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GENETICS, EVOLUTION, AND PHYLOGENY - REVIEW

Energy metabolism and its evolution in Microsporidia and allied taxa

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Abstract

The reduction and specialization of the energy metabolism system is a common trait in the evolution of intracellular parasites. One group of fungi-related parasites, the Microsporidia, seems to have developed this trait far more than other eukaryotes. As an extreme adaptation for a parasitic lifestyle, some of them have completely lost the ability to synthesize ATP, relying heavily upon the metabolic processes of host cells to ensure their own development and reproduction. For a long time, only fragmentary data on the functioning and evolution of the energy metabolism system in microsporidia was available. However, the recent discovery of microsporidia-related microorganisms, the Cryptomycota and Aphelida, alongside with the genome sequencing and new data about basal groups in the Microsporidia has shed new light on this problem. Here, we review recent data about functioning of the energy metabolism system in microsporidia and closely related organisms, and discuss possible evolutionary pathways in the group.

Keywords Energy metabolism · Microsporidia · Cryptomycota · Aphelida · Parasites · Evolution

Introduction

Microsporidia form a group of fungi-related unicellular obligate intracellular parasites with a wide range of hosts: from protists to mammals, including humans (Stentiford et al. 2016). All stages of their life cycle associated with growing and replication take place only inside the host cells, beyond which microsporidia can survive only as thick-walled spores. Adaptation to intracellular parasitism drives these parasites toward significant reduction and modification of their genome and functional apparatus (Cuomo et al. 2012). They have lost many metabolic pathways, including synthesis of many amino acids and nucleotides de novo, and hence are strongly dependent on substrates transported from infected host cells (Dean et al. 2016). The energy metabolism system of microsporidia shows the most striking example of this dependence. These parasites have lost canonical mitochondria and the oxidative phosphorylation pathway, so that glycolysis is the only way to generate ATP (Heinz et al. 2012; Corradi 2015). During the intracellular development stage, microsporidia apparently do not use their energy metabolism (Dolgikh et al. 2011) and instead satisfy their energy demands by "stealing" ATP from the host cell using unique nucleotide carriers acquired via horizontal transfer from bacteria (Tsaousis et al. 2008; Alexander et al. 2016).

Until recently, evolution of this group was enigmatic, and it was unclear when and how microsporidia acquired such unique adaptations to the parasitic lifestyle. The appearance of the first molecular phylogenetic data at the beginning of this century revealed the relationship of microsporidia with fungi (Hirt et al. 1999; Keeling et al. 2000). However, these data did not allow determination of how the evolution of microsporidia occurred, as this group has few common specific features with other groups of fungi except the presence of a chitin cell wall. A real breakthrough in the study of microsporidian evolution was made only in the last few years. At the beginning of this decade, a group called Rozellida (Lara et al. 2010) or Cryptomycota (Jones et al. 2011) was discovered, and proposed as the deepest branching clade of fungi. In addition to numerous environmental sequences, this lineage comprised a single described genus, *Rozella*—parasites of other fungi and algae characterized by phagotrophic nutrition and settlement by zoospores, previously considered to be chytridiomycetes. In parallel, the Microsporidia were also considered to be the deepest branching clade of fungi (Capella-Gutiérrez et al. 2012). In 2013, analysis of the *Rozella allomycis* genome

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demonstrated that the Microsporidia and Cryptomycota are close relatives, and the latter group is also characterized by a strong degree of reduction and modification of the genome and functional apparatus, although slightly less than those of microsporidia (James et al. 2013). This confirmed clustering Microsporidia and *Rozella* sp. in earlier phylogenies (James et al. 2006). At the same time, it was shown that Aphelida—another group of fungi with a previously unclear systematic position, was grouped together with microsporidia and cryptomycotans (Karpov et al. 2013, 2014). This taxon comprises phagotrophic zoosporic parasitoids of algae morphologically resembling *Rozella* sp.

