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Form and Function in the Trypanosomal Secretory Pathway

Jason S. Silverman and James D. Bangs*

Department of Medical Microbiology & Immunology, School of Medicine & Public Health,
University of Wisconsin-Madison, 1550 Linden Drive, Madison, WI, 53706

Abstract

Recent advances in secretory biology of African trypanosomes reveal both similarities and striking differences with other model eukaryotic organisms. Secretion is streamlined for rapid and selective transport of the major cargo, VSG. Selectivity in the early and post-Golgi compartments is dependent on glycosylphosphatidyl inositol anchors. Streamlining includes reduced organellar abundance, and close association of ER exit sites with Golgi and with unique flagellar cytoskeletal elements that govern organellar replication and segregation. These elements include a novel centrin containing bilobe structure. Innate signals for post-Golgi sorting of biosynthetic lysosomal cargo trafficking have been defined, as have pathways for both biosynthetic and endocytic trafficking to the lysosome. Less well-defined secretory organelles such as the multivesicular body and acidocalcisomes are receiving closer scrutiny.

Introduction

African trypanosomes (*Trypanosoma brucei* ssp., hereafter called trypanosomes) are parasitic protozoa that cause both human (Sleeping Sickness) and veterinary (Nagana) diseases throughout sub-Saharan Africa, wherever the insect vector, the tsetse fly, is found. Trypanosomes are representative of a broad group of parasites, the kinetoplastids, including South American *T. cruzi* and *Leishmania* species with world-wide distribution. The trypanosome life cycle alternates between pathogenic bloodstream forms (BSF) in the mammalian host and non-pathogenic forms found in the tsetse fly, the best understood being the procyclic midgut form (PCF). Critical to the pathogenesis of BSF trypanosomes is the process of antigenic variation in which variant surface glycoproteins (VSG) are sequentially expressed to avoid host immune responses [1]. Also critical to pathogenesis are core lysosomal and endosomal functions. BSF trypanosomes have an exceedingly rapid rate of endocytosis, primarily for nutritional purposes but also perhaps to eliminate potentially lytic surface immune complexes [2,3]. How the parasite makes and exports VSG to the cell surface, and how it regulates biosynthetic and endocytic trafficking to the lysosome are key cell biological issues that are central to the phenomenal success of trypanosomes as parasites.

Secretory Organelles and Cargo

Trypanosomes have an elongated shape conferred by tightly spaced sub-pellicular microtubules subtending the plasma membrane (Fig. 1A). Endocytic and exocytic

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*Corresponding author: Tel: 608-262-3110, jdbangs@wisc.edu.

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trafficking is restricted to the posterior flagellar pocket (FP). Nearby are the typical eukaryotic endomembrane organelles (Fig. 1B), including the endoplasmic reticulum (ER), Golgi, endosomes, and a single terminal lysosome. Defined markers include the ER chaperone BiP [4], Golgi glycosyltransferases and matrix proteins [5*,6], endosomal Rabs [7], and two lysosomal markers, cathepsin L (TbCatL) [8] and the membrane glycoprotein p67 [9]. An unusual extension of ER aligns with unique cytoskeletal elements below the flagellar adherence zone (Fig. 1C, FAZ:ER) and has recently defined molecular markers, CC2D and TbVAP [10*,11*]. Characterized trafficking machinery include the heavy chain and the AP-1 adaptor of clathrin coated vesicles [12-14], and the coat subunits of COP II vesicles for ER-to-Golgi transport [15**,16]. Additional machinery apparent in the genome includes COPI vesicles for recycling of ER content and components of the multivesicular body [17]. Overall, the trypanosomal secretory pathway is much like other eukaryotes, but its organellar components are reduced in complexity and copy number suggesting it is streamlined for efficient transport of major surface proteins and that it represents the basal requirements for secretory trafficking in eukaryotic cells.

