2p lacking ρ^- cells (Figure 3.33). $\Delta\Psi^{mito}$ was also shown to be increased with mutants of TOR pathway phosphatases and *TIP41* overexpression. We also showed that a high-copy plasmid carrying the *TIP41* allele is rescuing the petite-negative phenotype of Pde2p lacking ρ^- cells (Figure 3.33). Rescue by *ATP3-5* allele and overexpressed *TIP41* are both increasing the $\Delta\Psi^{mito}$ which supports the hypothesis of $\Delta\Psi^{mito}$ being the main reason for petite-negative phenotype.

Tpk1p, Tpk2p and Tpk3p are protein isoforms that are functioning as the catalytic subunit of PKA [182][183]. Two Tpk subunits join to the PKA tetramer complex along with to inhibitory subunits that are encoded by *BCY1* [184]. PKA is activated by cAMP which binds to the negative regulator Bcy1p. Activation of PKA leads to Tpk release to activate downstream targets. In order to block PKA activity we deleted Tpk1p, Tpk2p and Tpk3p in the background of *pde2*Δ ρ^- cells. However neither of the single deletions was able to rescue the petite-negative phenotype (Figure 3.33). The double deletion of each Tpk pairs is under construction and future plate assays will demonstrate whether deletion of Tpk pairs would rescue the proliferation of the *pde2*Δ ρ^- cells. Triple mutation is synthetically lethal [185], and will not be analyzed for future experiments.

3.3 Discussion

3.3.1 Raising the electrochemical potential across the inner membrane of mitochondria benefits the ρ^- cells and by itself can be the cure for petite-negative phenotype

Cells lacking mitochondrial DNA show reduced inner membrane electrochemical potential [5]. The lethality caused by deletion of the nuclear encoded petite-negative genes along with mtDNA depletion can be the $\Delta\Psi^{mito}$. [76][5]. We found that the rescue of the petite-negative phenotype showed an increased $\Delta\Psi^{mito}$ phenotype [78][158]. In this study we also showed that deletion of conserved phosphatases functioning under TOR pathway not only rescued the proliferation defect of petite-negative phenotype but also raised the $\Delta\Psi^{mito}$. The reason behind such increase in membrane potential remains a future goal to determine. Sit4p regulates many downstream pathways NPR kinase and GATA transcriptional activator Gln3p and also regulate G1 cyclin accumulation during cell cycle stage [133][151][149]. Possibly, Sit4p might be regulating another cell cycle protein or pathway since deletion of Sit4p has been shown to majorly effect the phosphoproteome levels [186]. Sit4p can raise the $\Delta\Psi^{mito}$ by an unknown mechanism activated during the cell cycle and supply the bud with mitochondria with high $\Delta\Psi^{mito}$. Elm1p also localizes to the bud neck during the cell division [187] and concomitant deletion of *sit4*Δ *elm1*Δ leads to synthetic lethality which can be due to their parallel roles in determining the fitness of mitochondria of the next generation. Also Sit4p shown to be in physical interactions with Atp3p, γ subunit of F_1F_O-ATP synthase, and Nde1p, mitochondrial external NADH dehydrogenase [188]. Such interactions can be inhibitory for Atp3p and Nde1p and removal of the Sit4p activity may prolong the phosphorylated, active state of these subunits that are functioning under complex which are generating membrane potential.

Furthermore, Sit4p or catalytic subunits of PKA may be directly controlling the phosphorylation state of proteins that are localizing to mitochondria. At least two SAPS have been identified to associate with highly purified mitochondria [189] also catalytic portion of PKA (C-PKA) were also shown to be localizing to mitochondria [121].

Deletion of these conserved phosphatases may in fact restrict them from cleaving the phosphorylation from the activated protein that is generating the $\Delta\Psi^{mito}$. Conversely reduction of PKA pathway can block the activation of a negative regulator of the $\Delta\Psi^{mito}$.

Moreover, the super-complex formation of ATP/ADP antiporter with F_1F_O-ATP synthase [73][74] can be under control of Sit4 protein's phosphatase function. Intriguingly, previous work showed that the major ATP/ADP antiporter Aac2p that is functioning in mitochondria, has been shown to contain phosphorylation residues [190]. Aac2p exchanges ATP^{4-} for ATP^{3-} across the mitochondrial inner membrane to generate $\Delta\Psi^{mito}$ [5]. Also *aac2*Δ ρ^- cells were unable to proliferate in the presence of *sit4*Δ and also *aac2*Δ ρ^- cells could not been rescued in any other known study. So far we still did not check overexpressed *PDE2* in this background. Downregulated TOR or PKA can change the ion mobility and distribution across the mitochondrial inner membrane and phosphorylation of the Aac2p may be the key for generation of $\Delta\Psi^{mito}$ in ρ^- cells. Alternatively, $\Delta\Psi^{mito}$ might be generated through more indirect fashion with a post-translational change in phosphorylation state and changing the substrate preference and/or efficiency of the mitochondrial import machinery [133], thereby altering the abundance of one or more ion transporters. We are currently investigating the phosphorylation state of Aac2p in different backgrounds containing *sit4* mutations. As a future goal we will perform a Phos-tag gel in which we will run Aac2p with a FLAG epitope attached to it in order to investigate whether the protein is phosphorylated in wildtype, *sit4*Δ, *sit4*Δ *snf1*Δ, or *sit4*Δ *snf1*Δ *mig1*Δ ρ^- cells.

3.3.2 The SNF1 is required for the full benefits of Sit4p removal in cells lacking mtDNA

Snf1p causes lethality after mtDNA loss and blocks the rescue of petite-negativity phenotype by Sit4p removal. We know from previous data that Sit4p cleaves the phosphorylation at threonine 210 in Snf1 [160], so whatever protein or pathway that is activated in conditions where Sit4p activity is repressed, is activated by Snf1p function. Also triple mutant *atp2*Δ *sit4*Δ *snf1*Δ ρ^- cells are still proliferating at a low rate compared to proliferation of *atp2*Δ *sit4*Δ ρ^- cells (Figure 3.35). The beneficial activity of Sit4p removal does not fully depend on Snf1p, which is also demonstrated with the increasing electrochemical potential in *sit4*Δ *snf1*Δ ρ^- cells or by using a mutated *ATP3-5* allele which hyperactivates F_1-ATPase in mtDNA lacking cells [158]. We have also investigated many other possible means by which Sit4 deletion benefits ρ^- cells. We used the substrates and regulators of Sit4p and also the transcriptome data generated in [158] to search for biological pathways and processes that may influence the rescue by Sit4p removal. However so far we couldn't isolate any specific interaction except for Snf1p. Also we have not yet investigated whether Snf1p is needed for the full benefits of the 2μ-*PDE2* rescue in *tim18*Δ ρ^- cells. Snf1p may be the converging point for TOR and PKA pathways and this experiment will remain as a future goal.

We will also investigate whether *snf1T210A* ρ^- cells show a petite-negative phenotype, and whether *snf1T210A* blocks the rescue of *atp2*Δ ρ^- cells by Sit4p removal. We will introduce a plasmid containing mutated *snf1* into a background deleted of SNF1 gene. Deletion of Reg1p acting on the SNF1 phosphorylation or deletion of upstream kinases of Snf1p in the background of *atp2*Δ *sit4*Δ ρ^- cells did not show any alteration in the proliferation of cells. Possible other roles of Snf1p independent of T210 phosphorylation can also be important in the absence of Sit4p. Mig1p is not the sole pathway for Snf1 activity in the Sit4p removed rescue since *snf1*Δ *mig1*Δ ρ^- cells are not viable. However deletion of Sit4p enables the proliferation of the petite-negativity phenotype of Snf1p deletion, meaning there are alternative roles of

Snf1p which is shown as the second branch of my model. Also Snf1p is found to localize to highly purified mitochondria which indicates a more direct role for Snf1p, as SAPs and catalytic portion of PKA, for influencing the mitochondrial biogenesis [189], this indicates a direct role for the SNF1 kinase for the modulation of mitochondrial function (Figure 3.35).

Inviable phenotype of *snf1*Δ *mig1*Δ ρ^- cells or *atp2*Δ *mig1*Δ ρ^- cells states that Sit4p removal is necessary for proliferation. Constitutive expression of a gene that is under transcriptional control of Mig1p (GCM) by removal of Mig1p is not sufficient to rescue the petite-negative phenotype. The need for Sit4p removal is maybe due to the requirement of prolonged phosphorylated state of GCM which may increase the membrane potential. Investigating GCMs may uncover the one branch of potential generation pathway and this is the third branch of my model (Figure 3.35).

3.3.3 The transcriptome, protein import and $\Delta\Psi^{mito}$ go back to wildtype state in mtDNA lacking cells in the absence of Sit4p or of Pph21p/Pph22p

Aside from the results that have been shown in this Chapter, there also other characterization that has been done by other authors about the mtDNA lacking cells with Sit4p or Pph21p/Pph22p deletions. The transcriptome data generated in *sit4Δ mrpl16Δ* and compared to *mrpl16Δ* also revealed that many of the genes that were upregulated/downregulated in the petite phenotype go back to the wildtype state in the absence of Sit4p [158]. Also quantitative detection of the $\Delta\Psi^{mito}$ by flow cytometry analysis revealed that ρ^- cells have increased mitochondrial electrochemical potential in the absence of Sit4p or in the presence of an *ATP3-5* allele that generates mitochondrial electrochemical potential [158]. Increased $\Delta\Psi^{mito}$ also rescued the import defects of the petite cells with fluorescent protein with N-terminal mitochondrial localization signal imported into the mitochondria in the absence of Sit4p and Pph21p/Pph22p [158].

3.4 Materials and Methods

3.4.1 Yeast strains and culture conditions: Yeast media recipes were prepared as previously (Adams et al., 1998a). Gene deletions were performed as in [192]. Strain genotypes and the details of their construction details, are listed in Table 3.1. Oligonucleotides used for strain construction are listed in Table 3.2. All strains are congenic with the S288C-based strains described in [193], except for strains CDD619, CDD631, and CDD632. EtBr (Thermo Fisher Scientific, Waltham, MA) was used at a concentration of 25 μg/ml to rapidly destroy mtDNA [10]. Serial dilution assays were performed growing the cultures to log-phase and then diluting these cultures to an OD600 of 0.1, then performing three additional five-fold dilutions. 4 μl of each dilution was then spotted to YEPD medium. Strains were tested for a petite-negative phenotype as in [78]; cells were incubated for 3 d (ρ+) or 4 d (ρ-) at 30°C, except where the temperature or incubation time is otherwise noted.

3.4.2 Plasmids: Plasmids M532, containing wild-type TAP42, and M533, encoding tap42-17 were constructed in the laboratory of Dr. Kim Arndt and provided by Dr. Maria Cardenas-Corona, Duke University. The tap42-17 allele in plasmid M533 was sequenced, and following mutations are obtained: T61N, L73Q, S100P, E192T, K241E, and E353K.

3.5 Figure Legends

Figure 3.1 *tap42-17* can suppress the petite negative phenotype. The temperature sensitive allele of *TAP42* gene enables the proliferation of mtDNA lacking cells with deleted F1β subunit of the ATP synthase. Strains CDD298 (*atp2Δ*) and CDD300 (*atp2Δ tap42-17*) were tested for the petite-negative phenotype, except that cells containing mtDNA were incubated for 2 d at 30°C.

Figure 3.2 Deletion of Tap42's client phosphatases rescues the petite-negative lethality. Deletion of Sit4p or Pph21p/Pph22p allows the proliferation of *atp2Δ ρ-* cells. Strains CDD275 (*atp2Δ*), CDD332 (*atp2Δ pph21Δ pph22Δ*), CDD273 (*atp2Δ pph3Δ*), CDD277 (*atp2Δ ppg1Δ*) and CDD296 (*atp2Δ sit4Δ*) were assayed for a petite-negative phenotype.

Figure 3.3 Deletion of individual adaptor proteins, SAPs, downstream of Sit4p does not suppress the petite-negative phenotype of *atp2Δ* cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD302 (*atp2Δ sap155Δ*), CDD303 (*atp2Δ sap185Δ*), CDD304 (*atp2Δ sap4Δ*) and CDD305 (*atp2Δ sap190Δ*) were tested for the petite-negative phenotype.

Figure 3.4 Double deletions of specific adaptor proteins, SAPs, downstream of Sit4p rescues the petite-negative phenotype of *atp2Δ* cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD417 (*atp2Δ sap4Δ sap155Δ*), CDD418 (*atp2Δsap155Δ sap190Δ*), CDD419 (*atp2Δ sap4Δ sap185Δ*), CDD420 (*atp2Δ sap155Δ sap185Δ*), CDD421 (*atp2Δ sap185Δ sap190Δ*) and CDD422 (*atp2Δ sap4Δ sap190Δ*) were tested for the petite-negative phenotype.

Figure 3.5 Simultaneous deletion of adaptor proteins Cdc55p and Rts1p functioning downstream of Pph21p/Pph22p rescues the proliferation of ρ^- cells lacking *atp2*Δ.

Strains CDD215 (*atp2Δ*), CDD332 (*atp2Δ pph21Δ pph22Δ*), CDD478 (*atp2Δ rts1Δ*), CDD479 (*atp2Δ cdc55Δ*) and CDD480 (*atp2Δ rts1Δ cdc55Δ*) were tested for the petite-negative phenotype.

Figure 3.6 SNF1 kinase is required to inactivate Mig1p for the full benefits of Sit4p deletion in *atp2Δ ρ-* cells. Strains CDD463 (*WT*), CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD458 (*atp2Δ sit4Δ snf1Δ*) and CDD614 (*atp2Δ sit4Δ snf1Δ mig1Δ*) were tested for the petite-negative phenotype.

Figure 3.7 Individual deletion of Snf1p-associated β-subunits does not block the rescue of *atp2Δ ρ-* cells with Sit4p removal. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD458 (*atp2Δ sit4Δ snf1Δ*), CDD626 (*atp2Δ sit4Δ sip1Δ*), CDD627 (*atp2Δ sit4Δ sip2Δ*) and CDD628 (*atp2Δ sit4Δ gal83Δ*) were tested for the petite-negative phenotype.

Figure 3.8 Deletion of upstream kinases activating Snf1p does not alter -activating kinases leaves the proliferation of *atp2Δ sit4Δ ρ-* cells unaffected. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD458 (*atp2Δ sit4Δ snf1Δ*), CDD629 (*atp2Δ sit4Δ sak1Δ*) and CDD630 (*atp2Δ sit4Δ tos3Δ*) were tested for the petite-negative phenotype.

Figure 3.9 Increasing $\Delta\Psi^{mito}$ by insertion of a mutated γ subunit of ATP synthase F1 sector rescues the petite-negative phenotype of *ρ-* cells lacking the SNF1 kinase. Strains CDD463 (*WT)* or CDD604 (*snf1Δ*) were plated as non-transformed and transformed with either plasmid M487, encoding *ATP3*, or plasmid M488, encoding *ATP3-5* (Dunn et al., 2006). Strains were then tested for a petite negative phenotype.

Figure 3.10 Deletion of the transcription factor Mig1p does not suppress the petite-negative phenotype of *atp2Δ* mutants. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*) and CDD351 (*atp2Δ mig1Δ*) were tested for a petite-negative phenotype.

Figure 3.11 Deletion of the regulatory subunit of protein phosphatase 1, Reg1p, does not allow *atp2Δ* ρ^- cells to survive. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*) and CDD611 (*atp2Δ reg1Δ*) were tested for the petite-negative phenotype.

Figure 3.12 Blocking retrograde signaling pathway by deletion of Rtg1p or Rtg2p does not suppress the lethality of *atp2Δ* ρ^- cells. Strains CDD284 (*rtg1Δ atp2Δ*), CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*), CDD292 (*atp2Δ*), CDD306 (*rtg2Δ atp2Δ*), CDD334 (*atp2Δ sit4Δ*) were tested for petite-negative phenotype.

Figure 3.13 Deletion of NPR1 protein kinase does not rescue the lethality of ρ^- cells lacking F1 subunit of ATP synthase. Strains CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*) and CDD287 (*npr1Δ atp2Δ*) were tested for petite-negative phenotype.

Figure 3.14 Blocking autophagy by deleting Atg1p does not suppress the proliferation defect or block the rescue of Sit4p in ρ^- cells lacking F_1 subunit of ATP synthase. Strains CDD291 (*atp2Δ atg1Δ*), CDD292 (*atp2Δ*), CDD294 (*atp2Δ sit4Δ atg1Δ*) and CDD334 (*atp2Δ sit4Δ*) were tested for petite-negative phenotype.

Figure 3.15 Cancellation of response to nitrogen limitations by deletion of Gat1p and Gln3p does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells. CDD292 (*atp2Δ*), CDD322 (*atp2Δ gln3Δ gat1Δ*) and CDD334 (*atp2Δ sit4Δ*) were tested for petite-negative phenotype.

Figure 3.16 Inhibition of cell cycle progression by deletion of Bck2p or of Swi4p does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD405 (*atp2Δ bck2Δ*) and CDD457 (*atp2Δ swi1Δ*) were tested for petite-negative phenotype.

Figure 3.17 Inhibition of pleotropic drug resistance pathway by combined deletion of *PDR1* and *PDR3* does not suppress the lethality of *atp2*Δ petite-negative phenotype. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*) and CDD423 (*atp2Δ pdr1Δ pdr3Δ*) were tested for petite-negative phenotype.

Figure 3.18 Inhibition of puridoxine metabolism by deletion of *SNO1* or of *SNZ1* does not suppress the lethality of *atp2*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD525 (*atp2Δ sno1Δ*) and CDD526 (*atp2Δ snz1Δ*) were tested for petite-negative phenotype.

Figure 3.19 Arginine auxotrophy does not allow the survival of *atp2*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*) and CDD586 (*atp2Δ arg3Δ*) were tested for petite-negative phenotype.

Figure 3.20 Deletion of vacuolar aminoacid transporter Rtc2p does not suppress the lethality of *atp2*Δ ρ^- cells. Strains CDD292 (*atp2Δ*), CDD334 (*atp2Δ sit4Δ*), CDD591 (*atp2Δ rtc2Δ*) and CDD526 (*atp2Δ snz1Δ*) were tested for petite-negative phenotype.

Figure 3.21 Deletion of paralog of *NPR1*, *PRR2* protein kinase does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells. Strains CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*) and CDD593 (*atp2Δ prr2Δ*) were tested for petite-negative phenotype.

Figure 3.22 Deletion of trans-aconitase methyltransferase, Tmt1p does not suppress the lethality of *atp2*Δ ρ^- cells. Strains CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*) and CDD595 (*atp2Δ tmt1Δ*) were tested for petite-negative phenotype.

Figure 3.23 Blocking of expression of iron starvation responding genes does not suppress the lethality of *atp2*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ*

sit4Δ), CDD608 (*atp2Δ aft1Δ*) and CDD616 (*atp2Δ aft2Δ*) were tested for the petite-negative phenotype.

Figure 3.24 Deletion of general aminoacid permease Gap1p genes does not suppress the lethality of *atp2*Δ ρ^- cells. Strains CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*) and CDD615 (*atp2Δ gap1Δ*) were tested for petite-negative phenotype.

Figure 3.25 Deletion of transcriptional factor Tec1 does not block the rescue of *atp2*Δ ρ^- cells by Sit4 removal. Strains CDD275 (*atp2Δ*), CDD295 (*atp2Δ sit4Δ tec1Δ*) and CDD296 (*atp2Δ sit4Δ*) were assayed for a petite-negative phenotype.

Figure 3.26 Blocking of inhibition of translation by eIF2α phosphorylation by deletion of *GCN2* and mutation of serine 51 to alanine in eIF2α does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD403 (*atp2Δ sit4Δ SUI2S51A*) and CDD404 (*atp2Δ sit4Δ gcn2Δ*) were tested for the petite-negative phenotype.

Figure 3.27 Deletion of Gcn4p, a transcriptional activator of amino acid biosynthetic genes, does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*) and CDD424 (*atp2Δ sit4Δ gcn4Δ*) were tested for the petite-negative phenotype.

Figure 3.28 Blocking of Rim15p, a protein kinase involved in cell proliferation and establishment of stationary phase does not rescue petite-negative phenotype or block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD488 (*atp2Δ sit4Δ rim15Δ*) and CDD546 (*atp2Δ rim15Δ*) were tested for the petite-negative phenotype.

Figure 3.29 Depletion of glycogen synthesis with simultaneous deletion of GSY1 and GSY2 genes does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells. Strains CDD215 (*atp2Δ*), CDD249 (*atp2Δ sit4Δ*), CDD458 (*atp2Δ sit4Δ snf1Δ*), CDD463 (*WT*) and CDD489 (*atp2Δ sit4Δ gsy1Δ gsy2Δ*) were tested for the petite-negative phenotype.

Figure 3.30 The major phosphate-regulated secreted acid phosphatase, Pho5p, is not required for Sit4p for the rescue of *atp2*Δ ρ^- cells. Strains CDD601 (*atp2Δ*), CDD598 (*atp2Δ sit4Δ*) and CDD590 (*atp2Δ sit4Δ pho5Δ*) were tested for the petite-negative phenotype.

Figure 3.31 Phm6p, a protein regulated by phosphate levels; removal does not block the proliferation of *atp2*Δ ρ^- cells lacking Sit4p. Strains CDD285 (*atp2Δ*), CDD286 (*atp2Δ sit4Δ*) and CDD592 (*atp2Δ sit4Δ phm6Δ*) were tested for petite-negative phenotype.

Figure 3.32 Deletion of hexokinase protein, Hxk1p, did not significantly alter the proliferation of *atp2*Δ ρ^- cells lacking Sit4p. Strains CDD587 (*atp2Δ*), CDD599 (*atp2Δ sit4Δ*) and CDD594 (*atp2Δ sit4Δ hxk1Δ*) were tested for petite-negative phenotype.

Figure 3.33 Increasing $\Delta\Psi^{mito}$ by hyperactivation of the ATP synthase F_1 sector rescues the petite-negative phenotype of ρ^- cells lacking the cyclic AMP phosphodiesterase. Strains CDD2 (*WT)* or CDD261 (*pde2Δ)* were transformed with either plasmid M487, encoding *ATP3*, or plasmid M488, encoding *ATP3-5* (Dunn et al., 2006). Transformants were then tested for a petite negative phenotype. ρ^+ and ρ^- cells were only cultured on solid media for 2 d.

Figure 3.34 Single Tpk deletions could not rescue the petite-negativity phenotype of *pde2*Δ ρ^- cells. Deletion of individual Tpks does not suppress the petite-negative phenotype of *pde2Δ* cells. Strains CDD2 (*WT*), CDD261 (*pde2Δ*), CDD751 (*pde2Δ*

tpk1Δ), CDD752 (*pde2Δ tpk2Δ*) and CDD753 (*pde2Δ tpk3Δ*) were tested for the petite-negative phenotype. ρ^+ and ρ^- cells were only cultured on solid media for 2 d.

Figure 3.35 Deletion of conserved phosphatases functioning under Tap42p increases the $\Delta\Psi^{mito}$ of cells lacking mtDNA. Tip41p inhibits Tap42p function and overexpression of Tip41p rescues the proliferation defect of *atp2*Δ petite-negative phenotype. Tap42p temperature sensitive allele also rescues the lethality at semi-permissive temperature and loss of Tap42 function leads to inhibition of Sit4p [150]. Blocking of activities of phosphatases Sit4p and Pph21p/Pph22p functioning under Tap42p increases the $\Delta\Psi^{mito}$ of ρ^- cells. Specific pairs of deletions of Sit4 associating proteins (SAPs) and concomitant deletion of adaptor proteins of Pph21p/Pph21p also rescued the proliferation defect of *atp2*Δ ρ^- cells. Snf1p is crucial in order to obtain full benefits of the Sit4p deletion, however Sit4p removal increases $\Delta\Psi^{mito}$ even in the absence of Snf1p. Snf1p itself has a role in the enhancement of $\Delta\Psi^{mito}$ which can be verified by the rescue of petite-negative phenotype of *snf1*Δ ρ^- cells in the presence of *ATP3-5* allele. Snf1p also increases expression of transcriptionally repressed targets (X) of Mig1p and quadruple mutant (*atp2*Δ *sit4*Δ *snf1*Δ *mig1*Δ) showed proliferation.

3.6 Figures

Figure 3.1 *tap42-17* can suppress the petite negative phenotype of mtDNA lacking cells with deleted F1β subunit of the ATP synthase.

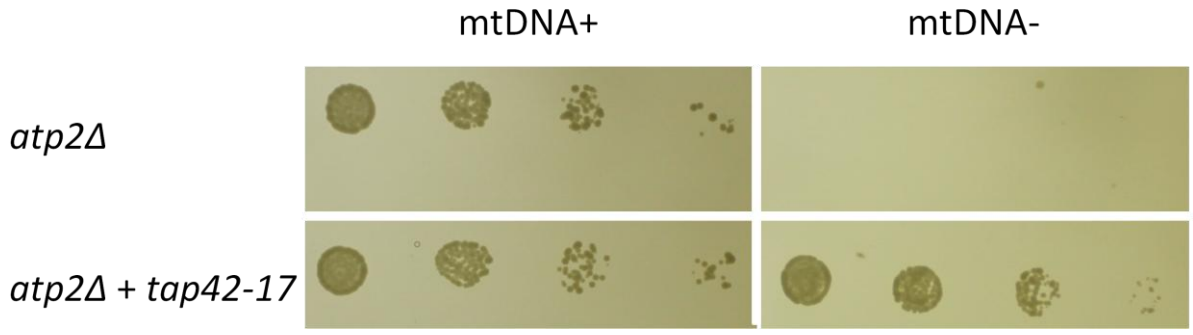

Figure 3.2 Deletion of Tap42's client phosphatases Pph21p/Pph22p and Sit4p rescues the petite-negative lethality.

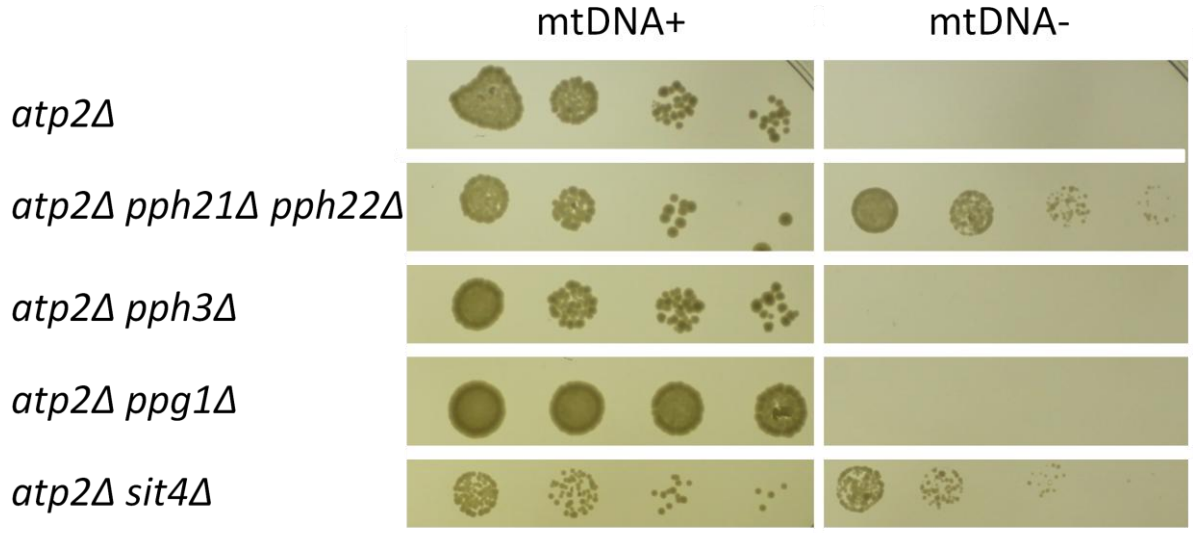

Figure 3.3 Deletion of individual Sit4p associating proteins, SAPs, downstream of Sit4p does not suppress the petite-negative phenotype of *atp2Δ* cells.

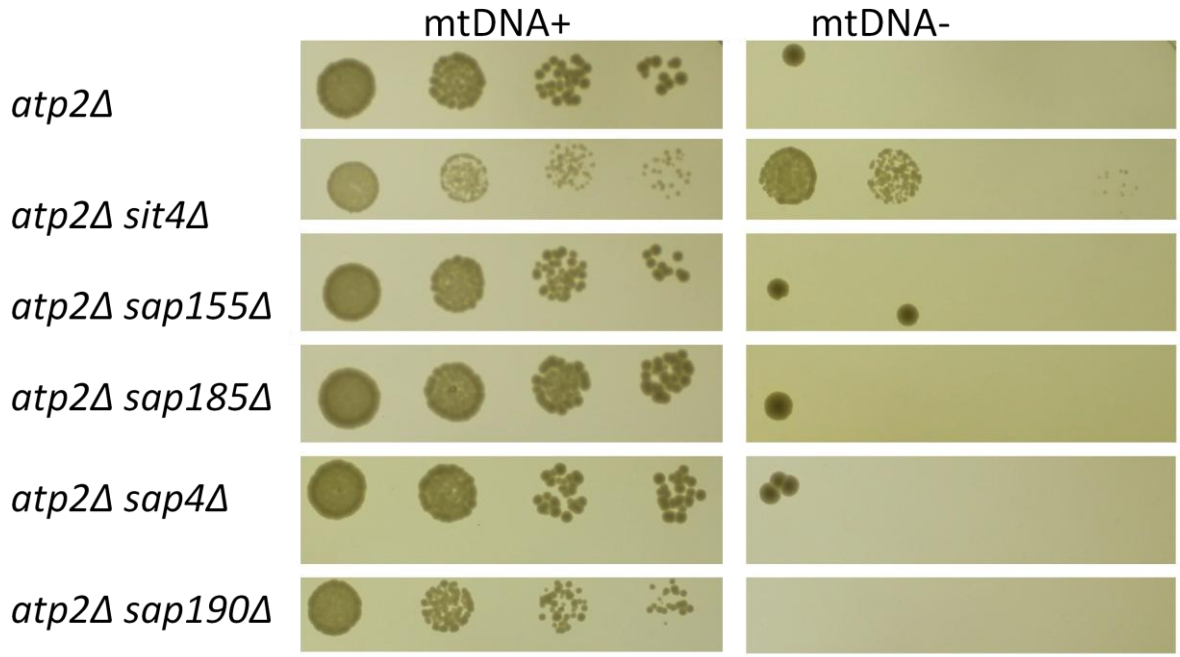

Figure 3.4 Double deletions of specific adaptor proteins, SAPs, downstream of Sit4p rescues the petite-negative phenotype of *atp2Δ* cells.

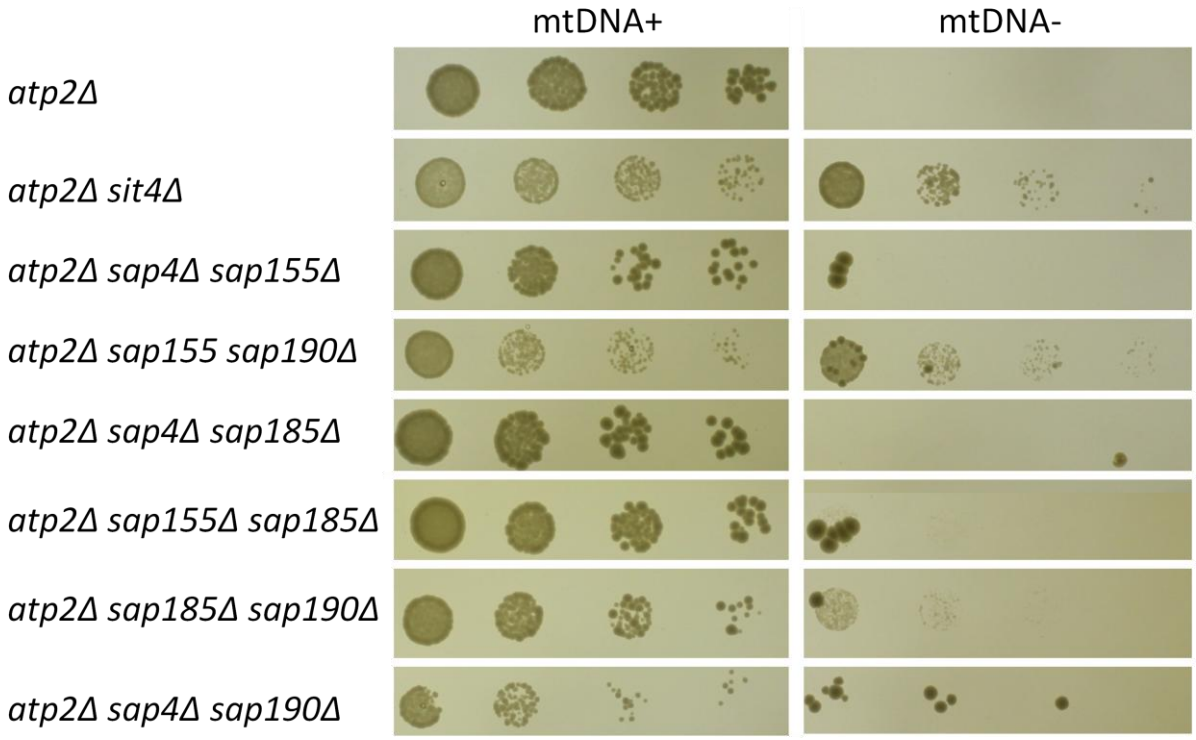

Figure 3.5 Simultaneous deletion of adaptor proteins Cdc55p and Rts1p functioning downstream of Pph21p/Pph22p rescues the proliferation of ρ^- cells lacking *atp2*Δ.

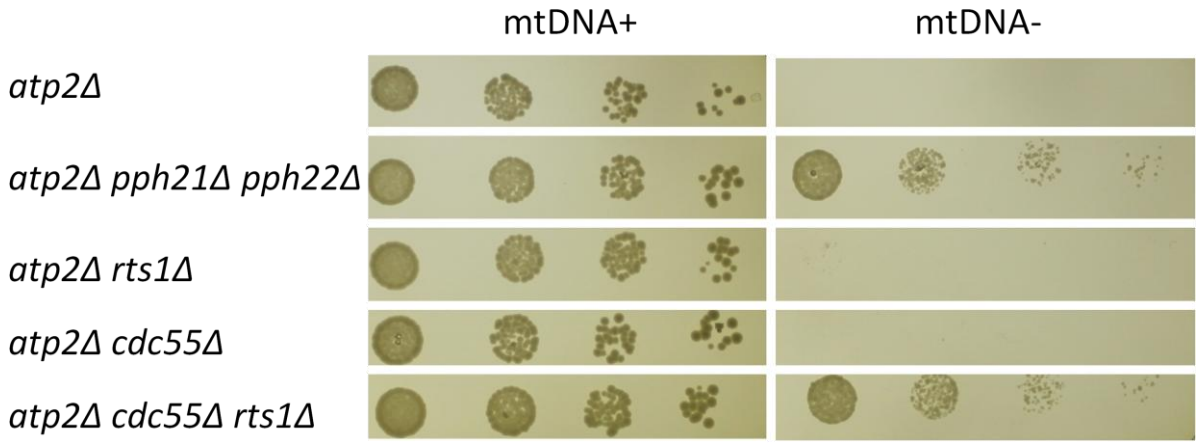

Figure 3.6 SNF1 kinase is required to inactivate Mig1p for the full benefits of Sit4p deletion in *atp2Δ ρ-* cells.

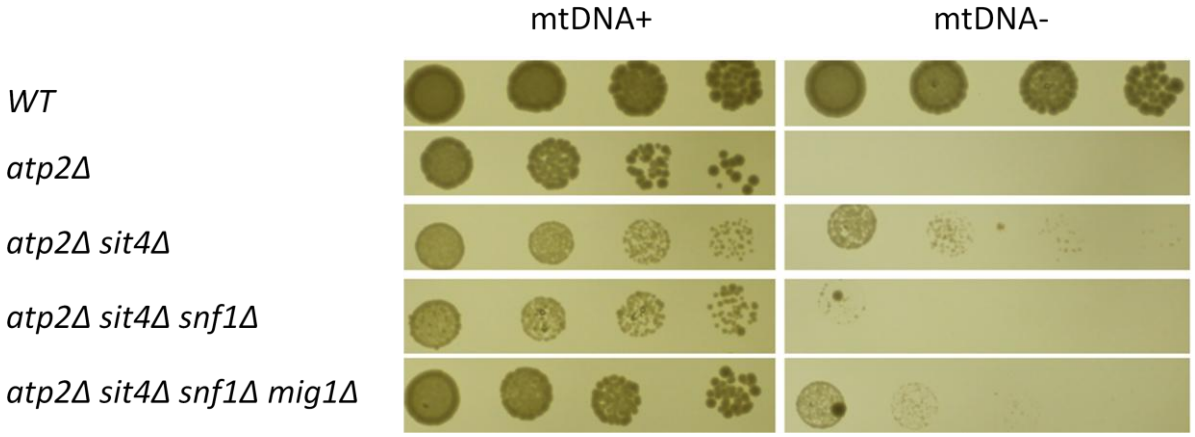

Figure 3.7 Individual deletion of Snf1p-associated β-subunits does not block the rescue of *atp2Δ ρ-* cells with Sit4p removal.

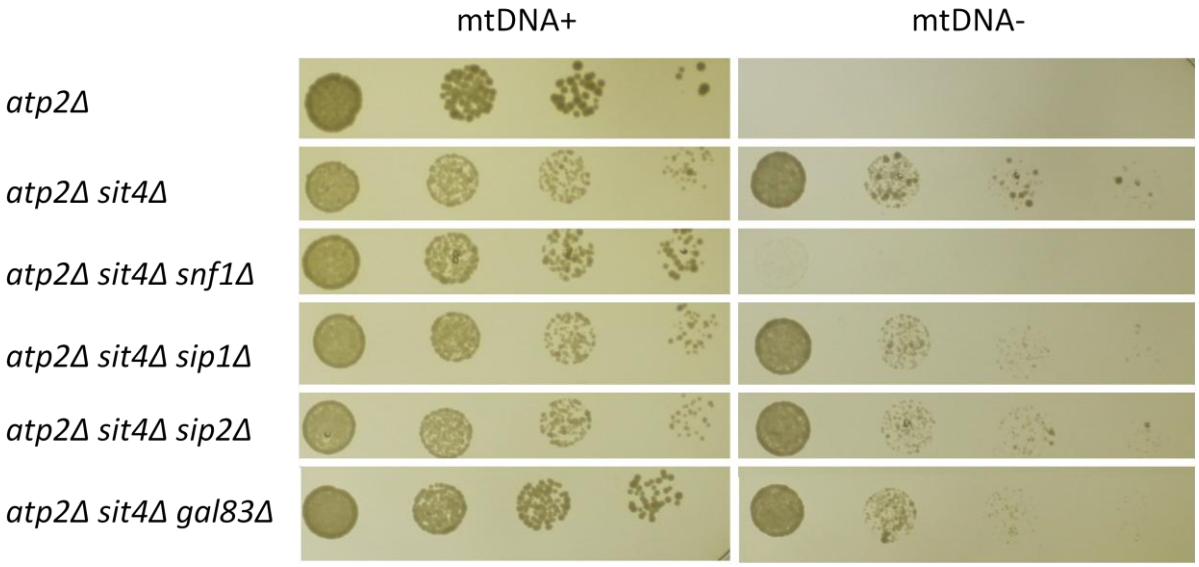

Figure 3.8 Deletion of upstream kinases activating Snf1p does not alter -activating kinases leaves the proliferation of *atp2Δ sit4Δ ρ-* cells unaffected.

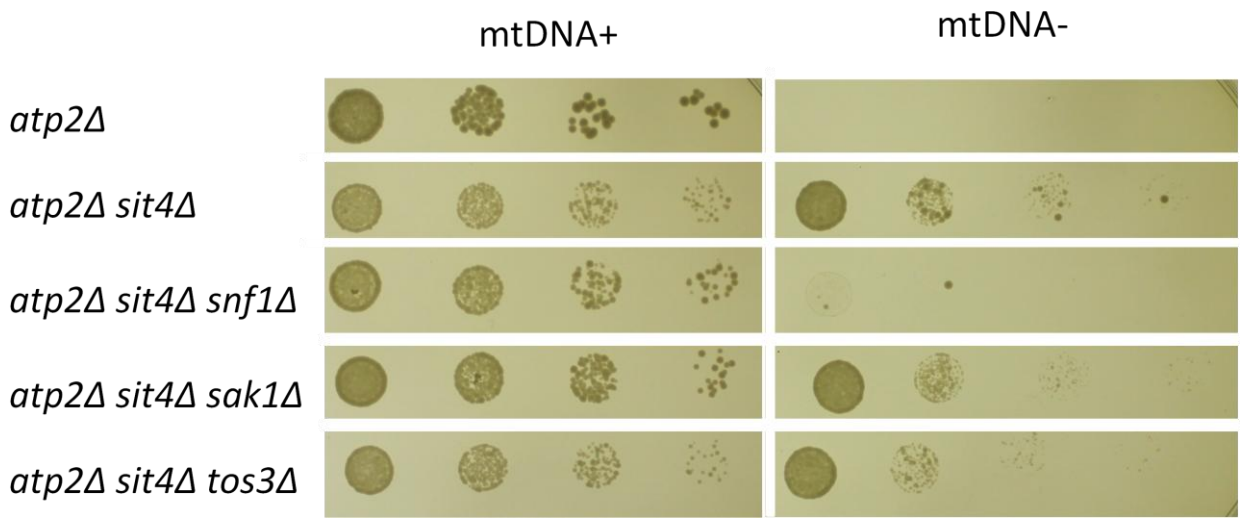

Figure 3.9 Increasing $\Delta\Psi^{mito}$ by insertion of a mutated γ subunit of ATP synthase F1 sector rescues the petite-negative phenotype of *ρ-* cells lacking the SNF1 kinase.