Soon after the discovery of Cryptomycota, it was shown that microsporidia-like organisms develop in nuclei of amoebae—*Paramicrosporidium* spp. (Michel et al. 2000, 2009) are also related to this group (Corsaro et al. 2014b). In addition, another species of intranuclear parasite of the amoeba *Nucleophaga amoebae* was redescribed, and it was shown that this species is related to *Paramicrosporidium* and also belongs to the rozellids (Corsaro et al. 2014a). In the same year, the description and genomic data of the unique *Daphnia* parasite *Mitosporidium daphniae* were published. This species has a microsporidia-like morphology and occupies an intermediate position between *R. allomycis* and microsporidia in the degree of reduction and specialization of the genome to a parasitic lifestyle (Haag et al. 2014). Subsequently, the genomes of the cryptomycotan *Paramicrosporidium saccamoebae* (Quandt et al. 2017) and the aphelid *Paraphelidium tribonemae* (Torruella et al. 2018), as well as two representatives of Metchnikovellidae—a basal group of the Microsporidia not previously studied from the point of molecular phylogeny, were published (Mikhailov et al. 2017; Galindo et al. 2018).

The discovery of the closest relatives of microsporidia, and the decoding of their genomes together with genomic data on the root groups of the Microsporidia itself, make it possible to take a fresh look at the evolution of this group. In this work, we present a generalized scheme of energy metabolism in microsporidia, review its modifications within the group, and hypothesize a possible evolutionary pathway in this system based on the functioning of the metabolic system in their closest relatives.

Current concepts of microsporidia phylogeny and terminology used in this article

In the frame of this article, we present a consensus scheme of phylogenetic relationships between the Microsporidia, Cryptomycota, and Aphelida groups and fungi, considering only species with sequenced genomes which are of interest from the point of view of their energy metabolism system and its evolution (Fig. 1). Placing these organisms into conditional groups, we were guided primarily by the structure of their respective energy metabolism systems for the purposes of this article. In this work, we will review the Aphelida, which was previously considered to comprise a monophyletic group with the Microsporidia according to initial data (Karpov et al. 2014). However, after decoding the genome of its representative *P. tribonemae*, the Aphelida is now considered to occupy an intermediate position between the Microsporidia and fungi (Torruella et al. 2018) (Fig. 1). For the Cryptomycota group, we will consider its "classical" representatives to be *R. allomycis*, as well as microsporidia-like organisms *P. saccamoebae* and *M. daphniae*. According to some authors, the last two organisms should be considered as basal microsporidia (Bass et al. 2018), indicated by the absence of a clear boundary between the two groups in Fig. 1. As the root group of the Microsporidia ("basal microsporidia"), we consider a unique group of hyperparasites, the Metchnikovellidae, two species of which have sequenced genomes: *Amphiamblys* sp. (Mikhailov et al. 2017) and *Metchnikovella incurvata* (Galindo et al. 2018). According to the latest data, the root position in the microsporidian phylogeny is occupied by another group, the Chytridiopsida (Corsaro et al. 2019), but we did not include it in this review due to the absence of any molecular data except the rDNA sequence. Finally, we conventionally designated other microsporidia as "canonical microsporidia," which includes dozens of species with genomic data.

Mitochondria reduction, glycolysis, and ATP stealing in canonical microsporidia

In general, the functioning of the energy metabolism system in canonical microsporidia can be described as follows: (1) at the stage of intracellular development, these parasites receive ATP by transporting it from infected host cells; and (2) at the spore stage, they synthesize this compound through glycolysis using glycerol-3-phosphate shuttle and an alternative oxidase for reoxidation of the reduction equivalents generated during this process (Fig. 2) (Williams et al. 2010).

