Movement of microbial pathogens plays an important role in pathogen biology, host-pathogen interaction, and disease pathogenesis. Microbial pathogens employ a variety of mechanisms for cell locomotion, including passive movement within their host's circulation, cooptation of host cytoskeletal and membrane transport pathways, and active self-propulsion through the action of flagellar, amoeboid, or gliding motility. The contribution of pathogen locomotion to virulence is well documented for bacterial and viral pathogens; it functions in chemotaxis, survival in the environment, host cell attachment and invasion, intracellular locomotion, colonization of host tissues, and dissemination within the host (46, 49, 53). In the case of protozoan pathogens, e.g., Plasmodium spp., Toxoplasma gondii, trypanosomes, and Leishmania spp., the contribution of cell motility to host-pathogen interactions has been largely unexplored. The complex life cycles of these organisms, generally requiring passage through multiple hosts, as well as the variety of hosts and host tissues that they colonize, provide numerous barriers to cell movement that must be overcome. Investigation of cell motility in these organisms therefore presents an opportunity not only for advancing our understanding of microbial pathogenesis but also for illuminating novel aspects of cellular locomotion.

Recent studies on Plasmodium and Toxoplasma have demonstrated a role for parasite motility in the mammalian host and/or insect vector (19–21, 52, 58, 70). For most protozoa, however, a specific requirement for active parasite movement remains strongly implied but not tested. Likewise, we have only just begun to understand the molecular mechanisms behind the diverse forms of motility employed by parasites to navigate within their environment. Some of these mechanisms resemble those employed for motility in other organisms, while others have features that represent unique adaptations to the demands imposed on a particular parasite. A more complete understanding of these mechanisms is therefore likely to facilitate identification of novel targets for therapeutic intervention in parasitic disease. Finally, protozoa also provide important model systems for investigating the fundamental mechanisms of cell locomotion. Examples include structural and functional studies of cilia and flagella in paramecia and trypanosomes (22, 68, 74) and of gliding motility in apicomplexan parasites (52).

This review will discuss biological and mechanistic aspects of cell motility in African trypanosomes, protozoan parasites that are the causative agent of African sleeping sickness. We will first discuss the importance of trypanosome cell motility for the interaction of the parasite with its mammalian host and insect vector. Next we will summarize what is known about the unusual and distinctive swimming behavior of trypanosomes. Finally, we will discuss the main structural features of the trypanosome motility apparatus and evidence for the requirement of these structures for normal cell motility. Emphasis will be placed on features that are unique to trypanosomes, and for the most part, we will restrict our discussion to Trypanosoma brucei, drawing comparisons to other kinetoplastid parasites where appropriate.

**FUNCTIONS FOR MOTILITY IN TRYPANOSOMES**

African trypanosomes, e.g., T. brucei and related subspecies, are unflagellated parasites that cause African trypanosomiasis in humans and in wild and domestic animals. T. brucei is the causative agent of human African trypanosomiasis, a fatal disease that is commonly referred to as "African sleeping sickness." These parasites are digenetic organisms, completing part of their life cycle in a mammalian host and part in an insect vector, the tsetse fly. T. brucei is transmitted to the bloodstream of a mammalian host through the bite of an infected tsetse fly. Once in the bloodstream, the parasites multiply extracellularly for a period of weeks to months. They eventually penetrate the blood vessel endothelium, spread within the connective tissues, and infiltrate the host's central nervous system (CNS), where they initiate a cascade of events that result in fatal sleeping sickness. Clinical manifestations of sleeping sickness are divided into an early stage, in which parasites are found in the blood and lymph, and a late stage, when parasites have invaded the CNS. The early and late stages of the disease are characterized by distinct clinical symptoms and respond very differently to antiparasitic drugs (57). If untreated, sleeping sickness is always fatal, and the fatal course of the disease is directly linked to the presence of parasites in the CNS (57). Hence, the pathogenic features of sleeping sickness are directly related to migration of the parasite to specific host tissues. Since T. brucei is extracellular at all stages of its life cycle, it is dependent upon its own vigorous cell motility for extravasation and dissemination within the host.

