Parasitol Res
DOI 10.1007/s00436-006-0329-2

REVIEW

Flagellar membrane trafficking in kinetoplastids

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Received: 26 August 2006 / Accepted: 29 August 2006

Introduction

The flagellum as an organelle

Trypanosomes are flagellated protozoa belonging to the order *Kinetoplastidae*. Three trypanosomes, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* are the causative agents of the human diseases: African sleeping sickness, Chagas disease, and leishmaniasis, respectively. These organisms have complex life cycles involving blood-sucking insects and vertebrates; humans become infected when infested insects feed on them. The trypanosome contains several unique organelles, one of the most important and conspicuous of which is the flagellum (Fig. 1). The trypanosome flagellum is a unique multifunctional organelle that plays critical roles in motility, chemotaxis, cell signaling, and host cell invasion (Landfear and Ignatushchenko 2001; Gull 2003; Hill 2003). In addition to containing a classical axonemal microtubular motor, the trypanosome flagellum also includes a paracrystalline structure called the paraflagellar rod (PFR), positioned alongside the axoneme. The flagellum emerges from the cell body from an invagination known as the flagellar pocket. As the cell body is constrained by a tight corset of cross-linked subpellicular microtubules (Fig. 1b), all vesicular trafficking into and out of the cell occurs at the non-microtubule-bound flagellar pocket, as does the trafficking of flagellar membrane-associated proteins between the cytoplasm and the flagellum (Bloodgood 1990; McConville et al. 2002). Because no new protein synthesis takes place in the flagellum, all protein components must be imported to the flagellum from the cell body, also via the flagellar pocket. To enter the flagellum, a protein must first pass through a cytoskeletal "neck" region within the flagellar pocket known as the flagellar pore complex (Rosenbaum and Witman 2002; Gull 2003). This complex, which contains structures including the basal body and transition zone filaments, is believed to act as a "stopper" for the flagellum, providing a selective barrier for protein entry (Vickerman and Preston 1976; Dentler and Adams 1992). Regulated flagellar protein import creates a flagellar microenvironment that is unique from that of the rest of the organism, where some of the protein and lipid components differ in composition from those in the cell body (Bloodgood 1990; Landfear and Ignatushchenko 2001).

Because of differences in composition and function, the surface membrane of a trypanosome is divided into three distinct but contiguous domains: the pellicular (cell body) membrane, flagellar membrane, and flagellar pocket membrane (Balber 1990; Landfear and Ignatushchenko 2001; Gull 2003). As the flagellum is not a "closed" membrane-bound organelle like the mitochondrion or a lysosome, it has this far

This review was presented at the International Symposium on Vesicle Trafficking in Parasitic Protozoa at Caxambu, Brasil, in November, 2005.

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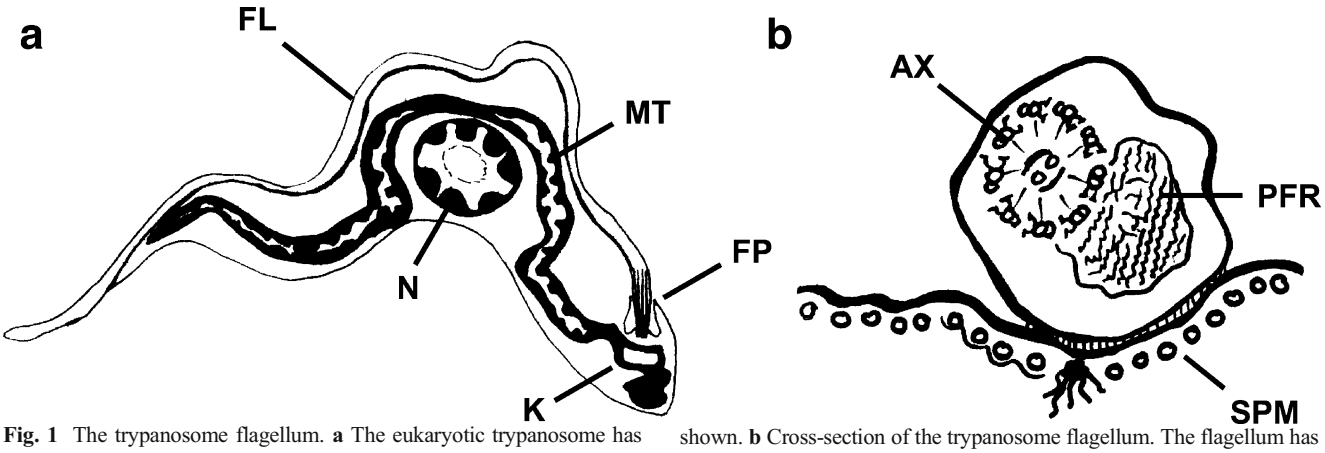

