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Eukaryotic flagella: variations in form, function, and composition during evolution

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Abstract

The microtubule axoneme is an iconic structure in eukaryotic cell biology and the defining structure in all eukaryotic flagella (or cilia). Flagella occur in taxa spanning the breadth of eukaryotic evolution indicating the organelle's origin predates the radiation of extant eukaryotes from a last common ancestor. During evolution the flagellar architecture has been subject to both elaboration and moderation. Even conservation of '9+2' architecture – the classic microtubule configuration seen in most axonemes – belies surprising variation in protein content. Classically considered as organelles of motility that support cell swimming or fast movement of material across a cell surface, it is now clear the functions of flagella are also far broader; for instance, the involvement of flagella in sensory perception and protein secretion is recently evident in both protists and animals. Here, we review and discuss in an evolutionary context recent advances in our understanding of flagellum function and composition.

Keywords: Chlamydomonas, centriole, cell biology, microtubules, Trypanosoma

Introduction

The origin of the eukaryotic flagellum (or cilium, terms referring to variants of essentially the same organelle) predates the radiation of known eukaryotes from a last common ancestor over a billion years ago (Roger and Simpson 2009). The presence of whip-like flagella or short, hair-like arrays of many cilia protruding from cell surfaces is a striking visual feature of many protists and animal cells **(figure 1)**. Historically, distinguishing between a 'flagellum' versus a 'cilium' was often made on the basis of organelle length (flagella are typically longer than cilia) or organelle number (many, often >100, 'cilia' can be arrayed across a cell surface, whereas examples of eukaryotes with more than eight 'flagella' are rare), but the defining architectural feature in these organelles is always a microtubule axoneme.

Motility is the feature most classically associated with flagella and is often central to cell swimming, fast movement of material across a cell surface, cell feeding, and reproduction by eukaryotic organisms. Yet, eukaryotic flagella also function as sensory antennae and in the green alga *Chlamydomonas reinhardtii*, at least, as a secretory organelle, too. Our understanding of the flagellum as a secretory organelle is in its infancy (Wood et al. 2013), but then it was only fifteen or so years ago that the importance of flagella in the perception of extracellular stimuli, particularly during animal development, began to be widely realised (Goetz and Anderson 2010). Now there is widespread appreciation that a wide variety of genetic syndromes are underpinned by defects in cilium assembly or function, and that the underlying pathology is often a consequence of altered sensory perception. Moreover, in some individuals defects in flagellum assembly or function contribute to a pre-disposition to some chronic conditions, including cancer and obesity; again connections between signalling dysregulation and disease are becoming evident. In animals, sensory perception is more commonly associated with immotile flagella, often referred to as primary cilia; most animal

cell-types are capable of building a single primary cilium in response to appropriate differentiation cues.

Discussions of how motile cilia beat and connections between ciliary function and human disease are provided in other overviews in this special edition (Brown and Witman 2014, Satir et al. 2014). Here, we consider some of the ways in which the architecture and function of the flagellum has been subject to surprising and potentially informative variation during eukaryotic evolution.

Eukaryotic flagella – ancient organelles unrelated to bacterial flagella

Since the release in April 1996 of a nuclear genome sequence for the bakers' yeast *Saccharomyces cerevisiae* (an aflagellate eukaryote), genetic blueprints for numerous eukaryotes have steadily been released. Using the wealth of information provided in annotated genome sequences, comparative analyses of protein-coding gene repertoires in taxa spanning the breadth of eukaryotic evolution indicate the hallmark characteristics of eukaryotic cell biology – a nucleus, endomembrane and vesicular trafficking systems, mitochondrial metabolism, a cytoskeleton based on actin and microtubules – were all surprisingly well advanced in the last common ancestor of extant eukaryotes (Koumandou et al. 2013). This includes the presence of a flagellum or flagella in that last common ancestor. From the outset, however, it is important to emphasize the architecture, composition, motility mechanism, and assembly of eukaryotic flagella are all fundamentally different from the non-homologous bacterial flagellum. Thus, (i) the number of proteins comprising a typical eukaryotic flagellum is far greater than in the bacterial organelle (several hundred different proteins in the case of the eukaryotic organelle versus less than 30 in the case of prokaryotic flagella); (ii) rather than utilising a rotary motor that sits at the base of the flagellum,

eukaryotic flagellar motility is dependent upon constrained, dynein-dependent microtubule sliding and the application of applied force into flagellum bending; (iii) the bacterial flagellum is a self-assembling filament, whereas synthesis of the eukaryotic flagellum is templated from a microtubule-based centriole to which flagellar proteins are first targeted, and from which the axoneme extends. The centriole is also often referred to as a flagellar basal body, although again the reader should not confuse this with the membrane-embedded bacterial basal body, which includes the rotary motor that drives motility and the pore through which flagellar proteins translocate in bacteria.

In the iconic, textbook '9+2' configuration, axonemes are formed from a ring of nine outer-doublet microtubules that surround two singlet central pair microtubules **(figure 2)**. A multitude of accessory structures decorate both the surface and the lumen of axonemal microtubules, but the obvious features evident from electron microscopy that are key to motility are the dynein ATPases, which are anchored to outer-doublet A tubules and, in most motile flagella, the radial spokes and central pair projections, which link central pair with the dynein ATPases and are essential for the regulated motility of flagella bearing these structures. If one looks longitudinally along the length of the flagellum, then a conserved 96 nm periodicity to the organisation of radial spokes, central pair projections, and the outer- and inner-arm dynein ATPases is evident. The dynein motor proteins form transient bridges between adjacent outer-doublet microtubules resulting in microtubule sliding, but because sliding is constrained by other connections between outer-doublet microtubules applied force is converted into flagellar bending.

Origin of nine-fold symmetry

The barrel-like microtubule organizing center (MTOC) which templates any axoneme is called a centriole or basal body, and is formed from a radially symmetrical arrangement of nine triplet microtubules from which the outer-doublet microtubules of the axoneme extend. In some eukaryotes, centrioles are multi-functional serving also to organize assembly of the mitotic spindle. The green alga *Chlamydomonas reinhardtii*, which since the 1950s has served as the principal model for biologists studying flagellum assembly and function, is a prime example: prior to cell division its flagellar basal bodies uncouple from their associated axonemes and migrate to the nuclear periphery, defining opposite poles of the mitotic spindle (Piasecki et al. 2008). Thus, to consider the origin of the flagellum compartment fully one might consider (i) the origin of the centriole; (ii) the origin of nine-fold symmetry; (iii) the evolution of an assembly pathway for flagellum biogenesis; (iv) the initial, and potentially the subsequent, evolutionary advantage(s) offered by a flagellum or flagella.

An early suggestion from the late Lynn Margulis that flagella evolved from an ancient symbiotic relationship between a eukaryotic ancestor and a spirochete bacterium, or an idea, based on experiments from the 1980s, that the centriole, like mitochondria and chloroplasts, was an organelle that possessed its own genome have been dismissed. Yet, while the autogenous origin of flagella is widely accepted, perhaps only speculation, rather than answers, can ever be offered for the functional advantage conferred by the prototypical flagellum. Even with respect to the centriole there is debate as to whether it perhaps evolved from a single triplet blade (Marshall 2009). However, recent bioinformatics interrogation of genome sequences suggests the structural complexity of the centriole is defined by only a handful of conserved proteins (Carvalho-Santos et al. 2011). Central to the definition of nine-fold triplet microtubule symmetry is SAS-6, a conserved protein present at the proximal end of centrioles **(figure 3)**. Structurally, SAS-6 has an N-terminal globular head domain and a C-terminal coiled-coil domain that mediates protein dimerization. *In vitro*, SAS-6 dimers self-

assemble into ring-like structures where the coiled-coil domains of nine SAS-6 dimers point outwards providing a hub-and-spoke arrangement similar in its dimensions to the cartwheel hub found at the proximal end of the immature (pro-) centrioles (Kitagawa et al. 2011). In the absence of SAS-6 assembly of the hub-and-spoke cartwheel fails and although triplet microtubule blades still assemble, nine-fold symmetry is lost (Nakazawa et al. 2007). Curiously, an exception that proves the rule is the SAS-6 protein from the nematode *Caenorhabditis elegans*, the organism from which SAS-6 was originally characterised. Here, SAS-6 still defines a nine-fold symmetry, but assembles as a tubular, rather than radial, spiral illustrating how when even using conserved component parts there can be significant evolutionary variation in the mechanisms through which core flagellar structures assemble (Hilbert et al. 2013).

