Swimming with protists: perception, motility and flagellum assembly

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Abstract | In unicellular and multicellular eukaryotes, fast cell motility and rapid movement of material over cell surfaces are often mediated by ciliary or flagellar beating. The conserved defining structure in most motile cilia and flagella is the '9+2' microtubule axoneme. Our general understanding of flagellum assembly and the regulation of flagellar motility has been led by results from seminal studies of flagellate protozoa and algae. Here we review recent work relating to various aspects of protist physiology and cell biology. In particular, we discuss energy metabolism in eukaryotic flagella, modifications to the canonical assembly pathway and flagellum function in parasite virulence.

Protists
Eukaryotes that cannot be classified as animals, fungi or plants. The kingdom Protista includes protozoa and algae.

Ciliates
A ubiquitous group of protists, members of which can be found in many wet environments. Ciliates are characterized by the hair-like covering of the cell body by hundreds of short cilia. Ciliary movement contributes to movement, cytokinesis and predation on other microbes.

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The canonical '9+2' microtubule axoneme is the principal feature of many motile cilia and flagella and is one of the most iconic structures in cell biology. The origin of the eukaryotic flagellum and cilium is ancient and predates the radiation, over 800 million years ago, of the lineages that gave rise to extant eukaryotes[1]. Therefore the absence of cilia and flagella from yeast, many fungi, red algae and higher plants are all examples of secondary loss.

In animals and microorganisms that have retained the ability to assemble a cilium or flagellum, the motility provided by these organelles (BOX 1) often plays a crucial role in survival, development, cell feeding and reproduction. Some protists, such as ciliates and the African trypanosome *Trypanosoma brucei*, always exist in a flagellate form, and for these protists flagellum assembly and motility are integral to cell morphogenesis and division. In other protists, such as the biflagellate green alga *Chlamydomonas reinhardtii*, the interchangeability of the flagellar basal body and the mitotic centriole is evident[2,3]. The importance of flagellar biology in sexual development is nicely illustrated in apicomplexan parasites[4]. For some apicomplexans (such as *Plasmodium* spp. and *Toxoplasma gondii*), but interestingly not for others (such as *Theileria* spp. and *Cryptosporidium hominis*), the sexual stage of the parasite life cycle requires *de novo* formation of flagella by male gametes. In these species, as well as in many other microorganisms, it is environmental changes that provide the cue for flagellum formation.

Cilium formation is essential for mouse embryonic development[5], and defects in ciliogenesis or ciliary function in humans are associated with a broad range of inherited pathologies (or ciliopathies), including infertility, chronic respiratory disease, polycystic kidney disease and syndromes that include Bardet–Biedl, Alstrom and Meckel syndrome[6–12]. Even illnesses such as cancer, diabetes and obesity have been linked to ciliary defects[6]. The biological basis for some ciliopathies is undoubtedly defective sensing (an essential function provided by cilia), rather than defects in the assembly process or motility. Yet understanding the role that defective ciliogenesis or ciliary function plays in the cell biology of complex human diseases is challenging, not least because it is difficult to propagate and maintain ciliated tissues and cells in culture. Many protist flagellates, by contrast, are more experimentally tractable, and for some (for example, *C. reinhardtii*, trypanosomes and the ciliates *Paramecium tetraurelia* and *Tetrahymena thermophila* (FIG. 1a–f)) key biological questions can be addressed using either reverse or forward genetics. In particular, stable transformation of *C. reinhardtii* and *T. brucei* to produce mutant cells in which target genes are subjected to either constitutive or inducible RNA interference (RNAi) provides a powerful approach to study gene function. Thus, using protists as model systems enables microbiologists to study aspects of flagellum function and assembly that are widely conserved. Such studies can be informative with regard to the study of human ciliopathies.

In this Review we consider new discoveries regarding flagellum assembly and function that pertain to various aspects of protist cell biology and physiology. We first describe the key features of a typical '9+2' axoneme, and then briefly discuss some variations that exist in protists. We will then focus on four topics: protist flagella as

Centriole
A barrel-shaped organelle that contains nine triplet-microtubules and that forms the basal body from which a flagellar axoneme is extended. In some eukaryotes, centrioles are required for mitosis. In *Chlamydomonas reinhardtii*, flagellar basal bodies are uncoupled from their associated axonemes to function as mitotic centrioles during division.

Apicomplexa
A phylum of obligate intracellular parasites that includes several important human pathogens, such as the malarial parasite *Plasmodium falciparum*, the opportunistic pathogen *Toxoplasma gondii* and the water-borne parasite *Cryptosporidium hominis*.

RNAi
A commonly used experimental tool to silence genes. In *Trypanosoma brucei*, RNAi is a very tractable reverse genetic approach for studying gene function. Less robust and often unstable RNAi systems are also available for motility studies in *Chlamydomonas reinhardtii*.

Reynolds number
A measure of the ratio of inertial forces to viscous forces that is used in fluid mechanics to quantify the relative importance of a type of force in a given flow condition.

Dyneins
Complex motor proteins that use the energy released from ATP hydrolysis to move towards the minus end of microtubules.

Diatoms
A major group of marine algae. Centric diatoms produce flagellate gametes.

Protozoa
Unicellular eukaryotes that do not possess the chitinous cell wall that is found in fungi. Protozoa are ubiquitous in aquatic and soil environments, where they make key ecological contributions. Several well-studied protozoa are parasites of medical, veterinary or agricultural significance.

Box 1 | Ciliary and flagellar beating

For swimming microorganisms and flagellate sperm, fluid viscosity, rather than inertia, is the dominant force affecting productive locomotion — that is, these cells swim at low Reynolds numbers[149]. For the purpose of this review, the differences in the definitions between flagella and cilia will not be considered. Many flagellate cells readily switch during swimming from using ciliary to flagellar waveforms, and *vice versa*[124,136,150]. However, the use of the term cilium perhaps helps to avoid confusion with bacterial flagella, which are structurally completely different organelles.

In motile axonemes, formation of transient bridges between dynein arms on outer-doublet A-tubules with the B-tubule of the adjacent outer-doublet microtubule results in microtubule sliding[15]. Sliding is constrained, however, due to other attachments, such as nexin links between adjacent outer-doublets, which convert applied force into bending. Ciliary style motility is characterized by asymmetric waveforms: an effective stroke provides thrust; the accompanying recovery stroke returns the cilium to its starting position with limited viscous resistance. For flagellates with small numbers of flagella (for example, 1, 2 or 4), ciliary waveforms are typically essentially planar. For organisms such as *Paramecium* or in ciliated animal tissues, in which fluids move rapidly across cell surfaces (such as, across the respiratory epithelium), energetically efficient[151] coordination of many closely opposed cilia (metachronal patterning) uses three-dimensional waveforms. In contrast to ciliary waveforms, a flagellar waveform is propagated with a flutter-kick-style motion. The phototactic behaviour of the flagellate alga *Chlamydomonas reinhardtii* in response to changing light intensity provides a classic paradigm for understanding how waveform changes are signalled[123,124]: base-to-tip ciliary beating propels *C. reinhardtii* forward, but a sudden increase in light intensity results in a brief Ca^{2+}-signalled switch to a planar symmetrical flagellar waveform, which mediates backwards swimming. By contrast, the characteristic symmetrical flagellar waveform in trypanosomatids is occasionally punctuated by wave-reversal and by the generation of a ciliary type beat[136].

'metabolic organelles'; diverse aspects of flagellum assembly and maintenance; protist flagella as sensory organelles; and exploiting motility perturbation as a novel drug target against the parasite that causes African sleeping sickness. Wider comparisons with animal cell biology are made when possible.

