Copper, ROS, and Mitochondrial Stress

Understanding the pathways that govern metabolic homeostasis

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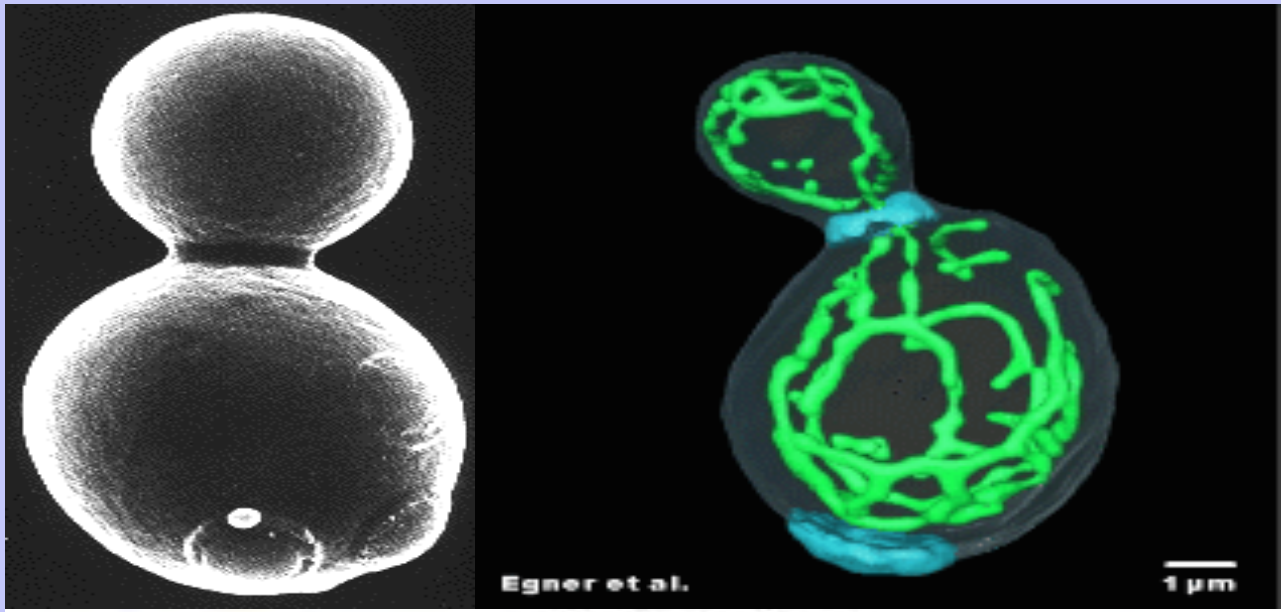
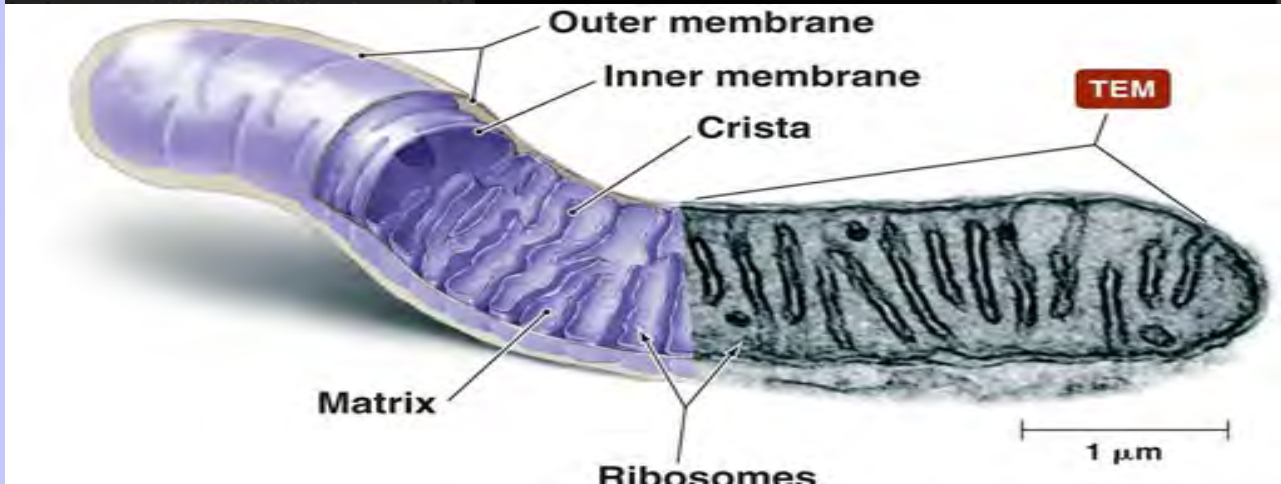
Overview of Topics

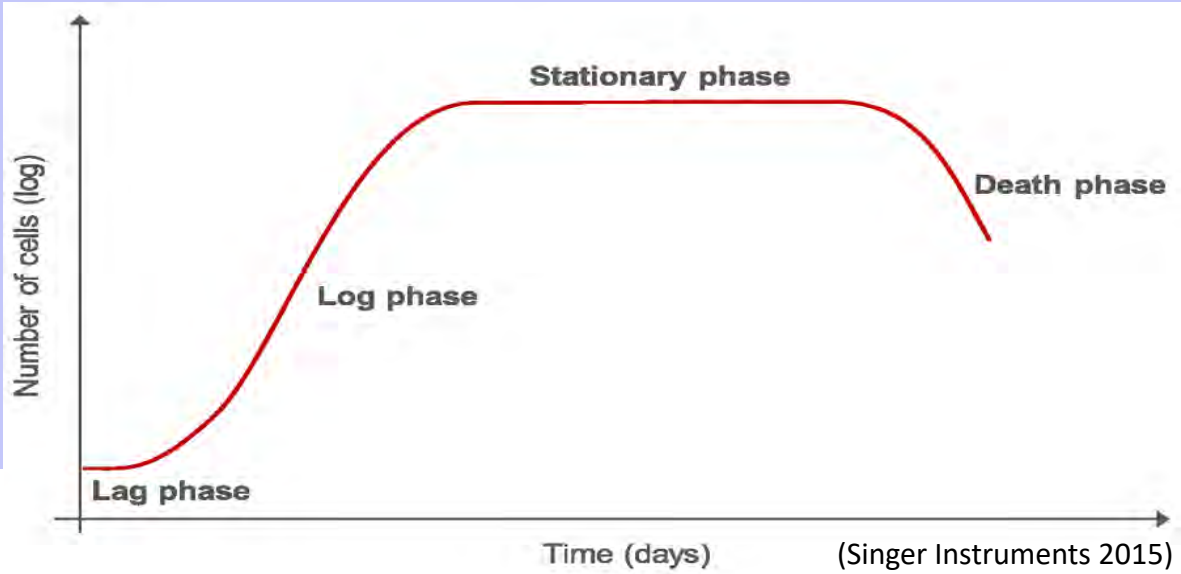
- Biology of model system (*S. cerevisiae)*
- Chemistry of reactive oxygen species (ROS) in metabolic stress
- Copper's protective role against ROS produced in the mitochondria
- Future Directions
- Clinical Implications