Axoneme assembly

Radiating out from the distal end of mature centrioles are transitional fibres to which axonemal proteins are first recruited prior to transport into the flagellar compartment, and thence to the distal tip of the flagellum where axoneme extension occurs (Deane et al. 2001). Transitional fibres also denote the proximal end of the transition zone, the name given to the most proximal region of a flagellum. The transition zone forms the boundary with the cytoplasm of the cell body (*i.e.* flagella are spatially distinct compartments that unusually are not entirely bounded by membrane) and a portal through which traffic of membrane and soluble proteins in and out of the flagellum is regulated (Czarnecki and Shah 2012).

Movement of membrane and axonemal proteins along the flagellum utilizes a conserved intraflagellar transport (IFT) pathway. Since the discovery of IFT in *Chlamydomonas* just over twenty years ago (Kozminski et al. 1993), the IFT pathway has been studied in many

flagellate eukaryotes and has featured prominently in the resurgence of interest in eukaryotic flagella and realization of the importance of these organelles for human health. At a molecular level, the IFT pathway involves three modular complexes, IFT-A, IFT-B, and the BBSome, that collectively assemble from over 30 different proteins. Kinesin and dynein motor proteins, respectively, are responsible for the forward (anterograde) and retrograde movement of the IFT complexes and their cargo along the axoneme. Phylogenetic reconstructions suggest IFT-A and the BBSome evolved via duplication of an IFT-B complex that served as the proto-typical IFT apparatus, and that IFT-B shares a common ancestry, at least in part, with protocoatomer proteins COPI-α, β', and ε (van Dam et al. 2013). The protocoatomer family of complexes are involved in vesicular trafficking within eukaryotic cells and nuclear pore complex assembly. A relationship between IFT and protocoatomer proteins also provides a basis for understanding how the involvement of IFT in the vesicular transport of some flagellar proteins to the centriole arose (Jekely and Arendt 2006), and provides another example of how the complexity of flagellum assembly is likely to have evolved from ancestral simplicity.

Initially an organelle of motility or sensory perception?

Motility is the characteristic most classically associated with eukaryotic flagella, but it does not necessarily follow that it was the archetypal flagellar function. Indeed, as awareness grows of how diverse flagellate protists use their flagella to interact with other cells and the environment (Bloodgood 2010), it appears that it will be a rare exception to find a flagellum that does not function in some capacity as a sensory antenna. Exploiting the flagellar membrane as a surface from which to detect extracellular ligands and using a slender flagellar antenna to concentrate (*i.e.* cluster) the protein players from signal transduction cascades provides opportunities for cells (or organisms) to respond rapidly and sensitively to even

small changes in the concentrations of stimulatory extracellular ligands. A compelling argument has been developed by Jékely and Arendt (2006) that suggests advantages offered from an enhanced capacity to sense and respond to environmental cues provided the driving force for evolution of the proto-flagellum in an early eukaryotic organism. It is a theory that also speaks to the evolution of the IFT system from other vesicular trafficking pathways (Jekely and Arendt 2006) and is compatible with the suggestion that the proto-centriole consisted of a single triplet blade, rather than a multi-triplet barrel. We will probably never know why flagella evolved in eukaryotes. However, should a novel mode of motility have provided the driving force for evolution of the organelle (the amoeboflagellate *Naegleria* provides a prime example of how a flagellate cell can swim a hundred times faster than an amoeboid cell can crawl), then this is also not inconsistent with a single triplet blade origin for the centriole since it has been suggested that the application of flagellar membrane adhesins to glide across a surface, rather than dynein-dependent microtubule sliding, was how flagella first conferred cell motility. In extant flagellate protists, gliding-based motility is common, and in *Chlamydomonas* gliding is also IFT-dependent (Shih et al. 2013), underscoring the centrality of IFT to multiple aspects of flagellum assembly and function.

Conservation of the protein inventory in motile flagella

How well does visual conservation of the iconic '9+2' microtubule ultrastructure align with the conservation of proteins that are incorporated into the '9+2' microtubule scaffold? Initial proteomic analyses of flagellum composition involved the analysis by two-dimensional gel electrophoresis of detergent-extracted flagella isolated from *Chlamydomonas* and indicated 250-300 different proteins adorn the outer-doublet and central pair microtubules, thereby contributing to the characteristic axonemal sub-architecture of dynein ATPases, radial spokes, and central pair projections.

Results from the early proteomic studies were reported in late 1970s and early 1980s (reviewed in (Luck 1984). 20-25 years on from those studies, and with nuclear genome sequences of model flagellates at hand, mass spectrometry-based proteomics of detergent-extracted flagella provided a candidate list of proteins not dissimilar in number to initial estimates (Broadhead et al. 2006, Pazour et al. 2005). Proteomics of intact flagella (*i.e.* combining axoneme, matrix, and flagellar membrane fractions), moreover, combined with results from genome comparisons between eukaryotes that build flagella with those that do not indicate that the number of proteins typically contributing to flagellum architecture and function is between 600 and 700 (Li et al. 2004, Pazour et al. 2005). Both comparative genomics and proteomics approaches have their limitations: for instance, the former cannot easily be used to identify flagellar protein kinases, phosphatases or other types of protein belonging to gene families where the functionality provided by the protein product is also used for core processes within the cell body of any eukaryote, and for the latter likely candidature of flagellar components identified by proteomics is always a function of the purity of biochemically fractionated material.

Flagellar candidacy of proteins identified by either *in silico* comparisons or proteomics is steadily being confirmed by experimental analyses of location and function. Unsurprisingly, given the importance of the structural conduit that extends from the central pair microtubules to both inner- and outer-dynein arms, flagellar proteomes are enriched with proteins containing domains that commonly function in mediating protein-protein interactions (*e.g.* WD and tetratricopeptide (TPR) repeats), as well as pfam domains associated with signal transduction cascades (Li et al. 2004, Pazour et al. 2005). What is surprising though are the number of candidate axonemal proteins in *Chlamydomonas*, *Trypanosoma brucei*, and the ciliate *Tetrahymena thermophila* (around a third of proteins in each flagellate) for which homologs in other organisms are not evident from sequence comparisons (Broadhead et al.

2006). Whilst these findings could be viewed as pointing towards lineage-specific novelties, it is important to remember structural studies of many protein classes indicate that primary sequence similarities between proteins sharing common ancestry diverge long before loss of shared protein folds. Indeed, comparisons of the major protein components from the eukaryotic and prokaryotic cytoskeletons (*i.e.* tubulin versus FtsZ and actin versus MreB family proteins) provide a prime illustration of this phenomenon (Wickstead and Gull 2011). Structural studies are therefore needed to gauge how closely conservation of '9+2' architecture across the breadth of evolution aligns with conservation of individual protein components. However, there are also very clear examples of variation in the evolutionary distribution of flagellar proteins with experimentally verified or, on a basis of their domain architecture, predicted regulatory functions.