Fig. 1 The trypanosome flagellum. **a** The eukaryotic trypanosome has three prominent cellular structures. The single tubular mitochondrion (*MT*) runs the length of the cell. Within the mitochondrion is a subcompartment called the kinetoplast (*K*) that houses the unusual catenated DNA network of the trypanosome cell, the kinetoplast DNA. The membrane-bound flagellum (*FL*) is physically connected to the kDNA and emerges from an invagination in the cell body called the flagellar pocket (*FP*), runs the length of the cell attached by an intermembrane junctional complex and emerges (in some species) from the anterior end of the cell as a free entity. The nucleus (*N*) is also shown. **b** Cross-section of the trypanosome flagellum. The flagellum has several conspicuous structures. The flagellar membrane and pellicular (cell body) membrane are attached by a junctional complex called the flagellar attachment zone. Within the flagellum proper reside the axonemal complex of microtubules (*AX*) and a semicrystalline structure called the paraflagellar rod (*PFR*). Proteins involved in flagellar signal transduction are believed to be located at the flagellar membrane, within the axoneme and within the paraflagellar rod. A tight corset of subpellicular microtubules (*SPM*) underlies the pellicular membrane

been viewed as a cell surface specialization or an extension of the cytoskeleton. However, as the flagellum performs specific functions and differs in structure and composition from the rest of the cell, it is now considered to be a distinct organelle, complete with a membrane, cytoskeletal, and soluble compartments (Bloodgood 1990). While the mechanisms for targeting proteins to membrane-bound organelles have been well characterized, the mechanisms for targeting proteins to flagella and cilia are just now being defined. A number of important signaling molecules, such as the somatostatin receptor, sonic hedgehog receptor, and PDGF receptor, localize to the ciliary membrane. Therefore, elucidating mechanisms of flagellar targeting and retention is crucial for our understanding of flagellar function in microorganisms and as well as ciliary function in human health.

Intraflagellar transport (IFT)

Intraflagellar transport is a motility process that moves the necessary flagellar protein precursors from the cell body into and out of the flagellum to build and maintain the organelle. Initially discovered and characterized in biflagellate algae *Chlamydomonas reinhardtii*, the IFT machinery is conserved in flagella and cilia of nearly all eukaryotic organisms including sea urchin, *Caenorhabditis elegans*, mammals (Rosenbaum and Witman 2002) and kinetoplastids (Kohl et al. 2003). To enter the flagellum, the cargo of proteins or protein precursors forms a "barge" with a flagellar-bound IFT protein complex. The barge is then transported in the anterograde direction along the flagellar microtubules by motor proteins kinesin II or in the retrograde direction by cytoplasmic dynein 1b, respectively, maintaining contact with both the axoneme and the flagellar plasma membrane as it transits the length of the flagellum to deliver the cargo (Rosenbaum and Witman 2002; Scholey 2003). The cargo can include both cytoskeletal components and membrane proteins (Rosenbaum and Witman 2002; Scholey 2003; Qin et al. 2005; Wang et al. 2006).

An intact IFT system is important for motility and for the control of flagellar length, cytokinesis, and a number of other processes (Rosenbaum and Witman 2002; Scholey 2003). In kinetoplastids, two *T. brucei* IFT components, TbDHC1b (dynein heavy chain) and TbIFT88 (cargo), are responsible for the control of flagellar length, cell polarity and size, and cytokinesis (Kohl et al. 2003). IFT is also involved in cell signaling processes within the flagella and cilia (Rosenbaum and Witman 2002; Scholey 2003). In *C. elegans*, IFT particles are important for transport and proper distribution of sensory signal-transducing channels, OSM-9 and OCR-2, membrane receptors which function in osmo- and chemo-compensation and mechanosensation (Qin et al. 2005). IFT can also directly organize a flagellum-generated signaling pathway during the fertilization of *C. reinhardtii*, both by transporting a flagellar membrane cGMP dependent protein kinase (CrPKG), into the flagellum, and by directly associating with it and causing its retention within a new flagellar compartment (Wang et al. 2006).

