The effect of low concentrations of copper on mitochondria and activity in yeast cells

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Introduction

Mitochondria are essential organelles in both yeast and human cells due to their role in metabolism, ATP production via the oxidative phosphorylation (OXPHOS) pathway, and other regulatory cellular processes. Using yeast as a model organism to study mitochondrial function in a chronological lifespan assay allows experiments to be conducted over a shorter timeframe and allows for connections to be made to human cells.[1]

Our aim is to investigate how exogenous copper in the mitochondria of yeast affects the production of reactive oxygen species (ROS), protein expression, and enzyme activity during yeast lifespan. Small amounts excess copper added to growth media (0.25 mM copper sulfate in restricted nutrient media) extend yeast chronological lifespan, but yeast lifespan is reduced when added copper levels are increased to 2.0 mM copper sulfate or higher. These results indicate that low levels of exogenous copper in the media is beneficial for yeast in these restricted media conditions.

To extend these findings, we assessed how added copper changes mitochondria within these cells over the course of the yeast lifespan (14 days growth). Using MitoTracker Green (MTG) and fluorescence detection we showed an increase in mitochondria in copper treated cells. This is consistent with previous studies showing mitochondria in mammal and yeast cells contain a labile copper pool located in the matrix, which is used in the metalation of the copper containing enzyme of the OXPHOS pathway Complex IV, Cytochrome C Oxidase (CcO), and superoxide dismutase (SOD1).[2,3] Our most recent work focuses on assessing CcO protein complex expression during yeast lifespan, specifically looking at cytochrome c oxidase subunits using western blotting; and assessing Sod1p activity using in-gel activity assays. The results of this research allow us to better understand the role of copper in mitochondrial activity across the lifespan of yeast.

Methods

14-Day Time Course: BY4743 wild type yeast cells were combined with ¼ YPD liquid culture. Three cultures were grown without added copper whereas 0.25 mM copper sulfate (CuSO4) was added to another three cultures. Cultures were shaken at 250 rpm at 30 ºC and 1 mL samples were collected throughout the time course (Figure 1). The yeast cell density was determined using a hemocytometer and 0.4% trypan blue (Sigma Aldrich) staining, as well as by determining the OD600.

Fluorescent Staining: BY4743 yeast cells collected from different time points were thawed on ice and washed with PBS. Cells were split into 2 separate test tubes. 10 uL of DMSO was added to one test tube while 10 uL of 14.3 mM DAPI (Sigma Aldrich) and 5 uL of 1 mM MTG (Invitrogen) were added to the other test tube. Test tubes were incubated at 30 ºC for 30 minutes and then centrifuged and supernatant was removed. Cells were centrifuged again with 1 mL of PBS and supernatant was removed. 500 uL of PBS was then added to the which were then imaged with the Zeiss Axio Observer Z1 inverted fluorescent microscope. Zeiss ZEN1 software was used to generate the overlay images of MTG and DAPI.

Mitochondrial Assays: CcO activity was assessed by monitoring the oxidation of reduced cytochrome c and reaction rates.[4] Reaction rates were normalized to total protein determined by the Bradford assay based on a standard curve generated by measuring the absorbance at 595 nm for known concentrations of BSA. SOD activity was visualized by an *in situ* gel assay through staining with nitro blue tetrazolium (NBT, Sigma Aldrich). Cells were subjected to non-denaturing gel electrophoresis (AnyKd gels and protein ladder, Bio-Rad), and the gels were stained for SOD activity.[5]

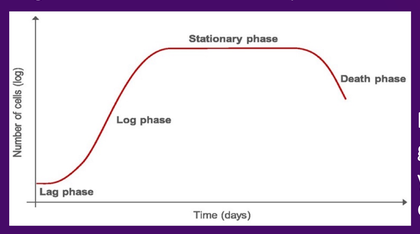

Figure 1. Normal growth curve for yeast cell growth over time in days.

Results

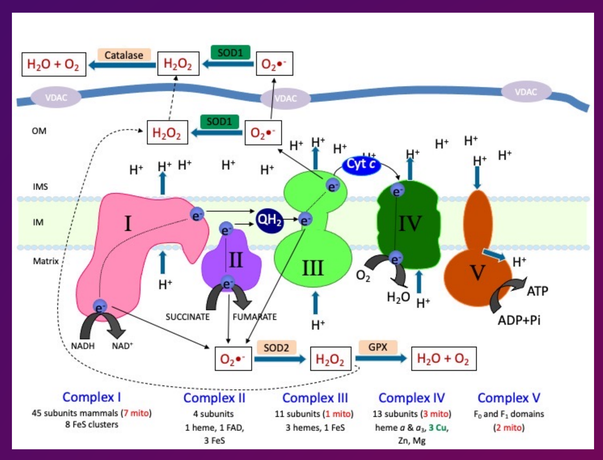

Figure 2. The mitochondrial respiratory chain – OXPHOS complexes and ROS.

