

Introduction

Mitochondria

- Double-membraned involved in ATP generation, cellular metabolism, and oxidative stress control
- Endosymbiotic origins
- Has its own circular DNA that encodes specific proteins and enzymes needed for mitochondrial pathways
- Relatively small DNA (~16,000-17,000 bp) that lacks introns



Figure 1. Source: Keith Porter/Science Source

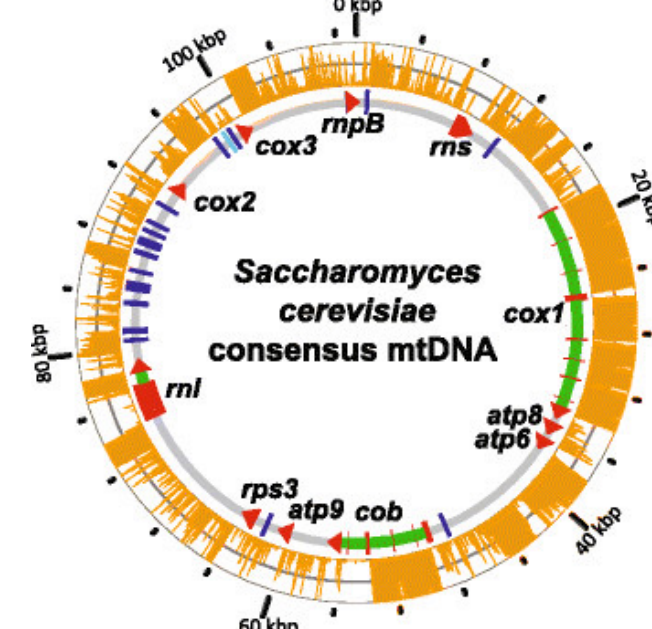
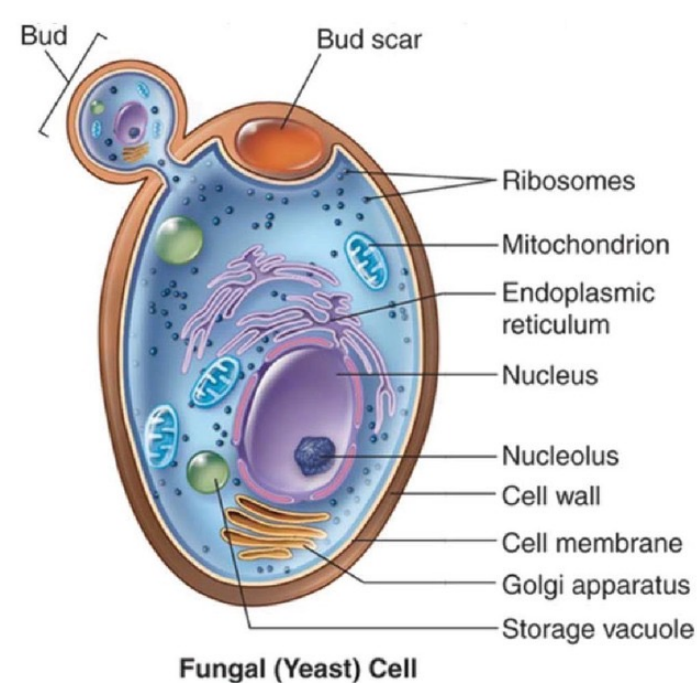


Figure 2. Source: Wolters et al., 2015.

Mitochondrial DNA

- High rate of polymorphisms and mutations
- Maternally inherited
- Regulates cellular metabolism
- Encodes proteins needed to regulate the oxidative phosphorylation cycle
- Mitochondrial transcription coordinates with nuclear transcription to carry out metabolic processes.



Saccharomyces Cerevisiae

- Commonly known as Brewer's Yeast
- Model organism used to experimentally evaluate disruptions to organism pathways
- Short generational lifespan- reproduces quickly

Figure 3. Source: Beetles, (2015).

Oxidative stress

- It is well known that yeast have developed antioxidant defenses against reactive oxidative species (ROS).
- ROS are vital for cell immunity and signaling in small doses, but in large amounts, leads to cellular mutagenesis.
- These adaptive responses to increased ROS are regulated at the transcriptional level.
- Transcription factors coordinate responses to oxidative stressors through the upregulation or repression of genes associated with antioxidant defenses.

Aims of Research

- Increase understanding of the expression of mitochondrial DNA and mtDNA transcriptional regulation in *S. Cerevisiae*.
- Evaluate the coordination between nuclear and mitochondrial transcription for the oxidative phosphorylation pathway.
- Better understand the effect of factors on mitochondrial transcription.
- Evaluate protein-DNA binding behavior and the production of reactive oxygen species (ROS).