In early ultrastructural studies, the absence of mitochondria in microsporidian cells was revealed, and the group was long considered to be primarily mitochondrial (see references in Keeling 2009). The absence of a mitochondrial genome in microsporidia was confirmed during the first sequencing of the genome of *Encephalitozoon cuniculi* (Katinka et al. 2001). However, genes related to some mitochondrial functions were found in the nuclear genome of this species, which suggested a secondary loss of mitochondria by these parasites. This was further confirmed by the discovery of mitosomes—mitochondrial remnants in the form of tiny two-membrane organelles devoid of a genome in the microsporidium *Trachipleistophora hominis* (Williams et al. 2002). The main

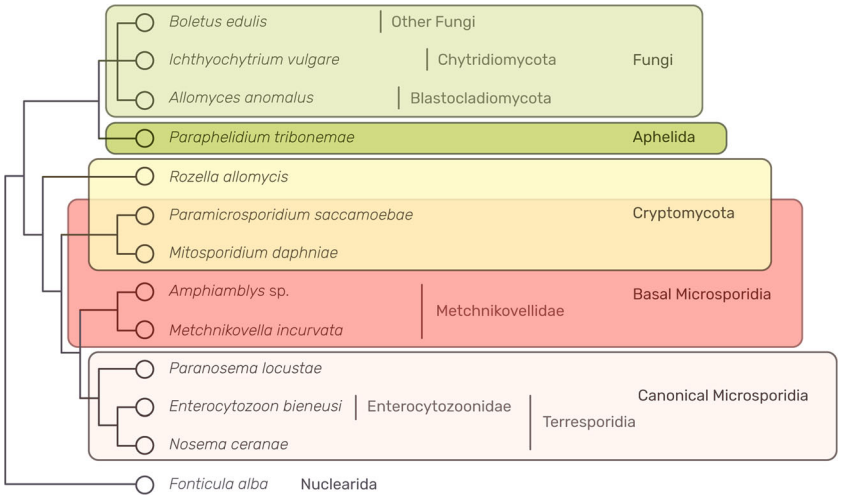

Fig. 1 A consensus cladogram reflecting evolutionary relationships of Microsporidia, Cryptomycota, Aphelida, and Fungi according to Bass et al. (2018), Galindo et al. (2018), Torruella et al. (2018), and Corsaro et al. (2019). Only species with genomic data and which are relevant in the context of this article were included. Intersecting coloring between Cryptomycota and basal Microsporidia indicates the questionable position of *P. saccamoebae* and *M. daphnia*, which are considered to be early branching Microsporidia according to Bass et al. 2018

function of these organelles in microsporidia is iron-sulfur cluster assembly (Goldberg et al. 2008). It has been shown that dozens of proteins localized in the mitosomes and cytoplasm are involved in this assembly, and that this process is highly conservative and seems to function in a similar way in all microsporidia (Freibert et al. 2017). After microsporidia had lost mitochondria and ability to generate ATP via oxidative phosphorylation, they acquired the ability to import ATP directly from the host cell cytoplasm during intracellular development (Tsaousis et al. 2008; Williams et al. 2014). Unique