Species-specific variation of regulatory networks

Changes in intraflagellar Ca^{2+} and cyclic nucleotide concentrations are ubiquitous regulators of motility, providing second messenger mediation of switches between ciliary- and flagellar-style waveforms and waveform reversal. A balance between the rates of synthesis and degradation regulates the steady-state cyclic nucleotide concentrations, whereas *trans*-membrane fluxes effect changes in intraflagellar Ca^{2+} concentration. The ecology of different flagellate cells influences the ways in which these ubiquitous second messengers regulate motility. For instance, in the freshwater- and soil-dwelling alga *Chlamydomonas*, intraflagellar Ca^{2+} concentrations underpin swimming behaviour in response to the alga's perception of changes in light intensity. In *Chlamydomonas*, the photo-response is all the more interesting because although the micro-compartmentalization of second messengers is key to dynamic amplification of signalling cascades, the phototactic response of *Chlamydomonas* requires transmission of signals initially detected at an eyespot located at an

equatorial position on the cell body, relative to the polar location of the flagella. This illustrates how holistic regulation of dynamic, transient changes to swimming patterns can extend far beyond the confines of the flagellar compartment. Photosynthetic activity is the physiological process in phototrophs most obviously influenced by changes in light intensity. The complexity of an ill-understood interplay between light-dependent and light-influenced processes in *Chlamydomonas* that takes place across different organelles (chloroplast and flagella) is further illustrated by the observation that the duration of the alga's photoresponse is influenced by photosystem I activity and NADPH:NADP turnover in the chloroplast (Wakabayashi and King 2006).

A more ubiquitous example of a recently characterised, but a still poorly understood regulatory network in flagella is ATP homeostasis. Movement of the IFT machinery, dynein-dependent sliding of outer-doublet microtubules, and cAMP synthesis are all energy-consuming processes. Yet, the evidence from proteomic datasets and biochemical studies for diverse ATP re-generating pathways operating within flagella of different protists during the 2000's still came as a surprise (Ginger et al. 2008). It indicates organelle assembly and motility cannot always rely on the diffusion of ATP from the cytosol. The regulation of dynein ATPase activity by ADP emphasizes further a requirement for intraflagellar nucleotide homeostasis although the environmental parameters under which flagellate protists need to make use of their intraflagellar ATP-regenerating systems is still not known.

Gene duplication and gene fusion provide a molecular basis through which species-specific elaboration of regulatory networks can occur, and again work with *Chlamydomonas* provides examples for each mechanism. Lineage-specific duplication of dynein heavy chains gives rise to differential localisation of individual isoforms along the flagellum (Yagi et al. 2009). The functional significance of proximal- and distal-specific dynein arms is not yet clear but points to a species-restricted, rather than a more widely distributed, regulatory network.

Chlamydomonas RSP2, a conserved radial spoke protein, provides a likely example of gene fusion. In most flagellate eukaryotes, RSP2 is a small protein (~120 amino acids) principally comprising of a Dpy-30 domain which forms a helix-loop-helix structure and is essential for radial spoke assembly. Yet, in *Chlamydomonas*, an additional C-terminal domain binds the ubiquitous Ca^{2+}-binding protein calmodulin, but is required only to support the normal helical swimming trajectory of *Chlamydomonas* under bright illumination (Gopal et al. 2012). In summary, as biologists mine through flagella proteome inventories it appears likely that structural approaches will be required to resolve just how many axonemal proteins are actually conserved across evolution, although examples of lineage-specific tinkering with regulatory proteins are also emerging.

Variation on a grander scale: extra-axonemal structures

Assembly of extra-axonemal structures occurs in a handful of flagellate eukaryotes, although insight into their function(s) and composition has relied on recent advances in reverse genetic and molecular approaches. Among protists, the lattice-like paraflagellar rod (PFR) seen in euglenozoan protists provides a well-studied, elegant example of an extra-axonemal structure. The Euglenozoa are a cosmopolitan group of unicellular flagellates, descended from a divergent, possibly even early-branching eukaryotic lineage, and include the parasitic trypanosomatid family (Tekle et al. 2009). Trypanosomatid species belonging to the genera *Trypanosoma* and *Leishmania* are responsible for several of the so-called 'neglected tropical diseases'. Composed of two major proteins PFR1 and PFR2 and ~30 proteins of lesser abundance, the PFR is a complex lattice of different filamentous structures that is attached to and runs alongside the axoneme (Portman and Gull 2010). It is also a multifunctional structure required for (i) motility, although it is difficult to distinguish whether motility is influenced more by the intrinsic biophysical properties of PFR structure or because a PFR

provides the platform into which regulatory enzymes are anchored; (ii) parasite attachment to epithelial surfaces within the insect vectors responsible for parasite transmission; and (iii) stable attachment of the flagellum to plasma membrane, which in some trypanosomatids is critical for normal cell morphogenesis (Ginger et al. 2013, Portman and Gull 2010).

Accessory structures are also found alongside the axoneme in mammalian sperm. In contrast to protists, and indeed other animal cells, where flagella represent *bona fide* organelles isolated from the rest of the cell body by the transition zone, mammalian sperm are better considered as a flagellate cell-type almost devoid of cytosol. Regarding the presence of accessory structures, however, an outer dense fibre is paired with each outer doublet microtubule along the length of the axoneme and a fibrous sheath surrounds the axoneme along the principal piece of the sperm tail (which accounts for ~3/4 of the flagellum length) (Eddy et al. 2003). Like the PFR, the fibrous sheath also acts as a scaffold into which enzymes assemble, most notably several glycolytic enzymes. Transgenic mice lacking the fibrous sheath-bound isoform of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the sole isoform of GAPDH found in sperm, complete spermatogenesis and build the fibrous sheath, but are infertile and exhibit severe motility defects, consistent with the idea that glycolytic flux provides the ATP necessary for sperm swimming (Miki et al. 2004). At least one fibrous sheath-bound glycolytic enzyme, enolase, however, is necessary for assembly of the accessory structure (Nakamura et al. 2013), as is the major sheath component AKAP4, which like the sheath components AKAP3 and TAKAP-80 serves as a protein-interaction partner for cAMP-dependent protein kinase A (Eddy et al. 2003, Miki et al. 2002). Thus, in very evolutionarily distant eukaryotes extra-axonemal structures are built in order to accommodate enzymes and regulatory proteins associated with energy homeostasis and signal transduction cascades. In the case of the fibrous sheath, scaffold integrity is dependent

upon the regulatory proteins that define its function; it is not yet clear if this is the case for the PFR.

Moderation of axoneme structure

Aside from extra-axonemal structures, variations on a canonical '9+2' microtubule architecture are also known. For instance, in many insect spermatozoa outer-doublet microtubules have an associated singlet microtubule (*i.e.* a '9+9+2' axoneme) (Dallai et al. 2006), whereas in spermatozoa from some arachnids three singlet central microtubules are seen (a '9+3' axoneme) (Michalik et al. 2003). In marine picoplankton of the genus *Micromonas* only a short 9+2 axoneme of ~1 μm length is built, but the central pair then extends for ~4 μm beyond the termination of the outer-doublet microtubules (Omoto and Witman 1981). However, variations on '9+2' architecture also occur via moderation of this iconic architecture. For instance, in moss, land plants that do produce flagellate sperm, the '9+2' axoneme is thought to lack outer dynein arms (Merchant et al. 2007). In flagellate gametes of the centric diatoms *Milosira* and *Thalassiosira*, radial spokes, central pair microtubules and inner dynein arms are absent (Idei et al. 2013), and in nodal cilia, which establish left-right asymmetry during the development of mammalian embryos, the central pair and radial spokes are also absent. More extreme is the change in underlying axonemal symmetry as seen, for example, in '6+0' and '3+0' axonemes built by male gametes of gregarine protists (Prensier et al. 2008, Schrevel and Besse 1975). Loss of the central pair microtubules and radial spokes often yields a helical waveform, rather than the ciliary or flagellar waveforms seen in flagella with a '9+2' axoneme. Yet, moderation without loss of a canonical '9+2' architecture is also evident in evolution, and malarial parasites provide a prime example.