The requirement for trypanosome cell motility is especially acute during transmission through the tsetse fly, where the parasite must undergo an ordered series of developmental transformations and directed migrations in order to achieve its goal of being delivered to a new, mammalian host (77, 79, 81, 85). Development within the tsetse fly has been extensively characterized by Vickerman (79, 81), Van Den Abbeele (77), and others (85) and is briefly summarized here. Following a blood meal, ingested quiescent bloodstream-form trypomastigotes first differentiate into actively dividing procyclic trypo-
mastiogotes and establish an infection in the tsetse fly midgut. The parasites then migrate from the midgut into the ectoperitrophic space and then through the proventriculus into the foregut, where they differentiate into elongated and asymmetrically dividing “postmesocyclic” epimastigotes (77). These elongated epimastigotes complete the journey through the proboscis and hypopharynx to reach the lumen of the salivary gland, where the final stage of development occurs. Parasites advancing to the foregut and proboscis exhibit dramatically increased motility compared to those found in the midgut (77). Once parasites are in the salivary gland, cell division is completed, generating short epimastigotes, which attach themselves to the gland epithelium through intricate membrane and cytoskeletal connections that are established between the parasite flagellum and the epithelial cell membrane (75, 79, 81). These attached epimastigotes differentiate into variant surface glycoprotein (VSG)-coated metacyclic trypomastigotes that detach from the epithelium and are now uniquely suited for survival in the mammalian bloodstream. Thus, migration of the parasite from the midgut to the salivary gland and the concomitant developmental changes that occur along the way are required for transmission to the mammalian host.

The importance of trypanosome motility for completion of the journey from the midgut to the salivary gland is obvious but remains to be tested experimentally. In addition, other important questions arise concerning development in the tsetse. Do changes in cell morphology and motility within specific compartments of the fly occur in response to environmental cues? Does the parasite arrive in the salivary gland by chance, or is this movement directed in response to chemotactic signals from the host? What is the nature of the highly structured attachment sites that form between the parasite flagellum and the salivary gland epithelium? Are these structures related to the desmosome-like adhesion junctions (see below) between the flagellum and the trypanosome cell body? The answers to these important questions await further investigation.

**PHYSIOLOGICAL ASPECTS OF TRYPANOSOME CELL MOTILITY**

The trypanosome cell body is roughly cylindrical in shape, approximately 10 to 20 μm long, with tapered anterior and posterior ends (Fig. 1A), though some developmental stages within the tsetse fly may be much longer (77). Cell motility is accomplished through the action of a single flagellum that emerges from the basal body apparatus near the posterior end of the cell. The flagellum is surrounded by its own membrane that is distinct from, but contiguous with, the plasma membrane (1). A specialized compartment called the “flagellar pocket” forms from an invagination of the plasma membrane at the position where the flagellum emerges from the cell (84). Unlike the situation in most flagellated cells, the trypanosome flagellum is attached to the cell not only through the basal body but also along the length of the flagellum. This attachment is mediated by a highly ordered array of transmembrane cross-links that form a unique cytoskeleton-membrane domain called the flagellum attachment zone (FAZ) (see below) (26, 80). Because of this unusual arrangement, movement of the cell body is tightly coupled to flagellar wave propagation, giving the appearance of an “undulating membrane” on one side of the cell when live parasites are examined by light microscopy. The possibility that undulations produced by cytoskeletal elements within the cell body, rather than the flagellum, also contribute to cell motility (34) is intriguing but difficult to test experimentally.