Flagellum structure and composition

The first description of axoneme structure was made by Manton and Clarke[13], and principal features that are evident in transverse sections through most '9+2' microtubule axonemes are highlighted in FIG. 1g. Notable features include the nexin links between outer doublet microtubules, inner- and outer-arm dynein ATPases, and radial spokes, which project from the A-tubule of each outer doublet microtubule towards the array of proteins and multiprotein complexes (or projections) that are found on the singlet central pair (CP) microtubules. However, our view of axoneme structure has recently been dramatically refined with three-dimensional reconstructions of flagellar dynein[14] and axonemes[15–18] using cryo-electron microscopy and tomography, respectively. From these studies physical evidence is emerging for the coordinate regulation of dyneins by a 'hard-wired circuit' (FIG. 2a); intriguingly, biochemical studies with *C. reinhardtii* have identified a candidate component of the outer–inner dynein arm link[19]. Detailed discussion of how ciliary and flagellar waveforms are, or might be, regulated is beyond the scope of this Review; discussions of these topics are available in various reviews[20–24] and other publications[25–29].

Although the '9+2' axoneme is an iconic structure, several variations in this structure have been described (FIG. 1h–k). Even in *C. reinhardtii*, historically the best studied flagellate, dynein arms are absent from outer-doublet 1, and instead the A-tubule of doublet 1 is linked to the B-tubule of doublet 2 in both flagella by two permanent bridges[30] (FIG. 1h,i). More extreme examples of natural variation include the '6+0' axoneme of male gametes of the Gregarine apicomplexan parasite *Lecudina tuzetae*[31] and the '9+0' axonemes that are assembled by male centric diatom gametes. The helical motility of such axonemes is independent of a CP–radial spoke regulatory system and is different from the waveforms that are generally associated with '9+2' axonemes. Interestingly, recent comparative genomic analyses have revealed that in the centric diatom *Thalassiosira pseudonana* gamete motility occurs in the absence of known inner-dynein-arm homologues, as well as in the absence of CP–radial spoke apparatuses[32,33].

The extent of architectural diversity is illustrated further with the observation that in some cell types and species the axoneme is buttressed by the presence of additional structures[34,35]. In the protozoa, the paraflagellar rod (PFR), which is found in many trypanosomatid parasites, is an easily identifiable and particularly elegant extra-axonemal structure[34]. The PFR is physically attached to the axoneme, usually at the point where the flagellum exits its flagellar pocket to emerge onto or beyond the cell body, providing a distinctive asymmetry to the flagellar ultrastructure (FIG. 1j,k). Failure to fully assemble the PFR in procyclic (the tsetse fly form) *T. brucei* or promastigote *Leishmania mexicana* results in non-productive motility, although the flagellum can still beat rudimentarily[36–40]. In pathogenic bloodstream forms of *T. brucei*, however, an inability to fully assemble the PFR results in a catastrophic failure of cytokinesis and is lethal[37,41]. Similar cytokinesis defects have been observed following RNAi-mediated ablation of other flagellar proteins in bloodstream trypanosomes[37,42], suggesting that inhibition of flagellar motility could constitute a novel target for designing new drugs to treat the sleeping sickness disease that is caused by African trypanosomes (see below). Thus, the phenotypes of the various 'PFR mutants' have been informative, but key questions remain, and these are most readily addressed through the application of molecular approaches. For instance, is the requirement for a PFR in trypanosome motility merely a consequence of the rod's mechano-structural properties or does PFR provide a platform

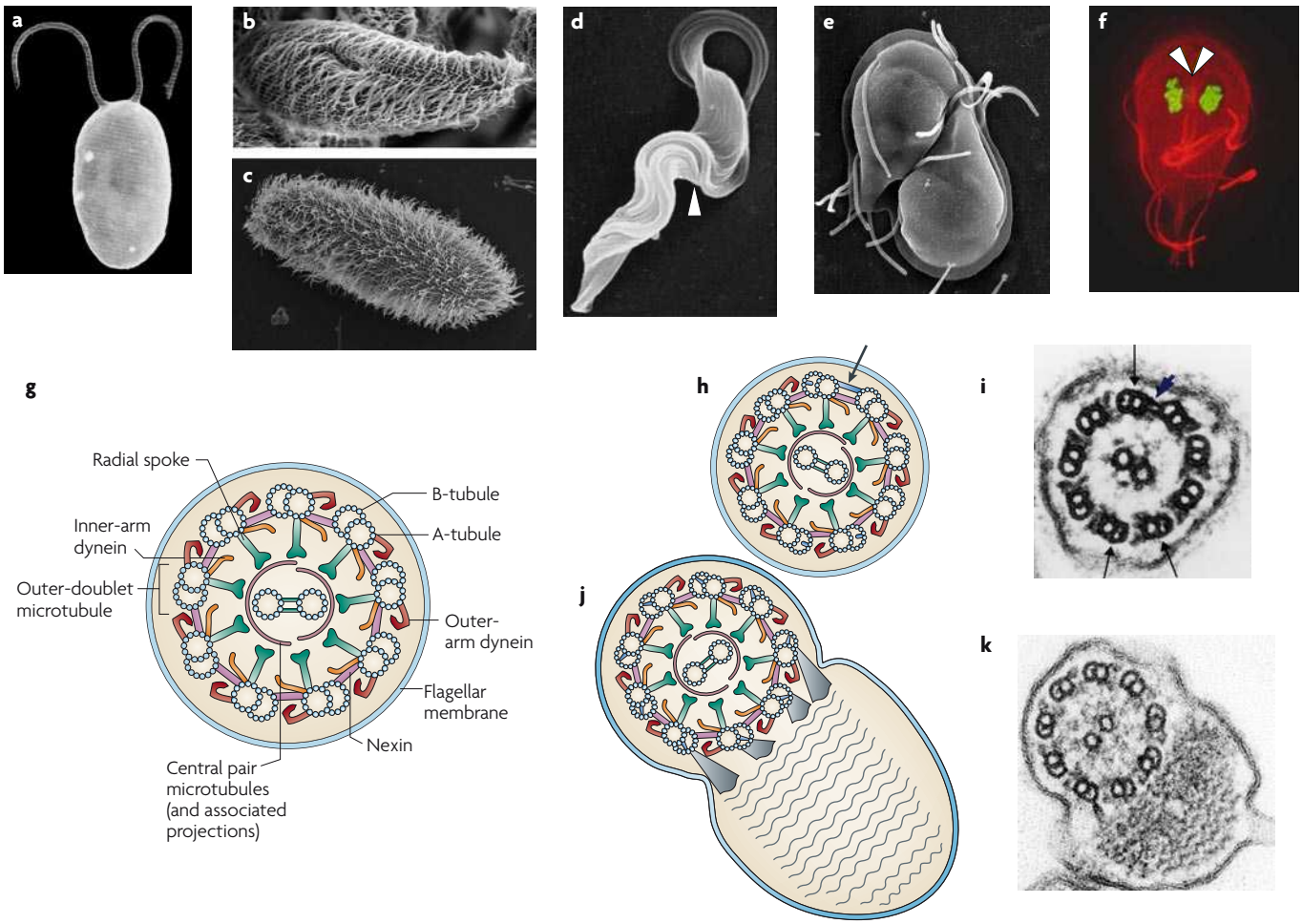


Figure 1 | **Flagellate diversity.** Morphology of: (**a**) *Chlamydomonas reinhardtii*; (**b**,**c**) *Paramecium tetraurelia*, (**b**) ventral and (**c**) dorsal views (the oral groove is evident in panel **b**); (**d**) *Trypanosoma brucei* bloodstream trypomastigote, illustrating elongation of the new flagellum (arrowhead) alongside the old during the cell-division cycle; (**e**,**f**) *Giardia lamblia*, which coordinates duplication and segregation of four flagella pairs during its cell-division cycle ((**f**) anti-tubulin staining indicates that flagella basal bodies (arrowheads) are positioned between the two nuclei (green) during interphase). Panel **g** shows a cartoon representation of a transverse section through a flagellum that contains the canonical '9+2' axoneme (diameter ~150 nm), viewed as looking towards the distal end of the flagellum. Panels **h**–**k** show common variations of the '9+2' axoneme: (**h**,**i**) in *C. reinhardtii*, doublets 1 and 2 are linked by a permanent bridge (arrow); (**j**,**k**) in trypanosomatids (and some related protists) a paraflagellar rod is attached along the length of the axoneme. Subtle elaborations include the presence of structures in the lumen of (**i**) some or (**k**) all B-tubules. Panel **a** reproduced with permission from REF. 89 © 2002 Nature Publishing Group. Panels **b** and **c** courtesy of: P. Dupuis-Williams and C. Fisch, INSERM, France; T. Blisnick, Institut Pasteur, France; F. Grillon, ENSMP, France. Panel **d** courtesy of S. Griffiths, University of Oxford, UK. Panels **e** and **f** courtesy of: J.J. Mancuso, W.Z. Cande, University of California, Berkeley, USA; S.C. Dawson, University of California, Davis, USA. Panel **i** reproduced with permission from REF. 30 © 1983 Rockefeller University Press.

