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More than one way to build a flagellum: comparative genomics of parasitic protozoa

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The membrane bound, microtubular axonemes of eukaryotic cilia and flagella are assembled at their distal tips. The discovery of intraflagellar transport (IFT) in *Chlamydomonas* provided an exciting and seemingly unifying framework for the formation of all eukaryotic cilia and flagella [1–3]. IFT operates in the space between the axoneme and the flagellar membrane to move particles from and to the cytoplasmic basal body and the assembly at the distal tip site. Anterograde movement is conducted via a kinesin II microtubule motor and retrograde movement via a specific IFT dynein. The particle cargo is likely to be a variety of proteins involved in axoneme structure and assembly and there is evidence that the basal body transitional fibres, which link the basal body to the membrane, act as docking sites for IFT particles [2,4]. The IFT system has been widely conserved in evolution and IFT mutations produce particular and medically relevant pathologies [3,5].

Recently the genomes of a number of parasitic protozoa have been sequenced. These organisms exhibit interesting variations in flagellum biology [6]. We employed the publicly available IFT protein sequences in bioinformatic interrogations for the signature of the IFT system in these genomes. We included flagellates, such as *Giardia,* kinetoplastid parasites (causing sleeping sickness, leishmaniasis and Chagas disease), and a range of apicomplexan parasites (such as those causing malaria, toxoplasmosis and coccidiosis).

We detected genes encoding the IFT particle proteins, the kinesin II and IFT dynein motors in the genome of *Trypanosoma brucei* and two other kinetoplastid parasites, *Leishmania major* and *T. cruzi.* We also detected IFT particle genes in *Giardia*. However, we noted the apparent complete absence of IFT genes in the completed genome of the malarial parasite *Plasmodium falciparum.* This apicomplexan produces a flagellated microgamete and might thus be expected to possess at least some IFT components [6]. We, therefore, analysed all available apicomplexan parasite genomes for the presence of IFT genes.

Using homology searches with full-length IFT protein sequences and short, highly conserved regions we reiteratively interrogated the genomes of *Eimeria*, *Toxoplasma, Plasmodium*, *Cryptosporidium* and *Theileria.* We identified IFT particle and the IFT kinesin II sequences in the partially sequenced *Toxoplasma* genome and found an IFT signature in *Eimeria.* However, there were no IFT genes in the genomes of the malarial parasites *P. falciparum, P. vivax, P. knowlesi, P. yoelii , P. chadaudi, P. berghei, and P. reichenowi*, nor in *Cryptosporidium* or two *Theileria* species. As a control, we searched for conserved cytoskeletal proteins, including δ-tubulin [7] and PF16 [8], which are characteristic of basal bodies and axonemes, respectively. Apicomplexan genomes contain α- and γ-tubulin, but PF16 and δ-tubulin are not present in *Cryptosporidium* or the two *Theileria* species. However, all were found in *Plasmodium, Toxoplasma* and *Eimeria*.

Our results reveal an intriguing evolutionary distribution of IFT components (Figure 1). An IFT cohort is found in the flagellates, *Trypanosoma* and *Giardia* and we found the footprint of an IFT signature in *Toxoplasma* and Eimeria, both of which have flagellated microgametes [6]. The absence of the IFT signature in *Cryptosporidium* and two *Theileria* species correlates with the fact that these organisms do not produce flagellated gametes nor possess centrioles.

Malaria parasites do not possess the IFT system, but they do build a flagellum. *Plasmodium* species produce flagellated microgametes in the mosquito. Basal bodies/centrioles, which are not found in the somatic stages, are formed and nucleate axonemes which are assembled in the cytoplasm [9]. Thus, although the parasite's ability to form a basal body and an axoneme is exemplified in its possession of δ-tubulin and PF16, flagellum formation cannot be IFT dependent. Our conclusions fit well with observations in *Drosophila* where mutations in kinesin II or the *Drosophila* IFT88 homologue NOMPB yield normal sperm flagella, but abnormal sensory cilia [10,11]. It has been conjectured that unusual features of *Drosophila* sperm may account for this lack of reliance on IFT [10,12]. For instance, the flagella are very long and form surrounded by cytoplasm rather than in direct contact with the plasma membrane. The *Plasmodium* flagellum is not unusually long, but intriguingly, *Plasmodium* microgamete flagella are also assembled in the cytoplasm. A further connection may be that *Drosophila* sperm and *Plasmodium* axonemes are nucleated on a single, instead of a paired, basal body. The single basal body may indicate a "primed/capped" state, which is able to nucleate assembly with alacrity in a cytoplasmic environment. Interestingly, in a recent comparative genomic analysis, the IFT genes were contained within a cohort of genes categorised as likely to be involved in "compartmentalised ciliogenesis" [13]. Hence, there are at least two ways of constructing a eukaryotic flagellum.

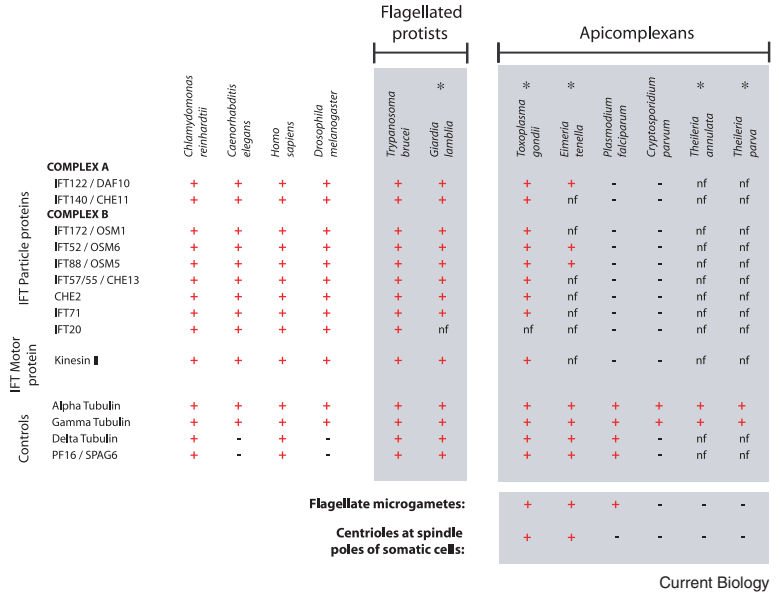

Figure 1. The evolutionary distribution of the intraflagellar transport (IFT) system. Candidate homologues of IFT proteins and controls were identified by a combination of BLAST and motif searches. True orthology was established by a detailed examination of candidate sequences, including reciprocal BLAST. Parasites for which the available genome sequence is incomplete (not formally published) are marked with an asterisk (*). 'nf' indicates that in these organisms the respective genes were not found.

The basal body transition fibres/membrane zone is envisaged as a docking or organising site for IFT particles. In this context, the biology of *Giardia* flagella is particularly intriguing. This parasite's flagella run, from a cluster of basal bodies, for a considerable distance through the cytoplasm before exiting the cell [14]. This may suggest an IFT independent, cytoplasmic mode of flagellum morphogenesis, but *Giardia* has an IFT system. Could two mechanisms operate in one cell? Also, do IFT particles dock at the basal body and run across the cytoplasm, or is there another docking option at the point of emergence? Further comparisons of flagellar biology and the evolutionary pattern of IFT gene occurrence/absence are likely to provide clarification of the diverse ways that eukaryotes build their flagella.

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