The major secretory cargo of BSF trypanosomes is glycosylphosphatidyl inositol (GPI) anchored VSG, which is transported rapidly to the cell surface and thereafter is constantly endocytosed and recycled with high efficiency through endosomal compartments back to the FP [2,18]. Deletion of the GPI anchor delays ER exit, and results in post-Golgi mis-sorting to the lysosome in BSF trypanosomes [19]. Other well-defined cargos are p67 and TbCatL. p67 is a membrane glycoprotein containing two cytoplasmic di-leucine motifs that are responsible for lysosomal targeting [9,20]. TbCatL is synthesized as a soluble proprotein that is activated by removal of the prodomain upon arrival in the lysosome [14]. Specific signals in the prodomain direct lysosomal targeting [21]. Over-expression results in secretion from PCF trypanosomes, as does knockdown of clathrin heavy chain and the AP-1 adaptor [14], suggesting that post-Golgi trafficking of TbCatL is mediated by a saturable clathrin-dependent sorting receptor.

GPIs and ER Exit

Secretory cargos leave the ER from defined ER exit sites (ERES) where they are loaded into COPII secretory vesicles [22]. Vesicles formation involves the deposition of COPII coat proteins - the inner Sec23/Sec24 heterodimer followed by the outer Sec31/Sec13 heterotetramer - leading to budding of mature secretory vesicles. Secretory cargo capture requires the interaction of specific cytoplasmic motifs in transmembrane proteins with the inner Sec23/Sec24 heterodimer. Trypanosomes have orthologues of all the COPII subunits, including two each for Sec23 and Sec24. These subunits form exclusive heterodimers (TbSec23.1/TbSec24.2 and TbSec23.2/TbSec24.1) all colocalizing to one or two ERES per cell that nucleate on the FAZ:ER [15**,16] (Fig. 1C). All the TbSec23 and TbSec24 subunits are essential in BSF trypanosomes, but silencing of any one has only minor effects on transport of TbCatL and p67 [15**]. Either the two obligate TbSec23/TbSec24 heterodimers are redundant for loading of these cargos, or they leave the ER by non-specific bulk flow. Silencing of the TbSec23.2/24.1 heterodimer, but not TbSec23.1/24.2, impairs transport of VSG implicating this heterodimer in selective loading of GPI-anchored proteins into COPII vesicles in trypanosomes. The essentiality of TbSec23.1/24.2 suggests that this heterodimer is responsible for selection of some other critical secretory cargo. Knockdown of both TbSec24s in PCF trypanosomes is not lethal, but does reduce transport of selected soluble and transmembrane cargo [16]. The effect on GPI-dependent cargo was not determined in PCF trypanosomes.

The dependence of VSG transport in BSF trypanosomes on the TbSec23.2/24.1 heterodimer presents a topology problem since it resides in the inner leaflet of vesicle membranes,

sequestered from the cytoplasmic COPII coat. In yeast loading of GPI anchored proteins is influenced by ceramide, but this is not the case in trypanosomes [23]. ER exit of GPI-anchored cargo in other systems is also dependent on p24s [24,25], a family of transmembrane receptors with luminal domains for cargo recognition and short cytoplasmic domains containing motifs for interaction with COPII coats for ER exit and COPI coats for ER retrieval [26]. There are multiple p24s in any species and trypanosomes have at least eight putative orthologues [15**] (authors' unpublished observations). The role of these proteins has yet to be defined, but one or more may provide the link between the phenomena of GPI-dependence and the unique COPII specificity of VSG exit from the ER in trypanosomes.

The ERES:Golgi Junction

Immediately downstream of the ER is the Golgi apparatus, a dynamic organelle responsible for carbohydrate processing and subsequent sorting of secretory and lysosomal proteins. It is typically a stack of flattened cisternae through/in which cargo are transported in a vectoral *cis-to-trans* manner [27]. Owing to their simplified architecture trypanosomes have increasingly been used to study Golgi biogenesis [28]. There are usually one or two Golgi in interphase PCF and BSF trypanosomes, respectively, each closely associated with an ERES and consequently aligned with the FAZ (Fig. 1C) as a larger ERES:Golgi Junction (EGJ) that replicates in concert [15**,29,30*]. There are stage specific differences in EGJ copy number, duplication, and segregation in PCF vs BSF trypanosomes (see Fig. 2). In addition, EGJ replication is tightly coupled to the cell cycle in PCF trypanosomes but quite promiscuous in BSFs [30*]. Strikingly, in arrested BSF trypanosomes duplication continues leading to multiple EGJ all aligned along the FAZ. All of these differences are likely reflective of broader stage specific differences in cell cycle regulation and organellar duplication in trypanosomes [31].

