Anaerobic peroxisomes in *Mastigamoeba balamuthi*

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Typical adaptation of protists to oxygen-poor environment includes a loss of oxygen-dependent energy metabolism in mitochondria that are transformed to various mitochondrial forms including hydrogenosomes and mitosomes. Anaerobes, with hydrogenosomes and mitosomes, such as *Trichomonas vaginalis* and *Giardia intestinalis*, respectively, also do not possess peroxisomes that are otherwise omnipresent eukaryotic organelles. The common functions of most peroxisomes that possibly raise a force for evolution of these organelles are scavenging of toxic oxygen compounds such as hydrogen peroxide and catalysis of fatty acid β-oxidation. However, beyond these general functions, peroxisomes contain enzymes for large scale of other metabolic processes that differ in different species and accordingly they get alternative names such as glyoxysome, glycosome or Woronin body. In spite of metabolic diversity, all peroxisomes are dependent on similar set of proteins required for their biogenesis called peroxins (Pex) that are highly conserved among eukaryotic lineages. Unexpectedly, analysis of the genome of *Mastigamoeba balamuthi*, an anaerobic free-living member of Amoebozoa supergroup revealed presence of a complete set of peroxins. Immunofluorescence microscopy showed co-localization of *Mb*Pex3, *Mb*Pex11, and MbPex14, in numerous vesicles that are distinct from other cellular organelles including hydrogenosomes, ER and Golgi apparatus. Heterologous expression of *Mastigamoeba* peroxisomal proteins in yeast revealed that they are specifically targeted to yeast peroxisomes and their delivery to the organelles is dependent on C-terminal peroxisomal targeting signal 1 (PTS1). Following combination of *in silico* and quantitative mass spectrometry screens revealed a list of twenty-eight potential peroxisomal proteins. Interestingly, some of them are shared with its pathogenic relative *Entamoeba histolytica*. Based on these analyses we constructed metabolic map of predicted peroxisomal pathways, indicating involvement in metabolism of purine, pyrimidine, CoA, nicotinamide, and pyruvate. In conclusion, we characterized the first peroxisomes functioning in anaerobic eukaryote.