

# Copper, ROS, and Mitochondrial Stress

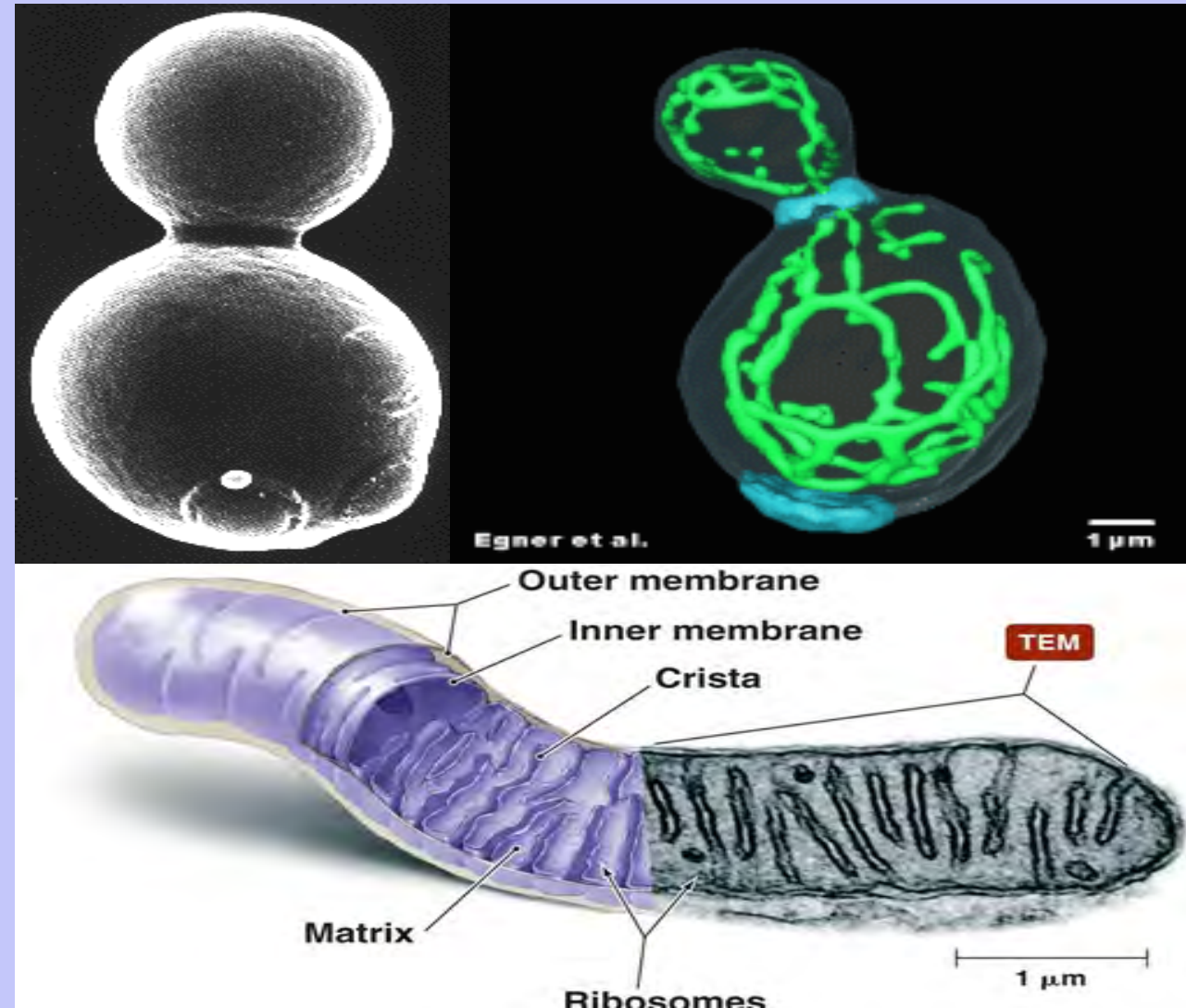
Understanding the pathways that govern  
metabolic homeostasis

Matthew Walser

Dr. Megan Bestwick,  
Linfield Chemistry Department

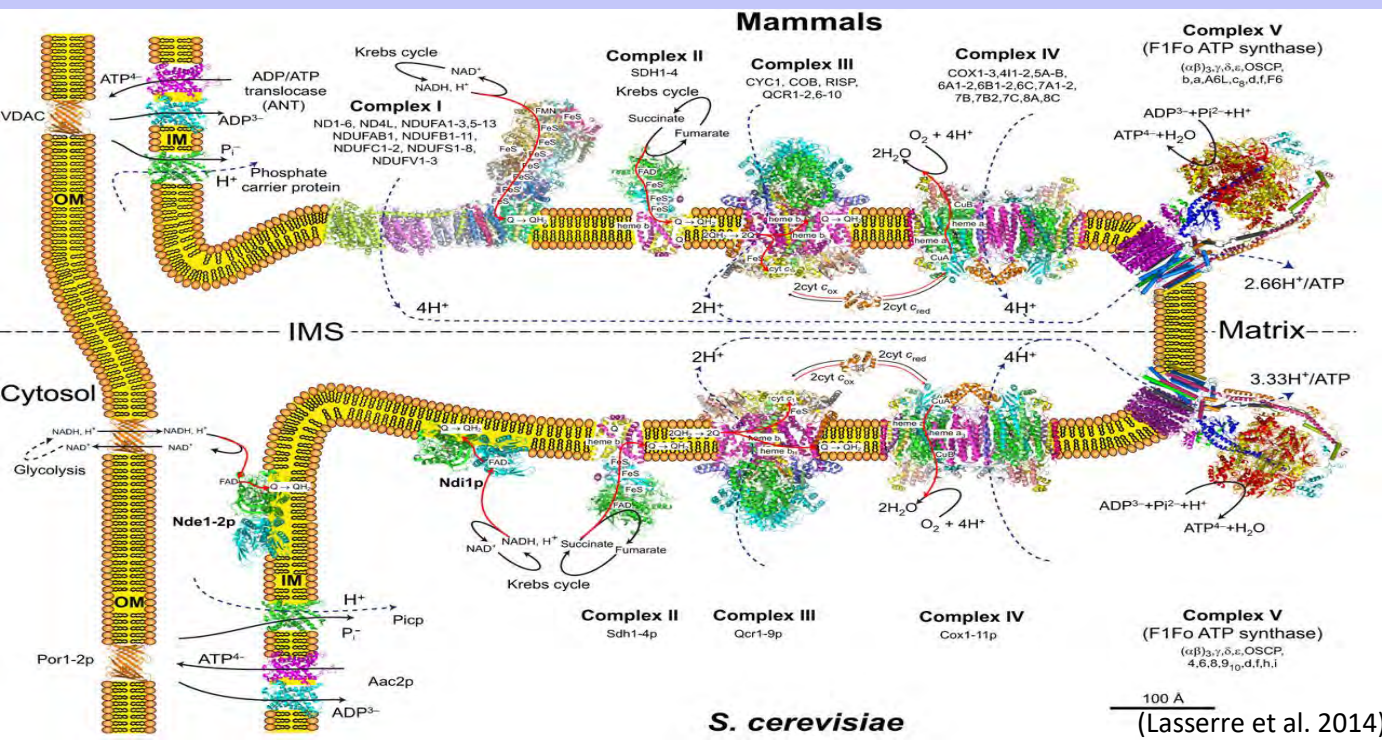
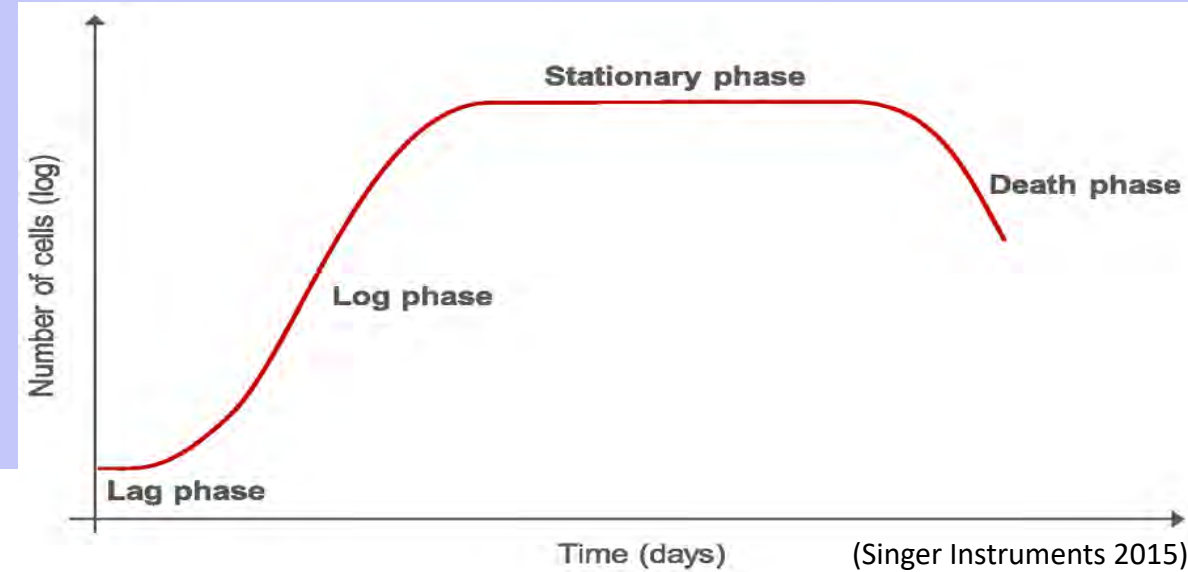
# Overview of Topics

