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Evolutionary Biology: Microsporidia Sex — A Missing Link to Fungi

The evolutionary origins of the microsporidia, a group of intracellular eukaryotic pathogens, have been unclear. Genome analysis of a sex locus and other gene clusters has now revealed conserved synteny with zygomycete fungi, indicating that microsporidia are true fungi descended from a zygomycete ancestor.

Paul S. Dyer

The microsporidia are an enigmatic group of organisms. Around 1,200 species are known, all of which are obligate, intracellular pathogens [1]. They infect a wide range of species from protozoa to invertebrate and vertebrate hosts. They are eukaryotic in nature, but have a number of unusual features, such as the lack of typical mitochondria, very compact genomes and the presence of a unique coiled organelle known as the 'polar tube' or 'polar filament' (Figure 1) [1–3]. The relatively small size of certain genes combined with rapid divergence and gene loss has hindered phylogenetic analysis. Consequently, the evolutionary origins and relatedness of the microsporidia to other eukaryotic groups has been difficult to resolve. As they report in this issue of *Current Biology*, Lee *et al.* [4] instead used a novel approach, based on analysis of genome structure, to investigate genetic relatedness: their results have revealed conservation of gene synteny with zygomycete fungi at an ancestral sex locus and over 30 other gene clusters, thereby providing significant insights into the placement of the microsporidia within the fungal kingdom and the broader evolutionary biology of these pathogens.

The microsporidia have been considered as ancient 'primordial' eukaryotes because of features such as the apparent absence of mitochondria, a reduced metabolic capacity, and relatively simple cellular organization [1]. Indeed, many texts and websites still refer to the microsporidia as primitive protozoa. Recent investigations, however, have found that all is not quite what it seems. Such research has been prompted in part by the growing medical importance of the microsporidia. The incidence of infections has risen considerably since the mid-1970s as a result of a rise in number of susceptible patients, with the emergence of AIDS and the use of immunosuppressant drugs. At least 13 species of microsporidia have been reported as human pathogens, causing a diversity of diseases affecting the digestive, urinary, respiratory and nervous systems [1].

The first key insight came from indications that mitochondria had been present in ancestors of microsporidia, with relic mitochondrial genes and remnant mitosomes being detected in extant microsporidia [2,5,6]. The second insight came from genome sequencing, which revealed surprisingly small genome sizes — most likely arising from gene loss and compaction [2,3]. Both of these observations suggested that the microsporidia were in fact descended from more complex eukaryotic ancestors. A third insight came from phylogenetic analysis of an increased number of genes, which indicated that microsporidia are closely allied with the fungal kingdom, either as one of the earliest diverging

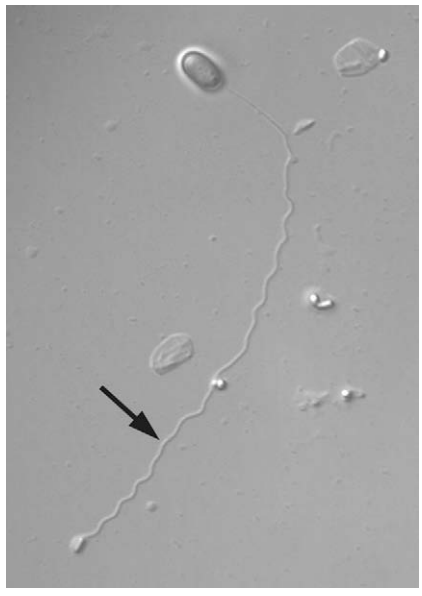

Figure 1. Spore and polar tube of *Antonospora locustae.*

DIC micrograph showing ejected polar tube (arrowed) and trailing spore (top left). The polar tube is discharged very rapidly and can pierce the membrane of potential host cells, thereby enabling infection [1]. (Photo courtesy of Patrick Keeling, University of British Columbia.)

branches or as a sister group [7,8]. But the exact evolutionary relationships of microsporidia to other fungi has been unclear [8].

Lee *et al.* [4] investigated whether conservation of genome structure could provide clues as to the closest fungal relatives of microsporidia. They drew on annotated genomes and experimentally derived sequence data from three distantly related microsporidia: *Encephalitozoon cuniculi*, a human pathogen and major cause of disease in rabbits; *Enterocytozoon bieneusithe*, a human pathogen causing intestinal infections and diarrhoea; and *Antonospora* (*Nosema*) *locustae*, an agent used for biological control of insect pests.

