BIOGENESIS OF A PRIMORDIAL COFACTOR: UNDERSTANDING IRON-SULFUR CLUSTER BIOGENESIS IN METHANOGENIC ARCHAEA.

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Iron-sulfur (Fe-S) clusters are important prosthetic groups used by enzymes involved in diverse processes essential to life, including photosynthesis, respiration, and DNA damage repair. Proteins utilizing Fe-S clusters are found in all three domains of life: *Eukarya*, *Bacteria*, and *Archaea*. It has been postulated that some of the most evolutionarily ancient metabolic pathways utilized Fe-S cluster proteins in reactions providing energy to early cellular life, in a so-called "iron-sulfur world." Fe-S cluster proteins continue to be used in the most complex extant forms of life. In spite of these facts, an understanding of *in vivo* Fe-S cluster synthesis is less than 20 years old. To date, three systems for Fe-S cluster biogenesis have been identified, called the Nif, Isc, and Suf systems. The Isc and Suf systems are the most broadly distributed, while the Nif system appears specialized for nitrogen-fixation. The Isc system is the primary system in the majority of bacteria and in the mitochondria of eukaryotes. The Suf system is also present in bacteria and in the chloroplasts of plants. Common to all systems is the use of cysteine as the source of sulfur, whereby a cysteine desulfurase liberates sulfur from cysteine and delivers it to a scaffold protein. The Fe-S cluster is assembled on the scaffold and then delivered to a target apo-protein. Despite the fact that the genomes of the strictly anaerobic methanogenic archaea (methanogens) code for more Fe-S cluster proteins than any other organisms, the Fe-S cluster biogenesis system(s) used by methanogens, and archaea in general, is unknown. Methanogens also comprise an ancient archaeal lineage; thus, an understanding of the Fe-S cluster biogenesis machinery in extant methanogens may provide insight into the origins and evolution of Fe-S cluster metabolism. Bioinformatic analyses indicate that the core components the Suf system are conserved in the genomes of all sequenced methanogens, and the core components of the Isc system are also present in the majority of species. Members of Order Methanosarcinales contain the core components of both Isc and Suf systems. For example, *Methanosarcina acetivorans* has three Isc operons, each encoding a cysteine desulfurase (IscS1-3) and a scaffold (IscU1-3) and two Suf operons, each encoding a SufBC scaffold. We show here that *M. acetivorans* is capable of using cysteine or sulfide as the sole sulfur source, consistent with either serving as a source of sulfur for Fe-S cluster biogenesis. To understand the importance of the Isc system, IscS2 and IscU2 were selected for biochemical characterization and the *iscSU2* gene cluster was deleted from the chromosome of *M. acetivorans*. Recombinant IscS2 and IscU2 were expressed in *E. coli* and purified under anaerobic conditions. UV-visible and EPR spectrometry, elemental analyses, and target protein activity assays indicate IscU2 is capable of binding and transferring Fe-S clusters to apo-proteins. Experimental analyses reveal IscS2 contains pyridoxal phosphate and has cysteine desulfurase activity. In the presence of iron and cysteine, IscS2 can mediate the assembly of a [4Fe-4S] cluster in IscU2, consistent with IscS2 and IscU2 functioning in Fe-S cluster biogenesis in *M. acetivorans*. Importantly, Δ*iscSU2* mutant cells exhibited decreased cysteine desulfurase activity and the mutant was impaired during growth with cysteine, but not sulfide, as the sole sulfur source. These results reveal that *M. acetivorans*, and likely additional methanogens, has a functional Isc system, which may be specific to the utilization of cysteine as the source of sulfur for Fe-S cluster biogenesis.