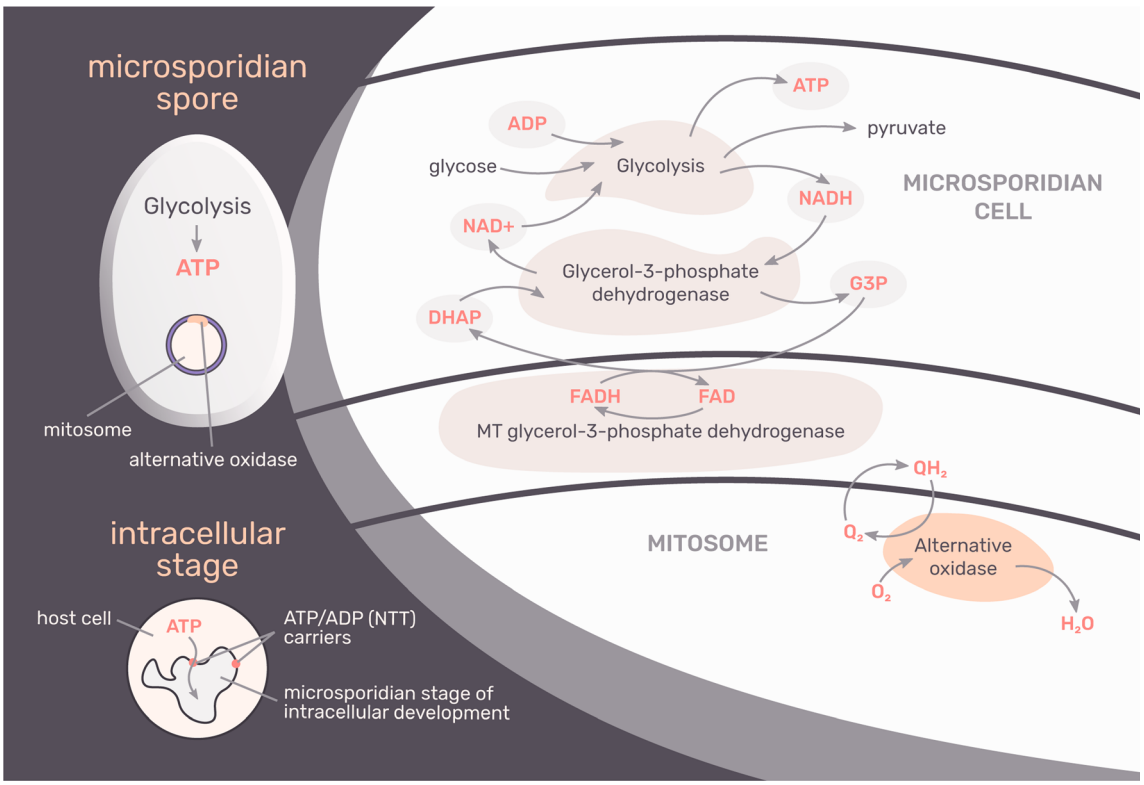

Fig. 2 Energy metabolic system in canonical microsporidia. Adopted from Williams et al. (2011, 2014) with modifications. At the stage of intracellular development, the parasite deactivates its own energy metabolism, and instead satisfies its energy needs by stealing ATP from the host cell using unique nucleotide carriers. At the spore stage, Microsporidia generate ATP through glycolysis using a glycerol-3-phosphate shuttle and an alternative oxidase for reoxidation of NADH generated during this process. This scheme is characteristic of all studied canonical Microsporidia except the Terresporidia group, which lost the alternative oxidase

nucleotide transport proteins (NTT) were discovered in the genomes of all studied canonical microsporidia species, which were obtained via horizontal gene transport (HGT) from intracellular parasitic bacteria (Cuomo et al. 2012). Studies performed on phylogenetically distant species of microsporidia—*E. cuniculi* and *T. hominis*—showed localization of these nucleotide transporters on the surface of the cytoplasmic membrane of the intracellular development stages. In addition, the ability of the transporters (expressed in *E. coli* cells) to transport ATP and other nucleotides from the environment was confirmed (Tsaousis et al. 2008; Dean et al. 2018).