High-speed cinematography and stroboscopic illumination studies have demonstrated that the flagellar waves in *T. brucei* and other trypanosomatids initiate at the distal tip of the flagellum and move toward the basal body (30, 31, 82, 83; M. E. J. Holwill, abstract from the 17th Meet. Soc. Protozool., J. Protozool. 11(Suppl.):40, abstr. 122, 1964). As a result, the direction of cell movement is toward the flagellar tip. Many trypanosomatid species are capable of reversing the direction of flagellar wave propagation and consequently the direction of cell movement (31, 74; Holwill, J. Protozool. 11(Suppl.):40, 1964), although this has not been demonstrated for *T. brucei*. Flagellar wave reversal has been examined in detail for the trypanosomatid *Crithidia oncopelti*, where it is used as an avoidance response and is influenced by the Ca2+ ion concentration (74). Tip-to-base wave propagation is unusual, since most flagellated cells propagate flagellar waves from base to tip (29). It remains to be determined whether the unusual directionality of wave propagation in trypanosomes results from specialized structural features of the trypanosome flagellum or the presence of specific regulatory factors. Tip-to-base wave propagation does not require an attached flagellum, since it is also observed in kinetoplastid parasites with a free flagellum, e.g., *Leishmania* spp. and *C. oncopelti* (31, 67).

The *T. brucei* flagellum wraps around the cell body in a left-handed helix as it extends from the posterior to the anterior end of the cell (Fig. 1A) (26, 80). As a result, beating of the flagellum produces a spiral waveform and causes the entire cell to rotate, driving it forward toward the flagellum tip in an “auger-like” motion (Fig. 1B) (82, 83). This spiral movement is a distinguishing feature of trypanosome cells. Indeed, the genus name *Trypanosoma* stems from the Greek word for auger, “trypon,” and translates as “auger cell.” Thus, the movement of the trypanosome cell through its environment resembles a corkscrew threading into a cork rather than a boat being driven forward by a twirling propeller or rowing oars. The unusual spiral motility of trypanosomes, also observed in treponemes and other spirochetes with attached flagella (7), is an extremely efficient means of cell locomotion and is thought to facilitate movement through very viscous environments (34), such as the bloodstream and connective tissues of the mammalian host. It remains to be determined experimentally whether the motility of *T. brucei* actively facilitates extravasation or influences disease pathogenesis.

Trypanosomes are vigorous swimmers, moving with a forward velocity as high as 20 μm/s, and are capable of highly directional cell motility, i.e., moving for extended periods in one direction. Careful observation of wild-type trypanosomes revealed an interesting aspect of this organism’s swimming behavior: the parasites occasionally stop their forward motion and tumble or spin in one location, then move forward again, often in a new direction (33). During this tumbling period, the trypanosome flagellum assumes a bent hook shape that is similar to the large curvature seen in sperm flagella during the transition from linear swimming to nonprogressive tumbling.
FIG. 1. *T. brucei* cell structure. (A) Scanning electron micrograph of *T. brucei*. The flagellum (arrow) is attached to the cell body along its length. The flagellum emerges from the posterior (p) end of the cell and charts a left-handed helical path as it extends toward the anterior end of the cell.
Propagation is presumed to operate in all eukaryotic organisms (42). The tumbling, or “hyperactivated” motility, of sperm cells occurs in response to Ca$^{2+}$ in vitro and as yet unidentified physiological cues in vivo (42). As discussed above, Ca$^{2+}$ also affects flagellar beat and cell movement in the trypanosomatid C. oncopelti (74). At present, it is not known whether environmental factors, such as Ca$^{2+}$, influence flagellar beat in T. brucei. The presence of calmodulin and other Ca-binding proteins (61, 86) in the flagellum of this parasite, together with the established role of Ca$^{2+}$ in regulating flagellar beat in other organisms (42, 74), makes this a likely possibility. The “run-and-tumble” motility of trypanosomes is also reminiscent of the chemotactic behavior of bacterial cells (73), and these observations together raise the intriguing possibility that trypanosomes may be capable of chemotaxis. Although chemotaxis has not yet been demonstrated in trypanosomes, such an idea is not inconsistent with the physiology of trypanosome infection in the mammalian host or with requirements for parasite development within the tsetse fly vector (see above).

**STRUCTURAL FEATURES OF THE TRYPANOSOME FLAGELLAR APPARATUS**

**Flagellar axoneme.** The axoneme is the basic unit of wave generation in the eukaryotic flagellum and consists of an estimated 250 proteins (23, 43) arranged in a core structure of 9 peripheral microtubule doublets surrounding 2 singlet microtubules (68). This canonical “9 + 2” arrangement is highly conserved throughout evolution, and electron microscopy studies indicate that it is the same in trypanosomes as it is in other eukaryotic organisms (Fig. 1C) (14, 68, 71, 80). Although biochemical analysis of the axoneme has not been conducted for trypanosomes, the same general mechanism for wave propagation is presumed to operate in all eukaryotic flagella. A simplified description of this general mechanism follows. For detailed reviews of axoneme structure and models for wave generation, see references 14, 68, and 71 and references therein.

