Cfd1p: A cytosolic FeS cluster assembly factor.

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In eukaryotes the maturation of FeS proteins takes place in different compartments. For example, regulation of iron-responsive gene expression by iron regulatory protein 1 (IRP1) requires the facile interconversion of IRP1 between an apo-RNA binding protein and a [4Fe-4S] aconitase in the cytosol of animal cells. Thus the regulation of IRP1 function depends on iron-responsive FeS cluster assembly in the cytosol. Maturation of cytosolic FeS proteins depends on the activity of both mitochondrial- and cytosol-specific factors. The nature of the cytosolic FeS cluster assembly machinery has only recently been revealed. We use a model yeast system, in which cell growth is dependent on the conversion of mammalian IRP1 to cytosolic aconitase, to investigate FeS cluster assembly in the cytoplasm. We recently identified the P-loop ATPase Cfd1p as a required cytosolic protein for FeS cluster assembly using this system. Cfd1p is conserved in eukaryotes and is essential for yeast viability. Non-lethal *cfd1* mutations impair cytosolic FeS cluster assembly, but do not affect cluster assembly in mitochondria. Mutation of Cfd1p also alters FeS cluster assembly in IRP1 in yeast cell-free extracts, suggesting that Cfd1p plays a direct role in cytosolic FeS cluster assembly. Analysis of Cfd1p through mutagenesis has revealed important domains for its function in cytosolic FeS cluster assembly. Mutation of predicted ATP binding residues impair the ability of Cfd1p to support FeS cluster assembly, as do mutations within the switch I and adjacent to the switch II regions of the protein. The switch I and II domains are important for transducing the effects of nucleotide binding and/or hydrolysis in the P-loop ATPase family of proteins. Mutation of residues within the conserved CX_2CX_2C domain reveals residues that are essential for Cfd1p to support cytosolic FeS cluster assembly but which are not essential for viability, which raises the question of whether Cfd1p participates in essential processes other than cytosolic FeS cluster assembly. Unlike mutations in FeS cluster assembly factors that are localized in mitochondria, *cfd1*mutations do not lead to elevated mitochondrial iron accumulation, nor does *cfd1* mutation itself result in elevated expression of Aft1/2 regulated genes. On the other hand, Cfd1p mutation alters the responsiveness of the Aft1/2 regulon in a way that suggests that Cfd1p is in a pathway that competes for a pool of iron sensed by Aft1/2. The implications of our results to cellular iron regulation and the role of FeS cluster assembly in this regulatory process will be discussed. (Supported by grant DK47281 from the NIH.)