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Motility and more: the flagellum of *Trypanosoma brucei*

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Abstract

A central feature of trypanosome cell biology and life cycle is the parasite's single flagellum, which is an essential and multifunctional organelle involved in cell propulsion, morphogenesis and cytokinesis. The flagellar membrane is also a specialized subdomain of the cell surface that harbors multiple parasite virulence factors with roles in signaling and host-parasite interactions. In this review, we discuss the structure, assembly and function of the trypanosome flagellum, including canonical roles in cell motility as well as novel and emerging roles in cell morphogenesis and host-parasite interaction.

INTRODUCTION

African trypanosomes, such as *Trypanosoma brucei* and related species, belong to the order kinetoplastida, which also includes the human pathogens *T. cruzi* and *Leishmania spp*. Together, these protozoan parasites cause significant human morbidity and mortality worldwide and limit economic development in some of the most impoverished regions of the world [1–3]. Here we focus on *T. brucei*, the causative agent of African trypanosomiasis <<Glossary>>, also known as sleeping sickness in humans. The disease is transmitted to humans by tsetse flies (box 1) and proceeds in two clinical stages: the hemolymphatic stage, during which parasites replicate in the blood and lymph, causing clinical manifestations of recurrent fever and general malaise; and the second, invasive phase, during which parasites move out of the bloodstream and into extravascular spaces, including cardiac tissue and the central nervous system (CNS). This second stage is marked by meningoencephalitis, headaches and severe neurological changes that disrupt the sleep-wake cycle, and if left untreated it is followed by coma and death [4]. Sleeping sickness poses a threat to an estimated 60 million people. No vaccine exists and treatments are antiquated, toxic and increasingly ineffective [5].

T. brucei has a single flagellum, which is present during all stages of development. The flagellum is essential for viability [6], is the sole means of motility and has emerged as a key player in multiple facets of development, transmission and pathogenesis (box 1). The distinctive auger-like motility of *T. brucei* (Video 1) in fact provided the basis for naming the genus. In 1843, in one of the earliest descriptions, Gruby [7] observed that the organism,

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which he identified in frog blood, "…*turns two or three times around its axis, like a drill or a corkscrew, which is why I propose to name this hematozoan 'Trypanosoma*'." The name combines the Greek words trypanon (auger) and soma (body), so literally translated it is an "auger body". Since then, trypanosome motility has captured the attention of many scientists and an "undulating membrane" [8] (now known to correspond to the flagellum) is a prominent feature of most descriptions.

Trypanosome movements to specific host tissues are defining events in pathogenesis and transmission, thus emphasizing the importance of the flagellum's motility function (box 1). In addition to its canonical role in motility, the *T. brucei* flagellum is important for morphogenesis and cell division. The flagellum is also a crucial host-parasite interface that mediates attachment to host tissues and provides a scaffold for the assembly of signaling proteins and virulence factors that function in host-parasite interactions. In this review, we outline *T. brucei* flagellum structure and assembly, and then discuss how this organelle functions in motility, morphogenesis and host-parasite interactions. Where relevant, we point out eukaryotic-conserved versus trypanosome-specific flagellum features.

FLAGELLUM STRUCTURE

Overview

The trypanosome flagellum emerges from the cell posterior and defines the anterior-posterior axis (Figure 1). It is built on a canonical 9 + 2 axoneme, the cytoskeletal core of the flagellum, that contains 9 doublet microtubules symmetrically arranged around a pair of singlet microtubules [9]. The axoneme is anchored in the cytoplasm at the cell's posterior via the basal body, a barrel-like structure containing 9 peripheral triplet microtubules and no central pair. As the basal body extends outward, triplet microtubules become doublets, forming the axoneme transition zone. Chalice-shaped filaments connect transition zone microtubules to the surrounding membrane, forming a ciliary necklace [10]. The transition zone ends at the basal plate, which marks the site where central pair microtubules and hence the 9 + 2 axoneme begin.

The transition zone and the first portion of the axoneme exit the cytoplasm through a specialized invagination of the plasma membrane, termed the flagellar pocket. The flagellar pocket corresponds to a gap in the subpellicular microtubules and is the exclusive site for endocytosis and secretion, making it a key portal for host-parasite interactions [11]. The *T. brucei* flagellar pocket is quite pronounced compared to what is observed in many other flagellated eukaryotes [12], which may reflect demands of a pathogenic lifestyle and the flagellar pocket's role in virulence and immune evasion [11]. The site where the axoneme exits the flagellar pocket is termed the flagellar pocket collar and is marked by a fibrous cytoskeletal structure that holds the flagellar membrane and cell membrane in close apposition [13]. From this point onward, the axoneme is surrounded by its own membrane, which has a lipid and protein composition distinct from that of the flagellar pocket and plasma membranes [14].

Most flagellated cells maintain flagellum connections to the plasma membrane only within the flagellar pocket [10]. The *T. brucei* flagellum however, is also laterally connected to the

cell body along almost its entire length, with just the distal tip extending free of the cell body (Figure 1). Lateral flagellum attachment is mediated by junctional complexes that employ proteins in the flagellum and cell body to hold the flagellum and plasma membranes in tight apposition [15–17]. These junctional complexes constitute a specialized "flagellum attachment zone" (FAZ) that extends from the flagellar pocket to the anterior end of the cell. The FAZ includes a cytoplasmic FAZ filament, [18, 19] together with a specialized quartet of subpellicular microtubules (MTQ) that are associated with a membranous reticulum [20]. The unique architecture of the trypanosome flagellum divides the cell surface into several discrete membrane subdomains (Figure 1) [21, 22]. Trafficking mechanisms that enable protein targeting to each of these specific subdomains remain enigmatic.

The Axoneme

The 9+2 axoneme is the fundamental unit of flagellum motility [23]. Each of the 9 outer doublet microtubules consists of an A-tubule and a B-tubule that provide a scaffold for the assembly of inner arm and outer arm dynein motors <<Glossary>>. Adjacent doublets are connected via the nexin-dynein regulatory complex (NDRC) (Figure 2) [24, 25]. Radial spokes extend inward from each outer doublet and converge on the central pair apparatus, which includes the central pair microtubules and several, mostly unknown components that extend in a sheath-like structure around the central pair [26] .

When viewed longitudinally, sub-structures on outer doublet microtubules are arranged in a repeating unit with a periodicity of approximately 96-nm that is well-conserved across vast evolutionary distances [27]. In *T. brucei* the axonemal repeat unit includes three radial spokes, four outer arm dyneins, inner arm dyneins and the NDRC (Figure 2) [28]. The stoichiometry of inner dyneins and the NDRC in *T. brucei* has not been determined. The basic architecture of the axoneme repeating unit is conserved among eukaryotes with motile flagella. However, there are a number of differences in *T. brucei* compared to *Chlamydomonas reinhardtii*, which is considered a reference organism for axoneme structure [27, 29]. These differences include three radial spokes instead of two, outer arm dyneins having two heavy chains each instead of three, a distinct repertoire of inner arm dynein heavy chains, specialized NDRC subunits, and central pair of microtubules that retain a fixed orientation relative to outer doublets [28, 30]. Assembly of these structures is an essential event in the parasite's cell cycle and crucial for its life cycle. Recent studies have begun to decipher how *T. brucei* flagellum assembly is achieved at the molecular level (box 2).

The paraflagellar rod

As the flagellum extends beyond the flagellar pocket, the paraflagellar rod (PFR) is formed [31]. The PFR is a lattice-like filament that runs alongside the axoneme and is connected to axonemal doublets 4 – 7. The PFR is restricted to kinetoplastids and a few related organisms. Its structure and function have remained enigmatic, although details are beginning to emerge indicating structural as well as regulatory roles. The PFR is essential for viability in *T. brucei* [32], though not in *Leishmania* [33] and is required for normal motility in both organisms. It imposes structural constraints on axonemal beating and can act as a biomechanical spring to absorb and transmit energy produced by flagellum beating and twisting [28]. The PFR is also a scaffold for assembly of Ca^{++} and cAMP regulatory

systems [34, 35], indicating a role in regulating flagellum functions. In cross section, the PFR presents three structural domains that are proximal (P), intermediate (I) and distal (D) to the axoneme-PFR interface (Figure 2). Three recent electron tomography studies report a repeating unit within the distal domain having 51–57 nm longitudinal periodicity, although these studies differ with respect to details of the repeating unit and description of the proximal and intermediate domains [15, 28, 36]. PFR connections to the axoneme were observed to occur with a longitudinal periodicity (56–57 nm), which corresponds well to the PFR repeat unit size and is a multiple of the 8-nm unit of a/b-tubulin dimers of the axoneme, suggesting that they might be elaborated from intrinsic structural repeats. The size and complexity of the PFR-axoneme super structure makes detailed structural studies technically demanding and more work is needed to allow a detailed structure to be defined.

FLAGELLUM MOTILITY

At its root level, flagellum motility is powered by ATP-dependent structural changes in axonemal dynein motors that are permanently attached to the A-tubule of each outer doublet microtubule. Dynein structural changes bring about reversible attachment to B-tubule of the neighboring doublet to drive sliding of adjacent doublets, while connections between doublets resist sliding, thus generating doublet bending [37].

Harnessing flagellum beating to drive propulsive parasite motility requires that localized bending of axonemal doublets be propagated along the length of the axoneme and this requires spatial and temporal regulation of thousands of dynein motors. The precise mechanisms for regulating axonemal dynein activity are not clear, but involve a variety of axonemal protein complexes [38, 39]. Among these, the NDRC is perhaps the best characterized [25]. The NDRC is a large (>1 MDa) complex that is present in virtually all motile axonemes and contains conserved as well as organism-specific subunits [40–42]. In *T. brucei* the NDRC includes trypanin [30] and component of motile flagella 70 protein (CMF70) [40], as well as candidate subunits, trypanin-related protein, CMF46, CMF40 and CMF22 [30, 41, 43]. Pioneering studies in *C. reinhardtii* demonstrate the NDRC operates in concert with the radial spokes and central pair apparatus as a reversible inhibitor of dynein [44–46]. Functional analysis of NDRC subunits supports a similar function in *T. brucei* [30, 40].

Flagellum beat direction in *T. brucei* and other trypanosomatids, is predominately tip-to-base [47, 48], which is opposite to that observed in most other eukaryotic flagella. Although specific signals that regulate flagellum beating have not been identified in *T. brucei*, studies in the related trypanosomatid, *Crithidia oncopelti*, demonstrate that tip-to-base beating happens at low (< 0.1 mM) Ca^{++} concentrations, and beat direction switches to base-to-tip at higher concentrations [49]. Reverse beating has also been observed in *T. brucei* [50] and is correlated with the parasite encountering obstacles [51]. Notably, NDRC subunits contain domains predicted to function in Ca^{++} signaling [41, 43]. In addition to the NDRC, the PFR has emerged as a potential player in beat regulation with the discovery that it houses proteins predicted to participate in Ca^{++} and cAMP signaling pathways, such as EF-hand calcium binding proteins and cAMP-specific phosphodiesterase [34, 35]. The precise role of these proteins is not yet known, but they function with second messengers known to regulate flagellum beat in trypanosomes and other organisms [49, 52]. Understanding the mechanisms

by which the NDRC, PFR and other regulatory complexes influence axoneme motility and respond to external cues presents the next big challenge for trypanosome flagellum motility research.

Flagellum-dependent parasite motility

Until recently, the basic view of trypanosome motility remained largely unchanged from original descriptions of 170 years ago [7]. Early work demonstrated that motility is driven by a flagellar wave that initiates at the flagellum tip and moves toward the flagellum base [47]. As the flagellar wave propagates along the cell, recoil against the surrounding fluid drives cell movement in the direction opposite to that of beat propagation. Hence, the cell moves forward with the flagellum tip leading (Video 1). Occasionally, beat direction reverses, causing tumbling, irregular and sometimes backward cell movement [50, 51, 53]. The flagellum follows a helical path around the cell and lateral attachment of the flagellum to the cell enables the flagellar wave to be directly transmitted to the cell body, causing the entire body to rotate as it moves, effectively turning the trypanosome into a microbial corkscrew. At low Reynolds numbers, viscous forces dominate and this type of helical motion becomes an efficient means of cell propulsion in high viscosity environments such as blood and tissues [54].

Two recent studies used high-speed video microscopy to revisit trypanosome cell movement. Rodriguez and colleagues [55] observed corkscrew movement of the cell body as it moved forward, consistent with earlier studies. The experiments further indicated that a helical waveform is an intrinsic characteristic of the flagellum beat, as observed in some other flagellated protists [56]. The authors also observed that rather than being uniformly left-handed (LH), the flagellar waveform is bihelical, alternating between left-handed and right-handed (RH) helical waves, causing the cell body to rotate alternately in a clockwise and counter-clockwise direction. Bihelical motility has also been observed for the plant pathogen *Spiroplasma melliferum* [57], which lacks a flagellum but similar to trypanosomes, needs to move through viscous host environments. A potential advantage for bihelical motility is the capacity to generate propulsive force with rotation in either direction. Build-up and release of torsional strain in the cell body might also provide a form of elastic energy to aid in cell propulsion.