A novel structure called the bilobe is closely associated with the posterior end of the FAZ filament at the mouth of the FP [30*,32-34**]. The bilobe contains orthologues of centrins [35-38], as well as the novel proteins TbMORN1 and TbLRRP1 [32,33]. In PCF trypanosomes the bilobe also aligns closely with the Golgi in all stages of the cell cycle and is thought to function in Golgi replication and as a cytoskeletal adaptor during cytokinesis [35,37] (Fig. 2). In contrast, in BSF trypanosomes the bilobe is spatially independent of the Golgi, suggesting that it is not directly involved in Golgi replication and segregation [30*]. Rather, segregation during cytokinesis appears to occur via the association of EGJ with the FAZ:ER and consequently with the flagellar cytoskeleton. This raises the possibility that the bilobe is not directly involved in PCF Golgi replication but only appears so due to the compressed volume of the nucleus-to-FP region relative to BSF cells [39]. Alternatively this may represent yet another stage-specific difference in trypanosome replication. Specific silencing of bilobe components in PCF trypanosomes has diverse pleomorphic effects including impaired Golgi duplication, nuclear replication, cytokinesis and FAZ biogenesis [33,35,37]. These results indicate at the very least that the bilobe is critical for cytoskeletal organization and cell division, a suggestion supported by the close association of the bilobe with structural elements at the mouth of the FP [34**].

Post-Golgi Trafficking

From the Golgi biosynthetic cargos are sorted to multiple destinations, including the lysosome and the cell surface. Neither route is well defined in trypanosomes, but they are likely to intersect at some point with the endocytic pathway. Endocytosis, which is greatly upregulated in BSF trypanosomes, occurs exclusively at the flagellar pocket and is clathrin mediated (Fig 1B) [40,41]. Endocytosed cargo is delivered to an endosomal system defined

by specific Rab proteins, small GTPase that regulate trafficking through specific compartments. These include TbRab5A/B (early endosome), TbRab11 (recycling endosome), and TbRab7 (late endosome) (reviewed in [7]). Studies with endocytic cargo (VSG, transferrin and lectins) in BSFs, in conjunction with RNAi silencing of TbRabs, reveal that cargo is initially delivered to the early endosome and then passed on to the late endosome and finally the lysosome [2]. Cargo such as VSG and transferrin receptor can be retrieved from early and late endosomes to the recycling endosome and hence returned to the cell surface via the FP. These findings largely reflect endosomal trafficking pathways in other eukaryotes but there are significant exceptions. In BSF trypanosomes TbRab7 does not regulate lysosomal trafficking of biosynthetic cargo such as p67 and TbCatL [42**], unlike in yeast and mammals where Rab7 controls both endocytic and biosynthetic routes. This finding suggests that post-Golgi lysosomal trafficking in *T. brucei* is distinct from other eukaryotic cells. Work on TbRab4 and TbRab28 further illustrate the complexity of post-Golgi pathways in trypanosomes. It is not clear exactly where these Rabs reside, but it is apparent that they influence trafficking through the endosomal compartment(s) [43,44].

A post-Golgi compartment of uncertain status in trypanosomes is the multivesicular body (MVB), defined in mammals as a Rab7⁺ late endosome that contains intraluminal vesicles [45,46]. Transmembrane proteins that are cytoplasmically tagged with ubiquitin are sorted into these intraluminal vesicles and targeted to the lysosome for degradation. Selection of ubiquitinated cargo and vesiculation are coordinately mediated by the cytoplasmic ESCRT machinery [46]. No structure with MVB morphology has ever been consistently documented in normal trypanosomes, although MVB-like structures have been seen in RNAi stressed cells [47,48]. Nevertheless, MVB type functionalities do exist in trypanosomes. Internalization and turnover of cell surface invariant glycoproteins ISG65 and ISG75 are directed by ubiquitination, and this process is mediated by orthologues of the ESCRT machinery [49,50*]. Imaging of the ESCRT components TbVps28 and TbVps23 are consistent with a late endosomal location, but direct colocalization with TbRab7 has not been performed. A conservative interpretation of these data would be that typical MVB functions are carried out in trypanosomes by the late endosome, but a classic MVB structure is absent from normal cells.

