Site Directed Mutagenes of COX17 Residues,
K46, F50, Y61, and E64

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Abstract
This research was performed to determine which amino acid residues were responsible for Cox17 copper binding. Cox17 mutants were transformed and expressed in yeast cells. Site directed mutagens of Cox17 occurred at positions 46 (K), 50 (F), 61(Y), and 64 (E). These mutants were not observed to interfere with copper binding.

Introduction
Electrons are carried from NADH-oxidoreductase to Cytochrome c oxidase, the terminal complex of the electron transport chain in mitochondria (Glerum et al. 1996).

Figure 1 The structure of Q-cytochrome c oxidoreductase. A helices are shown in blue, coppers in green and red, and heme groups in yellow.

The structure of cytochrome c oxidase includes a prominent region of membrane spanning helixes (figure 1) and contains two heme A groups and three copper ions (figure 2). Two copper ions, CuA/CuA, associate with two cysteine residues in the active site and mediate the first step of the electron transport system by accepting electrons (figure 3). The remaining copper ion, CuB, interacts with three to histidine residues and a
tyrosine residue (figure 4). Heme a and CuB transfer electrons to oxygen in the final step of the electron transport system (Punter and Glerum 2003).

Copper is essential for electron transfer to oxygen (George et al. 2001). In excess, copper can also be toxic. Copper reacts with oxygen creating superoxide radicals that damage DNA, proteins, and lipids. Therefore, cells have developed mechanisms to prevent copper from reacting with oxygen (Fridovich 1978). Cox17, Sco1, and Cox11 are metallochaperones that act to keep copper from reacting with oxygen. Transporting these proteins serve to transport ions from the cytoplasm to cytochrome c oxidase in specific locations in the cell (Rae et al. 1999). It has been suggested that Cox17 may serve to shuttle Cu ions into the mitochondria (Beers et al. 1997). Heaton et al. (2000) and Huffman and O’Halloran (2001) reported that 40% of all Cox17 was found in the cytoplasm and 60% within the inner membrane of Saccharomyces cerevisiae mitochondria. Additionally, Cox17 binds copper, and permits the growth of organism on non-fermentable carbon sources. Yeast strains with a Cox17 deletion, for example, are unable to grow on insert non-fermentable carbon sources here, but have been shown to
thrive when sub-toxic levels of Cu ions added to the growth media (Heaton et al. 2000, Huffman and O’Halloran 2001).

Figure 5. Several different species of Cox17 sequences. The colored residues were the sites of mutation.

These observations suggest that Cox17 may play an important role in the delivery of copper to cytochrome c oxidase. Mutations of Cox17 may lead to cytochrome oxidase deficiency. Previous studies have shown that specific amino acids are critical for Cox17 function (Heaton et al. 2000). However, not all Cox17 residues have been studied. The purpose of this study was to mutate amino acid residues, K46, F50, Y61, and E64 and to characterize their effects on Cox17. These four residues may not be highly conserved throughout all species but were worthy of research about the roles of each residue on Cox17 functionality (figure 5).

Methods

Using pRS316 as a template, DNA was isolated from a dam+ E.coli strain of two complimentary oligonucleotides containing the desired mutation flanked by unmodified nucleotide sequences were synthesized using Quick Change mutagenesis kit. PCR mutagenesis was carried out using the QuikChange mutagenesis kit Stratagene inc. according to the instructions of the manufacturer.
Following PCR amplification the plasmid was digested with Dpn I and transformed into DH5α cells. DH5α was grown on LB broth and on an LB agar plate at 37°C. The remaining DNA were used to transform XL 1-Blue supercompetent cells. Mutations were confirmed by DNA sequencing.

Strain pRS316Cox17α was obtained from an open biosystem for yeast transformation. Yeast were transformed using the method of Schiestl and Gietz (1998). Media for growth of the yeast was YPD broth and a drop-out mix synthetic minus Uracil w/o Yeast Nitrogen Base plate at 30°C. To determine if the DNA were functional, each Cox17 plasmid was inserted into a Cox17 deletion strain of *Saccharomyces cerevisiae* and tested for growth on plates containing glycerol as the only carbon source.

**Results**

Analysis demonstrated successful mutation of Cox17 at amino acid positions 46 (K46A), 50 (F50A), 61 (Y61A), and 64 (E64A) (tables 1-4). In each case, wild type residues were substituted with alanine.

Table 1. The transferred Cox17 mutant with wild type. The mutation at the 46
residue, Lysine (Lys, K) to Alanine (Ala, A). The mutation at the 50th residue,
Phenylalanine (Phe, F), to Alanine. The mutation at the 61st residue, Tyrosine (Tyr,
Y) to Alanine. The mutation at the 64th residue, Glutamate (Glu, E) to Alanine.

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Figure 6. The expression of *Saccharomyces cerevisiae* on glucose (a) and glycerol (b).

The transformed mutants were transported into yeast cells and expressed on glucose and glycerol plates to determine the function of Cox 17, and both plates were expressed. All mutated amino acid residues were functional on glycerol plates (figure 6). Since the amount of a colony streaked varied on each glucose and glycerol plate, whether negative or positive determined the functionality.

**Discussions**

The purpose of this study was to determine the functional amino acids of Cox17 by mutating some amino acid residues. The mutations, K46A, F50A, Y61A, and E64A were functional on the glycerol plate. These results suggest that the mutated residues were not essential in the function of the cytochrome oxidase complex. Therefore, these amino acids do not affect the structure, stability, copper binding, or the mitochondrial localization of protein. Alanine mutations do not appear to affect copper binding. This result was not unexpected since only cysteinyl residues appeared to be necessary for copper ligation. Heaton et al. (2000) revealed that three of the seven cysteinyl residues to be critical for Cox17 function. Three cysteinyl residues are present in a cys-cys-val-cys sequence motif which appear to be necessary for copper ligation (Heaton et al. 2000).
The residue cystein includes a reactive group which is a sulfhydryl group. Pairs of sulfhydryl goups may be able to form disulfide bonds to stabilize the protein (Berg et al. 2001). There is some evidence to suggest that the sulfhydryl groups in a cys-cys-val-cys sequence motif may bind to Cu ions though the exact nature of the association is unknown. The mutants of cystein in the sequence motif lose their ability to bind to Cu ions suggesting that the cystein residues are essential in normal copper binding. Thus, most residues except cysteiny1, cys-cys-val-cys sequence motif, may not affect the functional cytochrome oxidase complex. The further study needs to be investigated to ensure the functionality of Cox17 simple point mutation.
Work Cited