Heddergott and colleagues [51] employed high-speed video microscopy to study movement of bloodstream-form *T. brucei* in liquid cultures, as well as in conditions resembling the bloodstream environment. They also observed the cell body to rotate as it moved forward, but in this case rotation was uniformly in the counter-clockwise direction (LH waveform), as viewed looking posterior to anterior, which differs from the model of Rodriguez et al [55]. Using mathematical modeling, they further proposed that the flagellum beat itself is intrinsically planar, but that physical constraints imposed by attachment to the cell body cause the wave to become helical as it travels along the cell body. More work is needed to resolve the discrepancies between these two studies.

Trypanosome motility is influenced by the host environment

In suspension cultures, trypanosomes undergo periods of propulsive motility, interspersed with tumbling or irregular movements. Increasing viscosity of the culture medium to approximate that of blood increases propulsive velocity, and increases the proportion of trypanosomes undergoing propulsive motility [51]. Interestingly, when parasite motility was examined among pillars constructed out of silicone polymers, cell velocity was maximal when pillar spacing approximated that estimated for red blood cells in the bloodstream [51]. The results suggest that motility of bloodstream-form *T. brucei* is tuned to the environment encountered in the bloodstream of the mammalian host. Stationary pillars are different than the dynamic and heterogeneous nature of the bloodstream, but the results are a key step forward as they move to simulate natural environments encountered by trypanosomes and emphasize the impact that the extracellular environment has on microbial cell movements.

In its natural environment, *T. brucei* is in constant contact with host tissue surfaces, an environment that differs widely from suspension culture. For most microbes, life on a surface has dramatic influences on microbial physiology, motility, behavior and pathogenesis [58, 59]. Cultivation of *T. brucei* on surfaces induces a novel group behavior, termed social motility [60], in which individual parasites collect into multicellular communities that move en masse across the surface and divert their movements in response to as yet unidentified signals produced by nearby cells. *T. brucei* social motility is flagellum-mediated [60] and exhibits similarities to social motility and other surface-induced behaviors observed in a wide variety bacteria, including human pathogens such as *Vibrio parahaemolyticus* [61]. The discovery of social motility revealed a novel feature of trypanosome motility and behavior and offers new paradigms for understanding how trypanosomes alter their motility in response to external cues.

Motility in disease transmission and pathogenesis

T. brucei movement through specific host tissues is crucial for parasite development, transmission and pathogenesis (box 1). In the tsetse fly *T. brucei* moves in an ordered sequence through specific tissues, traveling from the midgut to the salivary gland where the parasites differentiate into VSG-coated metacyclic trypomastigotes [62]. Parasites traverse a distance equivalent to several tsetse body lengths and must penetrate tissue barriers such as the peritrophic matrix and proventriculus, indicating a requirement for active motility. These movements are important to the transmission cycle because VSG-coated metacyclics produced in the salivary gland are the only developmental stage within the tsetse fly capable of infecting a mammalian host [62]. However, a requirement for active parasite motility in tsetse transmission has not been directly tested and this presents a gap in our understanding of trypanosome motility and the disease transmission cycle.

Within the mammalian host, trypanosome motility is considered to be important for penetration of the blood brain barrier and invasion of the CNS [63]. Whether CNS invasion provides a specific advantage for the parasite is not clear. However, CNS invasion is a defining step in pathogenesis of sleeping sickness, as it marks the onset of the lethal stage of disease. CNS invasion also limits therapeutic intervention because parasites within this compartment are not affected by drugs that don’t cross the blood brain barrier and might

persist after drug treatment, thus constituting a source of relapse infection [64]. Two routes for CNS invasion have been proposed: In one model parasites first penetrate epithelial tight junctions of the blood-cerebrospinal fluid barrier at the choroid plexus to enter the cerebrospinal fluid and from there they invade the pia mater and ultimately the brain tissue [65, 66]. In the second model parasites penetrate endothelial tight junctions of the blood-brain-barrier in brain microvessels to directly invade the brain [67]. In both models the parasite's auger-like motility is hypothesized to facilitate tissue penetration, although this hypothesis has not yet been directly tested.

Parasite motility in the bloodstream is also hypothesized to facilitate defense against the host's humoral immune response [68]. *T. brucei* undergoes switching of its VSG surface antigens and rapid clearance of host immunoglobulin (Ig) bound to surface VSG to prevent immune clearance of the parasite population. Recent work showed that trypanosome propulsive motility drives clearance of host Ig-VSG immune complexes through a hydrodynamic flow-mediated mechanism [68]. In this model, parasite movement generates hydrodynamic forces that cause Ig-VSG complexes at the cell surface to be swept backward to the cell posterior where they are endocytosed, thus thwarting host efforts at opsonization and immune destruction.

Despite the expected importance of parasite motility, a direct test of the requirement for parasite motility in any aspect of host infection or pathogenesis has not been performed. Efforts to do so have been hampered because RNAi knockdown of flagellar proteins in bloodstream-form *T. brucei* is generally lethal even when parasites are grown in culture [50, 69, 70], thus precluding tests in animal models . This result had been interpreted to suggest that perturbing motility is lethal in bloodstream-form *T. brucei*. However, recent work has indicated this is not the case through the identification of axonemal dynein light chain 1 (LC1) point mutants that have defective motility but are viable [71], while LC1 knockdown is lethal [53] . Thus, the lethal phenotype appears to reflect pleotropic effects of knockdown, rather than a motility defect per se. Importantly, the availability of viable motility mutants in the mammalian infectious stage now provides a unique opportunity to directly investigate parasite motility in animal models of infection.

FLAGELLUM ROLE IN CELL MORPHOGENESIS AND CELL DIVISION

In addition to its canonical role in motility, the trypanosome flagellum is crucial for cell morphogenesis, cell division and inheritance of essential cellular structures (Figure 3), functions not widely recognized for flagella of other organisms. Presumably, this is due to physical and functional connections between the flagellum and other cellular structures, allowing the flagellum to define morphogenetic axes, control organellar inheritance and orchestrate cell division [72].

One of the first recognizable events in the trypanosome cell cycle is assembly of a new flagellum (Figure 3). Once the new flagellum reaches a specific length, cell cleavage occurs along a line (called the cleavage furrow) between the new and old flagellum. Several other single copy cell structures depend on flagellum assembly and segregation for their own assembly and/or segregation [72]. Perhaps the best characterized example of this dependence

is the mitochondrial genome, which is organized into a disc-like structure called the kinetoplast. Filaments extend from the kinetoplast, through the mitochondrial membrane and attach directly to the basal body [73]. During flagellum elongation, new and old basal bodies move apart (Figure 3) and basal body-kinetoplast connections allow this movement to drive separation of the new and old kinetoplasts so that subsequent cell cleavage leaves one basal body and one kinetoplast per daughter cell [74–76]. Mechanisms that drive basal body movements are not known, but the process requires microtubules and it is postulated that flagellum elongation pushes the new basal body toward the cell posterior after flagellum tip movement toward the cell anterior is halted (Figure 3) [74, 77].

Changes in cell size and form occur throughout parasite development (Figure 3). The biological roles of these changes are unknown, but the different morphotypes are defined by flagellum position relative to other cell structures, emphasizing the importance of the flagellum in cell morphogenesis. Within the tsetse fly, parasite developmental stages range from 15 to 30 μm long [78, 79] and cell length is closely correlated with FAZ filament length. Length of the FAZ filament, in turn is directly correlated with flagellum length and FAZ filament assembly requires flagellum elongation and lateral attachment of the flagellum to the cell body [6, 16, 17]. In the absence of flagellum elongation, cells ultimately fail to assemble a FAZ filament and are inviable [6]. When flagellum attachment is disrupted by knockdown of FAZ components within the cell body, assembly of the cytoplasmic FAZ filament is blocked and the phenotype is lethal [16, 19, 80]. In contrast, when attachment is disrupted by targeting FAZ components within the flagellum, a shortened FAZ filament assembles in the cytoplasm and cells divide with unaltered growth rate[17]. In cells with a shortened FAZ filament, cleavage furrow ingression initiates at the anterior end of the shortened FAZ, yielding cells that are shorter than wild type [6, 17, 19]. Together, these results suggest a model in which axoneme assembly controls FAZ filament assembly and the latter specifies the placement and path of the cleavage furrow and hence cell length [6, 17, 19, 81]. This model is supported by the finding that cell cycle regulators polo-like kinase and Aurora B-like kinase dynamically localize to the anterior end of the new FAZ and control cytokinesis in wild type *T. brucei* [82–85].

A prominent and so far unique feature in *T. brucei* of flagellum growth and cell morphogenesis is that the existing flagellum directs the path of assembly for the new flagellum. In procyclic cells, this is mediated by the flagellar connector (FC), a mobile molecular machine that tethers the tip of the new flagellum to the side of the existing flagellum and translocates with the new flagellum tip as it assembles alongside the old flagellum [86, 87]. FC composition and mechanisms of movement are not known. An FC is not apparent in bloodstream-form cells, although the new flagellum nonetheless follows the path of the old flagellum during assembly. As discussed above, assembly and segregation of the new flagellum is directly coupled to assembly, positioning and segregation of other cellular structures. As such, the capacity of the old flagellum to direct positioning and assembly of the new flagellum provides a remarkable example of “structural inheritance”, whereby an existing cellular structure dictates spatial relationships between nascent cellular structures in progeny cells [86, 88].

In addition to orchestrating cell division in proliferating forms, dramatic changes in flagellum length and position are associated with specific developmental transformations during development in the tsetse fly [62, 78, 89, 90], (Figure 1 and 3). Trypanosomes and other organisms employ specific regulatory mechanisms to control flagellum length, leading to the idea that control of flagellum length and hence FAZ length, might be a means to control cell size and morphology that mark *T. brucei* development [19, 91, 92].

FLAGELLUM ROLE IN HOST-PARASITE INTERACTIONS

The flagellum of eukaryotic cells is now recognized as a major center for sensing extracellular signals and transducing these into cellular responses [93]. Restricting signal perception and transduction machinery to a specific sub-compartment such as the cilium, provides increased sensitivity and control, and is a paradigm that applies to numerous signaling systems and cellular responses in vertebrates and protozoa [94–96].

An accumulating body of evidence indicates that the *T. brucei* flagellum functions as a sensory platform and interface for host-parasite interactions (Figure 4). A prime example is attachment to the tsetse salivary gland epithelium, which is mediated by extensive outgrowths of the flagellar membrane that interdigitate between host microvilli to form close contacts with the epithelial cell membrane. Plaques of electron dense material assemble inside the flagellum at sites where tips of microvilli contact the flagellum and are thought to fortify the host-parasite connection [97]. Flagellum adhesion events are observed in other flagellated protists, for example flagellum adhesion during mating in *C. reinhardtii* and *Paramecium* [98, 99]. However, *T. brucei* is distinguished by the extent of flagellum membrane expansion and restructuring, as well as the involvement of host cells. In another African trypanosome, *T. congolense*, the flagellum also mediates attachment to host blood vessel endothelial cells [100]. Signaling systems that direct flagellum membrane restructuring upon contact are not known, but presumably reside within the flagellum.

T. brucei flagellum attachment enables the parasite to establish a permanent infection in the salivary gland, which alters the fly's feeding behavior, favoring transmission to the mammalian host [101]. Flagellum attachment also correlates with parasite reentry into the cell cycle [89], expression of meiotic genes [102], and the onset of differentiation into mammalian infectious trypomastigotes [97]. Interestingly, differentiation can be achieved *in vitro* without flagellum attachment by overexpressing a specific RNA-binding protein [103]. It remains to be seen whether flagellum attachment contributes to signaling events that trigger meiosis or differentiation *in vivo*. In either case, flagellum attachment is a dramatic example of flagellum-dependent host interaction and a crucial step of the parasite transmission cycle.

Flagellar virulence factors

As discussed above, the *T. brucei* flagellar pocket is the sole site for exchange of macromolecules with the host and as such has a key role in host-parasite interactions. This is exemplified by its role in uptake of host transferrin [104] to meet cellular iron demands and uptake of trypanotoxic lytic factors that are present in human serum and dictate host-range specificity 105–107.

Additional determinants of host-parasite interactions associated with the flagellum include several flagellar proteins whose activities modulate virulence in the mammalian host. Glycosylphosphatidylinositol-Phospholipase C (GPI-PLC) is a bloodstream stage-specific enzyme that is concentrated in the flagellar membrane and promotes release of VSG [108]. Although the precise biological function of GPI-PLC-dependent VSG release is not known, the enzyme is required for full virulence of pleomorphic trypanosomes, as mice infected with GPI-PLC null mutants have extended survival and control parasitemia better than mice infected with control trypanosomes [109]. GPI-PLC also participates in VSG shedding during differentiation of bloodstream forms to procyclic forms [110].

Additional flagellar virulence factors include calflagins and metacaspase 4, both of which associate with the flagellar membrane through lipid modifications. Calflagins are calcium-binding proteins that are upregulated in bloodstream parasites and localize to lipid rafts of the flagellar membrane [111]. They are dispensable for proliferation, motility and surface antibody clearance in vitro. However, RNAi against calflagins in monomorphic trypanosomes results in attenuated parasitemia and prolonged survival of infected mice [112].