Globally, one of the leading causes of human mortality is malaria; the species responsible for much of the morbidity and mortality associated with the disease is *Plasmodium falciparum*. Malarial parasites undergo an obligatory sexual cycle during their life cycle that begins with gametocytogenesis in the host and culminates with fertilization within the mid-gut of a mosquito vector. Flagellum biogenesis in the male gamete is unusual not least because IFT-independent assembly of the axoneme occurs in the cytosol and the flagellum is produced from a rapid exflagellation (Sinden et al. 1976). Male gamete maturation, including axoneme assembly and exflagellation takes places within fifteen minutes following the perception of environmental cues within the mosquito mid-gut, with axoneme assembly taking as little as three minutes to occur. Unsurprisingly, axoneme assembly is error prone, but then again, fertilization is also completed soon after ingestion of an infected bloodmeal and the male gamete is only motile for up to 1 hour following exflagellation. Glycolysis as the major, if not sole, source of ATP to support motility is again evident (Sinden et al. 2010), but given the ongoing challenge of elucidating mechanisms through which flagellar motility is regulated, a very significant outcome from comparative genomic and proteomic analyses is the indication that very few conserved flagellar proteins are present in *Plasmodium* species (Broadhead et al. 2006, Sinden et al. 2010). This suggests assembly of a stripped-down '9+2' axoneme. From a systems biology perspective, *Plasmodium* parasites therefore provide a genetically tractable system from which to decipher a minimal regulatory system supporting motility of a flagellum with a '9+2' axoneme.

Moderation of axoneme ultrastructure is even evident within axonemes built by the model flagellate *Chlamydomonas*. Thus, in most flagellates, three radial spokes are present with 96 nm periodicity per outer-doublet microtubule along the length of the axoneme, but for more than thirty years it has been known that in *Chlamydomonas* only two spokes are present per 96 nm. The reason for this difference was unknown. In the last decade, the rise of cryo-

electron tomography as an experimental tool in cell biology has facilitated high resolution views of axoneme ultrastructure and led to the identification of new multi-protein complexes, which provide seemingly unbroken connectivity to the regulatory conduit that links outer and inner dynein ATPases with the central pair projections. Recently, electron tomographic views of *Chlamydomonas* axonemes provided an explanation for the enigma of missing radial spoke 3. Thus, in *Chlamydomonas*, in place of the third radial spoke there is instead a stump into which a third spoke could be anchored (Pigino et al. 2011). The parsimonious explanation for this observation is that degeneration of radial spoke 3 has occurred during evolution of flagellate green algae. Moreover, high resolution views of radial spoke architecture in another flagellate protist, *Tetrahymena thermophila*, which builds cilia possessing three radial spokes, indicates radial spoke 3 is likely to differ in its protein composition relative to spokes 1 and 2 (Pigino et al. 2011). To our knowledge, no examples of conserved axonemal proteins that are unexpectedly missing from *Chlamydomonas* have been reported. So, in the same way extensive moderation of malarial parasite '9+2' axonemes potentially informs on a minimal network for regulation of flagellar motility, further study of recently revealed differences in spoke composition and organisation in *Chlamydomonas*, relative to other flagellates, is likely to provide greater mechanistic understanding of how radial spokes 1, 2, and 3 each impact upon dynein activity, and thence waveform regulation.

'Ciliary' proteins in other guises

(a) Plants

The most extreme form of axoneme moderation is, of course, loss of the ability to build a flagellum. Secondary loss of flagella has occurred on multiple occasions during eukaryotic evolution, most obviously during evolution of the fungi, red algae, land plants, and many

amoebae. Recently constructed phylogenies indicate the secondary loss of flagella among plant and fungal lineages is surprisingly complex, and has occurred repeatedly during the evolution of these taxonomically diverse groups (Hodges et al. 2012, James et al. 2006). Loss of the capacity to build a flagellum offers an opportunity to ask the question of what happens to the several hundred genes encoding proteins required for assembly and function of a typical motile flagellum. Are they all lost from the genome or do alternative functions remain or evolve for the protein products encoded by some? With publicly available nuclear genome sequences accumulating for diverse aflagellate eukaryotes it is possible to begin to answer this question.

One recent genomic analysis led to the identification in angiosperms (land plants that possess neither flagella nor centrioles) of a small cohort of genes (~20) for which homologs are otherwise normally only found in flagellate eukaryotes (Hodges et al. 2011). Closer analysis of these genes suggests only one (a homolog of axonemal protein BUG22) owes its origin to a gene duplication event and subsequent divergence of the duplicates prior to loss of a capacity to build flagella, and thence the degeneration of flagellum-associated genes, in an angiosperm ancestor. Did the remaining 'flagellum-associated' genes retained in *Arabidopsis thaliana*, *Oryza sativa*, and *Populus trichocarpa* (the angiosperms analysed by Hodges et al. 2011) gain alternative, non-ciliary functions *prior to* or *following* the loss of cilia in a common ancestor? Intriguingly, high expression of some of the retained genes in pollen suggests these gene products still have gamete-associated function(s) and the experimental characterization of others indicates they are required for microtubule-associated cytoskeletal processes (Hodges et al. 2011).

The list of experimentally studied proteins includes a likely protein phosphatase 2A regulatory sub-unit encoded by the TONNEAU2/FASS gene and a phragmoplast-localised protein kinase, FUSED. The former is required for organization of the cortical cytoskeleton,

and is thus a determinant of cell morphology (Camilleri et al. 2002, Wright et al. 2009). Regarding the latter, the phragmoplast is a plant cell-specific microtubule array formed during mitosis and FUSED is required for cytokinesis (Oh et al. 2005).

A protein not reported as a candidate ciliary protein conserved in angiosperms, but is nonetheless clearly homologous to a protein associated with cilium assembly in flagellate eukaryotes is TONNEAU1. TONNEAU1 is most closely related to a centrosomal protein from animals called FOP, and failure to identify TONNEAU1 as a ciliary protein conserved in angiosperms perhaps merely serves to highlight the common limitation of using automated pipelines to identify candidate orthologous proteins (*i.e.* proteins encoded by genes that are separated only by speciation) that are divergent at the level of their primary amino acid sequence. It suggests further flagellum-associated proteins may await discovery in land plants that do not build flagella. TONNEAU1 is required for normal cell morphogenesis: it orchestrates organization of the cortical microtubule cytoskeleton, interacts with the classic MTOC protein centrin, and has most recently been detected in a protein complex containing TONNEAU2 (Azimzadeh et al. 2008, Spinner et al. 2010, Spinner et al. 2013). In other work, a screen for TONNEAU1-interacting proteins identified a novel family of 34 proteins that share in common six short sequence motifs; conservation of some of these motifs, including their order of appearance, is evident in another animal centrosomal protein, CEP350 (Drevensek et al. 2012). Collectively, recently published data are therefore rapidly revealing previously hidden evolutionary commonalities between the centrosome in animal cells and MTOCs of the plant cortical cytoskeleton.