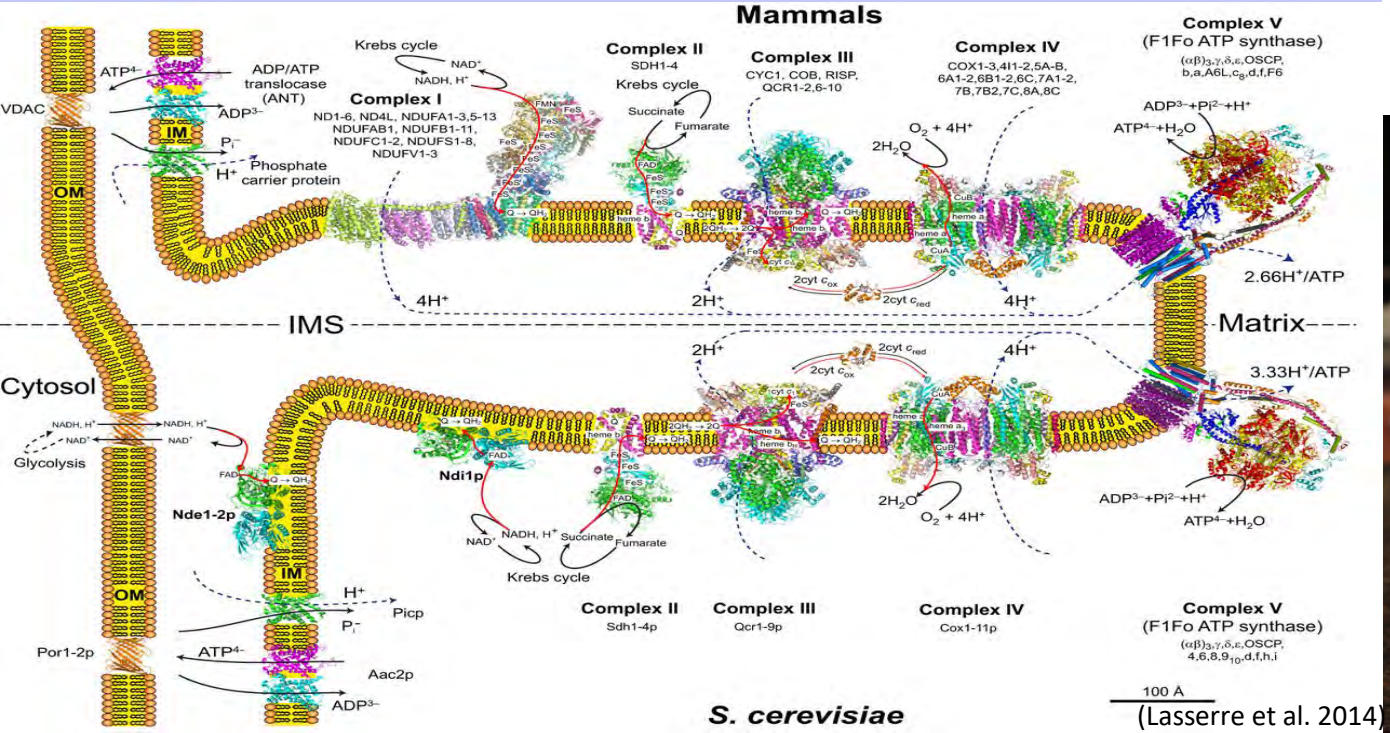


Biology of Our Yeast Model

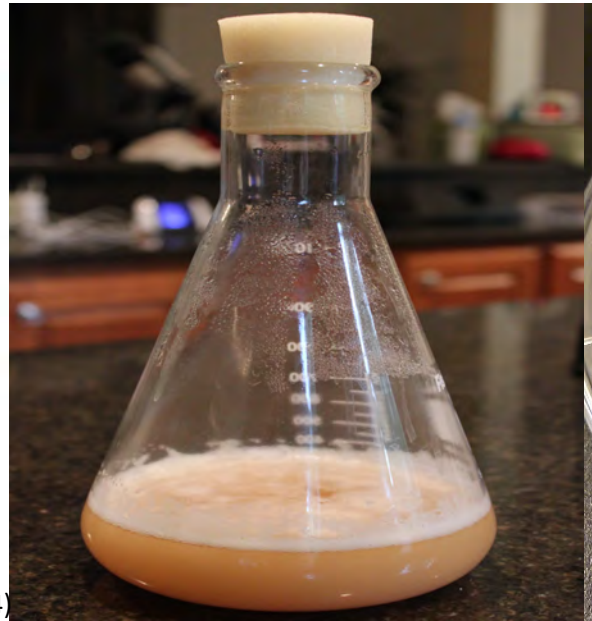
- Why yeast as a model organism?
  - Short life cycle
  - Grown in liquid cultures or on solid plates
  - Well established in literature
  - Many conserved gene homologs in humans



(Singer Instruments 2015)



(Lasserre et al. 2014)



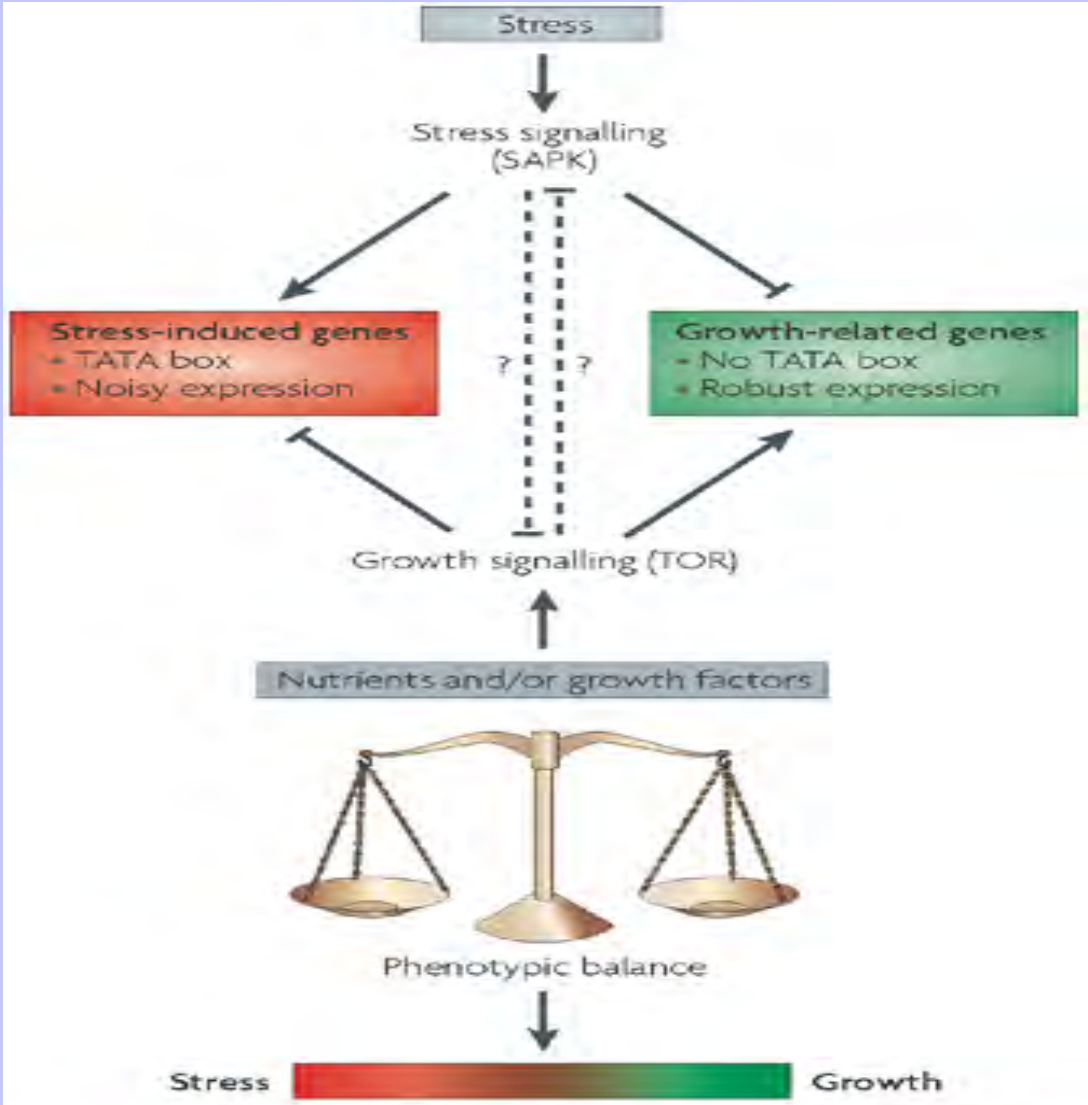
Environmental Stress Response (ESR)

Hallmarks of the ESR:

- Unfavorable environmental conditions are sensed
- Cell responds by altering expression of many general stress response genes (~900)

Common Stressors: temperature extremes, osmotic shock, DNA damage, oxidizing compounds **(ROS)**, and nutrient restriction **(Rapamycin)**

Cell Response: cell cycle arrest, slowed growth, metabolic shift **(fermentation → respiration)**, and upregulation of defensive proteins **(Sod1)**



(López-Maury et. al. 2008)

Reactive Oxygen Species as Mitochondrial Stressors

Common types of ROS:

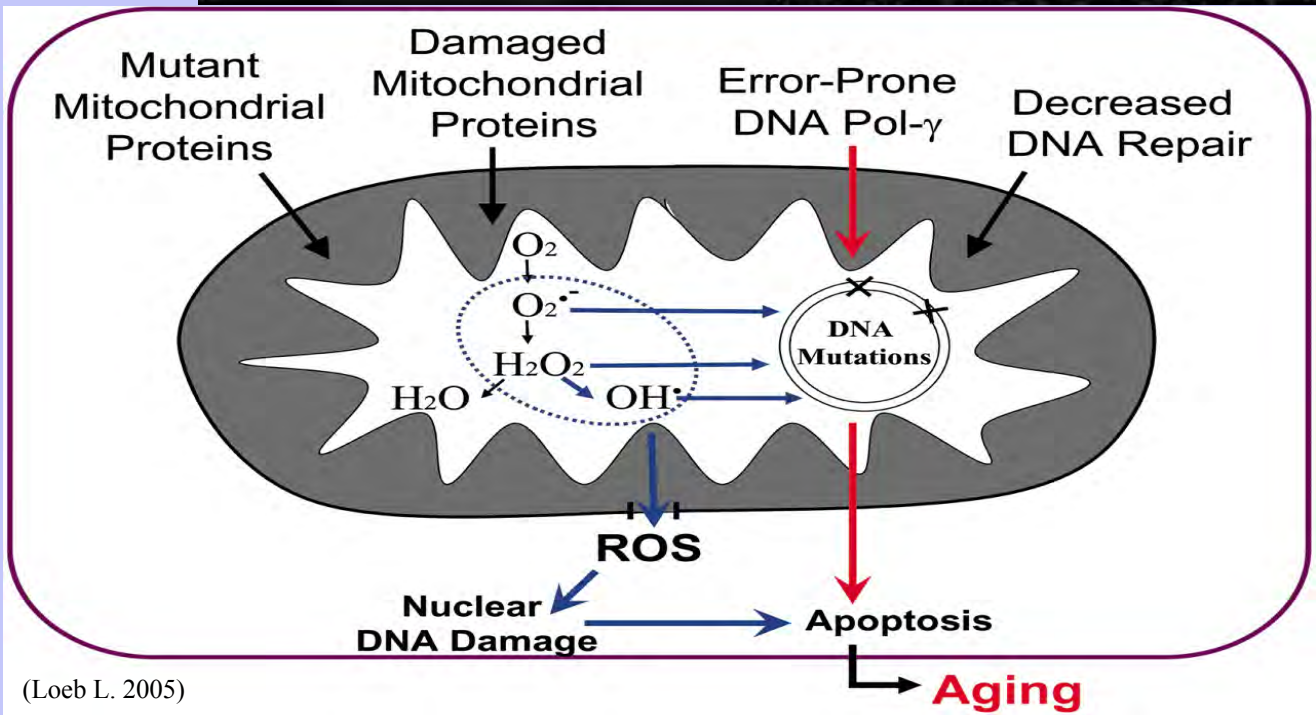
- superoxide anion ($O_2^{\bullet -}$)
- hydrogen peroxide (H_2O_2)
- hydroxyl radical ($HO^{\bullet}$)