At the spore stage, when they are surrounded by thick spore wall, microsporidia cannot steal ATP from the host. They are therefore forced to synthesize this compound by glycolysis (Williams et al. 2014). The most likely source of glucose in microsporidian spores is trehalose hydrolysis, because this sugar as well as enzyme trehalase and its activity were observed in spores of all studied species of microsporidia (Undeen and Vander Meer 1999; Dolgikh et al. 2011). To date, there are virtually no data concerning the physiological activity of resting spores of different microsporidia, or about their ATP needs at this stage. However, it has been shown that at least the spore extrusion process, by which the sporoplasm enters the infected cell, is ATP-dependent (Williams et al. 2014). In all sequenced genomes of microsporidia, with the exception of the highly specialized clade Enterocytozoonidae, genes encoding all enzymes involved in glycolysis were found, and their activity in spores was confirmed, for some species of microsporidia (Dolgikh et al. 1997; Dolgikh 2000; Williams et al. 2014). For a long time, it was unclear how reoxidation of the reduction equivalents formed during glycolysis, such as NADH, occurs in microsporidia in the absence of oxidative phosphorylation. To date, the following scheme of this process has been proposed, involving the glycerol-3-phosphate shuttle mechanism and alternative oxidase (see Fig. 2): (1) In the cytoplasm, the cytoplasmic form of glycerol-3-phosphate dehydrogenase oxidizes NADH to NAD^+, reducing dihydroxyacetone phosphate to glycerol-3-phosphate (G3P). (2) The latter compound enters the intermembrane space of the mitosome, where it is oxidized again to dihydroxyacetone phosphate (DHAP) due to the FAD-dependent mitosomal form of glycerol-3-phosphate dehydrogenase. (3) The reduced form of FADH2, in turn, transfers electrons to the ubiquinone (Q) pool located in the inner membrane of the mitosome. (4) The final step is the reoxidation of ubiquinone with the alternative oxidase enzyme using oxygen as the final electron acceptor, and reduction of the latter to water (Williams et al. 2010; Dolgikh et al. 2011). Thus, it is probable that the mitosomes of many microsporidia do not completely lose their role in the energy metabolism, and are in fact necessary to maintain glycolysis in the spores of these parasites.

This scheme of energy metabolism is characteristic of all studied canonical microsporidia, with the exception of the group, provisionally called the Terresporidia (Vossbrinck and Debrunner-Vossbrinck 2005). An alternative oxidase gene was not found in the genomes of representatives of this group, although both components of the glycerol phosphate shuttle were maintained (Williams et al. 2010). Immunolocalization of the mitosomal form of glycerol-3-phosphate dehydrogenase in *E. cuniculi* cultured in RK-13 cells showed that this protein is no longer localized in the mitosomes of microsporidian vegetative stages (Williams et al. 2008). It is possible that the glycerol-3-phosphate shuttle in Terresporidia cells continues to function in order to maintain glycolysis by transferring the electron to some other from the mitosome compartment of the parasite cell, different from oxygen final electron acceptor.

Some Terresporidia are characterized not only by the loss of alternative oxidase but also by glycolysis itself. Only two of 12 genes involved in glycolysis were found in the genome of *Enterocytozoon bieneusi* (Keeling et al. 2010). For a long time, this case of glycolysis loss was thought to be unique among microsporidia, until a recent study of other Enterocytozoonidae and closely related Hepatosporidae clades showed that this feature is typical for these groups, and losses occurred independently, since all five studied species lost a different set of glycolysis enzymes (Wiredu et al. 2017).

Transcriptome survey of *Paraphelidium tribonemae* shows no genome and metabolic system reduction in Aphelida

Aphelids are a small group of intracellular parasites or parasitoids of algae, with about 10 described species. Despite the fact that the genus *Aphelidium* was described in 1885, a classification for this taxon was proposed only in 2000, with class Aphelidea established as a part of the Rhizaria based on morphological and biological data (Gromov 2000). Further, molecular studies showed a close relationship of aphelids with the Microsporidia and Cryptomycota, and it was proposed to combine these three taxa into the superphylum Opistosporidia, a sister taxon to fungi (Karpov et al. 2013, 2014). However, the transcriptome sequence of the aphelid *P. tribonemae* and multigene phylogeny showed that the Aphelida is closer to the free-living fungi than to the Microsporidia and Cryptomycota (Torruella et al. 2018). Their energy metabolism system, as well as their whole genome, does not display reductive adaptation to intracellular parasitism. These organisms possess complete mitochondria and an ability to perform oxidative phosphorylation. Aphelids also retain an almost complete set of other metabolic pathways, such as synthesis of amino acids and nucleotides, which

are lost by microsporidia (Torruella et al. 2018). Thus, the evolution of this group is not shaped by the unique adaptations to a parasitic lifestyle evident in the Microsporidia.

