

ABSTRACT

Mitochondria carry out several important functions in eukaryotic cells such as energy metabolism, iron-sulfur cluster assembly, apoptosis, signaling pathways, protein quality control etc. Most mitochondrial proteins are synthesized on the cytosolic ribosomes and transported to the organelles by the cytosolic chaperones and mitochondrial protein import machinery based on specific targeting signals. Although, the basic principles of protein import have been explained, many questions remain unanswered, particularly for highly modified mitochondria such as hydrogenosomes. The aim of the study was to investigate protein translocation into hydrogenosomes of a human parasite, *Trichomonas vaginalis* (Tv) with a focus on the composition, function and structure of protein translocases and the role of targeting signals.

The translocase of the outer membrane (TOM) is responsible for the import of most proteins into the organelle. Even though, the presence of a TOM complex in trichomonad hydrogenosomes was predicted, its components were not known. Moreover, the generic structure of the mitochondrial TOM complex was not resolved. This study showed that the TvTOM complex is highly divergent consisting of two modified core subunits – channel-forming TvTom40 isoforms and a Tom22-like protein, and two lineage-specific subunits – Tom36 and Tom46 that most likely, function as receptors. Additionally, TvTOM forms a stable supercomplex with Sam50 that is involved in the biogenesis of β -barrel proteins. Electron microscopy revealed that the translocase has a triplet-pore structure with a unique skull shape.

Mitochondrial matrix preproteins carry an N-terminal targeting sequence (NTS). Interestingly, a glycolytic enzyme, ATP-dependent phosphofructokinase (ATP-PFK) that does not contain a predictable NTS localizes to hydrogenosomes. Localization experiments suggested that TvATP-PFK and its homologous ATP-PFKs from yeast and *E. coli* possess unknown internal targeting signal (ITS) that is possibly recognized by the protein import machinery. From an evolutionary perspective, the ability of mitochondria and hydrogenosomes to recognize proteins such as ATP-PFK may represent an ancient mechanism from the early phases of organelle evolution whereas, NTS-dependent import might have evolved later. *T. vaginalis* has several unique tail-anchored (TA) proteins, a class of integral membrane proteins that localize to the hydrogenosomal outer membrane, including the newly characterized TvTOM subunits. Analyses of physico-chemical properties

and localization experiments identified new traits for hydrogenosomal TA protein targeting such as higher net positive charges in the C-terminal segment which, otherwise are primarily for peroxisomal TA proteins in aerobic eukaryotes, and a slightly longer transmembrane domain when compared to mitochondrial TA proteins.

Taken together, these studies show that the protein import into hydrogenosomes is rather divergent compared to that of mitochondria. The triplet-pore TOM complex, composed of conserved core subunits was present in the last common eukaryotic ancestor while, the peripheral receptors evolved independently in different eukaryotic lineages. The changes observed in the protein translocases and the targeting signals most likely reflect the adaptation of hydrogenosomes to anaerobic conditions, particularly, the loss of respiratory chain complexes that resulted in low or absence of membrane potential.