ABSTRACT

Various protists from different eukaryotic groups are able to live in the oxygen-poor niches. Their metabolic adaptation to anaerobiosis is usually associated with loss of the typical mitochondrial functions, including the tricarboxylic acid cycle and oxidative phosphorylation. Anaerobic forms of mitochondria generate ATP exclusively by the substrate level phosphorylation in the hydrogen-producing hydrogenosomes, or the ATP synthesis is completely lost as observed in mitosomes. Consequently, the proteomes of such organelles are considerably reduced. It is a question of debate whether the anaerobic forms of mitochondria evolved directly from premitochondrial organelles that might be present in ancient anaerobic eukaryotes or during the secondary adaptation of aerobic eukaryotes to anaerobic niches.

The protist from super group Amoebozoa, *Mastigamoeba balamuthi*, is very attractive for study of mitochondria evolution, because it is closely related with two very different organisms: (i) the aerobic, free-living slime molds such as *Dictyostelium* that possesses classical aerobic mitochondria, as well as (ii) the anaerobic parasite *Entamoeba histolytica* that contains mitosomes, the most reduced form of mitochondria. The mitochondria derived organelles in anaerobic, free-living *M. balamuthi* could represent the intermediate stage between mitochondria and mitosomes. The functional analysis of *M. balamuthi* organelles revealed that these organelles are metabolically active and possess the hydrogen and ATP-generating pathway, which is typical for anaerobic energy metabolism in the hydrogenosomes. This pathway (extended glycolysis) includes the enzymes pyruvate:ferredoxin oxidoreductase (PFO), [FeFe]-hydrogenase, and ADP-forming acetyl-CoA synthetase (ACS). Interestingly, extended glycolysis is duplicated in the cytosol. The most conserved function of all mitochondria is the FeS cluster assembly, which is present also in Mastigamoeba hydrogenosomes. However, the typical mitochondrial ISC machinery is replaced by the bacterial NIF-like system , which is also duplicated in the cytosol. Based on functional and phylogenetic analysis we proposed that the transition of mitochondria-to-hydrogenosomes in *Mastigamoeba* included acquisition of genes encoded enzymes of anaerobic metabolism by lateral gene transfer. The products of these genes initially operated in the cytosol. After the gene duplication and acquisition of mitochondrial targeting signal, the pathways was parallelized in the mitochondria, whereas most of the mitochondrial pathways was lost.

The mitochondria of *Naegleria gruberi*, a member of Excavata supergroup, might represent another example of the aerobic-to-anaerobic transition of mitochondrial metabolism. Although these organelles possess the typical pathways of aerobic mitochondria including oxidative phosphorylation, it has been recently predicted that they also possess [FeFe]-hydrogenase, a typical enzyme of anaerobic metabolism. Using gas chromatography, we confirmed that aerobically grown *N. gruberi* indeed produces molecular hydrogen. However, the active hydrogenase was detected only in the cytosolic fraction, while no hydrogenase activity was associated with the mitochondria. This result supports our hypothesis that the cytosol is the primary site of hydrogenase upon the acquisition of corresponding gene by LGT and only in some organism, the hydrogenase was duplicated and targeted to the mitochondria that were transformed to the hydrogenosomes.