Role of Copper and Tor Signaling in Reactive Oxygen Species Induced Cell Aging

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Introduction

Chronological lifespan assays in yeast rely on promoting a culture's quiescent stationary phase by calorie restriction. In this phase, anaerobic fermentation of sugars is curtailed and aerobic respiration begins as a more efficient metabolic mechanism. This shift in glucose utilization can be accomplished by reduced TOR pathway signaling, a complex regulator of cell growth and cell cycle in which various environmental growth condition signals are integrated to activate or inhibit the Ser/Thr kinase activity of Tor1[3]. Optimal growth conditions promote TOR signaling causing macromolecule biosynthesis, sugar fermentation with increased metabolism, and progression through cell cycle[3]. Many downstream effects of TOR signaling can be silenced by cell treatment with Rapamycin, which binds to Tor1 and inhibits kinase domain function, drastically increasing a cell population's chronological lifespan.

Mitochondrial electron transport chain (ETC) machinery, whose activity is modulated by TOR signaling, is the primary site of superoxide formation. Superoxide is a reactive oxygen species (ROS) produced by premature electron leakage directly to oxygen, producing dangerous hydroxyl radicals via the Haber-Weiss reaction[2]. From here, the ROS can diffuse through the cell causing the damage to DNA, lipid peroxidation, and mitochondrial dysfunction associated with premature cell aging and death[2].

A primary defense of free radical damage as a respiratory by-product is the neutralization of superoxide by superoxide dismutase 1 (*SOD1*), which requires copper and zinc as cofactors in the conversion of superoxide to less harmful hydrogen peroxide[2]. Copper is additionally utilized in cytochrome c oxidase as an electron transferring group, and is required for the continuous movement of high energy electrons through the ETC, thereby limiting the potential for electron leakage and ROS[4].

A 2005 cell array study identified a set of yeast gene deletions involved in copper homeostasis, *SOD1* among them, that conferred resistance to the expected inhibition of growth and proliferation characterized by rapamycin treatment[1]. Copper, given its two roles in defending against ROS production and induced cell aging, is here modulated by extracellular supplementation to further elucidate its functionality in the context of Rapamycin treatment and *SOD1* deletion strains.

Conclusions and Future Directions

Chronological lifespan analysis of Rapamycin treatment on Δ*SOD1* yeast yields observable lifespan extension in the mutant strain, inconsistent with corresponding growth observations made by Xie MW et al. (Figure 5A). The normal outcome of lifespan extension conferred by Rapamycin, through TOR inhibition, is present in *SOD1* mutants. This suggests the notable possibility of Rapamycin treatment giving both advantageous growth and lifespan extension in these mutants by reducing the overall production of ROS over a yeast's metabolic lifetime.

Copper supplementation on Rapamycin treated *SOD1* mutants results in cell viability disadvantage over most of a chronological lifespan (Figure 5B). *SOD1* mutants' sensitivity to ROS can be aggravated by increased intracellular copper, leading to metal toxicity by copper's participation in the Fenton reaction to produce hydroxyl radicals that contribute to premature cell aging. However, Figure 5C shows copper supplementation to be clearly advantageous to lifespan in *SOD1* mutants when treated with Rapamycin. These data indicate a functional consequence of rapamycin treatment in suppressing overall metabolic activity, and therefore cytochrome C oxidase expression. In cells lacking Rapamycin treatment, as in Figure 5C, there could be a greater metabolic demand for copper to function in cytochrome C oxidase, and supplementation ensures that more ETC machinery is able to operate, lessening electron leakage and subsequent ROS production.

To further elucidate these findings, cytochrome C oxidase expression levels within rapamycin treated and control *SOD1* mutants can be assessed, along with trials of copper chelation, to determine whether a correlation exists between cytochrome C oxidase function and availability of copper. Additionally, ROS levels can be quantified at time points in Figure 5A's CLS assay to identify whether reduction in ROS production contributes to lifespan extension in *SOD1* mutants treated with Rapamycin.

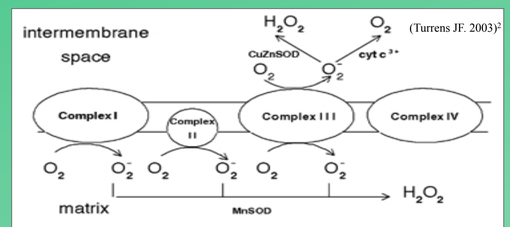

Figure 1. Electron transport chain formation and neutralization of superoxide.

Fe^{3+} + $O_2^{\bullet-}$ ⟷ Fe^{2+} + O_2
(Cu^{2+}) (Cu^{+})

Fe^{2+} + H_2O_2 ⟶ Fe^{3+} + $HO^{\bullet}$ + OH^-
(Cu^{+}) (Cu^{2+}) (Fenton reaction)

$O_2^{\bullet-}$ + H_2O_2 ⟷ O_2 + $HO^{\bullet}$ + OH^-
(Haber-Weiss reaction)

(Turrens JF. 2003)[2]

Figure 2. Haber-Weiss reaction of superoxide to produce hydroxyl radical by iron or copper metal catalysis.