Cryptomycota retain functional mitochondria but demonstrate independent tendency towards mitochondrial reduction

Unlike the aphelids, the close relationship of Cryptomycota with Microsporidia was confirmed by deeper study of this group. Sequencing of the *R. allomycis* genome showed that cryptomycotans and microsporidia possess shared signatures of parasitism inherited from a common endoparasitic ancestor (James et al. 2013). However, the reduction and specialization of the genome and functional apparatus in Cryptomycota do not reach the level attained by the Microsporidia. All cryptomycotans studied at the genomic level: *R. allomycis*, *M. daphniae*, and *P. saccamoebae*, apparently possess functional mitochondria (although for *P. saccamoebae*, these organelles have not yet been morphologically visualized, and only the mitochondrial genome of this species is available) (Haag et al. 2014; Quandt et al. 2017). Moreover, while *R. allomycis* and *M. daphnia* mitochondria lack the complex 1 of the oxidative phosphorylation pathway, the mitochondrial genome of *P. saccamoebae* preserves all necessary genes for this process (Quandt et al. 2017). In addition, the latter is the only species of known cryptomycotan which lacks the alternative oxidase gene characteristic of canonical microsporidia except Terresporidia.

It is quite surprising that the genome of *R. allomycis*, but not those of *M. daphniae* and *P. saccamoebae*, possesses plastid–bacterial ATP/ADP (NTT) carriers, similar to those used by microsporidia to steal ATP from host cells (James et al. 2013). Unlike microsporidia, it has not yet been proven that these carriers are localized on the cell membrane of *R. allomycis* when the parasite develops inside the infected cell, though their ability to transport ATP has already been experimentally confirmed (Dean et al. 2018). Thus, it is probable that *R. allomycis*, much like microsporidia, is capable of direct ATP transport from infected cells. This view is further supported by a concentration of host mitochondria around the developing pathogen (Powell et al. 2017).

Metchnikovellidae: Loss of mitochondria, an inability to undergo oxidative phosphorylation, and an absence of HGT-acquired ATP carriers

Metchnikovellidae (basal microsporidia) are unique microsporidia, infecting other unicellular parasites, specifically the gregarines (Apicomplexa) inhabiting marine invertebrates. Until recently, their phylogenetic position remained unclear. Fortunately, recent sequencing of *Amphiamblys* sp. and *Metchnikovella incurvata* genomes demonstrated that metchnikovellids are placed basally in relation to the other microsporidia (Mikhailov et al. 2017; Galindo et al. 2018). Like canonical microsporidia, these parasites do not possess classical mitochondria and have lost the ability to undergo oxidative phosphorylation, while maintaining a complete set of glycolysis enzymes. In the genomes of metchnikovellids, no alternative oxidase and mitochondrial glycerol-3-phosphate dehydrogenase (one of the two enzymes of the glycerol-3-phosphate shuttle) were found, so it remains unclear how oxidation of the reduction equivalents formed during glycolysis occurs in these organisms (Mikhailov et al. 2017; Galindo et al. 2018). An interesting fact is the presence in the genomes of both species of the enzyme mitochondrial malate dehydrogenase, which is absent in other microsporidia. It was shown that in some Apicomplexa, this enzyme can perform the function of lactate dehydrogenase (Boucher et al. 2014), which in turn can maintain the redox balance of glycolysis due to the conversion of lactate to pyruvate. The authors of *M. incurvata* genome annotation suggest that this enzyme in Metchnikovellidae may function in a similar way (Galindo et al. 2018).

Along with the inability to undergo oxidative phosphorylation, ATP/ADP (NTT) carriers, characteristic of other microsporidia, were not found in Metchnikovellidae. The authors suggest that carriers from mitochondrial carrier protein family (MCF) found in both *Amphiamblys* sp. and *Metchnikovella incurvata* genomes can play this function in these parasites, but this data has not yet been experimentally confirmed (Mikhailov et al. 2017; Galindo et al. 2018). As the evolution of new genes has taken place during the adaptation of microsporidia to their hosts (Galindo et al. 2018), this unique adaptation of Metchnikovellidae can be explained by the uniqueness of their unicellular parasitic hosts.

