2 Microsporidia – Highly Reduced and Derived Relatives of Fungi

Bryony A.P. Williams[1], Patrick J. Keeling[2]

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I. Introduction

Microsporidia are a diverse group of eukaryotic parasites with over 1200 described species. Most species infect animals, though some species infect other single-celled eukaryotes, for example the gregarine *Selenidium* and the amoeba *Vanella* (Leger and Dubosq 1909; Scheid 2007). However, microsporidia are increasingly important humans pathogens with 14 species now recognized as causing illness in the immunocompromised; and given the diversity of microsporidia there are likely many other species extant with the potential to infect humans given the right opportunity (Mathis et al. 2005). The spores of microsporidia are highly environmentally resistant and in humans are generally transmitted via the fecal–oral route, frequently causing intestinal infections. Other sites of infections are also possible and once in the body the parasites often migrate and can infect virtually any tissue of the body. These disseminated infections are rare, but generally far more serious and may result in death (Coyle et al. 2004).

The microsporidia have an interesting but not fully understood mechanism of cell invasion. The infective stage of microsporidia is the dormant spore, a characteristic structure dominated by a tightly coiled filament called the polar filament (Fig. 2.1; Kudo 1918). The germination of spores is triggered by a variety of environmental cues, which leads to expulsion of the polar filament, and its eversion to become a tube. The polar vacuole expands, forcing the contents of the spore through the tube into the host cell where it forms a rather amorphous cell, at this stage called a meront (Vávra and Larsson 1999). Expulsion of the polar tube is thought to be precipitated by an increase in pressure within the spore. This increase in pressure is likely produced by a rapid influx of water into the cell. Aquaporin proteins encoded in the *Encephalitozoon cuniculi* genome are suggested to provide the means of entrance of water in the cell across the plasma membrane (Frixione et al. 1997; Ghosh et al. 2006), the permeability of which may be controlled by changes in ion concentrations in the environment. It is also hypothesized that osmotic pressure may be increased inside the spore at the time of germination by the breakdown of the disaccharide sugar trehalose into less complex sugars, driving an influx of water. In line with this, it has been shown in some aquatic species of microsporidia that there is a rapid disappearance of trehalose and the appearance of smaller sugars at germination (Undeen and Vander Meer 1999).

Conventional theories suggest that triggers for germination are chemical and that the polar tube physically pierces through the host cell, and the spore contents pass directly into the host cell cytoplasm, where it can directly interact with

[1]Biosciences, Geoffrey Pope Building, College of Life and Environmental Sciences, University of Exeter, Stocker Road, Exeter, EX4 4QD, UK; e-mail: B.A.P.Williams@exeter.ac.uk
[2]Canadian Institute for Advanced Research, Botany Department, University of British Columbia, 3529–6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada; e-mail: pkeeling@interchange.ubc.ca

Evolution of Fungi and Fungal-Like Organisms, The Mycota XIV
S. Pöggeler and J. Wöstemeyer (Eds.)

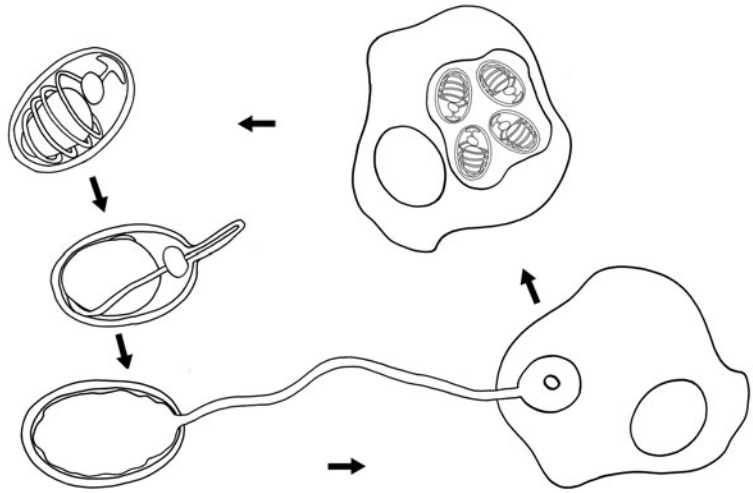

Fig. 2.1. Microsporidian generalized life cycle. The infection process starts with the expulsion of the polar tube forced out by expansion of the posterior vacuole. The tube pierces the host cell and the microsporidian passes its contents into the host cell cytoplasm to form a meront. These develop (sometimes within a vacuole – as shown here) into more spores that are released into the environment for further infection