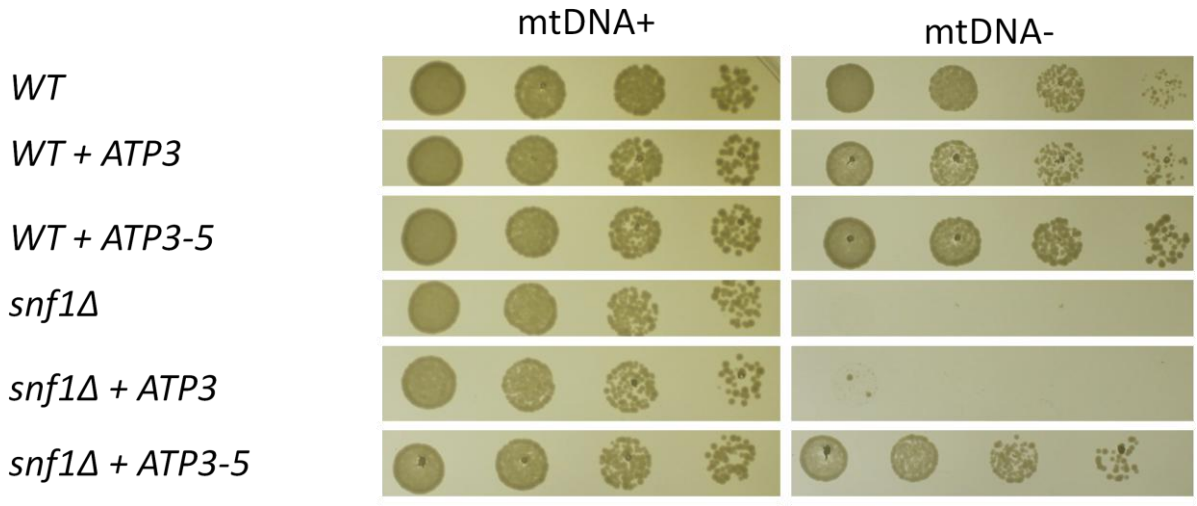

Figure 3.10 Deletion of the transcription factor Mig1p does not suppress the petite-negative phenotype of *atp2Δ* mutants.

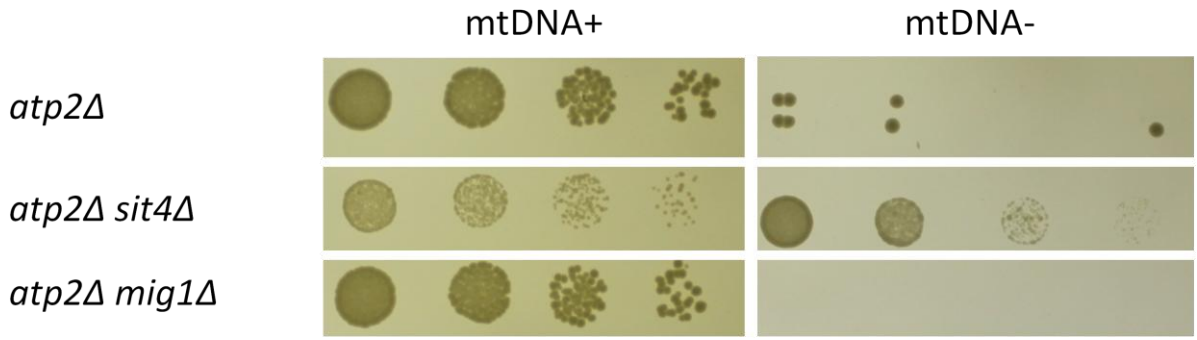

Figure 3.11 Deletion of the regulatory subunit of protein phosphatase 1, Reg1p, does not allow *atp2Δ* ρ^- cells to survive.

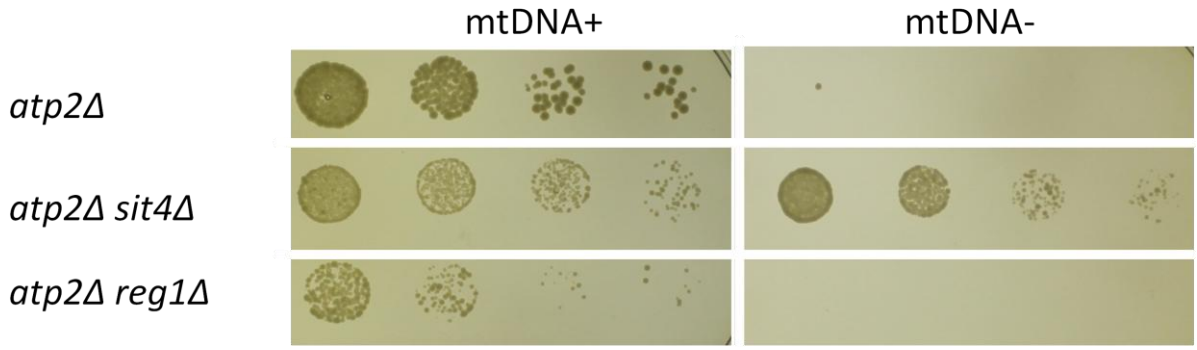

Figure 3.12 Blocking retrograde signaling pathway by deletion of Rtg1p or Rtg2p does not suppress the lethality of *atp2Δ* ρ^- cells.

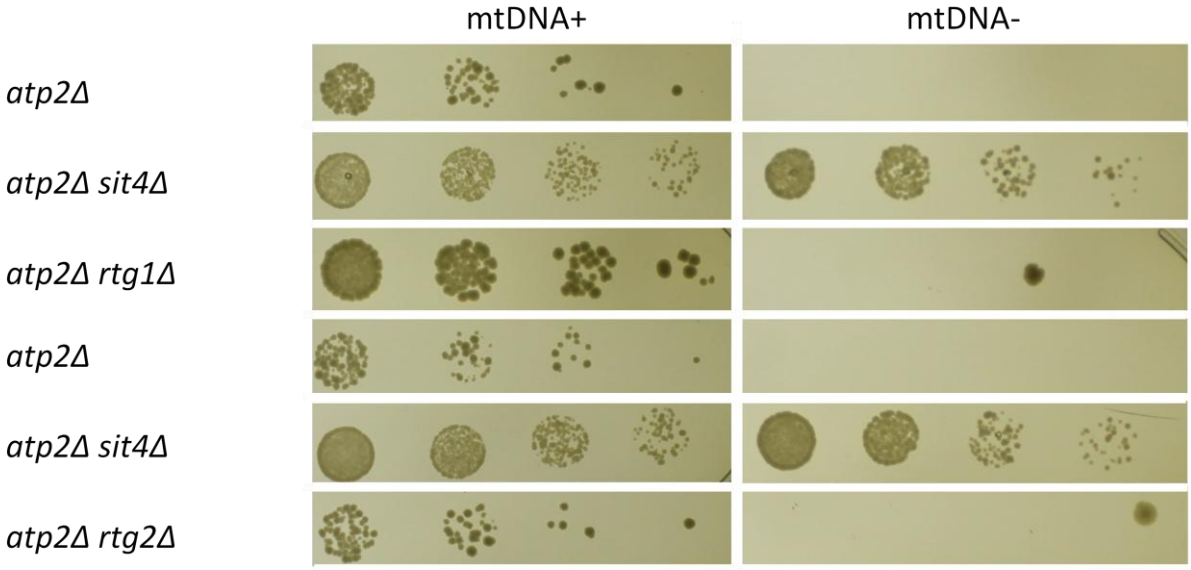

Figure 3.13 Deletion of NPR1 protein kinase does not rescue the lethality of ρ^- cells lacking F1 subunit of ATP synthase.

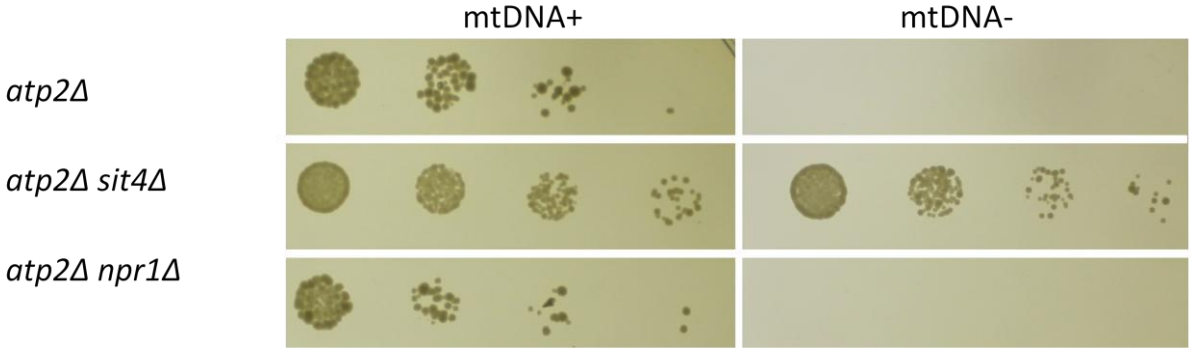

Figure 3.14 Blocking autophagy by deleting Atg1p does not suppress the proliferation defect or block the rescue of Sit4p in ρ^- cells lacking F_1 subunit of ATP synthase.

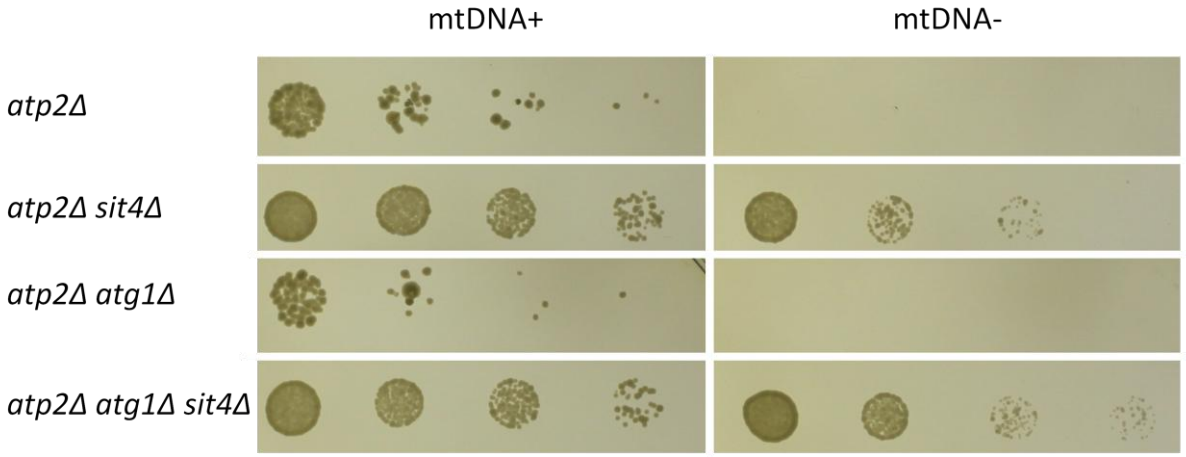

Figure 3.15 Cancellation of response to nitrogen limitations by deletion of Gat1p and Gln3p does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells.

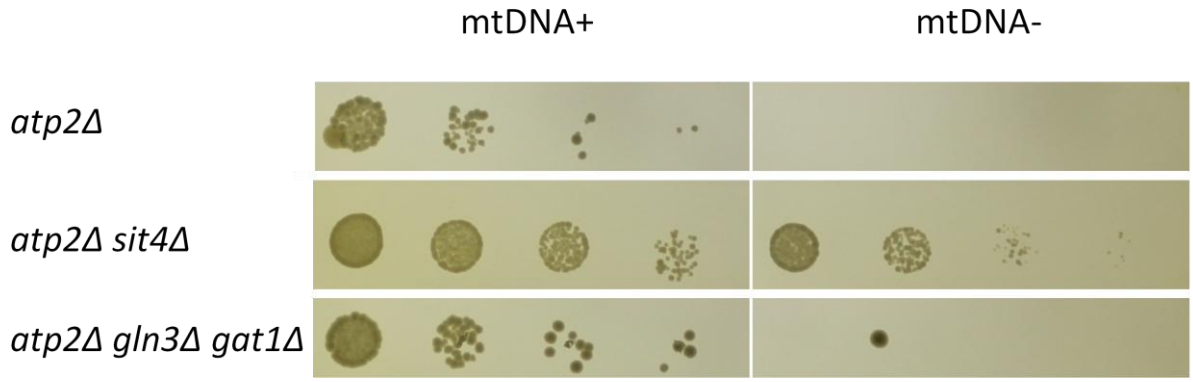

Figure 3.16 Inhibition of cell cycle progression by deletion of Bck2p or of Swi4p does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells.

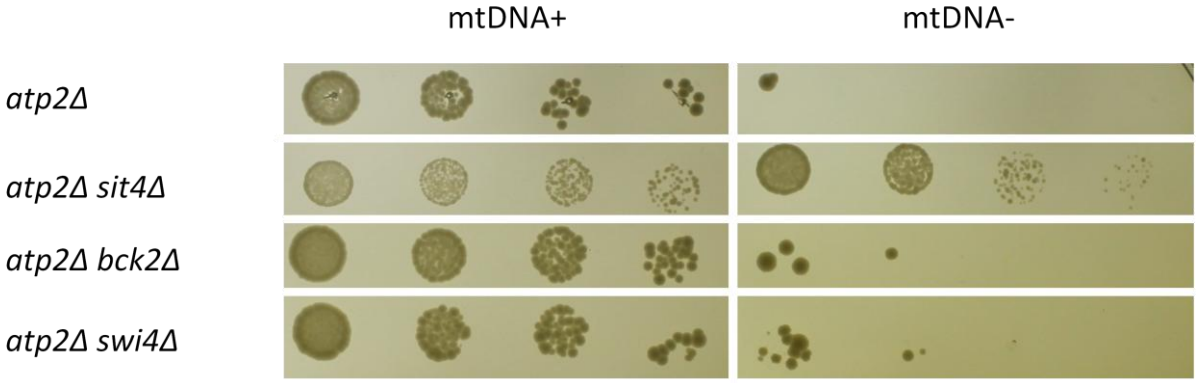

Figure 3.17 Inhibition of pleotropic drug resistance pathway by combined deletion of *PDR1* and *PDR3* does not suppress the lethality of *atp2*Δ petite-negative phenotype.

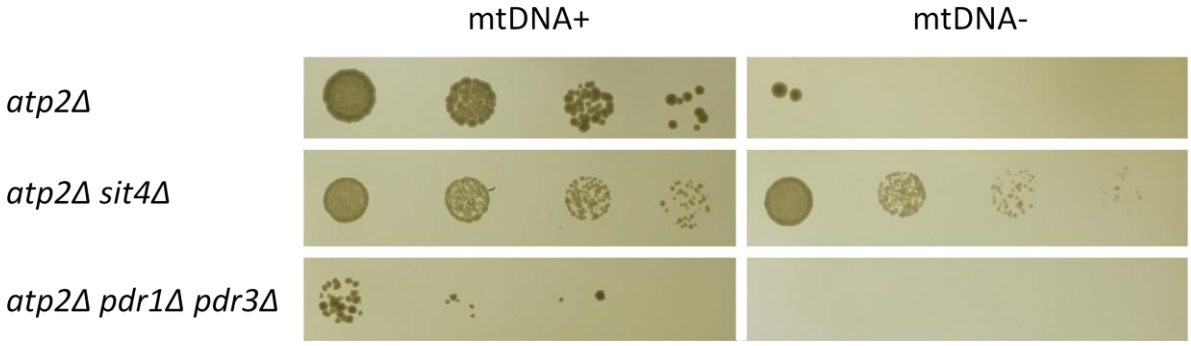

Figure 3.18 Inhibition of puridoxine metabolism by deletion of *SNO1* or of *SNZ1* does not suppress the lethality of *atp2*Δ ρ^- cells.

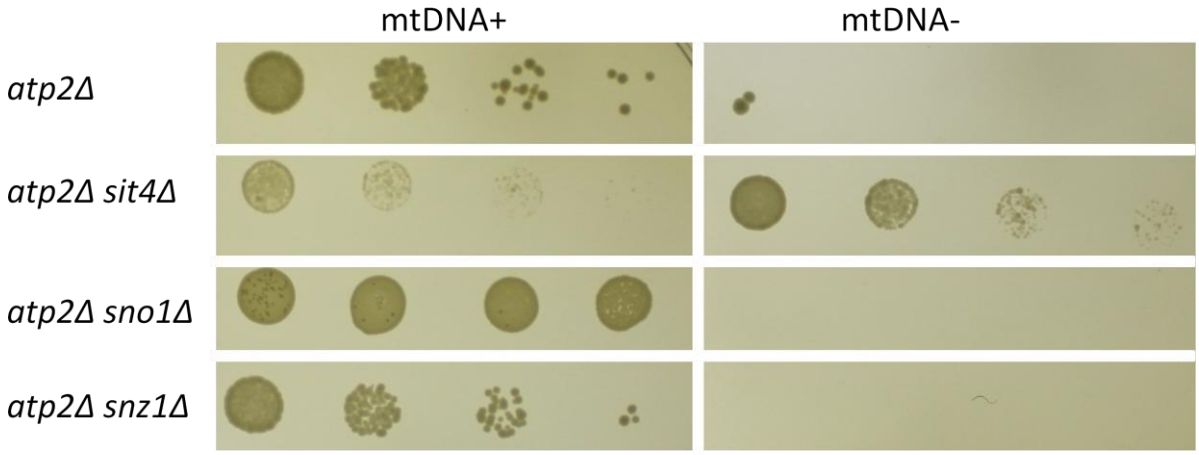

Figure 3.19 Arginine auxotrophy does not allow the survival of *atp2*Δ ρ^- cells.

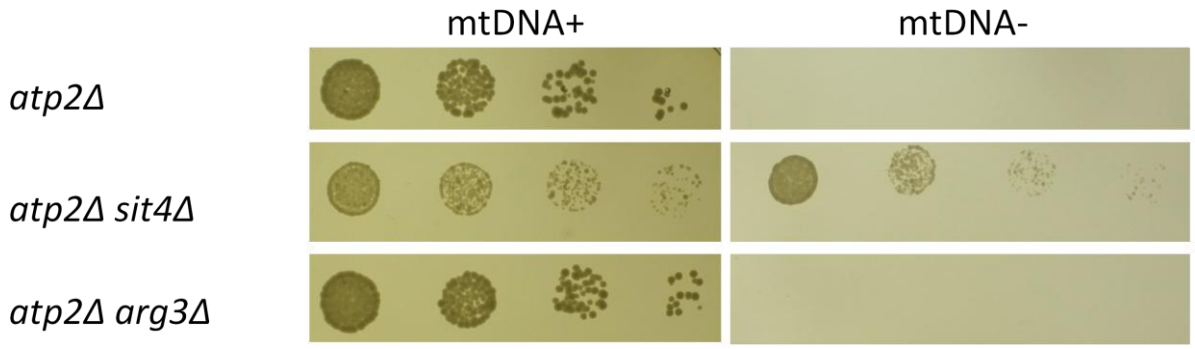

Figure 3.20 Deletion of vacuolar aminoacid transporter Rtc2p does not suppress the lethality of *atp2*Δ ρ^- cells.

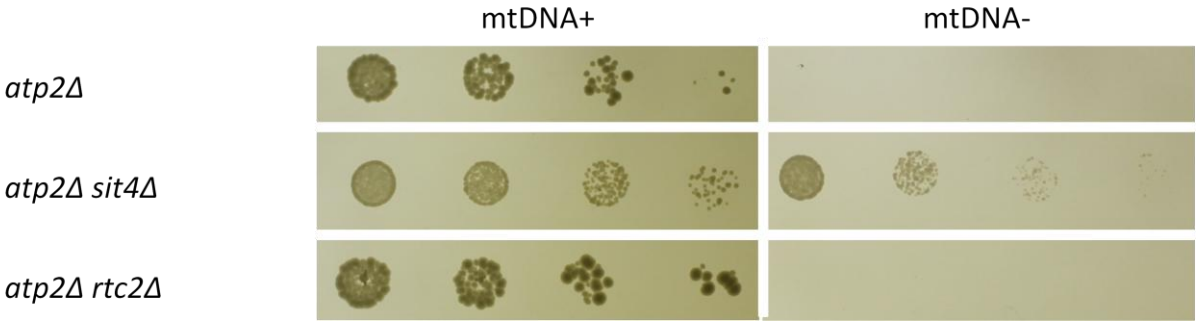

Figure 3.21 Deletion of paralog of *NPR1, PRR2* protein kinase does not rescue the petite-negative phenotype of *atp2*Δ ρ^- cells.

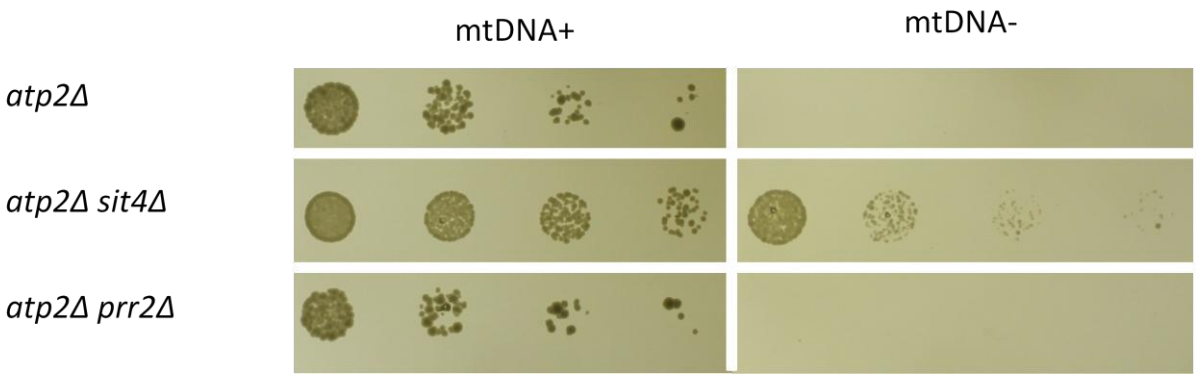

Figure 3.22 Deletion of trans-aconitase methyltransferase, Tmt1p does not suppress the lethality of *atp2*Δ ρ^- cells.

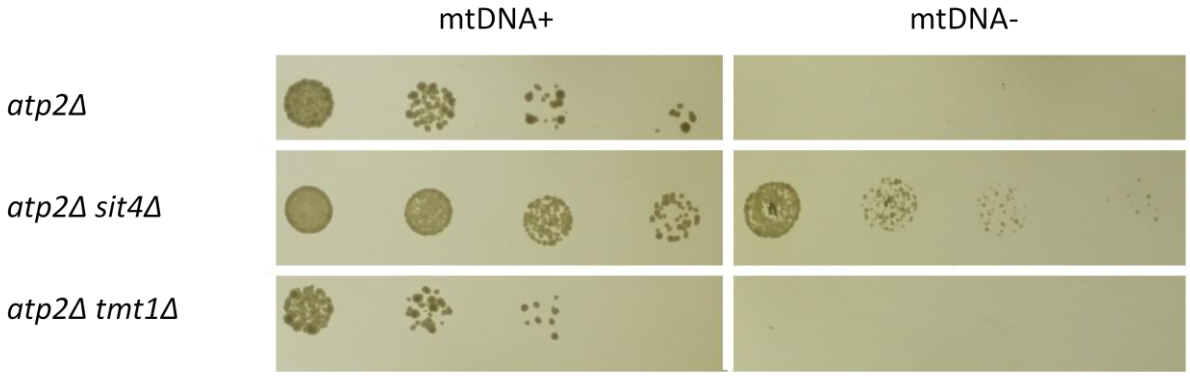

Figure 3.23 Blocking of expression of iron starvation responding genes does not suppress the lethality of *atp2*Δ ρ^- cells.

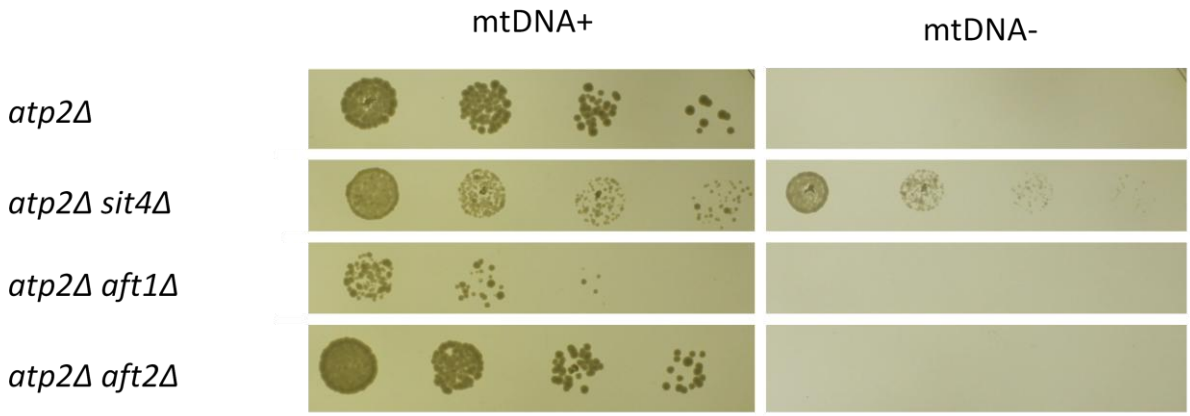

Figure 3.24 Deletion of general aminoacid permease Gap1p genes does not suppress the lethality of *atp2*Δ ρ^- cells.

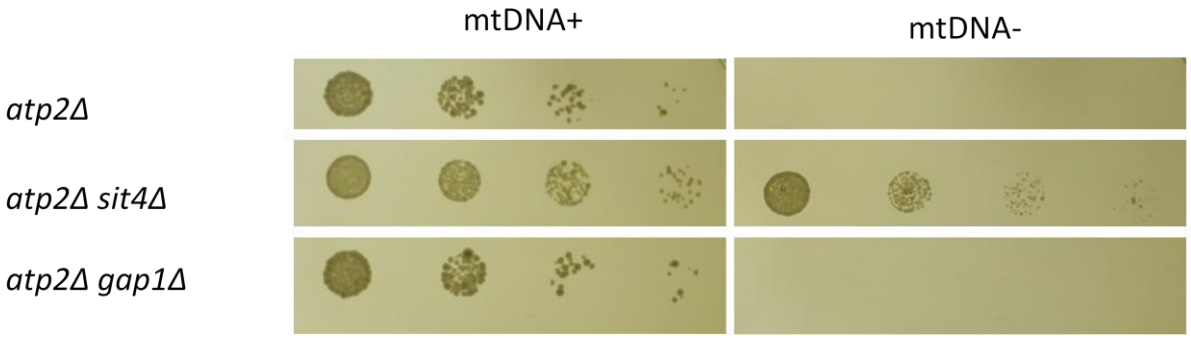

Figure 3.25 Deletion of transcriptional factor Tec1 does not block the rescue of *atp2*Δ ρ^- cells by Sit4 removal.

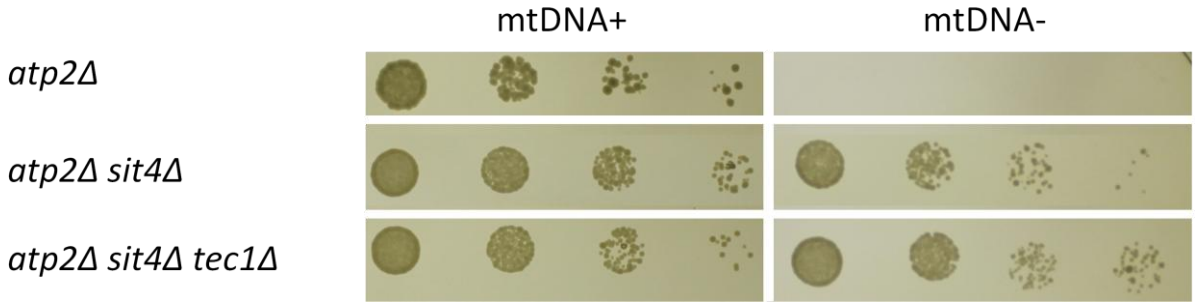

Figure 3.26 Blocking of inhibition of translation by eIF2α phosphorylation by deletion of *GCN2* and mutation of serine 51 to alanine in eIF2α does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells.

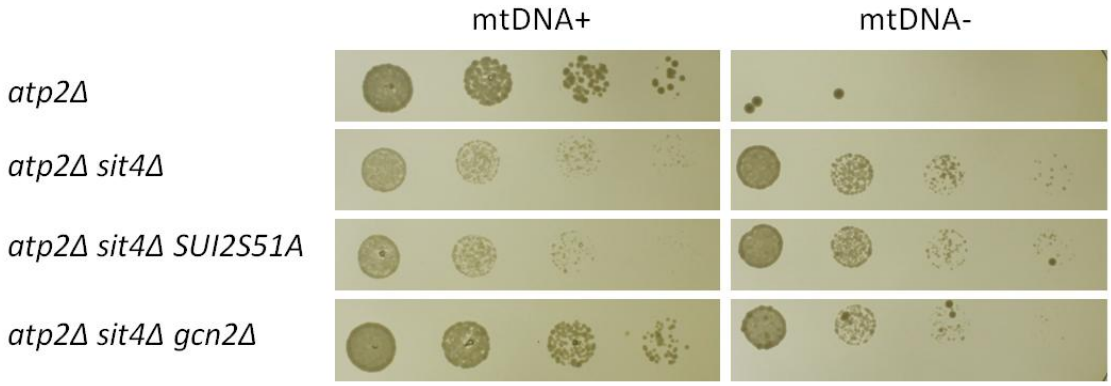

Figure 3.27 Deletion of Gcn4p, a transcriptional activator of amino acid biosynthetic genes, does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells.

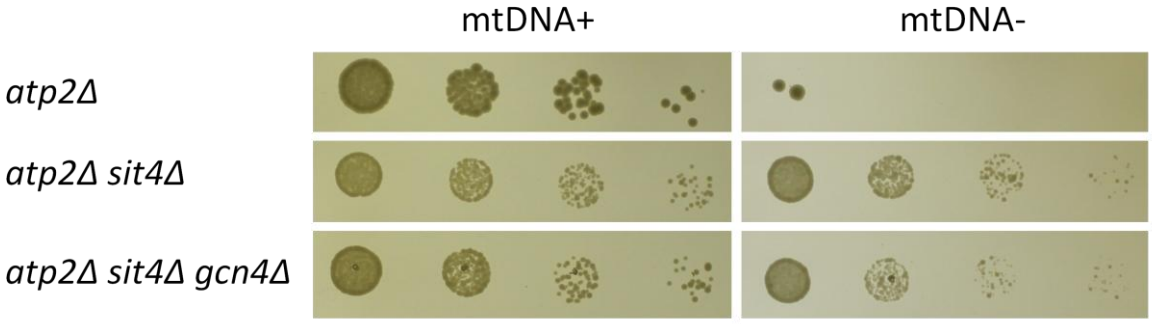

Figure 3.28 Blocking of Rim15p, a protein kinase involved in cell proliferation and establishment of stationary phase does not rescue petite-negative phenotype or block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells.

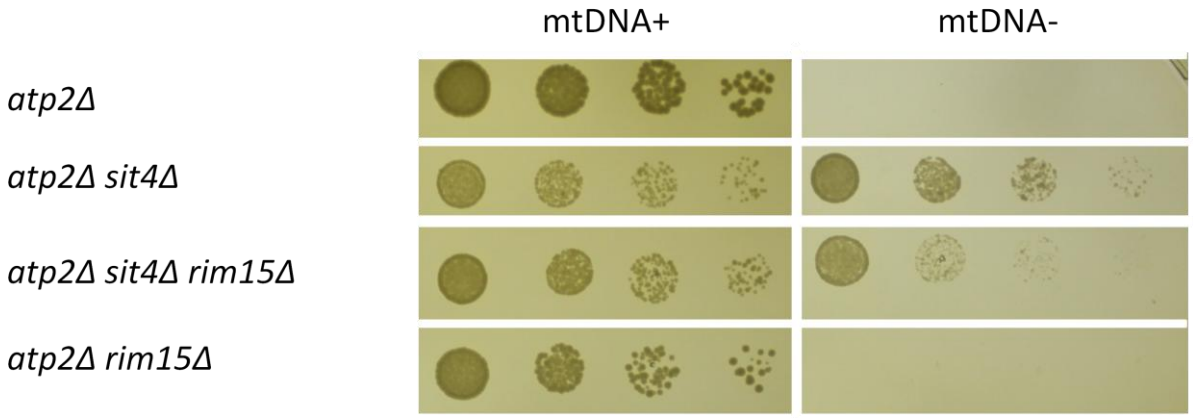

Figure 3.29 Depletion of glycogen synthesis with simultaneous deletion of GSY1 and GSY2 genes does not block the proliferation of *atp2*Δ *sit4*Δ ρ^- cells.

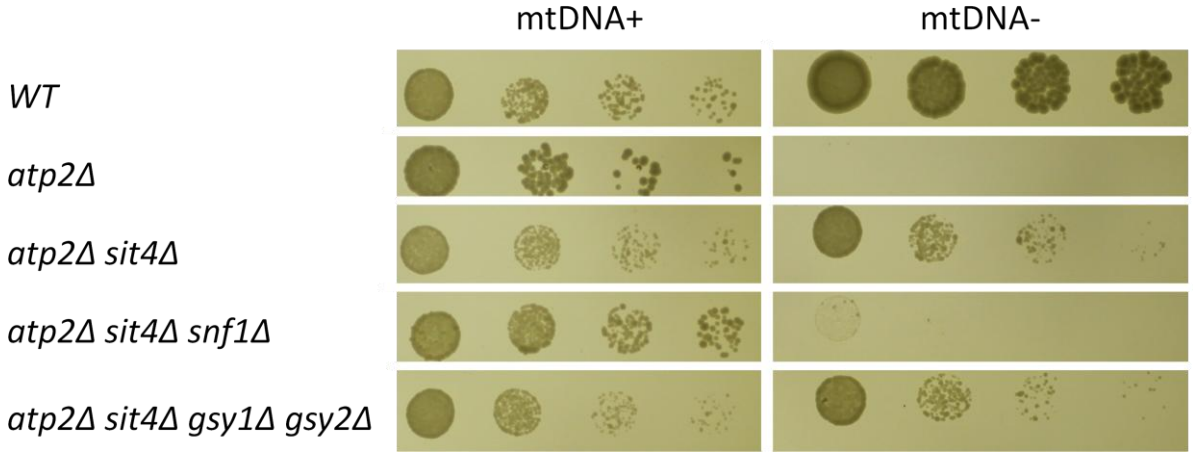

Figure 3.30 The major phosphate-regulated secreted acid phosphatase, Pho5p, is not required for Sit4p for the rescue of *atp2*Δ ρ^- cells.

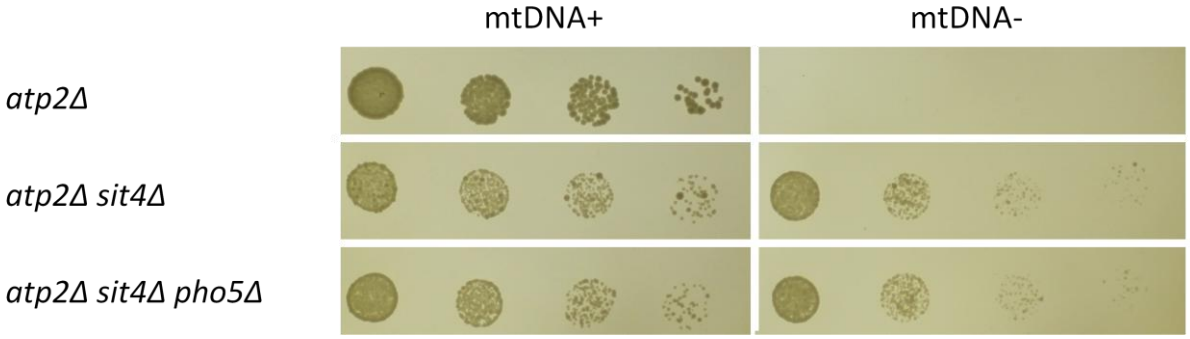

Figure 3.31 Phm6p, a protein regulated by phosphate levels; removal does not block the proliferation of *atp2*Δ ρ^- cells lacking Sit4p.

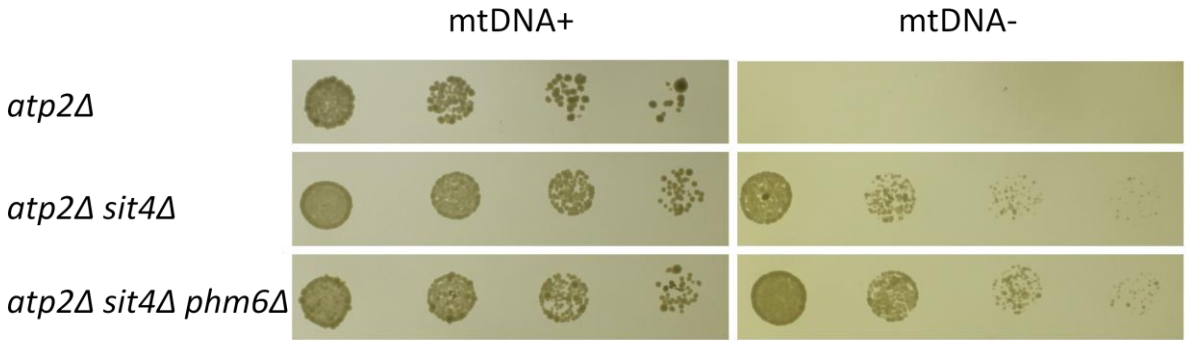

Figure 3.32 Deletion of hexokinase protein, Hxk1p, did not significantly alter the proliferation of *atp2*Δ ρ^- cells lacking Sit4p.

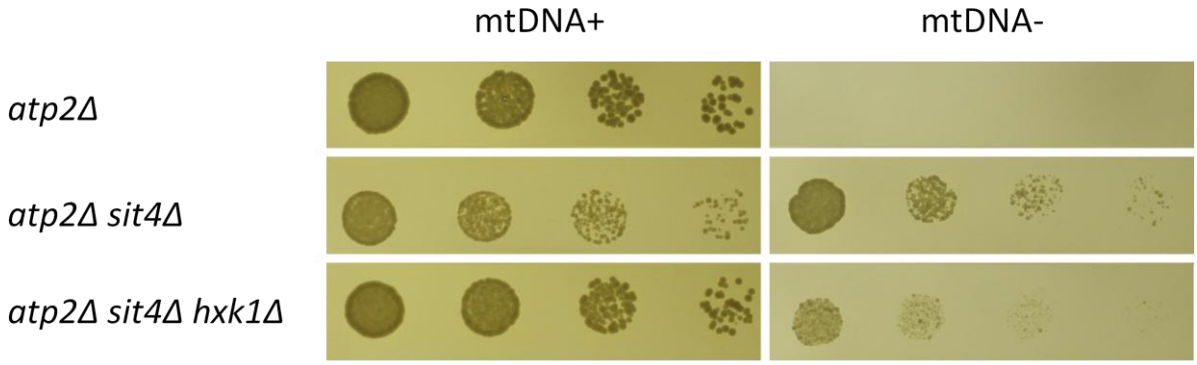

Figure 3.33 Increasing $\Delta\Psi^{mito}$ by hyperactivation of the ATP synthase F_1 sector rescues the petite-negative phenotype of ρ^- cells lacking the cyclic AMP phosphodiesterase.