- 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





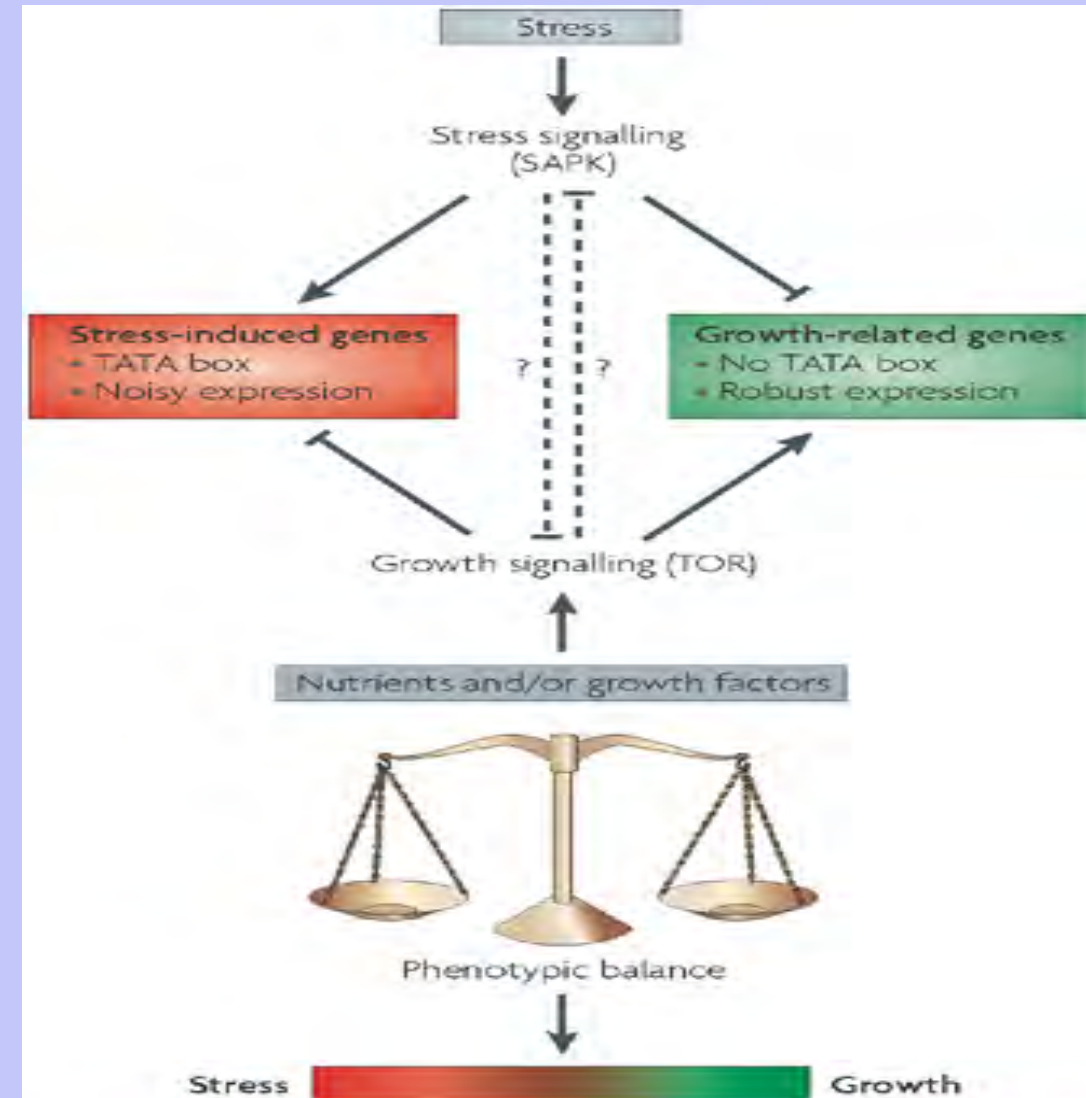
# Environmental Stress Response (ESR)

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**)