ROS react with nuclear DNA, mtDNA, proteins, and lipids creating mutations and dysfunctional machinery

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

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

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

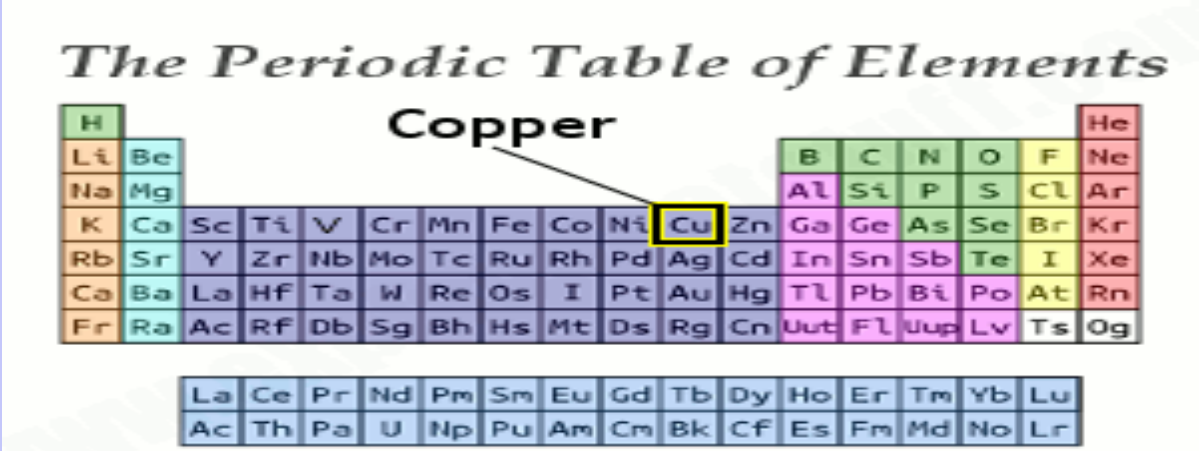


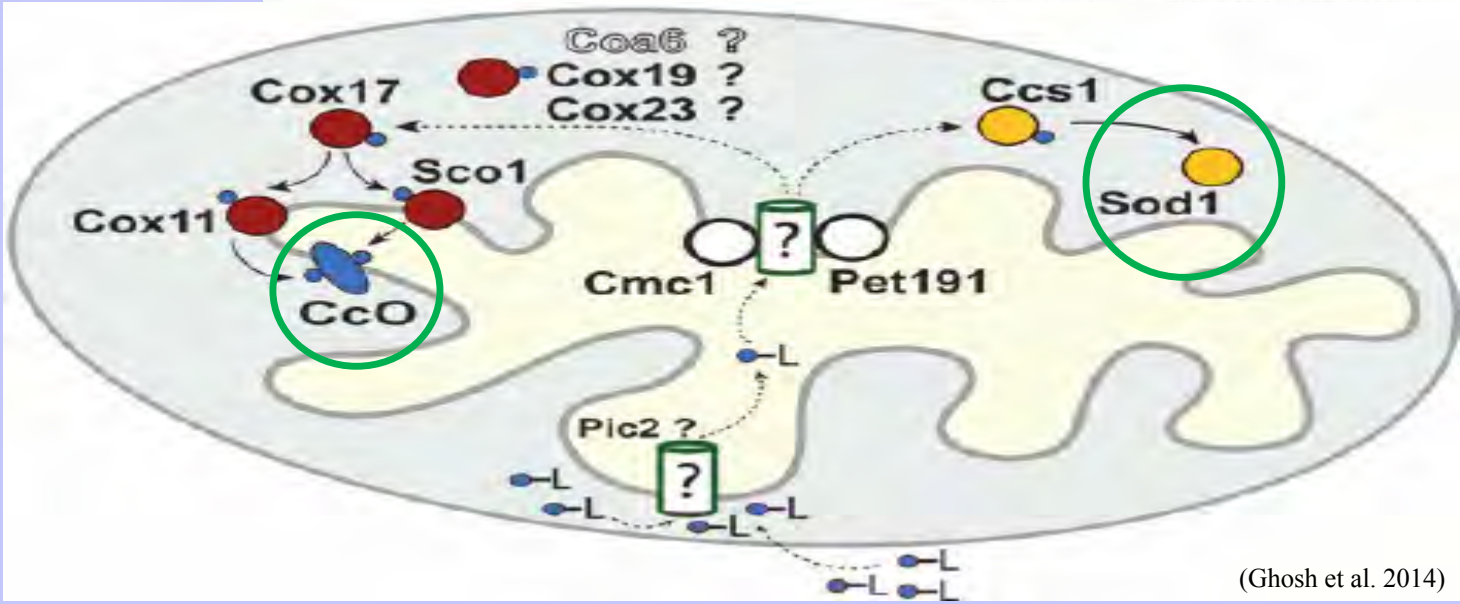
(Loeb L. 2005)

Protective Role of Copper against ROS

- **Original Goal: Investigate effect of copper treatment in mitochondrial oxidative damage**

- Why Copper?
  - Transition metal
  - Utility as redox cofactor
  - Main cellular use is in two key proteins involved in ROS homeostasis (CcO & Sod1)



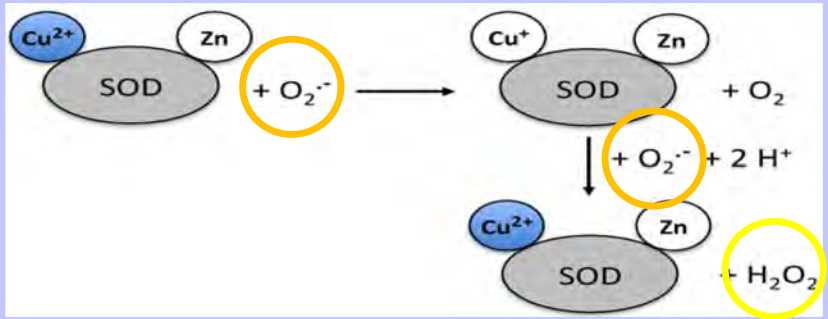


(Ghosh et al. 2014)