First they looked for the presence of so-called mating-type '*MAT*' genes, which regulate sexual reproduction in fungi [9–12]. These *MAT* genes, also termed '*sex*' genes [13], encode proteins that act as transcription factors, including high-mobility group (HMG), alpha-domain and homeodomain-type proteins [9]. In heterothallic (obligate outcrossing) species of fungi, the *MAT* genes are found at a characteristic 'mating-type' (*MAT*) locus [10–12]. This region, also termed the '*sex*' locus [13], is often bordered by one or two unrelated genes showing cross-species conservation of gene order — that is, synteny with the *MAT* genes — between related orders of fungi. This synteny is lost as more distantly related groups of fungi are compared. In the more highly evolved ascomycete fungi, the *MAT* locus, containing HMG or alpha-domain type genes [14,15], is bordered by DNA lyase and anaphase-promoting complex genes [14]. In contrast, in the 'lower' zygomycete fungi, the *sex* locus, containing solely HMG-type genes, is bordered by triose phosphate transporter (TPT) and RNA helicase genes [13]. When Lee *et al.* [4] examined the three microsporidia species, they found that all had a TPT gene present directly upstream of an HMG-type *MAT*/*sex* gene, and that two species, *E. cuniculi* and *E. bieneusithe*, had an RNA helicase gene downstream of the HMG gene — thus, the genome arrangement of the microsporidia directly resembled that of the *sex* locus in zygomycete fungi (Figure 2).

Second, an examination was made on a more genome-wide scale of possible conservation of gene order between fungi and the microsporidia. This revealed over 30 instances of syntenic gene pairs between the test microsporidial species and the zygomycete fungi *Phycomyces blakesleeanus* and *Rhizopus oryzae*, far more than would be predicted from randomized gene ordering [4]. Conservation of these gene pairings was limited almost exclusively to between microsporidia and zygomycete fungi; the synteny of only one pair of genes was conserved across all fungi. This was of importance in demonstrating that microsporidia and zygomycete fungi share a more recent common ancestor with one another than with any other fungal group [4].

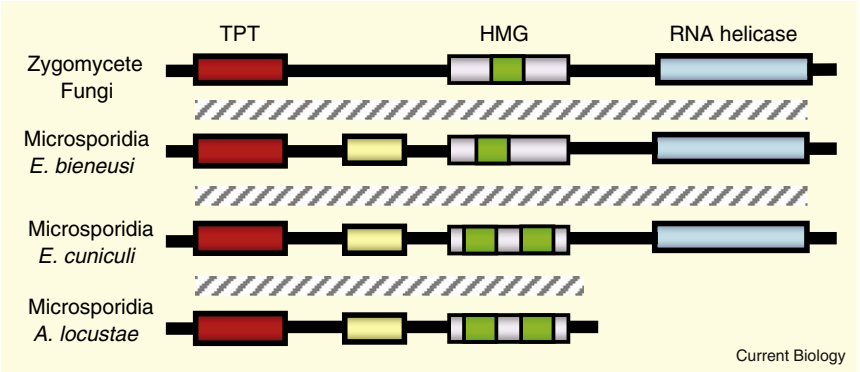

Figure 2. Genome arrangement of *sex* locus in microsporidia and zygomycete fungi.
Red box indicates triose phosphate transporter (TPT), yellow box indicates hypothetical protein, green box indicates HMG-encoding domain(s), blue box indicates RNA helicase gene. Hatched area indicates regions of conserved gene synteny. Gene orientation varies between the groups [4].

These results are of direct relevance to the central question of the phylogeny of the microsporidia, because they provide key 'missing link' evidence that the microsporidia are indeed true fungi, as shown by their genomic affinity with the basal zygomycete phylum of fungi. Furthermore, microsporidia can now be viewed as highly evolved life forms, exhibiting genome compaction linked to adoption of a parasitic intracellular life cycle, being derived from a common ancestor with the zygomycete fungi [4].