Lipid rafts

Another mechanism for establishing polarized membrane composition comes from the studies of lipid rafts. Lipid

rafts are heterogeneous specialized membrane microdomains enriched in cholesterol and sphingolipids that play crucial roles in processes such as cell signaling, lipid and protein sorting, and protein and lipid trafficking in polarized cells (Brown and Rose 1992; Simons and Ikonen 1997; Harder and Kuhn 2000; Simons and Toomre 2000; Pike 2003). Due to the sterol enrichment and tighter packing of the lipids, these regions are thought to constitute "liquid ordered" areas, as compared to the more "liquid disordered" surrounding membrane. This property makes rafts more resistant to extraction with nonionic detergents such as Triton X-100, with the membrane remnants that typically contain lipid rafts after such an extraction being termed detergent resistant membranes (DRMs). Rafts are also typically sensitive to cholesterol depletion, and due to higher cholesterol content, are buoyant on density gradients. They can be heterogeneous in size and stability, and it is possible for a single cell to contain several types of lipid rafts with differing protein and sterol compositions, and which may coalesce and break apart depending on the physiologic needs of the cell.

Because lipid rafts often act as platforms for signal transduction, a variety of proteins, especially those that are dually acylated, GPI-linked, or transmembrane, are typically recruited into rafts. For dually acylated proteins, fatty acid length can influence DRM association, as a myristate group (C14, saturated) is typically not long enough to associate with the DRM, but palmitate (C16, saturated) is sufficient (Schroeder et al. 1994; Benting et al. 1999; Melkonian et al. 1999).

In kinetoplastids, lipid rafts have been identified in *Leishmania* (Denny et al. 2001). Additionally, biochemical analyses of the *T. cruzi* membranes revealed that the flagellar membrane has a higher concentration of sterols compared to the pellicular membrane (Souto-Padron and de Souza 1983; Tetley 1986), suggesting that lipid rafts may be enriched within the flagellum. While trypanosomes may scavenge cholesterol, most life stages of the parasite synthesize their own 3-β-hydroxysterol, ergosterol (Beach et al. 1986, 1988; Urbina et al. 1991; Coppens and Courtoy 1995) which forms rafts that are larger and more thermostable than those comprised of cholesterol (Xu et al. 2001).

Known organelle-targeting mechanisms

How different membrane proteins target to distinct organelles or specific domains of contiguous membranes is a crucial question in cell biology. Cargo proteins transported into the flagellum by either IFT or other methods are likely to use targeting motifs for the process. To date, no universal targeting signal or sequence for membrane protein targeting to the flagellum has emerged in kinetoplastids, but several types of motifs and mechanisms have been elucidated. In general, trafficking of membrane and cytoskeletal proteins may take place via a simple linear targeting peptide or through a more complex three-dimensional structure comprised of amino acids from several regions of the protein. Alternatively, the protein may rely on a partner protein interaction to retain it in the proper location. All of these methods have, so far, been shown to be used for flagellar import in kinetoplastid protozoans. This review will focus primarily on what is known about targeting mechanisms of membrane proteins in kinetoplastid protozoans, although we will briefly discuss some aspects of the trafficking of cytoskeletal proteins as well.

Molecular determinants of flagellar targeting in kinetoplastids

Cytoskeletal flagellar targeting

Several motifs targeting cytoskeleton-associated proteins to the flagellum have been elucidated by examining paraflagellar rod (PFR) components in *T. brucei*. Bastin et al. used RNA interference to knock down the expression of PFRA, one of the two main proteins forming the PFR. The resulting phenotype, named *snl-2*, was characterized by paralyzed trypanosomes that are unable to assemble most of the PFR (Bastin and Gull 1999). The C-terminal amino acids 514–570 of PFRA were found to contain a sequence which is necessary but not sufficient for the exclusive flagellar localization of the protein, as the truncated protein was found in the cytoplasm, but the GFP reporter protein fused to this sequence localized both to the cytoplasm and the flagellum. The group also proposed that this region may help PFRA bind to the paraflagellar rod, and thus without it, the truncated protein would be poorly retained within the flagellum. Amino acids 563–570 were further shown to be necessary for incorporation of PFRA into the distal tip of the flagellum rather than only along its length (Bastin et al. 1999).