the host cell environment. However, recent data also suggest that infection may be mediated by a molecular anchoring of the spore to the host cell surface glycosaminoglycans prior to germination (Southern et al. 2006). This discovery of a molecular interaction between microsporidia and host before infection may provide a basis for understanding the host specificity of different microsporidia species. It has also been observed that the end of the everted polar tube, and subsequently the spore contents, can be endocytosed by the host after germination rather than being passed through the membrane via the polar tube (Schottelius et al. 2000). Once inside the cell, microsporidia life cycles are variable from species to species but generally there is a phase of meront cell division, followed by sporogony, in which the infection apparatus and spore wall develop, followed by maturation and release of the spores through the rupture of the host cell (Schottelius et al. 2000).

II. History of the Classification of Microsporidia

Microsporidia have been studied for over 150 years, but like many single-celled eukaryotic lineages, they have a long history of phylogenetic misplacement (see Chapter 1 in this volume). Suprisingly, when Nägeli first examined them in the 1800s (Nägeli 1857), he grouped microsporidia with the Schizomycete fungi, though at this time there was little information on the untrastructural differences between single-celled organisms. The Schizomycete fungi since turned out to be a collection of many unrelated species of eukaryotes and prokaryotes, and microsporidia were soon were re-assigned to their own group within the Sporozoa class of Protozoa (Balbiani 1882) and later grouped with the Myxozoa in the Cnidosporidia (Döflein 1901). This was based on the fact that both taxa share the characters of a polar filament and an ability to form spores, which were thought to be homologous traits. This grouping persisted for over 70 years until a greater understanding of the ultrastructure and biochemistry of the Cnidosporidia revealed fundamental differences in the two characters between various subgroups (Lom 1962), eventually leading to the microsporidia being moved into their own phylum (Sprague 1969, 1977).

In 1983, Cavalier-Smith placed microspora in the subkingdom Archezoa based on the perceived absence of mitochondria in the group. He hypothesized that microsporidia and other Archezoa (diplomonads, Parabasalia, Archamoebae) were primitively amitochondriate, having branched away from the main eukaryotic lineage before the mitochondrial endosymbiosis (Cavalier-Smith 1983). With the advent of molecular phylogeny, the source of characters for reconstructing protozoon phylogeny switched from ultrastructure and biochemistry to gene and amino acid sequences. The sequencing and phylogenetic analysis of the small subunit ribosomal RNA of the microsporidian *Vairimorpha necatrix* appeared to provide a stunning confirmation of

the hypothesis that microsporidia were very early-branching eukaryotes, because the phylogenies not only placed microsporidia at the base of eukaryotic tree (Vossbrinck et al. 1987), but also revealed the presence of fused 5.8S-LSU rRNA, a character shared with prokaryotes (Vossbrinck and Woese 1986). Subsequent phylogenies of EF1-α and EF-2 reinforced this, also showing microsporidia to branch early (Kamaishi et al. 1996). Indeed, microsporidia often branched even earlier than the other Archezoa, which together with other characters led to the idea that they may be the first branch of extant eukaryotes (Patterson 1994).

In 1996, the phylogenetic analysis of α- and β-tubulin sequences from *Antonospora* (*Nosema*) *locustae*, *Spraguea lophii*, Encephalitozoon *cuniculi*, and *Encephalitozoon hellem* suggested an alternative position for microsporidia as relatives of the Fungi (Edlind et al. 1996; Keeling and Doolittle 1996). This prompted re-analysis of the previously analysed data sets using methods correcting for potential artefacts and the sequencing of new phylogenetic markers in microsporidia (Fast et al. 1999; Hirt et al. 1999; Van de Peer et al. 2000). One particular source of artefact pervasive to molecular phylogenies of microsporidia is long-branch attraction.