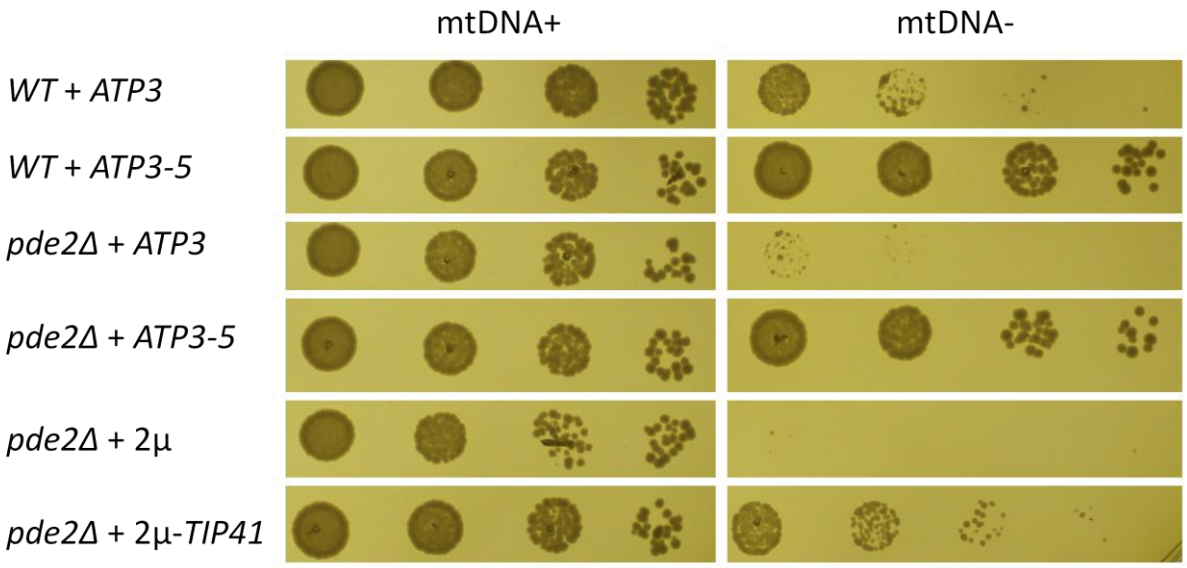

Figure 3.34 Single Tpk deletions could not rescue the petite-negativity phenotype of *pde2*Δ ρ⁻ cells.

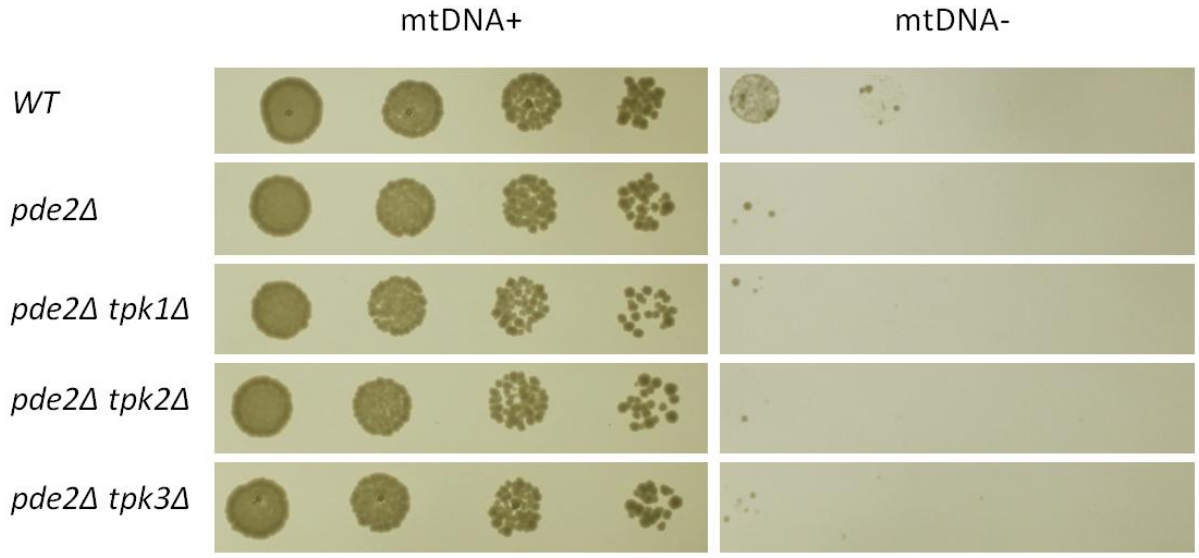

Figure 3.35 Deletion of conserved phosphatases functioning under Tap42p increases the $\Delta\Psi^{mito}$ of cells lacking mtDNA

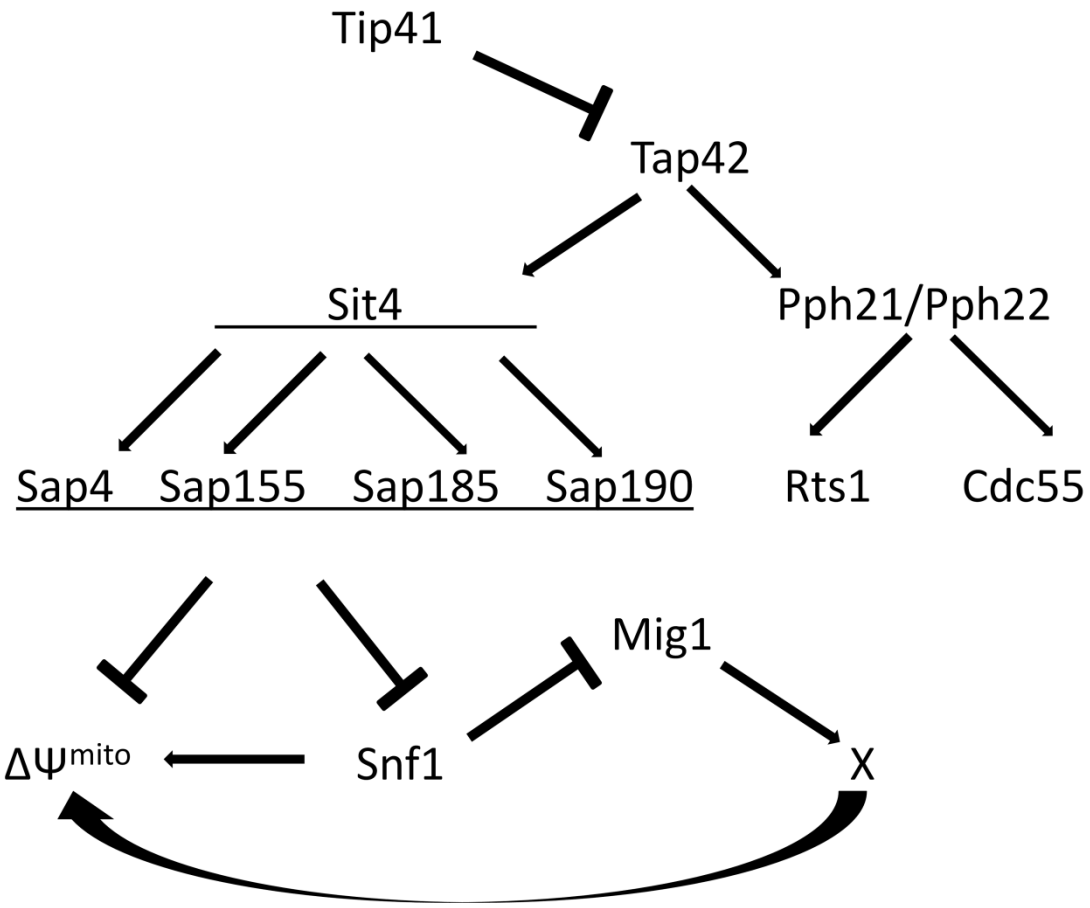

Table 3.1 List of strains used in Chapter 3

Strain	Genotype	Source	Parental strain(s)	Method Used
BY4741	*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1*	Brachman et al. (1998)		
BY4742	*MATα leu2Δ0 met15Δ0 ura3Δ0 his3Δ1*	Brachman et al. (1998)		
RJ1854	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4*	Dunn et al. (2008)		
RJ1921	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 tap42Δ::kanMX4/TAP42*	EUROSCARF Y20603		
RJ2288	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ200/his3Δ1 met15Δ0/met15Δ0 trp1Δ63/TRP1 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 vps16Δ::kanMX4/VPS16*	Garipler and Dunn (2013)		
CDD8	*MATα/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 yme1Δ::kanMX4/YME1*	EUROSCARF Y27144		
CDD24	*MATα/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 tom70Δ::kanMX4/TOM70*	EUROSCARF Y27244		

RJ97	*MATa cry1 lys1*	Robert Jensen		
CDD205	*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 tap42Δ::kanMX4 pLEU2-TAP42*	This study	RJ1921	transformed RJ1921 with plasmid M532 and sporulated
CDD208	*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 tap42Δ::kanMX4 pLEU2-tap42-17*	This study	RJ1921	transformed RJ1921 with plasmid M533 and sporulated
CDD215	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2*	This study	RJ2288	sporulation
CDD218	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2*		RJ2288	sporulation
CDD227	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 gap1Δ::kanMX4*	EUROSCARF Y17050		
CDD228	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 rtg2Δ::kanMX4*	EUROSCARF Y14619		
CDD229	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 rtg1Δ::kanMX4*	EUROSCARF Y11759		
CDD230	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 gln3Δ::kanMX4/GLN3*	EUROSCARF Y20173		

CDD231	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 npr1Δ::kanMX4*	EUROSCARF Y12029		
CDD233	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 pph21Δ::kanMX4/PPH21*	EUROSCARF Y23831		
CDD234	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pph3Δ::kanMX4*	EUROSCARF Y14010		
CDD235	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 ppg1Δ::kanMX4*	EUROSCARF Y15407		
CDD236	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mig1Δ::kanMX4*	EUROSCARF Y14403		
CDD239	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 atg1Δ::kanMX4*	EUROSCARF Y14547		
CDD240	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sds23Δ::kanMX4*	EUROSCARF Y14423		
CDD241	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tec1Δ::kanMX4*	EUROSCARF Y17155		
CDD246	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 pph21Δ::URA3/PPH21*	This study	CDD233	replaced *kanMX4* with URA3 using primers 115/116 and template

				pRS306
CDD247	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 gln3Δ::kanMX4/GLN3 gat1Δ::URA3/GAT1*	This study	CDD230	disrupted *GAT1* with *URA3* using primers 141/142 and template pRS306
CDD249	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3*	This study	RJ1854	sporulation
CDD250	*MATα met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 sit4Δ::HIS3*	Garipler and Dunn (2013)		
CDD251	*MATα met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 sit4Δ::HIS3*	Garipler and Dunn (2013)		
CDD254	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::HIS3*	This study	CDD218	replaced *LEU2* with *HIS3* using primers 136/137 and template pRS303
CDD255	*MATα/MATa leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 his3Δ1/his3Δ trp1Δ63/TRP1 atp2Δ::HIS3/ATP2 tap42Δ::kanMX4/TAP42 pLEU2-TAP42*	This study	CDD205, CDD254	mated CDD205 and CDD254
CDD256	*MATα/MATa leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 his3Δ1/his3Δ trp1Δ63/TRP1 atp2Δ::HIS3/ATP2 tap42Δ::kanMX4/TAP42 pLEU2-tap42-17*	This study	CDD208, CDD254	mated CDD208 and CDD254

CDD261	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pde2Δ::kanMX4*	EUROSCARF Y11657		
CDD264	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pph22Δ::kanMX4*	EUROSCARF Y13886		
CDD266	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sap155Δ::kanMX4*	EUROSCARF Y15854		
CDD267	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sap185Δ::kanMX4*	EUROSCARF Y11325		
CDD268	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sap4Δ::kanMX4*	EUROSCARF Y14596		
CDD269	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sap190Δ::kanMX4*	EUROSCARF Y17045		
CDD273	*MATa leu2Δ0 his3Δ ura3Δ0 met15Δ0 atp2Δ::LEU2 pph3Δ::kanMX4*	This study	CDD215, CDD234	crossed CDD215 and CDD234, followed by sporulation
CDD275	*MATa leu2Δ0 his3Δ ura3Δ0 met15Δ0 atp2Δ::LEU2*	This study	CDD215, CDD234	crossed CDD215 and CDD234, followed by sporulation
CDD277	*MATa leu2Δ0 his3Δ met15Δ0 ura3Δ0 atp2Δ::LEU2 ppg1Δ::kanMX4*	This study	CDD215, CDD235	crossed CDD215 and CDD235, followed by sporulation

CDD278	*MATα met15Δ0 lys2Δ0 his3Δ1 leu2Δ0 ura3Δ0 gln3Δ::kanMX4 gat1Δ::URA3*	This study	CDD247	sporulation
CDD284	*MATα leu2Δ0 his3Δ ura3Δ0 atp2Δ::LEU2 rtg1Δ::kanMX4*	This study	CDD229, CDD249	crossed CDD229 and CDD249, followed by sporulation
CDD287	*MATα leu2Δ0 ura3Δ0 his3Δ npr1Δ::kanMX4*	This study	CDD231, CDD249	crossed CDD231 and CDD249, followed by sporulation
CDD289	*MATα leu2Δ0 ura3Δ0 his3Δ*	This study	CDD236, CDD249	crossed CDD236 and CDD249, followed by sporulation
CDD290	*MATα leu2Δ0 ura3Δ0 his3Δ lys2Δ0 met15Δ0*	This study	CDD239, CDD249	crossed CDD239 and CDD249, followed by sporulation
CDD291	*MATa leu2Δ0 ura3Δ0 his3Δ lys2Δ0 met15Δ0 atg1Δ::kanMX4 atp2Δ::LEU2*	This study	CDD239, CDD249	crossed CDD239 and CDD249, followed by sporulation
CDD295	*MATα leu2Δ0 ura3Δ0 his3Δ met15Δ0 tec1Δ::kanMX4 atp2Δ::LEU2*	This study	CDD241, CDD249	crossed CDD241 and CDD249, followed by sporulation
CDD296	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 sit4Δ::HIS3 atp2Δ::LEU2*	This study	CDD241, CDD249	crossed CDD241 and CDD249, followed by sporulation

CDD298	*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ atp2Δ::HIS3 tap42Δ::kanMX4 pLEU2-TAP42*	This study	CDD255	sporulation
CDD300	*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ atp2Δ::HIS3 tap42Δ::kanMX4 pLEU2-tap42-17*	This study	CDD256	sporulation
CDD302	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap155Δ::kanMX4*	This study	CDD215, CDD266	crossed CDD215 to CDD266, followed by sporulation
CDD303	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap185Δ::kanMX4*	This study	CDD215, CDD267	crossed CDD215 to CDD267, followed by sporulation
CDD304	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap4Δ::kanMX4*	This study	CDD215, CDD268	crossed CDD215 to CDD268, followed by sporulation
CDD305	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap190Δ::kanMX4*	This study	CDD215, CDD269	crossed CDD215 to CDD269, followed by sporulation
CDD306	*MATα leu2Δ0 his3Δ met15Δ0 lys2Δ0 ura3Δ0 atp2Δ::LEU2 rtg2Δ::kanMX4*	This study	CDD228, CDD249	crossed CDD228 to CDD249, followed by sporulation
CDD307	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15 pph21Δ::URA3/PPH21 pph22Δ::kanMX4/PPH22*	This study	CDD246, CDD264	CDD246 was sporulated, and a *MATa URA3* spore was mated to CDD264

CDD308	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/met15Δ0 trp1Δ63/TRP1 pph21Δ::URA3/PPH21 pph22Δ::kanMX4/PPH22 atp2Δ::LEU2/ATP2*	This study	CDD307, CDD215	CDD307 was sporulated, and a *MATα URA3* spore that was geneticin-resistant was mated to CDD215
CDD319	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 pdr1Δ::URA3/PDR1*	This study	RJ1854	disrupted *PDR1* with *URA3* using primers 208/209 and template pRS306
CDD320	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 mig1Δ::URA3/MIG1*	This study	RJ1854	disrupted *MIG1* with *URA3* using primers 205/206 and template pRS306
CDD322	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 lys2Δ0 atp2Δ::LEU2 gln3Δ::kanMX4 gat1Δ::URA3*	This study	CDD278, CDD249	crossed CDD278 to CDD249, followed by sporulation
CDD324	*MATα/MATa met15Δ0/MET15 his3Δ200/his3-11,15 leu2Δ0/leu2-3,112 trp1Δ63/trp1Δ2 ura3Δ0/ura3-1 ade2-1/ADE2 can1-100/CAN1 sit4Δ::HIS3/SIT4 aac2Δ::URA3/AAC2*	Garipler and Dunn (2013)		
CDD325	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 sds23Δ::kanMX4/SDS23 sds24Δ::URA3/SDS24*	This study	BY4741, CDD240	crossed BY4741 to CDD240, then disrupted *SDS24* with *URA3* using primers 190/191 and template pRS306
CDD329	*MATα leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4*	Garipler and Dunn (2013)		

CDD332	*MATa leu2Δ0 his3Δ ura3Δ0 met15Δ0 pph21Δ::URA3 pph22Δ::kanMX4 atp2Δ::LEU2/ATP2*	This study	CDD308	sporulation
CDD333	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0*	This study	CDD215, CDD269	mated CDD215 and CDD269, then sporulated the resulting diploid
CDD335	*MATα his3Δ1 leu2Δ0 ura3Δ0 sds24Δ::URA3*	This study	CDD325	sporulation
CDD336	*MATa his3Δ1 leu2Δ0 ura3Δ0 sds23Δ::kanMX4 sds24Δ::URA3*	This study	CDD325	sporulation
CDD337	*MATα his3Δ1 leu2Δ0 ura3Δ0 sds23Δ::kanMX4 sds24Δ::URA3*	This study	CDD325	sporulation
CDD339	*MATα/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 vps16Δ::URA3/VPS16 end3Δ::LEU2/END3*	Garipler and Dunn (2013)		
CDD351	*MATa met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 mig1Δ::URA3*	This study	CDD320	sporulation
CDD352	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 trp1Δ63/TRP1 pph21Δ::URA3/PPH21 pph22Δ::kanMX4/PPH22 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4*	This study	CDD250, CDD332	mated CDD250 and CDD332

CDD353	*MATα/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 yme1Δ::kanMX4/YME1 sit4Δ::URA3/SIT4*	This study	CDD8	disrupted *SIT4* with *URA3* using primers 133/134 and template pRS306
CDD354	*MATα/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 tom70Δ::kanMX4/TOM70 sit4Δ::URA3/SIT4*	This study	CDD24	disrupted *SIT4* with *URA3* using primers 133/134 and template pRS306
CDD364	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4*	Garipler and Dunn (2013)		
CDD366	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2*	This study	CDD215, CDD333	mated CDD215 to CDD333
CDD377	*MATa leu2Δ0 ura3Δ0 his3Δ1 yme1Δ::kanMX4 sit4Δ::URA3*	This study	CDD353	sporulation
CDD378	*MATa leu2Δ0 ura3Δ0 his3Δ1 yme1Δ::kanMX4*	This study	CDD353	sporulation
CDD382	*MATa leu2Δ0 ura3Δ0 his3Δ1 tom70Δ::kanMX4 sit4Δ::URA3*	This study	CDD354	sporulation
CDD383	*MATα leu2Δ0 ura3Δ0 his3Δ1 tom70Δ::kanMX4*	This study	CDD354	sporulation

CDD397	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 SUI2-S51A::URA3/SUI2*	This study	RJ1854	from plasmid b66, a portion of *SUI2* and of *URA3* were amplified with primers 266/267, thereby introducing the sequence encoding a S51A mutation into the *SUI2* gene product. SUI2-S51A insertion at a SUI2 locus was confirmed by sequencing.
CDD403	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 SUI2-S51A::URA3*	This study	CDD397	Sporulation
CDD404	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 gcn2Δ::URA3*	This study	RJ1854	disrupted *GCN2* with *URA3* using primers 254/255 and template pRS306, then sporulated the resulting diploid
CDD405	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 bck2Δ::URA3*	This study	CDD366	disrupted *BCK2* with *URA3* using primers 225/226 and template pRS306, then sporulated the resulting diploid

CDD409	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 mrpl16Δ::URA3*	This study	RJ1854	disrupted *MRPL16* with *URA3* using primers 260/261 and template pRS306, then sporulated the resulting diploid
CDD410	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 mrpl16Δ::URA3*	This study	RJ1854	disrupted *MRPL16* with *URA3* using primers 260/261 and template pRS306, then sporulated the resulting diploid
CDD415	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 sit4Δ::HIS3 mrpl16Δ::URA3*	This study	RJ1854	disrupted *MRPL16* with *URA3* using primers 260/261 and template pRS306, then sporulated the resulting diploid
CDD417	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap155Δ::kanMX4 sap4Δ::URA3*	This study	CDD302	disrupted *SAP4* with *URA3* using primers 215/216 and template pRS306
CDD418	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap155Δ::kanMX4 sap190Δ::URA3*	This study	CDD302	disrupted *SAP190* with *URA3* using primers 221/222 and template pRS306

CDD419	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap185Δ::kanMX4 sap4Δ::URA3*	This study	CDD303	disrupted *SAP4* with *URA3* using primers 215/216 and template pRS306
CDD420	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap185Δ::kanMX4 sap155Δ::URA3*	This study	CDD303	disrupted *SAP155* with *URA3* using primers 217/218 and template pRS306
CDD421	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap185Δ::kanMX4 sap190Δ::URA3*	This study	CDD303	disrupted *SAP190* with *URA3* using primers 221/222 and template pRS306
CDD422	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sap190Δ::kanMX4 sap4Δ::URA3*	This study	CDD305	disrupted *SAP4* with *URA3* using primers 215/216 and template pRS306
CDD423	*MATα met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 pdr1Δ::URA3 pdr3Δ::TRP1*	This study	CDD319	disrupted *PDR3* with *TRP1* using primers 213/214 and template pRS304, then sporulated the resulting diploid
CDD424	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 gcn4Δ::URA3*	This study	RJ1854	disrupted *GCN4* with *URA3* using primers 47/48 and template pRS306, then sporulated the resulting diploid

CDD426	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 slt2Δ::URA3*	This study	RJ1854	disrupted *SLT2* with *URA3* using primers 257/258 and template pRS306, then sporulated the resulting diploid
CDD450	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 sap4Δ::URA3 sap190Δ::kanMX4 mrpl16Δ::URA3*	This study	CDD410, CDD422	mated CDD410 and CDD422, then sporulated the resulting diploid
CDD452	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 sap155Δ::kanMX4 sap190Δ::URA3 mrpl16Δ::URA3*	This study	CDD409, CDD418	mated CDD409 and CDD418, then sporulated the resulting diploid
CDD453	*MATα leu2Δ0 ura3Δ0 his3Δ lys2Δ0 met15Δ0 pph21Δ::URA3 pph22Δ::kanMX4*	This study	CDD307	disrupted *MRPL16* with *LEU2* using primers 260/261 and template pRS305, then sporulated the resulting diploid
CDD454	*MATα leu2Δ0 ura3Δ0 his3Δ lys2Δ0 met15Δ0 pph21Δ::URA3 pph22Δ::kanMX4 mrpl16Δ::LEU2*	This study	CDD307	disrupted *MRPL16* with *LEU2* using primers 260/261 and template pRS305, then sporulated the resulting diploid
CDD457	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 swi4Δ::URA3*	This study	CDD366	disrupted *SWI4* with *URA3* using primers 293/294 and template pRS306, then

				sporulated the resulting diploid
CDD458	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 snf1Δ::URA3*	This study	RJ1854	disrupted *SNF1* with *URA3* using primers 290/291 and template pRS306, then sporulated the resulting diploid
CDD463	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0*	This study	RJ1854	disrupted *SNF1* with *URA3* using primers 290/291 and template pRS306, then sporulated the resulting diploid
CDD478	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 rts1Δ::URA3*	This study	CDD366	disrupted *RTS1* with *URA3* using primers 310/311 and template pRS306, then sporulated the resulting diploid
CDD479	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 cdc55Δ::TRP1*	This study	CDD366	disrupted *RTS1* with *URA3* using primers 310/311 and template pRS306, then sporulated the resulting diploid

CDD480	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 rts1Δ::URA3 cdc55Δ::TRP1*	This study	CDD366	disrupted *RTS1* with *URA3* using primers 310/311 and template pRS306, then sporulated the resulting diploid
CDD488	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 rim15Δ::URA3*	This study	RJ1854	disrupted *RIM15* with *URA3* using primers 307/308 and template pRS306, then sporulated the resulting diploid
CDD489	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 gsy1Δ::URA3 gsy2Δ::TRP1*	This study	CDD366	disrupted *GSY1* with *URA3* using primers 284/285 and template pRS306, then disrupted *GSY2* with *TRP1* using primers 287/288 and template pRS304, then sporulated the resulting diploid
CDD521	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 avo2Δ::URA3*	This study	CDD364	disrupted *AVO2* with *URA3* using primers 334/335 and template pRS306

CDD522	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 bit61Δ::URA3*	This study	CDD364	disrupted *BIT61* with *URA3* using primers 337/338 and template pRS306
CDD523	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 tco89Δ::URA3*	This study	CDD364	disrupted *TCO89* with *URA3* using primers 299/300 and template pRS306
CDD524	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 sit4Δ::URA3*	This study	CDD364	disrupted *SIT4* with *URA3* using primers 133/134 and template pRS306
CDD525	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sno1Δ::URA3*	This study	CDD366	disrupted *SNO1* with *URA3* using primers 386/387 and template pRS306, then sporulated the resulting diploid
CDD526	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 snz1Δ::URA3*	This study	CDD366	disrupted *SNZ1* with *URA3* using primers 389/390 and template pRS306, then sporulated the resulting diploid
CDD528	*MATα leu2Δ0 his3Δ ura3Δ0 met15Δ0 trp1Δ63*	This study	CDD366	disrupted *SNO1* with *URA3* using primers 386/387 and template pRS306, then

			sporulated the resulting diploid
CDD533	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 arg3Δ::kanMX4*	EUROSCARF Y11335	
CDD535	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pho5Δ::kanMX4*	EUROSCARF Y13232	
CDD536	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 rtc2Δ::kanMX4*	EUROSCARF Y13286	
CDD537	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 phm6Δ::kanMX4*	EUROSCARF Y13640	
CDD538	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 prr2Δ::kanMX4*	EUROSCARF Y13912	
CDD539	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 hxk1Δ::kanMX4*	EUROSCARF Y15867	
CDD540	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 tmt1Δ::kanMX4*	EUROSCARF Y16171	
CDD543	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 cpa1Δ::kanMX4*	EUROSCARF Y17337	

CDD554	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 slt2Δ::URA3/SLT2 snf1Δ::TRP1/SNF1*	This study	CDD426, CDD528	mated CDD426 and CDD528, then disrupted *SNF1* with *TRP1* using primers 290/291 and template pRS304
CDD557	*MATα leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 snf1Δ::TRP1*	This study	CDD554	sporulation
CDD563	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 slt2Δ::URA3*	This study	CDD554	sporulation
CDD569	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 mig1Δ::URA3/MIG1 snf1Δ::TRP1/SNF1*	This study	CDD320	disrupted *SNF1* with *TRP1* using primers 290/291 and template pRS304
CDD578	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2ATP2 sit4Δ::HIS3/SIT4 snf1Δ::TRP1/SNF1*	This study	CDD463, CDD557	mated CDD463 and CDD557
CDD585	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 hap4Δ::kanMX4*	EUROSCARF Y14959		
CDD586	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 trp1Δ63 atp2Δ::LEU2 arg3Δ::kanMX4*	This study	CDD533, CDD249	mated CDD533 and CDD249, then sporulated the resulting diploid

CDD590	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 trp1Δ63 lys2Δ0 atp2Δ::LEU2 sit4Δ::HIS3 pho5Δ::kanMX4*	This study	CDD535, CDD249	mated CDD535 and CDD249, then sporulated the resulting diploid
CDD591	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 lys2Δ0 atp2Δ::LEU2 rtc2Δ::kanMX4*	This study	CDD536, CDD249	mated CDD536 and CDD249, then sporulated the resulting diploid
CDD592	*MATα leu2Δ0 ura3Δ0 his3Δ atp2Δ::LEU2 sit4Δ::HIS3 phm6Δ::kanMX4*	This study	CDD537, CDD249	mated CDD537 and CDD249, then sporulated the resulting diploid
CDD593	*MATα leu2Δ0 ura3Δ0 his3Δ atp2Δ::LEU2 prr2Δ::kanMX4*	This study	CDD538, CDD249	mated CDD538 and CDD249, then sporulated the resulting diploid
CDD594	*MATa leu2Δ0 ura3Δ0 his3Δ trp1Δ63 atp2Δ::LEU2 sit4Δ::HIS3 hxk1Δ::kanMX4*	This study	CDD539, CDD249	mated CDD539 and CDD249, then sporulated the resulting diploid
CDD595	*MATα leu2Δ0 ura3Δ0 his3Δ atp2Δ::LEU2 tmt1Δ::kanMX4*	This study	CDD540, CDD249	mated CDD540 and CDD249, then sporulated the resulting diploid
CDD600	*MATa leu2Δ0 ura3Δ0 his3Δ met15Δ0 trp1Δ63 atp2Δ::LEU2 cpa1Δ::kanMX4*	This study	CDD543, CDD249	mated CDD543 and CDD249, then sporulated the resulting diploid
CDD602	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 sit4Δ::HIS3 snfΔ::TRP1*	This study	CDD578	knock in *SNF1-MYC::kanMX6* using plasmid pFA6a-13Myc-kanMX6 (Longtine Yeast 14:953) and

				primers 423/424, then sporulated the resulting diploid
CDD604	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 snfΔ::TRP1*	This study	CDD578	knock in *SNF1-MYC::kanMX6* using plasmid pFA6a-13Myc-kanMX6 (Longtine Yeast 14:953) and primers 423/424, then sporulated the resulting diploid
CDD608	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 aft1Δ::URA3*	This study	CDD215	disrupted *AFT1* with *URA3* using primers 380/381 and template pRS306
CDD611	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 reg1Δ::URA3*	This study	CDD578	disrupted *REG1* with *URA3* using primers 428/429 and template pRS306, then sporulated the resulting diploid
CDD614	*MATa met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 mig1Δ::URA3 snf1Δ::TRP1*	This study	CDD569	sporulation
CDD615	*MATa leu2Δ0 ura3Δ0 his3Δ atp2Δ::LEU2 gap1Δ::kanMX4*	This study	CDD227, CDD249	mated CDD227 and CDD249, then sporulated the resulting diploid

CDD616	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 aft2Δ::URA3*	This study	CDD215	disrupted *AFT2* with *URA3* using primers 383/384 and template pRS306
CDD619	*MATa cry1 lys1 ρ-*	This study	RJ97	treated CDD49 with 25µg/ml EtBr, then struck for single colonies and tested for lethality on YEPGE
CDD626	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 sip1Δ::URA3*	This study	RJ1854	disrupted *SIP1* with *URA3* using primers 447/448 and template pRS306, then sporulated the resulting diploid
CDD627	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 sip2Δ::URA3*	This study	RJ1854	disrupted *SIP2* with *URA3* using primers 450/451 and template pRS306, then sporulated the resulting diploid
CDD628	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 gal83Δ::URA3*	This study	RJ1854	disrupted *GAL83* with *URA3* using primers 453/454 and template pRS306, then sporulated the resulting diploid

CDD629	*MATα leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 sak1Δ::URA3*	This study	RJ1854	disrupted *SAK1* with *URA3* using primers 431/432 and template pRS306, then sporulated the resulting diploid
CDD630	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 sit4Δ::HIS3 tos3Δ::URA3*	This study	RJ1854	disrupted *TOS3* with *URA3* using primers 444/445 and template pRS306, then sporulated the resulting diploid
CDD631	*MATα met15Δ0 his3 leu2 trp1 ura3 ade2-1 can1-100 sit4Δ::HIS3 aac2Δ::URA3*	This study	CDD324	sporulation
CDD632	*MATa met15Δ0 his3 leu2 trp1 ura3 aac2Δ::URA3*	This study	CDD324	sporulation
CDD653	*MATα met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 mig1Δ::URA3 snf1Δ::TRP1*	This study	CDD569	sporulation
CDD743	*MATα/a leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 pde2Δ::kanMX4*	This study	CDD1, CDD216	mated CDD1 and CDD216
CDD745	*MATα/a leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 pde2Δ::kanMX4 tpk1Δ::URA3*	This study	CDD743	disrupted *TPK1* with *URA3* using primers 589/590 and template pRS306, then

				sporulated the resulting diploid
CDD746	*MATα/a leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 pde2Δ::kanMX4 tpk2Δ::URA3*	This study	CDD743	disrupted *TPK2* with *URA3* using primers 592/593 and template pRS306, then sporulated the resulting diploid
CDD747	*MATα/a leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 pde2Δ::kanMX4 tpk3Δ::URA3*	This study	CDD743	disrupted *TPK3* with *URA3* using primers 595/596 and template pRS306, then sporulated the resulting diploid
CDD751	*MAT(?) leu2Δ0 lys2Δ0 MET15 ura3Δ0 his3Δ1 pde2Δ::kanMX4 tpk1Δ::URA3*	This study	CDD745	sporulation
CDD752	*MAT(?) leu2Δ0 lys2Δ0 MET15 ura3Δ0 his3Δ1 pde2Δ::kanMX4 tpk2Δ::URA3*	This study	CDD746	sporulation
CDD753	*MAT(?) leu2Δ0 lys2Δ0 MET15 ura3Δ0 his3Δ1 pde2Δ::kanMX4 tpk3Δ::URA3*	This study	CDD747	sporulation

Table 3.2 List of oligonucleotides used in Chapter 3.

Number	Sequence
47	CAATTTGTCTGCTCAAGAAAATAAATTAAATACAAATAAAAGATTGTACTGAGAGTGCAC
48	GAGAATGAAATAAAAAATATAAAATAAAAGGTAAATGAAACTGTGCGGTATTTCACACCG
115	GGATATAAATTATCGCATAAAACAATAAACAAAAAGAAAAAGATTGTACTGAGAGTGCAC
116	GTGAATATATATCTATATAGATGCATATATGTATACATACctgtgcggtatttcacaccg
133	TTGAAGCTCAAAAACATCCATAATAAAAGGAACAATAACAagattgtactgagagtgcac
134	TTATTTTTATTCGTCGAGTTAGGGAGGGCATGCCGTCGTGctgtgcggtatttcacaccg
136	TATACTGAATCTTTGAGAGAAAACAAAAATAAAAAAAAGAagattgtactgagagtgcac
137	ATTTCAAATTTTGCTTCCCTTGGTTTAAGCTTTATTTCTTctgtgcggtatttcacaccg
141	ATATATATAGGTGTGTGCCACTCCCGGCCCCGGTATTAGCagattgtactgagagtgcac
142	GACATGGAAAGAAGCGAGTACTTTTTTTTTTTTGGGGGATctgtgcggtatttcacaccg
190	AACACTTATAGAACAATTACCGTTTCTTCCTTCACACAACAGATTGTACTGAGAGTGCAC
191	AGGGAGAGGGAAACAGCGGGAAAAGACAACGCTGCAAGCCCTGTGCGGTATTTCACACCG
205	CGAGAGTTGAGTATAGTGGAGACGACATACTACCATAGCCAGATTGTACTGAGAGTGCAC
206	TCTTTTGATTTATCTGCACCGCCAAAAACTTGTCAGCGTACTGTGCGGTATTTCACACCG
208	CAGCCAAGAATATACAGAAAAGAATCCAAGAAACTGGAAGAGATTGTACTGAGAGTGCAC
209	GGAAGTTTTTGAGAACTTTTATCTATACAAACGTATACGTCTGTGCGGTATTTCACACCG
213	ATCAGCAGTTTTATTAATTTTTTCTTATTGCGTGACCGCAAGATTGTACTGAGAGTGCAC
214	TACTATGGTTATGCTCTGCTTCCCTATTTTCTTTGCGTTTCTGTGCGGTATTTCACACCG
215	GCTCTCTTGCTTTTCGATAAAGCTAAAATAAAATAAAAATAGATTGTACTGAGAGTGCAC

216	GGAAAAAAAAAGTCAAAATTGGTTTGACGACTTAACCTAACTGTGCGGTATTTCACACCG
217	GAAAAGAAAAAAAAGACGAAAACAACAAGCCAGAGTAAAGAGATTGTACTGAGAGTGCAC
218	ATATATATATACAAATTAAAGAAAAGTACAAAACAATGTACTGTGCGGTATTTCACACCG
221	CATTTCTTCATTTACTTAACTGCGAGAAGATTATAATAGCAGATTGTACTGAGAGTGCAC
222	TGAATAAAGGGTGAAAATGTGACAATGTGAATGTTTTAGTCTGTGCGGTATTTCACACCG
225	AGCAAGGCAGTCTTGATTTATCGCTCAAAACCAATAGCACAGATTGTACTGAGAGTGCAC
226	TTAGTTGCTATTATCAAAATAAAAAGACTGTAAATTATTACTGTGCGGTATTTCACACCG
254	TCAATAATTTTCCGTTCCCCTTAACACATACTATGTATAAAGATTGTACTGAGAGTGCAC
255	TTAACTGATGCGTTATAGCGCCGCACAGATCTTTAAAGGCCTGTGCGGTATTTCACACCG
257	AGTAGAAATAATTGAAGGGCGTGTATAACAATTCTGGGAGAGATTGTACTGAGAGTGCAC
258	GGTGATTCTATACTTCCCCGGTTACTTATAGTTTTTTGTCCTGTGCGGTATTTCACACCG
260	TACCTTTCACCAGAGGTACAGAGGAATAGAATTTTGCAACAGATTGTACTGAGAGTGCAC
261	CAATAGTTATATACATGTATACATGGTGTGTTCAATCCGTctgtgcggtatttcacaccg
266	GACAACATTGAAGGTATGATTCTACTAAGTGAATTGgctCGTAGACGTATTAGGTC
267	CCAATGACAGAAATCACTGCTTACAATGAATAAATTGTTCagattgtactgagagtgcac
284	GAAAAAGAAAATAAACTCAGACGCAGCATCACAGCGAAGTAGATTGTACTGAGAGTGCAC
285	GCTAAAAGAGTAAGATATGTTAGCAGAAGTTAAGATGGTTCTGTGCGGTATTTCACACCG
287	CTGTGATTGAAGTTTTGACTACCTCAGAGAAAAATTTTGAAGATTGTACTGAGAGTGCAC
288	TGGTAAGATTTTTTTAATACTGTTTATATCCTCATAGGATCTGTGCGGTATTTCACACCG
290	TTTGTAACAAGTTTTGCTACACTCCCTTAATAAAGTCAACAGATTGTACTGAGAGTGCAC
291	GGGAACTTCCATATCATTCTTTTACGTTCCACCATCAATTCTGTGCGGTATTTCACACCG

293	CTCTTGCGTAGTTCTAAAAGGTTGATTTATTCGAGAAATCAGATTGTACTGAGAGTGCAC
294	ATAATATAGTAAAAATTATTGGTACATTGTGAATTAAAATCTGTGCGGTATTTCACACCG
299	ATTGGGATATATCATATATCCTTACTGAGTAACTATAATTagattgtactgagagtgcac
300	TAGCTACTCTTTTAAACTGTGTGCTTCGTGTTGGTTGTTTctgtgcggtatttcacaccg
307	CTCATTTGATAGAATAGATAAGCCCAGTAGAGGAAGACAGagattgtactgagagtgcac
308	ATTCAGTTATTTTTTTTAATTATCTTTATCTTAAAATTTActgtgcggtatttcacaccg
310	ATCAATAGGCACGTGCTATTTTCGAACATCCACTTTCAATagattgtactgagagtgcac
311	AAACTTCCTCACTTCTTCGAGCTTGTAATGAATTGCTGTTctgtgcggtatttcacaccg
334	AAAAAACGCCCATTAGAAAAGAGGATATATTGAACAAAAGAGATTGTACTGAGAGTGCAC
335	ACGTAGTAGTCTTCTCAATCTTCTGCACGCGTGTTATTGCCTGTGCGGTATTTCACACCG
337	AAACGCCAATTGACCTCTGTAGAAATAAAGTTCTTTCATTAGATTGTACTGAGAGTGCAC
338	GATATTAAATAGGAGATTGAAATAAAGTGTTAGATGCTTTCTGTGCGGTATTTCACACCG
380	GGAAGGCTTCAATCCGGCTGACATAGAACATGCGTCACCGAGATTGTACTGAGAGTGCAC
381	ATGGACGAGAGATACGTCTAAGTTTGATTTCATCTATATGCTGTGCGGTATTTCACACCG
383	CTCTGGCGCAAATTAATCAAGAGAAAGCGACCCCCAAGAAAGATTGTACTGAGAGTGCAC
384	CGTGATACCGTTTTAATGAGTTGAAAACTAAATAATTAATCTGTGCGGTATTTCACACCG
386	TTCATTTCGTTAAATAGAAAGAAAAACCATATCTTAAAGTAGATTGTACTGAGAGTGCAC
387	AGGTTTTGGTAATATAAAAATGTGGAAAACCGGCGGTATTCTGTGCGGTATTTCACACCG
389	AGCAAATATACACAGTACTAATATTCAGTTAATTATCACGAGATTGTACTGAGAGTGCAC
390	GAAAAAGTGTTATAATGCTCAAAATACCTGTTCAAAGAAACTGTGCGGTATTTCACACCG
423	AAAACTAATTATGGAATTAGCCGTTAACAGTCAAAGCAATcggatccccgggttaattaa

424	AAAAAAGGGAACTTCCATATCATTCTTTTACGTTCCACCAgaattcgagctcgtttaaac
428	GCAAGCATATTGACGAAGACGAGATAAGAAAAATCCAAAAAGATTGTACTGAGAGTGCAC
429	TTTTTATTTTCTCTTCATGTTGACTTCAAAATTCTTTCTTCTGTGCGGTATTTCACACCG
431	GATAAAACGAAAAGAAGCATATTAATAAGGAGTTTTGAACagattgtactgagagtgcac
432	TAACATCGTAGTCCGATGGAAATTACTTTGAATTTTACACctgtgcggtatttcacaccg
444	GCGCACATATTCTGCATATAAAAAGGAAGCTTTGAAGAATAGATTGTACTGAGAGTGCAC
445	TCATATATTACATCTATTAAAATAATTTACATATATCATGCTGTGCGGTATTTCACACCG
447	GAAAGTTGAACTGTCATATTATATAGTTGTTGCAGCCGCCAGATTGTACTGAGAGTGCAC
448	GAATTAATAGAGTTCGTGAGAATCATTGCGAATTGAGATTCTGTGCGGTATTTCACACCG
450	ATTGTGCTTTTGGAAGGGTACTGAGAGCAGTGAAGTTTCTAGATTGTACTGAGAGTGCAC
451	AAATATATACAATACGCCTTTAAAAAGTAGGAATCCCGGACTGTGCGGTATTTCACACCG
453	GGTCGTTTCCTGCACAATAATAAATTATCTACACTGAAATAGATTGTACTGAGAGTGCAC
454	TTTATCTAAAAAGATGTAGCTATAAGCTCTATAATCATATCTGTGCGGTATTTCACACCG
589	GAATTATAGCTGATTGTGTGAAAGAATCTTTTTTTTGGGTAGATTGTACTGAGAGTGCAC
590	AATATAGATACGAGAGGAAAATACAACAAAACATTAGTCACTGTGCGGTATTTCACACCG
592	TTCACTCAGGTTAACTCACATACTGTTGAAAATTGTCGGTAGATTGTACTGAGAGTGCAC
593	GTACTTGAAAATTGTTTTTGTGTTTTTTGGTTCATGGAACCTGTGCGGTATTTCACACCG
595	ATATCGGTGGTTGTACAAGGAAAGAGCGAGCCTGCACAAAAGATTGTACTGAGAGTGCAC
596	CAATTATCCCACTGAACCTCCTTAAAATTCTTTCATTAAACTGTGCGGTATTTCACACCG

CHAPTER 4

A POTENTIAL RELATIONSHIP BETWEEN CYTOSOLIC PROTEOSIS AND MITOCHONDRIAL DYSFUNCTION

4.1 Introduction

mtDNA is not essential for *Saccharomyces cerevisiae.* This enables an easily modifiable background for studies on mitochondria related diseases. Cells that are subjected to 25 μg/ml ethidium bromide (EtBr) lose their mtDNA [10]. Unlike mtDNA, mitochondria itself is essential for yeast since it regulates many processes as heme biosynthesis, iron-sulfur cluster biogenesis, calcium sequestration, amino acid synthesis, lipid breakdown and ATP synthesis [195][196]. For regulation of these processes many proteins and metabolites has to be imported into the mitochondria even in the absence of mtDNA.