Within the axoneme, ATP-dependent binding and release of dyneins between adjacent peripheral microtubule doublets causes these microtubules to slide past one another. This sliding produces a localized bend that is propagated along the length of the flagellum through precisely coordinated activation and deactivation of dynein subsets, together with coordinated resistance forces on the side opposite the active dyneins (15, 68). This generalized model of flagellar wave generation and propagation is widely accepted and is presumed to operate in all flagellated eukaryotic cells. Four decades of careful biochemical and genetic studies with a variety of organisms have identified key structural and motor proteins and have provided a detailed picture of the arrangement of protein subunits within the axoneme (25, 68, 71). Many current efforts are therefore focused on the identification and characterization of regulatory proteins that are responsible for the precisely coordinated action of sliding and resisting forces necessary for wave propagation (60). The recent development of powerful tools for the molecular genetic analysis of trypanosomes (11, 41, 48, 76) makes them an excellent model system for investigating the molecular mechanisms of axoneme function. In particular, analysis of gene function through targeted gene disruption, RNAi knockdown of gene expression, and inducible ectopic gene expression in T. brucei will nicely complement the elegant genetic approaches that have been used with Chlamydomonas reinhardtii (23, 71).

**Paraflagellar rod.** In addition to the axoneme, the other major structural feature of the trypanosome flagellum is the paraflagellar rod (PFR), a large lattice-like filament that begins just anterior to the flagellar pocket and runs parallel to the axoneme within the flagellar membrane (3, 10, 44). Unlike the axoneme, which is a universal feature of all eukaryotic flagella, the PFR is observed only in kinetoplastids, euglenoids, and dinoflagellates (10). It is composed of two major protein subunits, designated PFRA and PFRF, in T. brucei (3, 44). The corresponding proteins are designated PFR2–PFRA and PAR2–PAR3 in Leishmania spp. and T. cruzi, respectively (3, 44). A few less-abundant components have recently been described (4), but most of the biochemical data on the PFR come from studies of its two main subunits. The T. brucei PFR has a diameter of approximately 150 nm and, when viewed in cross section, consists of three structurally distinct domains designated proximal, intermediate, and distal, based on their positions relative to the axoneme (Fig. 1C) (10). The PFR is connected to the axoneme by filaments between the PFR proximal domain and axonemal doublets 4 to 7 (10).

Until recently, a function for the PFR in cell motility was entirely speculative, since this enigmatic structure is not a universal feature of motile flagella or even of those of kinetoplastids (10). Nevertheless, independent studies of T. brucei and Leishmania mexicana have now unequivocally demonstrated that the PFR is required for normal cell motility (6, 32, 67). In L. mexicana, the PFR2 gene was deleted by conventional gene disruption (67). The resultant PFR2 knockout parasites were viable in culture but possessed an incompletely formed PFR and exhibited pronounced changes in flagellar waveform and a fourfold reduction in forward swimming velocity. Stroboscopic photomicroscopy revealed that the frequency and amplitude of flagellar beat were reduced, and previous studies indicated that such an effect could result from reduced flagellum rigidity (9, 31, 67). Similar effects on motility and flagellar beat were observed in PFRA knockout and PFR2 double-knockout L. mexicana mutants (45). Interestingly, the PFR2 homologue in T. brucei, PFRA, appears to be essential, since efforts to delete both PFRA alleles by conven-

(a). Bar, 1 μm. (B) Cartoon depicting the auger-like movement of the trypanosome cell. The dotted arrow indicates cell motion. (C) Schematic illustration of a transverse section through the FAZ, as viewed from the posterior end of the cell. The flagellum (top) contains a canonical 9 + 2 axoneme (1) and the PFR (2). A network of thin filaments (3) connects the axoneme and PFR to the cytoplasmic FAZ filament (4). Together with a specialized quartet of reticulum-associated microtubules (5), these filaments comprise the FAZ, which extends from the flagellar pocket to the anterior end of the cell. See the text for further details. The proximal (p), intermediate (i), and distal (d) regions of the PFR are indicated.

