The endocytic apparatus of the kinetoplastida. Part II: machinery and components of the system

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Endocytic systems within eukaryotic cells are a diverse set of intracellular transport pathways responsible for uptake, recycling, interaction with the exocytic system and degradation of molecules. Each of these pathways requires the interaction of distinct protein components that function in macromolecule sorting, control of transport rates and in membrane biogenesis. In the second of two articles on kinetoplastida endocytosis, the endocytic system in *Trypanosoma brucei* is considered as a model, and the molecules that control this system and the protein components of the endocytic pathway are discussed. We also consider novel mechanisms for sorting that have been proposed to operate in trypanosomes.

Endocytosis is the process through which material enters the cell through a membrane-vesicle-mediated process. At the same time as external molecules are being imported by endocytosis, cell surface components and secreted material is reaching the plasma membrane by exocytosis [1]. Kinetoplastids have evolved a cellular structure that concentrates endocytosis and exocytosis in the flagellar pocket, a small invagination at the base of the single flagellum. This polarization allows the remainder of the cell surface to be shielded from the environment in an appropriate manner. Most kinetoplastids exist in multiple environments, and must rapidly and completely change the bulk cell surface components in response to a change of host. The process of changing the cell surface is dependent on exocytosis and endocytosis, which are thus perceived as a target that could be exploited for therapy. In addition, kinetoplastids are distantly related to the crown group of eukaryotes (i.e. chordates, higher plants, fungi) and the degree to which the exocytic and endocytotic processes are conserved across the eukaryotes merits further investigation. The components of the endocytic pathway that have been identified in kinetoplastids, primarily *Trypanosoma brucei*, are discussed and tentative conclusions are drawn from this knowledge.

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Starting at the surface

The flagellar pocket has an exclusive role in endocytosis from the plasma membrane. This does not preclude receptors being distributed outside the flagellar pocket, but they must return there to be endocytosed. Generally, receptors are recycled back to the cell surface after being separated from their ligands and thus, in the steady state, many receptors are distributed between the cell surface and the underlying early endosomal system. The best characterized receptor in *T. brucei* is the transferrin receptor which, along with the low-density lipoprotein (LDL) receptor, was discussed in the first part of this review [1]. However, several other proteins have subcellular locations characteristic of receptors or components of the early endosomal system.

CRAM, BARP and ISG_{100}

The cysteine-rich acidic integral membrane protein (CRAM) is a surface protein confined to the flagellar pocket and closely connected compartments. CRAM contains 66 copies of a 12-amino acid motif and each copy contains one or two potential *N*-linked glycosylation sites. CRAM has a potential transmembrane domain close to the C-terminus and a potential N-terminal signal sequence [found in some copies of the gene in the genome sequence project as opposed to the original complementary DNA (cDNA) sequence [2]]. CRAM is more highly expressed in procyclics, but is present in bloodstream forms. The protein has been suggested to be a lipoprotein receptor on account of its weak homology to cysteine-rich motifs in the human LDL receptor family (restricted to the positions of cysteine residues) and the phenotype of CRAM null mutants. In these null mutants, there was a small decrease in the rate of high-density lipoprotein (HDL) uptake and a more significant loss of HDL binding in procyclics at 4°C. However, the viability of the CRAM null mutant argues that additional HDL uptake systems operate [3,4]. In the absence of direct binding data, it is possible that CRAM could be a structural component of the flagellar pocket, such that the alterations seen in HDL endocytosis might be secondary effects.

Bloodstream-stage acidic alanine-rich polypeptide (BARP), expressed exclusively in bloodstream forms, is an external glycosylphosphatidylinositol (GPI)-anchored protein, which was identified by expression cloning in mammalian cells [5]. BARP shares weak homology to the *Trypanosoma congolense* form of procyclin. Most significantly, BARP is clustered on the cell surface and is insoluble in cold 1% Triton X-100, suggesting that it might be a raft protein.

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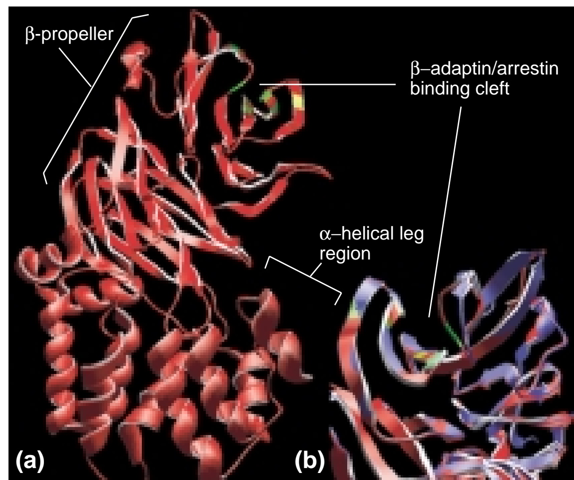