Conclusions

Trypanosomes have been found to be remarkably similar to the standard secretory model systems - yeast and vertebrate cells. Nevertheless, because of their streamlined architecture they offer unique opportunities to study general eukaryotic cell biology. Work from many laboratories over the last few years has revealed unusual features that are unique to trypanosomes. Future work should continue to provide new insights of both general and parasite specific interest. How are GPI-anchored proteins selectively loaded into COPII vesicles by the TbSec23.2/TbSec24.1 heterodimer? The putative p24 cargo receptors are prime candidates for this function [15**]. What is the composition of the FAZ:ER and how does it influence secretion and other processes in trypanosomes? The bilobe is an enigmatic structure that is likely to have multiple roles in regulating cell cycle, organellar segregation and cytokinesis. What are the post-Golgi pathways to the lysosome and the flagellar pocket, neither of which have been clearly delineated? The MVB is poorly defined in trypanosomes - how this organelle, or more likely its functional equivalent, is integrated into the endosomal system may illuminate critical aspects of post-Golgi lysosomal sorting. Finally, the acidocalcisome is a lysosome related organelle whose biogenesis is apparently dependent on protein sorting from the Golgi [51*]. How cargo destined for the acidocalcisome is sorted from lysosomal cargo in the *trans*-Golgi will be of great interest for future studies. These issues and more are certain to provide exciting new results in the future.

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References

1. Rudenko G. African trypanosomes: the genome and adaptations for immune evasion. *Essays Biochem.* 2011; 51:47–62. [PubMed: 22023441]
2. Engstler M, Thilo L, Weise F, Grünfelder CG, Schwarz H, Boshart M, Overath P. Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in *Trypanosoma brucei*. *J Cell Sci.* 2004; 117:1105–1115. [PubMed: 14996937]
3. Engstler M, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N, Overath P. Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell.* 2007; 131:505–515. [PubMed: 17981118]
4. Bangs JD, Uyetake L, Brickman MJ, Balber AE, Boothroyd JC. Molecular cloning and cellular localization of a BiP homologue in *Trypanosoma brucei*: divergent ER retention signals in a lower eukaryote. *J Cell Sci.* 1993; 105:1101–1113. [PubMed: 8227199]
- 5*. He CY, Ho HH, Malsam J, Chalouni C, West CM, Ullu E, Toomre D, Warren G. Golgi duplication in *Trypanosoma brucei*. *J Cell Biol.* 2004; 165:313–321. Despite being a older contribution this work is note worthy because it is the first published instance of real-time imaging in live immobilized trypanosomes. The replication of the Golgi in procyclic trypanosomes was followed using fluorescent protein tagged Golgi markers. This work established trypanosomes as a novel model system to study eukaryotic organellar replication, and laid the groundwork for subsequent discovery of the bilobe structure. [PubMed: 15138289]
6. Sutterwala SS, Hsu F-F, Sevova ES, Schwartz KJ, Zhang K, Key P, Turk J, Beverley SM, Bangs JD. Developmentally regulated sphingolipid synthesis in African trypanosomes. *Mol Microbiol.* 2008; 70:281–296. [PubMed: 18699867]
7. Ackers JP, Dhir V, Field MC. A bioinformatic analysis of the RAB genes of *Trypanosoma brucei*. *Mol Biochem Parasitol.* 2005; 141:89–97. [PubMed: 15811530]
8. Caffrey CR, Steverding D. Kinetoplastid papain-like cysteine peptidases. *Mol Biochem Parasitol.* 2009; 167:12–19. [PubMed: 19409421]
9. Alexander DL, Schwartz KJ, Balber AE, Bangs JD. Developmentally regulated trafficking of the lysosomal membrane protein p67 in *Trypanosoma brucei*. *J Cell Sci.* 2002; 115:3255–3263.
- 10*. 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*. *J Cell Sci.* 2011; 124:3848–3858. The FAZ:ER (Fig. 1C), an unusual extension of the ER that associates closely with the FAZ cytoskeleton, has been recognized since the earliest EM studies on trypanosomes. Recent work indicates that the FAZ:ER is the site of ERES formation and may function in ERES:Golgi segregation during cytokinesis. This work, along with the next citation [11], begins to unravel the molecular composition of the FAZ:ER and provides a starting point for future structure/function studies of this unusual endomembrane structure. [PubMed: 22114307]
- 11*. Lacomble S, Vaughan S, Deghelt M, Moreira-Leite FF, Gull K. A *Trypanosoma brucei* protein required for maintenance of the flagellum attachment zone and flagellar pocket ER domains. *Protist.* 2012 in press Along with the previous citation [10], this work begins to unravel the molecular composition of the FAZ:ER and provides a starting point for future structure/function studies of this unusual endomembrane structure. 10.1016/j.protis.2011.10.010
12. Morgan GW, Allen CL, Jeffries TR, Hollinshead M, Field MC. Developmental and morphological regulation of clathrin-mediated endocytosis in *Trypanosoma brucei*. *J Cell Sci.* 2001; 114:2605–2015. [PubMed: 11683388]
13. Qiao X, Chuang B-F, Jin Y, Muranjan M, Hung C-H, Lee P-T, Lee MG-S. Sorting signals required for trafficking of the cysteine-rich acidic repetitive transmembrane protein in *Trypanosoma brucei*. *Euk Cell.* 2006; 5:1229–1242.