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tional gene disruption were unsuccessful (32). Gull and colleagues circumvented this problem by using RNAi to knock down PFRA levels without completely blocking gene expression (6). The resultant PFRA-deficient mutants are viable but have only a rudimentary PFR and are paralyzed (5, 6). These experiments with L. mexicana and T. brucei not only provide the first demonstration of a motility function for the PFR but also clearly establish the utility of RNAi for determining the function of essential genes, for which conventional gene knockouts are not possible.

Interestingly, loss of PFRA also causes mislocalization of PFRC, which is deposited in the distal tip of the mature flagellum (6). Further analysis of PFRA-deficient and PFR1 PFR2 mutants has provided important information about the processes of flagellum biogenesis in trypanosomes (2, 5,45). This process appears to be related to intraflagellar transport (IFT), a motility process that provides a means for delivering axonemal subunits from the cytoplasm to the tip of the elongating axoneme (65). IFT was first discovered in flagella of the green alga C. reinhardtii (38, 64), where large IFT particles composed of an estimated 16 polypeptides (12, 59, 65) are transported bidirectionally along the flagellar axoneme between the outer doublet microtubules and the flagellar membrane (37, 65). These particles are hypothesized to carry axonemal subunits to the flagellum tip (anterograde movement), where they drop off their cargo, and then return to the cytoplasm (retrograde movement) to be reutilized (65). The identities of some IFT particle proteins in C. reinhardtii have been determined (12, 55, 56, 65), and mutations in the corresponding genes cause defects in flagellar assembly (8, 16, 54, 55, 65). IFT is dependent upon members of the kinesin (anterograde transport) (37) and dynein (retrograde transport) (56) families of molecular motors. Although the precise functions of individual IFT particle proteins are not known, the process is conserved in other eukaryotes, e.g., Caenorhabditis elegans (12) and mammals (54, 55), and the reader is referred to reference 65 for a detailed review of IFT in these organisms. Genes for putative homologues of IFT components are present in the T. brucei genome database, which can be accessed at http://www.sanger.ac.uk/Projects/T_brucei/(24). Direct analysis of IFT in trypanosomes promises to be a very exciting area of future investigation.

Flagellum attachment zone. In contrast to the situation in most flagellated cells, the flagellum of T. brucei is attached along its length to the cell body in a specialized cytoskeleton-membrane domain, the FAZ (Fig. 1C) (26, 69, 78, 80). The FAZ extends from the flagellar pocket to the anterior end of the cell, and within this region the flagellar membrane and plasma membrane are held in close juxtaposition by desmosome-like adhesion junctions (26, 69, 78, 80). The distal tip of the flagellum extends slightly beyond the anterior end of the cell, and the length of this “free” flagellar segment is different in different developmental stages.

The cytoplasmic side of the FAZ is defined by an electron-dense filament of unknown composition that subvents the plasma membrane and runs parallel to the long axis of the cell (26). Immediately to the left of this FAZ filament, as seen looking toward the anterior end of the cell, is a quartet of specialized microtubules that are associated with a membranous tubule. These four microtubules fractionate with the FAZ and flagellar cytoskeleton upon extraction with detergent and Ca2+, conditions that depolymerize the other microtubules of the subpellicular corset (36, 62). The function of this set of FAZ microtubules and the significance of their association with the membranous tubule are not known. Attachment of the flagellum to the cell body is mediated by a network of thin filaments that provide a physical link between the FAZ filament in the cytoplasm and the PFR and axoneme in the flagellum. These connecting filaments are assembled into regularly spaced, 25-nm-diameter attachment complexes that resemble desmosomes of mammalian cells and have a center-to-center period of 95 nm (26, 80). Transverse transmission electron microscopy sections through the FAZ show that there is extensive contact between the flagellar and plasma membranes outside the direct connections composed of these cytoskeletal filaments (26, 80). The nature of these membrane contacts and the composition of the cytoskeletal attachments are unknown.