Metacaspase 4 (MCA4) is a bloodstream-specific protein that is both flagellum-localized and secreted [113]. Secretion is concomitant with proteolytic processing by MCA3, whereas MCA4 itself is a pseudopeptidase that lacks detectable peptidase activity. In monomorphic *T. brucei*, MCA4 null mutants have a striking virulence phenotype characterized by multiple parasitemic waves and prolonged host survival [113]. Notably, the virulence defect can be rescued by expression of a mutant MCA4 that is secreted yet doesn't localize to the flagellum and isn't processed. It remains to be seen how calflagin and MCA4 influence host physiology to favor parasites and what the role is for flagellum localization of the proteins.

Perhaps the most in-depth insights into flagellum-mediated host-trypanosome interactions come from studies of expression site associated gene 4 (ESAG4). ESAG4 is bloodstream-specific and localizes to the flagellar membrane [114]. ESAG4 is member of a large family of adenylate cyclases (ACs) that catalyze conversion of ATP to cAMP. Expressing a dominant negative ESAG4 mutant reduced cellular cAMP levels by half [115]. Strikingly, this reduction of cAMP is tolerated during in vitro growth but leads to virulence defects such as reduced parasitemia and prolonged survival of infected mice. The authors provided mechanistic insights by elegantly showing that ESAG4 mutants are defective at inhibiting TNF-alpha production in host liver immune cells and provide evidence that reduction of trypanotoxic TNF-alpha by WT trypanosomes is mediated by host Protein Kinase A (PKA) [115]. The emerging model is that trypanosomes, upon lysis or phagocytosis by host immune cells, activate ACs to produce cAMP, which in turn activates host PKA to inhibit trypanotoxic TNF-alpha synthesis. This allows trypanosomes to resist the host's early innate immunity attack. Future studies will be needed to shed light on the molecular details. For example, is ESAG4 the only AC required for virulence? How does parasite-produced cAMP reach host PKA? And how does flagellum localization impact ESAG4 influence on host immune responses?

Signaling pathways at the flagellum membrane

A major limitation to studies of flagellum-dependent signaling in trypanosomes has been the lack of a flagellum preparation that retains an intact membrane. This poses a problem because the membrane is the direct host interface and signaling capacity is dictated by surface-exposed membrane proteins coupled to soluble components of signaling cascades in the flagellar matrix <<Glossary>>. The problem was recently overcome with purification of membrane-enclosed flagella from bloodstream-form *T. brucei* and subsequent proteomic analyses of the flagellum surface and flagellum matrix [116]. The identified proteins have a broad range of molecular functionalities and include several receptor and transporter proteins predicted to function in signaling and host-parasite interaction, as well as corresponding effector proteins in the matrix [116]. The flagellum surface proteome was enriched for proteins upregulated in the bloodstream life cycle stage, and contained many *T. brucei*-specific proteins. Thus, the trypanosome flagellum presents a diverse and dynamic host-parasite interface that is well-suited for host-parasite signaling. Notably, proteins identified were shown to be accessible to small molecules added to live parasites, indicating flagellum surface proteins could present novel targets for therapeutic intervention. Key now will be to test the function of flagellar signaling proteins for a requirement in parasite development and/or disease pathogenesis.

SUMMARY AND OUTLOOK

The *T. brucei* flagellum is typically recognized for its role in parasite motility, but increasing evidence demonstrates that it also functions in cell morphogenesis and host-parasite interactions. In all of these capacities, the flagellum is a key mediator of disease transmission and pathogenesis. Recent work has begun to identify molecules and mechanisms of flagellum biogenesis and function, whereas future studies are expected to reveal novel aspects of parasite biology and illuminate new targets for therapeutic intervention. Important areas of focus include examining the influence of parasite motility *in vivo*, defining mechanisms that regulate axoneme motility and mediate protein targeting to the flagellum, as well as elucidating mechanisms of action for flagellar virulence factors. More broadly, *T. brucei* has emerged as an excellent model system for studying the biology of the highly conserved eukaryotic flagellum, also called a cilium (box 3). Cilium defects cause a variety of heritable human diseases [117–119]. The importance of cilium motility and sensing to human development and physiology are now widely recognized (box 3). What is less appreciated however, is that cilia are an understudied yet prominent feature in a wide range of human pathogens that pose a tremendous global public health burden (box 3). The motility functions of cilia in these infectious organisms are self-evident, but their sensing functions and contribution to pathogenesis are largely unexplored. As such, the increasing importance of *T. brucei* as a powerful experimental system means that continued studies of the trypanosome flagellum will enhance understanding of a broad spectrum of inherited and infectious human diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Alvar J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012; 7:e35671. [PubMed: 22693548]
2. Barrett MP, et al. The trypanosomiases. Lancet. 2003; 362:1469–1480. [PubMed: 14602444]
3. Stuart K, et al. Kinetoplastids: related protozoan pathogens, different diseases. Journal of Clinical Investigation. 2008; 118:1301–1310. [PubMed: 18382742]
4. Kennedy PG. Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). Lancet Neurol. 2013; 12:186–194. [PubMed: 23260189]
5. Simarro PP, Jannin J, Cattand P. Eliminating human African trypanosomiasis: where do we stand and what comes next? PLoS Med. 2008; 5:e55. [PubMed: 18303943]
6. Kohl L, Robinson D, Bastin P. Novel roles for the flagellum in cell morphogenesis and cytokinesis of trypanosomes. Embo J. 2003; 22:5336–5346. [PubMed: 14532107] Demonstrates that the trypanosome flagellum is an essential organelle and depends on IFT for assembly.
7. Gruby M. Recherches et observations sur une nouvelle espèce d'hématozoaire, Trypanosoma sanguinis. Comptes rendus hebdomadaire des séances de l'Académie des Sciences, Paris. 1843; 17:1134–1136.
8. Valentin G. Uber ein Entozoon im Blute von Salmo fario. Arch. d. J. Muller. 1841:435–436.
9. Ralston KS, Kabututu ZP, Melehani JH, Oberholzer M, Hill KL. The Trypanosoma brucei flagellum: moving parasites in new directions. Annual Review of Microbiology. 2009; 63:335–362.
10. Gilula NB, Satir P. The ciliary necklace. A ciliary membrane specialization. Journal of Cell Biology. 1972; 53:494–509. [PubMed: 4554367]
11. Field MC, Carrington M. The trypanosome flagellar pocket. Nat Rev Microbiol. 2009; 7:775–786. [PubMed: 19806154] Comprehensive description of the trypanosome flagellar pocked, which is a key host-parasite portal.
12. Ghossoub R, Molla-Herman A, Bastin P, Benmerah A. The ciliary pocket: a once-forgotten membrane domain at the base of cilia. Biology of the Cell. 2011; 103:131–144. [PubMed: 21275905]
13. Bonhivers M, Nowacki S, Landrein N, Robinson DR. Biogenesis of the trypanosome endo-exocytotic organelle is cytoskeleton mediated. PLoS Biol. 2008; 6:e105. [PubMed: 18462016] Identification and functional analysis of BILBO as the first component of the flagellar pocket collar, demonstrating requirement of this structure for flagellar pocket formation.
14. Maric D, Epting CL, Engman DM. Composition and sensory function of the trypanosome flagellar membrane. Curr Opin Microbiol. 2010; 13:466–472. [PubMed: 20580599]
15. Hoog JL, Bouchet-Marquis C, McIntosh JR, Hoenger A, Gull K. Cryo-electron tomography and 3-D analysis of the intact flagellum in Trypanosoma brucei. J Struct Biol. 2012; 178:189–198. [PubMed: 22285651]
16. LaCount DJ, Barrett B, Donelson JE. Trypanosoma brucei FLA1 is required for flagellum attachment and cytokinesis. J Biol Chem. 2002; 277:17580–17588. [PubMed: 11877446]
17. Sun SY, Wang C, Yuan YA, He CY. An intracellular membrane junction consisting of flagellum adhesion glycoproteins links flagellum biogenesis to cell morphogenesis in Trypanosoma brucei. Journal of Cell Science. 2013; 126:520–531. [PubMed: 23178943] Discovery of a flagellum component of the FAZ and functional analyes that distinguish the FAZ role in flagellum attachment versus cell viability.

18. Vaughan S, Kohl L, Ngai I, Wheeler RJ, Gull K. A Repetitive Protein Essential for the Flagellum Attachment Zone Filament Structure and Function in Trypanosoma brucei. Protist. 2008; 159:127–136. [PubMed: 17945531]

19. Zhou Q, Liu B, Sun Y, He CY. A coiled-coil- and C2-domain-containing protein is required for FAZ assembly and cell morphology in Trypanosoma brucei. Journal of Cell Science. 2011; 124:3848–3858. [PubMed: 22114307]

20. Taylor AE, Godfrey DG. A new organelle of bloodstream salivarian trypanosomes. J. of Protozoology. 1969; 16:466–470.

21. Balber AE. The pellicle and the membrane of the flagellum, flagellar adhesion zone, and flagellar pocket: functionally discrete surface domains of the bloodstream form of African trypanosomes. Critical Reviews in Immunology. 1990; 10:177–201. [PubMed: 2085407]

22. Gadelha C, et al. Membrane domains and flagellar pocket boundaries are influenced by the cytoskeleton in African trypanosomes. Proc Natl Acad Sci U S A. 2009; 106:17425–17430. [PubMed: 19805090]

23. Satir P. Landmarks in cilia research from Leeuwenhoek to us. Cell Motil. Cytoskel. 1995; 32:90–94.

24. Gibbons IR. Studies on the Protein Components of Cilia from Tetrahymena Pyriformis. Proc Natl Acad Sci U S A. 1963; 50:1002–1010. [PubMed: 14082342]

25. Heuser T, Raytchev M, Krell J, Porter ME, Nicastro D. The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. Journal of Cell Biology. 2009; 187:921–933. [PubMed: 20008568] Seminal cryoET study of Chlamydomonas flagella showing that DRC is the long-sought nexin link, ideally positioned for its role in axonemal dynein regulation.

26. Carbajal-Gonzalez BI, et al. Conserved structural motifs in the central pair complex of eukaryotic flagella. Cytoskeleton (Hoboken). 2013; 70:101–120. [PubMed: 23281266]

27. Nicastro D, et al. The molecular architecture of axonemes revealed by cryoelectron tomography. Science. 2006; 313:944–948. [PubMed: 16917055]

28. Hughes LC, Ralston KS, Hill KL, Zhou ZH. Three-dimensional structure of the Trypanosome flagellum suggests that the paraflagellar rod functions as a biomechanical spring. PLoS One. 2012; 7:e25700. [PubMed: 22235240]

29. Pigino G, et al. Comparative structural analysis of eukaryotic flagella and cilia from Chlamydomonas, Tetrahymena, and sea urchins. J Struct Biol. 2012; 178:199–206. [PubMed: 22406282]

30. Ralston KS, Lerner AG, Diener DR, Hill KL. Flagellar Motility Contributes to Cytokinesis in Trypanosoma brucei and Is Modulated by an Evolutionarily Conserved Dynein Regulatory System. Eukaryotic Cell. 2006; 5:696–711. [PubMed: 16607017] Suppressor mutant analysis demonstrating NDRC function in regulation of T. brucei motility.

31. Cachon J, Cachon M, Cosson M-P, J C. The paraflagellar rod: a structure in search of a function. Biol. Cell. 1988; 63:169–181.

32. Bastin P, Sherwin T, Gull K. Paraflagellar rod is vital for trypanosome motility. Nature. 1998; 391:548. [PubMed: 9468133] One of the earliest demonstraties of a motility function for an extra-axonemal structure in trypanosomes.