Trypanosomatids
A family of flagellate parasites that includes monogenetic parasites of insects and digenetic parasites that are transmitted between mammalian or plant hosts by an invertebrate vector. Digenetic family members include the African sleeping sickness parasite *Trypanosoma brucei,* Chagas' disease parasite *Trypanosoma cruzi* and pathogenic *Leishmania* spp.

Procyclic trypomastigote
A morphological form of *Trypanosoma brucei* that migrates from the mid-gut of the tsetse fly vector. Like bloodstream trypanosomes, procyclic cells can be grown and genetically manipulated in culture. In both bloodstream and procyclic trypomastigotes, the flagellum emerges from the posterior end of the cell, is elongated in the direction of the anterior cell end and is attached along the length of the cell body.

onto which crucial proteins are assembled[43–45]? The two possibilities may be inextricably linked.

Before 2004, component complexity of the eukaryotic flagellum was only evident from classic two-dimensional gel electrophoreses of the type that were initially performed by Piperno, Luck and co-workers over 30 years ago[46]. With the application of comparative genomics and more recently with mass-spectrometry and proteomics, over 500 different candidate proteins that are required for flagellum construction and function have been identified[32,37,47–54]. These analyses also identified flagellar proteins that had previously been identified through classical biochemical studies with flagella, and although functional analyses support the flagellar candidature of some newly described proteins, the functions of the majority of the identified proteins remain unknown. Many candidate and *bona fide* flagellar proteins are flagellate specific (that is, homologues are not found in eukaryotes that cannot build flagella or prokaryotes), and there are numerous examples of proteins in these proteomes that probably function in regulatory signal-transduction cascades. Surprisingly, however, comparisons between protists reveals that numerous flagellar proteins are probably 'lineage specific' or at least fast-evolving, such that orthologues in other flagellates cannot be readily identified in sequence comparisons[37,52,55].

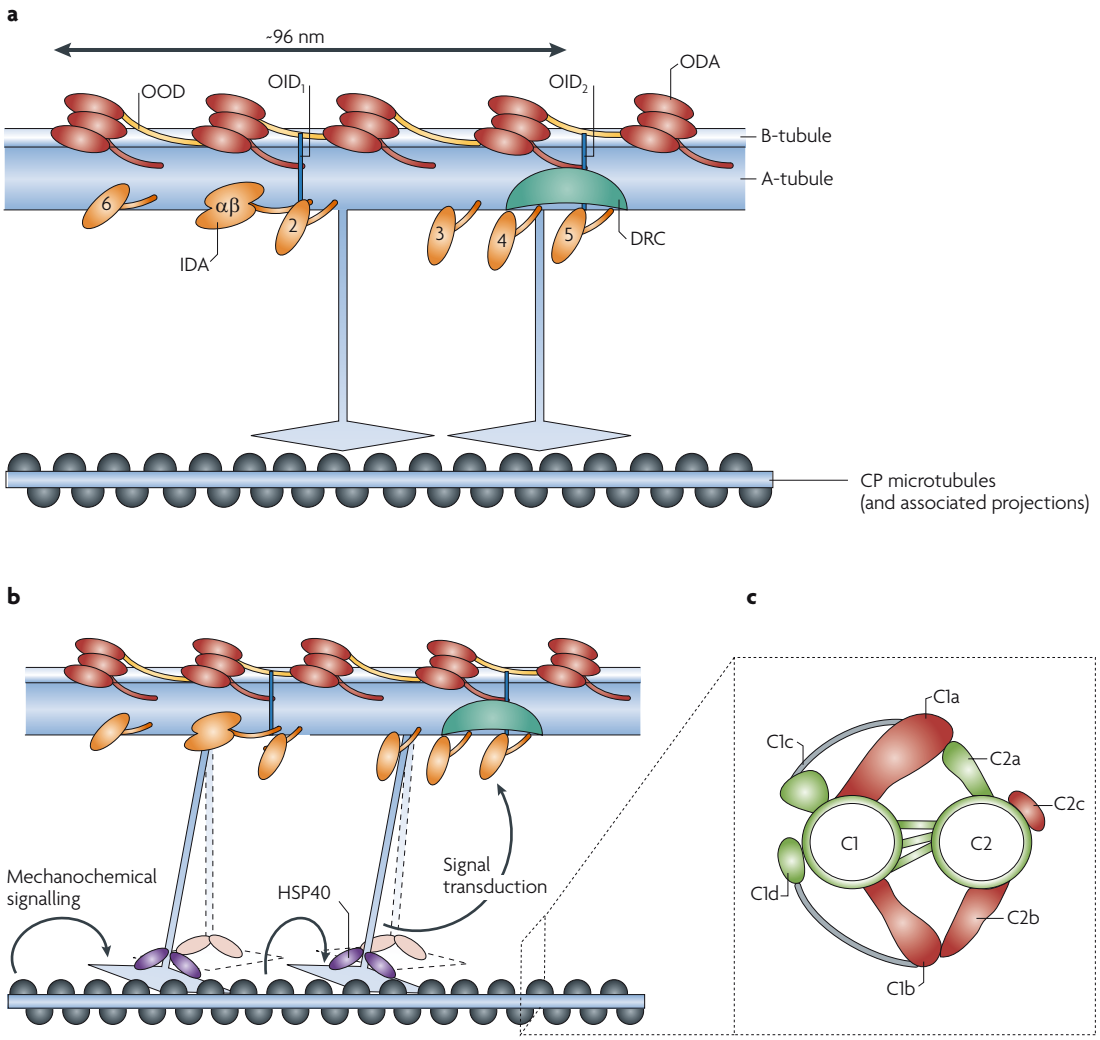


Figure 2 | **A structural network for motility regulation. a** | Longitudinal periodicities of outer dynein arm (ODAs) and multiple inner dynein arms (IDAs) along an outer-doublet A-tubule illustrate structural complexity in dynein organization (modified from REF. 17). Traditionally, ODA activity is viewed as a primary determinant of beat frequency: regulation of waveform shape and provision of additional power under high viscous load are functions ascribed to different IDAs[153,154]. '96-nm periodicity' is a classic paradigm in flagellar biology: either 2 or 3 radial spokes (RSs) are present per 96 nm (depending on the species in question); variations in spatial organization or in subunit composition of RSs and dyneins are common[16,33]. However, recent identification[17] of structural links between individual ODAs (outer-outer-dynein links (OODs)), and from individual OODs to αβ-IDA (OID_1) and the dynein regulatory complex (DRC) (OID_2) suggests regulation of microtubule sliding is 'hard wired'. Regulation of sliding velocities and waveform coordination requires the central pair (CP)–RS network, with second-messenger-stimulated phosphorylation or dephosphorylation providing a universal mechanism for regulating dynein motors[23]. **b** | Conformational switching provides a mechanism for signal transduction. Tilting and RS lengthening during transient interactions with CP projections occurs in mussel gill cilia[155], suggesting signal transduction from CP to dyneins is, in part, mediated by a conformational change of RS components. Characterization of a *Chlamydomonas reinhardtii* RS heat-shock protein 40 (HSP40) homologue[28] and of some CP proteins[26,29,73,156] provide widely conserved candidates for involvement in regulation by conformational switching. **c** | Asymmetric arrangement of projections in the *C. reinhardtii* CP. Projections containing conserved regulatory switch candidates are shown in red.

