# √Introduction

## Cellular aging and ROS, TOR 1 pathway, PCP1 function and its connection to CR and aging.

Aging of Saccharomyces cerevisiae cells is an invasive aspect of research that can potentially lead to several implications related to human aging diseases. There are many factors that may influence cellular aging in yeast such as reactive oxidative species (ROS), osmotic pressure, vacuole acidification and calorie restriction (CR). It has been proposed that a reduction in calorie intake slows aging and increases lifespan by suppressing the TOR signaling pathway. **(DIAGRAM)** Suppressing the TOR pathway consequently reduces endogenous ROS levels which are known to be the causative agents of cellular protein, lipid, and DNA damage as well as fragmentation of the mitochondria. Mitochondria play a critical role in life span extension effects of CR because it houses the electron transport chain which has been found to be the center of superoxide production. The research under study focuses on how CR alters the level of superoxide anions O2- in the ∆PCP1 cell and in turn how it affects the longevity of yeast cells. PCP1, a rhomboid serine protease, participates in mitochondrial dynamics and the processing of cytochrome c peroxidase (CCP1) which is involved in degradation of hydrogen peroxide species. Additionally PCP1 partakes in the processing of MGM1 a dynamin- like GTPase involved in mitochondrial fusion, fission and cristae formation. **(DIAGRAM)** We hypothesize thatdeletion of PCP1 will lead to an extended replicative life span (RLS) and lower growth fitness due to its influences on the endogenous levels of superoxide. If there is a ∆PCP1 gene then the cell will be dysfunctional in processing MGM1. Improper processing of MGM1 can lead to an irregular formation of the inner membrane of the mitochondria and thus affecting the function of the electron transport chain ETC. Ultimately an inefficient ETC along with CR should together decrease ROS levels and consequently increase the longevity of the cell.

# √Materials and Methods

In efforts to determine how calorie restriction and a ∆PCPP1 gene together influences superoxide level we first grew ∆Pcp1 mutant cells as well as BY4743 cells which served as the wild type control. A single colony from both were taken and grown separately at 30° degrees in tubes containing 20% glucose YPD liquid. Using two test tubes the ∆Pcp1 culture was exposed to 2% glucose which is normal nutrient conditions and .05% glucose which is calorie restricted conditions. The same was done for the wild type BY4743. From each media spin down 1ml of cells , then resuspend in 0.5ml PBS. Split to 2-5 eppendorf tubes for FACS set up, label the tubes as “no stain control”, “stained”, and strain name and incubation time. Spin down all tubes, re-suspend in 1ml of PBS. Add 1 ul of 5mM DHE stock in DMSO to a final concentration of 5uM  of DHE. After incubation for 10 min at 30 °C, cells were spun down , resuspend in 1ml PBS, and transfered to FACS tubes, keep them in boxes wrapped with aluminum foils. After briefly sonicating the suspension, intracellular superoxide anions were measured via dihydroethidium DHE (Molecular Probes) signals using a FACSCaliber2 flow cytometer (BD-Biosciences) with a 488-nm excitation laser. Signals from 25,000 cells/sample were captured in FL3 (>670 nm) at a flow rate of 5,000 cells/s. The DHE signals Data collected with the FACSCaliber2 flow cytometer were processed with Flowjo software (Tree Star) and quantified with WinList software (Verity Software House).

# √Results

## When comparing the wild type cells exposed to normal nutrient concentrations 2% glucose and restricted nutrient concentrations .5% glucose results indicate that CR suppress DHE signals. Contrarily Pcp1D has low DHE signals regardless of glucose concentration.

# Disucssion

## If CR works through H2O2 and endogenous H2O2 level are extremely low in pcp1D, then CR should not extend life span of pcp1D. A hypthoseiss that can be tested.

# References

# Figures and Tables

## Diagram of cellular aging, ROS, and PCP1 funciton (with hypothesis highlighted) (tell people what and why we are doing this project)

## DHE stain FACS data, BY47473 and pcp1D

## Table List of strains used