Protective Role of Copper Against ROS

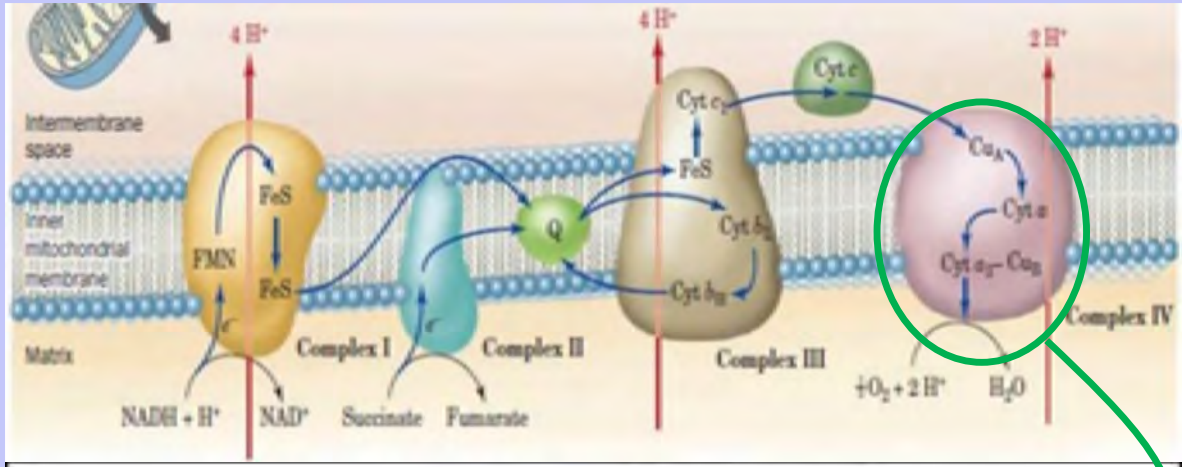
Curative Role: Redox cofactor for Sod1

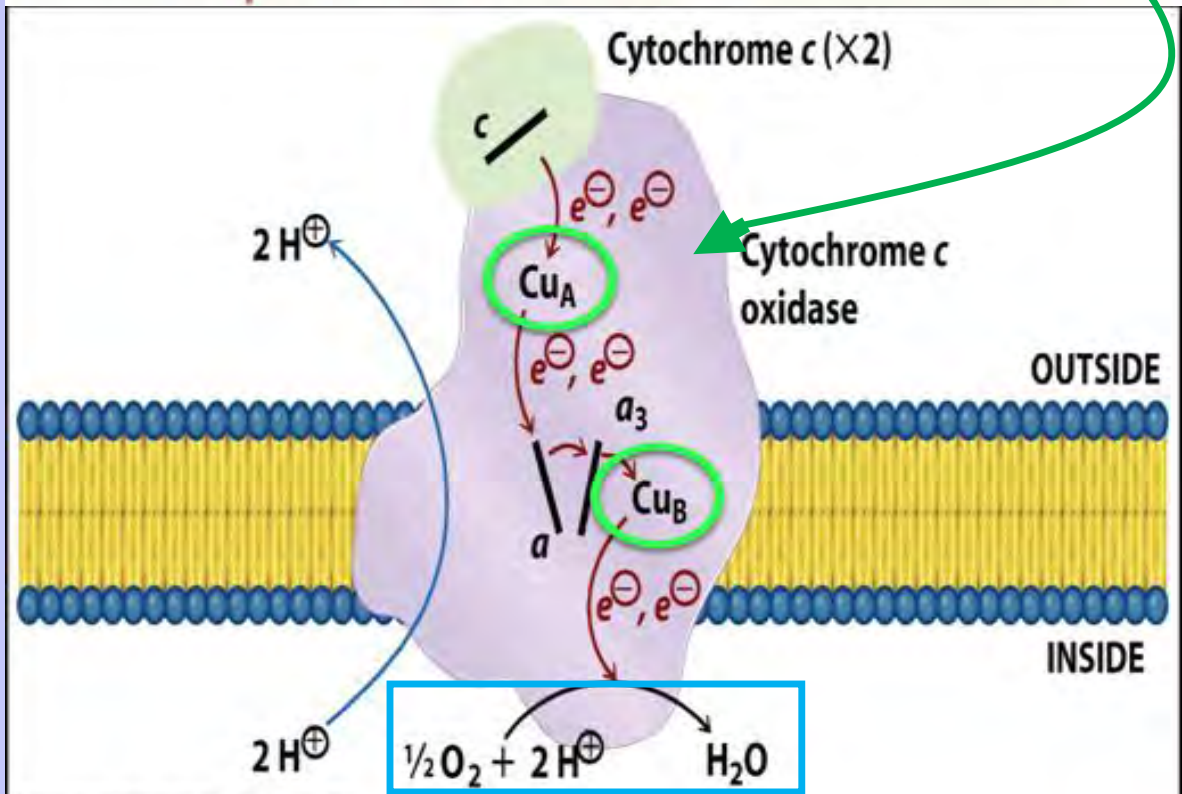
- Sod1 neutralizes two reactive superoxide radicals to oxygen and hydrogen peroxide



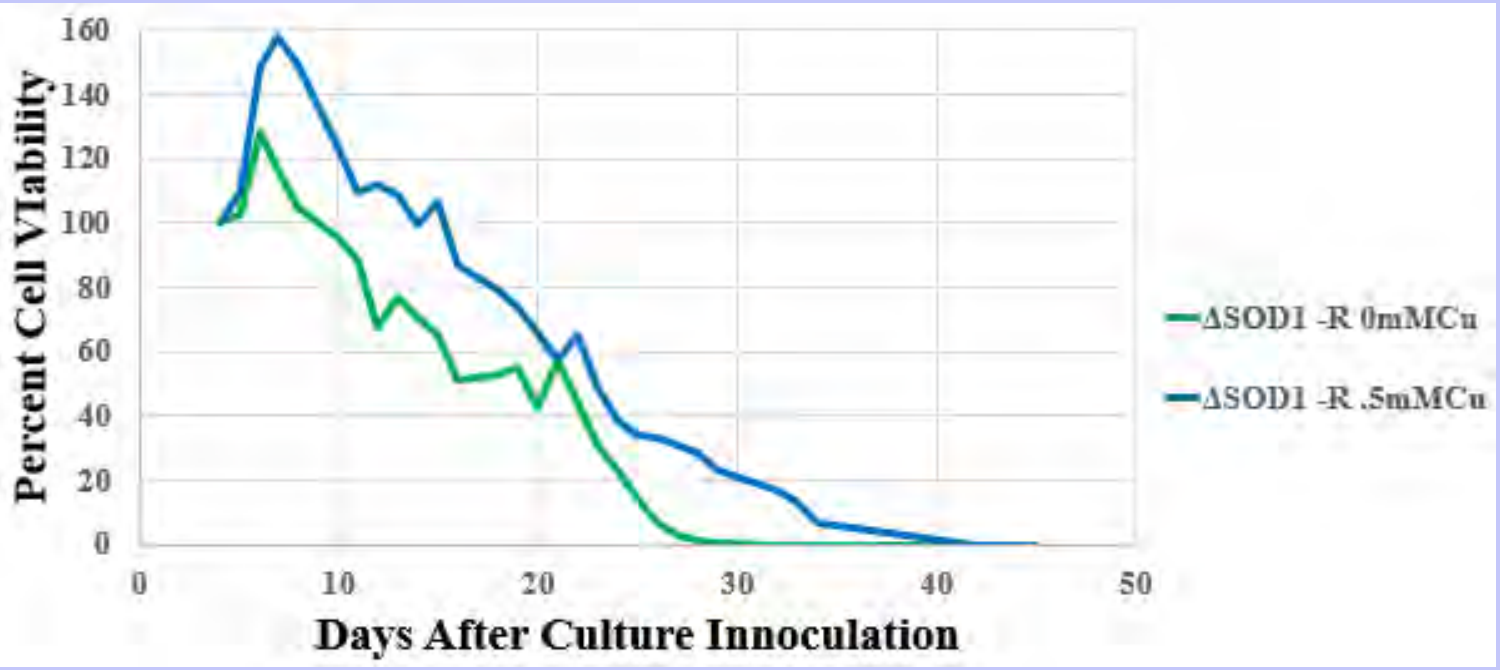
Preventative Role: Redox cofactor for cytochrome c oxidase (CcO) complex

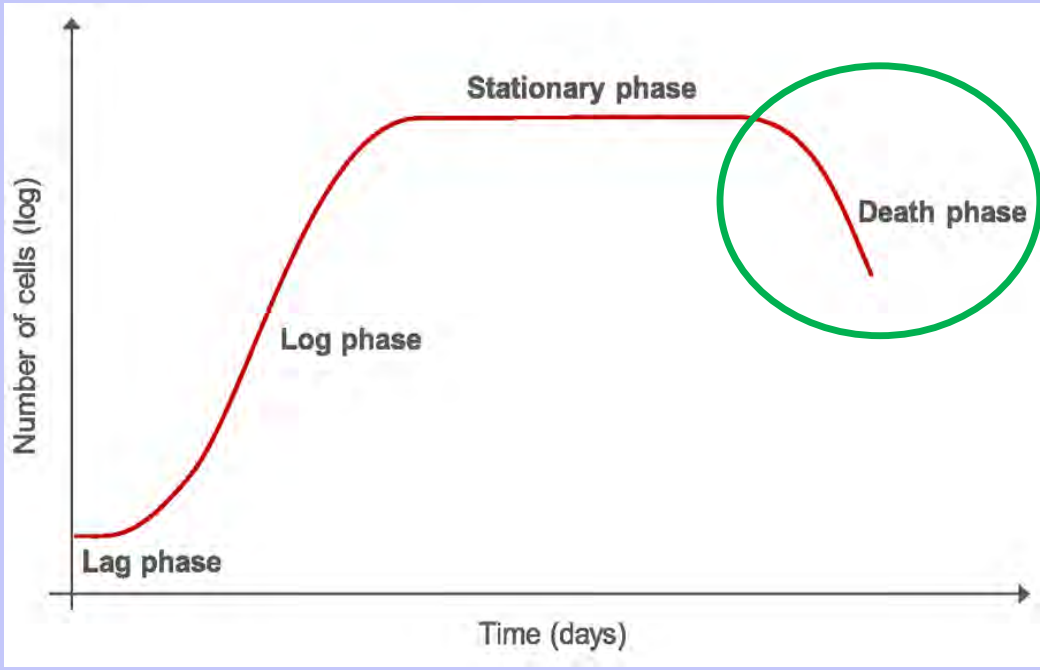
- CcO requires copper for overall function of electron transport chain (ETC) during respiration
- Transfers high energy electrons from the ETC to molecular oxygen at the terminal step