14. Tazeh NN, Silverman JS, Schwartz KJ, Sevova ES, Sutterwala SS, Bangs JD. The role of AP-1 in developmentally regulated post-Golgi trafficking in *Trypanosoma brucei*. *Euk Cell*. 2009; 8:1352–1361.
- 15*. Sevova ES, Bangs JD. Streamlined architecture and GPI-dependent trafficking in the early secretory pathway of African trypanosomes. *Mol Biol Cell*. 2009; 20:4739–4750. This paper confirmed the selectivity of GPI cargo loading into COPII vesicles in the early secretory pathway as being dependent on the obligate TbSec23.2/TbSec24.1 heterodimer. This in turn implicates the p24 proteins as possible cargo loading receptors and points the way to new directions in investigating the mechanism of GPI dependence of secretory trafficking in trypanosomes. It also demonstrated for the first time that trypanosome ER exit sites nucleate on the FAZ:ER, suggesting a model for organellar segregation by interaction with the flagellar cytoskeleton. [PubMed: 19759175]
16. Demmel L, Melak M, Kotisch H, Fendos J, Reipert S, Warren G. Differential selection of Golgi proteins by COPII Sec24 isoforms in procyclic *Trypanosoma brucei*. *Traffic*. 2011; 12:1575–1591. [PubMed: 21801288]
17. Engstler, M.; Bangs, JD.; Field, MC. Intracellular transport systems in trypanosomes: function, evolution and virulence. In: Barry, JD.; Mottram, JC.; McCulloch, R.; Acosta-Serrano, A., editors. *Trypanosomes - After the Genome*. Horizon Scientific Press; 2006. p. 281-317.
18. Bangs JD, Andrews N, Hart GW, Englund PT. Posttranslational modification and intracellular transport of a trypanosome variant surface glycoprotein. *J Cell Biol*. 1986; 103:255–263. [PubMed: 3722267]
19. Triggs VP, Bangs JD. Glycosylphosphatidylinositol-dependent protein trafficking in bloodstream stage *Trypanosoma brucei*. *Euk Cell*. 2003; 2:76–83.
20. Tazeh NN, Bangs JD. Multiple signals regulate trafficking of the lysosomal membrane protein p67 in African trypanosomes. *Traffic*. 2007; 8:1007–1017. [PubMed: 17521380]
21. Huete-Perez JA, Engel JC, Brinen JC, Mottram JC, McKerrow JH. Protease trafficking in two primitive eukaryotes is mediated by a prodomain protein motif. *J Biol Chem*. 1999; 274:16249–16256. [PubMed: 10347181]
22. Hughes H, Stephens DJ. Assembly, organization, and function of the COPII coat. *Histochemistry in Cell Biology*. 2008; 129:129–151.
23. Sutterwala SS, Creswell CH, Sanyal S, Menon AK, Bangs JD. De novo sphingolipid synthesis is essential for viability, but not transport of glycosylphosphatidylinositol-anchored proteins in African trypanosomes. *Euk Cell*. 2007; 6:454–464.
24. Takida S, Maeda Y, Kinoshita T. Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane. *Biochem J*. 2008; 409:555–562. [PubMed: 17927562]
25. Muñiz M, Nuoffer C, Hauri H-P, Reizman H. The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J Cell Biol*. 2000; 148:925–930. [PubMed: 10704443]
26. Strating JRPM, Martens GJM. The p24 family and selective transport processes at the ER-Golgi interface. *Biol Cell*. 2009; 101:495–509. [PubMed: 19566487]
27. Emr S, Glick BS, Linstedt AD, Lippincott-Schwartz J, Luini A, Malhotra V, Marsh BJ, Nakano A, Pfeffer S, Rabouille C, et al. Journeys through the Golgi - taking stock in a new era. *J Cell Biol*. 2009; 187:449–453. [PubMed: 19948493]
28. He CY. Golgi biogenesis in simple eukaryotes. *Cell Microbiol*. 2007; 9:566–572. [PubMed: 17223925]
29. Ho HH, He CY, de Graffenried CL, Murrells LJ, Warren G. Ordered assembly of the duplicating Golgi in *Trypanosoma brucei*. *Proc Natl Acad Sci USA*. 2006; 103:7676–7681. [PubMed: 16672362]
- 30*. Bangs JD. Replication of the ERES:Golgi junction in bloodstream form African trypanosomes. *Mol Microbiol*. 2011; 82:1433–1443. Unlike procyclic trypanosomes real-time imaging of immobilized bloodstream parasites has not been achieved. Consequently this manuscript resorted to quantitative enumeration of the ERES and Golgi throughout the cell cycle by IFA in fixed bloodstream cells. The results indicate significant differences compared to procyclics in how the