Why?

- Understanding these types of transcriptional regulatory processes provides insight into mitochondrial dysfunction related to disease and aging

Methods

Yeast Cultures

- 50 mL cultures
- Yeast Extract-Peptone-Dextrose Media

RNA isolation and imaging

- Cell lysis through douncing
- QIAGEN RNeasy RNA elution kits
- Run on 1% agarose in TBE (50V, 30 min)
- ~100 ng RNA per lane

Menadione

- The introduction of naphthoquinones such as menadione produces oxidative stress to ROS dependent pathways.
- ROS is generated by menadione through redox cycling.
- 10 uL of 50 mM Md in each Md+ culture

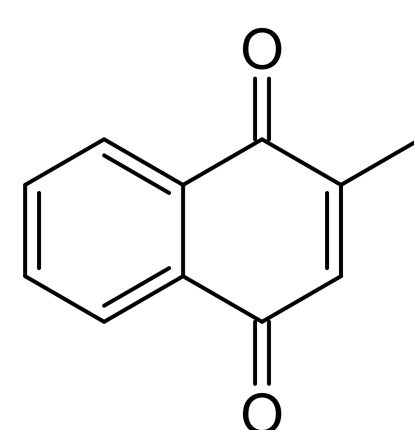


Figure 5. Chemical Structure of Menadione.

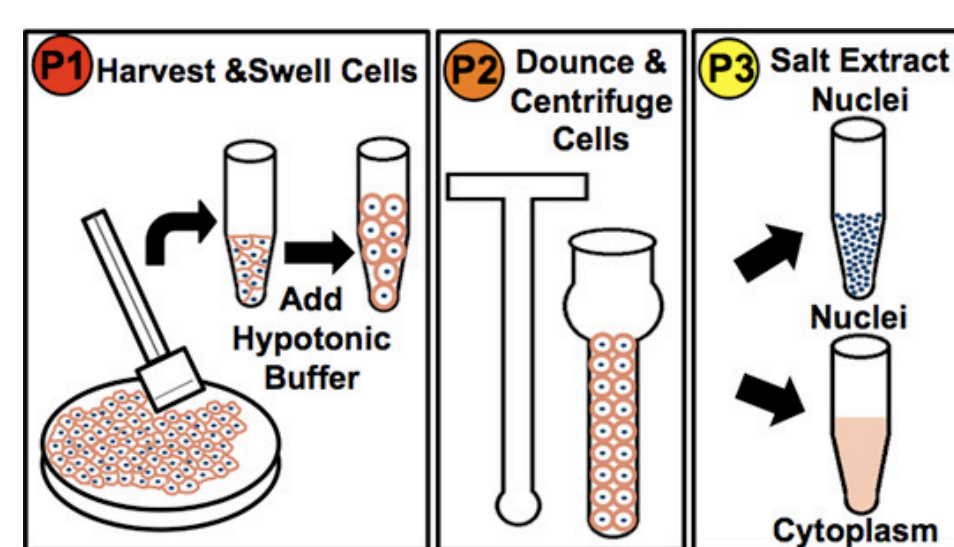


Figure 4. Source: Folco et al. (2012)