33. Santrich C, et al. A motility function for the paraflagellar rod of Leishmania parasites revealed by PFR-2 gene knockouts. Molecular and Biochemical Parasitology. 1997; 90:95–109. [PubMed: 9497035]

34. Oberholzer M, et al. The Trypanosoma brucei cAMP phosphodiesterases TbrPDEB1 and TbrPDEB2: flagellar enzymes that are essential for parasite virulence. Faseb J. 2007; 21:720–731. [PubMed: 17167070]

35. Portman N, Lacomble S, Thomas B, McKean PG, Gull K. Combining RNA interference mutants and comparative proteomics to identify protein components and dependences in a eukaryotic flagellum. Journal of Biological Chemistry. 2009; 284:5610–5619. [PubMed: 19074134]

36. Koyfman AY, et al. Structure of Trypanosoma brucei flagellum accounts for its bihelical motion. Proc Natl Acad Sci U S A. 2011; 108:11105–11108. [PubMed: 21690369]

37. Satir P. Studies on cilia. 3. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. J Cell Biol. 1968; 39:77–94. [PubMed: 5678451]

38. Heuser T, Dymek EE, Lin J, Smith EF, Nicastro D. The CSC connects three major axonemal complexes involved in dynein regulation. Mol Biol Cell. 2012; 23:3143–3155. [PubMed: 22740634]
39. Yamamoto R, et al. The MIA complex is a conserved and novel dynein regulator essential for normal ciliary motility. Journal of Cell Biology. 2013; 201:263–278. [PubMed: 23569216]
40. Kabututu ZP, Thayer M, Melehani JH, Hill KL. CMF70 is a subunit of the dynein regulatory complex. Journal of Cell Science. 2010; 123:3587–3595. [PubMed: 20876659]
41. Bower R, et al. The N-DRC forms a conserved biochemical complex that maintains outer doublet alignment and limits microtubule sliding in motile axonemes. Molecular Biology of the Cell. 2013; 24:1134–1152. [PubMed: 23427265]
42. Lin J, et al. Building blocks of the nexin-dynein regulatory complex in Chlamydomonas flagella. J Biol Chem. 2011; 286:29175–29191. [PubMed: 21700706]
43. Nguyen HK, Sandhu JS, Langousis G, Hill K. CMF22 is a broadly conserved axonemal protein and is required for propulsive motility in Trypanosoma brucei. Euk Cell. 2013 In Press.
44. Gardner LC, O'Toole E, Perrone CA, Giddings T, Porter ME. Components of a "dynein regulatory complex" are located at the junction between the radial spokes and the dynein arms in Chlamydomonas flagella. J Cell Biol. 1994; 127:1311–1325. [PubMed: 7962092]
45. Huang B, Ramanis Z, Luck DJ. Suppressor mutations in Chlamydomonas reveal a regulatory mechanism for Flagellar function. Cell. 1982; 28:115–124. [PubMed: 6461414]
46. Piperno G, Huang B, Luck DJ. Two-dimensional analysis of flagellar proteins from wild-type and paralyzed mutants of Chlamydomonas reinhardtii. Proc Natl Acad Sci U S A. 1977; 74:1600–1604. [PubMed: 266200]
47. Walker PJ. Organization of function in trypanosome flagella. Nature. 1961; 189:1017–1018. [PubMed: 13782746]
48. Walker PJ, Walker JC. Movement of trypanosome flagella. J. of Protozoology. 1963; 10(Suppl): 32.
49. Sugrue P, Hirons MR, Adam JU, Holwill ME. Flagellar wave reversal in the kinetoplastid flagellate *Crithidia oncopelti* . Biol Cell. 1988; 63:127–131. [PubMed: 3203154]
50. Branche C, et al. Conserved and specific functions of axoneme components in trypanosome motility. J Cell Sci. 2006; 119:3443–3455. [PubMed: 16882690]
51. Heddergott N, et al. Trypanosome motion represents an adaptation to the crowded environment of the vertebrate bloodstream. PLoS Pathog. 2012; 8:e1003023. [PubMed: 23166495] State of the art high-speed video microscopy analysis of T. brucei motilty, indicating that parasite flagellar motility is tuned to the bloodstream environment.
52. Salathe M. Regulation of mammalian ciliary beating. Annual Review of Physiology. 2007; 69:401–422.
53. Baron DM, Kabututu ZP, Hill KL. Stuck in reverse: loss of LC1 in Trypanosoma brucei disrupts outer dynein arms and leads to reverse flagellar beat and backward movement. J Cell Sci. 2007; 120:1513–1520. [PubMed: 17405810]
54. Jahn, TL.; Bovee, EC. Infectious Blood Diseases of Man and Animals. Weinman, D.; Ristic, M., editors. NY: Academic Press; 1968. p. 393-436.
55. Rodriguez JA, et al. Propulsion of African trypanosomes is driven by bihelical waves with alternating chirality separated by kinks. Proc Natl Acad Sci U S A. 2009; 106:19322–19327. [PubMed: 19880745] State of the art high-speed video microscopy analysis of T. brucei motilty, indicating that parasite motility occurs via bihelical flagellar waveforms.
56. Votta JJ, Jahn TL, Griffith DL, Fonseca JR. Nature of the flagellar beat in Trachelomonas volvocina, Rhabdomonas spiralis, Menoidium cultellus, and Chilomonas paramecium. Trans Am Microsc Soc. 1971; 90:404–412. [PubMed: 5112382]
57. Shaevitz JW, Lee JY, Fletcher DA. Spiroplasma swim by a processive change in body helicity. Cell. 2005; 122:941–945. [PubMed: 16179261]
58. Harshey RM. Bacterial motility on a surface: many ways to a common goal. Annu Rev Microbiol. 2003; 57:249–273. [PubMed: 14527279]
59. Dubey GP, Ben-Yehuda S. Intercellular nanotubes mediate bacterial communication. Cell. 2011; 144:590–600. [PubMed: 21335240]

60. Oberholzer M, Lopez MA, McLelland BT, Hill KL. Social motility in african trypanosomes. PLoS Pathog. 2010; 6:e1000739. [PubMed: 20126443] Demonstration that the generally solitary T. brucei engages in complex social behavior.

61. Trimble MJ, McCarter LL. Bis-(3'-5')-cyclic dimeric GMP-linked quorum sensing controls swarming in Vibrio parahaemolyticus. Proc Natl Acad Sci U S A. 2011; 108:18079–18084. [PubMed: 22006340]

62. Vickerman K. Developmental cycles and biology of pathogenic trypanosomes. Br Med Bull. 1985; 41:105–114. [PubMed: 3928017] A classic that provides a comprehensive description of the T. brucei life cycle, including role of the flagellum in host-parasite attachment.

63. Hill KL. Mechanism and biology of trypanosome cell motility. Euk Cell. 2003; 2:200–208.

64. Jennings FW, Whitelaw DD, Holmes PH, Chizyuka HG, Urquhart GM. The brain as a source of relapsing Trypanosoma brucei infection in mice after chemotherapy. Int J Parasitol. 1979; 9:381–384. [PubMed: 489242]

65. Mulenga C, Mhlanga JD, Kristensson K, Robertson B. Trypanosoma brucei brucei crosses the blood-brain barrier while tight junction proteins are preserved in a rat chronic disease model. Neuropathol Appl Neurobiol. 2001; 27:77–85. [PubMed: 11299005]

66. Wolburg H, et al. Late stage infection in sleeping sickness. PLoS One. 2012; 7:e34304. [PubMed: 22496723]

67. Frevert U, et al. Early invasion of brain parenchyma by African trypanosomes. PLoS One. 2012; 7:e43913. [PubMed: 22952808]

68. Engstler M, et al. Hydrodynamic Flow-Mediated Protein Sorting on the Cell Surface of Trypanosomes. Cell. 2007; 131:505–515. [PubMed: 17981118] Intriguing demonstration that trypanosome propulsive motility is an immune evasion mechanism that trafficks host antibodies to the parasite's endocytic organelle.

69. Broadhead R, et al. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature. 2006; 440:224–227. [PubMed: 16525475] One of the first proteomes of an eukaryotic axoneme and identification of human disease gene candidates.

70. Ralston KS, Hill KL. Trypanin, a Component of the Flagellar Dynein Regulatory Complex, Is Essential in Bloodstream Form African Trypanosomes. PLoS Pathogens. 2006; 2:873–882. e101.

71. Ralston KS, Kisalu NK, Hill KL. Structure-function analysis of dynein light chain 1 identifies viable motility mutants in bloodstream-form Trypanosoma brucei. Eukaryot Cell. 2011; 10:884–894. [PubMed: 21378260] The first identification of viable T. brucei motility mutants in the mammalian-infectious life cycle stage.

72. Vaughan S. Assembly of the flagellum and its role in cell morphogenesis in Trypanosoma brucei. Curr Opin Microbiol. 2010; 13:453–458. [PubMed: 20541452] Extensive review of the morphogenetic roles of the T.brucei flagellum

73. Ogbadoyi EO, Robinson DR, Gull K. A high-order trans-membrane structural linkage is responsible for mitochondrial genome positioning and segregation by flagellar basal bodies in trypanosomes. Mol Biol Cell. 2003; 14:1769–1779. [PubMed: 12802053]

74. Robinson DR, Gull K. Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. Nature. 1991; 352:731–733. [PubMed: 1876188] One of the first demonstrations that flagellum structures direct inheritance of other organelles in T. brucei.

75. Lacomble S, et al. Basal body movements orchestrate membrane organelle division and cell morphogenesis in Trypanosoma brucei. Journal of Cell Science. 2010; 123:2884–2891. [PubMed: 20682637]

76. Gluenz E, Povelones ML, Englund PT, Gull K. The kinetoplast duplication cycle in Trypanosoma brucei is orchestrated by cytoskeleton-mediated cell morphogenesis. Molecular and Cellular Biology. 2011; 31:1012–1021. [PubMed: 21173163]

77. Absalon S, et al. Flagellum elongation is required for correct structure, orientation and function of the flagellar pocket in Trypanosoma brucei. J Cell Sci. 2008; 121:3704–3716. [PubMed: 18940910]

78. Van Den Abbeele J, Claes Y, van Bockstaele D, Le Ray D, Coosemans M. *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. Parasitology. 1999; 118:469–478. [PubMed: 10363280]

79. Vickerman K, Tetley L, Hendry KA, Turner CM. Biology of African trypanosomes in the tsetse fly. Biol Cell. 1988; 64:109–119. [PubMed: 3067793]

80. Woods K, Nic a'Bhaird N, Dooley C, Perez-Morga D, Nolan DP. Identification and characterization of a stage specific membrane protein involved in flagellar attachment in Trypanosoma brucei. PLoS One. 2013; 8:e52846. [PubMed: 23335957]

81. Robinson DR, Sherwin T, Ploubidou A, Byard EH, Gull K. Microtubule polarity and dynamics in the control of organelle positioning, segregation, and cytokinesis in the trypanosome cell cycle. Journal of Cell Biology. 1995; 128:1163–1172. [PubMed: 7896879]

82. Hammarton TC, Kramer S, Tetley L, Boshart M, Mottram JC. Trypanosoma brucei Polo-like kinase is essential for basal body duplication, kDNA segregation and cytokinesis. Mol Microbiol. 2007; 65:1229–1248. [PubMed: 17662039]

83. Ikeda KN, de Graffenried CL. Polo-like kinase is necessary for flagellum inheritance in Trypanosoma brucei. Journal of Cell Science. 2012; 125:3173–3184. [PubMed: 22427687] This study provides evidence that the dynamic localization of polo-like kinase is required for duplication of flagellum-associated structures in T.brucei

84. Li Z, Umeyama T, Wang CC. The Aurora Kinase in Trypanosoma brucei plays distinctive roles in metaphase-anaphase transition and cytokinetic initiation. PLoS Pathog. 2009; 5:e1000575. [PubMed: 19750216]

85. Li Z, et al. Identification of a novel chromosomal passenger complex and its unique localization during cytokinesis in Trypanosoma brucei. PLoS One. 2008; 3:e2354. [PubMed: 18545648]

86. Moreira-Leite FF, Sherwin T, Kohl L, Gull K. A trypanosome structure involved in transmitting cytoplasmic information during cell division. Science. 2001; 294:610–621. [PubMed: 11641501]

87. Briggs LJ, et al. The flagella connector of Trypanosoma brucei: an unusual mobile transmembrane junction. J Cell Sci. 2004 Pt. Detailed charecterization of the structure through which the existing flagellum dictates the assembly path of the new flagellum in T.brucei.

88. Sonneborn TM. The Determinants and Evolution of Life. The Differentiation of Cells. Proc Natl Acad Sci U S A. 1964; 51:915–929. [PubMed: 14173011]

89. Rotureau B, Subota I, Buisson J, Bastin P. A new asymmetric division contributes to the continuous production of infective trypanosomes in the tsetse fly. Development. 2012; 139:1842–1850. [PubMed: 22491946]

90. Sharma R, et al. Asymmetric cell division as a route to reduction in cell length and change in cell morphology in trypanosomes. Protist. 2008; 159:137–151. [PubMed: 17931969]

91. Rotureau B, Subota I, Bastin P. Molecular bases of cytoskeleton plasticity during the Trypanosoma brucei parasite cycle. Cell Microbiol. 2011; 13:705–716. [PubMed: 21159115]

92. Sharma R, et al. The heart of darkness: growth and form of Trypanosoma brucei in the tsetse fly. Trends Parasitol. 2009; 25:517–524. [PubMed: 19747880]

93. Singla V, Reiter JF. The primary cilium as the cell's antenna: signaling at a sensory organelle. Science. 2006; 313:629–633. [PubMed: 16888132]

94. Berbari NF, O'Connor AK, Haycraft CJ, Yoder BK. The primary cilium as a complex signaling center. Current Biology. 2009; 19:R526–R535. [PubMed: 19602418]

95. Ogura A, Takahashi K. Artificial deciliation causes loss of calcium-dependent responses in Paramecium. Nature. 1976; 264:170–172. [PubMed: 995200]

96. Wang Q, Pan J, Snell WJ. Intraflagellar transport particles participate directly in cilium-generated signaling in Chlamydomonas. Cell. 2006; 125:549–562. [PubMed: 16678098]

97. Tetley L, Vickerman K. Differentiation in Trypanosoma brucei: host-parasite cell junctions and their persistence during acquisition of the variable antigen coat. J Cell Sci. 1985; 74:1–19. [PubMed: 4030903]

98. Goodenough, UW.; Thorner, J. Cell Interactions and Development. Yamada, K., editor. New York: Wiley Interscience; 1983. p. 29-75.

99. Miyake, A. Biochemistry and Physiology of Protozoa. Hunter, MLaSH., editor. New York: Academic Press; 1981. p. 126-198.