In 2001, Ersfeld and Gull reported that both PFRA and an unrelated actin-like protein of the cytoskeleton, TrypARP, share a peptide consensus sequence, HLA, that is essential for flagellar targeting of both proteins. In PFRA, the HLA domain is found within the C-terminal 563–570 amino acid region identified in the previous study (Ersfeld and Gull 2001); in TrypARP, the peptide is also found in the C terminus. In both cases, it was suggested that the peptide is positioned in a way that leaves it available to interact with other proteins. A screen for the HLA motif in flagellar proteins of kinetoplastids and other eukaryotes uncovered several other flagellar proteins with this sequence. Most notably, the HLA motif was present in the

IFT component dynein heavy chain isoform DHC1b of *C. reinhardtii*. In addition, within a family of three tektin proteins (tektins A, B, and C) involved in the formation of a connecting zone in the axoneme, only tektin C contains the HLA motif (Ersfeld and Gull 2001). As the tektins function together as a trimer, the authors speculated that the tektin A and B heterodimer enters the flagellum as part of a complex preassembled in the cytoplasm, and a single HLA targeting sequence on tektin C is sufficient to serve as a carrier for flagellar import of the entire complex. However, as the HLA motif is also found in non-flagellar proteins (Ersfeld and Gull 2001), it is necessary but not sufficient for targeting; other complex nonlinear motifs or structural requirements within HLA-containing proteins likely contribute to the process as well.

A proteomic analysis comparing the flagella of wild-type *T. brucei* to those of the paralyzed *snl-2* cell lines uncovered another novel cytoskeletal targeting sequence. Without a functional PFR, several proteins are unable to properly localize to the flagellum of the paralyzed cells. One such protein is the adenylate kinase A (*Tb*ADKA), which is normally incorporated into the PFR itself (Pullen et al. 2004). A 55-amino acid peptide from the N terminus of TbADKA was shown to target GFP to the flagellum, and deletion of this region from full-length *Tb*ADKA resulted in a loss of flagellar localization, demonstrating that this sequence alone is necessary and sufficient for flagellar protein localization. Homologous *N*-terminal peptides, containing the [YLxxxxxxIPxLxE] motif followed by two prolines, were found in human and *C. elegans* tandem adenylate kinases, which in turn, bear a similarity to other tandem kinase isoforms targeted to sperm flagella in sea urchins, *Chlamydomonas*, and mammalian sperm. These similarities suggest that this mechanism of targeting some cytoskeletal-anchored kinase isoforms is conserved in eukaryotic flagella (Pullen et al. 2004).

Flagellar membrane targeting

A number of flagellar membrane proteins have been identified in trypanosomes, including LDL receptors (Coppens et al. 1988), adenylate cyclases (Paindavoine et al. 1992), glucose transporters (Snapp and Landfear 1997), small myristoylated proteins (SMPs) (Tull et al. 2004), and calcium-binding proteins (Wu et al. 1992, 1994; Godsel 1997; Godsel and Engman 1999). Studies on trypanosomatid flagellar membrane proteins have identified several flagellar targeting and retention mechanisms, including the bipartite, possibly three-dimensional targeting sequence ofthe *Leishmania enrietti* glucose transporter isoform, ISO1, and the dual acylation targeting mechanisms of flagellar calcium binding protein (FCaBP) of *T. cruzi* and the small myristoylated protein 1 (SMP-1) of *Leishmania donovani*. In addition, there is evidence to indicate that lipid rafts may play a role in trafficking dually acylated proteins into the flagellum as well.