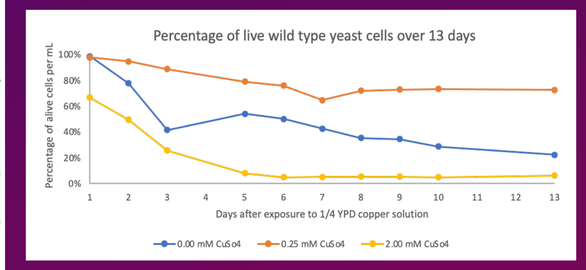

Figure 3. Addition of 0.25 mM copper sulfate to the media increases yeast lifespan of wild type cells. These data indicate that a small addition of copper to yeast media improves lifespan, but the higher concentration of 2.0 mM copper sulfate addition to media quickly killed most of the yeast cells.

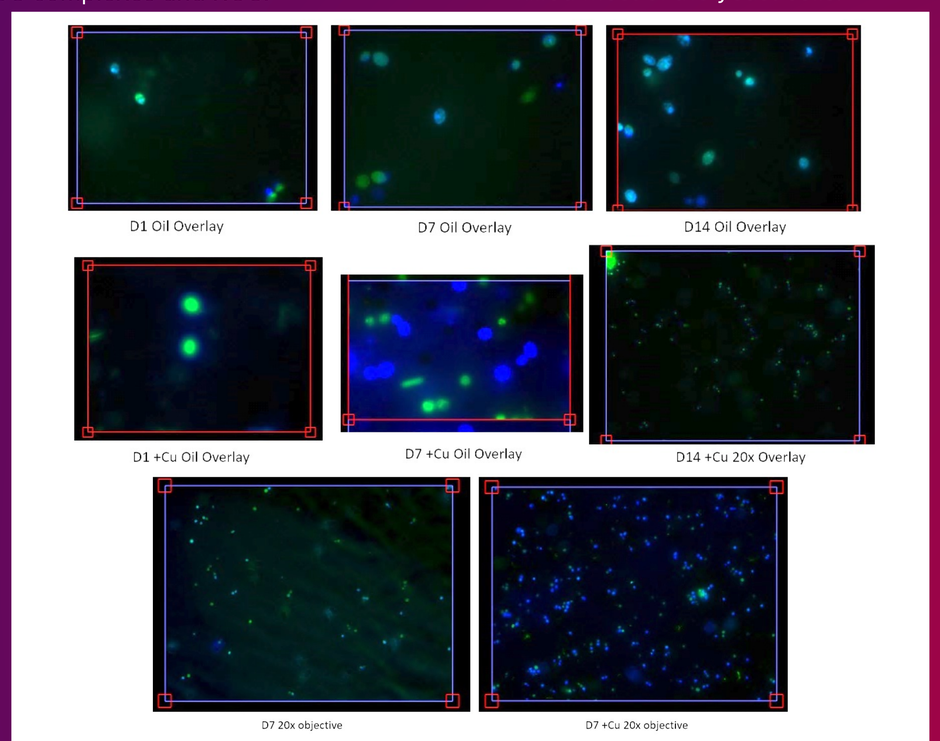

Figure 4. Fluorescent Microscope Images; 20x and 100x objective with oil overlay are indicated with multiple time points shown.

Figure 5. CcO activity of yeast cells +0.25 mM CuSO4 (A), or untreated (1). (n=2)

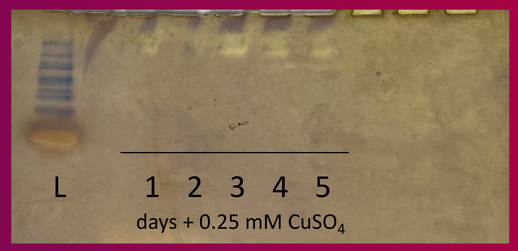

Figure 6. Preliminary SOD activity gels for cells grown in added copper. Timepoint days indicated and protein ladder (L).

Conclusions & Future Directions

Copper plays an important role in yeast cell growth and mitochondrial activity.

- Addition of 0.25 mM copper sulfate to restricted nutrient media increases yeast lifespan of wild type cells.
- In the presence of added copper, yeast cells multiplied faster than without added copper and had more mitochondria. The larger quantity of DAPI and MTG present in the +Cu samples suggests this as well as the +Cu cells having a higher fluorescent intensity.

Future Directions

- Continue to fluorescently stain yeast over the 14-day lifespan looking to multiplex DAPI, MTG and dihydroethiudium (DHE) which specifically stains ROS. We aim to continue utilizing fluorescent microscopy but to also analyze the stained cells via a fluorescence plate reader and fluorescent activated cell sorting (FACS) to quantitatively assess mitochondria and ROS changes in the presence and absence of added copper.
- Complete additional trials for mitochondrial activity assays, specifically the CcO activity. For the SOD activity assay non-denaturing electrophoresis gels that separate the Sod1 from Sod2 better need to be determined before continuing to assess activity changes over the 14-day lifespan.
- Complete western blots for CcO specific subunits in the presence and absence of added copper across the 14-day lifespan such Cox1, Cox2, Cox4, as well as Sod1.

References

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