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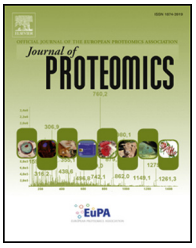

Compositional complexity of the mitochondrial proteome of a unicellular eukaryote (*Acanthamoeba castellanii*, supergroup Amoebozoa) rivals that of animals, fungi, and plants

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ABSTRACT

We present a combined proteomic and bioinformatic investigation of mitochondrial Q6 proteins from the amoeboid protist *Acanthamoeba castellanii*, the first such comprehensive investigation in a free-living member of the supergroup Amoebozoa. This protist was chosen both for its phylogenetic position (as a sister to animals and fungi) and its ecological ubiquity and physiological flexibility. We report 1033 *A. castellanii* mitochondrial protein sequences, 709 supported by mass spectrometry data (676 nucleus-encoded and 33 mitochondrion-encoded), including two previously unannotated mtDNA-encoded proteins, which we identify as highly divergent mitochondrial ribosomal proteins. Other notable findings include duplicate proteins for all of the enzymes of the tricarboxylic acid (TCA) cycle—which, along with the identification of a mitochondrial malate synthase–isocitrate lyase fusion protein, suggests the interesting possibility that the glyoxylate cycle operates in *A. castellanii* mitochondria. Additionally, the *A. castellanii* genome encodes an unusually high number (at least 29) of mitochondrion-targeted pentatricopeptide repeat (PPR) proteins, organellar RNA metabolism factors in other organisms. We discuss several key mitochondrial pathways, including DNA replication, transcription and translation, protein degradation, protein import and Fe–S cluster biosynthesis, highlighting similarities and differences in these pathways in other eukaryotes. In compositional and functional complexity, the mitochondrial proteome of *A. castellanii* rivals that of multicellular eukaryotes.

Abbreviations: BN-PAGE, blue native polyacrylamide gel electrophoresis; ERMES, ER-mitochondria encounter structure; ICL, isocitrate lyase; LC, liquid chromatography; ML, maximum likelihood; MRO, mitochondrion-related organelle; MalS, malate synthase; MEF, Q7 membrane-protein-enriched fraction; MS/MS, tandem mass spectrometry; mtDNA, mitochondrial DNA; nuDNA, nuclear DNA; MTS, mitochondrial targeting signal; ORF, open reading frame; PDH, pyruvate dehydrogenase; PFO, pyruvate:ferredoxin oxidoreductase; RNR, ribonucleotide reductase; SCX-HPLC, strong cation exchange high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEF, soluble-protein-enriched fraction; TCA, tricarboxylic acid.

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Biological significance

Comprehensive proteomic surveys of mitochondria have been undertaken in a limited number of predominantly multicellular eukaryotes. This phylogenetically narrow perspective constrains and biases our insights into mitochondrial function and evolution, as it neglects protists, which account for most of the evolutionary and functional diversity within eukaryotes. We report here the first comprehensive investigation of the mitochondrial proteome in a member (*A. castellanii*) of the eukaryotic supergroup Amoebozoa. Through a combination of tandem mass spectrometry (MS/MS) and in silico data mining, we have retrieved 1032 candidate mitochondrial protein sequences, 709 having MS support. These data were used to reconstruct the metabolic pathways and protein complexes of *A. castellanii* mitochondria, and were integrated with data from other characterized mitochondrial proteomes to augment our understanding of mitochondrial proteome evolution. Our results demonstrate the power of combining direct proteomic and bioinformatic approaches in the discovery of novel mitochondrial proteins, both nucleus-encoded and mitochondrion-encoded, and highlight the compositional complexity of the *A. castellanii* mitochondrial proteome, which rivals that of animals, fungi and plants.

