Unravelling the mechanism of [2Fe-2S] cluster biosynthesis using a functional reconstitution of the bacterial ISC machinery.

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Iron-sulfur (Fe-S) cluster are essential and ubiquitous prosthetic groups of proteins, biosynthesized by multi-protein machineries. The Iron-Sulfur Cluster assembly (ISC) machinery synthesizes [2Fe-2S] cluster on the scaffold protein IscU using sulfur provided in the form of a cysteine-bound persulfide by the cysteine desulfurase IscS, and electrons delivered by the ferredoxin-ferredoxin reductase couple Fdx-FdxR. Although several aspects of the mechanism have been elucidated, it is still unclear how [2Fe-2S] clusters are assembled. Here, we report the characterization of several key intermediates formed during the assembly process by the *E. coli* ISC machinery. Using an *in vitro* reconstituted machinery, our data revealed that the assembly of [2Fe-2S] clusters relies on the synthesis of [1Fe-1S] precursors on the scaffold protein IscU, which are fused into a bridging [2Fe-2S] cluster upon dimerization of IscU.

References

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