It is plausible that not all of the 'ciliary' proteins retained in angiosperms function in cytoskeletal processes. Then, the question would be how any gain of new function(s) occurred. Here, we provide two alternative views. First, if we consider another obvious example of multi-cellularity among eukaryotes, the animals, then their phenotypic complexity

is not explained simply by an increase in the number of protein-coding genes present in the genome, relative to near unicellular relatives. Instead, multiple mechanisms contribute to the generation of protein diversity including alternative splicing; in the case of the human genome over 90% of protein-coding genes are alternative spliced, often with tissue-specific dependency, resulting in protein isoforms that differ markedly with regard to function(s) and/or intracellular localisation(s) (Wang et al. 2008). In plants, alternative splicing has also emerged as an important mechanism for generating protein diversity (Reddy et al. 2013). Thus, alternative splicing could have readily provided the mechanism by which the function or localization of some ancestrally ciliary proteins changed in an angiosperm ancestor; following loss of the ability to build flagella during land plant evolution, alternatively spliced gene products conferring significant fitness benefits would provide a selective pressure for retention of a 'ciliary' gene.

Alternatively, retention and neo-functionalization in plants of proteins otherwise associated with ciliary function can also be explained by the concept of protein moonlighting. Thus, a classic reductionist view is that individual proteins typically have a single function, but examples of proteins fulfilling multiple, often very different, roles inside cells are becoming increasingly common (Copley 2012). The concept of moonlighting is different from the functional diversity that results from alternative splicing in that it describes the functionality of an individual polypeptide in multiple, distinct cellular processes. For moonlighters, functionality in different processes may occur as a consequence of conformational change induced by post-translational modification or utilise different regions of protein surface. For instance, moonlighting functions were first observed for abundant, soluble enzymes, such as those involved in the glycolytic pathway, where the active site used for catalysis represents only a small part of a protein surface. The prevalence of protein moonlighting is now such that it is no longer considered an oddity; indeed some consider that protein multi-

functionality is likely to contribute very significantly to cellular and organismal complexity (Copley 2012). If any ciliary proteins had assumed 'moonlighting' functions in an angiosperm ancestor, then fitness benefits conferred by some of those moonlighting activities could again explain the retention of genes encoding typically 'ciliary' proteins in land plants such as *Arabidopsis*.

(b) Apicomplexan parasites

We mentioned earlier the variations on typical '9+2' axoneme architecture seen in malarial and gregarine parasites. These protists belong to the phylum Apicomplexa. Over 5000 apicomplexan species have been described. Some exist as cysts in the environment and others undergo an obligatory sexual stage in order to complete often complex life cycles, but the apicomplexans are more generally thought of as a family of obligate intracellular parasites. They are thought to have evolved from free-living marine algae; among their closest extant relations are the recently discovered chromerid algae from the Australian Great Barrier Reef and the predatory colpodellids (Kuvardina et al. 2002, Moore et al. 2008). As with many other eukaryotic lineages some apicomplexans have secondarily lost the ability to build a flagellum, but as a group the apicomplexans owe their name to the unique, polarity-defining MTOC called the 'apical polar ring' from which sub-pellicular microtubules that define cell shape radiate. Structures homologous to the apical polar ring are evident in the chromerids and colpodellids, and recent studies suggest a flagellum-based origin for a structure that in the Apicomplexa is involved in cell invasion, as well as the definition of cell shape (Portman and Slapeta 2014).

In some apicomplexans, such as *Toxoplasma gondii*, an extremely successful parasite of vertebrates globally and opportunistic pathogen of the immunocompromised, a spiral-like arrangement of tubulin sheets folded in the shape of a cone, and known as the 'conoid' is also associated with the apical polar ring **(figure 4)**. Genetic tractability and easy visualization by

microscopy make *Toxoplasma* a well-used model for many general studies of apicomplexan cell biology. In the animal host, extracellular *Toxoplasma* parasites extend and retract the conoid through the apical polar ring, a process thought to contribute mechanically to the process of host cell invasion (Gubbels and Duraisingh 2012). Recently, it has been observed that following duplication of the parasite centrosome, which is associated with the nucleus, a fibrous connection extends from the duplicated centrosome to the apical polar ring providing a hard-wired link that helps orchestrate daughter cell budding. With regard to a possible connection with eukaryotic flagella, essential components of this fibrous connection appear homologous to proteins that form the rootlet fibres which link the flagellar basal bodies in *Chlamydomonas* (Francia et al. 2012).

Further evidence of a possible ancient relationship between the flagellar cytoskeleton and the cytoskeletal apparatus that facilitates cell invasion by *Toxoplasma* comes from the localisation of SAS6-like proteins to the conoid in *Toxoplasma* and to proximal end of the axoneme in trypanosomes (potentially to the basal plate from which the central pair microtubules are nucleated) (de Leon et al. 2013). Thus, the N-terminal region of SAS6, a protein integral to nine-fold symmetry of the centriole, provides the conserved domain annotated in the pfam database (pfam_B_2528) and is the feature also shared with SAS6-like proteins. Despite the large evolutionary distance between trypanosomes and apicomplexans (which spans major eukaryotic groups (Tekle et al. 2009)), and even though SAS-like orthologs are absent from animals and their nearest flagellate unicellular relatives (de Leon et al. 2013), the flagellar localization of SAS6-like protein in trypanosomes raises a possibility that during apicomplexan evolution flagellar SAS6-like protein found a novel function within the conoid structure as this unusual cytoskeletal structure evolved. If the view of a flagellum-based origin for the apical polar ring is correct, then not only does it provide an unexpected example of co-opting flagellar proteins into an alternative structure but it also provides an

intriguing comparison with retention and function of genes encoding 'ciliary' proteins in acentriolar plants, or indeed any other eukaryotes.

(c) other aflagellate eukaryotes

How many proteins with known or inferred flagellum functions will turn out to have homologs (or orthologs) in other eukaryotes that do not build flagella (*i.e.* most fungi, red algae, and a wide variety of heterotrophic protists) is really a topic for future investigations, although some intriguing observations have been recorded. For instance, miniature green alga belonging to the genus *Ostreococcus* are abundant oceanic picoplankton abundant and are believed to lack the capacity to build flagella; yet around forty proteins associated with flagellum function are still encoded within the nuclear genomes of different *Ostreococcus* species (Merchant et al. 2007). In contrast, using the same pipeline as for their identification of 'ciliary profile' proteins in land plants, Hodges et al. (2011) predict that the number of 'flagellar' proteins in fungi, at least, will be lower than in aflagellate land plants.

Closing summary

It is approaching 350 years since cilia or flagella were first described by Antoni van Leeuwenhoek as the 'little legs' on the protists he saw in rainwater using a homemade microscope (Leeuwenhoek 1677), a little over a 100 years since a sensory function was first suggested for the primary cilium (Zimmermann 1898), just over 60 years since the iconic '9+2' arrangement of axonemal microtubules was first predicted by Irène Manton and Bryan Clarke from their analysis by electron microscopy of zoospore ultrastructure in *Sphagnum* peat moss (Manton and Clarke 1952), and 50 years since Peter Satir and Ian Gibbons began to develop the dynein-dependent sliding filament model as the basis for flagellar and ciliary motility (Satir et al. 2014). It is during the last 30 or so years that molecular bases

underpinning the diversity in form and function of eukaryotic flagella, and their importance to human health have become apparent. As with other aspects of modern cell biology, our understanding of flagellum origins, function, and variation, and the ensuing paradigm revisions, have been hugely informed by advances in molecular biology and DNA techniques. Indeed, it is only recently apparent that the versatility of eukaryotic flagella as organelles of motility and sensory perception is exploited across the breadth of eukaryotic evolution (Bloodgood 2010, Brown and Witman 2014). Further surprises regarding flagellar (or ciliary) versatility continue to be revealed; for instance, the immunological synapse – a specialised surface membrane region formed when cytotoxic T lymphocytes recognise target cells and from which cytotoxic granules are secreted – has very recently been proposed to represent a highly modified cilium (de la Roche et al. 2013). With the new view of the flagellum as a secretory organelle (Wood et al. 2013), it might come as no surprise if opinion as to whether the eukaryotic flagellum ancestrally arose as either a sensory or motile organelle is called into question.