Mutation or deletion of mtDNA causes a decrease in the mitochondrial inner membrane (IM) electrochemical potential ($\Delta\Psi^{mito}$). Although import into mitochondria can partially occur through potential-independent ways, for potential-dependent proteins there occurs an import deficiency [152][3]. Cells with mtDNA mutations have growth defects because reduced import of nuclear DNA encoded, cytoplasmic translated proteins and metabolites into the mitochondria [60] [78]. Mitochondrial proteins that are not properly imported mainly localize to cytoplasm [194] and abundant

unimported precursors of mitochondrial proteins were found in cytoplasm [197]. One neuromuscular degeneration disease model, autosomal dominant progressive external ophthalmoplegia (adPEO), generated by Wang et. al. in yeast via a point mutation in ADP/ATP antiporter [197]. The phenotypes of this disease model are caused by low activity of ADP/ATP antiporter which results low ADP^{3-} levels and also generate low mitochondrial inner membrane potential [198][197]. The yeast ortholog of human *ant1A114P* mutation *aac2A128P* disrupts the structure of cytosolic gating region [199] causing the complex leak protons into mitochondria and uncouple the $\Delta\Psi^{mito}$ [200][197]. Decreased $\Delta\Psi^{mito}$ is also seen in mtDNA-lacking cells and aged cells [158][201] which may be the reason for mitochondrial degeneration in adPEO disease model [197]. These aged cells also show microcolony formation and stop dividing after a certain amount of divisions [128], similarly cells containing *aac2* mutant show lethality after tenth doubling [197].

Mutations of genes associating with nutrient signaling pathways, ribosomal biogenesis and protein genes were found to rescue the proliferation defect of cells lacking wildtype Aac2p activity [197]. Many studies have shown that reduction of cytosolic protein translation or calorie restriction benefits the proliferation and may delay the ageing phenotype [202][203]. Downregulation of branch of TOR pathway responsible for controlling ribosomal biogenesis, Sch9p, [128] and Gcn4 depletion of 60S subunits yeast ribosomal subunits also increases the replicative lifespan of yeast [204]. Moreover, ribosomal proteins Rpl10 and Rps6 were found as proteins affecting lifespan [205]. Lifespan of *aac2A128P* mutant containing cells were prolonged by at least 45% when protein synthesis is depreciated by mutations in pathways above [197] which highlights the importance of ribosomal biogenesis in mitochondrial disease model. We derived a hypothesis based on previous findings that these cytosolically translated mitochondrial proteins might be accumulating in the cytoplasm and cause cytotoxicity. Accumulation of these proteins may trigger more stress response proteins and possibly form aggregates with each other and possibly with other proteins.

Another mitochondrial disease model is the *Drosophila technical knockout* (*tko*^{25t}) which is a point mutation, at conserved leucine 85 to histidine, in the mitoribosomal protein S12, which furnishes the protein synthesis in mitochondria [206][79]. The mutant flies have side effects on respiratory chain deficiencies, paralytic seizures upon mechanical stress, stroke-like seizures, hearing loss, hyporeactivity upon physical stimulation and antibiotic sensitivity which are the phenotypes that are also seen in humans [79]. *tko*^{25t} only mildly effects the flies, which is the main reason it is used as a model disease phenotype to search for new interactions. However complete deletion of *tko* causes larval lethality [80]. Feeding of (1 mg/mL) mitochondrial protein translation inhibitor doxycyclin also gives the same growth retardation phenotype and bang sensitivity as *tko*^{25t} mutation [207]. As many other mitochondrial DNA mutations, *tko*^{25t} also causes oxidative phosphorylation deficiencies with Complex I, III and IV showing reduced activation in larval stage [79][80]. Lack of sufficient synthesis of mitochondrial genome encoded genes by small subunit mitochondrial ribosome also causes developmental retardation. One leading hypothesis state that since reduced OXPHOS activity leads to decreased conversion of food source to ATP which then leads to delay in the fly development [207].

Another phenotype of *tko*^{25t} mutants is the bang sensitivity phenotype. Flies that were subjected to mechanical stress through vortexing for 10 seconds experience paralytic seizures and takes a recovery time around 15 seconds to 4 minutes to get back to their normal movement [80]. There is no mechanism uncovered for the reason of bang sensitivity phenotype, however other mutations that are not linked to mitochondria also show bang sensitivity phenotype [208]. Citrate synthase deficiency is shown as one of the mitochondrial mutations that causes bang sensitivity phenotype [209]. Interestingly Drosophila ortholog of ATP/ADP antiporter *sesB* mutants also show bang sensitivity phenotype [210]. This protein is essential in yeast after mtDNA deletion to generate membrane potential and import ATP from cytosol into mitochondria [5] so there occurs a link between reduced OXPHOS activity, ATP synthesis and electrochemical potential and bang sensitivity phenotype. Deficiency of ATP synthesis

through OXPHOS in mechanoreceptor neurons are hypothesized to be one reason of bang sensitivity phenotype [79]. Mechanoreceptor failure also causes hearing loss in *tko*^{25t} flies [211]. The male specific courtship deficiency and low behavioral response to stimulation by *tko*^{25t} flies yet remain to be uncharacterized [80].

I used mtDNA lacking yeast and *tko*^{25t} flies to investigate whether the downregulation of ribosomal biogenesis is beneficial for different mitochondrial disease models. I also aimed to find whether there is a connection between heat shock protein expression and cytosolic protein stress in mtDNA lacking cells. Here, I represent the data obtained from this project so far and describe the outline of my future directions.

4.2 Results for experiments with *Saccharomyces cerevisiae*

4.2.1 Increased ribosomal biogenesis and protein translation decreases the proliferation of ρ^- cells

The regulation of ribosomal biogenesis is maintained through acetylation and deacetylation of the histones that are wrapping the genes for ribosomal biogenesis. Acetylation of histones removes the positive charge on lysine residue and decreases the attraction of negatively charged DNA by positively charged histones [212]. DNA is loosened when histones are acetylated and the locus is enabled for transcription. Deacetylation converts the residue back into its positively charged form and enables tight compaction [213]. DNA is disabled for transcription with deacetylation of histones. Rpd3 complex is a histone deacetylase complex that is controlling ribosome biogenesis related protein expression [214]. In order to experiment on mitochondria with damaged DNA, we treated cells with 25 μg/ml EtBr for 2 days to force mtDNA loss [10][215]. RPD3 complex mutations induce expression of high number of ribosome and proteins; therefore generating more cytotoxicity. Rpd3p is a subunit of RPD3 complex and *rpd3*Δ is shown to decrease the fitness of ρ^- cells (Figure 4.1).

Upon activation via nutrient signaling TORC1 activates the expression of ribosomal protein genes [216]. TORC1 activation causes the localization of histone acetylation protein Esa1p to promoter locus for ribosomal protein genes, and rapamycin treatment or downregulation of TOR activity leads to blocking of Esa1p function [216]. The acetylated regions are deacetylated by complexes regulated by RPD3 [214]. Rpd3p forms two different RPD3 complexes, Rpd3L and Rpd3S respectively [217][214] and furnishes the deacetylation and converts the region to transcriptional inactive state. Deletion of Rpd3p leads to ribosomal protein synthesis even in the presence of rapamycin and reduced TOR activity [216]. In order to determine the effect of two different RPD3 complexes on ribosomal biogenesis, subunits of these two complexes were deleted. Rpd3L complex deacetylates histones at the gene coding locus [218] and Sds3p is the catalytic histone deacetylase subunit of the Rpd3L deacetylase complex [219]. Sds3p is required for the structural integrity and catalytic activity of the complex and it is involved in transcriptional silencing. *sds3*Δ mutants showed similar proliferation rate as *rpd3*Δ with a decrease of fitness for cells lacking mtDNA but unlike *rpd3*Δ, there is no change for growth for ρ^+ cells. Rpd3S complex deacetylates the histones at the promoter regions [220] and Rco1p is a specific and essential component of Rpd3S complex and is required for silencing by deacetylase activity [221]. Unlike *sds3*Δ, *rco1*Δ did not cause any growth deficiency for the ρ^- cells. We found that mutations of Rpd3L but not Rpd3S components showed growth defect in the ρ^- cells (Figure 4.1).

Aside from deacetylation complex, there are also other transcriptional repressors, *STB3*, *DOT6*, *TOD6* that control the expression of ribosomal biogenesis and protein translation rate. Deletion of these transcriptional repressors cause expression of genes to trigger upregulation of ribosomal biogenesis [222][223]. Deletion of *tod6*Δ but not *stb3*Δ nor *dot6*Δ decreased the fitness of ρ^- cells and interestingly *stb3*Δ increased the fitness of the ρ^- cells (Fig. 4.2). Double deletion of *tod6*Δ *dot6*Δ or triple deletion of *tod6*Δ *stb3*Δ *dot6*Δ did not show significant difference compared single *tod6*Δ deletion so the effects seen were not additive.

As pointed previously in Chapter 3, ρ^- cells have lower $\Delta\Psi$ [158]. Upregulation of ribosomal biogenesis leaded to upregulation of protein translation which caused more accumulation of proteins in cytoplasm due to low import [3]. In order to revert the $\Delta\Psi$, we transformed all cells with plasmids containing either wildtype *ATP3* (as a control) or dominant *ATP3-5* allele [224]. The *ATP3-5* mutation increased the $\Delta\Psi$ by increasing ATP hydrolysis of F_1 sector of ATP synthase [225]. Interestingly reversion of the $\Delta\Psi$ by *ATP3-5* allele prevented the growth deficiency of *tod6*Δ *dot6*Δ and *tod6*Δ *dot6*Δ *stb3*Δ ρ^- cells (Figure 4.3). There is also a minor increase in the proliferation rate of *stb3*Δ ρ^- cells. Increased electrochemical potential enabled higher import into the mitochondria thereby reduced the cytoplasmic accumulation of proteins.

4.2.2 Decreased ribosomal biogenesis with a deletion of transcriptional activator partially rescues petite-negativity but not due to the cell size phenotype of *sfp1* mutation

Transcription factor Sfp1p also regulates RP gene expression in response to nutrients and stress. Sfp1p is localized to the nucleus and is a positive regulator of ribosomal protein gene expression by binding to the promoters of ribosomal protein genes [124]. Inhibition of TOR signaling is shown to be beneficial for mitochondrial disease phenotype [156] and blocking of phosphatase activity downstream of Tap42p is important for the beneficial effects on mtDNA lacking cells [158]. Inhibition of TOR also leads to translocation of Sfp1p out of the nucleus, where it no longer binds to the promoters of ribosomal protein genes [124]. This leads to low expression of ribosomal proteins and causes downregulation in the protein expression. In this aspect, we knocked out *SFP1* gene in the background of an *atp2*Δ in order to see whether downregulation of the ribosomal biogenesis is the cause of TOR inhibition. In fact, *sfp1*Δ deletion partially rescued the lethality in *atp2*Δ ρ^- cells (Figure 4.4). However growth seen in *sfp1*Δ *atp2*Δ was not comparable to TOR inhibition as we saw in (Figure 3.2) since *sfp1*Δ grows very slowly.

The colony size of the *sfp1*Δ was small since the protein translation is not high in cytoplasm. We tried to decrease the colony size of yeast cells by deleting the PEST ((P) Proline, (E) Glutamic acid, (S) Serine, (T) Threonine) sequence in C terminus of *CLN3* gene. PEST sequence controls the proteolysis of the Cln3p [226] and when it is deleted it causes the stability of the Cln3p [227]. When the proteolysis of protein is blocked, the protein will constantly be active and drive the entry into G1 phase of the cell division. CLN3 expresses an important G1 cyclin that activates cyclin-dependent kinases which is one of the checkpoints for entry into G1 [228]. Constant entry into G1, thus constant cell division, causes decreased cell size [229]. The small cell size phenotype was not the cause of the rescue since *CLN3*Δ*PEST* did not rescue the *atp2*Δ ρ^- cells.

4.2.3 Cytotoxicity generated by amino acid analogs canavanine and AZC does not change the proliferation of mtDNA lacking cells

As a next step, I aimed to generate more misfolded proteins by using canavanine and azetidine 2-carboxylic acid (AZC). Canavanine is an amino acid analog with a structural shape that is very similar to L-arginine [230] and it generates cytotoxicity by incorporating into the arginine's locus in proteins [231]. Such incorporation disrupts the structural integrity of 3D shape of the protein which increases the number of misfolded proteins and generates more cytotoxicity [232]. Another amino acid analog AZC was also used to incorporate into the locations of proline to disrupt 3D shape of the protein to induce misfolding [233]. I aimed to induce this cytotoxicity by subjecting wildtype (WT) ρ^+ and mtDNA lacking, ρ^-, yeast to different concentrations of canavanine and AZC.

Already there were some accumulated mitochondrial proteins in the cytoplasm of ρ^- cells because of the import deficiency, so triggering more misfolded proteins by canavanine and AZC was expected to generate further growth defects. In both drug

treated and untreated cases ρ^- cells have higher doubling times compared to the ρ^+ cells, however the difference between drug treated ρ^- cells to ρ^+ cells is not drastic compared to untreated samples to conclude the presence of any severe effect of AZC or canavanine on mtDNA lacking cells (Table 4.1).

4.2.4 Decreasing the cytosolic protein translation or mitochondrial membrane potential couldn't recover the heat shock protein induction in mtDNA damaged cells

Heat hock protein (HSP) overexpression and activation is a main consequence of mitochondrial DNA damage in various organisms [8][234][235][236]. HSPs induction is a stress reponse to temperature change or oxidative stress and it is a conserved process and demonstrate protection of many proteins, cell membranes and nuclear DNA [8] . HSPs furnish chaperone activity for unfolded proteins and also used in localization of proteins to specific compartments [237]. Importantly, thermotolerance supplied by HSP activity protects the mitochondrial ATPase activity [238], and effects seen after dissipation of membrane potential are reduced by HSP induction [8] promising unknown interaction between heat shock proteins and mitochondria.

One of the heat shock proteins that is activated in mtDNA lacking cells is Hsp26p which is also induced in heat shocked log-phase yeast cultures [3][158][239]. However it is also highly expressed in cells that are grown at stationary-phase at normal temperature and show more thermotolerance to heat shock compared to log-phase cultures [240][241]. Hsp26 show various localizations in different organisms under different stress conditions with showing presence in cytoplasmic aggregates in chicken cells [242], nuclear localization during heat shock in yeast [239] and in Drosophila [243] however in separate studies it also showed a cytoplasmic localization in Drosophila [244]. Like Hsp26p, Hsp12p is also another heat shock protein that is highly induced in log-phase heat shocked and mtDNA lacking cells (Garipler and Dunn, unpublished results)[245]. Hsp12p expressed in an unfolded state but forms helical

structure in order to interact with membranes to increase thermotolerance and impinge on certain negatively charged lipids [245]. Previous transcriptome data showed that, unlike iron starvation genes and RTG pathway elements, Hsp12p and Hsp26p that are activated in the absence of mtDNA, does not go back to the wildtype state in the presence of petite-negativity rescuing mutations [158][158]. In order to find the cause of the increase in heat shock factors in cells with damaged mitochondrial genome, we investigated the status of by western blotting for Hsp12p and Hsp26p with a 13MYC epitope tag attached to these proteins.

Since heat shock factors activate when there is an unfolded proteins accumulate during heat shock or other stress [246], and since we know that the proteins with mitochondrial localization sequence localize to the cytoplasm [78] and hypothetically defold or misfold; we hypothesized that decrease in the cytoplasmic translation would decrease the cytotoxicity and thus the heat shock protein expression in mtDNA damaged cells. Yeast cells were grown in YEPD containing 0.2 µg/mL cycloheximide (CHX) to decrease the cytoplasmic translation. CHX blocks cytosolic translation by interfering with the translocation step in protein synthesis. In the process of protein synthesis tRNAs move from A-site to P site and to the E-site to finish the addition of the amino acid that they were carrying [247]. Cycloheximide blocks the movement of tRNA molecules which leads to blocking of translation. CHX is used in many other studies as protein synthesis inhibitor. However CHX triggered an induction of Hsp12p and Hsp26p, even in ρ^+ cells (Figure 4.5). Cells showed a stress response to CHX or to some other consequence of protein translation inhibition to trigger HSP expression.

Next, we tried to increase the mitochondrial electrochemical potential by transforming *ATP3-5* allele containing plasmid into *HSP12-MYC* and *HSP26-MYC* strains. Previously we showed that cells carrying *ATP3-5* allele but not *ATP3* wildtype copy, can localize proteins with mitochondrial localization presequence to mitochondria even in ρ^- conditions [78]. Our aim was to recover the import defects of ρ^- cells to decrease the cytotoxicity. However the plasmid transformation required the cell to be grown under specific media lacking the amino acid that the plasmid was carrying.

Minimal media conditions affected the heat shock protein induction with no bands visualized from the extracts of ρ^- cells (Figure 4.6). The rich medium, YEPD, leads to higher protein production and generates more cytotoxicity when unfolded proteins accumulate in the cytoplasm. However when cells were grown under minimal media conditions, reduced protein translation benefits cells with mtDNA damage. Currently we are cloning the plasmid *ATP3* and *ATP3-5* into the yeast genomic DNA. Chromosomal *ATP3-5* will generate enough $\Delta\Psi$ for mitochondrial import and allow the usage of rich media for future experimentation.

Next, we wanted to impair the $\Delta\Psi$ by feeding CCCP to the yeast cells. CCCP dissipates the membrane potential by carrying protons into the negatively charged inner membrane [248]. However 20 mM CCCP did not cause significant increase for the Hsp12p, yet slightly increased the Hsp26p in ρ^- cells (Figure 4.7). However there was no increase in ρ^+ cells when they were grown in YEPD containing CCCP. Mitochondrial potential itself was not the cause of heat shock protein induction, as no wildtype ρ^+ cells were expressing similar levels of heat shock proteins as ρ^- cells (Figure 4.7).

4.2.5 Heat shock factors start to accumulate in 2nd day of liquid culture and then start to adapt to the conditions in 3rd day

Previous western blots were done on culturing the cells to YEPD after 2d treatment on YEPD+EtBr plate. We wanted to switch to a system to grow the cells for 24 hour periods until the log phase. For ρ^- cells, we grew them until the log phase for 24 hours in liquid YEPD containing 25 µg/mL EtBr. For second day the log phase culture was diluted again and grown in YEPD and same process was repeated for 3rd day. The timeline of HSP induction was important to determine since it is depending on accumulation of some target protein(s). We found that the second day is the peak expression of Hsp26p (Figure 4.8) and for the 3rd day the cell goes under adaptation or selection in culture. These experiments were performed in BY background and

experiments will be repeated in W303 background to determine whether there are any adaptation differences between the cells. Also day 4, 5, 6, 7 and 8 will be performed in cells with less Hsp26p expression to determine whether adaptation remains for upcoming days.

4.2.6 Levels of other heat shock proteins or AMP activated protein kinase remain stable in mtDNA damaged cells

Previous data showed that Hsp12p and Hsp26p were highly expressed in ρ^- cells, and we wanted to check other heat shock proteins. *HSP104* expresses a disaggregase which interferes with protein aggregates and also is activated in stress conditions such as heat, ethanol and radiation [249][250]. *SSA3* encodes a chaperone protein that is a member of a subfamily of HSP70 protein [251] and *SNF1* is discussed in previous chapter and there is no evidence showing an overexpression in ρ^- cells or in heat shock conditions. We have found that levels of both Hsp104p and Ssa3p but not Snf1p is highly activated in heat shock conditions (Figure 4.9) however the levels of Hsp104p and Ssa3p were only slightly affected in ρ^- cells compared to Hsp12p and Hsp26p. Snf1p is used as a control to show that it is constant in all conditions.

4.2.7 Deletion of transcriptional activators partially blocked the heat shock protein expression in mtDNA damaged cells

We deleted *MSN2* and *MSN4* in the background of *HSP12-MYC* and *HSP26-MYC* in order to determine whether the levels go down in ρ^- cells. Msn2p and Msn4p functions as a transcriptional activator for over 200 genes in the presence of a stress condition, such as heat shock, oxidative stress, ethanol and low pH [252][253]. *HSP104* and *MSN4* gene itself is also under control of *MSN2* and *MSN4* genes [254]. We also deleted *GIS1* in the background of *HSP12-MYC* and *HSP26-MYC*, which is another

transcriptional factor that is activated during heat shock and glucose starvation conditions [255][256]. Since it is an essential gene we did not delete *HSF1,* which is another transcriptional activator under heat shock conditions [257]. The truncated versions that were used in [258] were also inviable in our backgrounds and we are now currently constructing our own version.

Deletion of *MSN2*/*MSN4* completely blocked the expression of Hsp12p even in ρ^+ cells with 1 hour 42^{o}C heat shock (Figure 4.10). However *gis1*Δ did not alter the amount of Hsp12p in ρ^- cells nor in ρ^+ cells with 1h heat shock. Hsp26p still highly expressed in ρ^- cells in the presence of *msn2*Δ *msn4*Δ however the Hsp12p expression in wildtype ρ^- or ρ^+ HS was higher. *gis1*Δ also did not alter the amount of Hsp26p in ρ^- cells nor in ρ^+ cells with 1h heat shock (Figure 4.10).

4.2.8 ATP deficiency in ρ$^+$ cells showed significant Hsp26p expression

Deletion of mitochondrial DNA halts the respiration and the ATP synthesis through TCA cycle. We tried to mimic this condition only through deleting the $F_1\beta$ subunit of ATP synthase and blocking the production of ATP in mitochondria. *atp2*Δ cells will go under ATP deficiency since ATP is supplied only through cytoplasmic glycolysis as the conditions of ρ^- cells. *atp2*Δ did not significantly increased the Hsp12p levels in ρ^+ cells however the expression level in Hsp26p ρ^+ cells were similar to wildtype ρ^- cells (Figure 4.11).

4.3 Results for experiments with *Drosophila melanogaster*

4.3.1 Developmental time assay for *tko*^{25t} flies showed decreased average eclosion day for CHX fed flies

We also wanted to determine the effects of ribosomal biogenesis using a disease model in a higher eukaryote. We used developmental time assay which is based on the average eclosion day to determine the phenotype of *tko*^{25t} mutants since they are

eclosing with a delay of 3-4 days from the pupa [80]. Eclosion date shows the metamorphosis of egg cell to larvae to pupa and then to the eclosion of fly and used to determine the fitness of the fly under different conditions [79]. 1 day old 30 virgin females and 1 day old 15 males are crossed to each other for 24 hours in one vial and then transferred into 2nd vial for another 24 hour and then done again for a 3rd and 4th times. After spending 96 hours in 4 different vials, flies are discarded. After this point eclosion of flies are tracked with noting the amount of eclosion everyday for each vial until the last fly ecloses. Each eclosed fly must be discarded after it ecloses since it may again copulate inside the vial. Always there will be larval death with uneclosed pupils, so the number of enclosures (n) is important for each given drug concentration. The fly wildtype background, Oregon-R (OR) is used as control for the experiments and the average eclosion day is found as 9.0 for females and 9.2 for males. However *tko*^{25t} mutant females and males have average eclosion days of 12.7 and 13.3 days respectively (Table 4.2). Since *tko*^{25t} is on the X chromosome, homozygous mutants are selected for female flies; however there are also female heterozygous mutants with FM7 balancer in the homolog chromosome. These flies have an average eclosion day of 8.6 that is similar to OR flies.

The developmentally delayed *tko*^{25t} flies partially lose their translation abilities for mitochondrial genes and demonstrate impaired OXPHOS machinery [80]. This is a similar approach to mtDNA deleted yeast since in both there is an impaired mitochondrial protein translation. The developmental delay *tko*^{25t} flies can be due to the cytotoxicity, hence decreased protein production may alleviate the eclosion timeline. Cycloheximide is placed in the fly media with differential concentrations in order to reduce the amount of protein translation. CHX containing fly media is prepared containing 50 and 250 µg/mL for the first phase. Intriguingly, average day of eclosion for *tko*^{25t}/*tko*^{25t} and *tko*^{25t} /Y flies changed from 12.7 and 13.3 to 11.8 (n=77) and 12.4 (n=64) in 50 µg/mL CHX containing fly media. On contrary wildtype flies showed increasing average day of eclosion for females and males from 9.0 and 9.2 to 10.0 (n=162) and 9.8 (n=162) respectively in 50 µg/mL CHX. For *tko*^{25t} /*FM7*, there is also

an increase from 8.6 to 9.4 (n=95) (Table 4.2) with 50 μg/mL CHX. 250 μg/mL CHX containing fly media was almost lethal even for the wildtype flies with only 2 eclosion for females and 5 for males. However tko^{25t} flies had more eclosion (12 for females and 7 for males) in CHX compared to OR.

Secondary experiment is performed with different set of concentrations: 25, 75 and 125 μg/mL CHX. For each concentration there was a half-day decrease of average day of eclosion in the CHX containing media (Table 4.3). However, wildtype flies have half to two days of eclosion delay in different CHX concentrations. CHX concentration can be further optimized with more developmental time assays however the range that I have found can decrease the developmental time delay of *tko25t* mutants by a half day and severely affected the wildtype flies. This experiments were repeated (Kemppainen, unpublished) several times however a beneficial effect was not always obtained when tko^{25t} flies were subjected to CHX. However a reproducible data obtained which shows a resistance for tko^{25t} flies against CHX and also a growth delay obtained from wildtype flies whenever they were treated with CHX (Kemppainen, unpublished).

4.3.2 CHX fed *tko25t* fly paralysis showed partial suppression however the bang sensitivity was not alleviated to wildtype level

Aside from developmental delay, a main signature of tko^{25t} mutants is bang sensitivity [79]. Flies go under paralysis after a brief vibration, mainly through a vortex. This is mainly caused by failure of mechanoreceptor neurons [81]. Such failure is also seen in humans since the whole hearing process depend on the mechanoreceptor neurons that are located in inner ear [80].

Although wildtype flies have almost 2 to 5 second recovery time, *tko25t* flies show ranging bang sensitivities from 10 seconds to 4 minutes of recovery time (Table 4.4). This was also a known data from previous papers [80]. My aim was to determine whether decrease of the developmental delay by CHX uptake would correlate with decrease of bang sensitivity of tko^{25t} flies. The bang sensitivities of tko^{25t} flies decreased 28-52% in all CHX concentrations except for tko^{25t} / tko^{25t} females in 25 μg/mL CHX

which had a partial increase of bang sensitivity for homozygous *tko*^{25t} females. Although there was a partial decrease in the bang sensitivity of *tko*^{25t} mutants, the recovery time was still higher than CHX untreated OR and treated OR.

4.3.3 *minute* mutants were aimed to cross to *tko*^{25t} background to decrease ribosomal biogenesis

Minute mutants were identified 81 years ago by their unique phenotype with having eclosion delay, thick and small bristles, roughen eyes, reduced viability and fertility [259]. Also these minute mutations are commonly homozygous lethal. There are more than 50 genes whose mutations were identified to give a minute phenotype and these genes are all related with ribosomal protein or biogenesis [260]. The minute mutations also have reduced cell size due to their lack of proper ribosomal biogenesis and thus the protein translation machinery [261]. Our aim was to cross these minute mutations into the *tko*^{25t} background to determine whether impaired cytosolic protein translation would benefit flies with impaired mitochondrial protein translation. We picked 4 genes, *RpS3, RpS3A, RpL36* and *RpLP1* whose mutations yield to minute phenotype.

Rps3, Ribosomal protein S3, is a structural constituent of ribosome and it also has RNA binding function along with DNA-(apurinic or apyrimidinic site) lyase activity. Also it is involved in many cellular processes such as translation, mitosis and DNA repair [262][263]. Its mutation gives a strong minute phenotype with at least 2-3 days of developmental delay [264]. RpLP1, Ribosomal protein LP1, is also a structural constituent of ribosome but it is involved in the elongation step of protein synthesis [265]. Mutation of RpLP1 also gives a strong minute phenotype [266]. RpL36, Ribosomal protein L36e, is also a structural constituent of ribosome [267]. It is only known to be involved in translation and it is less characterized compared to other picked minutes but it has more severe minute phenotype than others with having the

least viability among all [262]. Also its eclosion is delayed by 3-4 days [264]. Rps3A is also another structural constituent of ribosome. It has involved in many cellular processes such as translation, oogenesis, mitotic spindle elongation and organization [268][269]. It has a medium minute phenotype with 2-3 day of developmental delay [264]. Homozygous mutants are lethal at embryonic stage since oogenesis role is essential for embryonic stage [270].

Drosophila genome has 4 chromosomes and unlike yeast, it is hard to manipulate the genome. Generating a mutation in fly background is not easy so usually the mutations are ordered from the sources such as Bloomington, FlyORF, VDRC and Kyoto. Minutes in this project are all ordered from Bloomington Stock Center (Table 4.6). In order to generate a double mutant minute and tko^{25t} in same background, flies had to be crossed to each other. The crossing scheme for RpLP1 mutant 6399, $RpLP1^{beo}$ is given in Figure 4.12 which is on the 2nd chromosome. The crossing scheme for three different RpS3 alleles, 527 ($RpS3^{1}$), 544 ($RpS3^{4}$), 1696 ($RpS3^{2}$), is given in Figure 4.13. RpS3 is located on 3rd chromosome. RpL36 is on the same chromosome with tko^{25t} and RpS3A is on the 4th chromosome (the smallest chromosome in Drosophila genome). These two genes weren't crossed to the tko^{25t} background, and only used in qPCR analyses.

4.3.4 Minutes couldn't recover the developmental delay caused by doxycyclin and are synthetically lethal with cycloheximide

Doxycyclin (DOX) hinders the mitochondrial translation and mimics the phenotype of tko^{25t} flies [80]. As a preliminary experiment, we wanted to observe whether minute mutations reduce the developmental delay for DOX-fed flies. $RpLP1^{beo}$ female and male flies are fed with 100 µg/mL and 500 µg/mL doxycyclin are tested for developmental delay. Results showed that minutes cannot recover the developmental delay caused by doxycyclin (Table 4.2). Untreated $RpLP1^{beo}$ flies had 1.2-1.4 day more

delay compared to Oregon-R, wildtype flies. However 100 µg/mL and 500 µg/mL doxycyclin fed minutes showed 3-4 days of delay compared DOX fed OR flies. Unfortunately, there was no rescue of doxycyclin phenotype by presence of a *minute* mutation and in fact, the *minute* background is more severely affected by doxycyclin than the wildtype background. However the affected background still had high viability with 70-80 eclosions even in the samples containing drugs.

As a side experiment we wanted to know how the minute flies respond to the cycloheximide conditions. The same concentrations of cycloheximide given to the tko^{25t} flies in the first experiment were also given to the minutes. $RpLP1^{beo}$ is found to be sensitive to the cycloheximide with showing zero eclosion in the presence of CHX (Table 4.2). Also, there was no larvae generation in the vials and further investigations can be done to analyze this synthetic interaction.

4.3.5 qPCR for various genes for OR, tko^{25t} and minute female, male and larvae was done to characterize the overexpression of six genes for future studies

The drastic changes in transcriptome of *Saccharomyces cerevisiae* were shown in Chapter 3 and such change was also seen in tko^{25t} background for different genes. Mitochondrial translation machinery malfunction in tko^{25t} background results overproduction of mitochondrial heat shock factor Hsp22 [271][272]. This protein is also shown to be highly abundant in aged Drosophila cells [273]. Although there is no mechanism showing the actual reason of upregulation of Hsp22, one explanation to upregulation can be the regulation of mitochondrial unfolded protein response to protect the mitochondria that is already facing translational defects [274]. Also impaired OXPHOS machinery or mitochondrial translation can remotely cause overexpression of Hsp22 [272][275]. Other evidence also shows that mitochondrial disease model in yeast requires chaperone activity to proliferate under impaired electrochemical potential conditions [197].

Insulin, insulin receptor (InR) and insulin-like peptides and growth factors determine the development of the organism with managing the disc growth and controlling organ and organism size [276][277]. Disc growth is essential for Drosophila since discs are hubs where the development of wing, antenna and legs of fly happen in larval stage [277]. It was important to determine the levels of insulin receptor (InR) and insulin-like peptides (Ilp3, Ilp5 and Ilp8) in tko^{25t} background since development of the fly is delayed and this can be observed in expression of InR and Ilps. Also future cross of minutes into tko^{25t} background may alter the expression of InR and Ilps if it is rescuing the developmental delay of tko^{25t} flies since there is high correlation between lifepsn extension and insulin-like growth factor signaling and ageing of Drosophila, C. elegans and human [278].

Thor is the Drosophila ortholog of S6K which regulates the ribosomal biogenesis under activation through TORC1 [279]. Thor expression is not altered in tko^{25t} flies [271]. However since we are also using *minutes* which have impaired cytosolic translation, tracking gene expression of S6K ortholog effecting ribosomal biogenesis is important and can be used in future experiments with tko^{25t} *minute* double mutants.

Expression of 6 different genes mentioned above tracked with measuring their mRNA expression in wildtype, tko^{25t} and *minutes* in different developmental stage and sexes. Hsp22, Thor, Ilp3, Ilp5, Ilp8 and InR were picked as the genes to be compared between the backgrounds. The main aim was to analyze whether we can decrease the effects seen in tko^{25t} with crossing the minutes into the tko^{25t} background. However this remained as a future direction since crosses seen in Figure 4.12 and 4.13 were not finished.

In this study we only characterized and compared the different backgrounds with each other. For Hsp22 gene, I found that tko^{25t}, $RpS3^{4}$, and *RpS3A57g* mRNA level were highly amplified in females and males, but was not accumulated in the larvae (Table 4.5). This is maybe due to high requirement for mitochondrial function in adult flies compared to larvae and expression of Hsp22 gene is ameliorating the impaired

OXPHOS and mitochondrial translation in *tko*^{25t} flies. Another minute allele of *RpS3*^{2} does not activate *HSP22* gene with showing 25% reduction compared to wildtype. Thor expression decreases around 20% for the *minutes* compared to wildtype but reaches to 1.5-2 fold for *tko*^{25t} flies. Ilp3, Ilp5, Ilp8 does not show significant variance between the samples except for larval overexpression of Ilp8 mRNA for *RpS3*^{2}, *RpS3A*^{57}*g* and *RpLP1*^{beo} which is increased by 5.12, 13.6, 32.0 fold of wildtype. Future qPCR results for *minute tko*^{25t} double mutants will show whether gene amplification changes to wildtype state or partially decreases to wildtype state.

4.4 Discussion

4.4.1 Decreased ribosomal biogenesis or increased mitochondrial potential was beneficial for mtDNA damaged cells

In chapter 3, downregulation of pKA and TORC1 is found to increase the fitness of cells lacking mtDNA. This may be due to the activation of ribosomal biogenesis and inactivation of transcriptional repressors Dot6p and Tod6p by TORC1 and PKA [280]. So deletion of both PKA and TORC1, aside from other possible rescue by parallel mechanism, can decrease the ribosomal biogenesis and protein synthesis via inability to inactivate transcriptional repressors. Downregulation of cytosolic protein synthesis reduced mitochondria degeneration phenotype [197]. This is also seen in Kluyveromyces lactis which can only survive in mtDNA depleted conditions by deletion of locus expressing ribosomal DNA [281]. Unimported precursor of nuclear encoded mitochondrial proteins localize to cytoplasm [3][78][158]. Aside from the electrochemical potential reduction in mtDNA lacking cells, one other theory is that unimported proteins localize to the mitochondrial inter membrane space but cannot be imported into the mitochondria and thus creating a membrane crowding [5][197]. This can explain the petite-negativity phenotype of mutant *yme1*, which is responsible for the quality control and degradation of proteins, and also the need for chaperone activity of prohibitins in the mitochondrial disease model *aac2A128P* [197]. Additionally mutation

of a gene responsible for synthesis of anionic phospholipids *PGS1,* also causes petite-negative phenotype [5]. Pgs1p mutation causes loss of functional integrity and structural deficiencies which leads to lethality [64].

The protein accumulations in the membrane can be enhanced with increasing ribosomal biogenesis by deletion of deacetylation complex Rpd3L which leads to ribosomal protein expression. Blocking of TOR activity by rapamycin deactivates ribosomal biogenesis by inhibiting Esa1p activity and upregulating RPD3 complex activity [216]. Conversely, deletion of transcriptional activator Sfp1 showed beneficial effects on cell lacking mtDNA and partially rescued the petite-negative phenotype. Proliferation defects in cells lacking deacetylation complex and fitness enhancement by deletion of ribosomal biogenesis transcriptional activator is consistent with the cytoplasmic accumulation and membrane crowding theory.

We tried to visualize aggregates with ThioflavinS, however we saw no aggregate formation in ρ^- cells. However ThioflavinS was only targeting specific aggregates called prions so a broader approach to purify all aggregates was used. We tried to purify aggregates as in [282], from ρ^+ and ρ^- cells and found more aggregates in ρ^- cells (Dunn, unpublished). As we will continue to investigate the total amount and components of the aggregates as a future goal, a question remains about how to revert this phenotype to wildtype state? One possible rescue for this proliferation deficiency can be found in mitochondrial potential since introduction of a mutated version of gamma subunit of ATP synthase generates higher $\Delta\Psi^{mito}$ and benefits ρ^- cells.