100. Hemphill A, Ross CA. Flagellum-mediated adhesion of Trypanosoma congolense to bovine aorta endothelial cells. Parasitology Research. 1995; 81:412–420. [PubMed: 7501641]

101. Van Den Abbeele J, Caljon G, De Ridder K, De Baetselier P, Coosemans M. Trypanosoma brucei modifies the tsetse salivary composition, altering the fly feeding behavior that favors parasite transmission. PLoS Pathog. 2010; 6:e1000926. [PubMed: 20532213]

102. Peacock L, et al. Identification of the meiotic life cycle stage of Trypanosoma brucei in the tsetse fly. Proc Natl Acad Sci U S A. 2011; 108:3671–3676. [PubMed: 21321215]

103. Kolev NG, Ramey-Butler K, Cross GA, Ullu E, Tschudi C. Developmental progression to infectivity in Trypanosoma brucei triggered by an RNA-binding protein. Science. 2012; 338:1352–1353. [PubMed: 23224556]

104. Salmon D, et al. A novel heterodimeric transferrin receptor encoded by a pair of VSG expression site-associated genes in *T. brucei* . Cell. 1994; 78:75–86. [PubMed: 8033214]

105. Kieft R, et al. Mechanism of Trypanosoma brucei gambiense (group 1) resistance to human trypanosome lytic factor. Proc Natl Acad Sci U S A. 2010; 107:16137–16141. [PubMed: 20805508]

106. Hager KM, et al. Endocytosis of a cytotoxic human high density lipoprotein results in disruption of acidic intracellular vesicles and subsequent killing of African trypanosomes. J Cell Biol. 1994; 126:155–167. [PubMed: 8027174]

107. Vanhollebeke B, et al. A haptoglobin-hemoglobin receptor conveys innate immunity to Trypanosoma brucei in humans. Science. 2008; 320:677–681. [PubMed: 18451305]

108. Sunter J, Webb H, Carrington M. Determinants of GPI-PLC Localisation to the Flagellum and Access to GPI-Anchored Substrates in Trypanosomes. PLoS Pathog. 2013; 9:e1003566. [PubMed: 23990786]

109. Webb H, et al. The GPI-phospholipase C of Trypanosoma brucei is nonessential but influences parasitemia in mice. J Cell Biol. 1997; 139:103–114. [PubMed: 9314532]

110. Grandgenett PM, Otsu K, Wilson HR, Wilson ME, Donelson JE. A function for a specific zinc metalloprotease of African trypanosomes. PLoS Pathog. 2007; 3:1432–1445. [PubMed: 17953481]

111. Emmer BT, et al. Identification of a palmitoyl acyltransferase required for protein sorting to the flagellar membrane. J Cell Sci. 2009; 122:867–874. [PubMed: 19240115] Identification of a mechanism for flagellum-specific targeting of membrane proteins.

112. Emmer BT, Daniels MD, Taylor JM, Epting CL, Engman DM. Calflagin inhibition prolongs host survival and suppresses parasitemia in Trypanosoma brucei infection. Eukaryot Cell. 2010; 9:934–942. [PubMed: 20418379]

113. Proto WR, et al. Trypanosoma brucei metacaspase 4 is a pseudopeptidase and a virulence factor. J Biol Chem. 2011; 286:39914–39925. [PubMed: 21949125]

114. Paindavoine P, et al. A gene from the variant surface glycoprotein expression site encodes one of several transmembrane adenylate cyclases located on the flagellum of Trypanosoma brucei. Mol Cell Biol. 1992; 12:1218–1225. [PubMed: 1545803]

115. Salmon D, et al. Adenylate cyclases of Trypanosoma brucei inhibit the innate immune response of the host. Science. 2012; 337:463–466. [PubMed: 22700656] Intriguing mechanistic insights into how trypanosomes employ cAMP to coopt host pathways and thereby thwart immunity.

116. Oberholzer M, et al. Independent analysis of the flagellum surface and matrix proteomes provides insight into flagellum signaling in mammalian-infectious Trypanosoma brucei. Mol Cell Proteomics. 2011; 10 M111 010538. Purification and proteomic analysis of intact flagella from T. brucei indicates the flagellum membrane is a dynamic host-parasite interface.

117. Gerdes JM, Davis EE, Katsanis N. The vertebrate primary cilium in development, homeostasis, and disease. Cell. 2009; 137:32–45. [PubMed: 19345185]

118. Sharma N, Berbari NF, Yoder BK. Ciliary dysfunction in developmental abnormalities and diseases. Current Topics in Developmental Biology. 2008; 85:371–427. [PubMed: 19147012]

119. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. Nat Rev Mol Cell Biol. 2007; 8:880–893. [PubMed: 17955020] Excellent review of the broad role of cilia in human development and physiology.

120. MacGregor P, Savill NJ, Hall D, Matthews KR. Transmission stages dominate trypanosome within-host dynamics during chronic infections. Cell Host Microbe. 2011; 9:310–318. [PubMed: 21501830] A seminal paper that illustrates host-parasite dynamics in the context of chronic T. brucei infection.

121. Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL. A motility in the eukaryotic flagellum unrelated to flagellar beating. Proc Natl Acad Sci U S A. 1993; 90:5519–5523. [PubMed: 8516294]

122. Witman GB. Cell motility: deaf Drosophila keep the beat. Curr Biol. 2003; 13:R796–R798. [PubMed: 14561418]

123. Absalon S, et al. Intraflagellar transport and functional analysis of genes required for flagellum formation in trypanosomes. Mol Biol Cell. 2008; 19:929–944. [PubMed: 18094047]

124. Buisson J, et al. Intraflagellar transport proteins cycle between the flagellum and its base. Journal of Cell Science. 2013; 126:327–338. [PubMed: 22992454] Real-time fluorescent imaging in flagella of live parasites reveals the complex dynamics of IFT particle trafficking in T.brucei.

125. Price HP, et al. A role for the vesicle-associated tubulin binding protein ARL6 (BBS3) in flagellum extension in Trypanosoma brucei. Biochimica et Biophysica Acta. 2012; 1823:1178–1191. [PubMed: 22609302]

126. Bastin P, Pullen TJ, Sherwin T, Gull K. Protein transport and flagellum assembly dynamics revealed by analysis of the paralysed trypanosome mutant *snl-1* . J. Cell Sci. 1999; 112:3769–3777. [PubMed: 10523512]

127. Demonchy R, et al. Kinesin 9 family members perform separate functions in the trypanosome flagellum. Journal of Cell Biology. 2009; 187:615–622. [PubMed: 19948486] Identification of a specific kinesin employed for paraflagellar rod assembly in T.brucei

128. Carvalho-Santos Z, Azimzadeh J, Pereira-Leal JB, Bettencourt-Dias M. Evolution: Tracing the origins of centrioles, cilia, and flagella. Journal of Cell Biology. 2011; 194:165–175. [PubMed: 21788366]

129. Yuan S, Sun Z. Expanding Horizons: Ciliary Proteins Reach Beyond Cilia. Annual Review of Genetics. 2013

130. Fritz-Laylin LK, et al. The genome of Naegleria gruberi illuminates early eukaryotic versatility. Cell. 2010; 140:631–642. [PubMed: 20211133]

131. Francia ME, et al. Cell division in Apicomplexan parasites is organized by a homolog of the striated rootlet fiber of algal flagella. PLoS Biol. 2012; 10:e1001444. [PubMed: 23239939]

132. Finetti F, et al. Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. Nature Cell Biology. 2009; 11:1332–1339.

133. Hodges ME, Wickstead B, Gull K, Langdale JA. Conservation of ciliary proteins in plants with no cilia. BMC Plant Biol. 2011; 11:185. [PubMed: 22208660]

134. Mitchell DR. The evolution of eukaryotic cilia and flagella as motile and sensory organelles. Advances in Experimental Medicine and Biology. 2007; 607:130–140. [PubMed: 17977465]

135. Bloodgood RA. Sensory reception is an attribute of both primary cilia and motile cilia. Journal of Cell Science. 2010; 123:505–509. [PubMed: 20144998]

136. Satir P, Christensen ST. Overview of structure and function of mammalian cilia. Annu Rev Physiol. 2007; 69:377–400. [PubMed: 17009929]

137. Inglis PN, Ou G, Leroux MR, Scholey JM. The sensory cilia of Caenorhabditis elegans. WormBook. 2007:1–22. [PubMed: 18050505]

138. Pazour GJ, Agrin N, Leszyk J, Witman GB. Proteomic analysis of a eukaryotic cilium. J Cell Biol. 2005; 170:103–113. [PubMed: 15998802]

139. Wirschell M, et al. The nexin-dynein regulatory complex subunit DRC1 is essential for motile cilia function in algae and humans. Nature Genetics. 2013; 45:262–268. [PubMed: 23354437]

140. Baron DM, Ralston KS, Kabututu ZP, Hill KL. Functional genomics in Trypanosoma brucei identifies evolutionarily conserved components of motile flagella. J Cell Sci. 2007; 120:478–491. [PubMed: 17227795] This study identifies and functionally validates in T. brucei more than 40 highly conserved flagellar proteins, providing insight into flagellum motility mechanisms.

141. McGraw EA, O'Neill SL. Beyond insecticides: new thinking on an ancient problem. Nat Rev Microbiol. 2013; 11:181–193. [PubMed: 23411863]

142. Haas BJ, et al. Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature. 2009; 461:393–398. [PubMed: 19741609]

143. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. Lancet. 2005; 365:1487–1498. [PubMed: 15850634]

144. Carlton JM, et al. Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science. 2007; 315:207–212. [PubMed: 17218520]

145. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006; 368:1106–1118. [PubMed: 16997665]

146. Desjardins CA, et al. Genomics of Loa loa, a Wolbachia-free filarial parasite of humans. Nature Genetics. 2013; 45:495–500. [PubMed: 23525074]

147. Morrison HG, et al. Genomic minimalism in the early diverging intestinal parasite Giardia lamblia. Science. 2007; 317:1921–1926. [PubMed: 17901334]

148. Bloodgood RA. From central to rudimentary to primary: the history of an underappreciated organelle whose time has come. The primary cilium. Methods in Cell Biology. 2009; 94:3–52. [PubMed: 20362083]

GLOSSARY

African trypanosomiasis	a lethal disease scourging sub-Saharan Africa. Two specific subspecies of *T. brucei,T. b. gambiense* and *T. b. rhodesiense*,cause disease in humans. A third subspecies,*T. b. brucei*,and the related trypanosomes *T. congolense* and *T. vivax* infect only non-primates,causing wasting disease,which limits economic development in endemic areas
Ciliary necklace	a specialized region of the flagellar/ciliary membrane surrounding the transition zone that is defined by chalice-shaped filaments that extend outward from the axoneme and form indentations in the ciliary membrane
Flagellum Attachment Zone (FAZ)	a membranous-cytoskeletal structure that mediates lateral connection of the flagellum to the cell body. The FAZ runs the entire length of flagellum attachment and is composed of filaments extending from the flagellum to the cell body,where they connect to a cytoplasmic FAZ filament. The FAZ also includes four specialized microtubules (see MTQ) that lie adjacent to the FAZ filament and are abutted by a region of the smooth ER
Flagellar matrix	luminal compartment of the flagellum. Although the matrix is contiguous with the cytoplasm,protein entry is restricted by a diffusion barrier at the flagellar base
Microtubule Quartet (MTQ)	four specialized subpellicular microtubules that extend from the basal body to the cell anterior and subtend the region of plasma membrane where the flagellum attaches to the cell body. These four microtubules constitute part of the flagellum attachment zone (FAZ),are associated with a subdomain of the smooth ER,and are antiparallel to the other subpellicular microtubules

Subpellicular Microtubules	a cage-like array of microtubules that subtend the plasma membrane (pellicle) and run parallel to the cell's long axis
Propulsive Parasite Motility	sustained, forward parasite movement. Propulsive motility is distinguished from general writhing of the parasite that is generated by unregulated beating of the flagellum
NDRC	nexin-dynein regulatory complex. A large megadalton protein complex,initially described as separate as "nexin link"and "dynein regulatory complex",that is tightly attached to the axoneme and connects neighboring outer doublet microtubules. The NDRC maintains outer double alignment and plays a central role in control of dynein activity both of which are required for productive axonemal beating
Reynolds Number	a dimensionless number that describes the relative contribution of inertial and viscous forces to cell movement. Microbes operate at low Reynolds numbers,e.g. $<10^{-3}$,at which viscous forces dominate.
VSG	variant surface glycoprotein. *T.brucei* encodes thousands of different VSGs. The surface of bloodstream-form *T. brucei* is covered with approximately 10^7 VSG molecules of a single variant and cells in the population periodically change to an alternate VSG variant,thereby avoiding destruction by the host immune system.
Choroid Plexus	network of vessels in the brain that produce the cerebrospinal fluid
Pia Mater	the innermost layer of membranous connective tissue that surrounds the brain and spinal cord
Kinetoplast	a small,disc-like structure that consists of highly-interconnected,mitochondrial DNA molecules and associated proteins that participate in DNA compaction and replication. The kinetoplast is located adjacent to the basal body of the flagellum and is connected to the basal body via a network of filaments
Pleomorphic	having many forms. The term is used to refer to those isolates of T. brucei that produce both long slender and short stumpy morphotypes during the mammalian bloodstream stage of the life cycle. Compare to "monomorphic"
Monomorphic	having a single form. The term is used to refer to those isolates of T. brucei that produce only a single morphotype during the mammalian bloodstream stage of the life cycle,i.e. they do not exhibit the long slender –to –short stumpy transition. Monomorphic forms generally arise through prolonged laboratory cultivation and tend to produce an acute,highly virulent mouse infection,marked by absence of multiple waves of parasitemia that typically are seen with field isolates. Compare to "pleomorphic"

Trypomastigote	parasite morphotype in which the basal body lies posterior to the nucleus
Epimastigote	parasite morphotype in which the basal body lies anterior to the nucleus
Outer Arm Dynein (OAD)	axonemal multisubunit dynein motor that sits on the outer face of the axoneme. Outer arm dyneins cooperate with inner arm dyneins to power axonemal beating
Inner Arm Dynein (IAD)	axonemal multisubunit dynein motor that sits on the inner face of the axoneme. Inner arm dyneins cooperate with outer arm dyneins to power axonemal beating
Radial Spokes	elongated structures extending inward from axonemal outer doublets to contact the central pair apparatus. Radial spokes operate in conjunction with the central pair and NDRC to regulate axonemal dyneins

Biographies

Gerasimos Langousis received his B.Sc. in Biology from the University of Athens, Greece. He then continued his studies at Uppsala University, Sweden where he obtained his M.Sc. in Evolutionary Biology. Gerasimos is now pursuing PhD studies at the University of California, Los Angeles studying motility and sensory functions of the trypanosome flagellum in the group of Kent Hill.