Acknowledgements

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References cited

Azimzadeh J, Nacry P, Christodoulidou A, Drevensek S, Camilleri C, Amiour N, Parcy F, Pastuglia M, Bouchez D. 2008. *Arabidopsis* TONNEAU1 proteins are essential for preprophase band formation and interact with centrin. Plant Cell 20: 2146-2159.

Bloodgood RA. 2010. Sensory reception is an attribute of both primary cilia and motile cilia. Journal of Cell Science 123: 505-509.

Broadhead R, et al. 2006. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature 440: 224-227.

Brown JM, Witman GB. 2014. Cilia and diseases. Bioscience.

Camilleri C, Azimzadeh J, Pastuglia M, Bellini C, Grandjean O, Bouchez D. 2002. The *Arabidopsis* TONNEAU2 gene encodes a putative novel protein phosphatase 2A regulatory subunit essential for the control of the cortical cytoskeleton. Plant Cell 14: 833-845.

Carvalho-Santos Z, Azimzadeh J, Pereira-Leal JB, Bettencourt-Dias M. 2011. Evolution: Tracing the origins of centrioles, cilia, and flagella. Journal of Cell Biology 194: 165-175.

Copley SD. 2012. Moonlighting is mainstream: paradigm adjustment required. Bioessays 34: 578-588.

Czarnecki PG, Shah JV. 2012. The ciliary transition zone: from morphology and molecules to medicine. Trends in Cell Biology 22: 201-210.

Dallai R, Lupetti P, Mencarelli C. 2006. Unusual axonemes of hexapod spermatozoa. International Review of Cytology 254: 45-99.

de la Roche M, Ritter AT, Angus KL, Dinsmore C, Earnshaw CH, Reiter JF, Griffiths GM. 2013. Hedgehog signaling controls T cell killing at the immunological synapse. Science 342: 1247-1250.

de Leon JC, Scheumann N, Beatty W, Beck JR, Tran JQ, Yau C, Bradley PJ, Gull K, Wickstead B, Morrissette NS. 2013. A SAS-6-like protein suggests that the *Toxoplasma* conoid complex evolved from flagellar components. Eukaryotic Cell 12: 1009-1019.

Deane JA, Cole DG, Seeley ES, Diener DR, Rosenbaum JL. 2001. Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. Current Biology 11: 1586-1590.

Drevensek S, et al. 2012. The *Arabidopsis* TRM1-TON1 interaction reveals a recruitment network common to plant cortical microtubule arrays and eukaryotic centrosomes. Plant Cell 24: 178-191.

Eddy EM, Toshimori K, O'Brien DA. 2003. Fibrous sheath of mammalian spermatozoa. Microscopy Research Techniques 61: 103-115.

Francia ME, Jordan CN, Patel JD, Sheiner L, Demerly JL, Fellows JD, de Leon JC, Morrissette NS, Dubremetz JF, Striepen B. 2012. Cell division in Apicomplexan parasites is organized by a homolog of the striated rootlet fiber of algal flagella. PLoS Biology 10: e1001444.

Ginger ML, Portman N, McKean PG. 2008. Swimming with protists: perception, motility and flagellum assembly. Nature Reviews Microbiology 6: 838-850.

Ginger ML, Collingridge PW, Brown RW, Sproat R, Shaw MK, Gull K. 2013. Calmodulin is required for paraflagellar rod assembly and flagellum-cell body attachment in trypanosomes. Protist 164: 528-540.

Goetz SC, Anderson KV. 2010. The primary cilium: a signalling centre during vertebrate development. Nature Reviews Genetics 11: 331-344.

Gopal R, Foster KW, Yang P. 2012. The DPY-30 domain and its flanking sequence mediate the assembly and modulation of flagellar radial spoke complexes. Molecular and Cellular Biology 32: 4012-4024.

Gubbels MJ, Duraisingh MT. 2012. Evolution of apicomplexan secretory organelles. International Journal for Parasitology 42: 1071-1081.

Hilbert M, et al. 2013. *Caenorhabditis elegans* centriolar protein SAS-6 forms a spiral that is consistent with imparting a ninefold symmetry. Proceedings of the National Academy of Sciences USA 110: 11373-11378.

Hodges ME, Wickstead B, Gull K, Langdale JA. 2011. Conservation of ciliary proteins in plants with no cilia. BMC Plant Biology 11: 185.

—. 2012. The evolution of land plant cilia. New Phytologist 195: 526-540.

Idei M, Osada K, Sato S, Nakayama T, Nagumo T, Mann DG. 2013. Sperm ultrastructure in the diatoms *Melosira* and *Thalassiosira* and the significance of the 9 + 0 configuration. Protoplasma 250: 833-850.

James TY, et al. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443: 818-822.

Jekely G, Arendt D. 2006. Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. Bioessays 28: 191-198.

Kitagawa D, et al. 2011. Structural basis of the 9-fold symmetry of centrioles. Cell 144: 364-375.

Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC. 2013. Molecular paleontology and complexity in the last eukaryotic common ancestor. Critical Reviews in Biochemistry and Molecular Biology 48: 373-396.

Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL. 1993. A motility in the eukaryotic flagellum unrelated to flagellar beating. Proceedings of the National Acadeny of Sciences USA 90: 5519-5523.

Kuvardina ON, Leander BS, Aleshin VV, Myl'nikov AP, Keeling PJ, Simdyanov TG. 2002. The phylogeny of colpodellids (Alveolata) using small subunit rRNA gene sequences suggests they are the free-living sister group to apicomplexans. Journal of Eukaryotic Microbiology 49: 498-504.

Leeuwenhoek AV. 1677. Observations, Communicated to the Publisher by Mr. Antony van Leewenhoeck, in a Dutch Letter of the 9th of Octob. 1676. Here English'd: concerning little animals by him obferved in rain- well- sea- and snow-water; as alfo in water wherein pepper had lain infufed. Philosophical Transactions 12: 821-831.

Li JB, et al. 2004. Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. Cell 117: 541-552.

Luck DJ. 1984. Genetic and biochemical dissection of the eucaryotic flagellum. Journal of Cell Biology 98: 789-794.

Manton I, Clarke B. 1952. An Electron Microscope Study of the Spermatozoid of *Sphagnum*. Journal of Experimental Botany 3: 265-&.

Marshall WF. 2009. Centriole evolution. Current Opinion in Cell Biology 21: 14-19.

Merchant SS, et al. 2007. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318: 245-250.

Michalik P, Gray MR, Alberti G. 2003. Ultrastructural observations of spermatozoa and spermiogenesis in *Wandella orana* Gray, 1994 (Araneae: Filistatidae) with notes on their phylogenetic implications. Tissue and Cell 35: 325-337.

Miki K, Willis WD, Brown PR, Goulding EH, Fulcher KD, Eddy EM. 2002. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. Developmental Biology 248: 331-342.

Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, Perreault SD, Eddy EM, O'Brien DA. 2004. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Proceedings of the National Academy of Sciences USA 101: 16501-16506.