Materials and Methods

Yeast strains and growth conditions:

Wild type and *SOD1* deletion S. *cerevisiae* were both of the BY4743 strain and were cultured (32° C) in standard YPD liquid media overnight prior to experimental culture inoculation. Experimental cultures of 1/5 YPD (50 mL, 1:5 media:flask volume) were inoculated (OD_{600} 0.1) and treated with cupric sulfate (.5 mM) and Rapamycin (30 nM in EtOH), with appropriate control flask combinations. All experimental cultures aged in a shaker (250 rpm, 32° C) for the duration of the lifespan assay.

Trypan blue staining:

Chronological lifespan was assayed by assessing percent viability of yeast cell cultures over time via Trypan Blue staining (4% Trypan Blue diluted 1:4) to visualize dead vs. live cells. Quantification of live cell density utilized hemocytometer slides to count average cell number within a fixed volume (average of five .2x.2x.1 mm square spaces) after even cell dispersal by vortex mixing (2800 rpm, 8 seconds).

Results

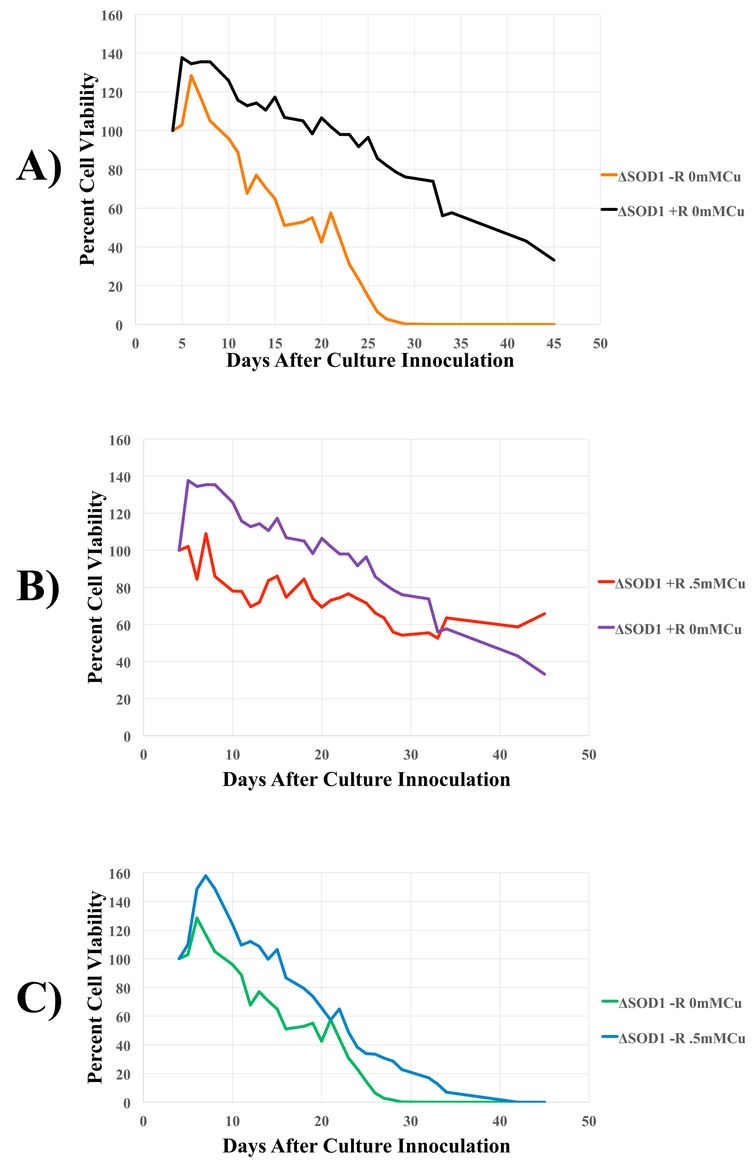

Figure 5. Chronological lifespan assays of experimental yeast cultures. A) lifespan of two *SOD1* deletion strain yeast cultures with no copper supplementation, and one with 30 nM Rapamycin treatment. B) Lifespan of two *SOD1* deletion strain yeast treated with 30 nM Rapamycin, and one with .5 mM copper supplementation. C) Lifespan of two *SOD1* deletion strain yeast without Rapamycin treatment, and one with .5 mM copper supplementation.

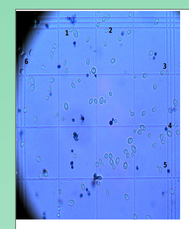

Figure 3. Trypan Blue stained yeast in hemocytometer microscopy cell viability assay.

Figure 4. Experimental cultures in temperature controlled shaker.

Literature Cited

[1]Xie MW et al. (2005) Insights into TOR function and rapamycin response: chemical genomic profiling by using a high-density cell array method. Proceedings of the National Academy of Sciences of the United States of America 102: 7215–7220.

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Acknowledgments

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For further information

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