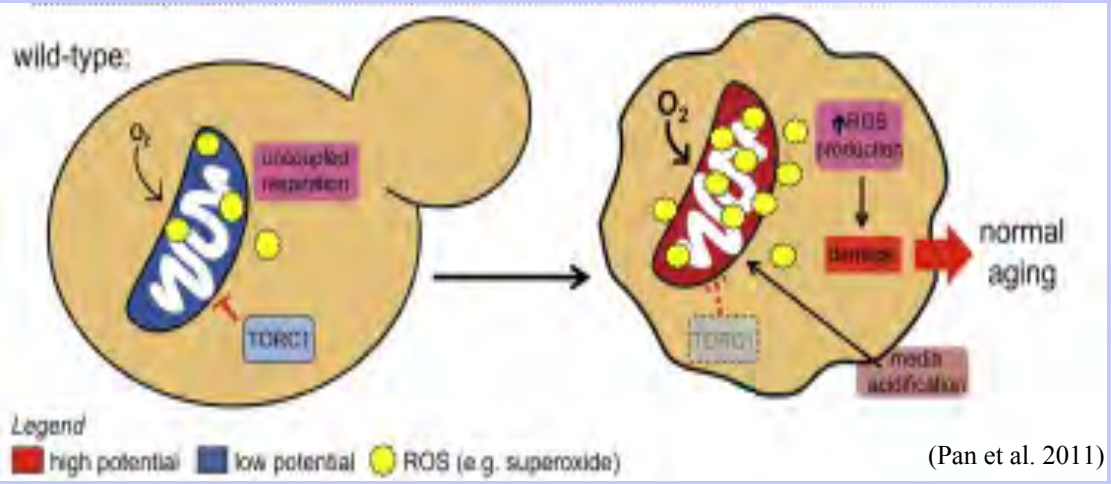




CLS Assay of Copper Treated Yeast







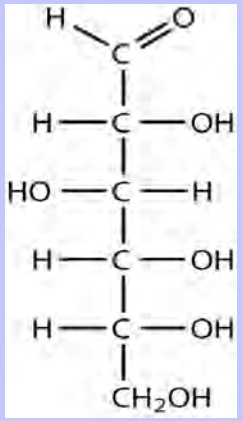
(Pan et al. 2011)

Goal: Investigate copper's effect on oxidative damage during yeast stationary phase lifespan

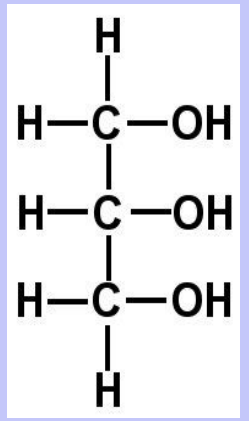
Growth Assays for Oxidative Stress

- **Goal: Observe relative oxidative stress, based on growth, between varying levels of copper treatment in different metabolic environments**

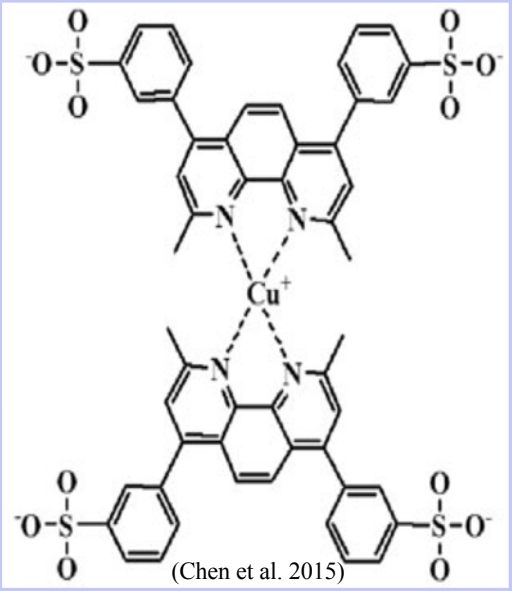
- Grow yeast on plates of varying conditions
  - Fermentable vs. nonfermentable carbon source
  - Copper treatment ($CuSO_4$) or chelation (BCS)
  - Treatment with Rapamycin (induces stress response & respiration)



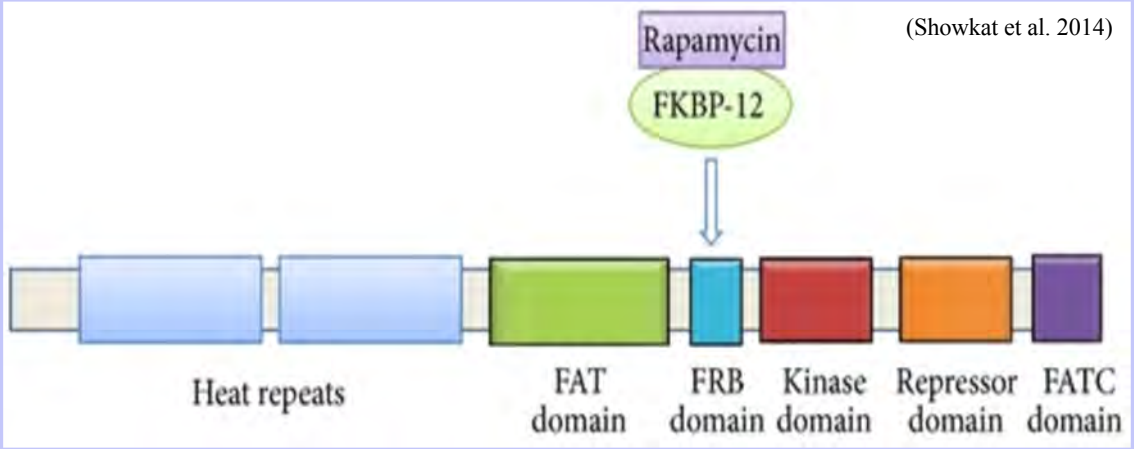
Fermentable
Glucose



Nonfermentable
Glycerol

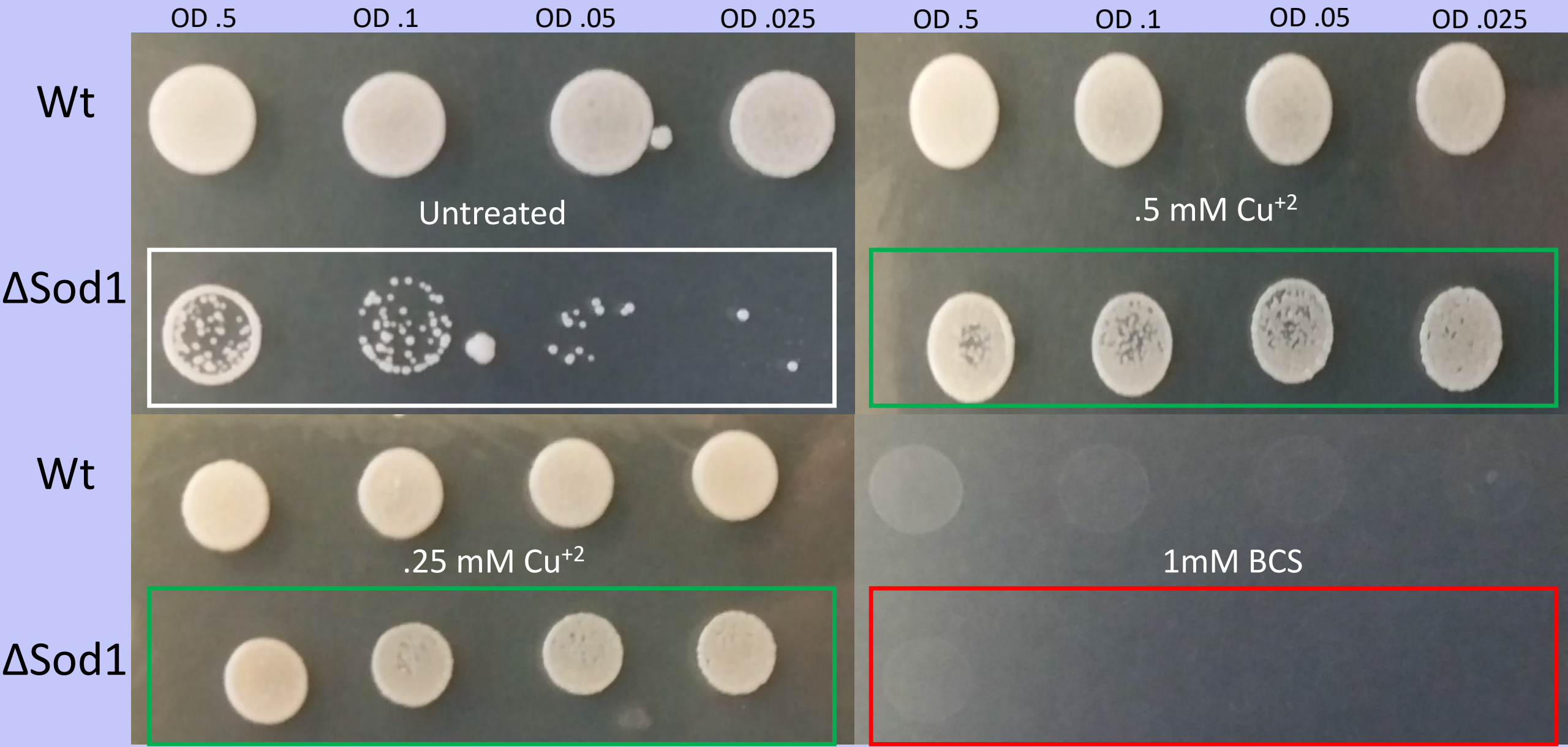


(Chen et al. 2015)