4.4.2 Heat shock proteins were highly produced in the presence of mitochondrial DNA damage as a defense mechanism

As a response to the mitochondrial DNA damage, the heat shock proteins are highly activated. Since their main aim was to perform chaperone activity, the proteostatis of ρ^- cells should be under jeopardy to cause such amplification in HSP levels. Reduction of chaperone levels in minimal media conditions and in increased

mitochondrial import conditions support this hypothesis. Matrix targeted mitochondrial proteins are associating with Hsp70p after they are translated in cytoplasm [237] and localization of these proteins to cytoplasm may also localize the Hsp70p to cytoplasm. Since cytoplasmic translation continues to proceed, there occurs a need for Hsp70p for associating with the precursor proteins. Transcriptional activation may play a role with driving the HSP expression and transcriptional activators may also express other HSPs, Hsp12p and Hsp26p, encoded in the genome since transcriptional activators are responsible for wide range of HSPs [252][258]. It is important to find the transcriptional drivers of the heat shock proteins in mtDNA damage conditions for potential therapeutic approaches. We deleted *MSN2* and *MSN4* and also *GIS1* in different strains containing Hsp12p-Myc and Hsp26p-Myc to investigate the potential transcriptional activator of the heat shock proteins. However did not recover full depletion of Hsp12p or Hsp26p in cells lacking mtDNA. We will continue to investigate this approach since other transcriptional activators like *HSF1* and *HAP4* are also important in chaperone production [258].

Also the interaction between Hsp70p and precursor proteins may be abolished which would generate an unfolded protein in the cytoplasm and require the presence of other HSPs to maintain chaperone activity. This can also lead to Hsp12p and Hsp26 overexpression. Furthermore oxidative stress shown in mitochondrial disease create transcriptional response along with showing responses to various other stresses [245][246]. Decreased electrochemical potential can drive HSP expression as a stress response or membrane crowding caused by low import due to low $\Delta\Psi^{mito}$ can overexpress Hsp12p which is a membrane bound heat shock protein [245].

ATPase activity is also crucial for mtDNA lacking cells with *atp2*Δ ρ^- cells showing petite-negative phenotype [5]. Heat shock activity is upregulated to protect ATPase activity in mtDNA lacking cells [8] and deletion of specific heat shock proteins caused lethality in oligomycin treated mtDNA lacking cells. This demonstrates a precautionary role for protecting the essential subunits after the mtDNA loss.

However the reason for HSP amplification can totally be different than what we are hypothesizing. ρ^- cells also go under ethanol stress and HSP26 is important for ethanol tolerance in Drosophila [283][250]. Yeast is used in making beer since it creates and secretes ethanol to the culture that it is growing in. Decreased respiration with impaired TCA cycle in ρ^- cells increases the ethanol levels in the cell and in the media. Preliminary experiments were done in our lab that showed increased Hsp26p production in media with increasing ethanol levels (Akdoğan, unpublished results) but further experiments showed no correlation between Hsp26p expression and ρ^- culture density. Although Hsp26p goes up in ethanol, we are still uncertain about whether that is the actual reason for expression in the presence of a mtDNA damage.

4.4.3 Decreased cytoplasmic translation is maybe beneficial for Drosophila mitochondrial mutant and partially alleviates the bang sensitivity

Aside from yeast studies the same hypothesis was made on Drosophila *tko*^{25t} mutant about cytotoxicity. The decreased protein translation via CHX fed *tko*^{25t} mutants gave promising results, and it was repeated 10 times by Howard T. Jacobs' lab, with showing no delay or half day eclosion for *tko*^{25t} mutant compared to 2-3 day eclosion delay for OR flies. The *tko*^{25t} mutants showed resistance against CHX however it is causing developmental delay for the OR flies. The one possibility is that PDR (pleiotropic drug resistance) genes are hyperactivated in mtDNA damage conditions [284] and this may lead to pumping of this drug out of the Drosophila cells. However compared to resistance, the beneficial effects are also seen with 11th day eclosions for *tko*^{25t} mutant when they are treated with CHX. This is unusual since we do not see any untreated *tko*^{25t} mutant eclosions at 11th day. Possibly, there can be a balance between reduced cytoplasmic translation and impaired mitochondrial translation which may in fact work in favor of the organism with a better stoichiometric balance.

Additionally CHX treatment can make a selection for flies with higher levels of mitochondrial RNA translation due to higher efficiency of the components or

mitochondrial DNA copy number. This will work in favor for the cells or organism with higher mitochondrial DNA copy numbers. Conversely, it will select against the ones that already have low copy mtDNA and further delayed. Selective killing of these low copy mtDNA carrying organism will subtract 14^{th}-16^{th} day eclosions from the average eclosion day and will give a statistically incorrect outcome.

However beneficial effects of the CHX treatment are also seen in the bang sensitivity phenotype. Treating CHX to Drosophila containing ATP/ADP antiporter mutation *sesB* mutants -which also have a bang sensitivity phenotype- can be a future direction in order to understand whether CHX is beneficial for general mitochondrial disease or only benefits the stoichiometry between nuclear and mitochondrial encoded subunits.

Unfortunately I never had the chance to cross *minute* mutants into the tko^{25t} background and the developmental timing of tko^{25t} *minute* double mutants remains as a question. Also the bang sensitivity and the mRNA levels of the stress genes in tko^{25t} mutant may go back or partially alleviated by the *minute* mutations.

4.5 Materials and Methods

4.5.1 *Saccharomyces cerevisiae* Media conditions and Strains: Yeast media were prepared as described previously [285]. The genes were manipulated according to [286]. Yeast strain genotypes and construction details are listed in Table 4.8. The primers used in yeast studies in Part II are listed in Table 4.9. EtBr (Thermo Fisher Scientific) was used at 25 µg/mL for serial dilution experiments [10]. Serial dilution assays were performed by logarithmic phase of each culture to OD600 of 0.1 followed by serially diluting the initial concentration to three additional five fold dilutions. 4 µL of each dilution were spotted on YEPD plates. Each background then tested on YEPD for their proliferation rates at 30^{o}C. Incubation time is 3 d for (ρ^{+}) and 4 d for (ρ^{-}), if not stated in figure legends differently, before plates were imaged using a Colony Doc-It system (UVP, Upland, CA).

4.5.2 Plasmids: Strains CDD567, CDD575 and CDD576 were transformed with pM487 (ATP3) or pM488 (ATP3-5) [225][194].

4.5.3 Proliferation assay for yeast: In order to obtain ρ^- cells, ρ^+ cells were streaked to YEPD + 25 µg/mL EtBr for 2d at 30°C. The cells were then cultured in YEPD and were grown overnight. Overnight cultures were diluted to an OD_{600} of 0.05 in YEPD medium containing different drug concentrations. The OD_{600} of samples are measured every two hours by a Mecasys Optizen POP UV-VIS spectrophotometer (Mecasys Co. Ltd., Daejeon, South Korea). Four to seven measurements in eight to fourteen hours were taken in two hour intervals. When the OD_{600} reached above 1.00, the measurements were terminated. Doubling time is calculated as the reciprocal of the slope for the graph which has X-axis values as log2 of OD_{600} measurements and Y-axis values as the minutes. The slope is calculated via Microsoft Excel 2007 (Microsoft Corp., Redmond, WA).

4.5.4 Growth conditions prior to protein extraction: For the solid medium followed by liquid medium approach, ρ^+ cells were grown on YEPD containing 25 µg/mL EtBr in order to obtain ρ^- cells. After 2 days on EtBr containing medium at 30°C, a streak of cells from EtBr plate was cultured into YEPD to grow overnight at 30°C. Then a diluted culture started from that overnight liquid culture into YEPD to perform protein extraction as soon as it reached to log phase (OD600 between 0.4-1.00). Liquid medium approach was intended to obtain more standardization since amount of streaked cells, the uptake of EtBr into the cells during 2 day period and reaching to a saturated overnight state were mixing unknowns into the experimentation. In secondary methodology, a freshly grown wildtype culture was diluted to OD600 around 10^{-6}-10^{-5} cells, in 5 mL YEPD containing 25 µg/mL EtBr for exactly 24 hours for every strain. After 24 hours, the OD600 arranged to $2x10^{-2}$-$5x10^{-4}$ in YEPD for growing another 24 hours until it reaches to the log phase to perform protein extraction.

4.5.5 Protein extraction and BCA assay: Protein extraction methodology is based on [287][288]. Cells grown at 5 mL cultures in glass tubes were dipped in 98^{o}C preheated water bath (WiseCircu, Wisd Laboratory Instruments). Tubes were swirled in water bath for 3 min and then left to cool to room temperature. After brief vortex to resuspend precipitated cells during cool down, cells were transferred to 15 mL conical to be centrifuged at 4.000 g (Allegra X-22R Centrifuge, Beckman Coulter) for 4 min. Supernatant is aspirated and pellet is resuspended in 200 µL 1xTE at pH 8.0 and transferred to a 1.5 mL microfuge tube. 200 µL 0.2M NaOH was added. Tube was then vortexed, incubated at room temperature for 5 min and then centrifuged at 10.000 g (Microfuge 22R centrifuge, Beckman Coulter) for 1 min. For Figures 4.5 and 4.6, the pellet was resuspended in the amount of [volume (5 mL) times 30 µL times OD600 unit of cells] 1xSDS-PAGE sample buffer. The tube is then boiled at 98^{o}C at heater (The Cube Dry Bath, Cleaver Scientific Ltd) and then centrifuged at 10.000g for 5 min. 20-30 µL was added for the gel.

For Figures 4.7 - 4.11, the pellet was resuspended in 100 µL 5% SDS in order to perform BCA assay. After 5% SDS was added and the tube is boiled at 98^{o}C at heater and then centrifuged at 10.000g for 5 min. 2 µL of supernatant was added on top of 1 mL Working Reagent (WR) (Reagent A:Reagent B (50:1)) (Pierce BCA Protein Assay Kit, Thermo Scientific). This solution is then vortexed and mixed for 20-25 min at 30^{o}C heater (Thermomixer comfort, Eppendorf). Also 1 mg/mL BSA, 5% SDS solution is prepared and 1, 2, 4, 8 and 10 µL of this solution was added to separate tubes containing same WR in order to calculate the unknown concentration of the extracted protein samples according to the linear regression plot calculated through known BSA concentrations. After 20-25 min at 30^{o}C, OD562 of the cultures were measured and samples were diluted with 5% SDS to the sample of lowest concentration. This solution is then mixed with 1:1 ratio with 2x SDS sample buffer and 20-30 µL was added for the gel.

4.5.6 Western Blotting: For big gels 20 mL separating gel (6.60 mL 30% Acrylamide-Bisacrylamide (29:1) (Sigma Life Sciences), 7.50 mL 1.0 M Tris-HCl (pH8.8), 200 µL 10% APS (in ddH_2O) (Sigma Life Sciences), 10 µL TEMED (Sigma Life Sciences), 5.69 mL ddH_2O) was poured into the plates and n-butanol was used to seal the top in order to avoid formation of bubbles. 10 mL stacking gel (1.7 mL 30% Acrylamide-Bisacrylamide (29:1), 1.26 mL 1.0M Tris-HCl (pH6.8), 100 µL 10% APS (in ddH_2O), 10 µL TEMED, 6.94 mL ddH_2O) was poured after the n-butanol was removed with a tissue paper. After the solidification 1xSDS Running Buffer (3.02 g Tris Base (Sigma Life Sciences), 14.4 g glycine (BioShop), 1 g SDS (Sigma Life Sciences), qs to 1 L ddH_2O) is prepared, 30 µL of protein extraction was loaded into the gels and run at 240V 70A for 2-3 hours. The proteins in the gel were then transferred onto a PVDF (Thermo Scientific) paper at a wet transfer machine in 1xTransfer Buffer (3.03 g Tris Base, 14.4 g glycine, 100 mL 100% Methanol (VWR), qs to 1L ddH_2O) or in semi dry blotting. When the transfer is complete, PVDF was hanged with a clip to dry. After it was dried, it was re-wet with 100% Methanol, and washed 3 times with ddH_2O. Ponceau S solution (Sigma Life Sciences) is used for visualizing the transfer and loading/concentration efficiency and then washed for 3 times. PVDF is then placed in 15 mL blocking solution for 3 times (0.75 g Nonfat dried milk powder (AppliChem), qs to 15 mL with TBST)**,** for 1h at room temperature. Primary antibody solution (0.15 g Nonfat dried milk powder, 15 mL TBST, primary antibody), is prepared with following antibodies (Table 4.10) for 2 hours at room temperature or overnight at 4^oC. After primary antibody, the PVDF was washed 3 times 10 min with TBST and then secondary antibody solution (0.15 g Nonfat dried milk powder, 15 mL TBST, primary antibody), is prepared with the following antibodies (Table 4.10). After two times 10 min washes with TBST and one time 10 min wash with TBS, PVDF is then exposed to the ECL reagent SuperSignal West Pico Chemiluminescent or SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific), and visualized under the Gel Logic 2200 Imaging System (Carestream Health) for 5 minutes or until saturation unless it is written differently in figure legends.

4.5.7 Drosophila melanogaster media conditions and strains: *tko*^{25t} and Oregon-R flies were kept at 25^{o}C as a balanced stock with FM7 for *tko*^{25t}. *minutes* were ordered from Bloomington Stock Center. Minute stocks were given in Table 4.6. In order to avoid Wolbachia infection, they were kept at quarantine for 3-4 weeks in order to avoid contamination to other fly backgrounds [289]. Wolbachia infection is mutualistic for flies since it increases the lifespan and reproducibility of the flies [290]. Moreover, it also increases the cytosolic protein expression levels, it was crucial to avoid such infection at the initial quarantine stages. After spending 3-4 generations in quarantine, minutes were then started to be crossed into *tko*^{25t} background as shown in Figure 4.12 and Figure 4.13.

Flies were maintained in fly media containing 1.5% sucrose (Merck), 3% glucose (Sigma), 3.5% Instant Dry Baker's Yeast (European), 1.5% maize flour (Oriola), 1% soya flour (Oriola), 1% agar (Oriola) and 3% Lyle's black treacle (Tate & Lyle), 0,1% methyl 4-hydroxybenzoate (Sigma) and 0.5% propionic acid (JT Baker) [291]. Differential amount of cycloheximide and doxycyclin were added to the fly media while it was still in liquid conditions and kept at room temperature to dry. All of the experiments were done in 25^{o}C incubator on a 12 hour light/12 hour dark cycle.

4.5.8 Developmental Time Assays: 30 virgin female and 15 males were picked from each given genotype and placed in a bottle containing different fly food for 24 hours. After 24 hours of copulating and laying eggs, these flies were then transferred into the next bottle containing same fly media conditions for another 24 hours and same process continues for 2 more times to yield 4 bottles. At the end of 4th day, flies were taken away and eclosion time for each fly is noted until the last fly ecloses.

4.5.9 Bang Sensitivity Assays: Bang sensitivity assay was performed one fly at a time so after a group of flies with same genotype were collected on CO_2 pad, flies were placed into different glass vials. After they were taken away from CO_2, 1 hour of

recovery time is given to the fly in order to lose the carbon dioxide that was taken into the system. After 1 hour in 25^{o}C, each fly was vortexed for 10 seconds. Flies were then flipped upside-down with their backs against the bottom glasss wall. The recovery time measured for a fly until it flips back and maintains proper mobility. This recovery time states the bang sensitivity of a fly. *tko*^{25t} flies mainly goes under seizure before waking up and even it reaches to normal position, it might still go under seizure which will still be included into the recovery time.

4.5.10 RNA extraction: 4 different biological replicates of 1 day old 30 virgin females or males and 20 larvae were placed in a microfuge tube and then stored at -80^{o}C. RNA extraction was done for these samples as in [291]. Frozen flies were crushed using a stick in 200 µL Trizol reagent (Invitrogen). After homogenizing the crushed flies in Trizol, another 800 µL of Trizol added and the solution was vortexed. The mixture was then incubated on ice for 5 min and then 200 µL chloroform was added on the mixture. After 3 min of incubation on ice, the microfuge tube was centrifuged for 15 min at 12.000 g at 4^{o}C. The upper phase was then transferred into a new microfuge tube and 500 µL of isopropanol was added in order to precipitate RNA for 10 min at 25^{o}C. The centrifugation was performed at 12.000 g for another 10 minutes at 4^{o}C. Pellet was then washed with 1 mL 75% ethanol then centrifuged again at 12.000g for 10 min at 4^{o}C. After sucking the ethanol, the pellet was air dried and resuspended in final mixture (89 µL DEPC-treated H_2O, 1 µL DNaseI (Amersham Biosciences), 10 µL DNaseI buffer (60 mM $MgCl_2$, 400 mM Tris-HCl, pH 7.5)) and incubated at 37^{o}C for 1 h for DNaseI activity. Phenol/chloroform extraction was increasing purity but decreasing the yield of RNA extraction so unlike [291], no phenol/chloroform extraction step used.

4.5.11 Quantitative RT-PCR: cDNA synthesis was performed with 2 µg of RNA samples which were denaturated at 90^{o}C. 1 µL of 10 mM dNTP mix (Finnzymes) and 1 µL of 0.0125 U/ µL random hexamers (Amersham Biosciences) were then added onto the RNA samples and mixture was placed on ice. 5 µL reaction buffer (250 mM Tris-

HCl, 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT, pH8.3) was added onto the solution along with 0.5 U/µL RNase inhibitor (Fermentas) and 2.5 µL of DEPC-treated water were added. After 10 minutes at 25°C, 2 µL of 20 U/µL MMuLV reverse transcriptase (Fermentas) was added and incubation continued at 25°C for 10 min and then 37°C for 1 h. Reaction was then halted with raising the temperature to 70°C for 10 min.

After the cDNA concentrations diluted to 100 ng/µL, the amplification reaction started in 20 µL containing 5 µL of sample, 10 µL of QuantiTect SYBR Green PCR Master Mix (Qiagen) and 1 µL of forward and reverse primers for each gene under investigation. Thermal cycle reaction was as follows: 15 min at 95°C, 45 cycles of [15 s at 95°C/ 30 s at 55°C / 30s at 72°C]. Flourescence was measured at the end of extension step at 72°C by Roche LightCycler 1.5. Expression of 6 different genes' mRNA levels were measured and compared to the housekeeping genes *GAPDH* and *RPL32*.

4.6 Figure Legends

Figure 4.1 Deletion of *rpd3*Δ and *sds3*Δ but not *rco1*Δ, decreases the fitness of cells lacking mtDNA. Serial dilution assays were performed for strains CDD2 (BY4742), CDD509 (*rpd3*Δ), CDD510 (*sds3*Δ) and CDD514 (*rco1*Δ) as in Materials and Methods. Incubation time is 3 d for (ρ^+) and 4 d for (ρ^-) at 30°C.

Figure 4.2 Deletions of transcriptional repressors reduce the proliferation of cells lacking mtDNA except for *stb3*Δ. Serial dilution assays were performed for strains CDD579 (WT), CDD 580 (*dot6*Δ), CDD581 (*tod6*Δ), CDD582 (*stb3*Δ), CDD567 (*dot6*Δ *tod6*Δ), CDD577 (*dot6*Δ *tod6*Δ *stb3*Δ) as in Materials and Methods. Incubation time is 2 d for (ρ^+) and 3 d for (ρ^-) at 30°C.

Figure 4.3 In order to increase the ΔΨ, plasmids containing either wildtype *ATP3* or dominant *ATP3-5* allele were transformed into different transcriptional suppressor mutations. Introduction of *ATP3-5* reversed the proliferation defects of CDD567 (*dot6*Δ

*tod6*Δ) double mutant. There was no effect on CDD575 (*stb3*Δ) or on CDD576 (*dot6*Δ *tod6*Δ *stb3*Δ). Incubation time is 2 d for (ρ^+) and 3 d for (ρ^-) at 30^{o}C.

Figure 4.4 Ribosomal biogenesis reduction partially rescues the petite-negativity. Serial dilution assays were performed for strains CDD463 (WT), CDD 215 (*atp2*Δ), CDD470 (*sfp1*Δ *atp2*Δ), CDD710 (*atp2*Δ *CLN3*Δ*PEST*) as in Materials and Methods. Incubation time is 3 d for (ρ^+) and 4 d for (ρ^-) at 30^{o}C.

Figure 4.5 Cells highly express Hsp12p and Hsp26p when they were grown in CHX containing media. CDD570 (*HSP12-13MYC*) and CDD571 (*HSP26-13MYC*) cells were grown in YEPD and YEPD+CHX. Solid medium followed by liquid medium approach was used. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used.

Figure 4.6 Hsp12p and Hsp26p shows no overexpression in minimal media conditions. CDD570 (*HSP12-13MYC*) and CDD571 (*HSP26-13MYC*) cells were transformed with b21 (*ATP3*) and b22 (*ATP3-5*) containing plasmids and cultured in SC-LEU. Untransformed cells were grown in YEPD. Protein extractions were performed in solid medium followed by liquid medium approach. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used.

Figure 4.7 Cells highly express Hsp26p when they were grown in CCCP containing media. CDD570 (*HSP12-13MYC*) and CDD571 (*HSP26-13MYC*) cells were grown in YEPD and YEPD+CCCP. Liquid medium approach was used. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used.

Figure 4.8 mtDNA damaged cells overexpress Hsp26p in day 2. CDD571 (*HSP26-13MYC*) cells were grown in EtBr containing media for 1 day and diluted to be grown in YEPD for 2nd day and 3rd day. Liquid medium approach was used. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used. After the visualization, the PVDF was washed 3 times with TBST and membrane blotted for Primary αF1β rabbit monoclonal IgG (1:10000) and followed by secondary antibody anti-rabbit IgG, HRP linked antibody (1:10000).

Figure 4.9 Hsp12p and Hsp26p were strongly, Hsp104p and Ssa3p were partially expressed in ρ^- cells. CDD570 (*HSP12-13MYC*), CDD571 (*HSP26-13MYC*), CDD756 (*HSP104-13MYC*), CDD757 (*SSA3-13MYC*) and CDD607 (*SNF1-13MYC*) cells were grown in EtBr containing media for 1 day and diluted to be grown in YEPD for 2nd day for liquid medium approach. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used. After the visualization, the PVDF was washed 3 times with TBST and membrane blotted for Primary αF1β rabbit monoclonal IgG (1:10000) and followed by secondary antibody anti-rabbit IgG, HRP linked antibody (1:10000).

Figure 4.10 *msn2*Δ *msn4*Δ strongly reduces Hsp12p and partially alleviates Hsp26p expression in ρ^- cells and in ρ^+ HS cells. CDD570 (*HSP12-13MYC*), CDD769 (*msn2*Δ *msn4*Δ *HSP12-13MYC*), CDD784 (*gis1*Δ *HSP12-13MYC*), CDD571 (*HSP26-13MYC*), CDD770 (*msn2*Δ *msn4*Δ *HSP26-13MYC*) and CDD785 (*gis1*Δ *HSP26-13MYC*), cells were grown in EtBr containing media for 1 day and diluted to be grown in YEPD for 2nd day for liquid medium approach. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used. After the visualization, the PVDF was washed 3 times with TBST and membrane blotted for Primary αF1β rabbit

monoclonal IgG (1:10000) and followed by secondary antibody anti-rabbit IgG, HRP linked antibody (1:10000).

Figure 4.11 Deletion of beta subunit of F1 sector of ATP synthase increases Hsp26p expression in ρ^+ cells. CDD783 (*HSP12-13MYC*), CDD787 (*atp2*Δ *HSP12-13MYC*), CDD786 (*HSP26-13MYC*) and CDD788 (*atp2*Δ *HSP26-13MYC*) cells were grown in EtBr containing media for 1 day and diluted to be grown in YEPD for 2nd day for liquid medium approach. Primary antibody c-MYC (9E10) mouse monoclonal IgG (1:5000) is used followed by secondary antibody anti-mouse IgG, HRP linked antibody (1:10000). 10 % seperating gel was used. After the visualization, the PVDF was washed 3 times with TBST and membrane blotted for Primary αF1β rabbit monoclonal IgG (1:10000) and followed by secondary antibody anti-rabbit IgG, HRP linked antibody (1:10000). The upper band visualized at *atp2*Δ *HSP26-13MYC* was probably a non-specific protein produced during heat shock since *atp2*Δ was proven to be a full deletion. Internal primers landing to ATP2 coding sequence verified that there was no ATP2 sequence since possible ATP2 duplications can be found in S288C background (Dunn C.D., PhD Thesis)[292].

Figure 4.12 The crossing scheme for $RpLP1^{beo}$ into tko^{25t} background. (i) tko^{25t} balancer FM7 is firstly crossed into a minute background to obtain a male minute with FM7 on its X chromosome. CyO curly wing balancer must be apart from the $RpLP1^{beo}$ since the sister 2nd chromosome will segregate. FM7 will give white eye color. So males with white eyes and normal wings will be picked. (ii) This male with FM7 on its X chromosome and $RpLP1^{beo}$ on its 2nd chromosome, is then crossed to tko^{25t} heterozygote with a curly wing gene on its 2nd chromosome. (iii) Flies containing bean shaped red eye (tko^{25t} /FM7), curly wings (CyO) and thick, tiny bristles ($RpLP1^{beo}$) was picked as females.White eyed (FM/Y), curly wings (CyO) and thick, tiny bristles ($RpLP1^{beo}$) was picked as males. Picked females and males kept as a stock since progeny from this vial yielded the same genotype as parents except FM7/FM7 females. (iv) tko^{25t} /FM7 ;

RpLP1^{beo} /CyO generated from cross (iii) will then be used to cross *tko*^{25t} /Y ; 2/CyO as an experimental cross. This cross will yield *tko*^{25t} males and *tko*^{25t} / *tko*^{25t} homozygous females with *RpLP1*^{beo} in its secondary chromosome. This experimental cross was not completed and remains as a future direction.

Figure 4.13 Crossing scheme for RpS3 mutants into *tko*^{25t} background. (i) White eyed (FM7/FM7) females crossed to males with double balancer (GFP protein and thick, tiny bristles) containing 3rd chromosome. (ii) FM7 males are then crossed to heterozygous tko25t, red, bean eyed fly. (iii) White eyed (FM7/FM7) with 3rd chromosome balancers then crossed to RpS3 mutants with only thick, tiny bristle balancer. RpS3 containing progeny, no matter which balancer it contains, will have thick tiny bristles due to minute phenotype. But only the GFP containing flies will be picked. GFP,Sb/Sb is embryonic lethal and eliminated from the progeny. (iv) As a side cross, heterozygous *tko*^{25t} containing GFP, Sb is crossed to FM7 males with GFP, Sb as a stock. (v) Progeny from (iv) will be crossed to (iii). This yielded RpS3 mutant cross into tko25t heterozygote and FM7 males. This cross is also kept as a stock since it gives the parental background. (vii) *tko*^{25t} heterozygote and *tko*^{25t} males are then crossed as the experimental cross which continuously gives *tko*^{25t} homozygous and tko25t males. This experimental cross was not completed and remains as a future direction.

4.7 Figures

Figure 4.1 Deletion of *rpd3*Δ and *sds3*Δ but not *rco1*Δ, decreases the fitness of cells lacking mtDNA.

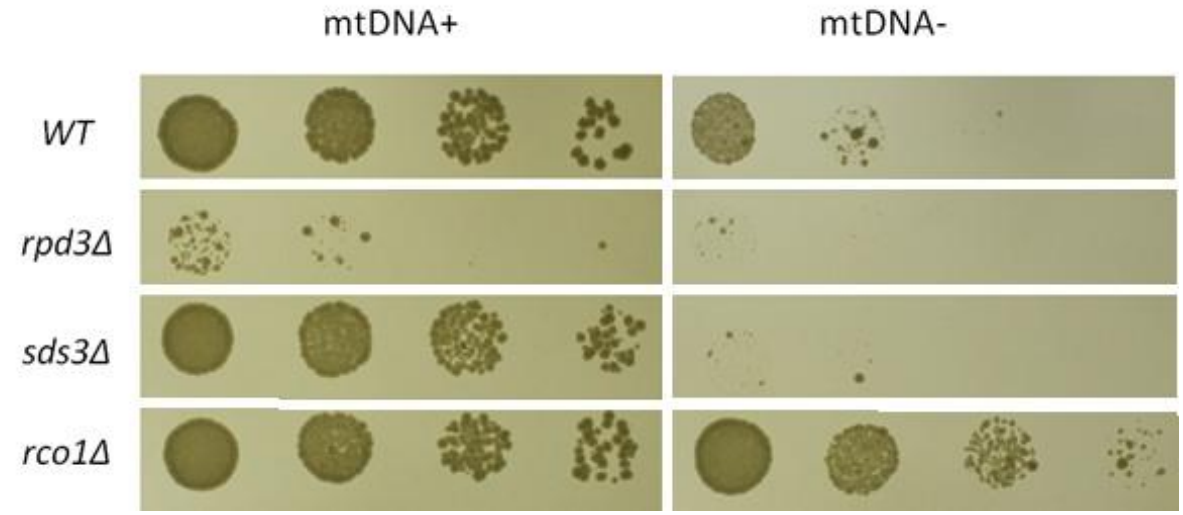

Figure 4.2 Deletions of transcriptional repressors reduce the proliferation of cells lacking mtDNA except for *stb3*Δ.

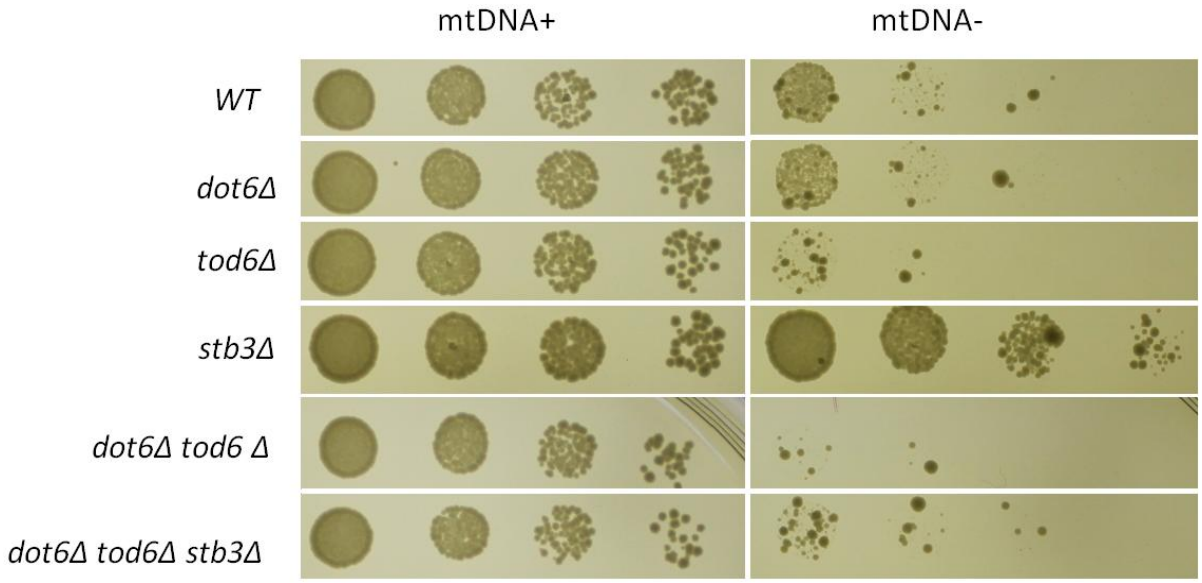

Figure 4.3 In order to revert the ΔΨ, plasmids containing either wildtype *ATP3* or dominant *ATP3-5* allele were transformed into different transcriptional suppressor mutations.

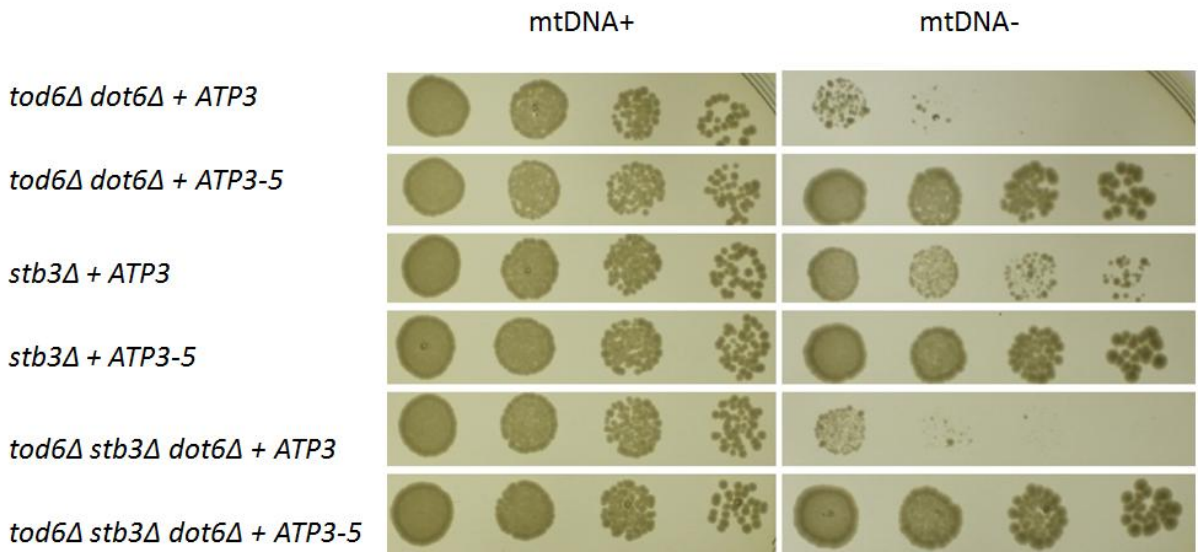

Figure 4.4 Ribosomal biogenesis reduction partially rescues the petite-negativity.

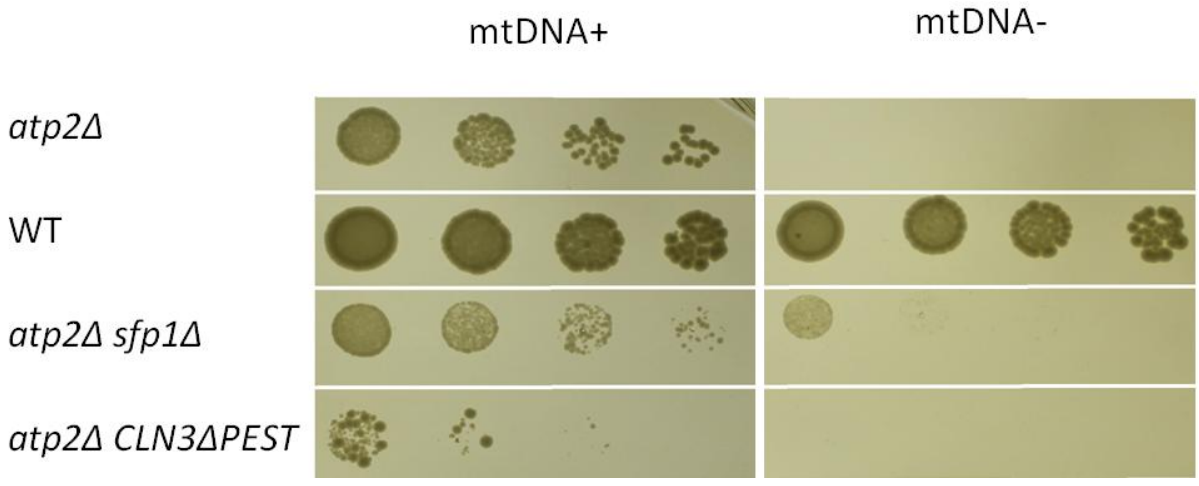

Figure 4.5 Cells highly express Hsp12p and Hsp26p when they were grown in CHX containing media.

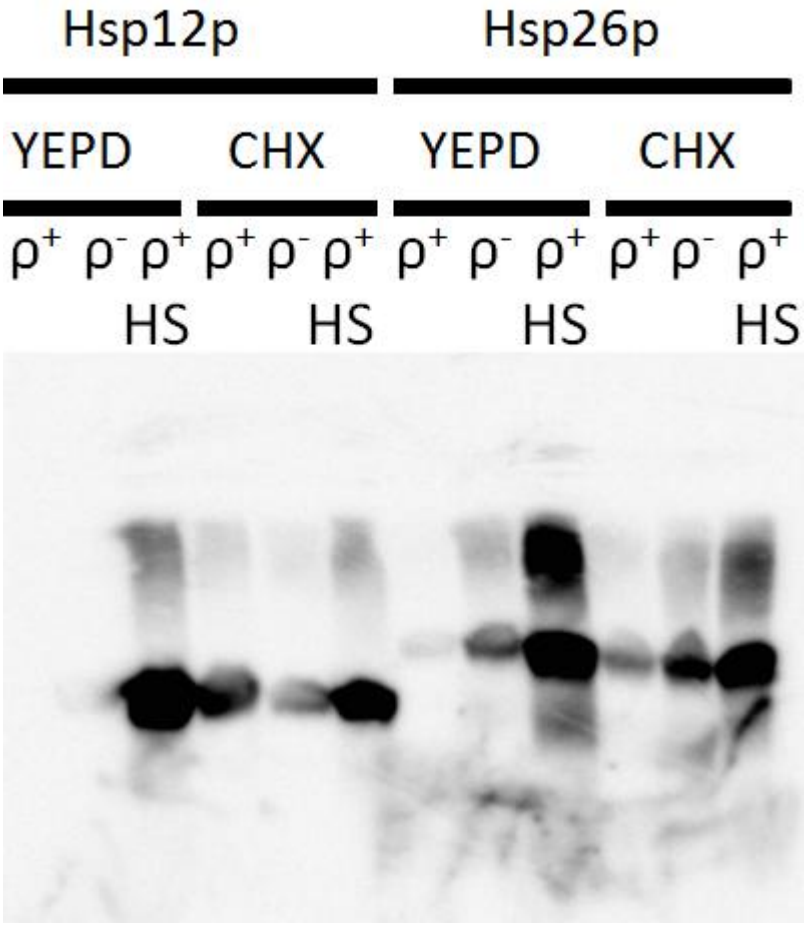

Figure 4.6 Hsp12p and Hsp26p shows no overexpression in minimal media conditions.

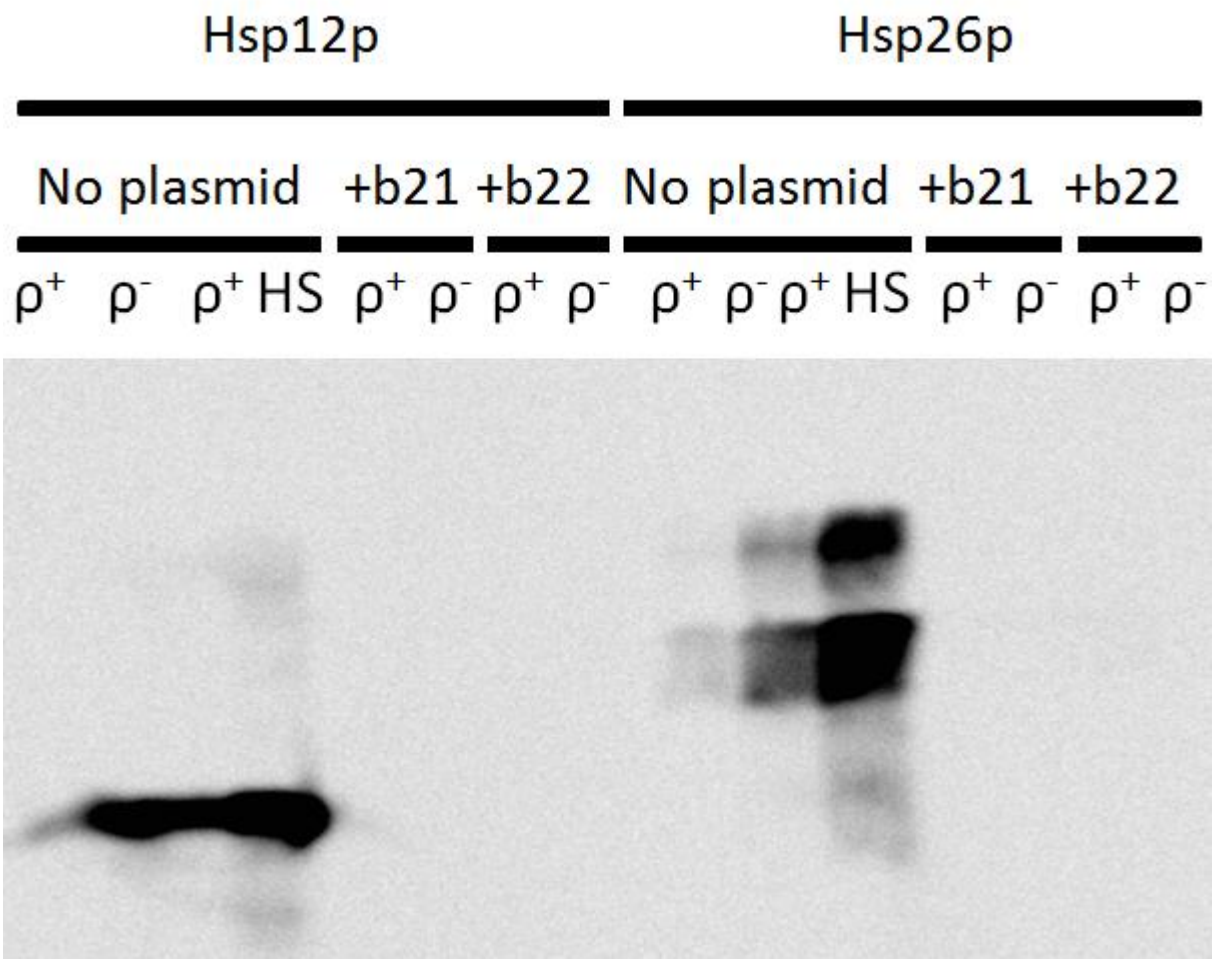

Figure 4.7 Cells highly express Hsp26p when they were grown in CCCP containing media.