The trypanosome flagellum and FAZ have been the subject of detailed ultrastructural analysis for some 40 years (66, 69, 78, 80). However, aside from the major structural proteins of the PFR and axoneme, little is known about the identities and characteristics of proteins that mediate flagellum attachment. Although antibodies raised against trypanosome cytoskeleton preparations have revealed a number of proteins that are localized to the FAZ (26, 35, 36), the identities and functions of these proteins are largely unknown. Recently, two proteins have been demonstrated experimentally to function in flagellum attachment in trypanosomes: GP72/FLA1 (13, 18, 40) and trypanin (33).

GP72 is a 72-kDa membrane-associated glycoprotein from T. cruzi that was originally identified as an immunodominant surface antigen (72). Indirect immunofluorescence localization studies indicate that GP72 in T. cruzi and FLA1, a GP72 homologue in T. brucei (51), are enriched along the flagellum and FAZ (18, 27, 51). Cross and colleagues (13) used conventional gene disruption to delete both alleles of the T. cruzi GP72 gene. The resultant GP72-null mutants exhibited a dramatic phenotype in which the flagellum is completely detached from the cell, except at the flagellar pocket (13, 18). GP72-null cells are viable in culture but display impaired motility and sediment to the bottom of the culture flask (13, 18). Viability was severely reduced in the insect vector, but no difference was observed in infection of cultured mammalian cells relative to that by wild-type parasites (18). These results provided the first demonstration that flagellum attachment is required for normal cell motility in trypanosomes.

Efforts to delete both alleles of the gene encoding the GP72 homologue, FLA1, in T. brucei were unsuccessful, suggesting that it is an essential gene (51). Once again, RNAi provided a means to overcome this problem. By using RNAi to block FLA1 expression, LaCount et al. (40) showed that loss of FLA1 causes a flagellum detachment and cell motility phenotype similar to that seen in GP72-null T. cruzi mutants. Importantly, the authors went on to show that loss of FLA1 also blocks cytokinesis, thus confirming that the FLA1 gene is essential (39). Expression of GP72 in FLA1-deficient T. brucei mutants does not rescue the flagellum attachment or cytokinesis defect (39). Therefore, despite the sequence similarity between GP72 and FLA1, these two proteins are not function-
ally interchangeable. Interestingly, ectopic expression of the *T. cruzi* GP72 gene in *T. brucei* causes flagellum detachment but does not block cytokinesis (39). The ability of GP72 to interfere with one FLA1 function (flagellum attachment), but not another (cytokinesis), suggests that the flagellum attachment and cytokinesis functions of FLA1 might be separable. A more complete understanding of FLA1 function will require further investigation. Of particular interest will be more precise localization of the protein in wild-type cells and ultrastructural analysis of FLA1-deficient mutants. Investigation of FLA2 (39), encoded by a gene related to FLA1, should also prove very informative.

The observation that PFRA and FLA1 are essential in *T. brucei*, but that the corresponding proteins in *L. mexicana* (PFR2) and *T. cruzi* (GP72) are dispensable, is intriguing and suggests that the PFR and FAZ participate in processes that are linked to cell division in *T. brucei*. Indeed, these results lend further support to the hypothesis that, in addition to their roles in cell motility, the *T. brucei* flagellum and FAZ provide important positional and directional information for cytokinesis and cell morphogenesis (26, 47, 50, 63).