Promastigote
Morphological form of *Leishmania* spp. that migrates through the digestive tract of the sandfly vector. A single free flagellum emerges from the anterior pole of the cell body in promastigotes.

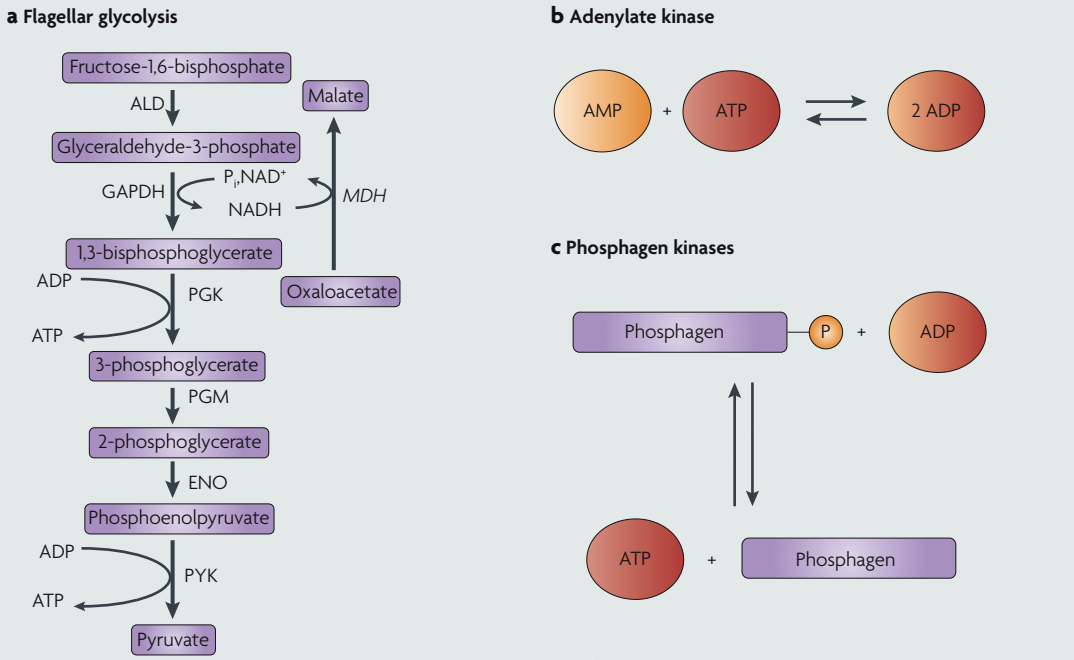
The diversity that is therefore apparent at a molecular level provides an interesting contrast with the general conservation of axoneme structure through eukaryotic evolution. In the proteomic inventories of *C. reinhardtii* and *T. brucei* are flagellum-specific isoforms of ubiquitous enzymes, and these discoveries[45,52,56] highlight the unexpected importance of protist flagella as metabolic organelles.

Metabolic pathways in protist flagella

Flagellum function requires significant amounts of ATP: the mechanistic basis for motility is dependent on the ATPase activity of the dynein heavy chain motor domain; transport of proteins along the length of the flagellum by the intraflagellar transport (IFT) machinery (see below) is also ATP dependent; and cyclic AMP (which is synthesized from ATP) is a second messenger in many

intraflagellar signalling pathways[57,58]. However, it has been suggested that the narrow aperture from the cytoplasm into the flagellum, coupled to the structural intricacy of this flagellar transition zone, plausibly restricts free diffusion of ATP from the cytoplasm into the flagellar compartment, and if the rate of ATP hydrolysis exceeds the rate of diffusion, then the distal region of the flagellum could readily become 'starved' for ATP[56]. The thin-section electron micrograph that is shown in FIG. 3a illustrates the portal that leads from the basal body into the flagellum in

Box 2 | **ATP production in flagella is catalysed by unusual enzymes**



Flagellar enzymes often have unusual features when compared to isoforms from other cellular compartments.

Chlamydomonas reinhardtii

In this alga (panel **a**), the early reactions of glycolysis (pre-aldolase) were reported to occur in the chloroplast, and the later reactions were thought to be cytoplasmic. Recently, a flagellar localization for the last three reactions of the glycolytic pathway, which yield one ATP per molecule of 3-phosphoglycerate, was reported[56]. In the *C. reinhardtii* proteome, other glycolytic enzymes and malate dehydrogenase are present[52]; absence of abundant cytoplasmic marker enzymes from the flagellar proteome demonstrates the authenticity of a partial flagellar glycolytic pathway. Generally, pyruvate kinase (PYK) exists as a homotetramer of 50 kDa; curiously, flagellar PYK is a large (>200 kDa) protein that contains 4 catalytic domains, suggesting that it functions as a monomer. Flagellar phosphoglycerate mutase (PGM) is vanadate sensitive, indicating it is not the cofactor-independent isoform that is present in *C. reinhardtii* and that has homologues in higher plants. ALD, aldolase, ENO, enolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDH, malate dehydrogenase; PGK, phosphoglycerate mutase.

Trypanosoma brucei

Here, two adenylate kinase (ADK) isoforms (panel **b**) are anchored along the length of the paraflagellar rod[45]. Predicted orthologues are present in other trypanosomatids, and would contribute to adenine-nucleotide homeostasis, either maximising energetic efficiency or contributing to dynein regulation. A third flagellar ADK is axonemal, but it is not obvious whether this protein is catalytically active[45,152]. Interestingly, trypanosomatid flagellar ADKs contain N-terminal extensions (~50-amino-acids long) that are: absent from ubiquitous cytoplasmic and mitochondrial ADKs; necessary and sufficient for flagellar targeting; and sufficient to identify other unusual ADKs isoforms, which are restricted in their evolutionary distribution to other eukaryotic flagellates[45].

Other protozoa

The likely functional significance of metabolic enzymes in the flagella of algae and trypanosomes will no doubt be informed by analysis of flagella and cilia from other protists. One other microbe for which there is relevant information is *Tetrahymena pyriformis*, in which a higher than usual molecular weight arginine kinase (~80 kDa, rather than ~40 kDa) was purified many years ago[66]. Classically, mobilization of high-energy phosphate from a phosphagen (such as arginine or creatine; panel **c**) maintains ATP levels in response to short periods of acute energy demand. Interestingly, a protein that contains two, rather than a single, arginine kinase catalytic domains (with a predicted molecular weight of ~83 kDa) is encoded in the *Tetrahymena thermophila* genome sequence, providing some parallels with the unusual triplicated creatine kinase activity that has been isolated from sea urchin spermatozoa[67] and the triple ADK that is present in *C. reinhardtii* flagella[60].

T. brucei; it probably provides a similarly restricted access point as that postulated for *C. reinhardtii*[56]. One solution to potential problems is to use intraflagellar metabolic networks to maintain adequate ATP levels.

Various reports over the past 40 years have documented the existence of enzyme activities associated with nucleotide homeostasis in flagellar preparations from various eukaryotes. The list of identified enzymes includes adenylate kinase, nucleotide diphosphate kinase and phosphagen kinases[59–67]. However, an obvious caveat when assaying the activities of abundant enzymes that are classically associated with many cellular compartments is that of confirming location specificity in individual biochemical fractions. Some clarity is provided, however, by observations that flagella-associated enzyme activities are often catalysed by novel, flagellum-localized enzyme isoforms (BOX 2). The issue that now needs attention is to understand when intraflagellar energy homeostasis becomes important for motility.

Metabolic enzymes, including adenylate kinase, creatine kinase and glycolytic enzymes, are found in the sperm tails of birds and mammals, and are sometimes physically associated with the insoluble flagellar ultrastructure[35,68]. However, an issue here is one of cell-type-specific expression (as sperm are essentially devoid of cytoplasm) versus differential targeting of enzyme isotypes (as flagella of protists can be viewed as organelles that are additional to the cytoplasm and organelles that are present in the cell body). The relevance of animal sperm to the current discussion is that metabolic repertoires in these cell types appear to be influenced by the ecological niche in which the sperm swim. Chicken and sea urchin spermatozoa, for example, swim in environments that are unlikely to provide a sustainable energy source and they therefore catabolize an internal fuel supply of fatty acids, shuttling ATP that is derived from mitochondrial oxidative phosphorylation along the axoneme using flagellar creatine kinase[69,70]. Mammalian sperm swim in a microaerophilic environment in which sugars provide a readily exploitable external carbon source, and it would appear that substrate-level phosphorylation from a flagellum-localized glycolytic pathway is essential for motility[71], although this possibility has recently been disputed[72].

