Iron Homeostasis Under Stress: Fe-S Cluster Biosynthesis and the *suf* Pathway in *E. coli*

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A key challenge of bioinorganic chemistry is to understand how homeostasis of essential metals is maintained when cells are stressed by adverse environmental conditions. Fe-S clusters can be perturbed under conditions that challenge iron and sulfur homeostasis. As a result organisms ranging from bacteria to plants possess multiple specialized pathways to construct Fe-S clusters under a variety of conditions in vivo. The gram-negative bacterium *E. coli* contains two operons that have been implicated in Fe-S cluster biosynthesis, the *iscRSUA* operon and the *sufABCDSE* operon. The *isc* operon is essential for assembly of a wide range of Fe-S clusters enzymes under normal growth conditions. In contrast, targeted mutagenesis reveals that the *suf* operon is required to assemble Fe-S clusters during iron starvation or oxidative stress, conditions known to disrupt Fe-S clusters in vivo.

A major goal is to determine the biochemical mechanisms used by the *suf* pathway to achieve this feat. This goal is being realized as we elucidate the biochemical functions of the gene products unique to the *suf* operon, namely SufB, SufC, SufD, and SufE. Work from our lab and that of Barras and Fontecave has shown that the SufE protein dramatically enhances sulfur donation by the SufS cysteine desulfurase enzyme by acting as a sulfur transfer partner. In addition, the sulfur transfer from SufS to SufE is highly shielded from disruption, thereby protecting a reactive persulfide intermediate from environmental perturbation. We also have found that SufB, SufC and SufD co-purify as a stable complex that has ATPase activity in vitro. The SufE dependent enhancement of SufS is further increased by the addition of the SufBCD complex. The SufC ATPase activity is not required for this enhancement, suggesting that the SufBCD complex has additional functions in vivo. Further genetic and biochemical analyses are being used to characterize the SufBCD complex with the goal of understanding its additional functions. This approach will increase our understanding of how the *suf* gene products are adapted to acquire iron and sulfur for construction of Fe-S clusters during iron starvation and oxidative stress.