Trypanin is a 54-kDa coiled-coil protein that is associated with the detergent- and calcium-insoluble flagellar fraction of the *T. brucei* cytoskeleton (28, 33). Biochemical fractionation studies demonstrate that trypanin is an integral component of the flagellar cytoskeleton (28), and indirect immunofluorescence studies demonstrate that the protein is localized along the flagellum and FAZ (33). The precise position of trypanin within this region awaits characterization by immunoelectron microscopy. Procyclic trypanosomes depleted of trypanin through RNAi exhibit a remarkable cell motility defect (33). Specifically, these mutants are completely incapable of directional cell motility. The vigorous motility of wild-type trypanosomes enables them to travel long distances at velocities as high as 20 μm/s (Fig. 2) (5, 33). In contrast, trypanin-deficient mutants spin and tumble uncontrollably, remaining primarily

![FIG. 2. Trypanin is required for directional cell motility. (A) Time-lapse video microscopy of trypanin-positive and trypanin-deficient trypanosomes. Elapsed time is shown in seconds, and the midpoint of each cell at time zero (white arrows) and at each successive time point (black arrows) is indicated. (B) Cartoon diagram depicting the typical cell motion of wild-type (WT) and trypanin mutant trypanosomes. Relative cell motion is indicated with an arrow, and the rotational axis of trypanin-deficient cells is indicated by a black dot or a vertical dotted line. (Reprinted from reference 33 with permission of the publisher.)](image-url)
in one location or occasionally moving backward (33). The most striking aspect of this motility defect is that trypanin-deficient cells are not paralyzed. Rather, they have an actively beating flagellum but can no longer harness flagellar beat to drive productive cell motility. Thus, without inhibiting cell motion per se, loss of trypanin prevents directional cell motility, i.e., the ability to move from point A to point B.

Evidence for trypanin’s involvement in flagellum attachment came from examination of whole cells by scanning electron microscopy (33), which revealed a partially detached flagellum in ~30% of trypanin-deficient cells. Similar regions of flagellum detachment are observed in wild-type cells, but at a much lower frequency, ~10%. The extent of flagellum detachment is relatively minor in intact cells but becomes more pronounced and more widespread (~60% of mutant cells versus 10% of wild-type cells) when cellular membranes are removed by detergent extraction (33). Time course experiments demonstrated that flagellum detachment parallels the loss of trypanin protein and the loss of cell motility. In transmission electron microscopy analysis of detergent-extracted trypanin-deficient cytoskeletons, the FAZ lacks the highly structured organization seen in wild-type cells. However, prior to detergent extraction, these structures appear unperturbed. This suggests that trypanin participates in the direct coupling of the flagellar cytoskeleton to the subpellicular cytoskeleton and that additional interaction between the flagellar and plasma membranes contributes to the overall stability of the complex. In the absence of trypanin, the cytoskeleton connection is destabilized, though not completely destroyed, and subsequent removal of the membrane connection leads to complete disruption of the attachment complex. This interpretation is consistent with earlier models for a bipartite attachment complex, consisting of both weak (membrane) and strong (cytoskeletal) components (27, 78).

Studies on GP72/FLA1 and trypanin demonstrate that the integrity of flagellum attachment complexes must be maintained for normal cell motility in T. brucei. As more of the genes for proteins involved in flagellum attachment are isolated, similar approaches, together with assays for protein-protein interactions, will make it possible to determine how these proteins function individually and collectively in flagellum attachment and cell motility. The existence of motility-deficient mutants will also provide an opportunity to investigate the relationship between cell motility, disease pathogenesis, and parasite development in the mammalian host and insect vector.

SUMMARY

Flagellum-mediated cell motility has captured the attention and imagination of biologists for more than 300 years (68). Trypanosomes provide an excellent experimental system for studying cell motility. These parasites are easily cultured in defined medium, and the ultrastructure of their flagellar apparatus and other cellular structures have been extensively characterized. Importantly, several state-of-the-art tools for molecular genetic manipulation of trypanosomes are now available. In particular, inducible silencing of gene expression via RNAi provides an extremely powerful method for determining the function of essential genes. As discussed above, these approaches have allowed the first glimpse of function for several prominent and enigmatic components of the trypanosome flagellar apparatus. Ongoing genome projects for T. brucei and related kinetoplastid parasites (17) will further enhance the utility of these organisms as experimental systems. In addition to presenting a fascinating biological phenomenon, cell motility plays an important role in the pathogenesis of infectious disease. In the case of trypanosomes and other protozoan pathogens, we are only now beginning to understand the nature of this relationship, and further study of both the biological and mechanistic aspects of cell motility are necessary before we can accurately describe the relationship between parasite and host.

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