If two taxa in a phylogeny have significantly longer branches than the other taxa, they have an increasing probability of being grouped together irrespective of their phylogenetic relationships as more characters are added to the data set (Felsenstein 1978). Another issue that has confounded microsporidian phylogenies is the problem of unequal site-rate variation. Not all sites vary freely or at the same rate across sequence alignments and some sites may not vary in any of the taxa. These sites can be hypothesised to be invariable, particularly in an alignment, which includes sequences representing highly diverse taxa, including eubacteria and diverse eukaryote lineages. If invariable sites are included in an analysis and among-site rate variation is not taken into account, the number of changes that have occurred at the fast evolving sites may be underestimated.

This can cause fast evolving sequences to branch together (Yang 1996).

After these sources of artefacts were corrected for, microsporidia were consistently found to branch within or at the base of the Fungi in most molecular markers previously used to support their basal position, except in the phylogeny of SSU rRNA where they remained basal to eukaryotes (Fast et al. 1999; Hirt et al. 1999; Van de Peer et al. 2000). The importance of long-branch attraction in analysis of microsporidian genes was recently highlighted by an analysis of 99 genes from the *E. cuniculi* genome. This showed a clear inverse correlation between the rate of substitution in a gene and its propensity to unite microsporidia and fungi in its phylogeny (Thomarat et al. 2004), which reinforces the conclusion that early phylogenies were hampered by long-branch attraction problems.

III. Microsporidia in or out of the Fungi?

Given that microsporidia are phylogenetically allied to the Fungi, the question is still open as to whether microsporidia evolved from a lineage within the Fungi or are a distinct lineage of eukaryotes branching at the base of the Fungi, therefore representing a sister group (see Chapter 1 in this volume). Apart from phylogenies, which may of course be vulnerable to artefactual relationships caused by poor taxon sampling, long-branch attraction, or lack of fit of the characters to the model of evolutionary change used to infer the phylogeny, are there any reliable synapomorphic characters that link microsporidia to the Fungi?

There are three characters that solidly place microsporidia within the opisthokonts (animals, fungi, and their closest protist relatives). One is a 10-amino-acid insertion within the EF1A protein that is unique to opisthokonts and is also present within microsporidia (Baldauf et al. 1996; Kamaishi et al. 1996). The second is the presence of two separate genes for dihydrofolate reductase and thymidylate synthase (Duffieux et al. 1998), which are fused in non-opithokont lineages (Stechmann and Cavalier-Smith 2002). Lastly, microsporidia and other opisthokonts also share a related holoarchaeal-type tyrosyl-tRNA synthetase, presumably laterally transferred into the ancestral lineages that gave rise to animals and fungi (Huang et al. 2005). These place microsporidia within the opithokonts but not specifically within the Fungi.

More specifically, one character that has been used to place microsporidia specifically outside or at the base of the Fungi is a two amino acid deletion, again in EF1A (Tanabe et al. 2002, 2005). This deletion exits in all fungi where EF1A has

been sequenced, but generally not in animals, nor does it occur in any microsporidia studied so far. This would suggest that microsporidia be placed toward the base of the Fungi, if not outside the group. However this character will probably never be definitive because some entomophagous fungi and many chytrid and fungi, including the important genus *Rozella* (see below), have lost the conventional EF1A gene in favour of a distantly related member of the same GTPase family, named EF-like or EFL (James et al. 2006a; Keeling and Inagaki 2004). Without EF1A in these lineages, we cannot determine whether this deletion was present in the most basal fungal lineages, and therefore whether any of these lineages branched before or concurrently with microsporidia.

None of these molecular characters place microsporidia specifically within the Fungi, however there are some characteristics of microsporidia that are typically fungal, for example the presence of closed intranuclear mitosis and the presence of chitin in the cell wall. Furthermore several relatively conserved homeotic and TALE genes have been identified in the *E. cuniculi* genome (Burglin 2003). These are characteristically found in plants, animals and fungi, controlling polarity, morphology and mating types. Two of these genes are adjacent in the *E. cuniculi* genome, reminiscent of a mating-type loci found in other fungi (Fraser and Heitman 2004). This does open an intriguing question about why a mating-type locus would occur in *E. cuniculi*, as sex has never been documented in this species, though molecular markers for meiosis are present (Gill and Fast 2007).

