Abstract

Iron is an essential element in nearly all organisms. It is present mainly as a component of iron sulfur (FeS) clusters or as a heme iron. These cofactors enable proteins to transfer electrons or diatomic gasses, signal sensing and enzyme catalysis. Numerous FeS and heme depending proteins are involved in photosynthesis and respiratory chain pathways, which are well described processes. However, there is still much to learn about more recently discovered pathways such as formation of FeS clusters in various cell compartments and about roles of novel FeS or heme proteins. Particularly, only limited information is available about how FeS clusters are assembled or how heme is used in anaerobic protists, in which cytochrome-dependent respiration and photosynthesis does not occur. We decided to focus on iron cofactors in anaerobic parasite *Giardia intestinalis*. This organism undergone dramatic reductive evolution that resulted in formation of one of the smallest eukaryotic genome and the most reduced form of mitochondria, the mitosome. We characterized some components of mitochondrial (ISC) and cytoplasmic (CIA) FeS assembly machineries. We have detected ISC components in mitosome by proteomic analysis. Furthermore we investigated the presence and subcellular localization of CIA proteins in *Giardia*. In the *Giardia* genome, we identified only some components of CIA machinery including Nbp35, Nar1, Cia1, Cia2 and two genes with similarity to Tah18. Moreover, we found that some CIA proteins display dual cytoplasmic/ mitosomal localization, which may facilitate the connection between ISC and CIA machineries.

Known set of *Giardia* hemoproteins is limited to a single flavohemoglobin and four cytochrome b_5 proteins. We showed that *Giardia* cytochromes b_5 (gCYTb_5) belong to yet uncharacterized group of proteins (cytb_5 type II). They lack typical C-terminal transmembrane domain and possess specific amino acid motif around essentials heme binding histidines. Moreover, we found, that cytb_5 type II are ubiquitously present in eukaryotes in contrary to typical cytochromes b_5 (type I), which are present only in aerobic eukaryotic cells. We showed that gCYTb_5 paralogues are all present in *Giardia* cytosol and we proved that recombinant gCYTb_5 proteins bind heme *in vitro*. Additionally, we demonstrated that *Giardia* can import extracellular heme and incorporate it into the cytoplasmic gCYTb_5-IV. Previously it was believed that anaerobic protists live entirely without heme. Therefore, our experiments demonstrated for the first time that heme is present and utilized by anaerobic protist *Giardia intestinalis* and most likely by other anaerobes possessing cytb_5.