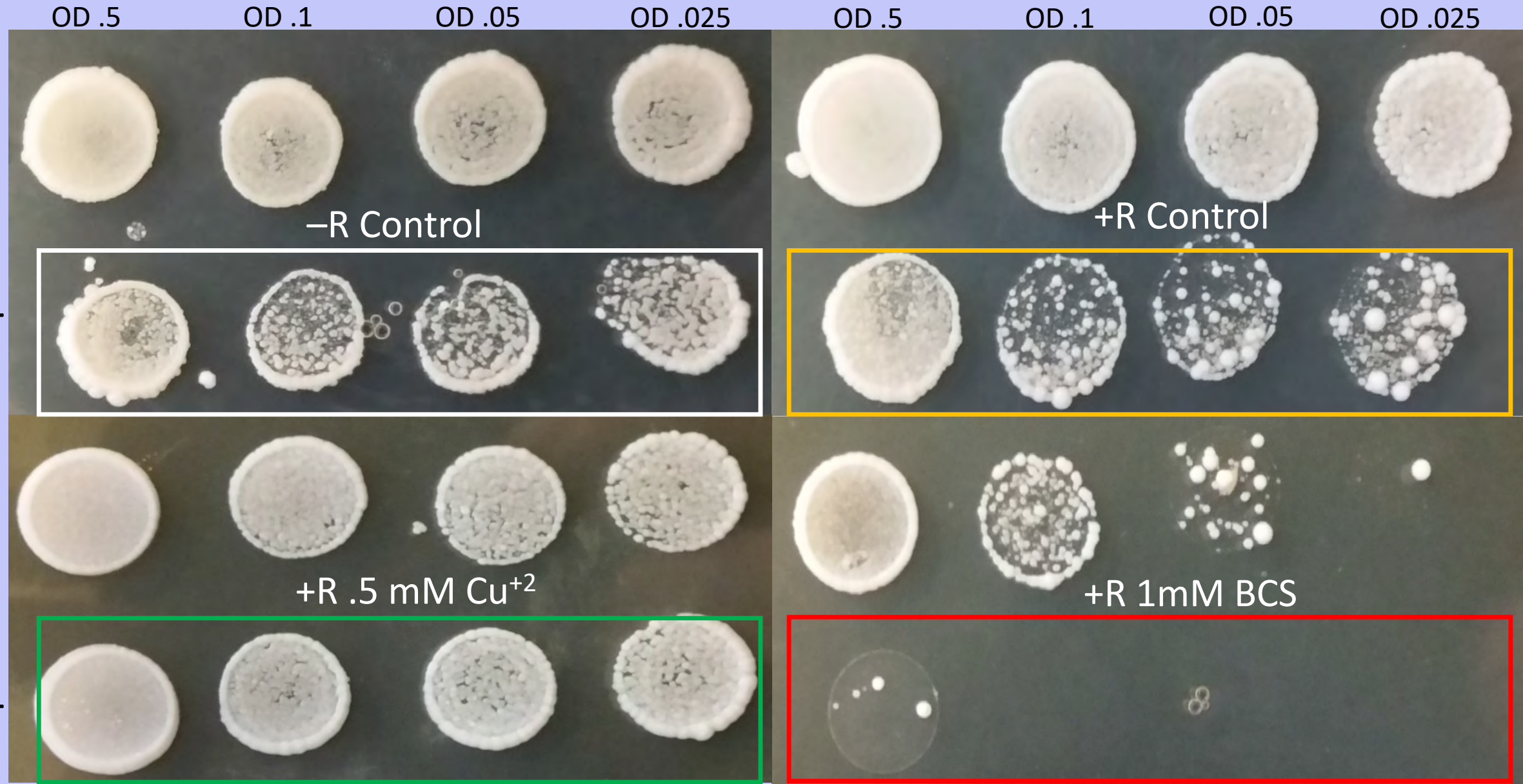


(Showkat et al. 2014)

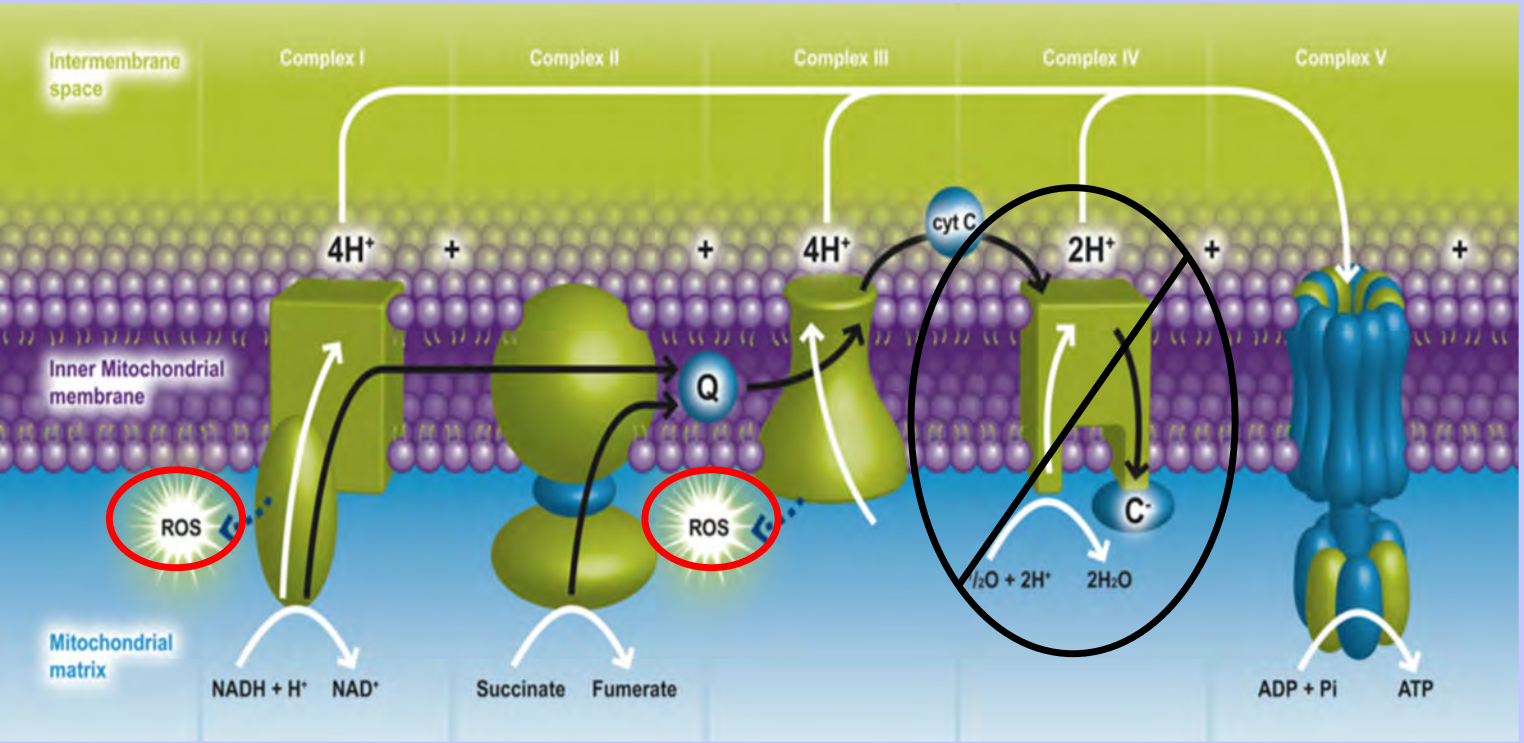
Nonfermentable Growth Assay Treated with Cu^{2+} or BCS



Fermentable Growth Assay with Rapamycin Treatment (R)



