Biochimica et Biophysica Acta 1833 (2013) 295–303

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Back to basics: A revealing secondary reduction of the mitochondrial protein import pathway in diverse intracellular parasites☆

Eva Heinz [a,b,*], Trevor Lithgow [a]

[a] Department of Biochemistry & Molecular Biology, Monash University, Clayton Campus, Melbourne 3800, Australia
[b] Institute of Cell and Molecular Biology, University of Newcastle, Newcastle upon Tyne, UK

ARTICLE INFO

Article history:
Received 29 December 2011
Received in revised form 9 February 2012
Accepted 9 February 2012
Available online 16 February 2012

Keywords:
Mitosome
Microsporidia
Parasite
Protein import
Reductive evolution

ABSTRACT

Mitochondria are present in all eukaryotes, but remodeling of their metabolic contribution has in some cases left them almost unrecognizable and they are referred to as mitochondria-like organelles, hydrogenosomes or, in the case where evolution has led to a great deal of simplification, as mitosomes. Mitochondria rely on the import of proteins encoded in the nucleus and the protein import machinery has been investigated in detail in yeast: several sophisticated molecular machines act in concert to import substrate proteins across the outer mitochondrial membrane and deliver them to a precise sub-mitochondrial compartment. Because these machines are so sophisticated, it has been a major challenge to conceptualize the first phase of their evolution. Here we review recent studies on the protein import pathway in parasitic species that have mitosomes: in the course of their evolution for highly specialized niches these parasites, particularly Cryptosporidia and Microsporidia, have secondarily lost numerous protein functions, in accordance with the evolution of their genomes towards a minimal size. Microsporidia are related to fungi, Cryptosporidia are apicomplexans and kin to the malaria parasite *Plasmodium*; and this great phylogenetic distance makes it remarkable that Microsporidia and Cryptosporidia have independently evolved skeletal protein import pathways that are almost identical. We suggest that the skeletal pathway reflects the protein import machinery of the first eukaryotes, and defines the essential roles of the core elements of the mitochondrial protein import machinery. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

One of the key characteristics of eukaryotic cells is mitochondria, perhaps best known as the 'energy factory' of the cell. Mitochondria are the remnant evidence of an ancient symbiotic relationship between a host cell and an alpha-proteobacterial endosymbiont [1,2]. For a long time, a group of unicellular eukaryotes referred to as Archezoa [3] were thought to have split from all other eukaryotes before this endosymbiotic event and to innately lack mitochondria. The Archezoa comprised a range of organisms including *Giardia*, *Trichomonas*, *Entamoeba*, and a group of obligate intracellular eukaryotes, the Microsporidia. These organisms are however all highly reduced eukaryotic parasites, whose strongly divergent sequences are known to pose challenges in phylogenetic analyses. With the development of more sophisticated phylogenetic methods, it was demonstrated that the Archezoa are not a monophyletic group: instead they are a collection of very distantly related organisms, and their collective placement at the root of the eukaryotic tree was a methodological artifact [4–7]. Later studies could prove that these supposedly pre-mitochondrial organisms not only encode homologs of mitochondrial proteins [8–11], but that they harbor organelles with double membranes that proved to be highly reduced mitochondria, which are now collectively referred to as mitosomes [8,12–14].

All mitosomes share a common ancestry with mitochondria but underwent extensive, independent secondary reduction [12,14–17], leaving them unrecognized for a long time due to both their drastic size reduction and the absence of characteristic mitochondrial cristae (Fig. 1). Mitosomes are also shrunken with respect to their protein content (Fig. 1, Table 1): they have lost almost all functions characteristic of mitochondria, most notably the generation of energy either by oxidative phosphorylation (mitochondria) or substrate-level phosphorylation (hydrogen-producing mitochondria called hydrogenosomes; [4,18]), and all mitosomes known to date lack a mitochondrial genome and the associated DNA replication, transcription and translation machinery. Mitosomes contain the proteins of the iron sulfur (Fe/S) cluster biosynthesis known as the ISC machinery [14,19], with the generation of Fe/S clusters being the only essential biosynthetic function in yeast

☆ This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

* Corresponding author at: Department of Biochemistry & Molecular Biology, Monash University, Clayton Campus, Building 77, Melbourne 3800, Australia. Tel.: +61 3 99020397; fax: +61 3 99053726.
E-mail address: Eva.Heinz@monash.edu (E. Heinz).