From functional mitochondria to complete loss of the energy metabolic system: a possible mechanism for energy metabolism evolution in the Cryptomycota–Microsporidia lineage

Current data suggest that microsporidia evolved from within the Cryptomycota; therefore, it is possible to trace the evolution of functional systems from basal cryptomycotans to the highly specialized microsporidia and suggest how these groups evolved from a common ancestor (Torruella et al. 2018; Bass et al. 2018; Corsaro et al. 2019). Since *P. saccamoebae*, which has complete mitochondria and a classical, unreduced system of energy metabolism, is present among cryptomycotans, this

system was probably characteristic of the common ancestor of cryptomycotans and microsporidia (Quandt et al. 2017) (Fig. 3). Most likely, the gene encoding the alternative oxidase was also present in the genome of this organism, which was independently lost by various cryptomycotans and microsporidia, or adapted to maintain the redox balance of glycolysis in canonical microsporidia. This enzyme is present in many fungal groups, including basal Aphelida (Torruella et al. 2018), where it performs various functions, for example, protecting cells from oxidative stress (Joseph-Horne et al. 2001), and was apparently characteristic of the common ancestor of fungi and the Cryptomycota–Microsporidia lineage (Williams et al. 2010). It is probable that, when transitioning into an intracellular parasitism lifestyle, this enzyme could not fulfill all its previous functions, which led to its independent loss in *P. saccamoebae* and the Metchnikovellidae. In the majority of canonical microsporidia, the alternative respiratory chain was adapted to maintain glycolysis and most likely was lost by representatives of the clade Terresporidia (Williams et al. 2010). The loss of this system may be associated with the adaptation of this group to parasitism in terrestrial hosts, a setting where gas exchange of spores with the external environment under dry conditions is much more complicated compared with that of the aquatic habitat.

Within the Cryptomycota group, a mitochondrial reduction is observed, which is reflected by the loss of oxidative phosphorylation complex 1, which occurred independently in *R. allomycis* and *M. daphniae*, but not in *P. saccamoebae* (Quandt et al. 2017). The full reduction of mitochondria to mitosomes and the loss of ability to undergo oxidative phosphorylation apparently occurred at the stage of divergence of the basal microsporidia. The key point in this adaptation remains a mystery. Earlier, such a strong reduction of the energy metabolism system in microsporidia was primarily associated with their HGT-acquisition of ATP/ADP carriers capable of transporting ATP from infected cells (Tsaousis et al. 2008; Heinz et al. 2014). However, these carriers are absent in basal microsporidia, but on the other hand are present in the cryptomycete *R. allomycis*, which has mitochondria with only an insignificant degree of reduction (James et al. 2013). It remains unclear when this adaptation was acquired within the Cryptomycota–Microsporidia lineage. Assuming that ATP/ADP (NTT) carriers were already present in the common ancestor of these groups (Heinz