In at least some zygomycete fungi, the mating type locus occurs as a syntenic cluster consisting of a high mobility group transcription factor flanked by a triose phosphate transporter and an RNA helicase. This gene cluster also occurs in both *E. cuniculi* and *E. bieneusi* and is partially conserved in *A. locustae* (Lee et al. 2008). In general the conservation of synteny between these microsporidia and zygomycetes is much higher than it is between microsporidia and other fungi (Lee et al. 2008). This provides a convincing character to place microsporidia within the Fungi and specifically suggests a relationship between microsporidia and zygomycetes.

This is interesting as re-analysis of α- and β-tubulin protein sequences with representation from several chytridiomycete, ascomycete, basidiomycete and zygomycete lineages also suggested some affinity to zygomycetes, and specifically a clade of entomophagous zygomycetes (Keeling 2003; Keeling et al. 2000). The analysis of tubulins is problematic, however, since evolutionary rates correlate to the presence of flagella. Both the microsporidia and most fungi lack flagella, and share long-branch tubulins, which could easily lead to an erroneous placement of microsporidia within such flagella-lacking fungi just as other genes erroneously placed microsporidia at the base of eukaryotes (Keeling 2003; Keeling et al. 2000). A concatenated phylogeny of eight protein-coding genes using a smaller taxon sample (but nevertheless including representatives of all four major fungal lineages) found microsporidia to branch at the base of the ascomycetes and basdiomycetes (Gill and Fast 2006). The authors of this study also suggest a possibility of long-branch attraction between the representative organisms within this clade and microsporidia, and that there are problems of taxon sampling as just a single representative for the chytrids was available for the eight-gene concatenation. More recently, the Fungal Tree of Life project attempted to resolve some of the major issues confounding fungal phylogenetics and classification by producing a multigene phylogeny with an unprecedented number of fungal lineages, including the microsporidia (James et al. 2006a). Here the microsporidia branch near the base of the fungal tree, and fascinatingly they form a clade with the parasite *Rozella allomycis* (James et al. 2006a). *Rozella* is not present in any other analysis including microsporidia, and indeed few genes have been sampled from both lineages, so this remains an intriguing avenue to follow.

This relationship between microsporidia and *Rozella* was proposed on the basis a phylogeny of two protein coding genes: RPB1 and RPB2. It is not well supported statistically, and several equally likely branching positions for microsporidia within the fungal tree of life were also proposed, one of which placed microsporidia basal to all fungi (James et al. 2006a). There are, however, a couple of conspicuous similarities between *R. allomycis* and microsporidia. Both are obligate intracellular parasites and therefore both require a mechanism for entering the host cell. In *Rozella* it is reported that the zoospore stage forms an attachment to the *Allomyces* host cell, which is followed by retraction of the flagellum, encystment,

proceeded by the formation of a germ tube. The parasite then injects its protoplast into the host cell, forced out of the cyst by a rapidly expanding vacuole (Held 1972). Of course, there is no evidence of a flagellum in microsporidia, or even any other 9+2 microtubular structures. However the need for an expanding vacuole is a somewhat reminiscent of the polar vacuole in microsporidia which forces out the sporoplasm (see above) and provides an interesting possible homology between these two unique fungal groups.

As with molecular data, there are few hints from morphology about the exact relationship between microsporidia and fungi. The relationship between microsporidia and entomophagous zygomycetes prompted some speculation that the microsporidian infection mechanism might be related to the mechanism used for zygospore dispersal in this group of insect parasites (Keeling 2003; Keeling et al. 2000), and interestingly a similar position among the Fungi was previously suggested based on possible homologies in cell biology and SSU ribosomal RNA phylogenies (Cavalier-Smith 2001). Cavalier-Smith (2001) hypothesized that there could be homology between the filamentous appendages in the infective trichospores of the harpellalean fungi and the polar tube of the microsporidia. In the spore stage of the harpellalean fungi, filamentous appendages develop and are coiled up within the cell. These are expelled as long filaments during germination (Lichtwar 1967). However their function is thought to be to allow entanglement of the fungus with the substrate and, although there are some morphological similarities to the microsporidian polar tubes, the two do not share functional similarity.