Fig. 1. Conservation of clathrin tertiary structure in trypanosomes. (a) Model of the clathrin heavy-chain (CLH) from a rat. This model focuses on the N-terminal β-propeller region and the end of the α-helical leg domain. The adaptin- and arrestin-binding sites are localized to a single groove in the propeller region, and the residues (highlighted in yellow and green) have been demonstrated by mutagenesis to be the crucial contact amino acids [40]. (b) The trypanosome sequence (blue) is threaded onto the rat CLH model (red). With the exception of a small displacement to a loop, the two tertiary structures are superimposable, suggesting that an identical mechanism underpins trypanosome clathrin and adaptin interactions. This model was prepared using the rat CLH X-ray crystal structure [14] and the online modelling tool at EXPASY. The figure was prepared using the SwissProt viewer.

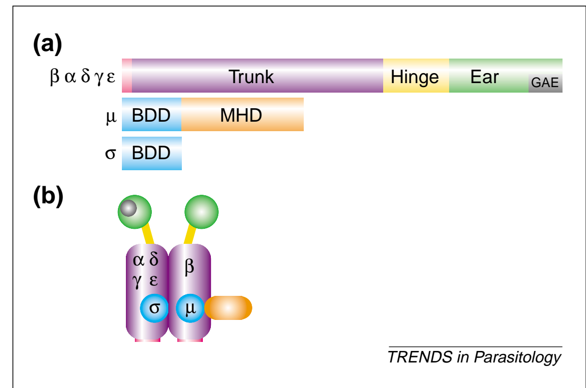

Fig. 2. (a) The structural relationship between different adaptin subunits. The five related, adaptin heavy chains (ADH) are indicated (β, α, δ, γανδ ε). Each subunit contains a N-terminal trunk domain (purple), which binds to the low molecular weight μ and σ subunits, and also interacts with the second ADH in the complex. All adaptins contain a hinge region (yellow) but, in some, a structure called the ear (green) is reduced, as observed in *Trypanosoma brucei* and *Saccharomyces cerevisiae*. Some of the protein domains are defined by homology, function and protein family (pfam) analysis. (b) The heterotetrameric adaptor protein complex, showing the protein domains described in (a), and using the same colour code. Where possible, protein–protein interactions are represented by subunit proximity. Abbreviations: BDD, β-binding domain; GAE, γ-adaptin C-terminus region; MHD, μ homology domain.

Invariant surface glycoprotein 100 (ISG_{100}) was identified by cell-surface iodination, and is a substantially glycosylated protein containing a large central domain of 72 copies of a serine-rich, 17-amino acid motif [6]. The exact orientation of ISG_{100} in the membrane has not been determined, but the fact that the single tyrosine residue close to the C-terminus was iodinated strongly suggests that the C-terminus is external. ISG_{100} is expressed in bloodstream forms, but not in procyclic forms, and is predominantly associated with vesicles located in the proximity of the flagellar pocket and also deeper endosomal structures including early endosomes [6,7]. The function of ISG_{100} is not known, but co-localization with *T. brucei* RAB (TbRAB) 5B suggests that the protein might have a role in endocytosis.

CRAM, BARP and ISG_{100} are all novel proteins, with no significant similarities to endosomal proteins of higher eukaryotes, suggesting the presence of novel functionality within the trypanosome endocytic system.

Getting into and around the cell

The identification of a range of trypanosomatid homologues of known higher eukaryotic proteins involved in intracellular transport has provided compelling evidence for the existence and designation of discrete intracellular compartments similar to those in other eukaryotes. In 1993, the first localized polypeptide marker to be described in trypanosomes was TbBiP, an abundant endoplasmic reticulum (ER) chaperone [8]. Since then, several TbRAB proteins have been described [7,9–12], together with components of the clathrin-dependent pathway [13] and the CB-1/p67 lysosomal protein. Antibodies raised against these markers have been used to track the progress of imported material through the endosomal system, principally by co-localization. Most of the protein families implicated in endocytosis in higher eukaryotes are present in trypanosomatids. Of the components characterized in kinetoplastida, those belonging to evolutionarily conserved families clearly retain major sequence and overall structural elements, suggesting that their modes of action are likely to be similar in trypanosomatids.