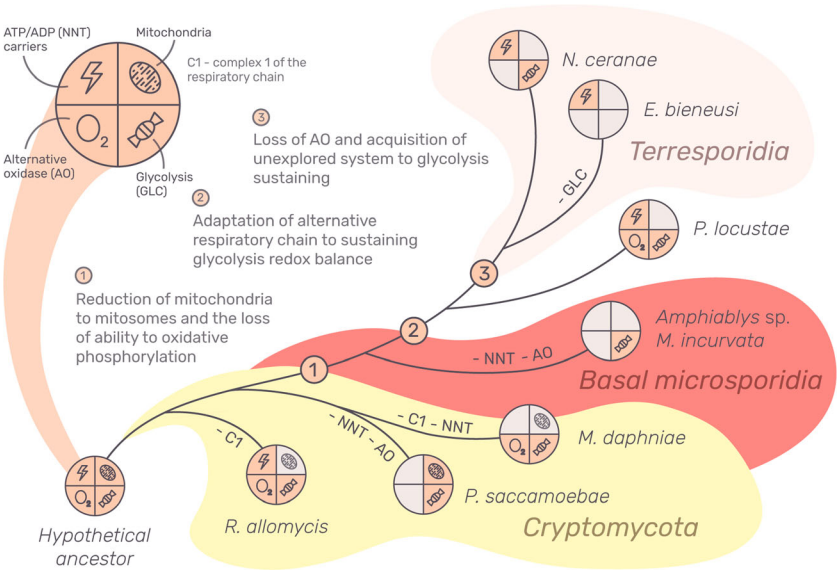

Fig. 3 Hypothetical scheme of energy metabolism evolution in the Cryptomycota–Microsporidia lineage from a hypothetical common ancestor of these groups to evolutionary advanced microsporidia—the Terresporidia. Evolutionary events in the group as a whole are marked on the main axis, while events occurring independently in the evolution of individual groups correspond to the lateral branches. The hypothetical endoparasitic ancestor of both groups, like a majority of fungi (the sister clade to the Cryptomycota–Microsporidia lineage), had a complete, unreduced system of energy metabolism, including a complete set of enzymes for glycolysis in the cytoplasm, oxidative phosphorylation in mitochondria, and an alternative respiratory chain. The hypothetical ancestor also had ATP/ADP carriers capable of transporting ATP from infected host cells. In different cryptomycotans, we can observe independent reduction events like the loss of complex 1 of the respiratory chain, alternative oxidase, or ATP/ADP transporters. Divergence of basal Microsporidia (Metchnikovellidae) was associated with a reduction of mitochondria to mitosomes and the loss of ability to perform oxidative phosphorylation. Adoption of an alternative respiratory chain for sustaining glycolysis redox balance can be observed at the transition to canonical Microsporidia, and the lack of this system corresponds to divergence of Terresporidia. Independent events in different Microsporidia lineages include acquisition of an unexplored system for sustaining glycolysis in basal Microsporidia and Terresporidia, and the loss of ability to generate ATP through glycolysis in the Enterocytozoonidae

et al. 2014; Dean et al. 2018), it is possible that they were independently lost by both cryptomycotans, *M. daphniae* and *P. saccamoebae*, and basal microsporidia. This seems unlikely, since it is difficult to explain why an intracellular parasite is driven to lose its ability to steal ATP from the host. On the other hand, it seems equally unlikely that these transporters were acquired independently by the closely related *R. allomycis* and canonical microsporidia, but by no other eukaryotic endoparasites. To date, there are only few studies devoted to the investigation and comparison of ATP transporters of *R. allomycis* and microsporidia, where the first hypothesis about the homology of NTT genes in these organisms is supported (Heinz et al. 2014; Dean et al. 2018), so we will adhere to it in the scope of this review (Fig. 3). At the time of this review preparation, a new family of ATP/ADP carriers (MFS transporters) was discovered with a potential ability to steal ATP from infected cells. The corresponding genes are present in the genomes of all studied cryptomycotans and microsporidia (Major et al. 2019), which indicates that our views on the uniqueness of HGT-acquired NTT transporters in the evolution of microsporidia may still change dramatically.

One way or another, by having lost canonical mitochondria, microsporidia were forced to satisfy their need for ATP synthesis due to glycolysis at the spore stage. It appears they did this by independently developing various systems to maintain the redox balance of this process. In canonical microsporidia, the alternative oxidase and glycerol-3-phosphate shuttle (Williams et al. 2010) are involved in sustaining glycolysis, while in metchnikovellids and Terresporidia, this process has not yet been fully studied. Representatives of the highly specialized Enterocytozoonidae and closely related Hepatosporidae clades, which lose their ability to undergo glycolysis and independent ATP synthesis, achieve the highest degree of reduction in the energy system in microsporidia and possibly in all eukaryotes. Apparently, these microsporidia acquired an adaptation that makes the parasite's existence completely ATP-independent at the spore stage. It is possible that such an adaptation could involve a unique mechanism for invading the host cell, perhaps by phagocytosis of the parasite spores by the host (Wiredu et al. 2017).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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