Study the mechanism of gene PCP1 in longevity of yeast cells

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Mitochondria play a central role in life span extension effects of calorie restriction (CR). PCP1, a mitochondrial rhomboid serine protease, participates in mitochondrial dynamics and the processing of cytochrome c peroxidase (CCP1) which is involved in degradation of ROS such as peroxides. Additionally PCP1 partakes in the processing of MGM11 a dynamin- like GTPase involved in mitochondrial fusion, fission and cristae formation. Deletion of PCP1 leads to extended replicative life span (RLS) and lower growth fitness. Therefore, we focused on the role of PCP1 to better understand the role of mitochondria in CR. We hypothesize that the effect of PCP1 on life span is due to its influences on the endogenous levels of superoxide. We test this hypothesis by comparing the superoxide levels between cells grown in normal conditions and calorie-restricted media, and then monitored the superoxide levels by dihydroethidium staining. Using flow cytometer, we found that CR suppresses superoxide levels in wild type cells. In contrast, cells of PCP1 deletion mutant show extremely low superoxide levels in both normal and CR media. These preliminary results suggest that the long RLS of pcp1∆ is due to its low levels of O2·-, and also suggest that CR would have no effect on the lifespan of pcp1∆. This study can potentially lead to more insights on ageing-related diseases in humans.

…..There are many factors that may influence aging such as reactive oxidative species, osmotic pressure ,vacuole acidification and calorie restriction. This research focuses on how calorie restriction alters oxidative stress levels in ∆PCP1 genes and in turn how it affects the longevity of yeast cells. It has been proposed that the reduction in calorie intake without reduction in adequate nutrition (CR) slows aging and increases lifespan by increasing the activity of sirtuin SIR2 a NAD- dependent deacetylase...........CONNECT TO OXID STRESS

Oxidative stress is the result of an occasional charged oxygen molecule being created (free radical) usually by the electron transport chain in the mitochondria. The charged oxygen molecule becomes very unstable and reactive. It then attacks other cells to seek electrons to pair with its missing electrons. If not neutralized by antioxidants they go on to create more volatile free radicals. The production of ROS such as peroxides and free radicals leads to fragmentation and damages all components of the cell, including proteins, lipids, and DNA (Frei 1997).

Pcp1...............

In efforts to determine how calorie restriction would affect a ∆PCP1 gene we 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 at 30° degrees in separate 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. Using a technique known as DHE

Results.........