Interpretation & Experimental Model



Results & Conclusions

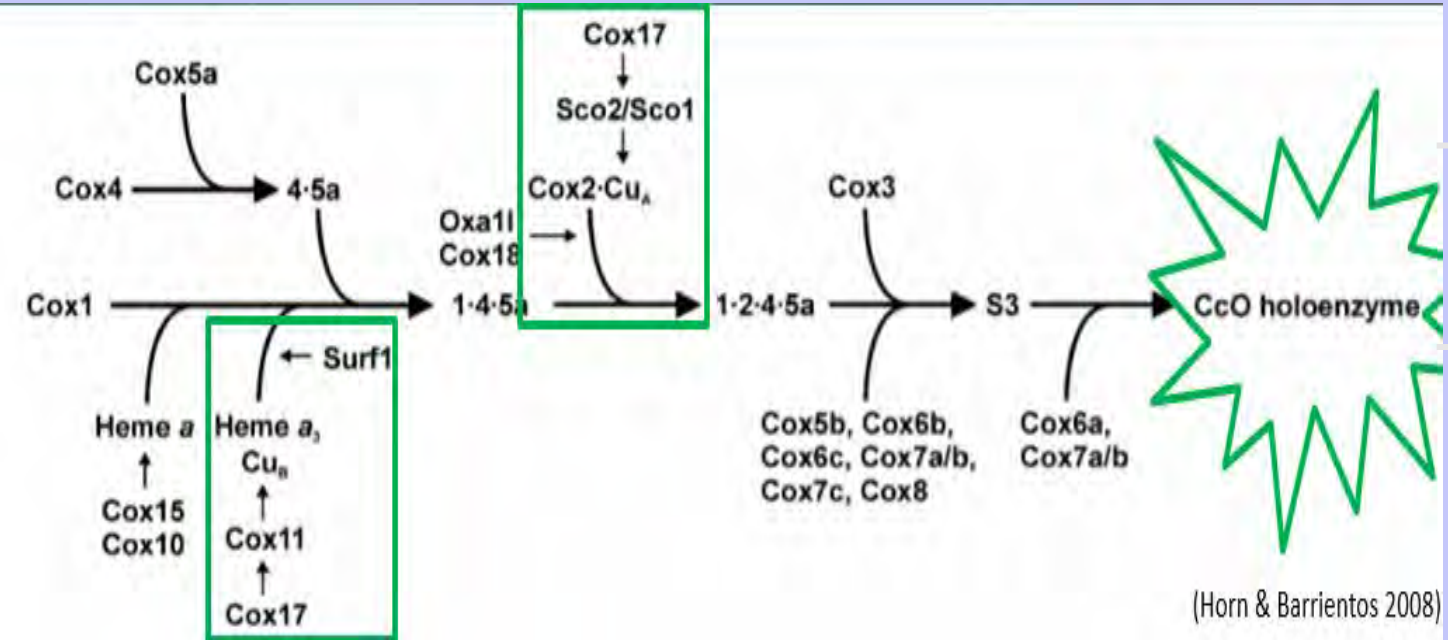
CLS Assay:

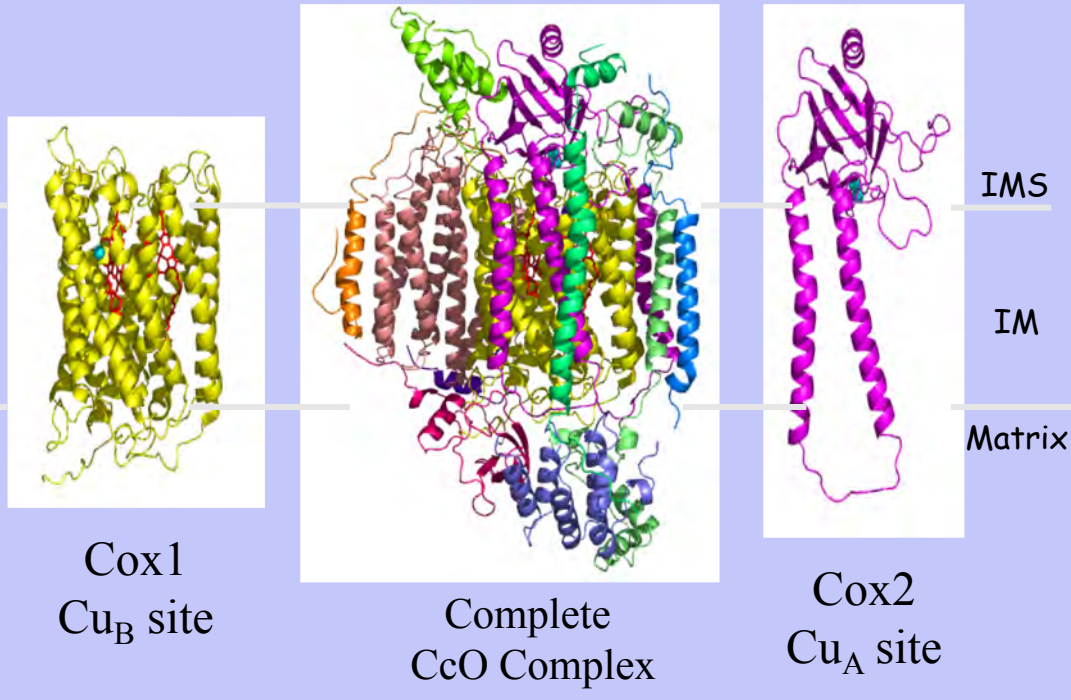
- Lifespan extension of copper treated yeast in all strains and culture conditions

Growth Stress Assays:

- Significant growth stimulation in copper treated cultures induced to respire
- Growth attenuation in respiring cultures treated with BCS and lacking available copper

Copper likely defends against ROS damage by limiting $O_2^{-\cdot}$ production in ETC





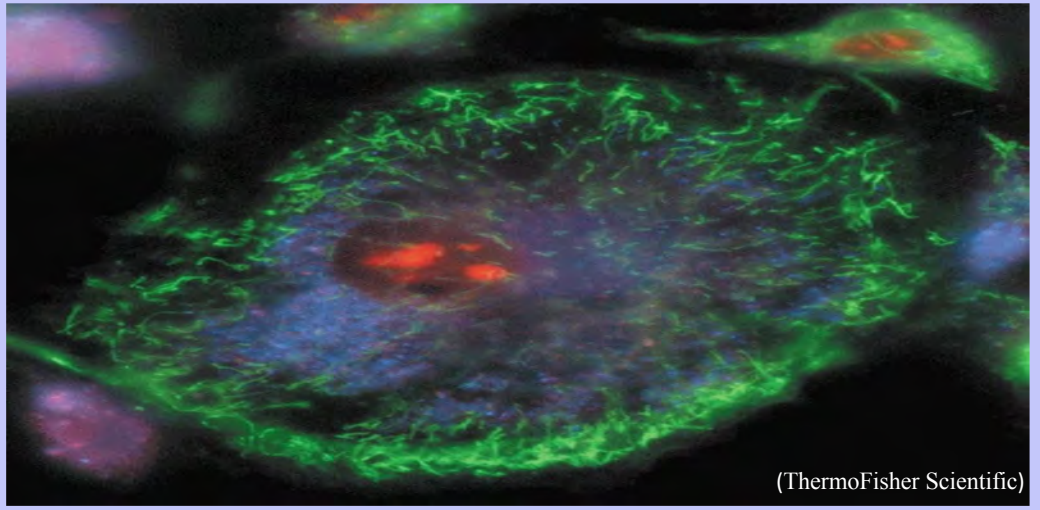
Future Directions

1) Image ROS production in live copper treated cultures via fluorescent dyes (Dihydroethidium)

2) Analyze mRNA expression of Cox2 in response to copper supplementation via RT-qPCR

3) Analyze Cox2 protein expression in response to copper supplementation via quantitative Western blot

1)



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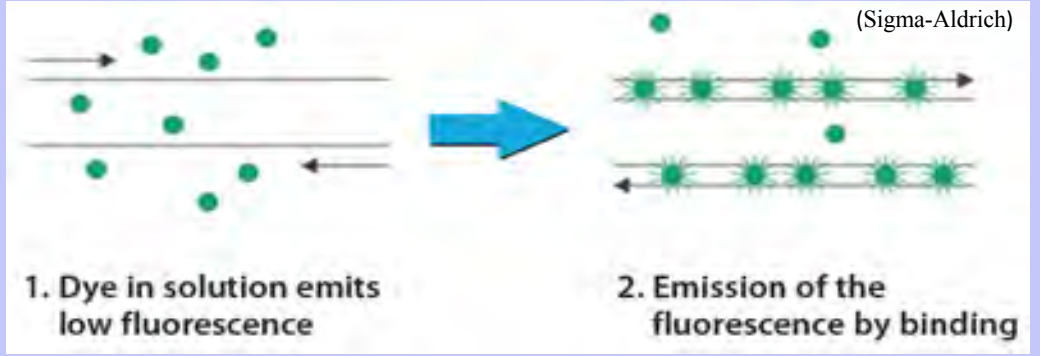

