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ScienceDirect
Experimental Parasitology 118 (2008) 10–16
Experimental Parasitology
www.elsevier.com/locate/yexpr

Minireview

Entamoeba histolytica mitosomes: Organelles in search of a function

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Received 5 March 2007; received in revised form 7 August 2007; accepted 8 August 2007
Available online 16 August 2007

Abstract

It has been more than eight years since the discovery of mitosomes (mitochondrial remnant organelles) in the intestinal human pathogen *Entamoeba histolytica*. Despite detailed knowledge about the biochemistry of this parasite and the completion of the *E. histolytica* genome sequencing project no physiological function has yet been unequivocally assigned to these organelles. *Entamoeba* mitosomes seem to be the most degenerate of all endosymbiosis-derived organelles studied to date. They do not appear to participate in energy metabolism and may have dispensed completely with the proteins required for iron–sulphur cluster biosynthesis. However, the large number of mitosomes found in *E. histolytica* trophozoites hints at a significant biological role for these organelles in their natural environment. Identifying the protein complement of mitosomes will provide answers as to their biological significance and the reason(s) for their retention in this parasite.

Index Descriptors and Abbreviations: Amoeba; Anaerobic metabolism; ATP transporter; Chaperone; *Cryptosporidium*; *Dictyostelium*; Energy metabolism; *Entamoeba*; *Giardia*; Hydrogenosome; Iron–sulphur; *Mastigamoeba*; Mitochondria; Mitosome; Organelle; Protist; Protein import; Protozoa; Pyruvate oxidation; *Trichomonas*; ATP, adenosine triphosphate; Cpn60, chaperonin 60; FMN, flavin mononucleotide; Isc, iron–sulphur cluster; *luc*, firefly luciferase gene; μm, micrometer; mtHsp70, mitochondrial-type Hsp70; NAD, nicotine adenine dinucleotide; NADP, nicotine adenine dinucleotide phosphate; NifS, cysteine desulphurase; NifU, iron-binding scaffold protein; NO_3, nitrate; PFO, pyruvate ferredoxin oxidoreductase; PNT, pyridine nucleotide transhydrogenase; rRNA, ribosomal ribonucleic acid

1. Introduction

Mitosomes are mitochondrion-related organelles found in a range of unicellular eukaryotic organisms that inhabit oxygen-poor environments. Although dissimilar in appearance from text-book mitochondria, mitosomes harbour a small number of mitochondrial marker proteins and are surrounded by a double membrane. These observations have been interpreted as evidence that mitosomes represent degenerate mitochondria and as a result these organelles are considered excellent models for the study of mitochondrial evolution.

Mitochondria are responsible for the efficient preservation of chemical energy in the form of ATP which allows the cell to carry out the myriad of biological functions required for its survival. All multicellular organisms possess mitochondria which in general can be classified into two different types: aerobic and anaerobic mitochondria. Aerobic mitochondria are the most widely studied energy-generating organelles – they utilise oxygen as the final acceptor of electrons during aerobic respiration. Anaerobic mitochondria are present in organisms that spend at least part of their life cycles under conditions of oxygen deprivation (anaerobiosis) and contain the biochemistry required to utilise organic (e.g., fumarate) or inorganic (e.g., NO_3) compounds as final acceptors of electrons during anaerobic respiration (Tielens et al., 2002; Risgaard-Petersen et al., 2006). The evolution of different types of aerobic and anaerobic mitochondria is thought to have been driven by selective pressures specific to diverse

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doi:10.1016/j.exppara.2007.08.004

environmental niches colonised by mitochondrion-containing organisms. Regardless of their diversity in form and function, a vast volume of experimental evidence supports the monophyletic nature of mitochondria (Gray et al., 1999).

In addition to the more visible multicellular eukaryotes, there is a large collection of nucleus-containing unicellular organisms representing a wide spectrum of eukaryotic diversity. Like their multicellular descendents, all aerobic microbial eukaryotes contain aerobic forms of mitochondria. However, anaerobic microbial organisms have traditionally posed a major challenge to biologists for two reasons: (i) sampling is usually problematic because they live mostly in anaerobic ecological niches that are difficult to access without sophisticated equipment (e.g., lake and ocean beds) or without disrupting their natural environment (in vertebrate and invertebrate digestive tracts) and (ii) they are either fastidious to grow or not amenable to axenic cultivation under laboratory conditions, making them difficult biological models for biochemical and genetic study. Traditional ultrastructural studies of anaerobic microbial organisms (e.g., *Entamoeba, Giardia*) consistently failed to identify cellular structures that could be construed as mitochondria. As a result, anaerobic microbial eukaryotes were for many years thought to be primitively amitochondrial – i.e., protoeukaryotic cells with nuclei hypothesised to have existed prior to the endosymbiotic acquisition of mitochondria – and to derive their biological energy exclusively by fermentation.