There are two main impediments to any attempt to clarify the relationship between microsporidia and fungi. First, data representing the full diversity of fungi and microsporidia are generally lacking (Bass et al. 2007; Gill and Fast 2006; James et al. 2006b; Keeling 2003). Even if the presently available phylogenetic markers were sufficient and the methods to extract phylogenetic signal from the data were adequate, we probably have not yet sampled some major fungal lineages and so may not yet have sampled from the fungal lineage most closely related to the microsporidia. Second, the microsporidia are so derived at all levels, from molecular sequences, to biochemistry, to morphology that it is possible that available markers and methods will not be sufficient to address the problem simply because their rapid adaptation and evolution have erased too much of their history. If this is the case, then one tack to take must be to identify the least divergent lineages of microsporidia. It is possible that early-diverging lineages of microsporidia were not subject to the same massive levels of change (similar to the recent discovery of an algal relative of apicomplexan parasites that retains many characters believed to be ancestral; Moore et al. 2008), and if so perhaps these lineages could be linked to their closest relatives more clearly. Further exploration of the diversity of both fungi and microsporidia is needed in order to know.

IV. Cellular and Genomic Reduction in the Microsporidia

Microsporidia were once regarded as ‘primitive’, but when they are re-evaluated as opithokonts, and perhaps fungi, we now see microsporidia as ‘reduced’ instead. Indeed, reduction and loss are two prevailing themes in just about every aspect of their biology. Reduction is seen in the simplification of many molecular systems (e.g., the ribosomes and ribosomal RNAs are both similar in size to those of prokaryotes rather than other eukaryotes), the absence of many components of some cellular and biochemical pathways, and the degeneration of organelles, such as the mitochondrion. Loss too is seen at all levels from the loss of genes, loss of introns, loss of complete biochemical pathways and perhaps even the complete loss of organelles (e.g., the peroxisome and flagella). It is interesting to ask whether this drastic reorganization of the cell and it molecular biology is really unique to the microsporidia, or are aspects of such reduction evident in other fungal lineages? In fact, many fungal lineages do exhibit many of the same characteristics that reduction and loss have generated in microsporidia, but the difference is that we do not see them altogether in one cell. Below, we will review some of the levels where microsporidian reduction is best studied and most severe, and we will compare a few such traits to corresponding characteristics of fungi.

A. Genome Reduction

Members of the microsporidia bear the smallest known eukaryotic nuclear genomes and are now model organisms for understanding genome compaction and reduction. The smallest documented microsporidian genome is 2.3 Mb and the largest estimated at 23 Mb, with a range of sizes in between (Belkorchia et al. 2008; Biderre et al. 1994; Méténier and Vivarès 2001). However, before the *E. cuniculi* genome was sequenced, *Saccharomyces cerevisiae* provided this model of genome compaction. Whereas *E. cuniculi* has a genome of just 2.9 Mb with an estimated 1997 protein coding genes (Katinka et al. 2001), baker's yeast has a 12-Mb genome with around 6000 genes (Dujon 1996): a proteome three times the size in a genome six times the size. In other fungi moderate genome reductions is also well known: the *Pneumocystis carinii* genome is just 6.5 Mb (Cushion and Smulian 2001) and the genome of the basiodiomycete *Malassezia* is around 9 Mb with 4285 genes (Xu et al. 2007). Although these are larger than the smallest microsporidian genome, they are within the range of microsporidian genome sizes, and with eukaryotic genomes ranging from 2.3 to 670000 Mb, they are also certainly at the small end of the spectrum in general. Moreover, smaller fungal genomes are still very likely to exist, given the number of species that have been investigated is a small proportion of what exists, so it is not inconceivable that a fungal genome smaller than the smallest microsporidian genome will be described. Overall, genome reduction appears to be a phenomenon that occurs in several lineages throughout the Fungi, though perhaps not the same extent as we see in the microsporidia, and perhaps not as consistently.

One of the notable traits of microsporidian genomes that arises as a consequence of this reduction is a high level of conservation of gene order between distantly related species.

This was initially observed between *A. locustae* and *E. cuniculi* and later between *A. locustae, E. cuniculi* and *Enterocytozoon bieneusi* (Corradi et al. 2007; Slamovits et al. 2004).

This is hypothesized to be a consequence of the difficulty in rearranging genes when intergenic spaces between genes are very small; the larger the gap between gene pairs the more likely they are to undergo a gene rearrangement event without disrupting a gene (Hurst et al. 2002; Slamovits et al. 2004). This is not unique to the microsporidia but also studied in yeast, which also have short distances between genes (Poyatos and Hurst 2007). However the number of shared syntenic genes between different species is exaggerated in the microsporidia where the spaces between genes are shortened to the extreme.

