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Cloning, Expression and Purification of the Mitochondrial Assembly Protein Sco2, Involved in the Pathogenesis of Fatal Infantile Cardioencephalopathy

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Structural and functional aberrations of mitochondria have been implicated in the pathogenesis of a number of neurodegenerative disorders. Cytochrome c oxidase (COX), the terminal component of the mitochondrial respiratory chain, is a thirteen subunit holoenzyme. Assembly of COX requires the function of a number of proteins (assembly proteins) encoded by nuclear genes. Recently, we have identified mutations in the gene of a human COX assembly protein Sco2. These mutations were detected in three unrelated infants suffering of fatal encephalomyopathy, hypertrophic cardiomyopathy and COX deficiency (*Nat. Genet. 23*: 333-337, 1999). Among these mutations (Q53X, E140K and S225F), a G to A transition at nt 1.541 converting Glu-140 to Lys (E140K) was found to be adjacent to a copper binding domain (CXXXC) of Sco2 protein. Sco2p is embedded in the inner mitochondrial membrane and has been implicated in the mitochondrial copper delivery (*J. Biol. Chem., 271* 20531-20535, 1996). The present study has been designed to explore whether the mutations found in the human gene *SCO2* affect the ability of mutated forms of Sco2p to bind and translocate copper into mitochondria. This copper binding capacity of Sco2 mutated proteins was compared to that of wild type Sco2p. We employed the IMPACT^{TM}-CN vector expression system and cloned, expressed and purified the recombinant mature wild type (Sco2p) as well as the mutated Sco2 proteins (E140K-Sco2p and S225F-Sco2p). Copper sulfate (1.4 mM $CuSO_4$) was added to the bacterial growth media one hour before cell harvest and the amount of copper bound to the isolated recombinant Sco2 protein was determined by flame atomic absorption spectrophotometry. Data accumulated thus far will be presented and discussed.