So, what then of flagellar metabolism in *C. reinhardtii* and *T. brucei*? In *C. reinhardtii*, several glycolytic enzymes were unexpectedly identified from proteomics of purified flagella. Although immunofluoresence data are lacking, a reasonable assumption, based in part on an observation that some of the flagellar enolase fraction co-purifies with the 1b projection of the CP[56], is that phosphoglycerate mutase, enolase, pyruvate kinase[56] and possibly other glycolytic enzymes[52] are present along the length of the flagellum. Is the activity of these flagellar glycolytic enzymes essential for normal motility? There is some experimental evidence supporting this possibility, and it centres on the incorporation of enolase in the C1b[56] CP projection (FIG. 2c). Loss of the C1b projection results in defective swimming and reduced beat frequency[73], but this can be restored in de-membranated cell models or purified axonemes by adding ATP. Thus, reduced availability of intraflagellar ATP could be responsible, at least in part, for the swimming defect of *cpc1* mutants, which lack the C1b projection[74]. Furthermore, reduced ATP concentrations might arise as a consequence of perturbing flagellar glycolysis because of the loss of a key enzyme enolase from the CP[56].

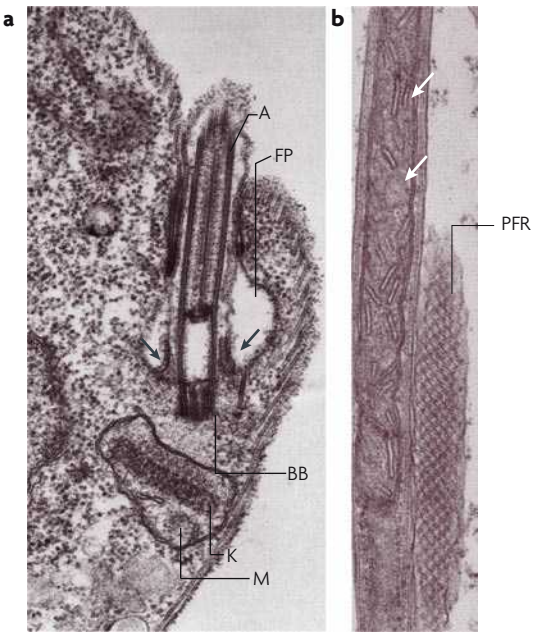


Figure 3 | **Trypanosome ultrastructure. a** | Thin-section electron micrograph of a longitudinal section through a procyclic trypanosome cell reveals the narrow aperture (transition zone; arrows) from the cytoplasm into the flagellar compartment. The flagellum can be seen exiting the flagellar pocket (FP) to emerge onto the cell surface. Structures indicated are the axoneme (A), basal body (BB), mitochondrion (M) and mitochondrial genome (otherwise known as the kinetoplast) (K). The scale bar represents 1 μm. **b** | A cristate mitochondrion is prominent in the slender cell bodies of parasites isolated from the guts of infected tsetse flies; the thin-section micrograph shows a longitudinal section through an anterior cell end. The scale bar represents 500 nm. PFR, paraflagellar rod. Panel **a** reproduced with permission from REF. 157 © 2007 Elsevier. Panel **b** courtesy of E. Gluenz, University of Oxford, UK.

There are, however, other interpretations for the available data. First, in addition to its incorporation into the CP, enolase also partitions into the flagellar matrix[56]. Second, enolase is one of several glycolytic enzymes for which moonlighting roles unrelated to carbohydrate metabolism are known[75–77], suggesting that irrespective of the enolase activity that purifies with the insoluble CP, enolase incorporation into C1b plausibly serves only a structural role. Third, the C1b projection contains two proteins that contain adenylate kinase motifs and could therefore contribute to adenine nucleotide homeostasis (see below), rather than provide structural or regulatory contributions to CP function[56,74]. Conceivably, the primary function of the glycolytic enzymes that are present in the *C. reinhardtii* flagellum is therefore not to sustain

motility under normal circumstances. Instead, we propose that these enzymes provide a necessary adaptation for sustaining motility during periods of environmental anoxia — a stress that is likely to be frequently encountered by unicellular oxygenic phototrophs, either as consequence of inadequate light availability or as a consequence of nutrient deprivation[78,79]. This hypothesis is supported by experimental evidence[80,81], and perhaps suggests that niche-specific flagellar energy metabolism exists in protist flagellates.

Thus, the draft genome sequence of *C. reinhardtii* reveals that, in comparison to many other unicellular eukaryotes, this alga presents an unprecedented capacity for metabolic flexibility[32,80,81]. The alga grows readily as a photoautotroph, but contains several enzymes that are classically associated with anaerobic metabolism and has an ability to ferment the glycolytic end-product pyruvate in numerous ways[80,81]. This suggests that *C. reinhardtii* is well placed in its natural soil and freshwater habitats to respond to environmental changes, such as onset of anoxia. Importantly, whereas oxygen-dependent changes to the *C. reinhardtii* metabolic network do occur at the level of gene expression[81], at least some enzymes that are classically associated with anaerobic metabolism (such as pyruvate formate lyase) are expressed in the light and dark under both aerobic and anaerobic conditions[80]. Such constitutive expression suggests metabolic pre-adaptation, a phenomenon that is noted in other eukaryotic microbes[82,83]. We speculate therefore that constitutive targeting of glycolytic enzymes to the flagellum provides not only a mechanism to ensure ATP homeostasis in the face of fluctuating environmental change over short timescales, but also facilitates rapid adaptation to anoxia. Thus, constitutive targeting of glycolytic enzymes conceivably allows intraflagellar ATP concentrations, and normal motility, to be efficiently maintained when respiratory O_2 uptake exceeds availability and when ATP production requires anaerobic fermentation of endogenous starch reserves, rather than photophosphorylation.

The discovery that the intracellular NADPH:NADP ratio and photosystem I activity influence both beat frequency and the duration of the photoshock response in *C. reinhardtii*[84] adds another layer of complexity to the link between cellular metabolism and motility, albeit one that is likely to reflect a phototroph-specific adaptation. By contrast, flagellar adenylate kinases in *T. brucei* perhaps provide another example of niche-driven, or putatively conditional, flagellar energy metabolism in a protist. To complete the transmission cycle, procyclic trypanosomes migrate from the mid-gut of the tsetse fly to the salivary glands. The tsetse digestive tract is thought to be devoid of readily exploitable carbohydrates (the presence of a key gluconeogenic enzyme, fructose-1,6-bisphosphatase, in trypanosomes provides further evidence that carbohydrate demand cannot always be satisfied by sugar uptake), and the parasite is not thought to accumulate a significant carbohydrate reserve. Thus, we can reason that glycolytic flux is unlikely to be significant for generating ATP inside the flagellum. In the absence of glucose, mitochondrial amino-acid metabolism and oxidative phosphorylation sustain ATP production in cultured procyclic *T. brucei*[85]. Although parasites that are isolated from infected tsetse flies are not readily amenable to direct biochemical analysis, the likely significance of mitochondrial metabolism is evidenced by the prominence of a cristate mitochondrion throughout the long, slender cell bodies of the highly motile trypanosomes that are found in the fore-gut (FIG. 3b). If intermediary metabolites that are suitable for cytoplasmic or intraflagellar substrate-level phosphorylation — that is, glycolytic intermediates — are in short supply, then the economy of energy management that adenylate kinase can supply[86] becomes a sensible strategy to preserve intraflagellar ATP concentrations. This may be particularly relevant for long slender trypomastigote and epimastigote forms in the anterior gut and in the proventriculus of the tsetse fly; here flagellum length exceeds 35 μm, and the prospect of 'ATP starvation' at the distal end is conceivably more acute than in shorter procyclic parasites, in which flagellum length is on average only ~20 μm[87].