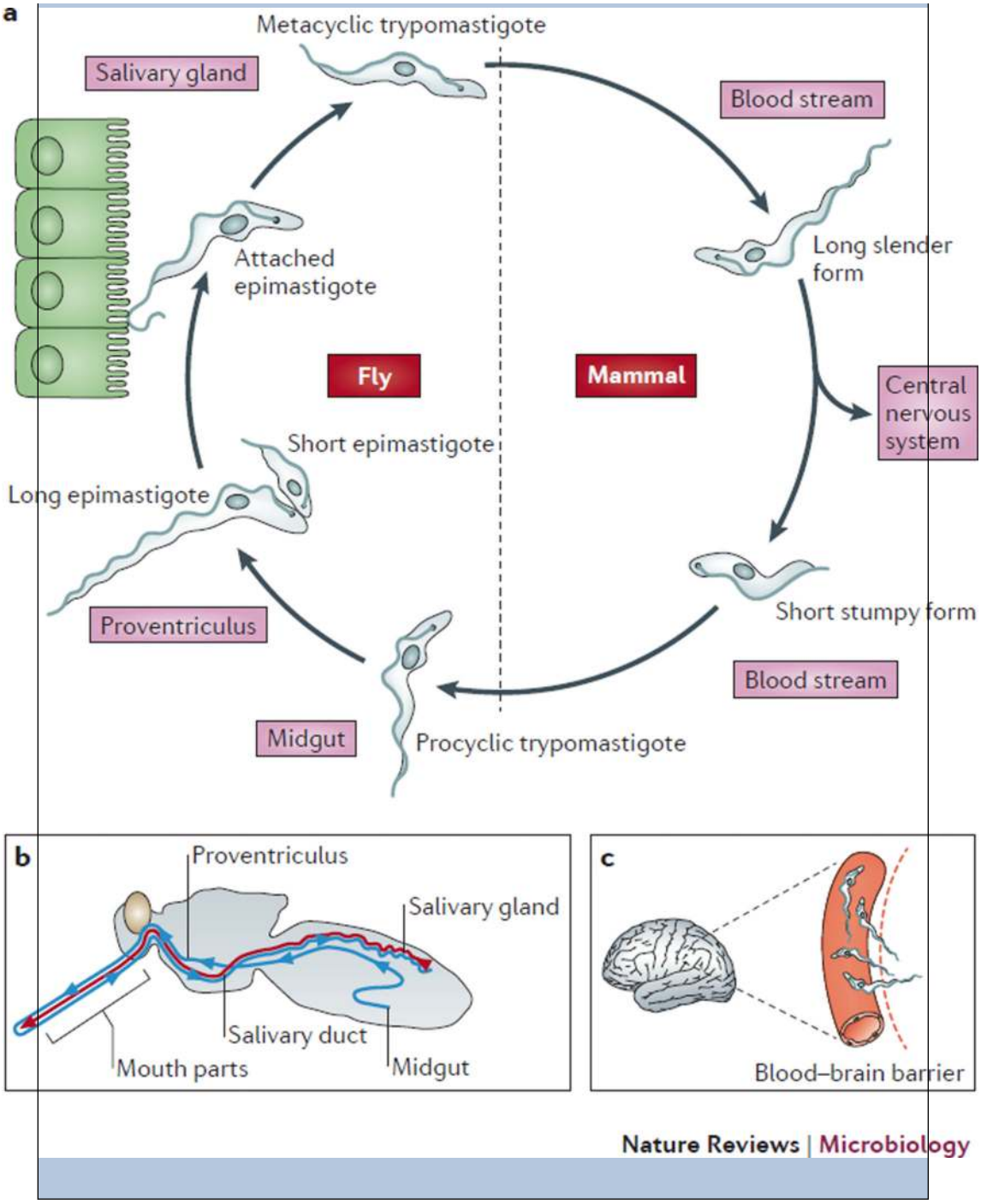
Kent Hill did his PhD training in Biochemistry with Professor Sabeeha Merchant at the University of California, Los Angeles. He then completed postdoctoral studies in yeast cell biology with Professor Lois Weissman at the University of Iowa followed by postdoctoral studies in trypanosome biology with Professor John Donelson at the University of Iowa, where he initiated his studies on flagellum biology in African trypanosomes. He is currently Professor of Microbiology, Immunology and Molecular Genetics at the University of California, Los Angeles.

Box 1

T. brucei life cycle

Albeit unicellular, *T. brucei* exhibits a complex life cycle with developmental transformations that are driven by specific gene expression programs and marked by cellular and metabolic differentiation. (A) Generalized life cycle. Infection of a mammalian host initiates when a tsetse fly bite delivers growth-arrested metacyclic trypomastigotes (MT) from the fly salivary gland to the mammalian bloodstream. Metacyclics then differentiate into proliferating long slender (LS) forms that establish and maintain a bloodstream infection. Parasites ultimately penetrate the blood vessel endothelium and invade extravascular tissues, including the CNS (panel C). Little is known about specific developmental forms present in extravascular compartments. In the bloodstream, a quorum sensing-like mechanism elicits differentiation of long slender forms into short stumpy (SS) forms that are cell-cycle arrested and pre-adapted for survival in the tsetse fly [120]. When an infected host is bitten by a tsetse fly, parasites are taken up with the bloodmeal into the midgut, where short stumpy forms differentiate into procyclic trypomastigotes (PT) that resume cell division and establish a midgut infection. Midgut procyclics then embark on an epic migration (panel B) that takes them through the peritrophic matrix, along the foregut to the proventriculus and from there, onward through the mouthparts, salivary ducts and ultimately into the salivary gland, where they attach to the gland epithelium (see panel B). In the proventriculus, procyclic trypomastigotes undergo extensive restructuring coupled to an asymmetric division to generate one long epimastigote (LE) and one short epimastigote (SE). The short epimastigote is the form that attaches to epithelial cells upon arrival in the salivary gland. Attached epimastigotes (AE) replicate and then complete the life cycle by differentiating into metacyclic trypomastigotes that detach from the epithelium and are uniquely adapted to survive in the mammalian host. (B and C) Cartoons illustrate parasite movements within the tsetse fly (B) and mammalian host (C). (B) Blue line depicts the route taken from midgut to salivary gland and the red line indicates route from salivary gland to mouthparts. (C) Parasites move out of blood vessels, penetrating the blood brain barrier (BBB) to enter the CNS.

BS: bloodstream. CNS: central nervous system. MG: midgut. PV: proventriculus. SG: salivary gland. SD: salivary duct. MP: mouthparts.



Box 2

Flagellum Assembly

T. brucei flagellum assembly depends on intraflagellar transport (IFT) [6], a bidirectional transport system that operates along outer doublets to deliver proteins into and out of the flagellum. Complexes of IFT proteins assemble into particles that mediate delivery of nascent flagellum subunits from their site of synthesis in the cytoplasm into the flagellum. Kinesin motors transport IFT particles and their cargo to the flagellar tip (anterograde transport), where cargo is released for assembly into the growing flagellum. IFT particles are returned to the base of the flagellum (retrograde transport) by dynein motors. IFT was originally identified in *C. reinhardtii* [121] and is now known to be a near universal feature of eukaryotic flagella [122]. *T. brucei* encodes a full cohort of IFT proteins that are required for flagellum assembly [123]. Live imaging of IFT in *T. brucei* (Video 2) suggest there are two pools of IFT particles at the flagellum base and that only one of these is actively participating in IFT [124]. These studies also revealed two distinct populations of anterograde IFT particles, each moving at different speeds. In addition to IFT, eukaryotes depend on a specialized protein complex, termed the BBSome, for trafficking of particular membrane proteins into and out of the flagellum. The BBSome is a multimeric complex of highly conserved proteins found only in ciliated organisms. In humans, mutations in BBSome genes result in Bardet Biedl Syndrome, a pleotropic disease caused by defective cilia. *T. brucei* possesses homologs of all BBSome subunits and there is indirect evidence connecting them to flagellum assembly [125], although their direct involvement has not been tested.

In addition to characteristics in common with other eukaryotes, *T. brucei* flagellum assembly exhibits parasite-specific features. First, the trypanosome flagellum is retained throughout the cell cycle, which differs from most other organisms studied, and assembly of the new and old flagellum appear to be controlled separately. Growth of each new flagellum follows a path defined by the old flagellum [86] and presence of the PFR appears to limit which outer doublet microtubules may be used for IFT [123]. Thus, *T. brucei* flagellum assembly must accommodate spatial constraints not seen in other organisms. The *T. brucei* flagellum also undergoes major changes in position and length throughout the parasite life cycle (Figures 1 and 3), indicating a need for plasticity in assembly systems that control organelle size and position. Furthermore, axoneme assembly must be coordinated with assembly of the PFR and FAZ (Figure 1). The regulatory systems responsible, as well as their relationships to canonical IFT and BBSome systems, remain to be discovered. PFR assembly depends on axoneme biogenesis but not vice versa, since IFT mutants fail to assemble both structures while PFR mutants assemble normal axonemes [6, 126]. Interestingly, PFR assembly requires the action of a kinesin 9 family protein that is devoted to construction of this extra-axonemal structure [127].

Box 3

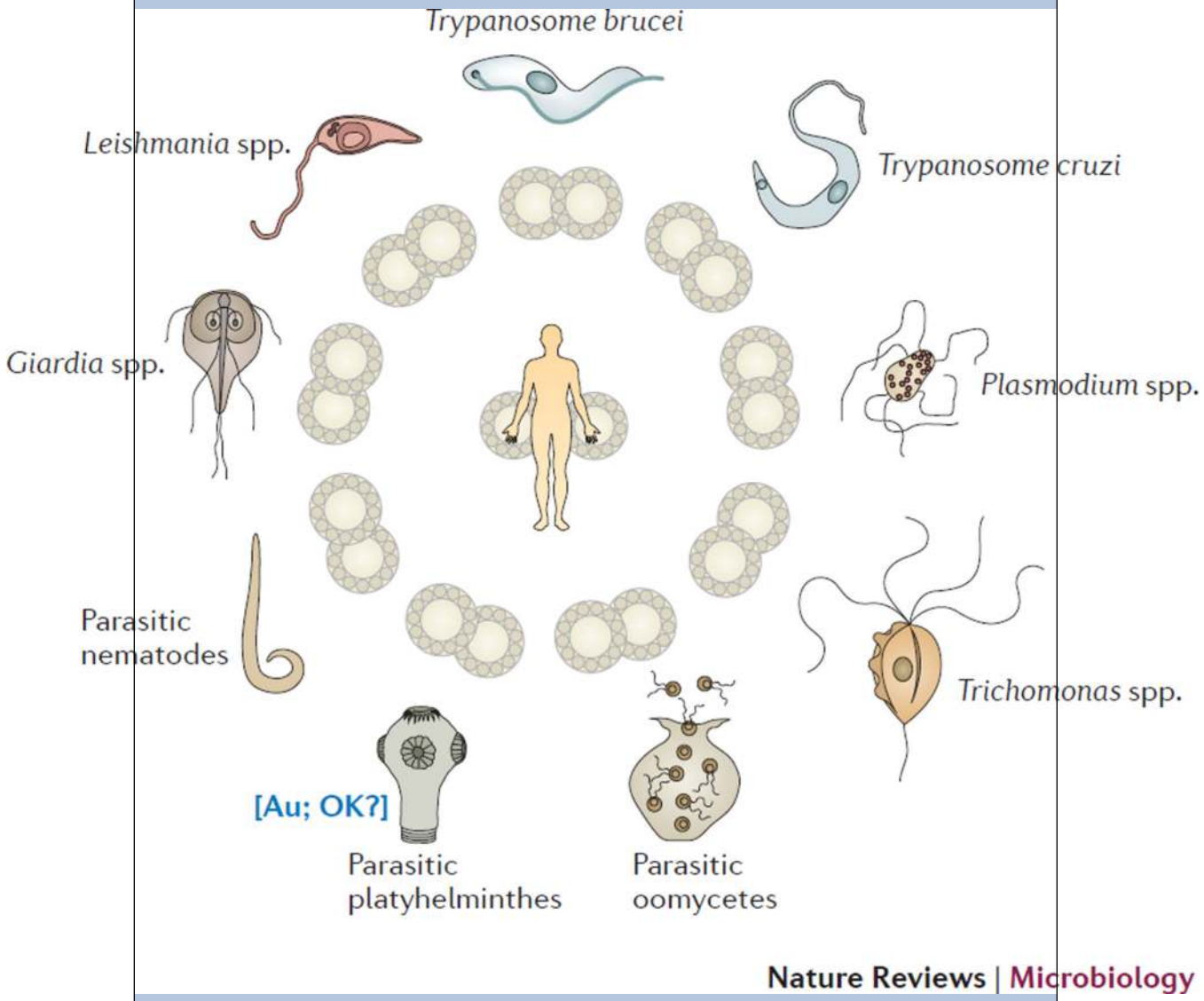
The eukaryotic flagellum is a conserved organelle of high significance to human health

The eukaryotic flagellum (synonymous with cilium) is an emblematic organelle, present in most extant lineages with notable exceptions being many amoebas, fungi and seed plants [128]. In contrast to typical organelles, the cilium protrudes into the environment and is surrounded by the extracellular milieu. The interface with the environment is the flagellar membrane that ensheaths the axoneme, the structural and functional core of the organelle. Axoneme structure is highly conserved across eukaryotes and generally exhibits a 9 + 0 or 9 + 2 arrangement of microtubules, although lineage-specific variations and extra-axonemal structures do exist [129]. Cilium structural conservation is mirrored at the molecular level, with a range of ciliary proteins that are broadly conserved in ciliated eukaryotes yet absent in non-ciliated species [130]. Ciliary proteins seem to have plastic functions since they can be harnessed for functions outside the cilium 131, 132 133.