Microsporidia have also been shown to have a messy system of transcription, apparently owing again to the fact that intergenic spaces are small, in this case so small that promoters and termination signals can no longer be contained within them (Corradi et al. 2008; Williams et al. 2005). Again this system is not exclusive to microsporidia and is found in other fungi with instances of overlapping transcription being found in mRNA transcripts from *S. cerevisiae* (Hurowitz and Brown 2003), but as with genome rearrangements, the effect is exaggerated in the extremely reduced genomes of microsporidia.

1. Reduced Microsporidian Biochemistry and Molecular Biology

Microsporidia have lost many metabolic pathways and molecular systems compared to their free-living relatives. The most obvious are the loss of the electron transport chain and the Krebs cycle. There are no components of the heme biosynthesis pathway encoded in the *E. cuniculi* genome. In the absence of mitochondrial cytochromes there are fewer heme-containing proteins and it is possible that heme is scavenged from the host rather than being synthesized within the microsporidia (Vivarès et al. 2002). There are no fatty acid or cholesterol synthesis pathway components, also suggesting a reliance on the host (Vivarès et al. 2002).

There are also many pathways that are not completely lost, but merely downsized, and whittled down to a core set of proteins. One well described example is the various DNA repair systems that exist in eukaryotic cells, which are of course essential systems in cell maintenance.

Reviewing the extent of loss of components the major DNA repairs systems in *E. cuniculi* compared to *S. cerevisiae*, including non-homologous end joining, homologous recombination repair, mismatch repair

(MMR), nucleotide excision repair, base excision repair and methyltransferase repair, showed all pathways were reduced to some extent in microsporidia, but some components of each individual process were also retained, suggesting the retention of a minimal DNA repair system (Gill and Fast 2007).

The network of protein kinases involved in signaling processes within the cell seems to be similarly reduced. Here there is a bias towards signaling pathways involved in cell maintenance, whereas signaling processes involved in sensing environmental change are more pared down, probably reflecting the protective and stable intracellular in which the microsporidia reside (Miranda-Saavedra et al. 2007).

B. Simplified Microsporidian Cell Structure: Variations on the Eukaryotic Theme

Microsporidia were long thought to be an example of the primitive cellular state of the eukaryotic cell. They were suggested to be primitively lacking mitochondria, Golgi and peroxisomes. Of course, in the context of microsporidia being closely related to fungi it makes no sense for them to be primitively lacking any of these organelles; instead they have been lost or transformed, and here we will discuss their evolution.

The mitochondrion has attracted much attention, in part due to the Archezoa hypothesis described earlier. A homologous organelle is now known to exist in microsporidia, but it is so highly reduced it was only identified through the immunolocalisation of an endosymbiont-derived mitochondrial Hsp70 protein (Williams et al. 2002). The complete genome of *E. cuniculi* (Katinka et al. 2001) revealed that the mitochondrion, termed mitosome, had lost its genome, lost its electron transport chain and the Krebs cycle and was predicted to exist mainly to allow iron–sulfur cluster assembly for export and integration into cytosolic iron–sulfur proteins, which is an essential function of mitochondria in yeast cells. This conclusion was substantiated by work that has localized proteins involved in iron–sulfur cluster in both *E. cuniculi* and *Trachipleistophora hominis* (Goldberg et al. 2008; Williams et al. 2008b). It has also been shown that some *E. cuniculi* proteins involved in iron–sulfur cluster assembly can complement yeast homologs. Interestingly, however, some of these predicted mitochondrial proteins do not seem to be localized to the mitosomes in all species of microsporidia. Goldberg et al. (2008) show that, in *E. cuniculi*, both the iron–sulfur cluster proteins ISCU1p and cysteine desulfurase localize to a mitosome whereas, in *T. hominis*, they appear to be cytoplasmic. Similarly, in *E. cuniculi*, ferredoxin, a typically mitochondrial protein in yeast cells, has a punctate distribution, consistent with a mitosomal location whereas the mitochondrial glycerol-3-phoshate dehydrogenase appears to have a cytoplasmic distribution (Williams et al. 2008b), but there is evidence that this protein is more likely mitosomal in *A. locustae* (Burri et al. 2006). This may indicate that mitosomes in different species of microsporidia may differ and be in different states of degeneration. This is also is reflected in a different complement of mitochondrial gene homologs in different microsporidia (Burri et al. 2006; Williams et al. 2008a).