1. Introduction

Mitochondria are organelles involved in a broad array of eukaryotic cellular processes, including energy generation, iron–sulfur (Fe–S) cluster biosynthesis, apoptosis and the metabolism of amino and fatty acids. Although mitochondria retain a distinct genome (mtDNA) that encodes a limited number of proteins (<70) and has been used to infer the α-proteobacterial origin of the mitochondrial genome [1,2], 95–99% of mitochondrial proteins are encoded in the nucleus and imported post-translationally [3]. As a result, the information encoded in mtDNA, although invaluable, provides only a limited insight into the overall function and evolution of the organelle, requiring the systematic identification and characterization of mitochondrial proteins encoded in nuclear DNA (nuDNA).

Methods that allow direct determination of the mitochondrial protein complement (i.e., via the characterization of purified mitochondria and/or submitochondrial compartments and complexes) are integral to the elucidation of mitochondrial function and evolution. In particular, peptide identification via tandem mass spectrometry (MS/MS) and the subsequent bioinformatic characterization of the proteins from which the peptides are derived have been especially valuable and popular [4,5]. Furthermore, direct proteomic approaches may be complemented by a number of bioinformatic techniques—often based on detecting the presence of N-terminal mitochondrial targeting signals (MTSs)—that are able to provide valuable auxiliary information about subcellular localization.

To date, the overwhelming majority of MS/MS-based mitochondrial proteomic studies has been carried out on mitochondria derived from animals [6–8], fungi [9–11], and land plants [12–15]. While these studies have provided important insights into mitochondrial evolution [16–19], and mitochondria-associated diseases [20], a more thorough understanding of the proteomes and the evolutionary histories of individual proteins from mitochondria of free-living single-celled eukaryotes (protists), which constitute the bulk of biodiversity within the eukaryotic lineage (domain Eucarya), is required to understand the evolutionary origin of the mitochondrial proteome and how similar it is among different eukaryotic groups. To this end, proteomic analyses of mitochondria from the ciliated protozoon *Tetrahymena thermophila* [21] and the green alga *Chlamydomonas reinhardtii* [22], along with recent proteomic investigations of mitochondria and mitochondrion-related organelles (MROs) from parasitic eukaryotes [23–28], have provided the first glimpses into the evolution of protist mitochondrial proteomes. These studies have reinforced an emerging concept of the mitochondrial proteome as an evolutionarily chimeric entity: a relatively small core set of proteins (~15%), largely associated with respiration and translation, is inferred to be α-proteobacterial in origin, whereas the rest of the proteome is encoded by genes of unresolved prokaryotic origin, conserved genes 'invented' early in eukaryotic evolution, and novel, lineage-specific additions [19,29–31]. Nonetheless, proteomic analysis of protist mitochondria is still very much in its infancy and much remains to be learned within a phylogenetically broad context about the evolution and metabolic capacities of these organelles.

In order to expand our understanding of mitochondrial proteome evolution among free-living protozoa, we have undertaken a proteomic investigation of the mitochondria of the amoeboid protozoon, *Acanthamoeba castellanii*, a cyst-forming soil and freshwater amoeba that feeds on fungi, other protists and bacteria [32]. Recently, species of the genus *Acanthamoeba* have received increasing attention because of their biomedical relevance as opportunistic human pathogens responsible for amoebic keratitis and granulomatous amoebic encephalitis, and as important reservoirs for bacterial pathogens [33]. As a member of the Amoebozoa supergroup that is sister to Opisthokonta (animals + fungi), *A. castellanii* occupies a key evolutionary position [34–36]. Limited analyses of its mitochondria have already uncovered several evolutionary and biochemical novelties [37–39].

A. castellanii mitochondria are a suitable target for a proteomic investigation as the mtDNA has been sequenced [40], and a high-coverage expressed sequence tag dataset is available, along with draft nuclear genome assemblies [41] (GenBank acc. nos. AEYA01000000 and AHJI01000000). Furthermore, techniques for isolating mitochondria from axenically-grown *A. castellanii* cultures have been described [42]. Here we present an analysis of the *A. castellanii* mitochondrial proteome, identifying a large number of authentic and candidate mitochondrial proteins and uncovering novel metabolic and evolutionary features of the organelle.

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