More broad observations may also be made from the work. The detection of a conserved *sex* locus will allow further investigations into the genetic basis of sexual compatibility in the microsporidia. It is intriguing that the HMG *sex* proteins of both *E. cuniculi* and *A. locustae* contained two distinct HMG domains, with possible implications for self-fertility (Figure 2) [4]. Many microsporidia lack a known sexual cycle [16], but detection of HMG *sex* gene(s) might suggest the capacity for sexual reproduction, in parallel with other 'asexual' fungi [17]. The reduced genome size of many microsporidia relative to other fungi can now be reconciled by the explanation that the intracellular parasitic habit has allowed (exerted?) considerable gene loss and specialization, an excellent paradigm for the effects of lifestyle on genome evolution. Recent sequencing projects have also revealed ways in which the genomes of symbiotic fungi appear to have adapted as a result of interactions with a host [18,19]. Finally, the presence of HMG genes within the putative *sex* locus of the microsporidia provides further evidence that HMG proteins are ancestral sex determinants in fungi [11–13].

The inclusion of the microsporidia as fungi is a welcome outcome to fungal biologists. This follows on from the 'loss' of the oomycetes as fungi, which phylogenetic analyses have shown to be derived from chromistan algae [20]. The oomycetes include the potato blight pathogen *Phytophthora infestans*, ironically often cited as the exemplar of plant fungal disease. Thus, molecular and genomic analyses can both give and take.

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Intercellular Junctions: Actin the PARt

Several distinct polarity complexes participate in the assembly of intercellular junctions. Two studies showing that some of the same polarity complexes are essential regulators of continued junctional integrity lead to a new appreciation of the relationships between assembly and maintenance of intercellular junctions and highlight unappreciated roles for endocytosis in these processes.

Le Shen and Jerrold R. Turner*

Specialized functions of complex organisms require compartmentalization. Both internal compartments and the external surface of the organism are covered by epithelial sheets that establish and maintain barriers while, in some cases, allowing polarized transport between adjacent compartments. Thus, the abilities of epithelial cells to stably interact with one another, seal the space between adjacent cells, and generate distinct plasma-membrane domains are essential for the survival of complex organisms. These fundamental processes are linked, as assembly of the apical junctional complex is a requisite step in apico-basal epithelial polarization.

Assembly of the adherens and tight junctions, which, in mammals, form the apical junctional complex that defines the apico-basolateral boundary, is a highly dynamic process that has been the subject of great scrutiny [1,2]. More recently, there has been a growing appreciation of the dynamic behavior of assembled intercellular junctions [3–5], although this has largely been considered to be independent of the signals that direct initial polarization and junctional assembly. Contrary to that hypothesis, a pair of studies published in this issue of *Current Biology* [6,7] now demonstrate that the polarity complex comprising Cdc42, Par6, and atypical protein kinase C (aPKC) as well as the effector pathways downstream of this complex play essential roles in adherens junction maintenance.

The development of epithelial polarity requires the participation of Partitioning Defective (PAR) proteins, which are conserved in *Caenorhabditis elegans*, *Drosophila*, and mammalian cells. In particular, three proteins, Par3 (also known as Bazooka in *Drosophila*), Par6, and atypical PKC (aPKC), are necessary for epithelial morphogenesis and polarization. However, Par3, Par6, and aPKC are not sufficient for polarization to occur and require input from additional polarity pathways [8]. Moreover, the cytoskeleton plays a critical role in epithelial polarization and the correct localization of Par3 requires cues from both microtubules and the perijunctional microfilament ring [9].

Although relatively little is known of the mechanisms that direct perijunctional microfilament organization, studies in *Drosophila* have implicated a protein termed Bitesize in this process [10]. In the absence of Bitesize, the adherens junction is unstable, probably due to defective perijunctional microfilament organization; the adherens junction protein E-cadherin is initially recruited normally, but then fails to be retained at the adherens junction. While the mechanisms of E-cadherin removal following depletion of Bitesize in *Drosophila* are not known, studies in mammalian systems have demonstrated that actin reorganization induced by small GTPases, such as Rac and Cdc42, results in clathrin-mediated E-cadherin endocytosis [5,11].

To better define the mechanisms that participate in the maintenance of adherens junctions, Georgiou *et al.* [6] and Leibfried *et al.* [7] initially studied the effects of somatic Cdc42 mutation on adherens junction organization and E-cadherin localization in the