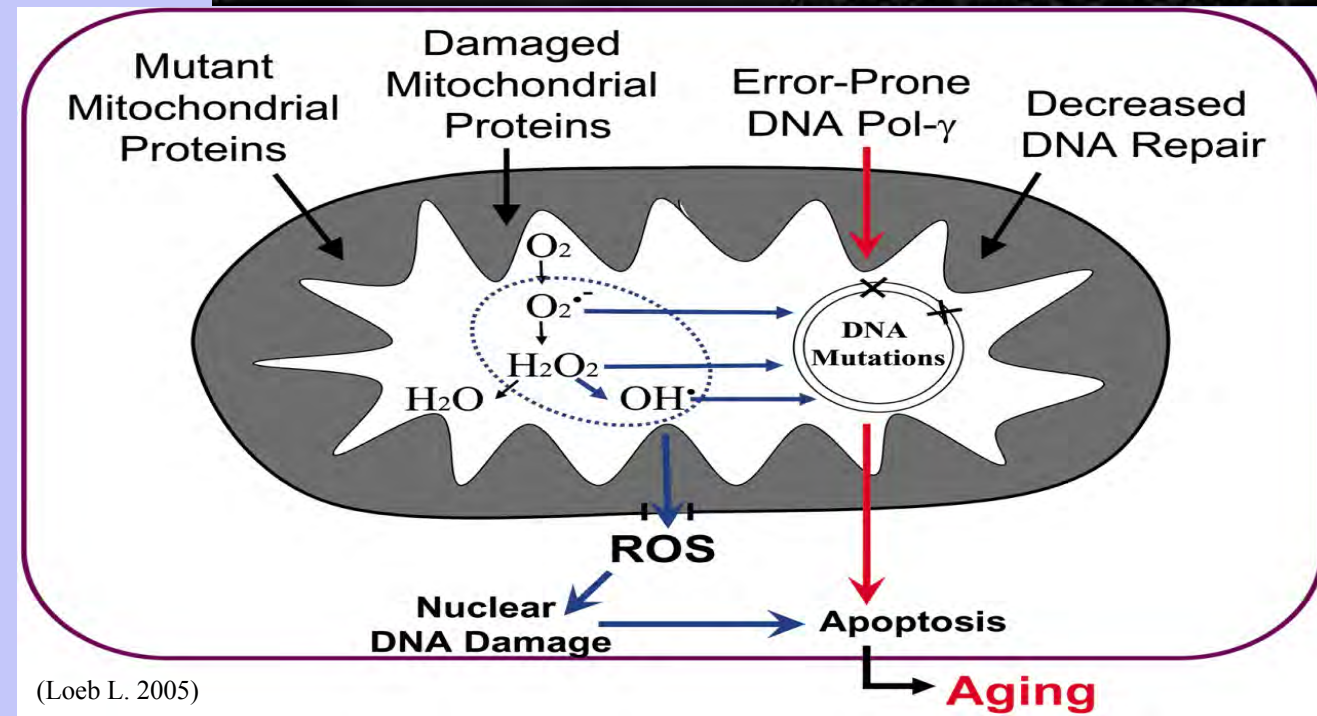
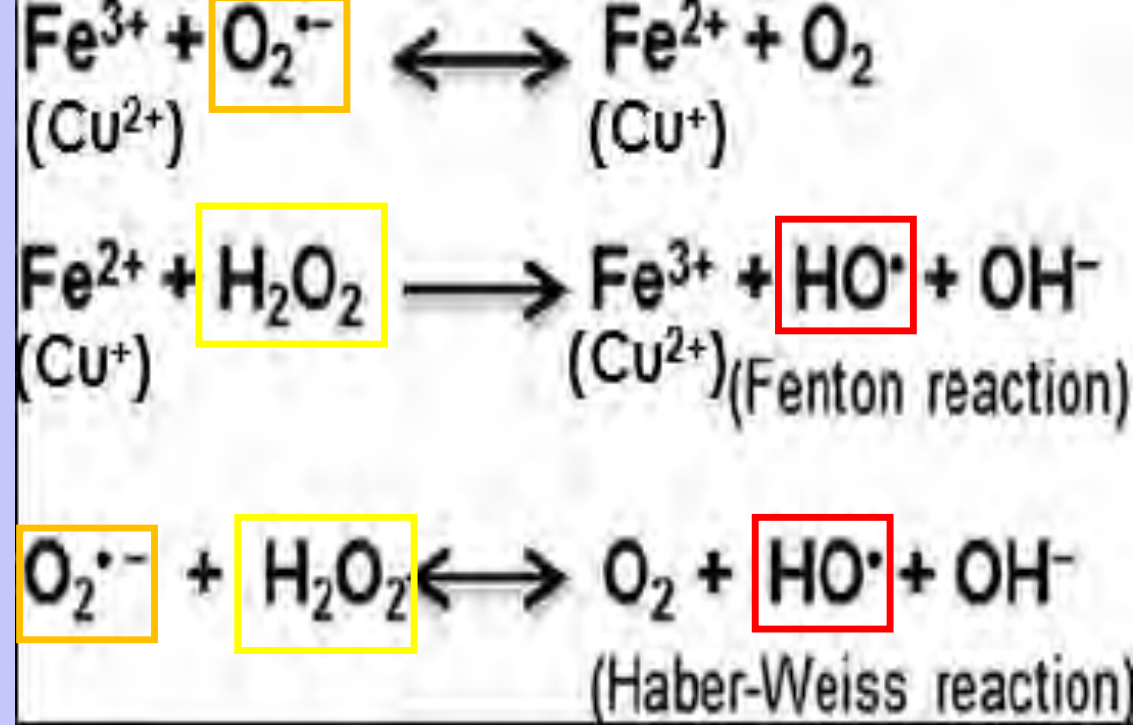


# Reactive Oxygen Species as Mitochondrial Stressors

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



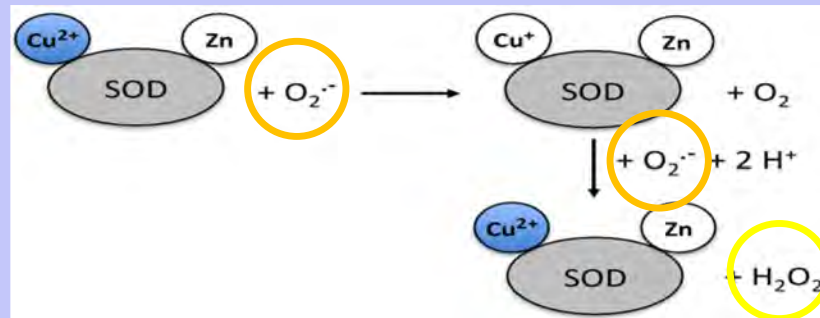




# Protective Role of Copper Against ROS

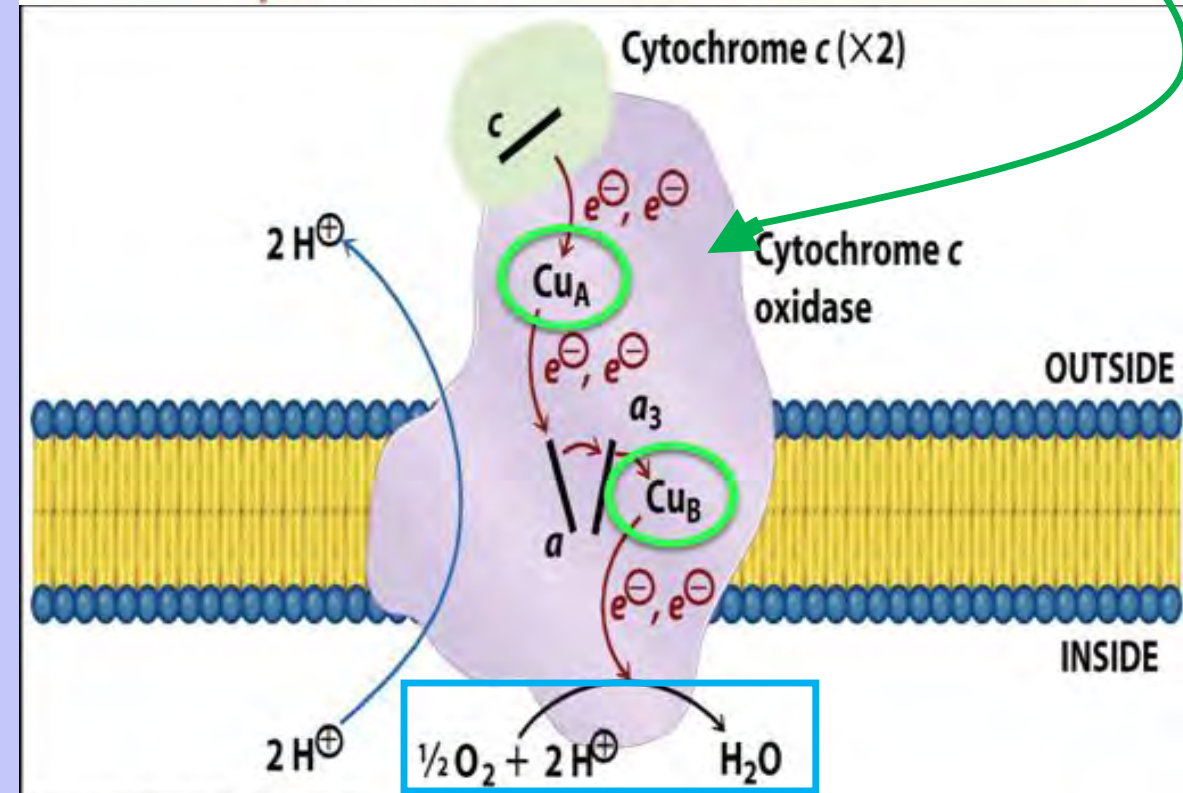
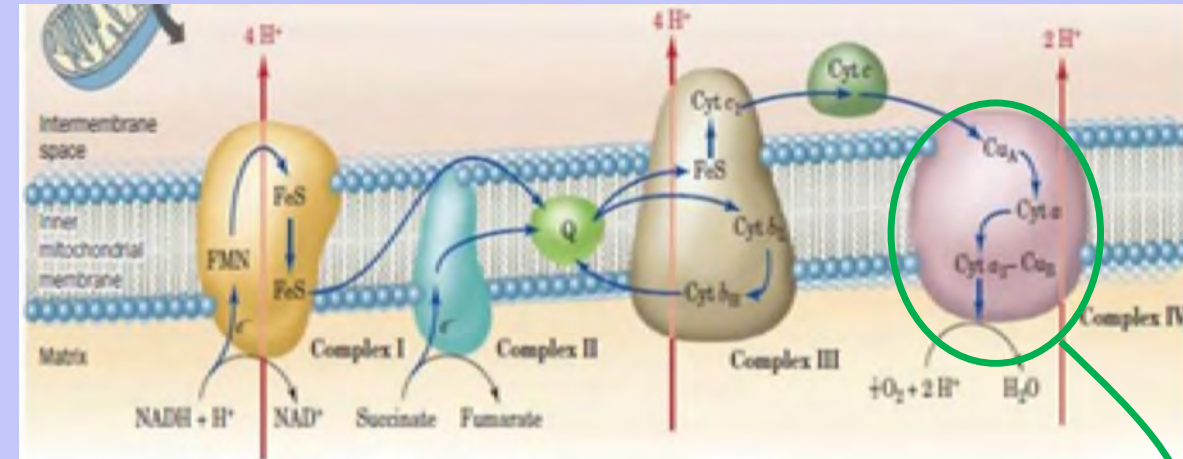
## Curative Role: Redox cofactor for Sod1