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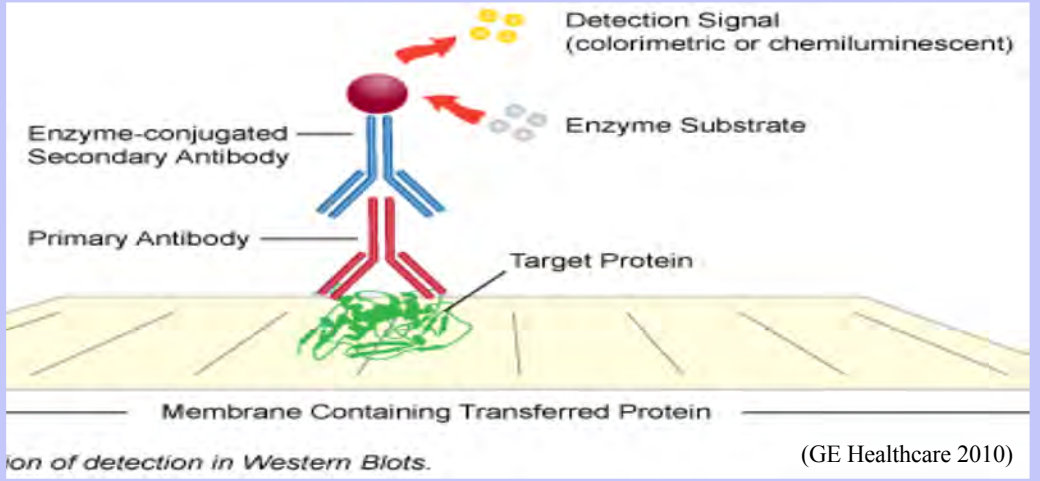
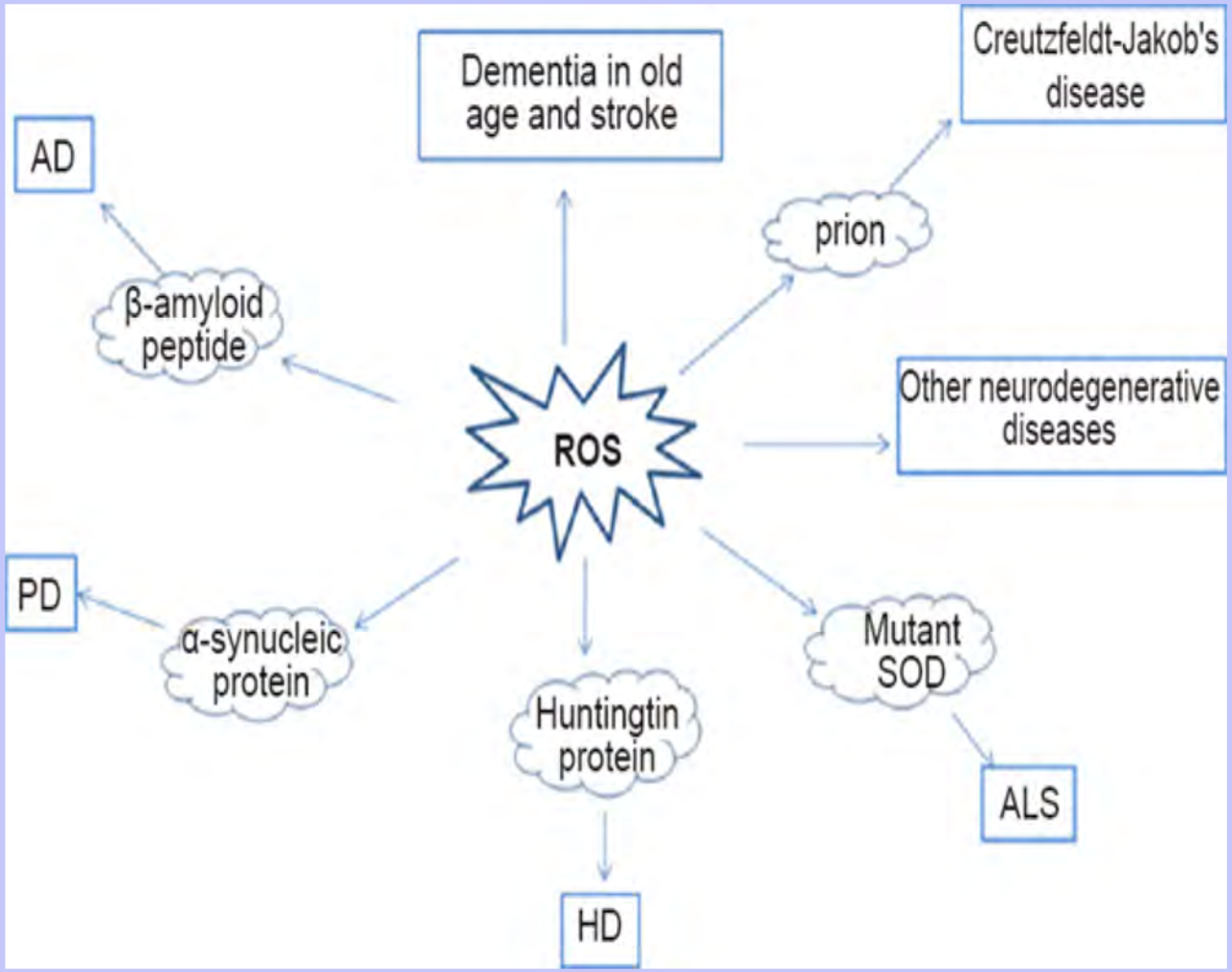
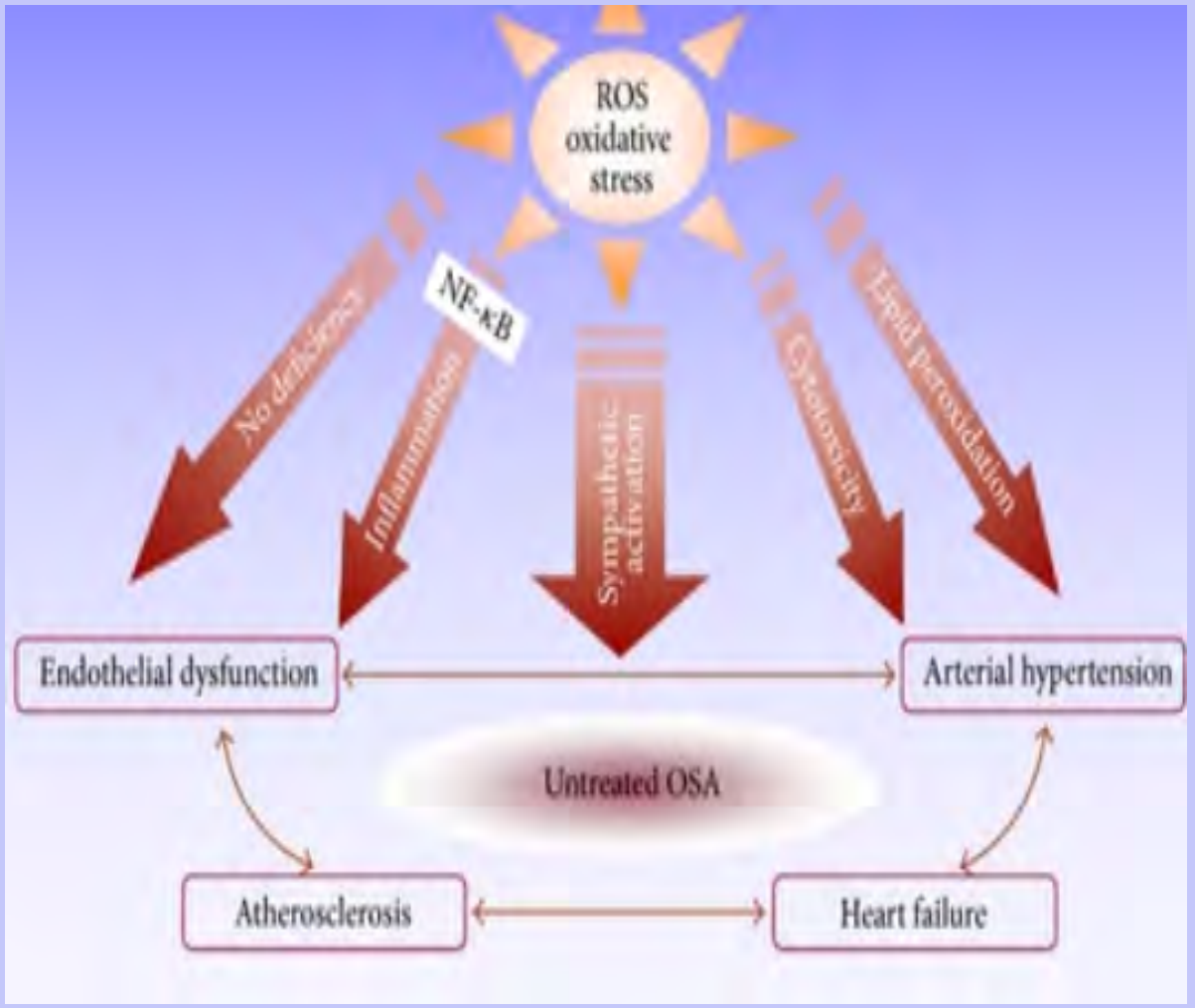


Clinical Relevance





Acknowledgements

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Questions?