combined ERES:Golgi Junction is replicated and segregated. In addition, based on spatial considerations it appears that the bilobe structure is not physically involved in these processes in bloodstream form parasites. Rather the results emphasize the importance of EGJ nucleation on the FAZ:ER. [PubMed: 22026408]

31. Hammarton TC, Monnerat S, Mottram JC. Cytokinesis in trypanosomatids. *Cur Opin Microbiol.* 2007; 10:520–527.
32. Morriswood B, He CY, Sealey-Cardona M, Yelnik JT, Pypaert M, Warren G. The bilobe structure of *Trypanosoma brucei* contains a MORN-repeat protein. *Mol Biochem Parasitol.* 2009; 167:95–103. [PubMed: 19445968]
33. Zhou Q, Gheiratmand L, Chen Y, Lim TK, Zhang J, Li S, Xia N, Liu B, Lin Q, He CY. A comparative proteomic analysis reveals a new bi-lobe protein required for bi-lobe duplication and cell division in *Trypanosoma brucei*. *PLoS one.* 2010; 5:e9660. [PubMed: 20300570]
- 34**. Esson HJ, Morriswood B, Yavuz S, Vidilaseris K, Dong G, Warren G. Morphology of the trypanosome bilobe, a novel cytoskeletal structure. *Euk Cell.* 2012 in press This manuscript builds on earlier work from the Warren and He laboratories that first defined and characterized the bilobe as a novel trypanosomal structure. The fine ultrastructure of the bilobe in procyclic trypanosomes is investigated using a suite of electron microscopy techniques. The results provide a detailed and elegant model of how bilobe components define specific subdomains that in turn interact physically with the FAZ cytoskeleton and the flagellar collar at the mouth of the flagellar pocket. This is structurally perhaps the most complex, yet poorly understood, region of the trypanosome cell. The proposed model is a significant contribution that will illuminate future studies in this area. 10.1128/EC.05287-05211
35. He CY, Pypaert M, Warren G. Golgi duplication in *Trypanosoma brucei* requires centrin2. *Science.* 2005; 310:1196–1198. [PubMed: 16254149]
36. Selvapandiyan A, Kumar P, Morris JC, Salisbury JL, Wang CC, Nakhasi HL. Centrin 1 is required for organelle segregation and cytokinesis in *Trypanosoma brucei*. *Mol Biol Cell.* 2007; 18:3290–3301. [PubMed: 17567955]
37. Shi J, Franklin JB, Yelnik JT, Ebersberger I, Warren G, He CY. Centrin4 coordinates cell and nuclear division in *T brucei*. *J Cell Sci.* 2008; 121:3062–3070. [PubMed: 18768932]
38. Wang M, Gheiratmand L, He CY. An interplay between centrin2 and centrin4 on the bi-lobed structure in *Trypanosoma brucei*. *Mol Microbiol.* 2012 in press. 10.1111/j.1365-2958.2012.07998.x
39. Matthews KR, Sherwin T, Gull K. Mitochondrial genome repositioning during the differentiation of the African trypanosome between life cycle forms is microtubule mediated. *J Cell Sci.* 1995; 108:2231–2239. [PubMed: 7673343]
40. Langreth SG, Balber AE. Protein uptake and digestion in bloodstream and culture forms of *Trypanosoma brucei*. *J Protozool.* 1975; 22:40–53. [PubMed: 1117436]
41. Allen CL, Goulding D, Field MC. Clathrin-mediated endocytosis is essential in *Trypanosoma brucei*. *EMBO J.* 2003; 22:4991–5002. [PubMed: 14517238]
- 42**. Silverman JS, Schwartz KJ, Hajduk SL, Bangs JD. Late endosomal Rab7 regulates lysosomal trafficking of endocytic but not biosynthetic cargo in *Trypanosoma brucei*. *Mol Microbiol.* 2011; 82:664–678. The existence of the Rab7⁺ late endosome in trypanosomes has been accepted for many years, but this is the first study to investigate its role in biosynthetic and endocytic trafficking to the lysosome. Knockdown of TbRab7 in bloodstream trypanosomes blocks delivery of endocytic cargo to the terminal lysosome, but has no effect on trafficking of newly synthesized lysosomal proteins. This latter finding is in complete contrast to the function of Rab7 in other eukaryotic model systems. In addition, conditional knockout in procyclics indicate the requirement for TbRab7 in this stage is so low as to be almost non-essential. Future efforts should focus on defining the pathway of post-Golgi sorting to the lysosome that may or may not bypass the late endosome. [PubMed: 21923766]
43. Hall BS, Pal A, Goulding D, Field MC. Rab4 is an essential regulator of lysosomal trafficking in trypanosomes. *J Biol Chem.* 2004; 279:45047–45056. [PubMed: 15284229]
44. Lumb JH, Leung KF, Dubois KN, Field MC. Rab28 function in trypanosomes: interactions with retromer and ESCRT pathways. *J Cell Sci.* 2011; 124:3771–3783. [PubMed: 22100919]