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

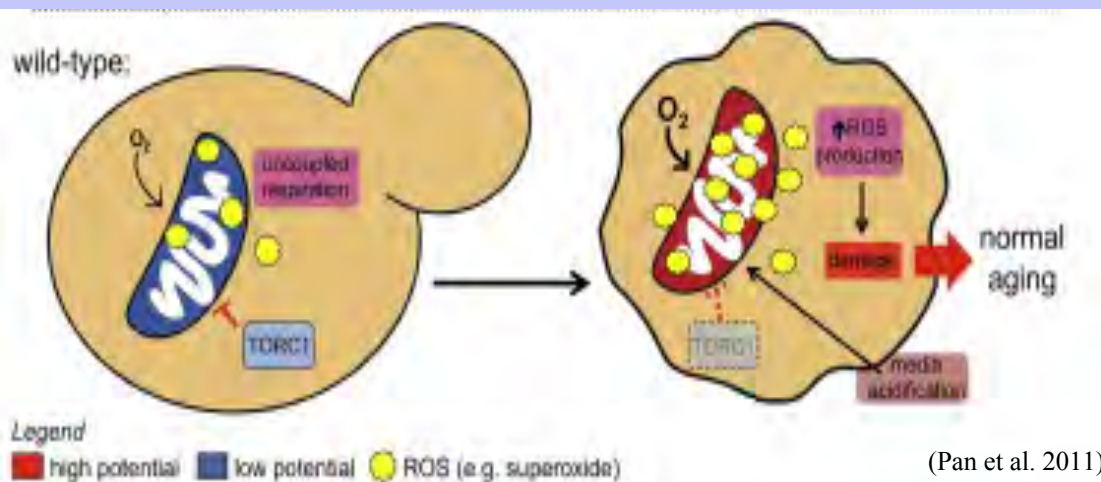
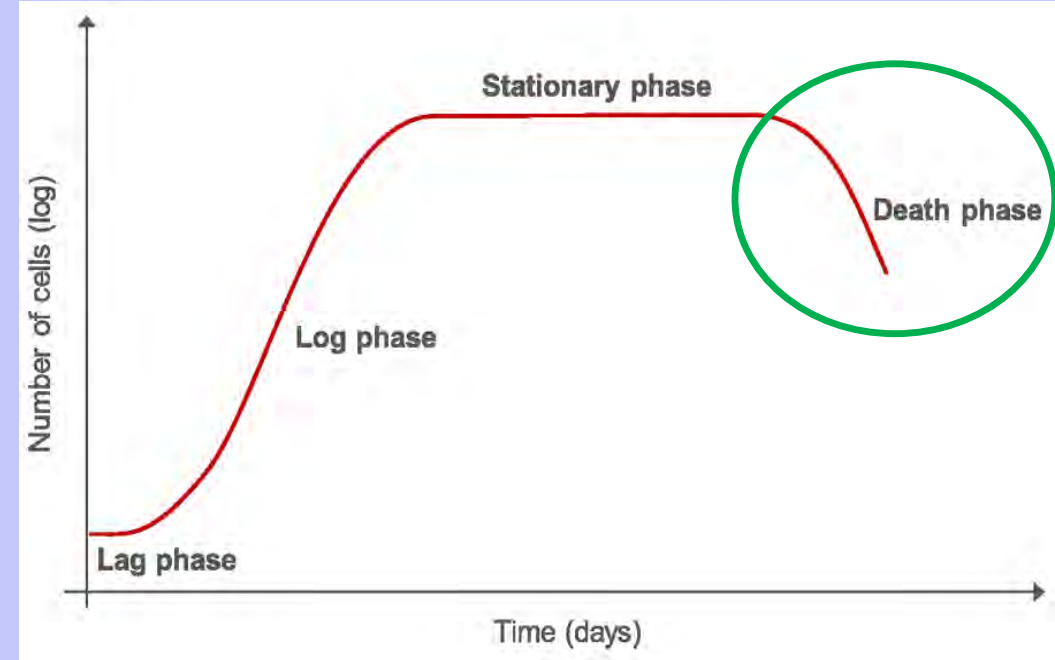
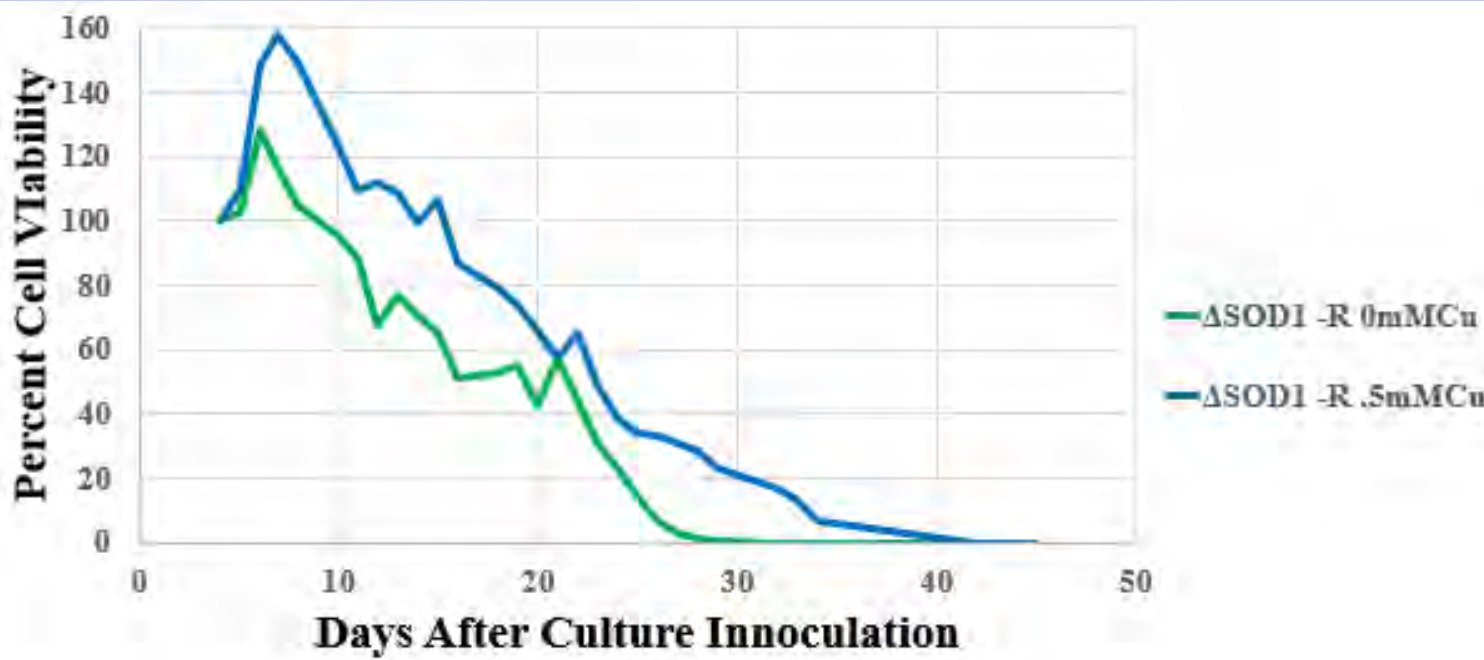