Flagellum assembly and maintenance

Axonemes are built by extension from the distal tip, and in most cases the growing axoneme is surrounded by its ciliary membrane — that is, assembly occurs by flagellum elongation. However, flagella are ribosome-free compartments. Thus, for flagellum elongation to occur, coordinated targeting and transport of components from the cytoplasm (the site of protein synthesis) into the flagellum is necessary; this transport into and along the flagellum is dependent on an evolutionarily conserved mechanism, IFT, that was first identified in *C. reinhardtii*[88]. IFT moves flagellar proteins along axonemal microtubules from the base of a flagellum to its tip (anterograde transport, powered by specific kinesin motor complexes), where axoneme extension occurs, and from tip-to-base (retrograde transport, facilitated by dynein motor activity)[89,90].

Flagellar length control is also an important aspect of flagellate biology[91]. As illustrated by the response of *C. reinhardtii* to the amputation of a single flagellum[92], cells actively monitor flagellar length. Flagellum and cilium length also varies enormously between different eukaryotic species, and evidence suggests that length control is a complex affair that is influenced by a balance between IFT-dependent assembly and disassembly, kinesin-dependent microtubule de-polymerization and protein kinase and phosphatase signalling cascades[91,93–99].

Several amino-acid sequences, which are either necessary or sufficient to confer flagellar targeting of reporter proteins, have also been identified in several organisms[45,100–104]. However, in contrast to our broad understanding of motility regulation, good insight into how proteins are targeted into the flagellum is lacking, and addressing this shortcoming is a challenge for potential further study. As illustrated by studies with mammalian cells, at least one component of the IFT machinery, IFT20, may be used for recruitment of flagellar membrane proteins from the Golgi[105]. Studies with

Kinesins
Motor proteins that couple ATP hydrolysis to plus-end-directed movement along microtubules. In flagella, kinesins are required for intraflagellar transport and a kinesin-like protein is a component of the central pair microtubules.

ift46 mutants in *C. reinhardtii* have also recently linked individual subunits from IFT particle complex B with transport and axonemal assembly of specific cargoes[106]. In *C. reinhardtii*, full length IFT46 is required for IFT of outer-dynein arms (ODAs), but not for other axonemal components, including the ODA-docking complex. This result is all the more intriguing given that earlier results suggest that incorporation of ODAs onto axonemes was IFT independent[107]. As the authors of the *ift46* study comment, however[106], published data are readily reconciled if multiple kinesin motors are required for anterograde IFT in *C. reinhardtii*, as they are in some other eukaryotes[90]. An unresolved challenge for future study is to determine whether targeting signals that have been identified thus far confer flagellar localization because they present motifs that are identified as 'recognition handles' by the IFT machinery or whether they alternatively function in assembly of axonemal subcomplexes (such as, radial spokes and ODAs) that are built in the cell body.

The control of the assembly of multiprotein complexes in the cytoplasm probably also ensures the fidelity of flagellum construction. The predominant protein required for flagellum assembly is heterodimeric α/β-tubulin (the building blocks of microtubules) and recent results using trypanosomes as a model system suggests that the status of tubulin heterodimers is monitored before IFT-mediated transport[108]. Following identification of a specific tubulin cofactor C (TBCC)-domain-containing protein in the published trypanosome flagellar proteome, Stephan *et al.*[108] showed specific localization of this protein (designated RP2) to transitional fibres on the mature basal body from which the axoneme is built; RNAi-mediated knockdown of RP2 causes axonemal microtubule defects, but has no effect on other microtubule populations in the cell, and comparative bioinformatics revealed that RP2 belongs to a family of related TBCC-domain-containing proteins whose phylogenetic occurrence is restricted to eukaryotes that build flagella. Biochemical characterization of the human orthologue of trypanosome TBCC suggests that the protein acts to assess the GTPase competency of tubulin[109], an important consideration given that hydrolysis of GTP bound to β-tubulin is closely coupled to dynamic microtubule polymerization. Overall, the data suggest that a TBCC isoform evolved to meet the specific challenges of tubulin provision in the context of cilia and flagella. Looking further at the flagellar protein catalogues from trypanosomes and *C. reinhardtii* it will be interesting to learn whether the unexpected flagellar localization of ecotin homologues, cyclophillins and various chaperone proteins serve merely structural functions, whether they are required for post-translational modifications during axoneme construction or whether they contribute some dynamic aspect to motility regulation. In that regard, it is intriguing that the flagellar heat-shock protein 40 at the juncture between the radial spoke head and the stalk (FIG. 2b) is required in *C. reinhardtii* to couple power and recovery strokes in ciliary waveforms and to sustain normal beat frequency[28].

IFT particles
Core components of the IFT machinery. Two large multisubunit IFT particle complexes have been characterized: complex A (classically associated with retrograde IFT) and complex B (associated with anterograde IFT). Each of these complexes contains its own unique set of widely-conserved protein subunits.

Challenging the canonical assembly mechanism

The studies discussed above provide a brief overview of the processes that are required for flagellum assembly, and highlight areas where knowledge is currently lacking. Interestingly, glimpses into the genome sequences of various protists and some unusual cell biology have either revealed streamlining of the IFT pathway or have provided other challenges to our understanding of assembly mechanisms.

Intriguingly, malarial parasites have lost the IFT pathway. All known IFT genes are absent from the complete genome sequences of various *Plasmodium* species[51,110], and flagella assembly occurs in the cytoplasm during gametogenesis from single, rather than paired, basal bodies[111]. Here, loss of the IFT pathway is all the more interesting because an IFT footprint is present in other apicomplexan parasites (such as *T. gondii* and *Eimeria tenella*) which produce flagellate male gametes[33,110]. Interestingly, *T. gondii* lacks a homologue of the DYNC2 dynein motor, which is necessary for retrograde IFT[33]. A DYNC2 homologue is also absent from the genome of the centric diatom *T. pseudonana*, as are the components of the retrograde IFT-associated complex A[32,33]. Whereas the absence of anterograde IFT in ciliated animal tissues and many protists results in failure to assemble flagella, perturbation to retrograde IFT produces a spectrum of phenotypes. In trypanosomes and *C. reinhardtii*, loss or perturbation of retrograde IFT results in the assembly of short flagella. Anterograde transport can still occur in the absence of IFT complex A or of its associated dynein motor, thus explaining the assembly of short flagella, but in these organelles anterograde IFT complex B components accumulate at the distal tip[89,90,112]. In the case of *T. brucei*, this IFT perturbation results in loss of growth as flagellum-templated cell morphogenesis is lost[112]. In the nematode *Caenorhabditis elegans,* zebrafish and the ciliate *T. thermophila*, cilium assembly also occurs in the absence of retrograde IFT components, albeit that changes in cilium length and cilium distribution, as well as various physiological phenotypes, are evident[113–115]. Clearly, therefore, one might anticipate that evolutionary moderations to the retrograde part of IFT-dependent flagellum assembly are feasible; thus, it will be intriguing to discover why DYNC2 and apparently retrograde IFT are not needed in *T. gondii* and diatoms, respectively.