Various levels of mitochondrial reduction also occur in other fungal mitochondria. For example, mitochondrial genomes have been completely lost in the anaerobic chytrid fungi in the family Neocallimastigaceae. Here the mitochondria have been transformed into hydrogenosomes: mitochondrion-derived organelles that produce ATP via decarboxylation of pyruvate and generate hydrogen gas and, as in microsporidia, rely exclusively on proteins encoded in the nuclear genome (Yarlett et al. 1986; van der Giezen et al. 2002). Diverse yeast lineages, the so-called petite-positive yeast species, can also exist with a genome-less mitochondrion, and the ability to survive without mitochondrial DNA is a widespread trait across the yeasts (Fekete et al. 2007) and in some basiodiomycetes (Kuscera et al. 2000).

One specific factor that may have allowed the degeneration of the mitochondria in the microsporidia is the capacity of the parasite cell to import ATP from the host cell. This ability has known of for quite some time, and was identified through the measuring relative levels of ATP consumption in blue crabs, infected or uninfected with microsporidia (Weidner and Trager 1973). The genetic basis for this trait was identified in the *E. cuniculi* genome as a family of ATP/ADP translocases that otherwise exist in only in the eubacterial energy parasites of the *Rickettsia* and *Chlamydia* lineages, and in plastids where they import ATP into storage plastids to drive biosynthetic processes (Katinka et al. 2001; Neuhaus and

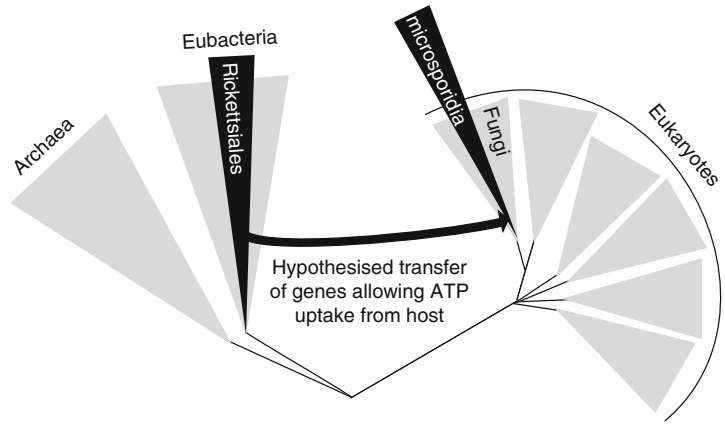

Fig. 2.2. Lateral transfer of the energy parasite ATP/ADP translocase. The ability to exploit host ATP may have been acquired by microsporidia through the lateral gene transfer of the gene encoding an ATP/ADP translocase from the lineage of proteobacteria containing *Rickettia* parasites (adapted from Richards et al. 2003)

Emes 2000; Tsaousis et al. 2008). Homologous genes have now been found in the genomes of *E. cuniculi*, *E. bieneusi*, *S. lophii* (Corradi et al. 2007; Hinkle et al. 1997; Katinka et al. 2001) and *A. locustae* (http://gmod.mbl.edu/perl/site/antonospora01). This gene is thought to have moved between lineages by lateral gene transfer (Tyra et al. 2007), and it appears possible that the microsporidia proteins may also have been acquired by lateral gene transfer, perhaps from either a rickettsial or chlamydial parasite (Fig. 2.2; Richards et al. 2003).

Another organelle of note that is absent from microsporidia is a classical stacked Golgi dictyostome. This is not to say there is no requirement for protein sorting in microsporidia; indeed, proteins inserted into the spore wall and into the polar tube appear to be trafficked much like secreted proteins in other eukaryotes. The microsporidian Golgi exists as a mass of vesicular tubules, which is present throughout the life cycle, but is particularly apparent during sporogony when the spore wall and polar tube are being formed.