## Preventative Role: Redox cofactor for cytochrome c oxidase (CcO) complex

- 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

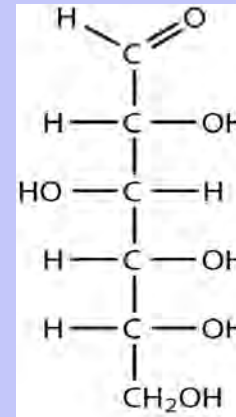


**Goal: Investigate copper's effect on oxidative damage during yeast stationary phase lifespan**

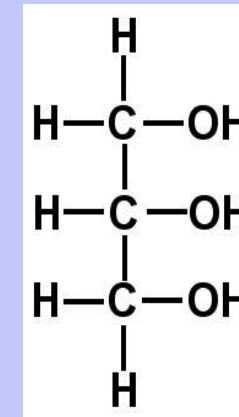


# Growth Assays for Oxidative Stress

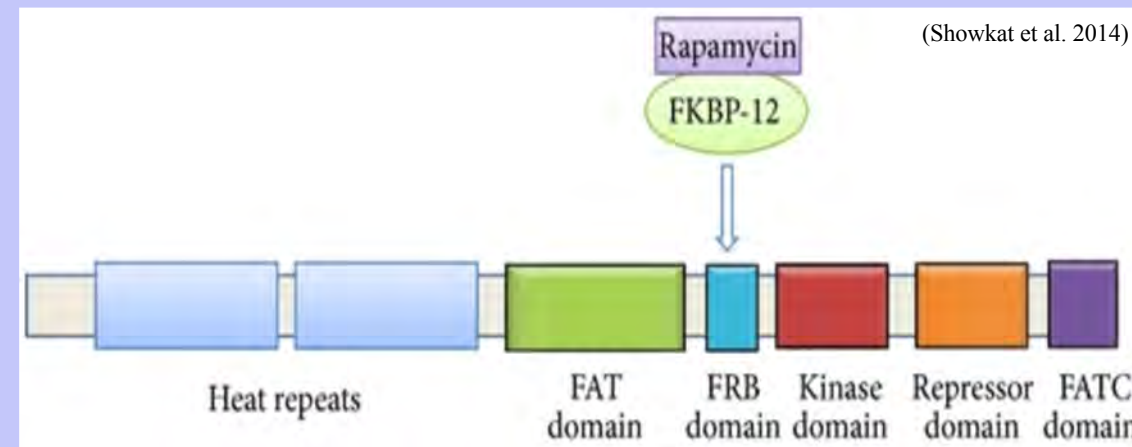
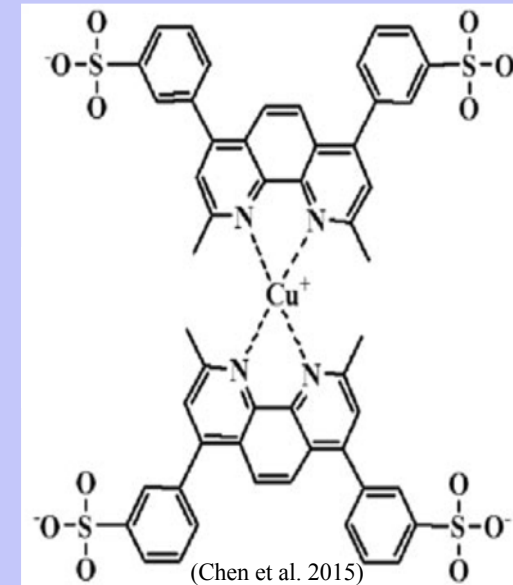
- **Goal: Observe relative oxidative stress, based on growth, between varying levels of copper treatment in different metabolic environments**
- Grow yeast on plates of varying conditions
  - Fermentable vs. nonfermentable carbon source
  - Copper treatment ( $\text{CuSO}_4$ ) or chelation (BCS)
  - Treatment with Rapamycin (induces stress response & respiration)