Moore RB, et al. 2008. A photosynthetic alveolate closely related to apicomplexan parasites. Nature 451: 959-963.

Nakamura N, Dai Q, Williams J, Goulding EH, Willis WD, Brown PR, Eddy EM. 2013. Disruption of a spermatogenic cell-specific mouse enolase 4 (eno4) gene causes sperm structural defects and male infertility. Biology of Reproduction 88: 90.

Nakazawa Y, Hiraki M, Kamiya R, Hirono M. 2007. SAS-6 is a cartwheel protein that establishes the 9-fold symmetry of the centriole. Current Biology 17: 2169-2174.

Oh SA, Johnson A, Smertenko A, Rahman D, Park SK, Hussey PJ, Twell D. 2005. A divergent cellular role for the FUSED kinase family in the plant-specific cytokinetic phragmoplast. Current Biology 15: 2107-2111.

Omoto CK, Witman GB. 1981. Functionally significant central-pair rotation in a primitive eukaryotic flagellum. Nature 290: 708-710.

O'Toole ET, Giddings TH Jr, Porter ME, Ostrowski LE. 2012. Computer-assisted image analysis of human cilia and *Chlamydomonas* flagella reveals both similarities and differences in axoneme structure. Cytoskeleton 69: 577-590.

Pazour GJ, Agrin N, Leszyk J, Witman GB. 2005. Proteomic analysis of a eukaryotic cilium. Journal of Cell Biology 170: 103-113.

Piasecki BP, LaVoie M, Tam LW, Lefebvre PA, Silflow CD. 2008. The Uni2 phosphoprotein is a cell cycle regulated component of the basal body maturation pathway in *Chlamydomonas reinhardtii*. Molecular Biology of the Cell 19: 262-273.

Pigino G, Bui KH, Maheshwari A, Lupetti P, Diener D, Ishikawa T. 2011. Cryoelectron tomography of radial spokes in cilia and flagella. Journal of Cell Biology 195: 673-687.

Pigino G, Maheshwari A, Bui KH, Shingyoji C, Kamimura S, Ishikawa T. 2012. Comparative structural analysis of eukaryotic flagella and cilia from *Chlamydomonas*, *Tetrahymena*, and sea urchins. Journal of Structural Biology 178: 199-206.

Portman N, Gull K. 2010. The paraflagellar rod of kinetoplastid parasites: from structure to components and function. International Journal for Parasitology 40: 135-148.

Portman N, Slapeta J. 2014. The flagellar contribution to the apical complex: a new tool for the eukaryotic Swiss Army knife? Trends in Parasitology 30: 58-64.

Prensier G, Dubremetz JF, Schrevel J. 2008. The unique adaptation of the life cycle of the coelomic gregarine *Diplauxis hatti* to its host *Perinereis cultrifera* (Annelida, Polychaeta): an experimental and ultrastructural study. Journal of Eukaryotic Microbiology 55: 541-553.

Reddy AS, Marquez Y, Kalyna M, Barta A. 2013. Complexity of the alternative splicing landscape in plants. Plant Cell 25: 3657-3683.

Roger AJ, Simpson AG. 2009. Evolution: revisiting the root of the eukaryote tree. Current Biology 19: R165-167.

Satir P, Heuser T, Sale WS. 2014. A structural basis for how motile cilia beat. Bioscience.

Schrevel J, Besse C. 1975. A functional flagella with a 6 + 0 pattern. Journal of Cell Biology 66: 492-507.

Shih SM, Engel BD, Kocabas F, Bilyard T, Gennerich A, Marshall WF, Yildiz A. 2013. Intraflagellar transport drives flagellar surface motility. Elife 2: e00744.

Sinden RE, Canning EU, Spain B. 1976. Gametogenesis and fertilization in *Plasmodium yoelii nigeriensis*: a transmission electron microscope study. Proceedings of the Royal Society London Series B Biological Sciences 193: 55-76.

Sinden RE, Talman A, Marques SR, Wass MN, Sternberg MJ. 2010. The flagellum in malarial parasites. Current Opinion in Microbiology 13: 491-500.

Spinner L, Pastuglia M, Belcram K, Pegoraro M, Goussot M, Bouchez D, Schaefer DG. 2010. The function of TONNEAU1 in moss reveals ancient mechanisms of division plane specification and cell elongation in land plants. Development 137: 2733-2742.

Spinner L, et al. 2013. A protein phosphatase 2A complex spatially controls plant cell division. Nature Communications 4: 1863.

Tekle YI, Parfrey LW, Katz LA. 2009. Molecular Data are Transforming Hypotheses on the Origin and Diversification of Eukaryotes. Bioscience 59: 471-481.

van Dam TJ, Townsend MJ, Turk M, Schlessinger A, Sali A, Field MC, Huynen MA. 2013. Evolution of modular intraflagellar transport from a coatomer-like progenitor. Proceedings of the National Academy of Sciences USA 110: 6943-6948.

Wakabayashi K, King SM. 2006. Modulation of *Chlamydomonas reinhardtii* flagellar motility by redox poise. Journal of Cell Biology 173: 743-754.

Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. 2008. Alternative isoform regulation in human tissue transcriptomes. Nature 456: 470-476.

Wickstead B, Gull K. 2011. The evolution of the cytoskeleton. Journal of Cell Biology 194: 513-525.

Wood CR, Huang K, Diener DR, Rosenbaum JL. 2013. The cilium secretes bioactive ectosomes. Current Biology 23: 906-911.

Wright AJ, Gallagher K, Smith LG. 2009. discordia1 and alternative discordia1 function redundantly at the cortical division site to promote preprophase band formation and orient division planes in maize. Plant Cell 21: 234-247.

Yagi T, Uematsu K, Liu Z, Kamiya R. 2009. Identification of dyneins that localize exclusively to the proximal portion of *Chlamydomonas* flagella. Journal of Cell Science 122: 1306-1314.

Zimmermann KW. 1898. Beiträge zur Kenntniss einiger Drüsen und Epithelien. Archiv für mikroskopische Anatomie 52: 552-706.

Figure Legends

Figure 1. *Diversity of cilia and flagella. (a) Cilia or flagella project as whip-like structures from the cell surface; a cross-section through a flagellum reveals the arrangement of outer-doublet (arrowheads) and central pair (asterisk) microtubules in a motile axoneme. (b) motile cilia lining the human respiratory tract. (c) Human sperm. (d) Chlamydomonas sp., a biflagellate green alga and principal model system for many studies of flagellar biology. (e) The voracious predator Didinium sp. devouring its favoured prey Paramecium; note the two bands of cilia that support the motility of this ciliate. (f-g) In other ciliates, e.g. Paramecium caudatum, cilia are arrayed across the cell surface. Cilia are also present along the oral groove and are used to waft food into the oral cavity (arrowhead). In the image shown in (g) the ciliates are on a mat of false-coloured filamentous Oscillatoria cyanobacteria. (h) The intestinal parasite and possibly ancient eukaryote Giardia lamblia; its basal bodies are located deep in the cytoplasm and the axonemes of eight flagella traverse through the cytosol before emerging from different exit points on the cell surface. (i) The uniflagellate human sleeping sickness parasite Trypanosoma brucei shown amongst red blood cells. All images used with permission from Encyclopaedia Britannica ImageQuest.*

Figure 2. *Axoneme ultrastructure. (a) Cartoon schematic of a transverse section through a typical '9+2' microtubule axoneme with key architectural features highlighted. (b) A transmission electron micrograph of a transverse section through a detergent-extracted axoneme isolated from human respiratory epithelium; image averaging through 110 doublet microtubules allows the individual protofilaments of outer-doublet microtubules to be seen. (c) Electron tomographic averaging of the 96 nm periodicity in axonemes from the ciliate Tetrahymena thermophila. Images in (b) are reproduced with permission from Figure 1B and*

1D in O'Toole et al. 2012; image in (c) reproduced with permission from Figure 6D in Pigino et al. 2012.

