The Cytosolic Iron-Sulfur protein Assembly machinery: Characteriation of the Cfd1p-Nbp35p complex

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Iron-sulfur proteins are key proteins involved in several electron transfer and metabolic pathways. Recently, four proteins specifically involved in extra-mitochondrial iron-sulfur (Fe-S) protein assembly have been identified in *Saccharomyces cerevisiae*: Cfd1p, a putative P-loop ATPase [1]; Nbp35p, a close homologue of the latter which has a 54 residue *N*-terminal extension harbouring 4 conserved cysteines [2]; Nar1p, a protein with a remarkable homology to bacterial Fe-only hydrogenases [3]; and Cia1p, a WD40-repeat protein which occurs as a fusion protein with Cfd1p in *Schizosaccharomyces pombe* (Balk, unpublished). Here, we present data on the physical interaction between the two P-loop ATPases Nbp35p and Cfd1p. Studies on complex formation show that both proteins co-precipitate *in vivo* in yeast and form a stable hetero-tetrameric complex when co-expressed in *E. coli*. An initial biophysical investigation of the complex properties revealed that Nbp35p binds an Fe-S cluster at the N-terminus and the efficiency of Fe-S cluster binding to Nbp35p is improved in the complex. Cfd1p does not stably bind Fe^{2+} or an Fe-S cluster despite the presence of a cysteine-rich, putative Fe-S binding motif at the C-terminus. Additionally, complex formation of point mutants of Cfd1p with Nbp35p was investigated. The residues C202 and C204, but not C207 are important for complex formation. Finally, the purified and chemically reconstituted complex exhibits an ATPase activity with a K_m value for ATP of ~ 0.5 mM.

References:

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