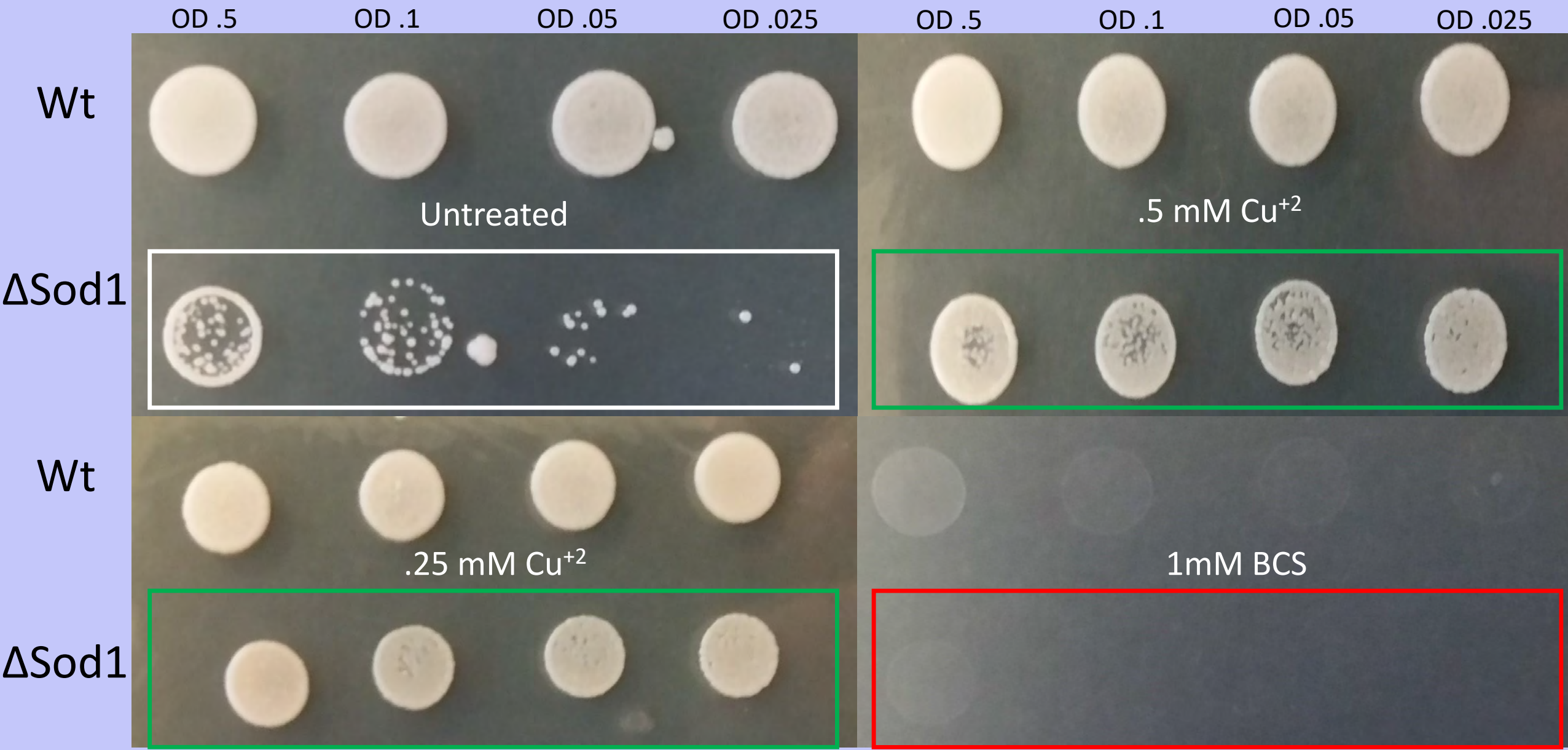
Fermentable  
Glucose



Nonfermentable  
Glycerol

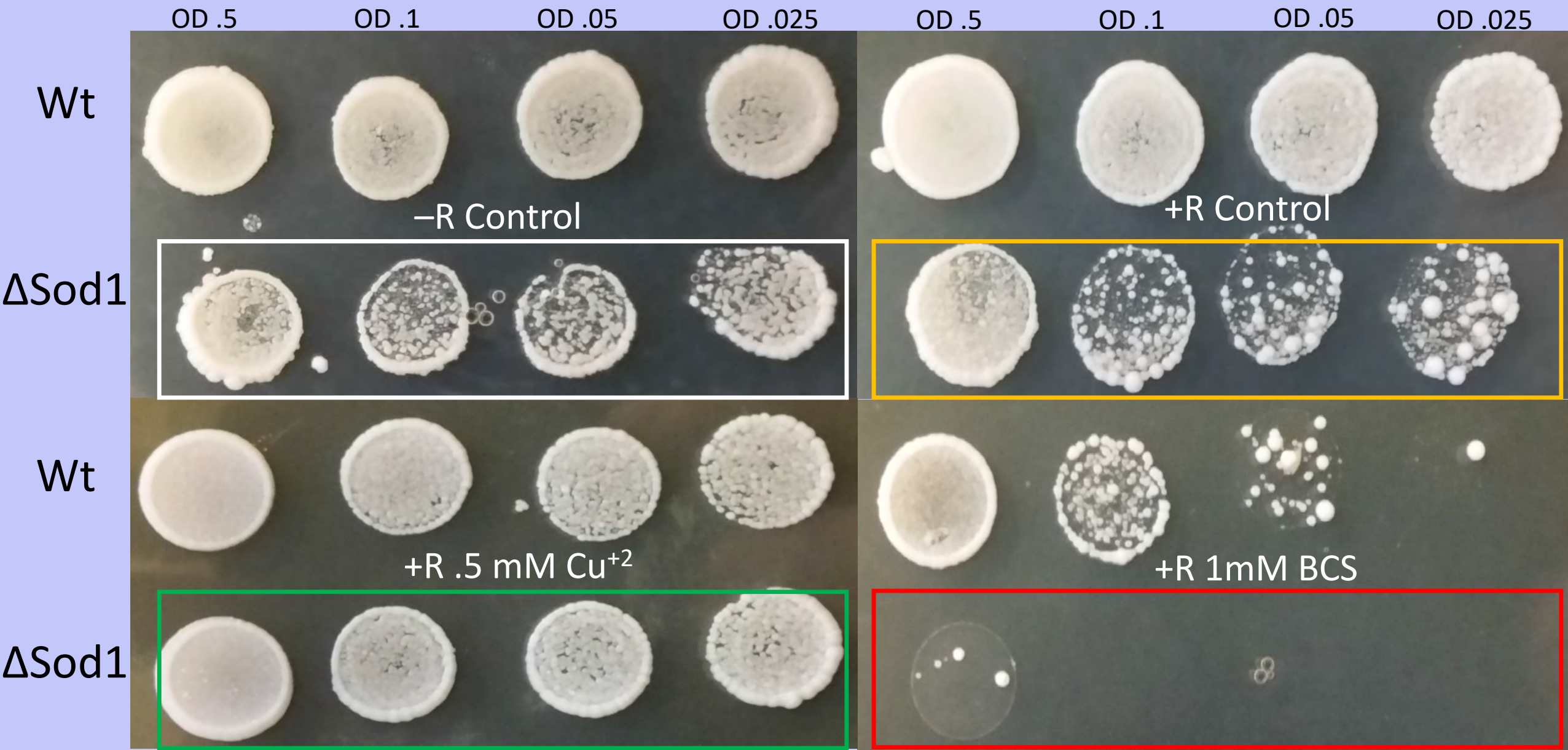


# Nonfermentable Growth Assay Treated with $\text{Cu}^{2+}$ or BCS



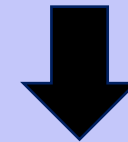
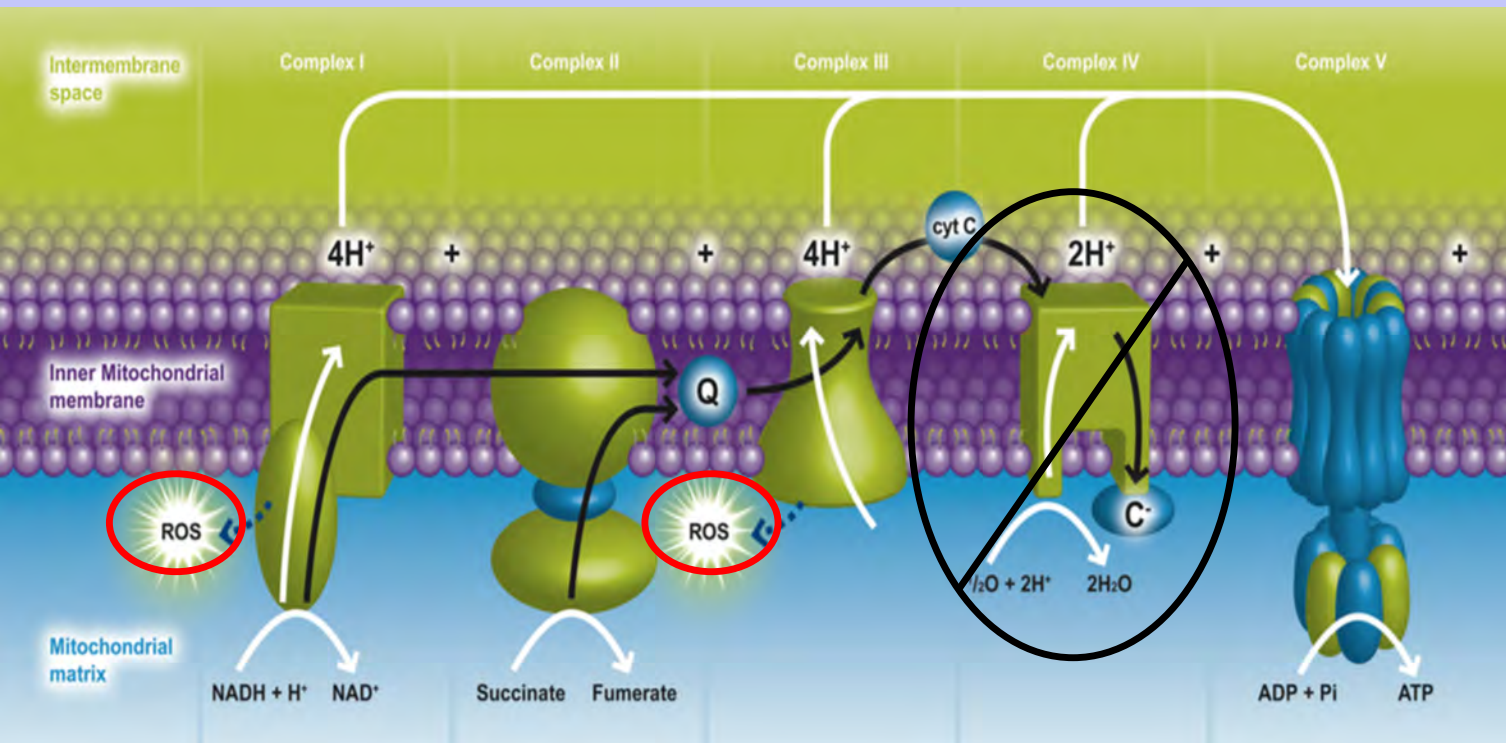