Figure 3. *SAS-6 and nine-fold radial symmetry of the centriole. (a) A transmission electron micrograph of a longitudinal section through the basal body (centriole) and proximal region of a Chlamydomonas flagellum – stacks of cartwheel structures that define the proximal pole of the centriole are arrowed. (b) A transmission electron micrograph of a transverse section through the proximal pole of the Chlamydomonas basal body showing the 'hub and spoke' arrangement of the cartwheel structure. (c) Structural model showing the assembly of nine SAS-6 homodimers into a nine-fold symmetrical cartwheel structure. (d) Cartoon showing the SAS-6-based cartwheel within the context of the triplet microtubules of the basal body/centriole. Images in (a) and (b) are reproduced from Figure 2C in Nakazawa et al. 2007 ; images in (c) and (d) are reproduced from Figures 5B and 7, respectively in Kitagawa et al. 2011 under the terms of a Creative Common Attribution 3.0 unported licence.*

Figure 4. *Apical polar ring – flagellum connections? (a) Cartoons of Toxoplasma gondii tachyzoites (which invade and replicate within host cells) and flagellate micro-gametes – the centriole-containing centrosome is located close to the nucleus distant from the apical polar ring (APR); the closed conoid (CC) is topped by preconoidal rings (PCR), and the rhoptries are secretory organelles that release their contents during cell invasion. Note the very different morphologies of tachyzoites versus biflagellate micro-gametes, and an absence of the APR and associated structures in the latter. (b) Toxoplasma tachyzoites expressing SAS-6 protein fused to yellow fluorescent protein, grown within human fibroblasts, and decorated for immunofluorescence microscopy with anti-sera recognizing SAS-6 like protein: SAS-6 is*

found only within the centrioles and SAS-6 like protein is present only at the apical end of the cell; the central position of the nucleus is also shown. Images in (a) and (b) are reproduced from Figures 6A and 1B, respectively in de Leon et al. 2013 under the terms of a Creative Common Attribution 3.0 unported licence.

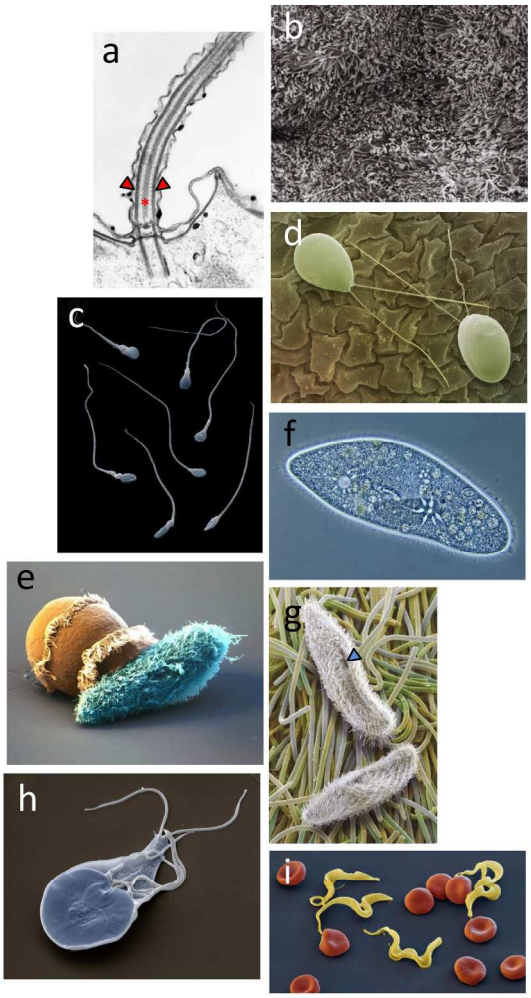

Figure 1

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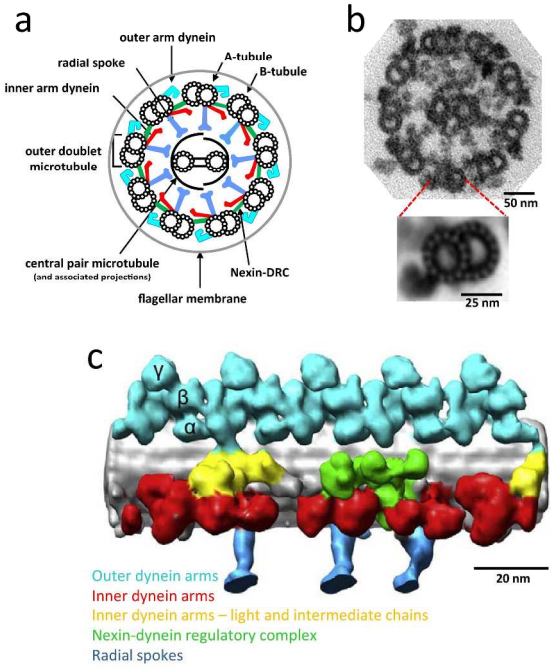

Figure 2

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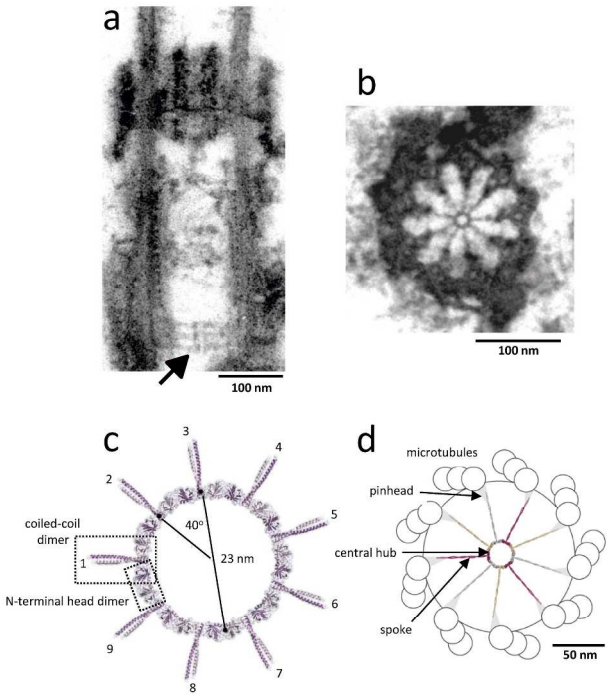

Figure 3

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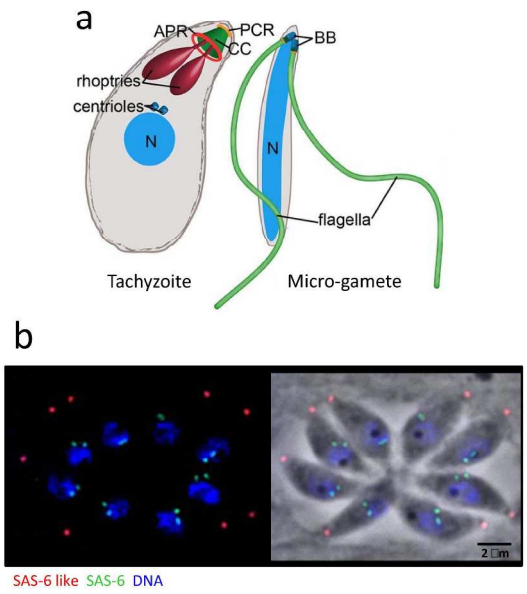

Figure 4

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