Transitional fibres radiate from flagellar basal bodies to the plasma membrane, marking the boundary between cytoplasmic and flagellar compartments, and are considered to provide a docking zone for IFT particles before transport up the flagellum[116,117]. *Giardia lamblia* therefore provides an unusual challenge to our conception of IFT and flagellar assembly, as although the IFT genes are present and its eight axonemes are extended from clustered basal bodies[110,118], the basal bodies are located deep in the cytoplasm and axonemes run a considerable distance through the cytoplasm to the exit point from the cell body (FIG. 1f). There are three possibilities: two modes of flagellar assembly operate in *G. lamblia* (IFT-independent cytoplasmic

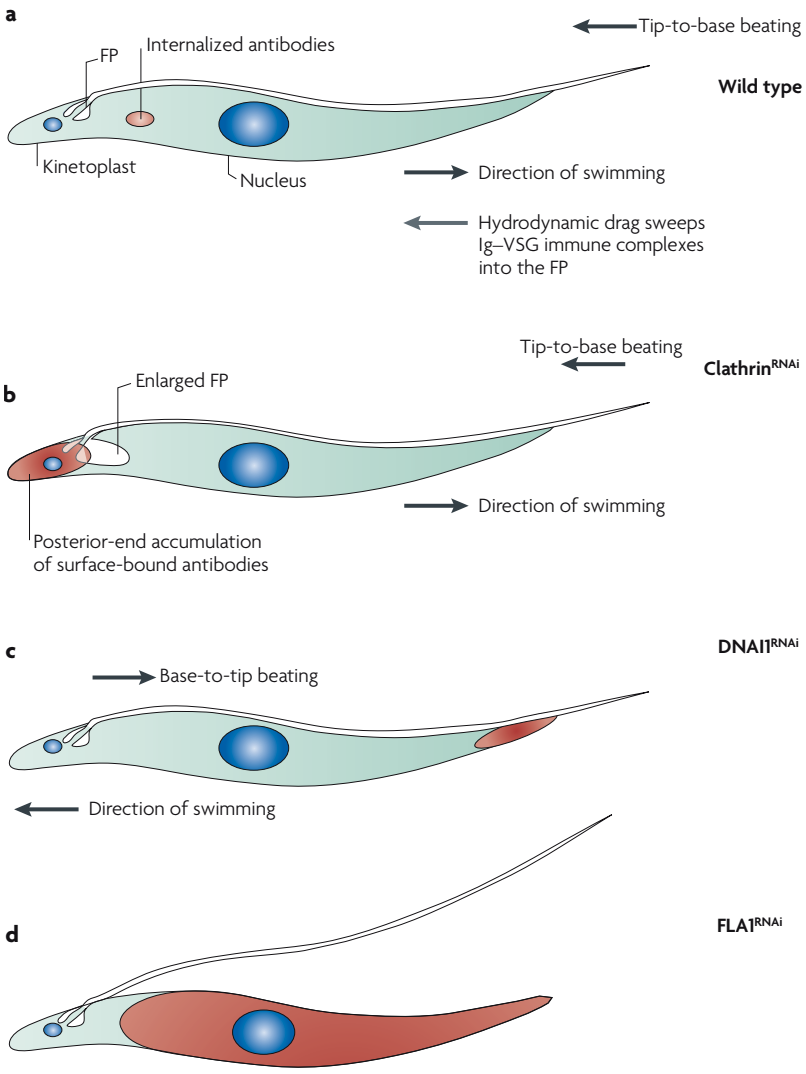


Figure 4 | **Motility and immune evasion. a** | In bloodstream *Trypanosoma brucei* the normal flagellum beat is from tip-to-base, and surface-bound antibodies (Ig) that recognize the variant surface glycoprotein (VSG) coat are rapidly endocytosed at the flagellar pocket (FP). **b** | When expression of clathrin is silenced by RNA interference (RNAi), the size of the FP increases because endocytosis is blocked. Surface-bound anti-VSG antibodies accumulate at the posterior end of the cell, but cannot be internalized in the absence of clathrin. **c** | RNAi-mediated depletion of dynein intermediate chain 1 results in the loss of outer dynein arms (ODAs), and the flagellum can only beat base-to-tip (wave reversal). Accumulation of anti-VSG antibodies at the anterior cell end indicates that directional movement of surface-bound immune complexes is dependent on flagellum beating. **d** | In the absence of flagellum attachment ($FLA1^{RNAi}$), no directional movement of surface-bound molecules is possible; loss of surface-bound antibodies at the posterior cell end is due only to diffusion and endocytosis at the FP. Figure modified from REF. 143.

extension, followed by IFT-dependent extension from the cell body); IFT particles dock at the basal body and then run along the cytoplasm; or an alternative docking site for IFT is present distal to the basal bodies at the point where axonemes are extended out of the cell body. Preliminary analysis of GFP-tagged IFT kinesins, IFT81–GFP and IFT140–GFP (two conserved IFT components) suggests that these possibilities need not be mutually exclusive[118].

Sensory perception and protist flagella

Many aspects of animal physiology require cilia to function in sensory capacities[6–12,119,120]: the retina, inner ear and nasal epithelium are all dependent on cilium function or assembly; in nematodes the only ciliated cells are the sensory neurons, which produce immotile '9+0' axonemes[113]; an immotile sensory (or primary) '9+0' cilium is also extended from many quiescent or differentiated vertebrate cells; and, finally, during embryogenesis, motile '9+0' nodal cilia are crucial for establishing left–right asymmetry. Functions that are associated with such cilia include the perception of environmental changes (for example, by recognizing chemical stimuli or by detecting, as a consequence of cilium bending, extracellular fluid flow rates (mechanosensing)), and, in the example of nodal cilia, establishment of a morphogen gradient. Recognition of chemical cues and the physical response of primary cilia to fluid flow result in second messenger activation, and thus effect specific cellular responses through various signalling networks. Extension of sensory ciliary membranes into the extracellular milieu provides a mechanism by which the area within which, and sensitivity with which, cells and tissues can respond to environmental cues is increased; it is therefore perhaps easy to appreciate why defects in cilium assembly or function are now so often implicated in human disease. What about flagellate protozoa?

On the one hand, the signal-transduction cascades that regulate ciliary and flagellar waveforms and beat frequency depend on the same second messengers (for example, Ca^{2+} and cyclic nucleotides) as the signalling networks that are activated in primary cilia. This suggests that for any protist using motile flagella to directly sense the environment, changes in waveform or beat frequency could presumably be regulated concurrently with the amplification of any signal-transduction cascade, causing a biological response in the cell body. On the other hand, in some protists, for instance malarial parasites and the amoebo flagellate *Naegleria gruberi*, environmental change actually provides the stimulus to build a flagellum or to become motile for a short time[121,122]. Similarly, in *C. reinhardtii* photo-behavioural responses are dependent on flagellum-localized ion-channel-gated Ca^{2+} influx, but the changes in light intensity that trigger Ca^{2+}-dependent changes in motility are registered by the eye-spot on the cell body[123,124]. However, protist flagella do function at the initial step of sensory perception, and even when motility changes might be viewed as the downstream consequence of sensory perception (for example, in photo-behavioural responses), the involvement of flagellar components in the downstream steps of associated signal-transduction pathways are probably crucial, as is exemplified by the identification of the proteins Agg2 and Agg3 in the proximal regions of *C. reinhardtii* flagella[125].

Further defining the sensory functions of protist flagella will ultimately allow interested parties to understand whether the 'sensing' that is associated with this organelle is generally mediated by proteins or pathways that are conserved across the animal and protist kingdoms. Interestingly, insulin-receptor-like proteins have been detected in ciliary membranes of *T. thermophila*[126],

but again recent studies with *C. reinhardtii* are also relevant. A prime example of flagellum sensing is mating in *C. reinhardtii*, in which the sexual cycle follows adhesion of flagella from gametes of opposite mating types[127]; adhesion occurs due to interactions between sex-specific agglutinins that are exposed on gamete flagella[128]. Following adhesion, the IFT machinery facilitates the essential re-localization of a crucial signal-transduction component of the mating pathway, a flagellar membrane cyclic GMP-dependent protein kinase (PKG)[129]. Following Ca^{2+}-dependent activation of PKG kinase (occurring through PKG phosphorylation), adenylate cyclase activity increases, thereby increasing cAMP concentrations in the flagellum; these steps represent the earliest events in the signal-transduction cascade that eventually results in cell fusion. Thus, upon nitrogen starvation, *C. reinhardtii* differentiates, forming gametes and exposing mating determinants on the outer face of the flagellar membranes. Adhesion of the two flagella then initiates a flagellar signal-transduction cascade, which ultimately results in signal-amplification in the cell body and leads to cell–cell fusion.