A flagellar localization epitope

Two glucose membrane transporter isoforms, ISO1 and ISO2, have been characterized in *L. enriettii*. The ISO2 isoform is found in the pellicular membrane, while ISO1 is found in the flagellar membrane (Piper et al. 1995). This difference in targeting is due exclusively to differences in the *N*-terminal domains of the two isoforms, which are positioned on the cytosolic face of the membrane. The *N*-terminal domain of ISO1 is 130 amino acids long and is completely different in sequence from the 46 amino acid *N*-terminal domain of ISO2. The first 130 amino acids of the ISO1 isoform are sufficient to target the pellicular membrane protein D2 hexose transporter to the flagellar membrane (Snapp and Landfear 1997). Furthermore, two regions within the ISO1 N terminus, containing amino acids 84–100 and 110–118, appear to be essential for targeting this isoform to the flagellum (Nasser and Landfear 2004). Comparison of the *N* termini of *L. enriettii* ISO1 and *Leishmania mexicana* glucose transporter LmGT1, which also localizes to the flagellar membrane, did not reveal any significant sequence identity. However, the ISO1 domain does contain a high density of positively and negatively charged residues—a property shared by LmGT1 (Nasser and Landfear 2004). As the charged bipartite sequences are not arranged in a linear motif, they may instead comprise a three-dimensional structure which may mediate the interaction between ISO1 or LmGT1 and another protein that is a part of the flagellar targeting machinery, such as IFT "barges" or lipid-raft associated proteins.

Small myristoylated proteins (SMPs)

A family of SMPs has been found in *Leishmania major* (Tull et al. 2004). One, SMP-1, is both myristoylated and palmitoylated at the N terminus and localizes to the flagellar membrane. Studies of myristoylation and palmitoylation mutants showed that the dual acylation is required for flagellar membrane targeting of SMP-1. SMP-1 was initially identified as a major protein in the buoyant Triton X-100 insoluble membrane fraction, indicating that it is raft-associated. Treating cells with myriocin, which inhibits sphingolipid biosynthesis (Miyake et al. 1995), and ketoconazole, which inhibits sterol biosynthesis (Beach et al. 1988), reduced the levels of the most abundant sphingolipid, inositolphosphorylceramide, and sterols by 70 and 50%, respectively. While neither myriocin nor ketoconazole affected the cellular growth rate or the flagellar localization of SMP-1, ketoconazole treatment did increase

the solubility of SMP-1 in cold 1% Triton X-100, indicating that a decrease in sterol concentration resulted in a change in the physical properties of the DRMs. Treatment of cells with both myriocin and ketoconazole caused the flagellum to retract and dilate at the point where it emerged from the flagellar pocket. Interestingly, the localization of SMP-1 was not affected by these perturbations of the flagellum, leading the authors to conclude that flagellar localization is not dependent upon interactions with the axoneme. This suggests that flagellar targeting of SMP-1 is independent of the IFT mechanism.

Flagellar calcium binding protein (FCaBP)

FCaBP is a 26-kDa protein that localizes to the flagellum of *T. cruzi* (Engman et al. 1989). FCaBP contains four canonical calcium-binding EF hand domains, of which two, EF3 and EF4, bind calcium (Maldonado et al. 1999). FCaBP is modified at the N terminus by the addition of myristate and palmitate and requires this dual acylation to localize to the flagellar membrane (Godsel and Engman 1999). The *N*-terminal 24 amino acids of FCaBP are sufficient to localize GFP marker protein to the flagellum (Godsel and Engman 1999). The palmitoyl moiety of FCaBP is essential for the association of FCaBP with lipid rafts (Buchanan, manuscript in preparation). It has also been shown that the association of FCaBP with the flagellar membrane may also be due to a calcium-dependent interaction with a partner protein (Buchanan et al. 2005). It is possible that FCaBP may selectively partition into the flagellar membrane via association of its acyl groups with lipid raft domains in the flagellar membrane, as is seen in the *T. brucei* homologues of FCaBP, the calflagins.