# Fermentable Growth Assay with Rapamycin Treatment (R)





# Interpretation & Experimental Model



# Results & Conclusions

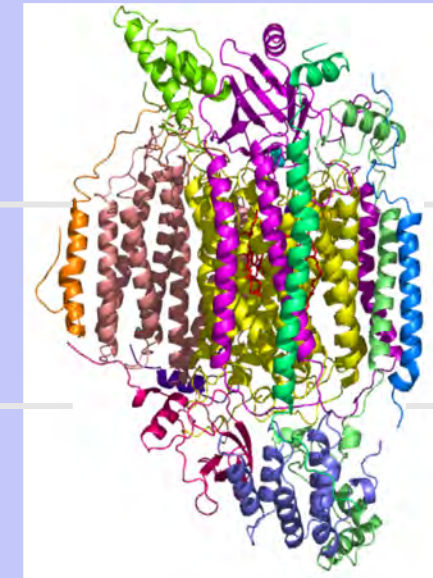
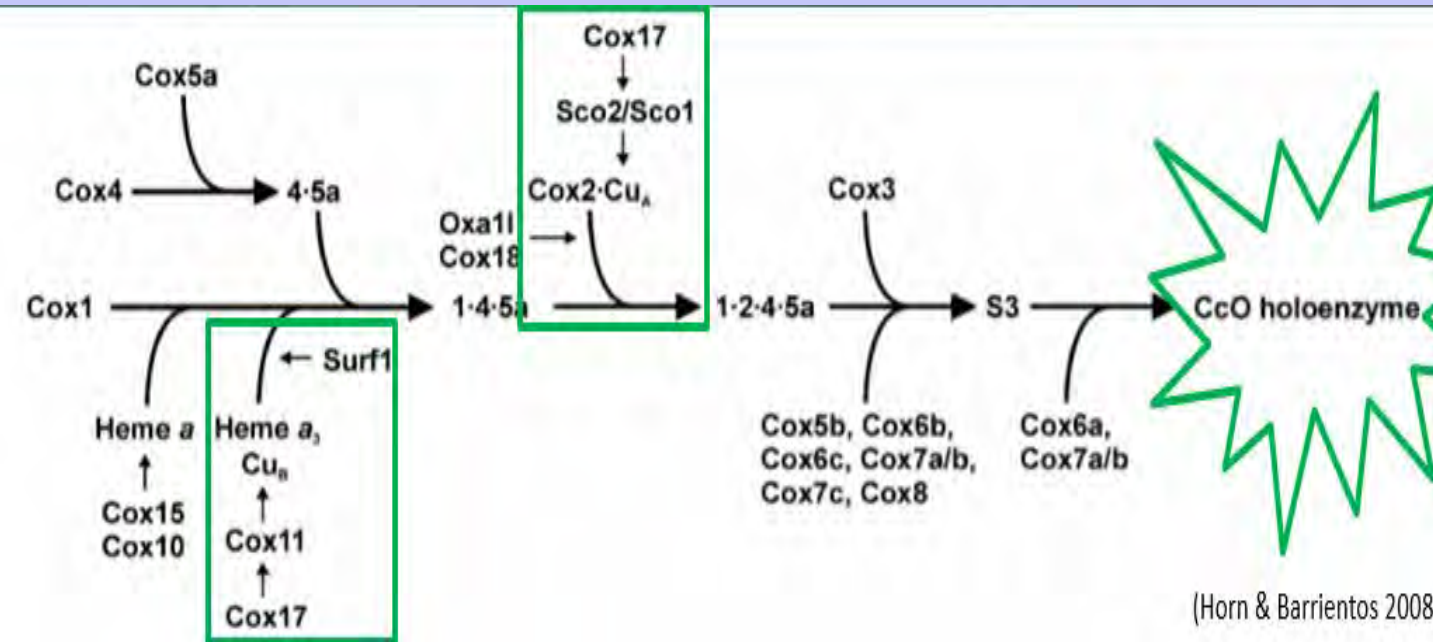
## CLS Assay:

- 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  $O_2^-$  production in ETC**



IMS

IM

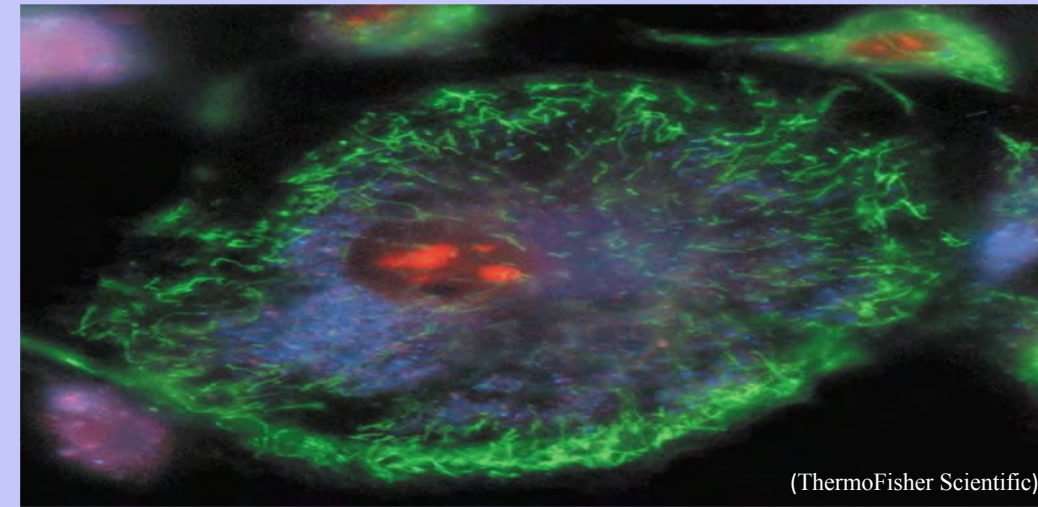
Matrix



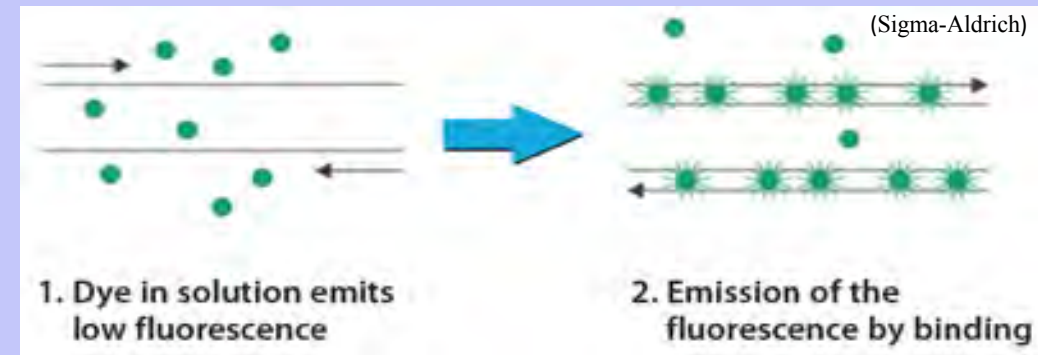
# Future Directions

- 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

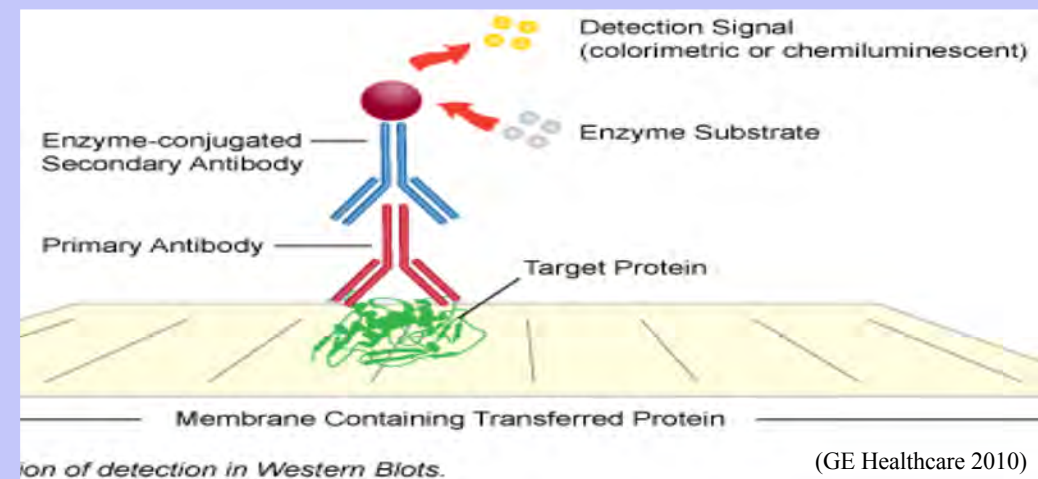
1)