Over the past few years however it has become apparent that this interpretation is incorrect. All those anaerobic microbial eukaryotes studied in sufficient detail so far have been found to contain mitochondrion-related organelles known as hydrogenosomes or mitosomes, depending on whether or not they evolve molecular hydrogen as a product of their metabolism. The discovery of mitochondrion-related organelles in these microbial organisms has provided biologists with much needed biological models to investigate the evolutionary origins of mitochondria and the early evolution of the eukaryotic cell. Comparative studies aimed at identifying morphological, physiological and biochemical differences/similarities between mitosomes, hydrogenosomes, aerobic and anaerobic mitochondria are currently in progress in many laboratories worldwide. These studies are fuelled by genome wide comparative surveys in the postgenomic era. Experimental data emanating from such investigations have challenged traditional views of eukaryogenesis and are informing the design of alternative testable hypotheses to explain the origins of the eukaryotic cell.

Over the past few years the rate of progress in the field has been unprecedented. Several review articles and at least two books have been written recently about the biology and evolutionary significance of mitochondrion-related organelles and their contribution to our understanding of the early evolution of the eukaryotic cell (Embley et al., 2003; Williams and Keeling, 2003; Hirt and Horner, 2004; van der Giezen and Tovar, 2005; van der Giezen et al., 2005b; Embley, 2006; Embley and Martin, 2006; Martin and Müller, 2007). The interested reader will find in those references a wider and more in depth exploration of this area of study. In this minireview we will focus attention on what is known about the biology of *Entamoeba histolytica* mitosomes with comparative reference to mitosomes and hydrogenosomes from other parasitic protists where appropriate.

2. *Entamoeba* phylogeny and mitosome discovery

Entamoeba histolytica is an intestinal parasite of humans that colonises the large intestine. Although most infections are asymptomatic a small proportion (~10%) of infected individuals develop amoebic colitis as a direct result of *E. histolytica* infection (Stanley, 2003). Under conditions that are not yet fully understood the parasite may occasionally penetrate the intestinal wall and reach internal organs, particularly the liver, causing abscesses that can be fatal if untreated. During its life cycle the parasite undergoes cyclical morphological transformations between a colonising trophozoite and an environmentally resistant infective cyst, a process of differentiation that is essential for parasite infectivity and disease transmission. Of the various *Entamoeba* species known to colonise the human intestine, namely *Entamoeba dispar, Entamoeba coli, Entamoeba histolytica, Entamoeba moshkovskii, Entamoeba polecki* and *Entamoeba chattoni,* only *E. histolytica* has been unambiguously shown to cause symptoms of disease. *Entamoeba* species however are closely related. Phylogenetic analysis of ribosomal RNA gene sequences has demonstrated the monophyletic nature of the *Entamoeba* genus (Silberman et al., 1999). Initially no free-living amoebas appeared to be related to the lineage leading to *Entamoeba* but it is now known through multigene phylogenetic analysis that *Mastigamoeba* and *Dictyostelium*, two free-living amoebas, are relatives of *Entamoeba* (Bapteste et al., 2002). As a result, the amoebid lineages Entamoebidae (represented by *Entamoeba*), Mastigamoebidae (represented by *Mastigamoeba*) and Eumycetozoa (represented by *Dictyostelium*) are now grouped together in the Cluster Amoebozoa (Adl et al., 2005).

An early phylogenetic tree based on the small subunit ribosomal RNA genes found *Entamoeba* to be a postmitochondrial branch, diverging from the main eukaryotic trunk well after mitochondrion-containing lineages such as Euglenoids and Kinetoplastida (Sogin, 1991). Although the early and late branches of this tree were subsequently shown to be the result of methodological artifact (Gribaldo and Philippe, 2002), this finding was taken as evidence that the absence of recognisable mitochondria in *Entamoeba* was likely due to organelle decay or loss and not to primitive absence. Support for this view came from the work of Clark and Roger (1995) who amplified and cloned from the *Entamoeba* genome two genes encoding mitochondrial marker proteins, chaperonin 60 (Cpn60) and pyridine