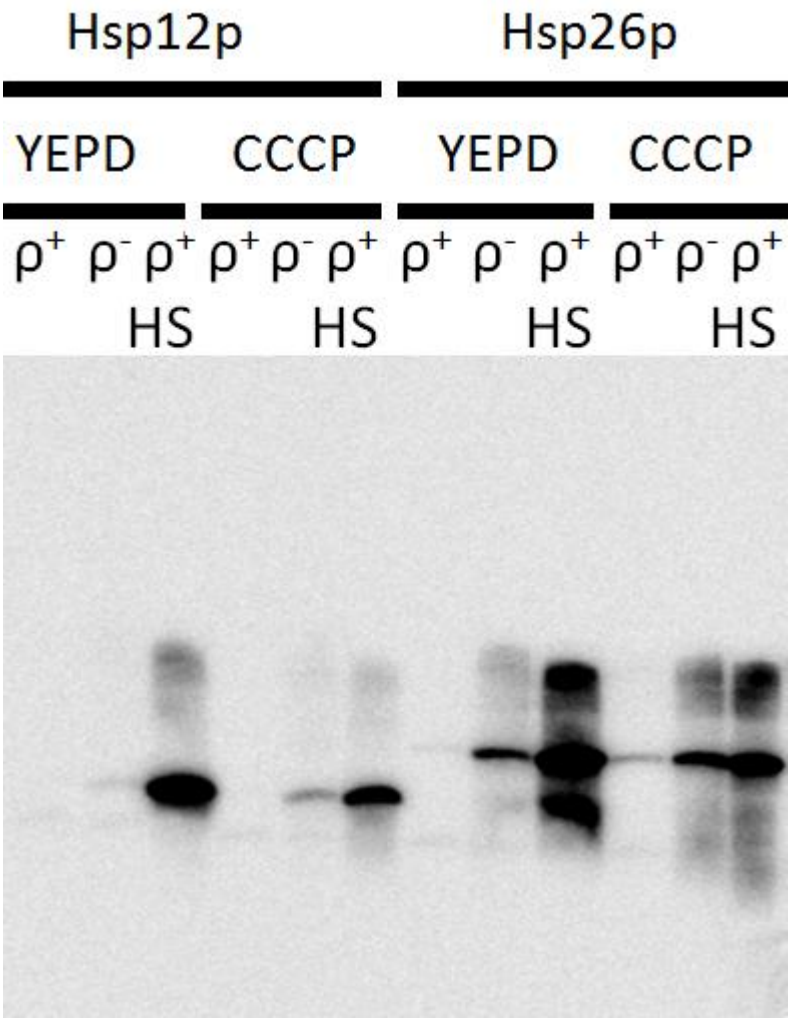

Figure 4.8 mtDNA damaged cells overexpress Hsp26p in day 2.

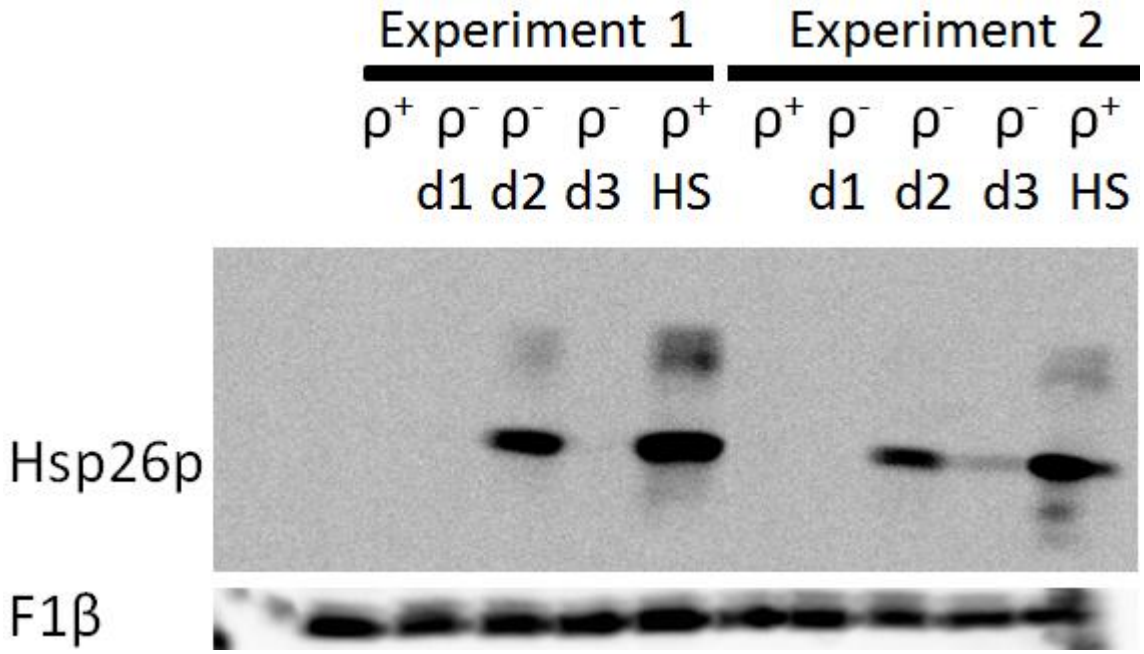

Figure 4.9 Hsp12p and Hsp26p were strongly, Hsp104p and Ssa3p were partially expressed in ρ^- cells.

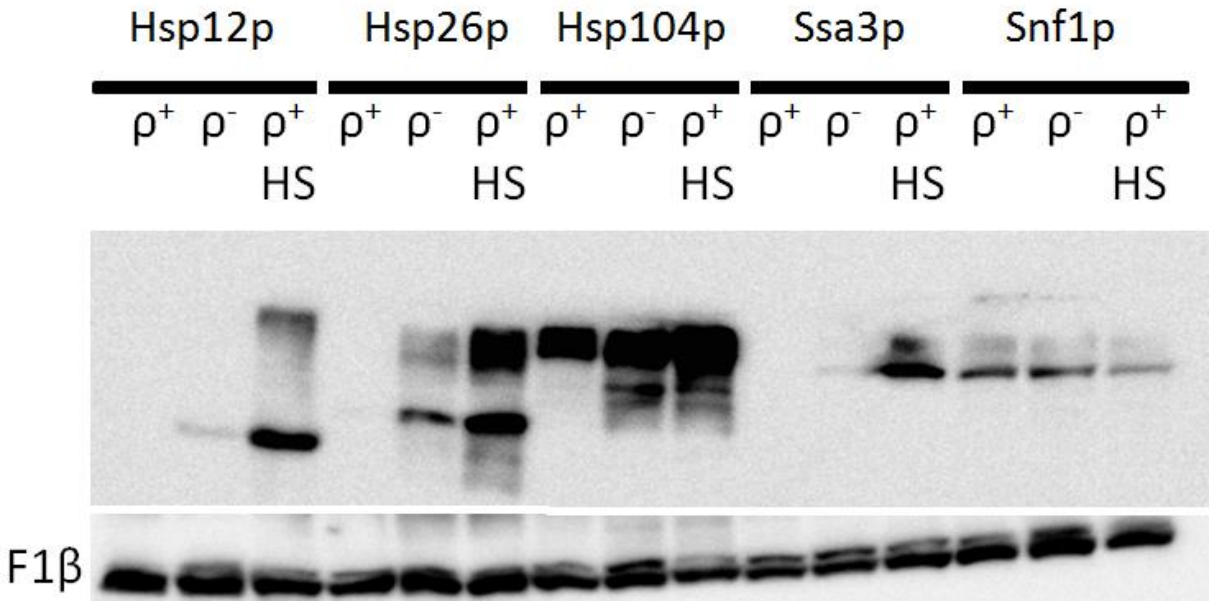

Figure 4.10 *msn2*Δ *msn4*Δ strongly reduces Hsp12p and partially alleviates Hsp26p expression in ρ^- cells and in ρ^+ HS cells.

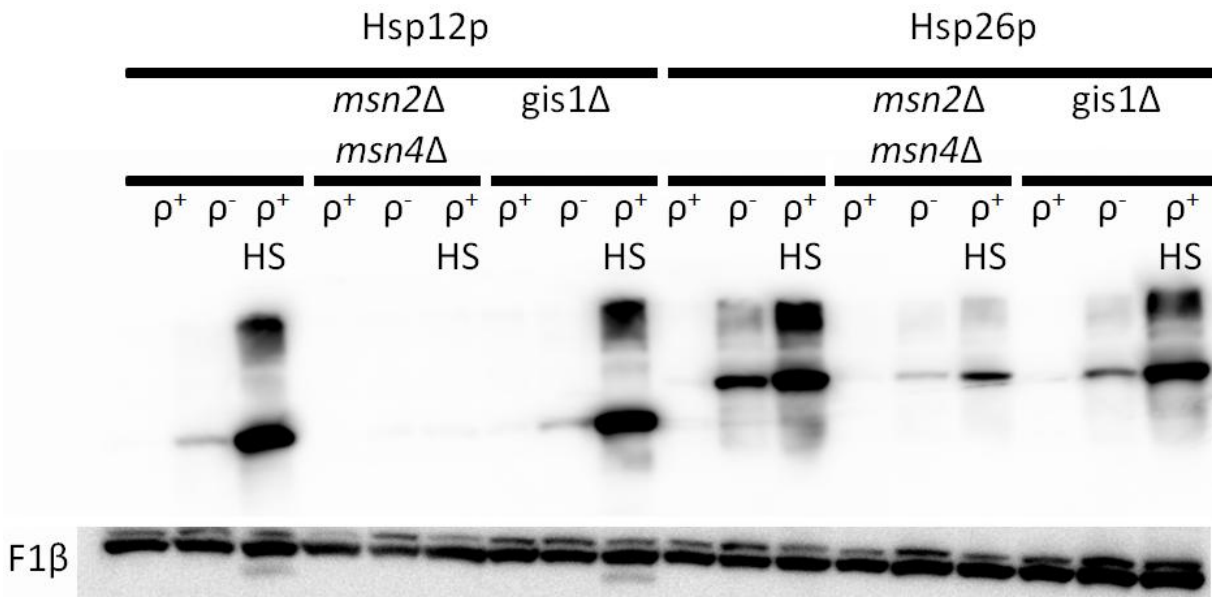

Figure 4.11 Deletion of beta subunit of F1 sector of ATP synthase increases Hsp26p expression in ρ^+ cells.

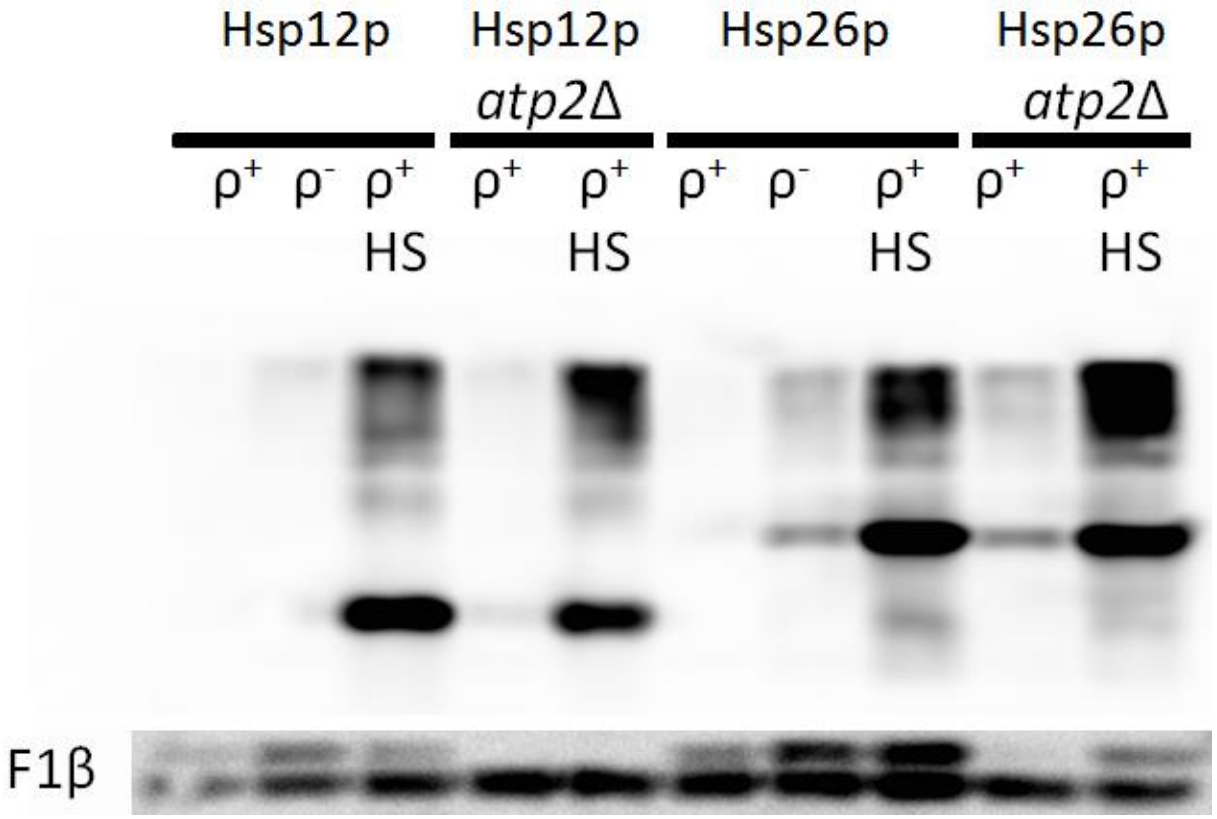

Figure 4.12 The crossing scheme for *RpLP1*^{beo} into *tko*^{25t} background.

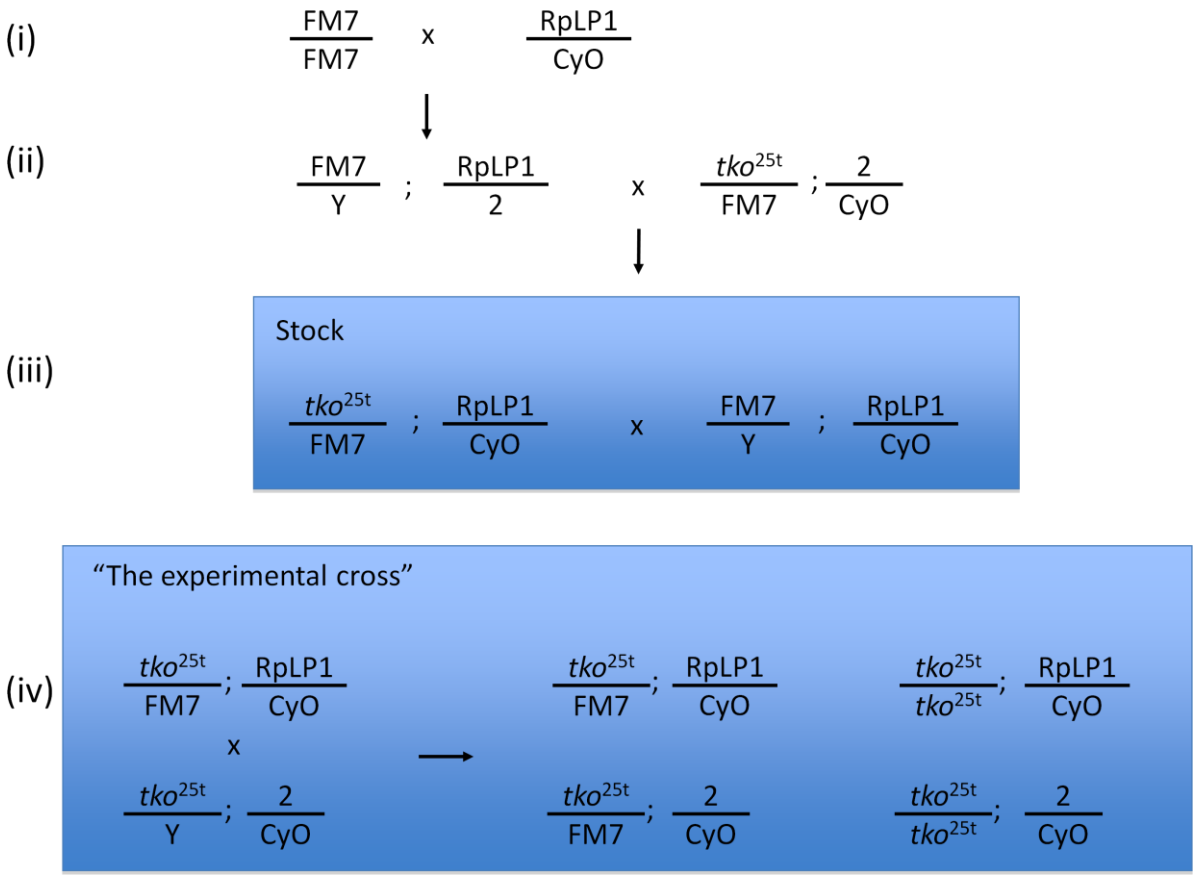

Figure 4.13 Crossing scheme for RpS3 mutants into *tko*^{25t} background.

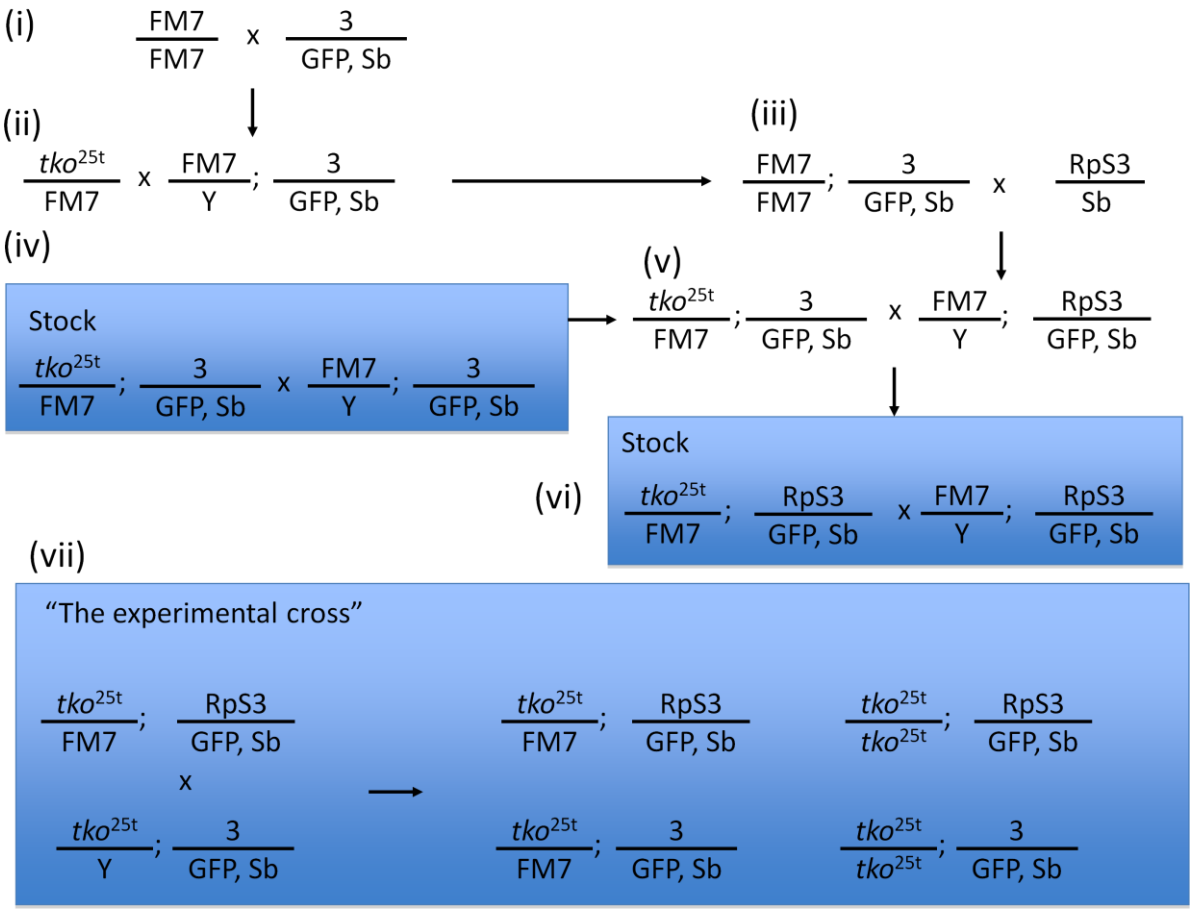

Table 4.1 Treatment of ρ^+ and ρ^- cells with various concentrations of canavanine and AZC did not significantly change the doubling time difference between ρ^+ and ρ^- cells. CDD2 (ρ^+) and CDD2 (ρ^-) are started at 0.05 OD_{600} and grown for 600 and 1400 minutes respectively. OD_{600} units are measured every 2 hours and doubling time is calculated as in [78]. Treated samples have higher doubling time and right column shows the fold of wildtype for doubling time of treated sample in order to compare ρ^+ and ρ^-.

		Doubling Time		Fold of wildtype	
Wildtype	Conc.	Canavanine	AZC	Canavanine	AZC
ρ^+	0 mM	87,42	87,42	1	1
	2,5 mM	104,91	148,08	1,2	1,69
	7,5 mM	147,39	330,26	1,69	3,78
	12,5 mM	260,67	365,71	2,98	4,18
	17,5 mM	321,11	464,73	3,67	5,32
ρ^-	0 mM	141,44	141,44	1	1
	2,5 mM	174,95	169,37	1,24	1,2
	7,5 mM	231,73	491,04	1,64	3,47
	12,5 mM	344,88	1155,16	2,44	8,17
	17,5 mM	483	894,51	3,41	6,32

Table 4.2 Average eclosion day for wildtype (OR), *tko*^{25t} and *RpLP1*^{beo} female and male flies under media containing different drug concentrations. Average day of eclosion (Av. Day of Ec) is the mean day for all flies that have eclosed from 4 different vials. Standard deviation (St. Dev) is given, and it is high since eclosion of flies is distributed to couple days. Number of flies eclosed (n) is given and there are less or no eclosions for different diets.

	Normal media			50 µg/mL CHX			250 µg/mL CHX			100 µg/mL DOX			500 µg/mL DOX		
	Av. Day of Ec	St. Dev.	N	Av. Day of Ec	St. Dev.	n	Av. Day of Ec	St. Dev.	n	Av. Day of Ec	St. Dev.	n	Av. Day of Ec	St. Dev.	n
OR (X/Y)	9,2	0,18	483	9,78	0,07	162	11	0	2	13,02	0.14	134	10,2	0,09	138
OR (X/X)	9	0,22	574	9,97	0,08	162	12,5	2,12	4	13,06	0,15	126	10,24	0,06	142
tko^{25t} / *tko*^{25t}	12,72	0,37	467	11,75	0,25	77	12,94	0,92	12	19	0,49	74	14,65	0,41	98
tko^{25t} /FM7	8,59	0,27	746	9,42	0,12	95	10,5	0,87	7	11,22	0,21	168	10,05	0,11	168
tko^{25t} /Y	13,32	0,42	315	12,44	0,11	64	12,9	0,14	7	19,42	1,23	19	15,51	0,45	57
RpLP1^{beo} (X/Y)	10,47	0,35	242	0	0	0	0	0	0	14,27	0,46	83	13,98	1	88
RpLP1^{beo} (X/X)	10,44	0,36	222	0	0	0	0	0	0	13,85	0,43	71	13,78	0,77	76

Table 4.3 Average eclosion day *tko*^{25t} female and male flies showed reduced average eclosion days under media containing 3 different CHX concentrations. Average day of eclosion (Av. Day of Ec) is the mean day for all flies that have eclosed from 4 different vials. Standard deviation (St. Dev) is given, and it is high since eclosion of flies is distributed to couple days. Number of flies eclosed (n) is given and there are less or no eclosions for different diets.

	Normal media			25 µg/mL CHX			75 µg/mL CHX			150 µg/mL CHX		
	Av. Day of Enc	St. Dev.	n	Av. Day of Enc	St. Dev.	n	Av. Day of Enc	St. Dev.	n	Av. Day of Enc	St. Dev.	N
OR (X/Y)	9,44	0,49	442	9,91	0,62	440	10,34	0,78	61	10,95	0,55	19
OR (X/X)	9,32	0,51	494	9,93	0,67	490	10,65	0,97	62	11,42	0,51	23
tko^{25t} / *tko*^{25t}	13,07	0,34	163	12,56	0,69	103	12,54	0,63	150	12,57	0,8	21
tko^{25t} /FM7	9,31	0,35	240	9,74	0,51	111	10,11	0,69	108	10,67	0,28	30
tko^{25t} /Y	13,66	0,32	108	13,13	0,44	73	13,1	0,61	116	13,22	0,26	13

Table 4.4 CHX does not suppress the bang sensitivity phenotype of *tko*^{25t} flies. The recovery time after bang sensitivity assay is performed for OR and *tko*^{25t} flies that have eclosed from the developmental time assay vials with CHX containing media. Recovery time is measured in terms of seconds. Standard Deviation (St. Dev.) and the number of flies underwent the assay (N) is given in the table.

		Recovery Time(s)	St. Dev.	N
Normal Diet	OR (X/Y)	5,3	4,5	20
	OR (X/X)	2,4	1,8	20
	tko^{25t} / *tko*^{25t}	18	30,5	50
	tko^{25t} /FM7	1,7	2	20
	tko^{25t}/Y	22,1	27,6	50
25 µg/mL CHX	*tko*^{25t} / *tko*^{25t}	21,2	32,8	40
	tko^{25t}/Y	13,1	19	30
75 µg/mL CHX	*tko*^{25t} / *tko*^{25t}	12,9	18,2	50
	tko^{25t}/Y	14,5	22,8	50
150 µg/mL CHX	*tko*^{25t} / *tko*^{25t}	9,3	14,6	9
	tko^{25t}/Y	10,6	12,2	7

Table 4.5 qPCR results in terms of Average Fold Change (Av. FC) for female, male and larvae Oregon R, *tko*^{25t} and minutes. Average of the reads obtained from the Roche LightCycler 1.5 was taken for *RPL32* and *GAPDH*. This average was then subtracted from the read from an investigated gene; this value is ΔCt for that read. Average ΔCt is calculated from the average of ΔCt of Oregon R reads. This value is then subtracted from the ΔCt of each gene to yield ΔΔCt. 2 to the power of negative ΔΔCt gives the fold change for that particular sample. In order to find average fold change, the average of four biological replicates was taken. The standard deviation is calculated according to the Microsoft Excel 2007 (Microsoft Corp., Redmond, WA).

		HSP22		**THOR**		**ILP3**		**ILP5**		**ILP8**		**InR**	
		Av. FC	**St. Dev.**	**Av. FC**	**St. Dev.**	**Av. FC**	**St. Dev.**	**Av. FC**	**St. Dev.**	**Av. FC**	**St. Dev.**	**Av. FC**	**St. Dev.**
Females	**OR**	2,33	2,55	1,01	0,13	1,00	0,09	1,00	0,03	1,01	0,13	1,00	0,11
	***tko*^{25t}**	11,88	8,02	1,47	0,28	0,84	0,09	1,00	0,05	0,67	0,11	2,48	0,44
	***RpS3*^{4}**	3,52	2,71	1,02	0,11	1,36	0,09	1,50	0,03	1,13	0,82	2,11	0,36
	***RpS3A*^{57g}**	4,75	1,32	0,95	0,08	1,06	0,07	1,19	0,02	1,00	0,44	1,85	0,14
	***RpS3*^{2}**	0,42	0,56	0,98	0,10	1,22	0,07	1,15	0,05	0,62	0,31	1,59	0,25
	***RpLP1*^{beo}**	1,45	0,77	0,70	0,09	0,85	0,06	0,92	0,08	0,37	0,10	1,34	0,40
Males	**OR**	2,35	2,88	1,01	0,17	1,00	0,02	1,00	0,03	1,25	0,78	1,01	0,18
	***tko*^{25t}**	7,14	2,76	2,08	1,29	0,69	0,17	0,69	0,11	0,56	0,10	1,56	0,74
	***RpS3*^{4}**	8,45	7,59	1,03	0,17	1,28	0,13	1,44	0,09	1,02	0,51	1,41	0,34
	***RpS3A*^{57g}**	9,35	0,60	0,85	0,10	0,87	0,13	1,13	0,21	0,67	0,23	1,48	0,22

	RpS3^{2}	0,75	0,07	0,92	0,15	1,12	0,09	1,00	0,13	0,61	0,26	1,26	0,14
	RpL36^{y} 74k24.1	1,63	1,40	1,28	0,10	1,35	0,19	1,72	0,13	1,16	0,38	1,51	0,22
	RpLP1 beo	3,21	3,21	0,83	0,00	0,64	0,03	0,82	0,00	0,45	0,08	1,24	0,23
Larvae	**OR**	1,73	2,27	1,01	0,16	1,03	0,25	1,05	0,34	1,13	0,72	1,02	0,22
	tko^{25t}	2,45	0,75	1,50	0,20	1,78	0,25	1,32	0,33	2,56	1,40	1,26	0,41
	RpS3^{4}	1,34	0,24	0,60	0,06	1,14	0,10	1,01	0,11	1,62	0,48	0,64	0,21
	RpS3A 57g	1,89	0,51	0,82	0,08	1,37	0,20	1,44	0,16	13,55	12,86	0,92	0,08
	RpS3^{2}	0,76	0,27	0,37	0,05	1,12	0,23	1,10	0,26	5,12	8,19	0,68	0,37
	RpL36^{y} 74k24.1	0,81	0,50	0,61	0,07	1,64	0,20	1,31	0,06	31,97	35,48	1,04	0,13

Table 4.6 Minute mutations ordered from Bloomington Stock Center.

Minute Genes	Bloomington Stock Number	Annotation in this chapter	Genotype
RpS3	527	*RpS3*^{1}	*l(3)ac*^{1} *e*^{s} *RpS3*^{1}*/LVM*
RpS3	544	*RpS3*^{4}	*RpS3*^{4}*/In(3R)C, Sb*^{1} *e*^{1} *l(3)e*^{1}
RpS3	1696	*RpS3*^{2}	*RpS3*^{2}*/TM2*
RpS3A	1229	*RpS3A*^{57}*g*	*w*^{1118}*; RpS3A*^{57g}*/In(4)ci*^{iD}*, ci*^{iD} *pan*^{ciD}
RpL36	3922	*RpL36*$^{y74k24.1}$	*C(1)DX, y*^{1} *w*^{1} *f*^{1}*/Df(1)y74k24.1, RpL36*$^{y74k24.1}$*/Dp(1;Y)y*^{2}*61l*
RpLP1	6399	*RpLP1*^{beo}	*w*^{1118}*; RpLP1*^{beo}*/CyO*

Table 4.7 Primers used for drosophila experiments in Chapter 4.

Name	Sequence
Hsp22 F	CAATGCGTTCCTTACCGATG
Hsp22 R	GGTAGCGCCACACTCCAAAC
InR F	AAAACAGCGCTTTCGGTGTC
InR R	GTGCGACACCATGTTCCTGA
Ilp3 F	CCATACACACAGAGCTTGGAGAGA
Ilp3 R	CCAGGCCACCATGAAGTTGT
Ilp5 F	CCACTTGGCGGATTTGGA
Ilp5 R	AAATCGCGCCGAATGCT
Ilp8 F	CCACTTGGCGGATTTGGA
Ilp8 R	AAATCGCGCCGAATGCT
Thor F	ATCCAGATGCCCGAGGTGTA
Thor R	TCATGAAAGCCCGCTCGTAG

Table 4.8 List of yeast strains used in Chapter 4.

Strain	Genotype	Source	Parental Strains	Method Used
BY4742	*MATα hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0*	(Brachmann et al., 1998)		
BY4743	*MATα/MATa hisΔ1/hisΔ1 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 ura3Δ0/ura3Δ0*	(Brachmann et al., 1998)		
CDD215	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2*	(Garipler et al., 2014)		
CDD232	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 msn2Δ::kanMX4/MSN2*	EUROSCARF Y27117		
CDD245	*MATα/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4*	RJ1854		

CDD248	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 msn2Δ::kanMX4/MSN2 msn4Δ::URA3/MSN4*	This study	CDD232	disrupted *MSN4* with *URA3* using primers 144/145 and template pRS306, then sporulated the resulting diploid
CDD281	*MATa met15Δ0 his3Δ1 leu2Δ0 msn2Δ::kanMX4 msn4Δ::URA3*	This study	CDD248	sporulation
CDD366	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2*	(Garipler et al., 2014)		
CDD463	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0*	(Garipler et al., 2014)		
CDD470	*MATa leu2Δ0 his3Δ200 met15Δ0 trp1Δ63 ura3Δ0 tco89Δ::LEU2*	This study	RJ1854	sporulation
CDD509	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 rpd3Δ::kanMX4*	EUROSCARF Y11114		
CDD510	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 sds3Δ::kanMX4*	EUROSCARF Y11475		

CDD514	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 rco1Δ::kanMX4*	EUROSCARF Y16209		
CDD515	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 dot6Δ::kanMX4*	EUROSCARF Y16393		
CDD516	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 tod6Δ::kanMX4/TOD6*	EUROSCARF Y23080		
CDD546	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2/ATP2 rim15Δ::URA3/RIM15*	This study	CDD366	disrupted *RIM15* with *URA3* using primers 307/308 and template pRS306, then sporulated the resulting diploid
CDD556	*MATα/MATa met15Δ0/MET15 lys2Δ0/LYS2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 dot6Δ::kanMX4/DOT6 tod6Δ::kanMX4/TOD6*	This study	CDD515, CDD516	crossed CDD515 and CDD516 spore, followed by sporulation
CDD567	*MATa his3Δ1 leu2Δ0 ura3Δ0 dot6Δ::kanMX4 tod6Δ::kanMX4*	This study	CDD556	sporulation
CDD570	*MATα hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0 HSP12-13MYC::KanMx6*	This study	BY4742	knock in *HSP12-13MYC::kanMX6* using plasmid pFA6a-*13MYC-kanMX6* (Longtine Yeast 14:953) and primers 405/406

CDD571	*MATα hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0 HSP26-13MYC::KanMx6*	This study	BY4742	knock in *HSP26-13MYC::kanMX6* using plasmid pFA6a-*13MYC-kanMX6* (Longtine Yeast 14:953) and primers 408/409
CDD575	*MATa met15Δ0 lys2Δ0 his3Δ1 leu2Δ0 ura3Δ0 stb3Δ::URA3*	This study	CDD556	disrupted *STB3* with *URA3* using primers 402/403 and template pRS306, then sporulated the resulting diploid
CDD576	*MATa met15Δ0 his3Δ1 leu2Δ0 ura3Δ0 dot6Δ::kanMX4 tod6Δ::kanMX4 stb3Δ::URA3*	This study	CDD556	disrupted *STB3* with *URA3* using primers 402/403 and template pRS306, then sporulated the resulting diploid
CDD577	*MATα his3Δ1 leu2Δ0 ura3Δ0 dot6Δ::kanMX4 tod6Δ::kanMX4 stb3Δ::URA3*	This study	CDD556	disrupted *STB3* with *URA3* using primers 402/403 and template pRS306, then sporulated the resulting diploid
CDD578	*MATα/MATa leu2Δ0/leu2Δ0 his3Δ/his3Δ met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 sit4Δ::HIS3/SIT4 snf1Δ::TRP1/SNF1*	(Garipler et al., 2014)		
CDD579	*MATα his3Δ1 leu2Δ0 ura3Δ0*	This study	CDD556	sporulation

CDD580	*MATa his3Δ1 leu2Δ0 ura3Δ0 dot6Δ::kanMX4*	This study	CDD556	sporulation
CDD581	*MATα his3Δ1 leu2Δ0 ura3Δ0 tod6Δ::kanMX4*	This study	CDD556	sporulation
CDD582	*MATα his3Δ1 leu2Δ0 ura3Δ0 stb3Δ::URA3*	This study	CDD556	disrupted *STB3* with *URA3* using primers 402/403 and template pRS306, then sporulated the resulting diploid
CDD607	*MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 SNF1-13MYC::kanMX6*	This study	CDD578	knock in *SNF1-13MYC::kanMX6* using plasmid pFA6a-13MYC-kanMX6 (Longtine Yeast 14:953) and primers 423/424, then sporulated the resulting diploid
CDD710	*MAT(?) met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2 CLN3ΔPEST-13MYC::kanMX6*	This study	CDD245	knock in *MYC* and disrupted *PEST* sequence in *CLN3* using primers 522/523 and plasmid pFA6a-*13MYC-kanMX6* (Longtine Yeast 14:953), then sporulated the resulting diploid
CDD756	*MAT(?) hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0 HSP104-13MYC::kanMX6*	This study	BY4743	knock in *HSP104-13MYC::kanMX6* using plasmid pFA6a-*13MYC-kanMX6* (Longtine Yeast 14:953) and primers 604/605, then sporulated the

				resulting diploid
CDD757	*MAT(?) hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0 SSA3-13MYC::kanMX6*	This study	BY4743	knock in *SSA3-13MYC::kanMX6* using plasmid pFA6a-*13MYC-kanMX6* (Longtine Yeast 14:953) and primers 607/608, then sporulated the resulting diploid
CDD769	*MAT(?) his3Δ1 leu2Δ0 lys2Δ0 msn2Δ::kanMX4 msn4Δ::URA3 HSP12-13MYC::KanMx6*	This study	CDD281, CDD570	crossed CDD281 and CDD570, followed by sporulation
CDD770	*MAT(?) his3Δ1 leu2Δ0 lys2Δ0 msn2Δ::kanMX4 msn4Δ::URA3 HSP26-13MYC::KanMx6*	This study	CDD281, CDD571	crossed CDD281 and CDD571, followed by sporulation
CDD783	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 HSP12-13MYC::KanMx6*	This study	CDD546, CDD570	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation
CDD784	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 gis1Δ::HIS3*	This study	CDD546, CDD570	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation

CDD785	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 gis1Δ::HIS3 HSP26-13MYC::KanMx6*	This study	CDD546, CDD571	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation
CDD786	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 gis1Δ::HIS3*	This study	CDD546, CDD571	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation
CDD787	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 atp2Δ::LEU2 HSP12-13MYC::KanMx6*	This study	CDD546, CDD570	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation
CDD788	*MATa leu2Δ0 lys2Δ0 his3Δ trp1Δ63 ura3Δ0 atp2Δ::LEU2 HSP26-13MYC::KanMx6*	This study	CDD546, CDD571	crossed CDD546 and CDD570, disrupted *GIS1* with *HIS3* using primers 618/619 and template pRS303, followed by sporulation

Table 4.9 List of oligonucleotides used in yeast studies in Chapter 4

Number	Sequence
144	TTCGGCTTTTTTTTCTTTTCTTCTTATTAAAAACAATATAagattgtactgagagtgcac
145	TAGCTTGTCTTGCTTTTATTTGCTTTTGACCTTATTTTTTctgtgcggtatttcacaccg
307	CTCATTTGATAGAATAGATAAGCCCAGTAGAGGAAGACAGagattgtactgagagtgcac
308	ATTCAGTTATTTTTTTTAATTATCTTTATCTTAAAATTTActgtgcggtatttcacaccg
313	ATTTTTATTTTTCTTCGAAACAGGAAAAGCTTATCTGAGTagattgtactgagagtgcac
314	ATAGCATACTAAATCAATTCAGTAAAGAAACAATTATATCctgtgcggtatttcacaccg
402	ATTTTGAATAAAAAGACATTATTTCAGACTACCACTAATAAGATTGTACTGAGAGTGCAC
403	TACTGTTTTTTTGTTATTTCATGGAACTGGTAAAGAATTCCTGTGCGGTATTTCACACCG
405	TTCCGGTCGTGTCCACGGTGAAGAAGACCCAACCAAGAAGcggatcccccgggttaattaa
406	TAAAGAAAAAACCATGTAACTACAAAGAGTTCCGAAAGATgaattcgagctcgtttaaac
408	CAAGAAGATTGAGGTTTCTTCTCAAGAATCGTGGGGTAACcggatcccccgggttaattaa
409	GGTCCTCGCGAGAGGGACAACACTATAGAGCCAGGTCACTgaattcgagctcgtttaaac
423	AAAACTAATTATGGAATTAGCCGTTAACAGTCAAAGCAATcggatccc

	cgggttaattaa
424	AAAAAAGGGAACTTCCATATCATTCTTTTACGTTCCACCAgaattcgagctcgtttaaac
522	TTCACAAGAATTGGAAACCATGTACAATACTATCTTTGCTcggatccccgggttaattaa
523	CATTTGAGTAAACACAAGTCAATGAATCGCTGTCAAAGGAgaattcgagctcgtttaaac
604	CGATAATGAGGACAGTATGGAAATTGATGATGACCTAGATcggatcccgggttaattaa
605	ATTCTTGTTCGAAAGTTTTTAAAAATCACACTATATTAAAgaattcgagctcgtttaaac
607	AGGTGGAGAAGATACAGGTCCAACAGTGGAAGAGGTTGATcggatcccgggttaattaa
608	AACATAAAAAGTAGCTAAATAGAACACTATAGAAGAATAAgaattcgagctcgtttaaac
618	GAAGAATAGCTACAAAAACAGACTACAATGGAAATCAAGCagattgtactgagagtgcac
619	GAACCCATTTTGTATATCATTTTCTTGACCTATGATTCAGctgtgcggtatttcacaccg

Table 4.10 Antibodies used in Chapter 4

Antibody	**Company/Origin**	**Details**
Primary c-MYC (9E10) mouse monoclonal IgG	Santa Cruz Biotechnology	Lot #J2413
Primary αFLAG mouse monoclonal IgG	EMBO	G882x 25% glycerol
Primary αF1β rabbit monoclonal IgG	Thanks to Rob Jensen lab at Johns Hopkins University	JH2617cx
Secondary Anti-rabbit IgG, HRP linked antibody	Cell Signaling	#7074S
Secondary Anti-mouse IgG, HRP linked antibody	Cell Signaling Technologies	CST-7076

BIBLIOGRAPHY

[1] Y. Kraytsberg, E. Kudryavtseva, A. C. McKee, C. Geula, N. W. Kowall, and K. Khrapko, "Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons.," *Nat. Genet.*, vol. 38, no. 5, pp. 518–20, May 2006.

