Copper uptake by CMC1 Deletion in Saccharomyces Cerevisiae

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Abstract
The CMC1 deletions appears to uptake less copper when media is supplement with .04% copper sulfate or higher.

Introduction
Cytochrome c oxidase (CcO) or complex IV is the terminal component of the electron transport chain. In eukaryotic organisms, CcO is composed of 12-13 subunits. The core of eukaryotic CcO contains three mitochondrial encoded subunits that comprise the catalytic complex of the several gene products encoded for by the nucleus (1). Essential to the redox function of CcO are several critical cofactors: two hemes and two copper centers (2). The crystal structure of CcO has led us to several insights about its structural components and catalytic activity (3). However, a large set of nuclear gene products are essential for CcO activity that are not part of the structural machinery of the complex (4,5). These components have been implicated in various stages of CcO assembly including, heme processing and insertion (6), Cu(II) and Cu(0) site delivery and insertion (7), subunit processing and subunit assembly (8,9). Among them are well characterized CcO assembly factors that involve the biogenesis of the CuA and CuB sites in CcO. Sco1, Cox17, Cox23 are all factors that involve the biogenesis of the Cu centers during Cox1 maturation in yeast cytochrome oxidase biogenesis that facilitates the maturation of Cox1.

Materials and Methods
A 20% glucose solution and minimal media solution (5 g of ammonium sulfate, 1.7 g of yeast nitrogen base without amino acids, 0.73 g of amino acid dropout mix (CSM-DE-TRP)) were made. Poured 45 mL of minimal media into separate 250 mL Erlenmeyer flasks and then autoclaved the minimal media and the 20% glucose solutions. After the solutions cooled down, added 5 mL of the 20% glucose solution to the Erlenmeyer flasks. Inoculated one of the 250 mL Erlenmeyer flasks with the wild type cells and another flask with the CMC1 cells and allowed them to grow for less than 24 hours in a 30C incubator rotating at 250 rpm. Once OD reached 0.3 (1:10 dilution)—UV-Vis spectrometer measurement—transferred the calculated amount of cells to the shock series flasks and allowed to grow for less than 24 hours. Once OD reached 0.3 (1:10 dilution)—UV-Vis spectrometer measurement—from the shock series flasks, the corresponding copper percent was added and the shock series started with 15 minute 1 mL samples taken for up to two hours (0.5 M Cu solution was prepared from 19.95 g of CuSO4 in a 250 mL volumetric flask).

The 1 mL samples were centrifuged in 2 mL micro centrifuge tubes twice with the second centrifuge done with the removal of the supernatant. Removed 0.5 mL of these different cell solutions, added them to separately appropriately labeled 50 mL conical tubes to be run on the AAS. Transferred these solutions to appropriately labeled 50 mL conical tubes to be run on the AAS. Made 2% nitric acid solutions and made 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm, 2.5 ppm, and 3 ppm Cu standards (The blank was the 2% nitric acid solution with no Cu added). After calibrating the AAS, the samples were run.

Results
Discrepancies in the amount of Cu uptake between the two (CMC1 & Wild), over the 2hr time period, was only observed at the upper limit of the Cu% (0.04% and 0.05% Cu) added. Though the 0.04% Cu series only showed minor differences in the amount of Cu uptake the trend drastically increased at the 0.05% Cu series.

Flame Atomic Absorption Spectroscopy (AAS)

Discussion
The discrepancy in the uptake of Cu at the two upper limits (0.04% & 0.05% Cu), in the Cu concentration series, may be attributed to the buildup of a Cu concentration within the cytoplasm of the cell. Other factors play a role in the high Cu limit of transporting Cu out of the cytoplasm, due to the mitochondria’s inability to uptake copper in the CMC1 cells. Comparison of the wild type suggests that the wild type cells, at the two upper limits of the Cu concentration series, do not invoke this mechanism due to the lack of buildup of Cu within the cytoplasm as the available transport mechanism of Cu into the mitochondria is available.

This mechanism provides and explanation as to the presence of a higher Cu concentration outside of the CMC1 cells at the two upper limits of copper concentration. The validity of the mechanism requires that a similar analysis should be done to measure the amount of Cu within the cytoplasm.