Calflagin and lipid rafts

The calflagins, a family of *T. brucei* calcium-binding proteins with amino acid sequence homology to *T. cruzi* FCaBP, are also significantly enriched in the flagellar membrane (Wu et al. 1992, 1994). Like FCaBP and SMP-1, calflagins contain the myristoylation and palmitoylation consensus sequences, and are therefore, also likely to be acylated at the N terminus, which may target them to the flagellum. Recent work in our lab provided evidence that the flagellum of *T. brucei* is enriched in lipid rafts (Tyler et al., manuscript in preparation). Using laurdan, a fluorescent lipophilic probe that measures the relative order of the membrane based on the order of degrees of freedom of water surrounding it to examine the order of the *T. brucei* membrane (Dietrich et al. 2001a,b), we found that membrane order is greatly increased in the flagellum as compared to the rest of the cell. In addition to the calflagins, fluorescence and immunofluorescence assays showed that hydroxysterols and sphingolipids, two canonical components of rafts, are enriched in the flagellum as well. In both procyclic and bloodstream forms, calflagin form Tb1.7 localizes to the detergent-resistant, buoyant fractions of a discontinuous OptiPrep density gradient following cell lysis with cold Triton X-100, suggesting that calflagin is a raft associated protein. Pretreatment of bloodstream-stage parasites with methyl-β-cyclodextrin (MBCD), a cholesterol sequestering agent widely used to disrupt DRMs (Cobb et al. 1990; Regev et al. 1999), disrupts calflagin association with the detergent resistant membrane fraction. Immunofluorescence microscopy studies showed that in cells washed with ice-cold 1% Triton X-100, calflagin remains associated with the flagellum but is washed out of the cell after pretreatment with MBCD. These lines of evidence suggest that association of the acyl moieties with lipid rafts may be responsible for targeting calflagin to the flagellar membrane.

Possible IFT involvement in lipid rafts

Interestingly, when *T. brucei* cells examined by scanning electron microscopy after extraction with cold 1% Triton X-100, small patches of membrane complexes are observed along the flagellar axoneme, but not along the PFR. These complexes are not scattered randomly on the axoneme but follow an almost straight line progressing to the distal tip of the flagellum. We hypothesize that these patches may represent DRM remnants. In general, the patches appear larger in ergosterol-rich procyclic forms than in cholesterol-rich bloodstream forms, which may be explained by the fact that ergosterol has been shown to form larger and more thermostable rafts than cholesterol (Xu et al. 2001). The linear distribution of the detergent-insoluble particles observed on the axoneme is reminiscent of the arrangement of the IFT barges on the axoneme described by Rosenbaum et al., and may represent the membrane components of intraflagellar transport particles. Kohl et al. have already reported that a functional IFT system exists in *T. brucei* and that homologues of IFT particles have been annotated the genome databases of *T. cruzi* and *Leishmania* (Kohl et al. 2003). Structures morphologically resembling *Chlamydomonas* IFT particles have previously been observed in cross-sections of the *T. brucei* flagella by electron microscopy as well (Sherwin and Gull 1989; Bastin et al. 2000).

Future directions

To date, there is no single mechanism for flagellar targeting in kinetoplastids. However, a number of mechanisms, including linear epitopes such as the HLA motif in PFR and TrypARP, three-dimensional epitopes such as the

charged stretches of residues in the *Leishmania* glucose ISO1 transporter, and interactions of these motifs with IFT particles or lipid raft-associated molecules have all been proposed. While dual acylation is emerging as one possible mechanism for targeting flagellar membrane-associated proteins, the extent to which this mechanism is used remains unknown. Not all dually acylated proteins are bound for the flagellum. The *T. brucei* cytoskeletal-associated calpain-like protease CAP5.5, which is both myristoylated and palmitoylated, localizes exclusively to the cell body, associating with pellicular microtubules and possibly the cell membrane (Hertz-Fowler et al. 2001). However, for FCaBP, a 24-amino acid region containing the acylation sequence is both necessary and sufficient for flagellar targeting (Godsel and Engman 1999). Further work is necessary to determine whether a specific binding sequence within the 24-amino acid residues may help FCaBP associate with a partner protein and/or whether the protein is able to associate with a preassembled complex outside of the flagellar pore by palmitoylation. Conversely, CAP5.5 may contain additional sequences which may help anchor it to cytoskeletal or plasma membrane proteins and retain it within the pelliculum. Identification of other acylated proteins and their binding partners in trypanosomes and analyses of protein domains of FCaBP, the calflagins, and the SMPs which may mediate partner protein interaction, should begin to unravel whether acylation may be a default flagellar trafficking mechanism.