[2] A. Trifunovic, A. Wredenberg, M. Falkenberg, J. N. Spelbrink, A. T. Rovio, C. E. Bruder, M. Bohlooly-Y, S. Gidlöf, A. Oldfors, R. Wibom, J. Törnell, H. T. Jacobs, and N.-G. Larsson, "Premature ageing in mice expressing defective mitochondrial DNA polymerase.," *Nature*, vol. 429, no. 6990, pp. 417–23, May 2004.

[3] J. R. Veatch, M. A. McMurray, Z. W. Nelson, and D. E. Gottschling, "Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect.," *Cell*, vol. 137, no. 7, pp. 1247–58, Jun. 2009.

[4] B. A. I. Payne, I. J. Wilson, C. A. Hateley, R. Horvath, M. Santibanez-Koref, D. C. Samuels, D. A. Price, and P. F. Chinnery, "Mitochondrial aging is accelerated by anti-retroviral therapy through the clonal expansion of mtDNA mutations.," *Nat. Genet.*, vol. 43, no. 8, pp. 806–10, Aug. 2011.

[5] X. J. Chen and G. D. Clark-Walker, "The petite mutation in yeasts: 50 years on.," *Int. Rev. Cytol.*, vol. 194, pp. 197–238, Jan. 2000.

[6] Z. Liu and R. A. Butow, "A Transcriptional Switch in the Expression of Yeast Tricarboxylic Acid Cycle Genes in Response to a Reduction or Loss of Respiratory Function," *Mol. Cell. Biol.*, vol. 19, no. 10, pp. 6720–6728, Oct. 1999.

[7] M. V Miceli, J. C. Jiang, A. Tiwari, J. F. Rodriguez-Quiñones, and S. M. Jazwinski, "Loss of mitochondrial membrane potential triggers the retrograde response extending yeast replicative lifespan.," *Front. Genet.*, vol. 2, p. 102, Jan. 2011.

[8] B. S. Polla, S. Kantengwa, D. Francois, S. Salvioli, C. Franceschi, C. Marsac, and A. Cossarizza, "Mitochondria are selective targets for the protective effects of heat shock against oxidative injury.," *Proc. Natl. Acad. Sci.*, vol. 93, no. 13, pp. 6458–6463, Jun. 1996.

[9] G. C. Kujoth, A. Hiona, T. D. Pugh, S. Someya, K. Panzer, S. E. Wohlgemuth, T. Hofer, A. Y. Seo, R. Sullivan, W. A. Jobling, J. D. Morrow, H. Van Remmen, J. M. Sedivy, T. Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh, and T. A. Prolla, "Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging.," *Science*, vol. 309, no. 5733, pp. 481–4, Jul. 2005.

[10] E. S. Goldring, L. I. Grossman, D. Krupnick, D. R. Cryer, and J. Marmur, "The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide.," *J. Mol. Biol.*, vol. 52, no. 2, pp. 323–35, Sep. 1970.

[11] L. Margulis, “Origin of Eukaryotic Cells: Lynn Margulis,” 1970. [Online]. Available: http://www.amazon.com/Origin-Eukaryotic-Cells-Lynn-Margulis/dp/0300013531. [Accessed: 08-Jul-2014].

[12] E. T. Bui, P. J. Bradley, and P. J. Johnson, “A common evolutionary origin for mitochondria and hydrogenosomes.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 93, no. 18, pp. 9651–6, Sep. 1996.

[13] A. V Goldberg, S. Molik, A. D. Tsaousis, K. Neumann, G. Kuhnke, F. Delbac, C. P. Vivares, R. P. Hirt, R. Lill, and T. M. Embley, “Localization and functionality of microsporidian iron-sulphur cluster assembly proteins.,” *Nature*, vol. 452, no. 7187, pp. 624–8, Apr. 2008.

[14] E. E. Gill, S. Diaz-Triviño, M. J. Barberà, J. D. Silberman, A. Stechmann, D. Gaston, I. Tamas, and A. J. Roger, “Novel mitochondrion-related organelles in the anaerobic amoeba Mastigamoeba balamuthi.,” *Mol. Microbiol.*, vol. 66, no. 6, pp. 1306–20, Dec. 2007.

[15] B. A. P. Williams, R. P. Hirt, J. M. Lucocq, and T. M. Embley, “A mitochondrial remnant in the microsporidian Trachipleistophora hominis.,” *Nature*, vol. 418, no. 6900, pp. 865–9, Aug. 2002.

[16] M. W. Gray, “Mitochondrial evolution.,” *Cold Spring Harb. Perspect. Biol.*, vol. 4, no. 9, p. a011403, Sep. 2012.

[17] J. E. Feagin, “Mitochondrial genome diversity in parasites.,” *Int. J. Parasitol.*, vol. 30, no. 4, pp. 371–90, Apr. 2000.

[18] D. B. Sloan, A. J. Alverson, J. P. Chuckalovcak, M. Wu, D. E. McCauley, J. D. Palmer, and D. R. Taylor, “Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates.,” *PLoS Biol.*, vol. 10, no. 1, p. e1001241, Jan. 2012.

[19] S. Anderson, A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young, “Sequence and organization of the human mitochondrial genome,” *Nature*, vol. 290, no. 5806, pp. 457–465, Apr. 1981.

[20] M. J. Bibb, R. A. Van Etten, C. T. Wright, M. W. Walberg, and D. A. Clayton, “Sequence and gene organization of mouse mitochondrial DNA,” *Cell*, vol. 26, no. 2, pp. 167–180, Oct. 1981.

[21] R. W. Taylor and D. M. Turnbull, “Europe PMC Funders Group MITOCHONDRIAL DNA MUTATIONS IN HUMAN DISEASE,” vol. 6, no. 5, pp. 389–402, 2007.

[22] S. Anderson, M. H. de Bruijn, A. R. Coulson, I. C. Eperon, F. Sanger, and I. G. Young, “Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome.,” *J. Mol. Biol.*, vol. 156, no. 4, pp. 683–717, Apr. 1982.

[23] E. Richly, P. F. Chinnery, and D. Leister, “Evolutionary diversification of mitochondrial proteomes: implications for human disease.,” *Trends Genet.*, vol. 19, no. 7, pp. 356–62, Jul. 2003.

[24] M. W. Gray, “Evolution of organellar genomes,” *Curr. Opin. Genet. Dev.*, vol. 9, no. 6, pp. 678–687, Dec. 1999.

[25] T. Gabaldón and M. A. Huynen, “Shaping the mitochondrial proteome.,” *Biochim. Biophys. Acta*, vol. 1659, no. 2–3, pp. 212–20, Dec. 2004.

[26] N. Wiedemann, A. E. Frazier, and N. Pfanner, “The protein import machinery of mitochondria.,” *J. Biol. Chem.*, vol. 279, no. 15, pp. 14473–6, Apr. 2004.

[27] I. J. Holt, A. E. Harding, and J. A. Morgan-Hughes, “Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies.,” *Nature*, vol. 331, no. 6158, pp. 717–9, Feb. 1988.

[28] D. Wallace, G. Singh, M. Lott, J. Hodge, T. Schurr, A. Lezza, L. Elsas, and E. Nikoskelainen, “Mitochondrial DNA mutation associated with Leber’s hereditary optic neuropathy,” *Science (80-.).*, vol. 242, no. 4884, pp. 1427–1430, Dec. 1988.

[29] Y. Goto, S. Horai, T. Matsuoka, Y. Koga, K. Nihei, M. Kobayashi, and I. Nonaka, “Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): A correlative study of the clinical features and mitochondrial DNA mutation,” *Neurology*, vol. 42, no. 3, pp. 545–545, Mar. 1992.

[30] D. D. de Vries, B. G. van Engelen, F. J. Gabreëls, W. Ruitenbeek, and B. A. van Oost, “A second missense mutation in the mitochondrial ATPase 6 gene in Leigh’s syndrome.,” *Ann. Neurol.*, vol. 34, no. 3, pp. 410–2, Sep. 1993.

[31] F. M. Santorelli, S. Shanske, A. Macaya, D. C. DeVivo, and S. DiMauro, “The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh’s syndrome.,” *Ann. Neurol.*, vol. 34, no. 6, pp. 827–34, Dec. 1993.

[32] J. M. Shoffner, M. T. Lott, A. M. Lezza, P. Seibel, S. W. Ballinger, and D. C. Wallace, “Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation.,” *Cell*, vol. 61, no. 6, pp. 931–7, Jun. 1990.

[33] C. Casali, F. M. Santorelli, G. Damati, P. Bernucci, L. Debiase, and S. Dimauro, “A Novel mtDNA Point Mutation in Maternally Inherited Cardiomyopathy,” *Biochem. Biophys. Res. Commun.*, vol. 213, no. 2, pp. 588–593, Aug. 1995.

[34] A. Suomalainen, L. Peltonen, A. Paetau, H. Leinonen, A. Majander, and H. Somer, “Inherited idiopathic dilated cardiomyopathy with multiple deletions of mitochondrial DNA,” *Lancet*, vol. 340, no. 8831, pp. 1319–1320, Nov. 1992.

[35] D. C. Wallace, “Mitochondrial Diseases in Man and Mouse,” *Science (80-.).*, vol. 283, no. 5407, pp. 1482–1488, Mar. 1999.

[36] S. DiMauro and E. A. Schon, “Mitochondrial DNA mutations in human disease.,” *Am. J. Med. Genet.*, vol. 106, no. 1, pp. 18–26, Jan. 2001.

[37] A. S. Reichert and W. Neupert, “Mitochondriomics or what makes us breathe.,” *Trends Genet.*, vol. 20, no. 11, pp. 555–62, Nov. 2004.

[38] S. Züchner, I. V Mersiyanova, M. Muglia, N. Bissar-Tadmouri, J. Rochelle, E. L. Dadali, M. Zappia, E. Nelis, A. Patitucci, J. Senderek, Y. Parman, O. Evgrafov, P. De Jonghe, Y. Takahashi,

S. Tsuji, M. A. Pericak-Vance, A. Quattrone, E. Battaloglu, A. V Polyakov, V. Timmerman, J. M. Schröder, J. M. Vance, and E. Battologlu, "Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A.," *Nat. Genet.*, vol. 36, no. 5, pp. 449–51, May 2004.

[39] H. Koutnikova, V. Campuzano, F. Foury, P. Dollé, O. Cazzalini, and M. Koenig, "Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin.," *Nat. Genet.*, vol. 16, no. 4, pp. 345–51, Aug. 1997.

[40] C. Alexander, M. Votruba, U. E. Pesch, D. L. Thiselton, S. Mayer, A. Moore, M. Rodriguez, U. Kellner, B. Leo-Kottler, G. Auburger, S. S. Bhattacharya, and B. Wissinger, "OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28.," *Nat. Genet.*, vol. 26, no. 2, pp. 211–5, Oct. 2000.

[41] P. Chinnery, K. Majamaa, D. Turnbull, and D. Thorburn, "Treatment for mitochondrial disorders.," *Cochrane database Syst. Rev.*, no. 1, p. CD004426, Jan. 2006.

[42] J. Liu and L. Wang, "Mitochondrial enhancement for neurodegenerative movement disorders: a systematic review of trials involving creatine, coenzyme Q10, idebenone and mitoquinone.," *CNS Drugs*, vol. 28, no. 1, pp. 63–8, Jan. 2014.

[43] M. H. Parkinson, J. B. Schulz, and P. Giunti, "Co-enzyme Q10 and idebenone use in Friedreich's ataxia.," *J. Neurochem.*, vol. 126 Suppl , pp. 125–41, Aug. 2013.

[44] C. Shults, "Coenzyme Q10 in Neurodegenerative Diseases," *Curr. Med. Chem.*, vol. 10, no. 19, pp. 1917–1921, Oct. 2003.

[45] M. Mattiazzi, C. Vijayvergiya, C. D. Gajewski, D. C. DeVivo, G. Lenaz, M. Wiedmann, and G. Manfredi, "The mtDNA T8993G (NARP) mutation results in an impairment of oxidative phosphorylation that can be improved by antioxidants.," *Hum. Mol. Genet.*, vol. 13, no. 8, pp. 869–79, Apr. 2004.

[46] D. S. Kerr, "Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade.," *Mol. Genet. Metab.*, vol. 99, no. 3, pp. 246–55, Mar. 2010.

[47] R. N. Lightowlers, P. F. Chinnery, D. M. Turnbull, and N. Howell, "Mammalian mitochondrial genetics: heredity, heteroplasmy and disease," *Trends Genet.*, vol. 13, no. 11, pp. 450–455, Nov. 1997.

[48] S. E. Calvo, A. G. Compton, S. G. Hershman, S. C. Lim, D. S. Lieber, E. J. Tucker, A. Laskowski, C. Garone, S. Liu, D. B. Jaffe, J. Christodoulou, J. M. Fletcher, D. L. Bruno, J. Goldblatt, S. Dimauro, D. R. Thorburn, and V. K. Mootha, "Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing.," *Sci. Transl. Med.*, vol. 4, no. 118, p. 118ra10, Jan. 2012.

[49] T. B. Haack, K. Danhauser, B. Haberberger, J. Hoser, V. Strecker, D. Boehm, G. Uziel, E. Lamantea, F. Invernizzi, J. Poulton, B. Rolinski, A. Iuso, S. Biskup, T. Schmidt, H.-W. Mewes, I. Wittig, T. Meitinger, M. Zeviani, and H. Prokisch, "Exome sequencing identifies ACAD9

mutations as a cause of complex I deficiency.," *Nat. Genet.*, vol. 42, no. 12, pp. 1131–4, Dec. 2010.

[50] A. H. V Schapira, "Mitochondrial disease.," *Lancet*, vol. 368, no. 9529, pp. 70–82, Jul. 2006.

[51] M. F. Marusich, B. H. Robinson, J.-W. Taanman, S. J. Kim, R. Schillace, J. L. Smith, and R. A. Capaldi, "Expression of mtDNA and nDNA encoded respiratory chain proteins in chemically and genetically-derived Rho0 human fibroblasts: a comparison of subunit proteins in normal fibroblasts treated with ethidium bromide and fibroblasts from a patient with mtDNA de," *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1362, no. 2–3, pp. 145–159, Dec. 1997.

[52] B. Ephrussi, "Action de l'acriflavine sur les levures .I. La mutation petite colonie," *Ann. Inst. Pasteur*, 1949.

[53] B. Ephrussi, "Action de l'acrifiavine sur les levures. II. Etude genetique du mutant "petite colonie," *Ann. Inst. Pasteur*, 1949.

[54] J. Tavlitzki, "Action de l'acriflavine sur les levures. III. Etude de la croissance des mutants 'petite colonie.,'" *Ann. Inst. Pasteur*, 1949.

[55] P. Silonimski, "Action de l'acriflavine sur les levures. IV. Mode d'utilisation du glucose par les mutants petite colonie," *Ann. Inst. Pasteur*, 1949.

[56] G. Schatz, E. Haslbrunner, and H. Tuppy, "Deoxyribonucleic acid associated with yeast mitochondria," *Biochem. Biophys. Res. Commun.*, vol. 15, no. 2, pp. 127–132, Mar. 1964.

[57] M. Rabinowitz and H. Swift, "Mitochondrial nucleic acids and their relation to the biogenesis of mitochondria," *Physiol Rev*, vol. 50, no. 3, pp. 376–427, Jul. 1970.

[58] P. P. Slonimski, G. Perrodin, and J. H. Croft, "Ethidium bromide induced mutation of yeast mitochondria: complete transformation of cells into respiratory deficient non-chromosomal 'petites'.," *Biochem. Biophys. Res. Commun.*, vol. 30, no. 3, pp. 232–9, Feb. 1968.

[59] D. C. Wallace, "Mitochondrial DNA mutations in disease and aging.," *Environ. Mol. Mutagen.*, vol. 51, no. 5, pp. 440–50, Jun. 2010.

[60] P. E. Thorsness, K. H. White, and T. D. Fox, "Inactivation of YME1, a member of the ftsH-SEC18-PAS1-CDC48 family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in Saccharomyces cerevisiae.," *Mol. Cell. Biol.*, vol. 13, no. 9, pp. 5418–5426, Sep. 1993.

[61] L. Lefebvre-Legendre, J. Vaillier, H. Benabdelhak, J. Velours, P. P. Slonimski, and J. P. di Rago, "Identification of a nuclear gene (FMC1) required for the assembly/stability of yeast mitochondrial F(1)-ATPase in heat stress conditions.," *J. Biol. Chem.*, vol. 276, no. 9, pp. 6789–96, Mar. 2001.

[62] O. Kerscher, N. B. Sepuri, and R. E. Jensen, "Tim18p is a new component of the Tim54p-Tim22p translocon in the mitochondrial inner membrane.," *Mol. Biol. Cell*, vol. 11, no. 1, pp. 103–16, Jan. 2000.

[63] C. D. Dunn and R. E. Jensen, “Suppression of a Defect in Mitochondrial Protein Import Identifies Cytosolic Proteins Required for Viability of Yeast Cells Lacking Mitochondrial DNA,” *Genetics*, vol. 165, no. 1, pp. 35–45, Sep. 2003.

[64] J. Subík, J. Kolarov, and L. Kovác, “Obligatory requirement of intramitochondrial ATP for normal functioning of the eucaryotic cell.,” *Biochem. Biophys. Res. Commun.*, vol. 49, no. 1, pp. 192–8, Oct. 1972.

[65] J. Kolarov and M. Klingenberg, “The adenine nucleotide translocator in genetically and physiologically modified yeast mitochondria.,” *FEBS Lett.*, vol. 45, no. 1, pp. 320–3, Sep. 1974.

[66] C. H. Dupont, J. P. Mazat, and B. Guerin, “The role of adenine nucleotide translocation in the energization of the inner membrane of mitochondria isolated from rho + and rho degree strains of Saccharomyces cerevisiae.,” *Biochem. Biophys. Res. Commun.*, vol. 132, no. 3, pp. 1116–23, Nov. 1985.

[67] L. E. Marbella, H. S. Cho, and M. M. Spence, “Observing the translocation of a mitochondria-penetrating peptide with solid-state NMR.,” *Biochim. Biophys. Acta*, vol. 1828, no. 8, pp. 1674–82, Aug. 2013.

[68] J. A. Johnson and M. Ogbi, “Targeting the F1Fo ATP Synthase: modulation of the body’s powerhouse and its implications for human disease.,” *Curr. Med. Chem.*, vol. 18, no. 30, pp. 4684–714, Jan. 2011.

[69] M. F. Giraud and J. Velours, “ATP synthase of yeast mitochondria. Isolation of the F1 delta subunit, sequence and disruption of the structural gene.,” *Eur. J. Biochem.*, vol. 222, no. 3, pp. 851–9, Jun. 1994.

[70] S. Dean, M. K. Gould, C. E. Dewar, and A. C. Schnaufer, “Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 36, pp. 14741–6, Sep. 2013.

[71] D. P. Nolan and H. P. Voorheis, “The mitochondrion in bloodstream forms of Trypanosoma brucei is energized by the electrogenic pumping of protons catalysed by the F1F0-ATPase.,” *Eur. J. Biochem.*, vol. 209, no. 1, pp. 207–16, Oct. 1992.

[72] A. Schnaufer, G. D. Clark-Walker, A. G. Steinberg, and K. Stuart, “The F1-ATP synthase complex in bloodstream stage trypanosomes has an unusual and essential function.,” *EMBO J.*, vol. 24, no. 23, pp. 4029–40, Dec. 2005.

[73] C. Chen, Y. Ko, M. Delannoy, S. J. Ludtke, W. Chiu, and P. L. Pedersen, “Mitochondrial ATP synthasome: three-dimensional structure by electron microscopy of the ATP synthase in complex formation with carriers for Pi and ADP/ATP.,” *J. Biol. Chem.*, vol. 279, no. 30, pp. 31761–8, Jul. 2004.

[74] S. Detke and R. Elsabrouty, “Identification of a mitochondrial ATP synthase-adenine nucleotide translocator complex in Leishmania.,” *Acta Trop.*, vol. 105, no. 1, pp. 16–20, Jan. 2008.

[75] M. van der Laan, N. Wiedemann, D. U. Mick, B. Guiard, P. Rehling, and N. Pfanner, “A role for Tim21 in membrane-potential-dependent preprotein sorting in mitochondria.,” *Curr. Biol.*, vol. 16, no. 22, pp. 2271–6, Nov. 2006.

[76] E. R. Weber, R. S. Rooks, K. S. Shafer, J. W. Chase, and P. E. Thorsness, “Mutations in the Mitochondrial ATP Synthase Gamma Subunit Suppress a Slow-Growth Phenotype of yme1 Yeast Lacking Mitochondrial DNA,” *Genetics*, vol. 140, no. 2, pp. 435–442, Jun. 1995.

[77] X. J. Chen and G. D. Clark-Walker, “Specific mutations in alpha- and gamma-subunits of F1-ATPase affect mitochondrial genome integrity in the petite-negative yeast Kluyveromyces lactis,” *EMBO J.*, vol. 14, no. 13, pp. 3277–86, Jul. 1995.

[78] G. Garipler and C. D. Dunn, “Defects associated with mitochondrial DNA damage can be mitigated by increased vacuolar pH in Saccharomyces cerevisiae.,” *Genetics*, vol. 194, no. 1, pp. 285–90, May 2013.

[79] H. T. Jacobs, D. J. M. Fernández-Ayala, S. Manjiry, E. Kemppainen, J. M. Toivonen, and K. M. C. O’Dell, “Mitochondrial disease in flies.,” *Biochim. Biophys. Acta*, vol. 1659, no. 2–3, pp. 190–6, Dec. 2004.

[80] J. M. Toivonen, K. M. C. O’Dell, N. Petit, S. C. Irvine, G. K. Knight, M. Lehtonen, M. Longmuir, K. Luoto, S. Touraille, Z. Wang, S. Alziari, Z. H. Shah, and H. T. Jacobs, “technical knockout, a Drosophila Model of Mitochondrial Deafness,” *Genetics*, vol. 159, no. 1, pp. 241–254, Sep. 2001.

[81] J. E. Engel and C.-F. Wu, “Altered mechanoreceptor response in Drosophila bang-sensitive mutants,” *J. Comp. Physiol. A*, vol. 175, no. 3, Sep. 1994.

[82] R. Zoncu, A. Efeyan, and D. M. Sabatini, “mTOR: from growth signal integration to cancer, diabetes and ageing.,” *Nat. Rev. Mol. Cell Biol.*, vol. 12, no. 1, pp. 21–35, Jan. 2011.

[83] T. Noda and Y. Ohsumi, “Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast.,” *J. Biol. Chem.*, vol. 273, no. 7, pp. 3963–6, Feb. 1998.

[84] T. P. Neufeld, “NIH Public Access,” vol. 22, no. 2, pp. 157–168, 2011.

[85] J. Avruch, X. Long, S. Ortiz-Vega, J. Rapley, A. Papageorgiou, and N. Dai, “Amino acid regulation of TOR complex 1.,” *Am. J. Physiol. Endocrinol. Metab.*, vol. 296, no. 4, pp. E592–602, Apr. 2009.

[86] Y. Li, Y. Wang, E. Kim, P. Beemiller, C.-Y. Wang, J. Swanson, M. You, and K.-L. Guan, “Bnip3 mediates the hypoxia-induced inhibition on mammalian target of rapamycin by interacting with Rheb.,” *J. Biol. Chem.*, vol. 282, no. 49, pp. 35803–13, Dec. 2007.

[87] E. M. Smith, S. G. Finn, A. R. Tee, G. J. Browne, and C. G. Proud, “The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses.,” *J. Biol. Chem.*, vol. 280, no. 19, pp. 18717–27, May 2005.

[88] S. Schleich and A. A. Teleman, “Akt phosphorylates both Tsc1 and Tsc2 in Drosophila, but neither phosphorylation is required for normal animal growth.,” *PLoS One*, vol. 4, no. 7, p. e6305, Jan. 2009.

[89] F. Dubouloz, O. Deloche, V. Wanke, E. Cameroni, and C. De Virgilio, “The TOR and EGO protein complexes orchestrate microautophagy in yeast.,” *Mol. Cell*, vol. 19, no. 1, pp. 15–26, Jul. 2005.

[90] M. Binda, M.-P. Péli-Gulli, G. Bonfils, N. Panchaud, J. Urban, T. W. Sturgill, R. Loewith, and C. De Virgilio, “The Vam6 GEF controls TORC1 by activating the EGO complex.,” *Mol. Cell*, vol. 35, no. 5, pp. 563–73, Sep. 2009.

[91] K. Hara, Y. Maruki, X. Long, K. Yoshino, N. Oshiro, S. Hidayat, C. Tokunaga, J. Avruch, and K. Yonezawa, “Raptor, a Binding Partner of Target of Rapamycin (TOR), Mediates TOR Action,” *Cell*, vol. 110, no. 2, pp. 177–189, Jul. 2002.

[92] D. D. Sarbassov, S. M. Ali, D.-H. Kim, D. A. Guertin, R. R. Latek, H. Erdjument-Bromage, P. Tempst, and D. M. Sabatini, “Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton.,” *Curr. Biol.*, vol. 14, no. 14, pp. 1296–302, Jul. 2004.

[93] C. F. Bentzinger, K. Romanino, D. Cloëtta, S. Lin, J. B. Mascarenhas, F. Oliveri, J. Xia, E. Casanova, C. F. Costa, M. Brink, F. Zorzato, M. N. Hall, and M. A. Rüegg, “Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy.,” *Cell Metab.*, vol. 8, no. 5, pp. 411–24, Nov. 2008.

[94] D. M. Gwinn, D. B. Shackelford, D. F. Egan, M. M. Mihaylova, A. Mery, D. S. Vasquez, B. E. Turk, and R. J. Shaw, “AMPK phosphorylation of raptor mediates a metabolic checkpoint.,” *Mol. Cell*, vol. 30, no. 2, pp. 214–26, Apr. 2008.

[95] E. Vander Haar, S.-I. Lee, S. Bandhakavi, T. J. Griffin, and D.-H. Kim, “Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40.,” *Nat. Cell Biol.*, vol. 9, no. 3, pp. 316–23, Mar. 2007.

[96] K. G. Foster, H. A. Acosta-Jaquez, Y. Romeo, B. Ekim, G. A. Soliman, A. Carriere, P. P. Roux, B. A. Ballif, and D. C. Fingar, “Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation.,” *J. Biol. Chem.*, vol. 285, no. 1, pp. 80–94, Jan. 2010.

[97] C. He and D. J. Klionsky, “Regulation mechanisms and signaling pathways of autophagy.,” *Annu. Rev. Genet.*, vol. 43, pp. 67–93, Jan. 2009.

[98] J. T. Cunningham, J. T. Rodgers, D. H. Arlow, F. Vazquez, V. K. Mootha, and P. Puigserver, “mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex.,” *Nature*, vol. 450, no. 7170, pp. 736–40, Nov. 2007.

[99] X. M. Ma and J. Blenis, “Molecular mechanisms of mTOR-mediated translational control.,” *Nat. Rev. Mol. Cell Biol.*, vol. 10, no. 5, pp. 307–18, May 2009.

[100] B. M. Zid, A. N. Rogers, S. D. Katewa, M. A. Vargas, M. C. Kolipinski, T. A. Lu, S. Benzer, and P. Kapahi, "4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila.," *Cell*, vol. 139, no. 1, pp. 149–60, Oct. 2009.

[101] D. G. Hardie, "AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy.," *Nat. Rev. Mol. Cell Biol.*, vol. 8, no. 10, pp. 774–85, Oct. 2007.

[102] H. Zong, J. M. Ren, L. H. Young, M. Pypaert, J. Mu, M. J. Birnbaum, and G. I. Shulman, "AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 99, no. 25, pp. 15983–7, Dec. 2002.

[103] S.-P. Hong and M. Carlson, "Regulation of snf1 protein kinase in response to environmental stress.," *J. Biol. Chem.*, vol. 282, no. 23, pp. 16838–45, Jun. 2007.

[104] M. P. DeYoung, P. Horak, A. Sofer, D. Sgroi, and L. W. Ellisen, "Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling.," *Genes Dev.*, vol. 22, no. 2, pp. 239–51, Jan. 2008.

[105] M. Høyer-Hansen, L. Bastholm, P. Szyniarowski, M. Campanella, G. Szabadkai, T. Farkas, K. Bianchi, N. Fehrenbacher, F. Elling, R. Rizzuto, I. S. Mathiasen, and M. Jäättelä, "Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2.," *Mol. Cell*, vol. 25, no. 2, pp. 193–205, Jan. 2007.

[106] S.-P. Hong, F. C. Leiper, A. Woods, D. Carling, and M. Carlson, "Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 100, no. 15, pp. 8839–43, Jul. 2003.

[107] R. R. McCartney, E. M. Rubenstein, and M. C. Schmidt, "Snf1 kinase complexes with different beta subunits display stress-dependent preferences for the three Snf1-activating kinases.," *Curr. Genet.*, vol. 47, no. 6, pp. 335–44, Jun. 2005.

[108] S.-P. Hong, M. Momcilovic, and M. Carlson, "Function of mammalian LKB1 and Ca2+/calmodulin-dependent protein kinase kinase alpha as Snf1-activating kinases in yeast.," *J. Biol. Chem.*, vol. 280, no. 23, pp. 21804–9, Jun. 2005.

[109] K. Hedbacker, S.-P. Hong, and M. Carlson, "Pak1 protein kinase regulates activation and nuclear localization of Snf1-Gal83 protein kinase.," *Mol. Cell. Biol.*, vol. 24, no. 18, pp. 8255–63, Sep. 2004.

[110] K. Elbing, R. R. McCartney, and M. C. Schmidt, "Purification and characterization of the three Snf1-activating kinases of Saccharomyces cerevisiae.," *Biochem. J.*, vol. 393, no. Pt 3, pp. 797–805, Feb. 2006.

[111] E. M. Rubenstein, R. R. McCartney, and M. C. Schmidt, "Regulatory domains of Snf1-activating kinases determine pathway specificity.," *Eukaryot. Cell*, vol. 5, no. 4, pp. 620–7, Apr. 2006.

[112] M.-D. Kim, S.-P. Hong, and M. Carlson, "Role of Tos3, a Snf1 protein kinase kinase, during growth of Saccharomyces cerevisiae on nonfermentable carbon sources.," *Eukaryot. Cell*, vol. 4, no. 5, pp. 861–6, May 2005.

[113] P. Sanz, G. R. Alms, T. A. Haystead, and M. Carlson, “Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase.,” *Mol. Cell. Biol.*, vol. 20, no. 4, pp. 1321–8, Feb. 2000.

[114] R. R. McCartney and M. C. Schmidt, “Regulation of Snf1 kinase. Activation requires phosphorylation of threonine 210 by an upstream kinase as well as a distinct step mediated by the Snf4 subunit.,” *J. Biol. Chem.*, vol. 276, no. 39, pp. 36460–6, Sep. 2001.

[115] K. Hedbacker and M. Carlson, “Regulation of the nucleocytoplasmic distribution of Snf1-Gal83 protein kinase.,” *Eukaryot. Cell*, vol. 5, no. 12, pp. 1950–6, Dec. 2006.

[116] J. L. DeRisi, V. R. Iyer, and P. O. Brown, “Exploring the metabolic and genetic control of gene expression on a genomic scale.,” *Science*, vol. 278, no. 5338, pp. 680–6, Oct. 1997.

[117] K. Hedbacker and M. Carlson, “SNF1/AMPK pathways in yeast.,” *Front. Biosci.*, vol. 13, pp. 2408–20, Jan. 2008.

[118] O. Vincent, R. Townley, S. Kuchin, and M. Carlson, “Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism.,” *Genes Dev.*, vol. 15, no. 9, pp. 1104–14, May 2001.

[119] K. Hedbacker, R. Townley, and M. Carlson, “Cyclic AMP-Dependent Protein Kinase Regulates the Subcellular Localization of Snf1-Sip1 Protein Kinase,” *Mol. Cell. Biol.*, vol. 24, no. 5, pp. 1836–1843, Feb. 2004.

[120] A. Affaitati, L. Cardone, T. de Cristofaro, A. Carlucci, M. D. Ginsberg, S. Varrone, M. E. Gottesman, E. V Avvedimento, and A. Feliciello, “Essential role of A-kinase anchor protein 121 for cAMP signaling to mitochondria.,” *J. Biol. Chem.*, vol. 278, no. 6, pp. 4286–94, Feb. 2003.

[121] Z. Technikova-Dobrova, A. M. Sardanelli, F. Speranza, S. Scacco, A. Signorile, V. Lorusso, and S. Papa, “Cyclic Adenosine Monophosphate-Dependent Phosphorylation of Mammalian Mitochondrial Proteins: Enzyme and Substrate Characterization and Functional Role †,” *Biochemistry*, vol. 40, no. 46, pp. 13941–13947, Nov. 2001.

[122] L. Hlavatá, H. Aguilaniu, A. Pichová, and T. Nyström, “The oncogenic RAS2(val19) mutation locks respiration, independently of PKA, in a mode prone to generate ROS.,” *EMBO J.*, vol. 22, no. 13, pp. 3337–45, Jul. 2003.

[123] J. Urban, A. Soulard, A. Huber, S. Lippman, D. Mukhopadhyay, O. Deloche, V. Wanke, D. Anrather, G. Ammerer, H. Riezman, J. R. Broach, C. De Virgilio, M. N. Hall, and R. Loewith, “Sch9 is a major target of TORC1 in Saccharomyces cerevisiae.,” *Mol. Cell*, vol. 26, no. 5, pp. 663–74, Jun. 2007.

[124] P. Jorgensen, I. Rupes, J. R. Sharom, L. Schneper, J. R. Broach, and M. Tyers, “A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size.,” *Genes Dev.*, vol. 18, no. 20, pp. 2491–505, Oct. 2004.

[125] G. Thomas and M. N. Hall, “TOR signalling and control of cell growth.,” *Curr. Opin. Cell Biol.*, vol. 9, no. 6, pp. 782–7, Dec. 1997.

[126] M. Mahalingam and D. J. Templeton, “Constitutive activation of S6 kinase by deletion of amino-terminal autoinhibitory and rapamycin sensitivity domains.,” *Mol. Cell. Biol.*, vol. 16, no. 1, pp. 405–13, Jan. 1996.

[127] I. Pedruzzi, F. Dubouloz, E. Cameroni, V. Wanke, J. Roosen, J. Winderickx, and C. De Virgilio, “TOR and PKA signaling pathways converge on the protein kinase Rim15 to control entry into G0.,” *Mol. Cell*, vol. 12, no. 6, pp. 1607–13, Dec. 2003.

[128] M. Kaeberlein, R. W. Powers, K. K. Steffen, E. A. Westman, D. Hu, N. Dang, E. O. Kerr, K. T. Kirkland, S. Fields, and B. K. Kennedy, “Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients.,” *Science*, vol. 310, no. 5751, pp. 1193–6, Nov. 2005.

[129] T. Schmelzle and M. N. Hall, “TOR, a central controller of cell growth.,” *Cell*, vol. 103, no. 2, pp. 253–62, Oct. 2000.

[130] C. J. Di Como and K. T. Arndt, “Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases.,” *Genes Dev.*, vol. 10, no. 15, pp. 1904–16, Aug. 1996.

[131] K. Düvel, A. Santhanam, S. Garrett, L. Schneper, and J. R. Broach, “Multiple roles of Tap42 in mediating rapamycin-induced transcriptional changes in yeast.,” *Mol. Cell*, vol. 11, no. 6, pp. 1467–78, Jun. 2003.

[132] Y. Jiang and J. R. Broach, “Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast.,” *EMBO J.*, vol. 18, no. 10, pp. 2782–92, May 1999.

[133] A. Schmidt, T. Beck, A. Koller, J. Kunz, and M. N. Hall, “The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease.,” *EMBO J.*, vol. 17, no. 23, pp. 6924–31, Dec. 1998.

[134] S. Inui, H. Sanjo, K. Maeda, H. Yamamoto, E. Miyamoto, and N. Sakaguchi, “Ig receptor binding protein 1 (alpha4) is associated with a rapamycin-sensitive signal transduction in lymphocytes through direct binding to the catalytic subunit of protein phosphatase 2A.,” *Blood*, vol. 92, no. 2, pp. 539–46, Jul. 1998.

[135] J. Chen, R. T. Peterson, and S. L. Schreiber, “Alpha 4 associates with protein phosphatases 2A, 4, and 6.,” *Biochem. Biophys. Res. Commun.*, vol. 247, no. 3, pp. 827–32, Jun. 1998.

[136] W. Görner, E. Durchschlag, J. Wolf, E. L. Brown, G. Ammerer, H. Ruis, and C. Schüller, “Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor.,” *EMBO J.*, vol. 21, no. 1–2, pp. 135–44, Jan. 2002.

[137] T. Beck and M. N. Hall, “The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors.,” *Nature*, vol. 402, no. 6762, pp. 689–92, Dec. 1999.

[138] M. E. Cardenas, N. S. Cutler, M. C. Lorenz, C. J. Di Como, and J. Heitman, “The TOR signaling cascade regulates gene expression in response to nutrients.,” *Genes Dev.*, vol. 13, no. 24, pp. 3271–9, Dec. 1999.

[139] E. Jacinto, B. Guo, K. T. Arndt, T. Schmelzle, and M. N. Hall, “TIP41 Interacts with TAP42 and Negatively Regulates the TOR Signaling Pathway,” *Mol. Cell*, vol. 8, no. 5, pp. 1017–1026, Nov. 2001.

[140] A. C. Gingras, B. Raught, and N. Sonenberg, “Regulation of translation initiation by FRAP/mTOR.,” *Genes Dev.*, vol. 15, no. 7, pp. 807–26, Apr. 2001.

[141] H. Wang, X. Wang, and Y. Jiang, “Interaction with Tap42 Is Required for the Essential Function of Sit4 and Type 2A Phosphatases,” vol. 14, no. November, pp. 4342–4351, 2003.

[142] A. A. Sneddon, P. T. Cohen, and M. J. Stark, “Saccharomyces cerevisiae protein phosphatase 2A performs an essential cellular function and is encoded by two genes.,” *EMBO J.*, vol. 9, no. 13, pp. 4339–46, Dec. 1990.

[143] A. Sutton, D. Immanuel, and K. T. Arndt, “The SIT4 protein phosphatase functions in late G1 for progression into S phase.,” *Mol. Cell. Biol.*, vol. 11, no. 4, pp. 2133–48, Apr. 1991.

