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Analysis of Human HCCS-Mediated Cytochrome c Biogenesis

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Cytochrome c (cyt c), found in the intermembrane space of mitochondria, is an essential component of cellular processes such as energy production and apoptosis. It acts as an electron carrier, and thus, defects in cyt c or its production, impair respiration. Holocyt c synthase (HCCS), a membrane-associated enzyme, is responsible for mediating the attachment of heme to cyt c. The stability and function of cyt c relies on attachment of heme via two thioether linkages at conserved cysteine residues ($Cys_{15}XxxXxxCys_{18}His_{19}$). We characterized HCCS-mediated synthesis of several cyt c variants containing mutations in and around the conserved motif to understand specific mechanisms of cyt c biosynthesis. Based on previous data from the Kranz lab regarding an enhanced cyt c product release feature of HCCS E159A, we successfully produced several cyt c variants in quantities suitable for biochemical characterization. My first study suggests that heme attachment preferentially begins at Cys18. We also show that mutations at Cys18 result in a cyt c species modified by an oxygen adduct, explaining the absence of Cys18 cyt c variants in nature. For my second related study, we show that residues outside the cyt c heme attachment motif and in the N-terminal alpha helix-1 position Cys15 of cyt c. We provide evidence that deletion of Met13 in alpha helix-1 displaces Cys15 away from heme 2-vinyl, thus preventing Cys15 thioether formation. In support of this conclusion, we show that further mutations to shift the positioning of Cys15 restore the two thioether conformation. My third study explores *in vitro* reconstitution of System III cyt c biogenesis to identify some optimal conditions for HCCS activity and required cyt c domains for HCCS recognition and attachment. My studies have contributed to understanding the mechanism of HCCS, an enzyme required by most eukaryotes.