45. Vanlandingham PA, Ceresa BP. Rab7 regulates late endocytic trafficking downstream of multivesicular body biogenesis and cargo sequestration. *J Biol Chem.* 2009; 284:12110–12124. [PubMed: 19265192]
46. Hurley JH, Hanson PI. Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat Rev Mol Cell Biol.* 2010; 11:556–566. [PubMed: 20588296]
47. Allen CL, Liao D, Chung W-L, Field MC. Dileucine signal-dependent and AP-1-independent targeting of a lysosomal glycoprotein in *Trypanosoma brucei*. *Mol Biochem Parasitol.* 2007; 156:175–190. [PubMed: 17869353]
48. Peck RF, Shiflett AM, Schwartz KJ, McCann A, Hajduk SL, Bangs JD. The LAMP-like protein p67 plays an essential role in the lysosome of African trypanosomes. *Mol Microbiol.* 2008; 68:933–946. [PubMed: 18430083]
49. Leung KF, Dacks JB, Field MC. Evolution of the multivesicular body ESCRT machinery; retention across the eukaryotic lineage. *Traffic.* 2008; 9:1698–1716. [PubMed: 18637903]
- 50*. Leung KF, Riley FS, Carrington M, Field MC. Ubiquitylation and developmental regulation of invariant surface protein expression in trypanosomes. *Euk Cell.* 2011; 10:916–931. Here the authors show that invariant trypanosome trans-membrane cell surface proteins require ubiquitination for correct localization and turnover. Along with work demonstrating orthologues and function of mammalian ESCRT machinery [49], this work suggests that a functional equivalent of the mammalian multivesicular body is present in *T. brucei*.
- 51*. Huang G, Fang J, Sant'Anna C, Li Z-H, Wellems DL, Rohloff P, Docampo R. Adaptor protein-3 (AP-3) complex mediates the biogenesis of acidocalcisomes and is essential for growth and virulence of *Trypanosoma brucei*. *J Biol Chem.* 2011; 286:36619–36630. Heretofore, investigations of clathrin-dependent vesicular trafficking in trypanosomes have focused on the AP-1 adaptin complex, which is implicated in post-Golgi sorting of lysosomal cargo. In this work the role of the AP-3 adaptin complex is studied by RNAi knockdown. The results indicate that AP-3 is essential in bloodstream trypanosomes, and plays a critical role in the biogenesis of the acidocalcisome. The findings confirm the status of the acidocalcisome as a lysosome-related organelle, and raise many interesting question about selective cargo sorting at the *trans*-Golgi in trypanosomes. [PubMed: 21880705]
52. Grünfelder CG, Engstler M, Wieise F, Schwarz H, Stierhof Y-D, Morgan GW, Field MC, Overath P. Endocytosis of a glycosylphosphatidylinositol-anchored protein via clathrin-coated vesicles, sorting by default in endosomes and exocytosis via RAB11-positive carriers. *Mol Biol Cell.* 2003; 14:2029–2040. [PubMed: 12802073]
53. Briggs LJ, McKean PG, Baines A, Moreira-Leite F, Davidge J, Vaughan S, Gull K. The flagella connector of *Trypanosoma brucei*: an unusual mobile transmembrane junction. *J Cell Sci.* 2004; 117:1641–1651. [PubMed: 15075226]
54. Tyler KM, Matthews KR, Gull K. Anisomorphic cell division by African trypanosomes. *Protist.* 2001; 152:367–378. [PubMed: 11822664]