[144] H. Ronne, M. Carlberg, G. Z. Hu, and J. O. Nehlin, “Protein phosphatase 2A in Saccharomyces cerevisiae: effects on cell growth and bud morphogenesis.,” *Mol. Cell. Biol.*, vol. 11, no. 10, pp. 4876–84, Oct. 1991.

[145] W. van Zyl, W. Huang, A. A. Sneddon, M. Stark, S. Camier, M. Werner, C. Marck, A. Sentenac, and J. R. Broach, “Inactivation of the protein phosphatase 2A regulatory subunit A results in morphological and transcriptional defects in Saccharomyces cerevisiae.,” *Mol. Cell. Biol.*, vol. 12, no. 11, pp. 4946–4959, Nov. 1992.

[146] Y. Shu, H. Yang, E. Hallberg, and R. Hallberg, “Molecular genetic analysis of Rts1p, a B’ regulatory subunit of Saccharomyces cerevisiae protein phosphatase 2A.,” *Mol. Cell. Biol.*, vol. 17, no. 6, pp. 3242–53, Jun. 1997.

[147] A. M. Healy, S. Zolnierowicz, A. E. Stapleton, M. Goebl, A. A. DePaoli-Roach, and J. R. Pringle, “CDC55, a Saccharomyces cerevisiae gene involved in cellular morphogenesis: identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase.,” *Mol. Cell. Biol.*, vol. 11, no. 11, pp. 5767–80, Nov. 1991.

[148] M. Luke, F. Della Seta, C. Di Como, H. Sugimoto, R. Kobayashi, and K. Arndt, “The SAP, a new family of proteins, associate and function positively with the SIT4 phosphatase,” *Mol. Cell. Biol.*, vol. 16, no. 6, pp. 2744–2755, Jun. 1996.

[149] M. J. Fernandez-Sarabia, A. Sutton, T. Zhong, and K. T. Arndt, “SIT4 protein phosphatase is required for the normal accumulation of SWI4, CLN1, CLN2, and HCS26 RNAs during late G1.,” *Genes Dev.*, vol. 6, no. 12a, pp. 2417–2428, Dec. 1992.

[150] M. Kong, D. Ditsworth, T. Lindsten, and C. B. Thompson, “Alpha4 is an essential regulator of PP2A phosphatase activity.,” *Mol. Cell*, vol. 36, no. 1, pp. 51–60, Oct. 2009.

[151] P. G. Bertram, J. H. Choi, J. Carvalho, W. Ai, C. Zeng, T. F. Chan, and X. F. Zheng, “Tripartite regulation of Gln3p by TOR, Ure2p, and phosphatases.,” *J. Biol. Chem.*, vol. 275, no. 46, pp. 35727–33, Nov. 2000.

[152] G. Reid and G. Schatz, “Import of proteins into mitochondria. Yeast cells grown in the presence of carbonyl cyanide m-chlorophenylhydrazone accumulate massive amounts of some mitochondrial precursor polypeptides,” *J. Biol. Chem.*, vol. 257, no. 21, pp. 13056–13061, Nov. 1982.

[153] C. D. Dunn, “The genetic requirements of budding yeast lacking a mitochondrial genome,” Johns Hopkins School of Medicine, 2006.

[154] P. Sass, J. Field, J. Nikawa, T. Toda, and M. Wigler, “Cloning and characterization of the high-affinity cAMP phosphodiesterase of Saccharomyces cerevisiae.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 83, no. 24, pp. 9303–7, Dec. 1986.

[155] E. A. Schon, S. DiMauro, M. Hirano, and R. W. Gilkerson, “Therapeutic prospects for mitochondrial disease.,” *Trends Mol. Med.*, vol. 16, no. 6, pp. 268–76, Jun. 2010.

[156] S. C. Johnson, M. E. Yanos, E.-B. Kayser, A. Quintana, M. Sangesland, A. Castanza, L. Uhde, J. Hui, V. Z. Wall, A. Gagnidze, K. Oh, B. M. Wasko, F. J. Ramos, R. D. Palmiter, P. S. Rabinovitch, P. G. Morgan, M. M. Sedensky, and M. Kaeberlein, “mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome.,” *Science*, vol. 342, no. 6165, pp. 1524–8, Dec. 2013.

[157] R. K. Dagda, A. M. Gusdon, I. Pien, S. Strack, S. Green, C. Li, B. Van Houten, S. J. Cherra, and C. T. Chu, “Mitochondrially localized PKA reverses mitochondrial pathology and dysfunction in a cellular model of Parkinson’s disease.,” *Cell Death Differ.*, vol. 18, no. 12, pp. 1914–23, Dec. 2011.

[158] G. Garipler, N. Mutlu, N. A. Lack, and C. D. Dunn, “Deletion of conserved protein phosphatases reverses defects associated with mitochondrial DNA damage in Saccharomyces cerevisiae.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 4, pp. 1473–8, Jan. 2014.

[159] Y. Jiang, “Regulation of the cell cycle by protein phosphatase 2A in Saccharomyces cerevisiae.,” *Microbiol. Mol. Biol. Rev.*, vol. 70, no. 2, pp. 440–9, Jun. 2006.

[160] A. Ruiz, X. Xu, and M. Carlson, “Roles of two protein phosphatases, Reg1-Glc7 and Sit4, and glycogen synthesis in regulation of SNF1 protein kinase.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 16, pp. 6349–54, Apr. 2011.

[161] M. A. Treitel, S. Kuchin, and M. Carlson, “Snf1 Protein Kinase Regulates Phosphorylation of the Mig1 Repressor in Saccharomyces cerevisiae,” *Mol. Cell. Biol.*, vol. 18, no. 11, pp. 6273–6280, Nov. 1998.

[162] A. Komeili, K. P. Wedaman, E. K. O’Shea, and T. Powers, “Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors.,” *J. Cell Biol.*, vol. 151, no. 4, pp. 863–78, Nov. 2000.

[163] K. P. Byrne and K. H. Wolfe, “The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species.,” *Genome Res.*, vol. 15, no. 10, pp. 1456–61, Oct. 2005.

[164] T. Schmelzle, T. Beck, D. E. Martin, and M. N. Hall, "Activation of the RAS/Cyclic AMP Pathway Suppresses a TOR Deficiency in Yeast," *Mol. Cell. Biol.*, vol. 24, no. 1, pp. 338–351, Dec. 2003.

[165] J. A. Coffman, R. Rai, T. Cunningham, V. Svetlov, and T. G. Cooper, "Gat1p, a GATA family protein whose production is sensitive to nitrogen catabolite repression, participates in transcriptional activation of nitrogen-catabolic genes in Saccharomyces cerevisiae.," *Mol. Cell. Biol.*, vol. 16, no. 3, pp. 847–58, Mar. 1996.

[166] M. N. Miranda, C. A. Masuda, A. Ferreira-Pereira, E. Carvajal, M. Ghislain, and M. Montero-Lomelí, "The serine/threonine protein phosphatase Sit4p activates multidrug resistance in Saccharomyces cerevisiae.," *FEMS Yeast Res.*, vol. 10, no. 6, pp. 674–86, Sep. 2010.

[167] P. A. Padilla, E. K. Fuge, M. E. Crawford, A. Errett, and M. Werner-Washburne, "The highly conserved, coregulated SNO and SNZ gene families in Saccharomyces cerevisiae respond to nutrient limitation.," *J. Bacteriol.*, vol. 180, no. 21, pp. 5718–26, Nov. 1998.

[168] Y.-X. Dong, S. Sueda, J.-I. Nikawa, and H. Kondo, "Characterization of the products of the genes SNO1 and SNZ1 involved in pyridoxine synthesis in Saccharomyces cerevisiae," *Eur. J. Biochem.*, vol. 271, no. 4, pp. 745–752, Feb. 2004.

[169] S. Vidan and A. P. Mitchell, "Stimulation of yeast meiotic gene expression by the glucose-repressible protein kinase Rim15p.," *Mol. Cell. Biol.*, vol. 17, no. 5, pp. 2688–97, May 1997.

[170] E. L. Braun, E. K. Fuge, P. A. Padilla, and M. Werner-Washburne, "A stationary-phase gene in Saccharomyces cerevisiae is a member of a novel, highly conserved gene family.," *J. Bacteriol.*, vol. 178, no. 23, pp. 6865–72, Dec. 1996.

[171] F. Messenguy, "Regulation of arginine biosynthesis in Saccharomyces cerevisiae: isolation of a cis-dominant, constitutive mutant for ornithine carbamoyltransferase synthesis.," *J. Bacteriol.*, vol. 128, no. 1, pp. 49–55, Oct. 1976.

[172] A. Jézégou, E. Llinares, C. Anne, S. Kieffer-Jaquinod, S. O'Regan, J. Aupetit, A. Chabli, C. Sagné, C. Debacker, B. Chadefaux-Vekemans, A. Journet, B. André, and B. Gasnier, "Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109, no. 50, pp. E3434–43, Dec. 2012.

[173] M. C. V Donaton, I. Holsbeeks, O. Lagatie, G. Van Zeebroeck, M. Crauwels, J. Winderickx, and J. M. Thevelein, "The Gap1 general amino acid permease acts as an amino acid sensor for activation of protein kinase A targets in the yeast Saccharomyces cerevisiae.," *Mol. Microbiol.*, vol. 50, no. 3, pp. 911–29, Nov. 2003.

[174] J. O. De Craene, O. Soetens, and B. Andre, "The Npr1 kinase controls biosynthetic and endocytic sorting of the yeast Gap1 permease.," *J. Biol. Chem.*, vol. 276, no. 47, pp. 43939–48, Nov. 2001.

[175] S. Brückner, S. Kern, R. Birke, I. Saugar, H. D. Ulrich, and H.-U. Mösch, "The TEA transcription factor Tec1 links TOR and MAPK pathways to coordinate yeast development.," *Genetics*, vol. 189, no. 2, pp. 479–94, Oct. 2011.

[176] V. A. Cherkasova and A. G. Hinnebusch, “Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2.,” *Genes Dev.*, vol. 17, no. 7, pp. 859–72, Apr. 2003.

[177] T. E. Dever, L. Feng, R. C. Wek, A. M. Cigan, T. F. Donahue, and A. G. Hinnebusch, “Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast.,” *Cell*, vol. 68, no. 3, pp. 585–96, Feb. 1992.

[178] W. Jablonka, S. Guzmán, J. Ramírez, and M. Montero-Lomelí, “Deviation of carbohydrate metabolism by the SIT4 phosphatase in Saccharomyces cerevisiae.,” *Biochim. Biophys. Acta*, vol. 1760, no. 8, pp. 1281–91, Aug. 2006.

[179] Y. Oshima, “The phosphatase system in Saccharomyces cerevisiae.,” *Genes Genet. Syst.*, vol. 72, no. 6, pp. 323–34, Dec. 1997.

[180] N. Ogawa, J. DeRisi, and P. O. Brown, “New components of a system for phosphate accumulation and polyphosphate metabolism in Saccharomyces cerevisiae revealed by genomic expression analysis.,” *Mol. Biol. Cell*, vol. 11, no. 12, pp. 4309–21, Dec. 2000.

[181] R. B. Walsh, G. Kawasaki, and D. G. Fraenkel, “Cloning of genes that complement yeast hexokinase and glucokinase mutants.,” *J. Bacteriol.*, vol. 154, no. 2, pp. 1002–4, May 1983.

[182] G. M. Santangelo, “Glucose signaling in Saccharomyces cerevisiae.,” *Microbiol. Mol. Biol. Rev.*, vol. 70, no. 1, pp. 253–82, Mar. 2006.

[183] J. BROACH, “RAS genes in Saccharomyces cerevisiae: signal transduction in search of a pathway,” *Trends Genet.*, vol. 7, no. 1, pp. 28–33, Jan. 1991.

[184] L. Barrett, M. Orlova, M. Maziarz, and S. Kuchin, “Protein kinase A contributes to the negative control of Snf1 protein kinase in Saccharomyces cerevisiae.,” *Eukaryot. Cell*, vol. 11, no. 2, pp. 119–28, Feb. 2012.

[185] A. Smith, M. P. Ward, and S. Garrett, “Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation.,” *EMBO J.*, vol. 17, no. 13, pp. 3556–64, Jul. 1998.

[186] B. Bodenmiller, S. Wanka, C. Kraft, J. Urban, D. Campbell, P. G. Pedrioli, B. Gerrits, P. Picotti, H. Lam, O. Vitek, M.-Y. Brusniak, B. Roschitzki, C. Zhang, K. M. Shokat, R. Schlapbach, A. Colman-Lerner, G. P. Nolan, A. I. Nesvizhskii, M. Peter, R. Loewith, C. von Mering, and R. Aebersold, “Phosphoproteomic analysis reveals interconnected system-wide responses to perturbations of kinases and phosphatases in yeast.,” *Sci. Signal.*, vol. 3, no. 153, p. rs4, Jan. 2010.

[187] N. Bouquin, Y. Barral, R. Courbeyrette, M. Blondel, M. Snyder, and C. Mann, “Regulation of cytokinesis by the Elm1 protein kinase in Saccharomyces cerevisiae.,” *J. Cell Sci.*, vol. 113 (Pt 8, pp. 1435–45, Apr. 2000.

[188] “SGD Project.” [Online]. Available: http://www.yeastgenome.org/analyze#enrichment.

[189] A. Sickmann, J. Reinders, Y. Wagner, C. Joppich, R. Zahedi, H. E. Meyer, B. Schönfisch, I. Perschil, A. Chacinska, B. Guiard, P. Rehling, N. Pfanner, and C. Meisinger, "The proteome of Saccharomyces cerevisiae mitochondria.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 100, no. 23, pp. 13207–12, Nov. 2003.

[190] J. Reinders, K. Wagner, R. P. Zahedi, D. Stojanovski, B. Eyrich, M. van der Laan, P. Rehling, A. Sickmann, N. Pfanner, and C. Meisinger, "Profiling phosphoproteins of yeast mitochondria reveals a role of phosphorylation in assembly of the ATP synthase.," *Mol. Cell. Proteomics*, vol. 6, no. 11, pp. 1896–906, Nov. 2007.

[191] A. Adams, D. Gottschling, C. Kaiser, and T. Stearns, "Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual," 1998.

[192] R. S. Sikorski and P. Hieter, "A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces cerevisiae," *Genetics*, vol. 122, no. 1, pp. 19–27, May 1989.

[193] C. B. Brachmann, A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter, and J. D. Boeke, "Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications.," *Yeast*, vol. 14, no. 2, pp. 115–32, Jan. 1998.

[194] C. D. Dunn, M. S. Lee, F. A. Spencer, and R. E. Jensen, "A genomewide screen for petite-negative yeast strains yields a new subunit of the i-AAA protease complex.," *Mol. Biol. Cell*, vol. 17, no. 1, pp. 213–26, Jan. 2006.

[195] H. Lange, G. Kispal, and R. Lill, "Mechanism of Iron Transport to the Site of Heme Synthesis inside Yeast Mitochondria," *J. Biol. Chem.*, vol. 274, no. 27, pp. 18989–18996, Jul. 1999.

[196] A. Chacinska, C. M. Koehler, D. Milenkovic, T. Lithgow, and N. Pfanner, "Importing mitochondrial proteins: machineries and mechanisms.," *Cell*, vol. 138, no. 4, pp. 628–44, Aug. 2009.

[197] X. Wang, X. Zuo, B. Kucejova, and X. J. Chen, "Reduced cytosolic protein synthesis suppresses mitochondrial degeneration.," *Nat. Cell Biol.*, vol. 10, no. 9, pp. 1090–7, Sep. 2008.

[198] A. Spinazzola and M. Zeviani, "Disorders of nuclear-mitochondrial intergenomic signaling.," *Gene*, vol. 354, pp. 162–8, Jul. 2005.

[199] E. Pebay-Peyroula, C. Dahout-Gonzalez, R. Kahn, V. Trézéguet, G. J.-M. Lauquin, and G. Brandolin, "Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside.," *Nature*, vol. 426, no. 6962, pp. 39–44, Nov. 2003.

[200] M. D. Brand, J. L. Pakay, A. Ocloo, J. Kokoszka, D. C. Wallace, P. S. Brookes, and E. J. Cornwall, "The basal proton conductance of mitochondria depends on adenine nucleotide translocase content.," *Biochem. J.*, vol. 392, no. Pt 2, pp. 353–62, Dec. 2005.

[201] C.-Y. Lai, E. Jaruga, C. Borghouts, and S. M. Jazwinski, “A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae.,” *Genetics*, vol. 162, no. 1, pp. 73–87, Sep. 2002.

[202] N. Tavernarakis, “Ageing and the regulation of protein synthesis: a balancing act?,” *Trends Cell Biol.*, vol. 18, no. 5, pp. 228–35, May 2008.

[203] J. C. Jiang, E. Jaruga, M. V Repnevskaya, and S. M. Jazwinski, “An intervention resembling caloric restriction prolongs life span and retards aging in yeast.,” *FASEB J.*, vol. 14, no. 14, pp. 2135–7, Nov. 2000.

[204] K. K. Steffen, V. L. MacKay, E. O. Kerr, M. Tsuchiya, D. Hu, L. A. Fox, N. Dang, E. D. Johnston, J. A. Oakes, B. N. Tchao, D. N. Pak, S. Fields, B. K. Kennedy, and M. Kaeberlein, “Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4.,” *Cell*, vol. 133, no. 2, pp. 292–302, Apr. 2008.

[205] A. Chiocchetti, J. Zhou, H. Zhu, T. Karl, O. Haubenreisser, M. Rinnerthaler, G. Heeren, K. Oender, J. Bauer, H. Hintner, M. Breitenbach, and L. Breitenbach-Koller, “Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span.,” *Exp. Gerontol.*, vol. 42, no. 4, pp. 275–86, Apr. 2007.

[206] Z. H. Shah, K. M. O’Dell, S. C. Miller, X. An, and H. T. Jacobs, “Metazoan nuclear genes for mitoribosomal protein S12.,” *Gene*, vol. 204, no. 1–2, pp. 55–62, Dec. 1997.

[207] J. M. Toivonen, S. Manjiry, S. Touraille, S. Alziari, K. M. C. O’Dell, and H. T. Jacobs, “Gene dosage and selective expression modify phenotype in a Drosophila model of human mitochondrial disease.,” *Mitochondrion*, vol. 3, no. 2, pp. 83–96, Oct. 2003.

[208] S. Benzer, “From the Gene to Behavior,” *JAMA J. Am. Med. Assoc.*, vol. 218, no. 7, p. 1015, Nov. 1971.

[209] T. Fergestad, B. Bostwick, and B. Ganetzky, “Metabolic disruption in Drosophila bang-sensitive seizure mutants.,” *Genetics*, vol. 173, no. 3, pp. 1357–64, Jul. 2006.

[210] Y. Q. Zhang, J. Roote, S. Brogna, A. W. Davis, D. A. Barbash, D. Nash, and M. Ashburner, “stress sensitive B encodes an adenine nucleotide translocase in Drosophila melanogaster.,” *Genetics*, vol. 153, no. 2, pp. 891–903, Oct. 1999.

[211] D. F. Eberl, G. M. Duyk, and N. Perrimon, “A genetic screen for mutations that disrupt an auditory response in Drosophila melanogaster.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, no. 26, pp. 14837–42, Dec. 1997.

[212] D. E. Sterner and S. L. Berger, “Acetylation of Histones and Transcription-Related Factors,” *Microbiol. Mol. Biol. Rev.*, vol. 64, no. 2, pp. 435–459, Jun. 2000.

[213] A. Eberharter and P. B. Becker, “Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics.,” *EMBO Rep.*, vol. 3, no. 3, pp. 224–9, Mar. 2002.

[214] S. E. Rundlett, A. A. Carmen, R. Kobayashi, S. Bavykin, B. M. Turner, and M. Grunstein, "HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription," *Proc. Natl. Acad. Sci.*, vol. 93, no. 25, pp. 14503–14508, Dec. 1996.

[215] P. P. Slonimski, G. Perrodin, and J. H. Croft, "Ethidium bromide induced mutation of yeast mitochondria: Complete transformation of cells into respiratory deficient non-chromosomal 'petites,'" *Biochem. Biophys. Res. Commun.*, vol. 30, no. 3, pp. 232–239, Feb. 1968.

[216] J. R. Rohde, M. E. Cardenas, and N. Carolina, "The Tor Pathway Regulates Gene Expression by Linking Nutrient Sensing to Histone Acetylation," vol. 23, no. 2, pp. 629–635, 2003.

[217] D. Yeheskely-Hayon, A. Kotler, M. Stark, T. Hashimshony, S. Sagee, and Y. Kassir, "The roles of the catalytic and noncatalytic activities of Rpd3L and Rpd3S in the regulation of gene transcription in yeast.," *PLoS One*, vol. 8, no. 12, p. e85088, Jan. 2013.

[218] M. J. Carrozza, L. Florens, S. K. Swanson, W.-J. Shia, S. Anderson, J. Yates, M. P. Washburn, and J. L. Workman, "Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex.," *Biochim. Biophys. Acta*, vol. 1731, no. 2, pp. 77–87; discussion 75–6, Nov. 2005.

[219] D. Vannier, D. Balderes, and D. Shore, "Evidence That the Transcriptional Regulators SIN3 and RPD3, and a Novel Gene (SDS3) with Similar Functions, Are Involved in Transcriptional Silencing in S. cerevisiae," *Genetics*, vol. 144, no. 4, pp. 1343–1353, Dec. 1996.

[220] M. J. Carrozza, B. Li, L. Florens, T. Suganuma, S. K. Swanson, K. K. Lee, W.-J. Shia, S. Anderson, J. Yates, M. P. Washburn, and J. L. Workman, "Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription.," *Cell*, vol. 123, no. 4, pp. 581–92, Nov. 2005.

[221] M. Crauwels, M. C. V. Donaton, M. B. Pernambuco, J. Winderickx, J. H. de Winde, and J. M. Thevelein, "The Sch9 protein kinase in the yeast Saccharomyces cerevisiae controls cAPK activity and is required for nitrogen activation of the fermentable-growth-medium-induced (FGM) pathway," *Microbiology*, vol. 143, no. 8, pp. 2627–2637, Aug. 1997.

[222] A. Huber, S. L. French, H. Tekotte, S. Yerlikaya, M. Stahl, M. P. Perepelkina, M. Tyers, J. Rougemont, A. L. Beyer, and R. Loewith, "Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L.," *EMBO J.*, vol. 30, no. 15, pp. 3052–64, Aug. 2011.

[223] M. S. Singer, A. Kahana, A. J. Wolf, L. L. Meisinger, S. E. Peterson, C. Goggin, M. Mahowald, and D. E. Gottschling, "Identification of high-copy disruptors of telomeric silencing in Saccharomyces cerevisiae.," *Genetics*, vol. 150, no. 2, pp. 613–32, Oct. 1998.

[224] D. J. Kominsky, M. P. Brownson, D. L. Updike, and P. E. Thorsness, "Genetic and Biochemical Basis for Viability of Yeast Lacking Mitochondrial Genomes," *Genetics*, vol. 162, no. 4, pp. 1595–1604, Dec. 2002.

[225] E. R. Weber, R. S. Rooks, K. S. Shafer, J. W. Chase, and P. E. Thorsness, “Mutations in the Mitochondrial ATP Synthase Gamma Subunit Suppress a Slow-Growth Phenotype of yme1 Yeast Lacking Mitochondrial DNA,” *Genetics*, vol. 140, no. 2, pp. 435–442, Jun. 1995.

[226] S. Rogers, R. Wells, and M. Rechsteiner, “Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis,” *Science (80-.).*, vol. 234, no. 4774, pp. 364–368, Oct. 1986.

[227] F. R. Cross and C. M. Blake, “The yeast Cln3 protein is an unstable activator of Cdc28.,” *Mol. Cell. Biol.*, vol. 13, no. 6, pp. 3266–71, Jun. 1993.

[228] L. Shi and B. P. Tu, “Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote entry into the cell division cycle in Saccharomyces cerevisiae.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 18, pp. 7318–23, Apr. 2013.

[229] R. Nash, G. Tokiwa, S. Anand, K. Erickson, and A. B. Futcher, “The WHI1+ gene of Saccharomyces cerevisiae tethers cell division to cell size and is a cyclin homolog.,” *EMBO J.*, vol. 7, no. 13, pp. 4335–46, Dec. 1988.

[230] J. B. Walker, “ARTICLE : CANAVANINE AND HOMOARGININE AS ANTIMETABOLITES OF ARGININE AND LYSINE IN YEAST AND ALGAE,” 1955.

[231] J. Tu and M. Carlson, “REG1 binds to protein phosphatase type 1 and regulates glucose repression in Saccharomyces cerevisiae.,” *EMBO J.*, vol. 14, no. 23, pp. 5939–46, Dec. 1995.

[232] J. Kiefer, S. Rase, E. Schneider, H. Straaten, G. Kraft, and H. Liesem, “Heavy Ion Effects on Yeast Cells: Induction of Canavanine-resistant Mutants,” *Int. J. Radiat. Biol.*, vol. 42, no. 6, pp. 591–600, Jan. 1982.

[233] E. W. Trotter, L. Berenfeld, S. a Krause, G. a Petsko, and J. V Gray, “Protein misfolding and temperature up-shift cause G1 arrest via a common mechanism dependent on heat shock factor in Saccharomycescerevisiae.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 98, no. 13, pp. 7313–8, Jun. 2001.

[234] E. K. Hudson, B. A. Hogue, N. C. Souza-Pinto, D. L. Croteau, R. M. Anson, V. A. Bohr, and R. G. Hansford, “Age-associated change in mitochondrial DNA damage,” *Free Radic. Res.*, vol. 29, no. 6, pp. 573–579, Jan. 1998.

[235] T. Finkel and N. J. Holbrook, “Oxidants, oxidative stress and the biology of ageing.,” *Nature*, vol. 408, no. 6809, pp. 239–47, Nov. 2000.

[236] E. Fehrenbach and H. Northoff, “Free radicals, exercise, apoptosis, and heat shock proteins.,” *Exerc. Immunol. Rev.*, vol. 7, pp. 66–89, Jan. 2001.

[237] U. Feige and B. S. Polla, “Hsp70--a multi-gene, multi-structure, multi-function family with potential clinical applications.,” *Experientia*, vol. 50, no. 11–12, pp. 979–86, Nov. 1994.

[238] E. J. Patriarca and B. Maresca, “Acquired thermotolerance following heat shock protein synthesis prevents impairment of mitochondrial ATPase activity at elevated temperatures in Saccharomyces cerevisiae.,” *Exp. Cell Res.*, vol. 190, no. 1, pp. 57–64, Sep. 1990.

[239] J. M. Rossi and S. Lindquist, “The intracellular location of yeast heat-shock protein 26 varies with metabolism.,” *J. Cell Biol.*, vol. 108, no. 2, pp. 425–39, Feb. 1989.

[240] C. E. Deutch and J. M. Parry, “Sphaeroplast Formation in Yeast During the Transition from Exponential Phase to Stationary Phase,” *J. Gen. Microbiol.*, vol. 80, no. 1, pp. 259–268, Jan. 1974.

[241] A. Schenberg-Frascino and E. Moustacchi, “Lethal and mutagenic effects of elevated temperature on haploid yeast,” *MGG Mol. Gen. Genet.*, vol. 115, no. 3, 1972.

[242] N. C. Collier, J. Heuser, M. A. Levy, and M. J. Schlesinger, “Ultrastructural and biochemical analysis of the stress granule in chicken embryo fibroblasts.,” *J. Cell Biol.*, vol. 106, no. 4, pp. 1131–9, Apr. 1988.

[243] A. P. Arrigo and C. Ahmad-Zadeh, “Immunofluorescence localization of a small heat shock protein (hsp 23) in salivary gland cells of Drosophila melanogaster.,” *Mol. Gen. Genet.*, vol. 184, no. 1, pp. 73–9, Jan. 1981.

[244] F. L. J. L. Duband, “Expression and localization of hsp-23 in unstressed and heat-shocked Drosophila cultured cells,” *Can. J. Genet. Cytol.*, vol. 28, no. 6, pp. 1088 – 1092, 2011.

[245] S. Welker, B. Rudolph, E. Frenzel, F. Hagn, G. Liebisch, G. Schmitz, J. Scheuring, A. Kerth, A. Blume, S. Weinkauf, M. Haslbeck, H. Kessler, and J. Buchner, “Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function.,” *Mol. Cell*, vol. 39, no. 4, pp. 507–20, Aug. 2010.

[246] M. Haslbeck, S. Walke, T. Stromer, M. Ehrnsperger, H. E. White, S. Chen, H. R. Saibil, and J. Buchner, “Hsp26: a temperature-regulated chaperone.,” *EMBO J.*, vol. 18, no. 23, pp. 6744–51, Dec. 1999.

[247] C. R. Johnson, T. Jiffar, U. M. Fischer, P. P. Ruvolo, and W. D. Jarvis, “Requirement for SAPK-JNK signaling in the induction of apoptosis by ribosomal stress in REH lymphoid leukemia cells.,” *Leukemia*, vol. 17, no. 11, pp. 2140–8, Nov. 2003.

[248] F. Legros, A. Lombès, P. Frachon, and M. Rojo, “Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins.,” *Mol. Biol. Cell*, vol. 13, no. 12, pp. 4343–54, Dec. 2002.

[249] D. A. Parsell, A. S. Kowal, M. A. Singer, and S. Lindquist, “Protein disaggregation mediated by heat-shock protein Hsp104.,” *Nature*, vol. 372, no. 6505, pp. 475–8, Dec. 1994.

[250] Y. Sanchez, J. Taulien, K. A. Borkovich, and S. Lindquist, “Hsp104 is required for tolerance to many forms of stress.,” *EMBO J.*, vol. 11, no. 6, pp. 2357–64, Jun. 1992.

[251] M. Werner-Washburne, D. E. Stone, and E. A. Craig, “Complex interactions among members of an essential subfamily of hsp70 genes in Saccharomyces cerevisiae.,” *Mol. Cell. Biol.*, vol. 7, no. 7, pp. 2568–77, Jul. 1987.

[252] M. T. Martínez-Pastor, G. Marchler, C. Schüller, A. Marchler-Bauer, H. Ruis, and F. Estruch, "The Saccharomyces cerevisiae zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE).," *EMBO J.*, vol. 15, no. 9, pp. 2227–35, May 1996.

[253] H. C. Causton, B. Ren, S. S. Koh, C. T. Harbison, E. Kanin, E. G. Jennings, T. I. Lee, H. L. True, E. S. Lander, and R. A. Young, "Remodeling of yeast genome expression in response to environmental changes.," *Mol. Biol. Cell*, vol. 12, no. 2, pp. 323–37, Feb. 2001.

[254] A. P. Gasch, P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown, "Genomic expression programs in the response of yeast cells to environmental changes.," *Mol. Biol. Cell*, vol. 11, no. 12, pp. 4241–57, Dec. 2000.

[255] S. Hohmann and W. H. Mager, *Yeast Stress Responses*, vol. 1. Berlin, Heidelberg: Springer Berlin Heidelberg, 2003.

[256] C. Klockow, F. Stahl, T. Scheper, and B. Hitzmann, "Induction of Stress Genes in Saccharomyces cerevisiae during Starvation," *Eng. Life Sci.*, vol. 8, no. 4, pp. 431–437, Aug. 2008.

[257] A. Yamamoto, Y. Mizukami, and H. Sakurai, "Identification of a novel class of target genes and a novel type of binding sequence of heat shock transcription factor in Saccharomyces cerevisiae.," *J. Biol. Chem.*, vol. 280, no. 12, pp. 11911–9, Mar. 2005.

[258] S. Nicholls, D. M. MacCallum, F. A. R. Kaffarnik, L. Selway, S. C. Peck, and A. J. P. Brown, "Activation of the heat shock transcription factor Hsf1 is essential for the full virulence of the fungal pathogen Candida albicans.," *Fungal Genet. Biol.*, vol. 48, no. 3, pp. 297–305, Mar. 2011.

[259] C. B. Bridges, 1889-1938, T. H. Morgan, and 1866-1945, "third-chromosome group of mutant characters of Drosophila melanogaster," 1923.

[260] S. Saeboe-larssen and A. Lambertsson, "A Novel Drosophila," 1996.

[261] G. Morata and P. Ripoll, "Minutes: Mutants of Drosophila autonomously affecting cell division rate," *Dev. Biol.*, vol. 42, no. 2, pp. 211–221, Feb. 1975.

[262] J. Alonso and J. F. Santarén, "Characterization of the Drosophila melanogaster ribosomal proteome.," *J. Proteome Res.*, vol. 5, no. 8, pp. 2025–32, Aug. 2006.

[263] D. M. 3rd Wilson, W. A. Deutsch, and M. R. Kelley, "Drosophila ribosomal protein S3 contains an activity that cleaves DNA at apurinic/apyrimidinic sites.," *J. Biol. Chem.*, vol. 269, no. 41, pp. 25359–25364, Oct. 1994.

[264] A. Ferrus, "PARAMETERS OF MITOTIC RECOMBINATION IN MINUTE MUTANTS OF DROSOPHILA MELANOGASTER," *Genetics*, vol. 79, no. 4, pp. 589–599, Apr. 1975.

[265] T. J. Parry, J. W. M. Theisen, J.-Y. Hsu, Y.-L. Wang, D. L. Corcoran, M. Eustice, U. Ohler, and J. T. Kadonaga, "The TCT motif, a key component of an RNA polymerase II transcription system for the translational machinery.," *Genes Dev.*, vol. 24, no. 18, pp. 2013–8, Sep. 2010.

[266] S. J. Marygold, J. Roote, G. Reuter, A. Lambertsson, M. Ashburner, G. H. Millburn, P. M. Harrison, Z. Yu, N. Kenmochi, T. C. Kaufman, S. J. Leevers, and K. R. Cook, "The ribosomal protein genes and Minute loci of Drosophila melanogaster.," *Genome Biol.*, vol. 8, no. 10, p. R216, Jan. 2007.

[267] K. N. Rugjee, S. Roy Chaudhury, K. Al-Jubran, P. Ramanathan, T. Matina, J. Wen, and S. Brogna, "Fluorescent protein tagging confirms the presence of ribosomal proteins at Drosophila polytene chromosomes.," *PeerJ*, vol. 1, p. e15, Jan. 2013.

[268] M. van Beest, "Drosophila RpS3a, a novel Minute gene situated between the segment polarity genescubitus interruptus and dTCF," *Nucleic Acids Res.*, vol. 26, no. 19, pp. 4471–4475, Oct. 1998.

[269] G. Goshima, R. Wollman, S. S. Goodwin, N. Zhang, J. M. Scholey, R. D. Vale, and N. Stuurman, "Genes required for mitotic spindle assembly in Drosophila S2 cells.," *Science*, vol. 316, no. 5823, pp. 417–21, Apr. 2007.

[270] G. M. Farnsworth and A. W. Nordskog, "Breeding for Egg Quality: 3. Genetic Differences in Shell Characteristics and Other Egg Quality Factors," *Poult. Sci.*, vol. 34, no. 1, pp. 16–26, Jan. 1955.

[271] D. J. M. Fernández-Ayala, S. Chen, E. Kemppainen, K. M. C. O'Dell, and H. T. Jacobs, "Gene expression in a Drosophila model of mitochondrial disease.," *PLoS One*, vol. 5, no. 1, p. e8549, Jan. 2010.

[272] S. Chen, M. T. Oliveira, A. Sanz, E. Kemppainen, A. Fukuoh, B. Schlicht, L. S. Kaguni, and H. T. Jacobs, "A cytoplasmic suppressor of a nuclear mutation affecting mitochondrial functions in Drosophila.," *Genetics*, vol. 192, no. 2, pp. 483–93, Oct. 2012.

[273] G. Morrow, M. Samson, S. Michaud, and R. M. Tanguay, "Overexpression of the small mitochondrial Hsp22 extends Drosophila life span and increases resistance to oxidative stress.," *FASEB J.*, vol. 18, no. 3, pp. 598–9, Mar. 2004.

[274] C. M. Haynes and D. Ron, "The mitochondrial UPR - protecting organelle protein homeostasis.," *J. Cell Sci.*, vol. 123, no. Pt 22, pp. 3849–55, Nov. 2010.

[275] C. E. J. Dieteren, P. H. G. M. Willems, H. G. Swarts, J. Fransen, J. A. M. Smeitink, W. J. H. Koopman, and L. G. J. Nijtmans, "Defective mitochondrial translation differently affects the live cell dynamics of complex I subunits.," *Biochim. Biophys. Acta*, vol. 1807, no. 12, pp. 1624–33, Dec. 2011.

[276] D. Weinkove and S. J. Leevers, "The genetic control of organ growth: insights from Drosophila.," *Curr. Opin. Genet. Dev.*, vol. 10, no. 1, pp. 75–80, Feb. 2000.

[277] S. J. Leevers, "Perspectives: cell biology. All creatures great and small.," *Science*, vol. 285, no. 5436, pp. 2082–3, Sep. 1999.

[278] A. Metaxakis, L. S. Tain, S. Grönke, O. Hendrich, Y. Hinze, U. Birras, and L. Partridge, "Lowered insulin signalling ameliorates age-related sleep fragmentation in Drosophila.," *PLoS Biol.*, vol. 12, no. 4, p. e1001824, Apr. 2014.

[279] C. De Virgilio and R. Loewith, "Cell growth control: little eukaryotes make big contributions.," *Oncogene*, vol. 25, no. 48, pp. 6392–415, Oct. 2006.

[280] S. I. Lippman and J. R. Broach, "Protein kinase A and TORC1 activate genes for ribosomal biogenesis by inactivating repressors encoded by Dot6 and its homolog Tod6.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 47, pp. 19928–33, Nov. 2009.

[281] R. Maleszka and G. D. Clark-Walker, "A petite positive strain of Kluyveromyces lactis has a 300 kb deletion in the rDNA cluster.," *Curr. Genet.*, vol. 16, no. 5–6, pp. 429–32, Dec. 1989.

[282] A. Koplin, S. Preissler, Y. Ilina, M. Koch, A. Scior, M. Erhardt, and E. Deuerling, "A dual function for chaperones SSB-RAC and the NAC nascent polypeptide-associated complex on ribosomes.," *J. Cell Biol.*, vol. 189, no. 1, pp. 57–68, Apr. 2010.

[283] A. A. Awofala, S. Jones, and J. A. Davies, "The Heat Shock Protein 26 Gene Is Required for Ethanol Tolerance in Drosophila," *J. Exp. Neurosci.*, p. 31, Mar. 2011.

[284] N. Mutlu, G. Garipler, E. Akdoğan, and C. D. Dunn, "Activation of the Pleiotropic Drug Resistance Pathway Can Promote Mitochondrial DNA Retention by Fusion-Defective Mitochondria in Saccharomyces cerevisiae.," *G3 (Bethesda).*, p. g3.114.010330–, May 2014.

[285] A. Adams, D. Gottschling, C. Kaiser, and T. Stearns, "Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual," 1998.

[286] R. S. Sikorski and P. Hieter, "A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces cerevisiae," *Genetics*, vol. 122, no. 1, pp. 19–27, May 1989.

[287] V. V Kushnirov, "Rapid and reliable protein extraction from yeast.," *Yeast*, vol. 16, no. 9, pp. 857–60, Jun. 2000.

[288] M. Orlova, L. Barrett, and S. Kuchin, "Detection of endogenous Snf1 and its activation state: application to Saccharomyces and Candida species.," *Yeast*, vol. 25, no. 10, pp. 745–54, Oct. 2008.

[289] S. L. Dobson, E. J. Marsland, and W. Rattanadechakul, "Mutualistic Wolbachia infection in Aedes albopictus: accelerating cytoplasmic drive.," *Genetics*, vol. 160, no. 3, pp. 1087–94, Mar. 2002.

[290] S. Chen, M. T. Oliveira, A. Sanz, E. Kemppainen, A. Fukuoh, B. Schlicht, L. S. Kaguni, and H. T. Jacobs, "A cytoplasmic suppressor of a nuclear mutation affecting mitochondrial functions in Drosophila.," *Genetics*, vol. 192, no. 2, pp. 483–93, Oct. 2012.

[291] E. Kemppainen, D. J. M. Fernández-Ayala, L. C. A. Galbraith, K. M. C. O'Dell, and H. T. Jacobs, "Phenotypic suppression of the Drosophila mitochondrial disease-like mutant tko(25t) by

duplication of the mutant gene in its natural chromosomal context.," *Mitochondrion*, vol. 9, no. 5, pp. 353–63, Sep. 2009.

[292] M. Takeda, H. Katayama, T. Satoh, and T. Mabuchi, "Three copies of the ATP2 gene are arranged in tandem on chromosome X in the yeast Saccharomyces cerevisiae.," *Curr. Genet.*, vol. 47, no. 5, pp. 265–72, May 2005.

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