0167-4889/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2012.02.006

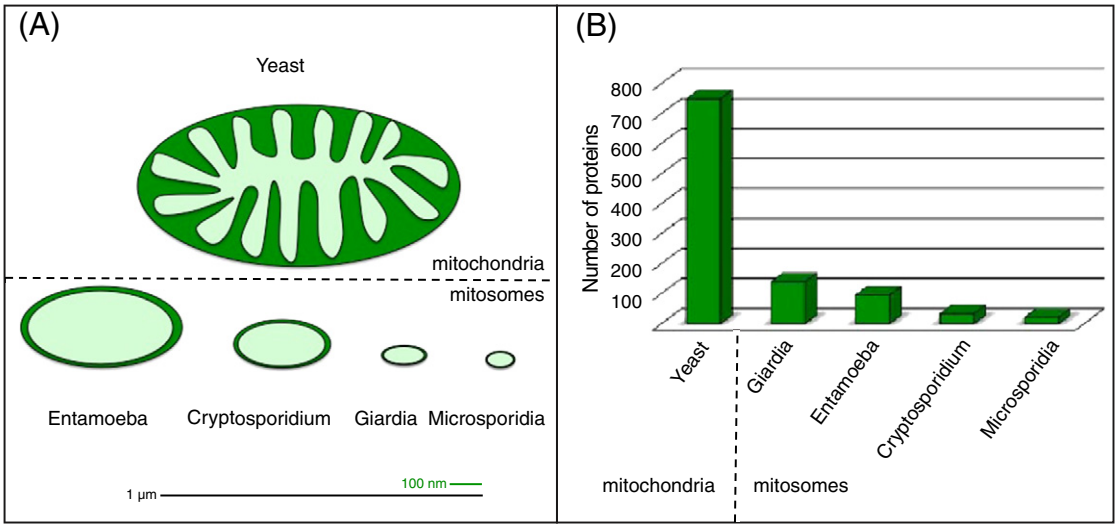

Fig. 1. A drastic reduction in size and protein content of mitosomes. (A) Diagram represents the relative size of organelles as defined by microscopy: 50×90 nm in Microsporidia [12], 140×65 nm in *Giardia* [14], 150–300 nm diameter in *Cryptosporidium* ([89], shown with a 300 nm diameter), 150–500 nm diameter in *Entamoeba* ([15,90]; shown with a 500 nm diameter), in contrast to approximately 1 µm diameter in yeast and 1–7 µm in human mitochondria (not shown). Black scale bar, 1 µm; green scale bar, 100 nm. (B) A graphical representation of the complexity of the organelle proteome reported in the literature, references are provided in the text. Detailed protein numbers are provided in Table 1.

mitochondria [20,21]. The location of Isc and other proteins in mitosomes and in no other compartment of the cells demonstrates that these proteins have to be targeted to and imported into mitosomes, and it has become clear now that this import pathway is composed of homologs of the proteins mediating protein import into mitochondria. Indeed, the observation that the protein import pathway is conserved between mitosomes and mitochondria stands as important evidence for the monophyletic origins of mitochondria and mitosomes. In this review, we focus on four organisms which are phylogenetically very distant, but are all unicellular parasites whose genomes have undergone drastic reduction with respect to their predicted proteome. All these organisms have reduced their mitochondria to mitosomes, and their protein import machineries represent a drastically simplified version of the well-described systems of known model organisms such as *Saccharomyces cerevisiae* or *Homo sapiens*.

2. Microsporidia: a sister group to the fungi

Microsporidia are highly derived obligate intracellular organisms whose genomes have been streamlined towards extreme forms of reduction [22,23]. In terms of comparative cell biology and genome evolution, Microsporidia are of great interest and significance because of their close relationship with fungi. They are a large group of more than 1200 species with a wide host range and are known as important pathogens infecting insects, fish and mammals including humans. Microsporidia have been known for a long time to cause economic loss in the silk industry, are of increasing economic significance in the fishing industry, and are associated with honey bee colony collapse disorder [24–26]. The number of reported Microsporidia infections of humans increased drastically during the AIDS epidemic, and there is now a growing awareness of infections in humans in non-AIDS patients [27,28]. All Microsporidia described so far have a complex life cycle where they alternate between two life stages: the infectious, extracellular spore stage that is metabolically inactive, which, once a eukaryotic host cell has been invaded, germinates into a metabolically active meront. After several rounds of replication meronts sporulate and leave the host cell, and a new infectious cycle begins [29].

The difficulty of purifying sufficient Microsporidia DNA from co-cultures with their host cells or directly from infected host organisms has long hampered the analyses of Microsporidia genomes. However, with the rise of new sequencing techniques, six (partial) Microsporidia genomes have been released in the last 10 years, and several new species and strains are being investigated (the currently listed ongoing genome sequencing projects at the NCBI BioProjects database comprise 14 different Microsporidia species). The first microsporidian genome to be analyzed was that of *Encephalitozoon cuniculi* [30], an opportunistic human pathogen that can also proliferate in a range of other vertebrates, most predominantly in rabbits [30,31]. *Encephalitozoon cuniculi* (2.9 Mb) and its close relative *Encephalitozoon intestinalis* (2.3 Mb, [32]), are of high interest as they represent the smallest eukaryotic genomes known to date. They both encode less than 2000 genes, and the drastic reduction can be observed not only by the loss of genes, but also shortening of these in length and the loss of targeting signals [30]. The following section will discuss the highly reduced protein import machinery of Microsporidia, focusing on *Encephalitozoon cuniculi* as the best-described organism, as well as drawing comparisons with other Microsporidia species whose draft genomes have been released. Microsporidia species differ widely in various aspects including their host range, details of their life cycles, and their genome sizes, but the number of conserved genes seems to be relatively stable between the *Encephalitozoon* sp. and species with larger genomes sequenced so far [33]: *Nosema ceranae* (7.8 Mb, [34]), which infects honey bees and is suspected to contribute to bee colony collapse disorder; *Antonospora locustae* (5.3 Mb, *Antonospora locustae* Genome Project, Marine Biological Laboratory

Table 1
Mitosomes contain a highly reduced number of proteins compared to mitochondria. The protein numbers are based on mass spectrometry experiments of purified organelles for *S. cerevisiae* [93], *G. lamblia* [83] and *E. histolytica* [80]; and on numbers of proteins predicted to be in the mitosomes based on the genome sequences for *Cryptosporidium* [65] and the Microsporidia [30], where no mass spectrometry data are available yet. The first proteomic analysis for yeast was chosen to facilitate a comparison of equivalent data.

	Number of proteins	Based on prediction alone?
Yeast	750	
Giardia	139	
Entamoeba	95	
Cryptosporidium	34	Yes
Microsporidia	22	Yes