Volume 13

Washington University
Undergraduate Research Digest

Spring 2018

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Determining the Recognition Requirements for Heme Attachment in Prokaryotic Cytochrome *c* Biogenesis

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Cytochromes *c* (cyt *c*) play an essential role in many electron transport chains, making it important to understand how this protein is matured. Cyt *c* maturation requires the covalent attachment of heme, via two thioether bonds, at the cysteine thiols of a conserved CXXCH motif. Three pathways, two prokaryotic (Systems I and II) and one eukaryotic (System III), can mature cyt *c*. I focused on System II, in which two integral membrane proteins (CcsB and CcsA) function as the holocyt *c* synthetase. CcsBA is proposed to transport heme from the cytoplasm to the periplasm and then attach it to apocyt *c* at two conserved CXXCH motifs in the di-heme cyt *c*4. The purpose of this project was to determine the recognition capabilities of CcsBA for apocyt *c* in order to reveal new information about the requirements for heme transfer in prokaryotes. The ability of CcsBA to recognize cyt *c* CXXCH motif variants with altered spacing (CXCH, CXAXCH) or altered amino acid composition (SXXCH, CXXSH, SXXCH, CXXCA) was determined by co-expressing these variants with CcsBA and monitoring the maturation of cyt *c*. We concluded that full length cyt *c* cannot be matured at wildtype levels when either motif is mutated and that variation of one motif does not prevent maturation of the other motif. These results demonstrate, for the first time, that the order of heme attachment is not fixed (neither motif is preferentially matured) and that CcsBA has stringent recognition requirements for heme attachment. Similar variants and experimental techniques will be used to explore cyt *c* recognition requirements for the more complex System I synthetase (CcmF/H), allowing the two prokaryotic cyt *c* maturation processes to be compared and contrasted.