Results

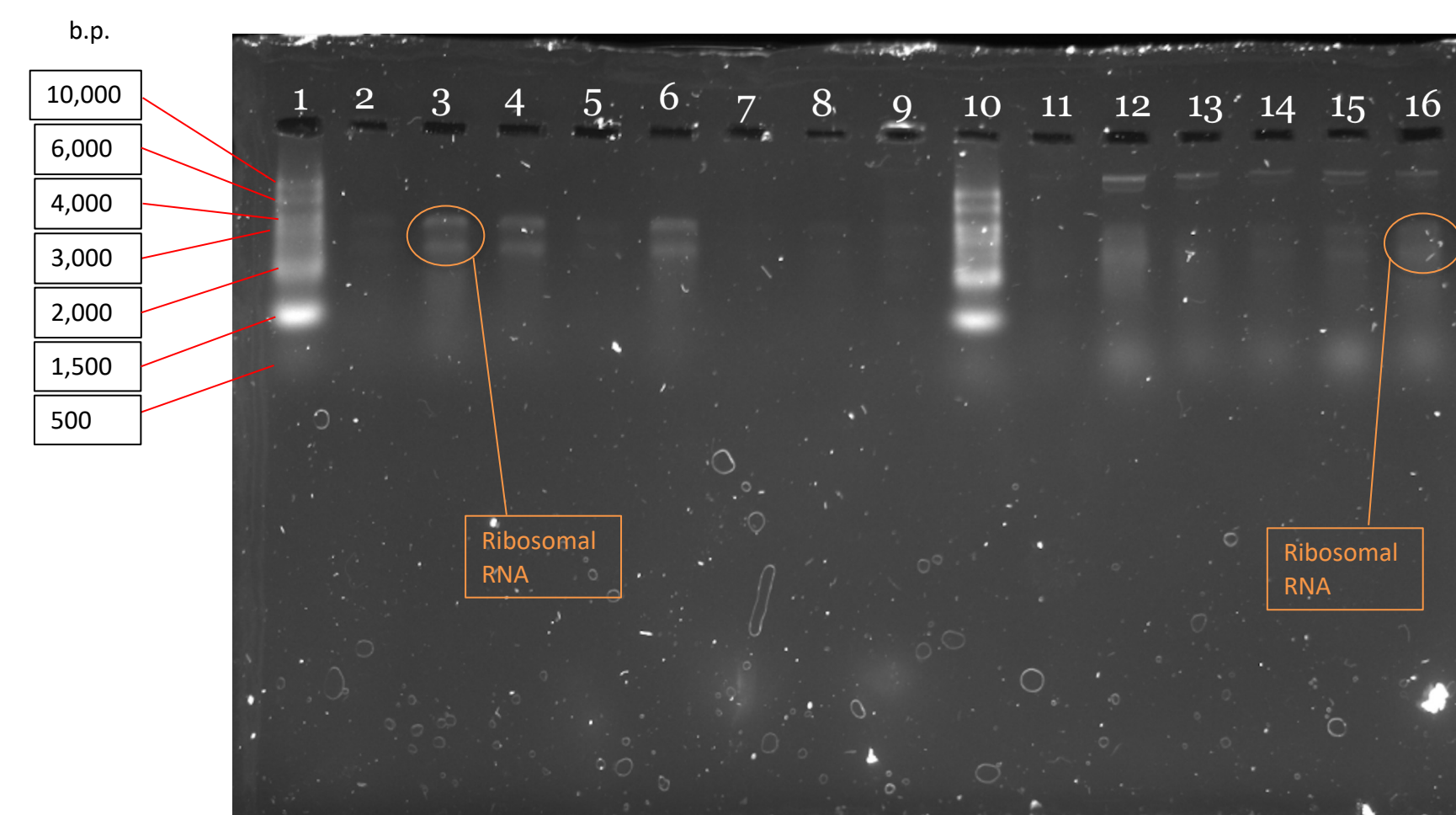


Figure 6. *S. Cerevisiae* RNA banding patterns for the cultures with menadione (lanes 2-9) and untreated cells (lanes 11-16) against RNA transcript marker (Sigma-Aldrich) (lanes 1 & 10).

S. Cerevisiae Documented Ribosomal RNA sizes

- 18S – 2.0 kb
- 26S – 3.8 kb

Ribosomal RNA

- Presence of 26S ribosomal RNA at ~4000 bp
- Low presence of 18S ribosomal RNA at ~2000 bp

Smear

- We observed a difference in the RNA transcripts isolated from mitochondria in treated and untreated cells.
- This is mainly apparent in the RNA smear below 2000 bp, which is brighter in the untreated cells, suggesting that there are more transcripts present.

Discussion

Analysis

- In conditions of high oxidative stress, we expect increased reactive oxidative species (ROS), and we saw different RNA banding patterns for our md+ cells when compared to the untreated cells.
- There was a brighter smear in the untreated yeast mtRNA, suggesting the presence of many different transcripts from the mitochondria when the organism is not under stress.
- These data suggest the possibility of an organism response to oxidative stress at the mitochondrial level.
- It is not surprising that an organism under oxidative stress would devote its resources to important transcripts (such as rRNA), rather than the broad range of transcripts that we see in the smear of untreated cells.

Conclusions

- It is necessary for organisms to be able to sense and respond to external stimuli. We expect cells to express certain genes depending on the stimuli present.
- In response to oxidative stress, such as that triggered by the presence of menadione, changes were observed in the expression of ribosomal RNA.
- Antioxidative defenses are speculated to occur in *S. Cerevisiae* at the transcriptional level in mitochondria.

Future work

- RNAseq using purified RNA samples to determine sequence and abundance of transcripts under different conditions.
- Assess mitochondrial protein levels for treated and untreated cells.
- Look for mtDNA damage and mtDNA counts, which could affect transcription.

References

Atig et al. (2009), Daum et al. (1982), Farrugia et al. (2012), Folco et al. (2012), Loor et al. (2010), Mattmiller (2017), Wolters et al. (2015),

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