Mitochondrial DNA Deletions and ROS Scavengers

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The purpose of this experiment is to observe how deletion of genes that are involved in the electron transport chain cause mitochondrial damage and an increase in reactive oxygen species and if antioxidants could minimize the effects of oxidation. *Saccharomyces* cerevisiae is ideal for this study as it is used for research with chronological aging. Chronological aging is the survival during the stationary phase after nutrients and space becomes limited and has been used to study neurons in the central nervous system, oxidative stress, and changes in morphology. Deletions of mitochondrial DNA and the increase of reactive oxygen species over time has been linked to a decline in the production of ROS scavengers. ROS scavengers serve as a defense against the oxidation of various cells by neutralizing the reactive oxygen species. These include antioxidants such as Vitamin-C, Vitamin-E, and flavonoids. For this experiment, genes from the yeast *Saccharomyces* cerevisiae will be removed using gene knockout, which will inactivate the genes of interest. The genes of interest are Cox 1, 2, 3, 5a, 12, 23 and SOD 1 (Superoxide Dismutase) and 2, OPA 1, and Atg32. These genes are important in the electron transport chain, fission, fusion, and mitophagy. The mutated yeast will then be placed into a ROS scavenger media containing Vitamin-C and incubated overnight. Assays that will be used include Rhodamine 123 which determines membrane potential and proton flow from the inner membrane to the matrix, Janus Green which reveals alterations in the electron transport chain and amount of oxygen available, Cytochrome c Oxidase assay which can determine cytochrome c activity and outer membrane stress, and dihydrorhodamine to indicate ROS levels. Using fluorescent dyes such as Rhodamine 123 will allow the cells to be observe through a microscope and observe the amount of damage and fission that has been produced by the mutations and the effects of the antioxidants on the destruction of the mutated cells.

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