Recently, the characterization of a Ca^{2+}-permeable, non-selective cation channel TRPP2 homologue (PKD2) provided further insight into the flagellum-specific organization of this signal-transduction cascade. PKD2 is a membrane-associated protein, and its abundance in flagella is higher in gametes than it is in vegetative cells. RNAi-mediated knockdown of PKD2 results in reduced phosphorylation of PKG, and the extent to which phosphorylation of PKG declines correlates with decreased mating efficiency. Importantly, mating competency following RNAi-mediated knockdown of PKD2 was rescued when signalling was restored downstream of PKG phosphorylation by addition of cAMP analogues[130]. Interestingly, in humans, mutations to TRPP2 (also known as polycystin 2) are a primary cause of polycystic kidney disease[12].

C. reinhardtii is a free-living flagellate, seemingly able to respond flexibly to environmental change (see above), but many flagellates are parasites. Thus, the question of flagellar sensing is again interesting because, much like metabolic streamlining, it could be argued that adaptation to parasitism is likely, in comparison to free-living species, to result in moderation of sensory capabilities. Again it is the trypanosomatids (*T. brucei* and *Leishmania* spp.), which swim with their flagellum leading, that provide the clearest examples of how flagellum sensing is conceivably a feature of parasite biology.

One possibility for sensing relates to the attachment of trypanosomatid parasites to epithelial surfaces in their respective invertebrate vectors. Attachment is mediated via the length of the flagellum[131] and provides an opportunity for signalling-dependent orchestration of parasite–vector interactions. In the host, immotile *Leishmania* amastigotes assemble a short flagellum, which possibly facilitates closure of the flagellar pocket. Although this short flagellum barely extends onto the cell surface, an intriguing possibility is that targeting of flagellar membrane proteins defines a polarity that could be used by the parasite to orientate itself in the macrophage phagolysosomes. The best evidence, however, for flagellum-localized sensory function is perhaps provided by the flagellar localization of a low-affinity glucose transporter in promastigote *L. mexicana*[132,133]. The flagellum-localized transporter, encoded by *GT1*, appears to play a minor role in the uptake of glucose as a carbon source for cellular metabolism[132], but as cell swimming occurs with the flagellum leading[134–138], this integral membrane protein represents a candidate sensor protein for contributing signalling functions in a similar manner to GLUT1 (REF. 139), an archetypal mammalian transporter, or various plasma-membrane localized glucose-transporter-like proteins that are found in fungi[140]. A flagellar-localized aquaglyceroporin from *Leishmania major* was also characterized recently and this molecule may have a sensory role in mediating osmotaxis[141].

Flagellum function and parasite viability

A role for flagellum-mediated motility during the migration of *T. brucei* from the mid-gut to the salivary glands of its tsetse fly vector is perhaps easy to appreciate. Yet, until recently it was unclear what role motility might play in extracellular bloodstream form trypanosomes. The turbulence and shear force of blood flow make directed motility unlikely, but two unusual and essential roles for flagellum function have recently been reported. Antigenic variation of a protective glycosylphosphatidylinositol-anchored variant surface glycoprotein (VSG) coat, which covers the entire cell surface of *T. brucei*, provides the strategy through which bloodstream parasites evade humoral immunity[142]. VSGs are highly immunogenic molecules; Engstler and co-workers recently discovered that at low antibody titres and in the absence of VSG switching, trypanosomes avoid antibody-dependent opsonization or antibody-mediated complement lysis by using flagellum-dependent motility to sweep surface-bound immune complexes backwards towards the flagellar pocket where they are endocytosed[143] (FIG. 4). A high rate of endocytosis in the flagellar pocket[144] ensures that this continual 'self preening' process works efficiently as an immune-evasion strategy.

Coupling motility to immune evasion is an elegant, evolved tactic, but other studies have revealed that motility is also fundamental for cell division in bloodstream parasites[37,39,41]. During each cell division, cycle trypanosomes elongate a new flagellum, which follows the same left-handed helical pitch as the adjacent mature, old flagellum (FIG. 1d). Following mitosis, a cell-cleavage furrow ingresses, and for procyclic trypanosomes, provided the flagellum remains attached to the cell body and can at least beat rudimentarily, cells generally grow robustly in liquid culture[36,37,39] (although they may be paralyzed or incapable of vectorial movement). Bloodstream trypanosomes, however, are

Amastigote
Pathogenic, immotile morphological forms of *Leishmania* spp. that replicate in acidic phagolysosomes of a host macrophage.

Kinetoplast
The mitochondrial genome in trypanosomatids. A kinetoplast consists of several thousand catenated circular DNA molecules, is replicated once per cell cycle and is attached to the flagellar basal body.

Chemical genetics
The use of small molecules, rather than genetic mutations, to interfere directly with protein function. In the case of trypanosome motility, small molecules could inhibit the activity of signalling enzymes, disrupt essential protein–protein interactions or be active against any of the 200 plus 'trypanosomatid specific' flagellum proteins that have been described.

exquisitely sensitive to perturbation of flagellar motility: knockdown of numerous flagellar targets, using RNAi that yields only motility phenotypes in procyclic *T. brucei*, are lethal in the bloodstream form[37,42]. These bloodstream cells fail to complete cytokinesis. There is no checkpoint control that recognizes this failure, and the mutants subsequently enter into further rounds of the cell-division cycle, ultimately producing multinucleate, contorted cells[37,42]. Even before the defect in cytokinesis is evident — that is, when cells contain a normal complement of DNA-containing organelles (nucleus and kinetoplast) — RNAi-mediated knockdown of flagellar targets leads to defective motility, providing persuasive evidence that the subsequent failure of cytokinesis is due to the perturbation of motility[41].

Although the sensitivity of bloodstream *T. brucei* to flagellum perturbation came as a surprise, the use of motility in cell division is not without precedent. For example, *T. thermophila* uses ciliary beating to contribute to the final stages of cytokinesis, when the two daughter cells are connected by a thin cytoplasmic bridge. Rotation of one daughter cell around its longitudinal axis provides the force which breaks the connecting bridge[145,146]. In *T. thermophila* mutants that cannot assemble cilia, cytokinesis defects due to problems during this final stage commonly arise. Interestingly, agitation of cultures provides a mechanical force that is often sufficient to compensate for a lack of ciliary-driven rotation during cytokinesis[147]. Crucially, in the case of *T. brucei*, however, neither shear force nor blood-pressure turbulence — externally applied mechanical forces that parasites experience during growth in the mammalian bloodstream — rescues the cytokinesis defect of the bloodstream-form of parasites, in which flagellar motility has been perturbed by depletion of proteins using RNAi[41]. Instead, induction of protein ablation *in vivo* using RNAi results in the rapid clearance of parasites by the host immune system. The onus will now be to use chemical genetics to see whether proteins involved in flagellum function might be possible drug targets for sleeping sickness.

Closing comments

The current widespread interest in the cell biology of the eukaryotic flagellum and, moreover, the significance of flagellum function in health and disease is epitomized by a glance at the research presented at a 2007 FASEB-sponsored meeting on the biology of cilia and flagella[148]. Undoubtedly, the systems-based challenges of understanding cilium assembly and function will continue to be informed by experimental studies with flagellate protists. Fundamental topics we did not discuss, such as basal body and centriole formation and duplication, are also readily addressed using protist systems[49,50]. As we have attempted to illustrate here, however, nuances of flagellum assembly or function that are particular to individual protist species are interesting in their own right. Although recent experimental observations have perhaps been dominated by studies of *C. reinhardtii* and trypanosomes, the insights afforded by mining genome sequences of other flagellates often challenge the paradigms that have been established from studies with tractable model systems. In the case of the African trypanosome, small molecule intervention of flagellum function might eventually afford new possibilities for drug design.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
cpc1
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Chlamydomonas reinhardtii | *Giardia lamblia* | *Tetrahymena thermophila* | *Toxoplasma gondii* | *Trypanosoma brucei* | *Paramecium tetraurelia* | *Plasmodium*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alstrom | Bardet–Biedl | Meckel

FURTHER INFORMATION
Michael Ginger's homepage: http://biol.lancs.ac.uk/bs/people/teach/mlg.htm
Paul McKean's homepage: http://biol.lancs.ac.uk/bs/people/teach/pgm.html

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