Given the structural and molecular conservation across diverse phyla and the absence of similarities to prokaryotes and viruses, the cilium is postulated to be an early eukaryotic innovation and present in the last eukaryotic common ancestor. The initial function of the cilium is uncertain but in extant lineages it has assumed both motility and sensory capabilities [134]. Motile cilia propel single cells and extracellular liquids, and also contribute sensory functions [135]. On the other end of the spectrum, nematode neuronal cilia and mammalian primary cilia are immotile and solely devoted to environmental sensing [136, 137]. The relevance of cilia in human development and physiology is paramount, as cilium defects lead to a broad class of inherited diseases, termed ciliopathies, which include primary ciliary dyskinesia, polycystic kidney disease, nephronophthisis, Bardet-Biedl syndrome and Meckel syndrome [117–119]. These diseases exhibit diverse clinical manifestations, such as respiratory malfunction, infertility, body axis defects, mental retardation, polydactyly, retinopathy, renal failure and obesity, thus showcasing the multiple roles that the cilium integrates. Understanding of these roles has long been aided by protozoan model systems such as *C. reinhardtii* [121, 138, 139] and *T. brucei*. The *T. brucei* flagellum is one of the best characterized, can be manipulated by potent molecular genetic tools, and has provided valuable insights into how cilia assemble and move [69, 124, 140].

Recent findings have reinforced the appreciation of the *T. brucei* flagellum as a crucial host pathogen interface. This will hopefully raise the awareness of the cilium's importance in several additional pathogens of medical and economic importance. A plethora of unicellular and multicellular eukaryotic pathogens rely on cilia to complete their life cycle, yet the contribution of their cilia to virulence and pathogenesis remains largely unstudied. These pathogens cause tremendous human suffering, pose a significant threat to global public health, and contribute to the destruction of human resources [141, 142]. Examples are shown in the figure and include kinetoplastid parasites [3], causative agents of malaria [143], trichomoniasis [144], schistosomiasis [145], filariasis [146], epidemic diarrhea [147] along with the potato blight pathogen [142]. Effective vaccines are

not available for any of these pathogens and new ways to combat the diseases that they cause are desperately needed. It will be thus crucial to apply the paradigm of the cilium as host-pathogen interface in these parasites and to test the putative roles of cilia in virulence and pathogenesis. Similar to the renaissance of mammalian cilia research [148], such studies will undoubtedly yield valuable insights into pathogen biology and might uncover novel avenues for therapeutic intervention.



Online Summary

- *Trypanosoma brucei* is a unicellular pathogen that causes lethal sleeping sickness in humans, a devastating and neglected tropical disease endemic to vast regions of Africa. *T. brucei* also infects wild and domestic livestock, limiting sustainable development and is thus considered both cause and consequence of poverty.
- *T. brucei* possesses a single flagellum that is present throughout the parasite's cell and life cycle. The flagellum emerges from a membrane invagination at the posterior end of the cell and remains attached to the cell body for most of its length.
- The flagellum contains cytoskeletal structures ensheathed by a specialized flagellar membrane that interfaces with the external environment and exhibits distinct protein and lipid composition from the rest of the cell surface. The *T. brucei* flagellum has multiple functions and is essential for parasite motility, viability, transmission and pathogenesis.
- Flagellum-mediated motility is powered by the axoneme, a biological machine that converts dynein motor structural changes into flagellum beating and parasite propulsion. *T. brucei* motility is crucial for movement through host tissues and provides a surprising immune evasion mechanism.
- In addition to motility, the *T.brucei* flagellum is a critical morphogenetic hub that controls cell shape and size, directs organelle segregation and governs cell division. These functions can be modulated during developmental transitions of the parasite and are achieved by direct or indirect physical connections to other cellular elements.
- The flagellum is a crucial host pathogen interface with important roles in parasite transmission and virulence. Flagellar proteins mediate attachment to host tissues, perform uptake of host growth factors and promote parasite survival against host immunity.
- *T. brucei* is an excellent model system to study the biology of the highly conserved eukaryotic flagellum, offering valuable insights into how flagella assemble, move and sense the environment. Continued studies of the *T.brucei* flagellum hold the promise of great impact on human health since flagella are paramount in human development and physiology and are salient yet unexplored features of many human pathogens.

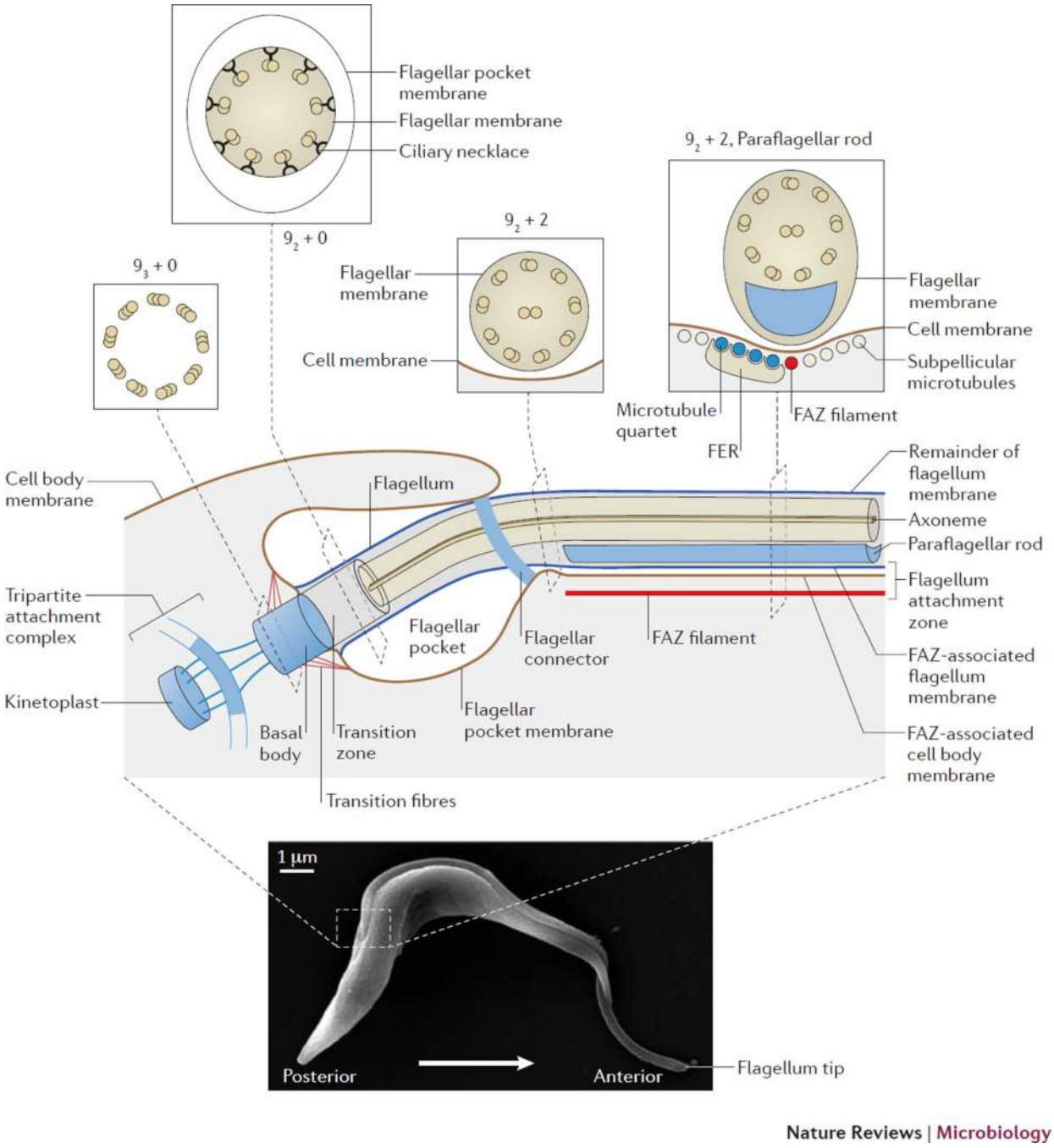


Figure 1. *T. brucei* flagellum overview

Bottom shows cartoon diagram of a *T. brucei* cell with flagellum in black. Cell movement (arrow) is with the flagellum tip leading. Anterior (A) and posterior (P) of the cell are labeled. Top shows a cartoon diagram of flagellum emergence from the flagellar pocket (FP) at the posterior end of the cell (boxed region in bottom panel). The flagellum is built around a core of microtubules that are arranged in characteristic patterns, as shown above the corresponding cross sections. The flagellar axoneme (AX) emanates from the basal body (BB) via the transition zone (TZ) and is laterally connected to the cell membrane (CM) via

the flagellum attachment zone (FAZ). Extra-axonemal structures inside the flagellar membrane (FM) include the paraflagellar rod (PFR) and the ciliary necklace (CN). A tripartite attachment complex (TAC) links the basal body to the kinetoplast (KP) and transition fibers (TF) connect the basal body to the flagellar pocket. The distinctive architecture of the trypanosome flagellum is dictated by specialized membrane/cytoskeletal features, such as the flagellar pocket collar (FPC) and the FAZ filament (FAZF). Adjacent to the FAZ filament are the subpellicular microtubules (SPM), microtubule quartet (MTQ) and FAZ ER (FER). Numbered magenta lines indicate specific subdomains of the parasite cell surface, corresponding to the cell body membrane (1), flagellum (2) and cytoplasmic (3) sides of the flagellar pocket membrane (FPM), FAZ-associated flagellum (4) and cell body (5) membranes, the flagellum tip (6), and the remainder of the flagellum membrane (7)

