

ABSTRACT

Iron is an essential nutrient for the parasitic protist *Trichomonas vaginalis* as a component of iron-sulfur (FeS) proteins that are indispensable for energy metabolism of the parasite. The FeS clusters are formed by FeS cluster (ISC) assembly machinery that resides, together with a number of FeS proteins, in *Trichomonas* hydrogenosomes. These double-membrane bound organelles, which are related to mitochondria, metabolize malate and pyruvate and produce ATP and molecular hydrogen. To obtain more complete information about hydrogenosomal pathways with particular focus on FeS proteins and ISC machinery, we participated on analysis of *T. vaginalis* genome sequence. To predict hydrogenosomal localization of putative gene products, we developed an application “Hunter” for the *in silico* searching for N-terminal presequences that are required for protein targeting into the hydrogenosomes. This approach substantially contributed to annotate genes coding for hydrogenosomal proteins that provided base for construction of novel map of hydrogenosomal metabolism as well as for following proteomic studies.

Investigation of hydrogenosomal proteins led to identification of three members of Hyd machinery that is required for the maturation of the specific FeS cluster of hydrogenases named H cluster. *T. vaginalis* is, thus far, the second eukaryotic organism in which the complete set of Hyd proteins have been described. We further provided evidence that the acquisition of [FeFe] hydrogenases and Hyd maturases occurred once during eukaryotic evolution.

Iron availability modulates expression of a number of proteins that are important for the establishment of *Trichomonas* infections, proliferation and virulence. We utilized transcriptomic as well as proteomic approach to compare changes between *T. vaginalis* cultivated under iron-rich and iron-restricted conditions. The transcriptome analysis was based on the combination of two powerful methods: oligonucleotide microarrays and comparative expressed sequence tag (EST) sequencing of cDNA libraries. In the proteomic analysis we focused specifically on hydrogenosomes, because the main changes associated with iron availability occur in these organelles. Both surveys revealed important changes in the physiology of the pathogen; mainly proteins of energy metabolism and ISC assembly system were affected by iron availability. One of the most striking observations from these investigations was the differential regulation of individual copies of the expanded gene groups, with only some of the copies showing iron-dependent regulation. This

finding might reflect the functional diversification of individual gene copies after gene duplication.