Highlights

- The trypanosome secretory pathway is streamlined for efficient transport.
- GPI anchors influence selective transport in both early and post-Golgi compartments.
- ER exit sites and associated Golgi nucleate on unique flagellar cytoskeletal elements.
- A novel bilobe structure plays multiple roles in cytoskeletal and organellar replication.
- Unusual pathways of biosynthetic trafficking to the lysosome exist.

Figure 1. Trypanosome morphology and subcellular structure

A. The classic trypomastigote morphology is shown with the flagellum emerging from the posterior flagellar pocket, adhering to the cell body, and extending in the anterior direction. BSF trypanosomes are $\sim 3 \times 25 \mu\text{m}$. The direction of motility is indicated (arrow). B. Diagram of the post-nuclear region where most secretory and endocytic organelles are located. Arrows indicate major transport pathways. Uncertainties in pathways are indicated (?). As indicated, clathrin-coated vesicles function at three steps: 1) uptake at the flagellar pocket [12]; 2) RE-to-LE transport [52]; and 3) Golgi-to-lysosome transport [14]. COPII vesicles mediate ER-to-Golgi transport (II) [15]. Retrograde Golgi-to-ER transport (not indicated) has not been formally demonstrated but is presumed to require COPI machinery. The bilobe, and the distinct positions of the ERES:Golgi junction (EGJ) in PCF and BSF trypanosomes are indicated in Fig. 2. C. Enlarged cross-section of the flagellar attachment zone transecting an EGJ. ER, endoplasmic reticulum; FAZ, flagellar attachment zone; ERES, ER exit site; TGN, *trans*-Golgi network; EE, early endosome; RE recycling endosome; LE, late endosome; MT, microtubule; GRASP, Golgi reassembly stacking protein; GT, glycosyltransferase.

Figure 2. Stage specific models for EGJ replication

These models take into account documented stage-specific morphological differences in the trypanosome cell cycle including: a) connection of the flagellar tip during outgrowth of the daughter flagellum in PCF cells [53]; b) relative positioning of nuclei and kinetoplasts in post-mitotic cells [54]; c) compressed nucleus-to-FP region in PCF trypanosomes [39]. In both stages the bilobe is closely associated with the posterior end of the FAZ filament. Also in each stage the ERES portion of the EGJ nucleates on the FAZ:ER. In PCF cells (top) the single EGJ replicates early in S-phase and is redistributed to the new FAZ of the outgrowing flagellum. Thereafter, cells progress through mitosis and a single EGJ segregates to each daughter cell during cytokinesis. Each EGJ is closely associated with the bilobe throughout the cell cycle. In BSF cells (bottom) there are two EGJ, one of which realigns to the new FAZ early in S-phase. Each duplicates to give two EGJs aligned to each FAZ in post-mitotic cells, and these segregate to each daughter cell during cytokinesis. At no time is the bilobe closely associated with any of the EGJ. Genomic configurations (K, kinetoplast; N, nucleus) are indicated at each stage of the cell cycle. Reproduced with permission from [30].