2)

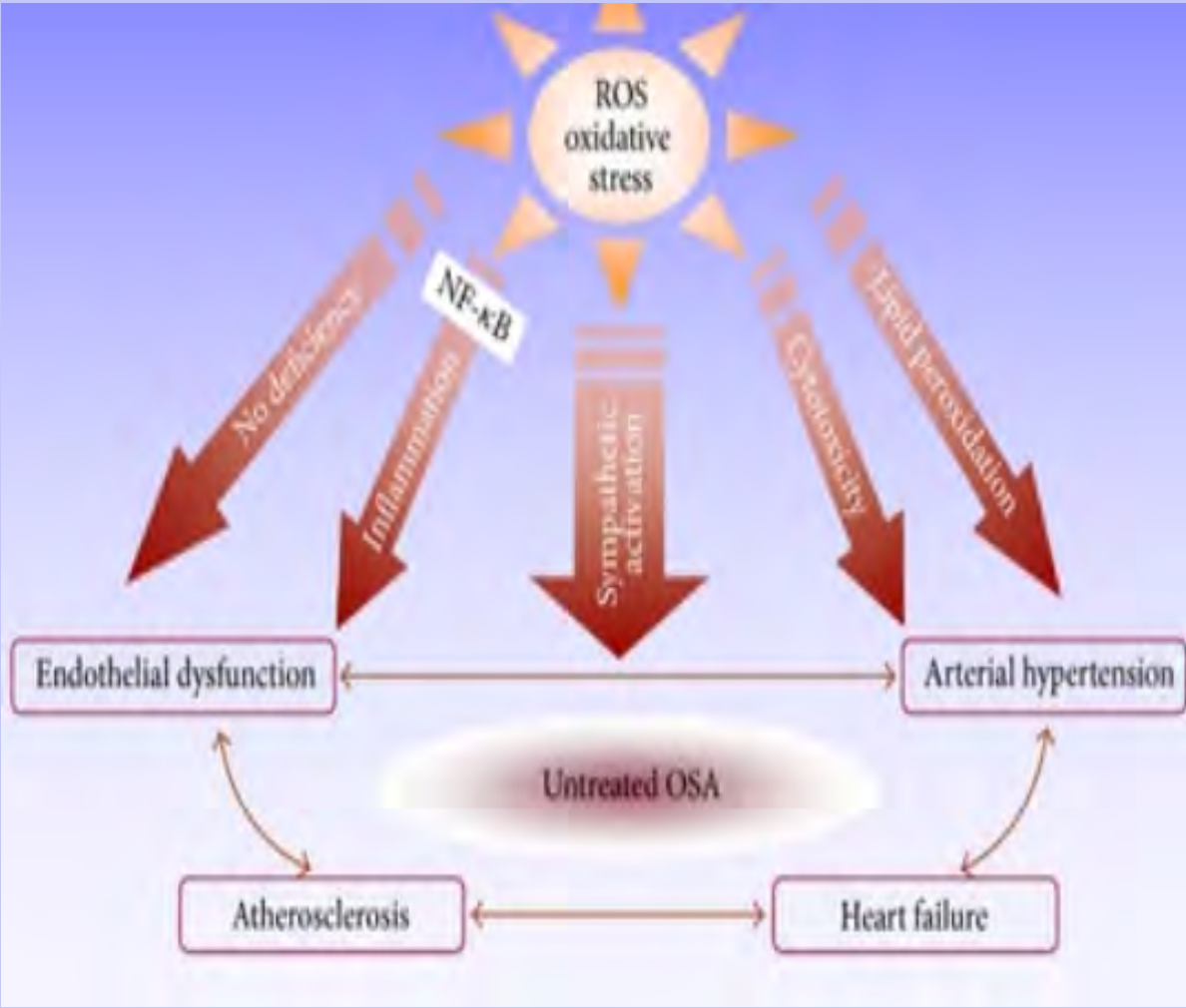
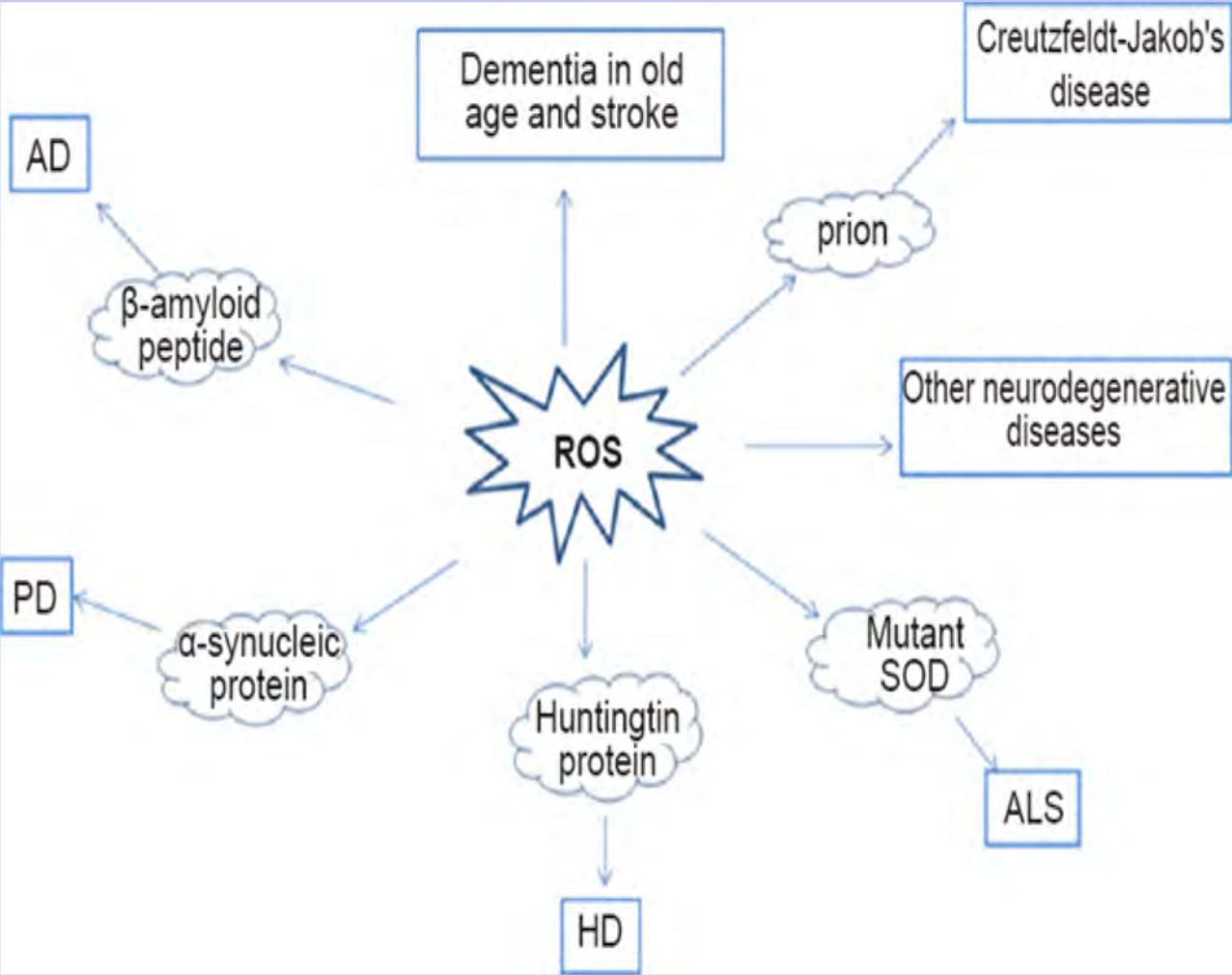


3)





# Clinical Relevance



# Acknowledgements

- Dr. Megan Bestwick, PhD
- The Linfield Chemistry and Biology Departments
- The Linfield College Student Faculty Collaborative Research Grant
- The National Science Foundation
- The Murdock College Research Program for Natural Sciences
- Kelly Schultz, Kelsey Bruce, Sarah Rempelos, Shae Reece



Questions?