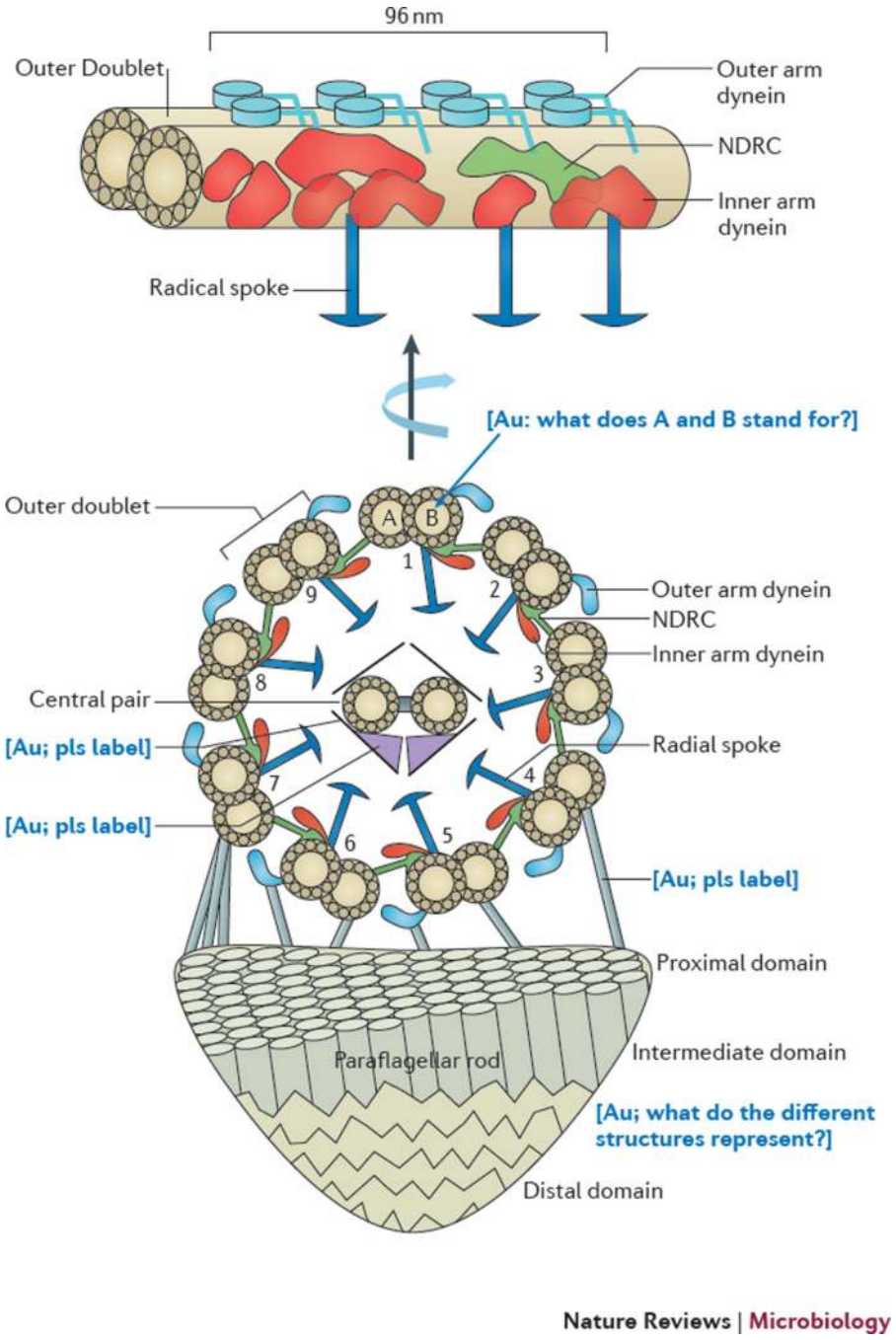


Figure 2. Axoneme and PFR structure

(Bottom) Cartoon showing transverse section detail of axoneme and PFR, as viewed looking posterior to anterior. (Top) Cartoon showing longitudinal view of the axonemal repeating unit on one outer doublet microtubule, oriented with the basal body end toward the left. Prominent axonemal structures are labeled and include outer doublet (OD) and central pair microtubules, outer arm dyneins (OAD) and inner arm dyneins (IAD), radial spokes (RS) and the nexin-dynein regulatory complex (NDRC). Outer doublet microtubules are

numbered 1 – 9, as indicated. Proximal (P), intermediate (I) and distal (D) domains of the PFR are shown.

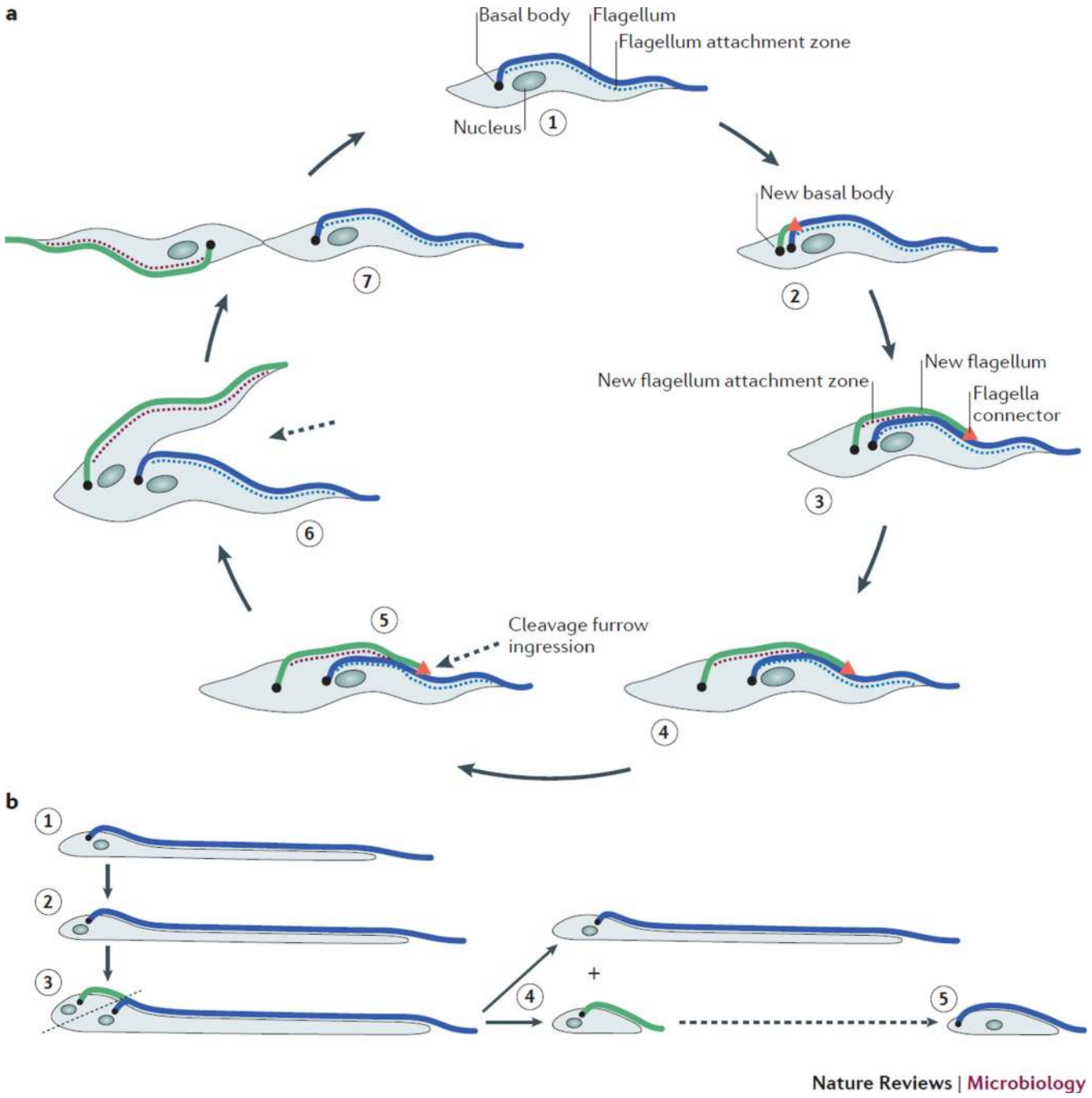


Figure 3. The flagellum in cell division and cell morphogenesis

(A) Cartoon diagram showing the relationship of the flagellum and flagellum-associated structures to key steps of the cell division cycle in procyclic form trypomastigotes. (i) In G1 the cell has a single flagellum, FAZ , basal body and nucleus. (ii) The first recognizable event in cell division is formation of a new basal body, which moves posterior to the old basal body and elongates to form the new flagellum. The tip of the new flagellum is attached to the old flagellum by the flagella connector. (iii) The new flagellum elongates in parallel with a new FAZ and is guided by attachment to the old flagellum via the flagella connector.

(iv) Elongation of the flagellum and FAZ continue until the flagella connector reaches a specific stop point, approximately 0.6 lengths of the old flagellum, at which point the two basal bodies separate fully. (v) Mitosis ensues and the new nucleus moves to lie between the new and old basal body. (v - vi) Cytokinesis initiates with cleavage furrow ingression beginning at the tip of the new flagellum and FAZ (red arrow) and progressing anterior to posterior along a line between the two flagella. Strikingly, cleavage furrow ingression does not depend on an actomyosin contractile ring, which governs membrane abscission in other eukaryotic cells. (vii) Ultimately, daughter cells are connected at their posterior ends and oriented in opposite directions, with their flagella exerting rotational and pulling forces that facilitate final cell separation to yield two G1 cells[30, 50]. Events for division of bloodstream form cells are essentially the same, except that there is no flagella connector and both nuclei remain anterior to both kinetoplasts[72]. (B) Major morphological variants that occur during the developmental cycle within the tsetse are shown. Restructuring and repositioning of the flagellum is a prominent feature of cellular differentiation events that give rise to these developmental forms. (i) A mesocyclic trypomastigote is generated from a procyclic cell through elongation of the flagellum and cell body. (ii) Long epimastigotes are formed via repositioning of the nucleus and basal body. (iii – iv) An asymmetric division gives rise to one long and one short epimastigote. The short epimastigote arises via cleavage furrow initiation at the tip of a shortened flagellum and FAZ (arrow in iii). (v) Metacyclic trypomastigotes are formed through an asymmetric division that again repositions the nucleus and basal body.

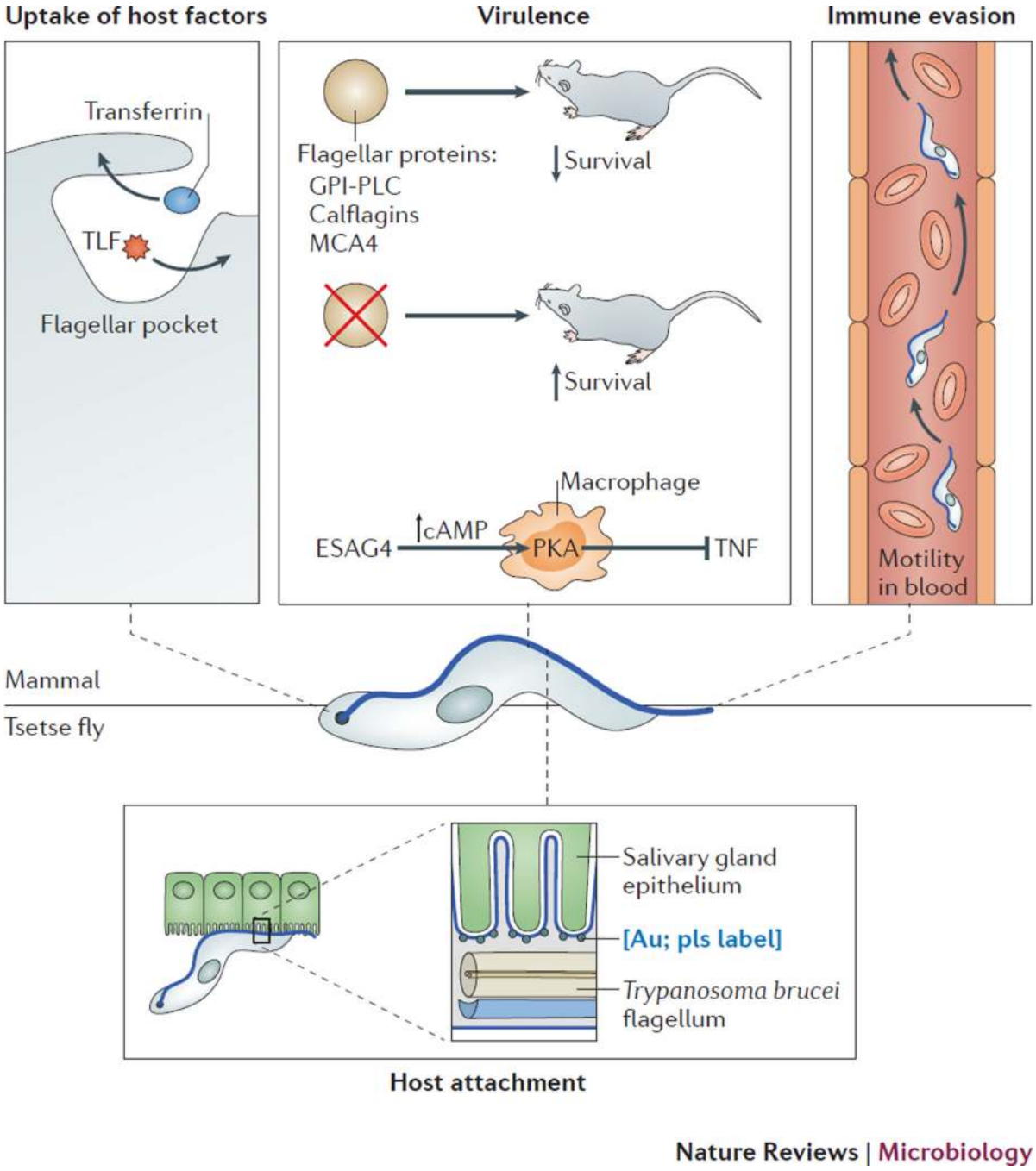


Figure 4. The flagellum as a platform for host-pathogen interactions

Boxes highlight flagellum features that contribute to host-parasite interaction. In the mammalian host, receptors on the trypanosome flagellar pocket mediate uptake of host factors that are essential for parasite survival, such as transferrin (Tf), as well as those that dictate host-range specificity, such as trypanosome lytic factor (TLF). Several flagellar proteins contribute to virulence in the mammalian host. Loss of flagellar proteins GPI-PLC, calflagins (CFs)or metacaspase 4 (MCA4)reduces parasite virulence and prolongs mouse survival through unknown mechanisms. Flagellar adenylate cyclase (ESAG4) interferes with

the host's early innate immune response to promote parasite virulence. Flagellum-dependent motility facilitates immune evasion through clearance of host immunoglobulin from the parasite surface. In the tsetse fly, the flagellum-mediated attachment to the salivary gland epithelium enables parasite persistence and marks the final stage of differentiation into mammalian infectious forms.