Microsporidia genomes have a reduced complement of Rabs, SNAREs and vesicle coat proteins (Dacks and Field 2007; Katinka et al. 2001). Unlike a typical Golgi, the microsporidian Golgi is also suggested not to have COPI and COPII vesicles, and therefore require a direct connection somewhere between the ER and the Golgi (Beznoussenko et al. 2007). So again we see a typical eukaryotic system present in microsporidia, but very much pared down.

The peroxisome is an organelle that has not been seen in the microsporidian cell, though this may not be so surprising since peroxisomes are small organelles and might be easily overlooked in cells as small as those of microsporidia. However, a gene for one of one of the hallmark enzymes of the organelle, catalase, is definitely absent from the complete *E. cuniculi* genome. An alternative catalase is present in the genome of the insect parasite *A. locustae* (Fast et al. 2003), however this is a kind of catalase that is not normally localized to a peroxisome in other organisms, but rather can be secreted into the spore wall in certain fungi, possibly to protect the spore from an oxidative burst from the host (Paris et al. 2003). Furthermore it appears to be derived by lateral gene transfer from an, obviously, organelle lacking proteobacterium (Fast et al. 2003).

Catalase-like activity has been observed within the polar vacuole in the fish-infecting microsporidian *S. lophii*. In the presence of catalase, diaminobenzidine (DAB) reacts with H_2O_2 to form a dark precipitate that can be visualized with by electron microscopy. Such a reaction was observed around the polar vacuole in *S. lophii*, and based on this it was suggested that the polar vacuole is a highly derived peroxisome (Findley et al. 2005), though there is not yet any evidence for a gene encoding a peroxisomal catalase in microsporidia (Katinka et al. 2001). An alternative possibility is that this vacuole is related to the vacuoles of *S. cerevisiae* and other fungi, which are considered analogs of mammalian lysosomes (Weisman 2003). Similarly, in the infection strategy of the rice blast fungus *Magnaporthe grisea,* the vacuole plays a key role and acts as the site of breakdown of lipid reserves to create the high turgor pressure needed for the fungus to penetrate leaf surfaces (Weber et al. 2001).

V. Diversity of Microsporidia

Most of what we know of the molecular biology of microsporidia has been inferred from two species where extensive genomic data have been available for several years, *A. locustae* and *E. cuniculi*. While it is always tempting to generalize from model species, it is likely that these two species represent only a small fraction of the variability within microsporidia. Indeed, a genome sequence survey of two other species, *Brachiola algerae* and *Edhazardia aedis*, shows that the gene complement of these is likely similar to the better-studied *E. cuniculi* and *A. locustae*, but the overall architecture of these gene-sparse genome is completely different (Williams et al. 2008c). Now, two further draft genomes for *N. ceranae* and *E. bieneusi* are available (Akiyoshi et al. 2009; Cornman et al. 2009). In the case of *E. bieneusi*, it has demonstrated even further metabolic reduction than *E. cuniculi* with some key pathways, such as glycolysis and trehalose metabolism seemingly degenerated. Additional new genome projects for microsporidia with larger genomes are underway (e.g., Belkorchia et al. 2008; Xu et al. 2006) and as these data emerge they will undoubtedly reveal still more diversity within microsporidia. Given the large number of described species, we are likely to see a degree of variation in a wide range of different cellular characteristics, and perhaps we may even pinpoint characteristics that link the microsporidia to other fungi.

VI. Conclusion

The exact phylogenetic position of the microsporidia remains an open question: it is clear they are not a basal eukaryotic lineage and are somehow related to fungi. Current data is converging on the idea that that they are a true fungal group and potentially allied to a zygomycete lineage. However the discovery of their true sister group requires further analysis and more data.

The *E. cuniculi* genome not only provided a wealth of direct evidence for many conclusions on various aspects of microsporidian cell and molecular biology, but it also acted as a catalyst for other research questions, which are still transforming our view of many characteristics of this parasite. At the same time, however, greater sampling from across the taxonomic breadth of the group is and will continue to challenge generalizations which are based on *E. cuniculi* or any other single species; so one of the major lessons from recent microsporidian research is to expect the unexpected. Much of what we know about the biology of these parasites relates to their bending or breaking the 'rules' of biology, so perhaps it is not surprising that the rules of microsporidian biology are not easily predictable.

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