It is also currently unknown which trypanosome proteins the IFT delivers to the flagellum, what motifs mediate the IFT–cargo interaction, and which proteins may be targeted independently of the IFT system in kinetoplastids. Disruption of IFT transport by targeting the dynein and cargo molecules by RNAi in *T. brucei* results in a short-flagellum phenotype similar to that of IFT dynein deletion seen in *C. reinhardtii* and *T. brucei* (Pazour et al. 1999; Porter et al. 1999; Kohl et al. 2003). IFT component dynein and cytoskeletal proteins PFR and TrypARP, all share a C-terminal HLA signal domain which is necessary but not sufficient for flagellar targeting (Ersfeld and Gull 2001), suggesting that these proteins may be trafficked via the IFT. The loss of the PFR, in turn, disrupts the flagellar localization of *Tb*ADKA, which uses a different targeting sequence found on its N terminus (Pullen et al. 2004). On the other hand, in *L. major*, disrupting membrane composition by ketoconazole and myriocin also results in a damaged PFR and axoneme and a truncated flagellum; however, the membrane protein, SMP-1, is nonetheless targeted into the flagellum, suggesting that SMP-1 transport is IFT independent (Tull et al. 2004).

It is likewise possible that changing the lipid composition of the membrane may disrupt flagellar assembly by disrupting IFT particle recruitment and trafficking. Recently, we have visualized small membrane patches which associate with the axoneme in an orderly fashion and may represent DRMs; these patches disappear when the cells are treated with MBCD, a cholesterol-sequestering agent predicted to disrupt lipid rafts. In their arrangement along the axoneme, these patches resemble the IFT barges described by Rosenbaum et al. As rafts have also been shown to be involved in sorting and trafficking proteins to appropriate membranes and cell signaling, these remnants raise the possibility that there may be a connection between the membrane-associated components of IFT and lipid rafts. It is interesting to recall that in the process of adhesion during fertilization of *Chlamydomonas*, IFT is directly involved in organizing a flagellum-generated signaling pathway by transporting and retaining the flagellar membrane adhesion signaling protein CrPKG within a novel flagellar compartment (Wang et al. 2006). In trypanosomes, the flagellum is also viewed as a signaling organelle. Studies determining potential co-localization of calflagin or FCaBP with IFT particles within the DRM patches and identification of other IFT and lipid raft-associating processes will shed further light on the possibility of IFT–lipid raft interactions.

The specific composition and stability of DRMs may also affect how tightly a given protein associates with the lipid raft. For example, while sphingolipids are generally thought to be necessary for raft formation, this is not true of all rafts. In two studies in *Leishmania*, the genes encoding serine palmitoyltransferase (SPT), the first committed enzyme in sphingolipid synthesis were deleted (Zhang et al. 2003; Denny et al. 2004; Denny and Smith 2004). In the SPT null cell lines, while the formation of early rafts in the endoplasmic reticulum is initially delayed (Denny et al. 2004), lipid rafts do eventually form even in the absence of sphingolipids (Zhang et al. 2003; Denny et al. 2004; Denny and Smith 2004). This may occur either because the ergosterol synthesized by kinetoplastids tends to form more stable rafts than does cholesterol or because of the abundance of GPI-linked proteins and ether lipids, which may create a "permissive environment" in the membrane which maintains rafts even in the absence of sphingolipids (Zhang et al. 2003). On the other hand, the inhibition of sterol biosynthesis does disrupt the association of SMP-1 with lipid rafts, but neither the inhibition of sterol nor sphingolipid biosynthesis affects the flagellar association of this dually acylated protein (Tull et al. 2004). In *T. brucei* bloodstream forms, which scavenge cholesterol but do not synthesize ergosterol, DRM disruption results in removal of calflagin from the flagellar membrane (Tyler et al., manuscript in preparation). Identification of more raft-associated flagellar membrane proteins will help us better understand the connection between lipid rafts and membrane protein trafficking in trypanosomes.

Acknowledgements Research described in this review was supported by funds from NIH grant AI46781. A. Fridberg and K. T. Buchanan were supported, in part, by predoctoral fellowships from the American Heart Association, Greater Midwest Chapter. Kevin Tyler and Cheryl Olson contributed to unpublished research described in this review. We thank Kent Hill for helpful comments on this review.

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