Clathrin and adaptins

In yeast and metazoans, a major route from the cell surface is mediated by the clathrin and adaptin system. Clathrin contains a heavy (CLH) and a light chain (CLL). The CLH of *T. brucei* (TbCLH) is ~38% identical to human CLH. The binding site for β-arrestins and β-adaptins [14] is highly conserved in kinetoplastids, as is the trimerization domain necessary for assembly of clathrin cages and thus vesicle formation. Molecular modelling suggests that the trypanosomal homologue has a structure essentially indistinguishable from mammalian clathrin (Fig. 1). The N-terminal domain of the *T. brucei* CLL (TbCLL) is divergent from the human homologue; however, sequences required for regulation of clathrin self-assembly [15] are conserved in TbCLL. The 100 residues of the C-terminal are well conserved, having ~38% identity with human CLL. Overall, this degree of identity suggests that clathrin assembly is similar to higher eukaryotes.

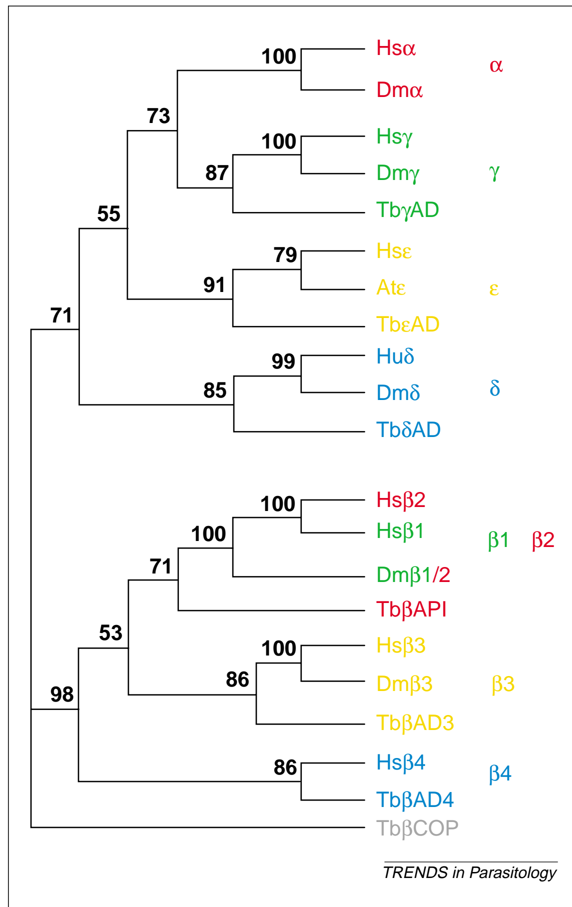

Fig. 3. The sequence relationships and phylogenetic reconstructions indicate three adaptin (AP) complexes in *Trypanosoma brucei*. The rooted phylogenetic reconstruction displays the evolutionary relationships of the large adaptin subunit proteins from *Drosophila melanogaster* (Dm), *Homo sapiens* (Hs) and *T. brucei* (Tb). Note the clear bifurcation of the tree into a β-branch and all other subunits, consistent with recent views of adaptin evolution from a single heavy chain [41]. For non-β-subunits, there is a clear homologue of each of the metazoan subunits in trypanosomes, with high bootstrap values, with the exception that no trace of an α-subunit could be retrieved from the *T. brucei* sequence databases, suggesting the absence of an AP2 complex. This conclusion is strengthened by the presence of three β-adaptins in the database; two of these are clear homologues of higher eukaryote β-subunits (TbβAD3 and TbβAD4 most probably being β3 and β4, respectively), whereas the third trypanosome β-adaptin (TbβAP1 in [13]) clusters with the β1/2 grouping and, by sequence analysis, cannot be assigned. Additional data indicate that TbβAP1 is present on the Golgi complex; hence, it is most likely a β1 orthologue, consistent with the absence of the AP2 α-subunit. Protein sequence alignment was performed with ClustalX, following exhaustive database searches during November 2001 using BLAST, and assembly of complete open reading frames where possible. The phylogenetic tree was generated using PAUP (default parameters) with a branch and bound initial topology calculation followed by 1000 bootstrap reiterations. Numbers in bold indicate the bootstrap values in percent. The *T. brucei* coatamer protein, β-COP, (a distant adaptin relative) was used as the outgroup (grey), and the *Arabidopsis thaliana* ε-subunit was include to tighten the ε-branch. Key; blue, AP4; green, AP1; red, AP2; yellow, AP3.

Adaptins are heterotetrameric complexes that act as a bridge between the cytoplasmic domains of extracellular receptors and clathrin. Adaptins have a highly conserved domain structure (Fig. 2) and this has allowed the identification, from the genome sequencing project, of genes encoding adaptin heavy chain subunits β1, β3, β4, γ, δ and ε, sufficient to constitute three distinct adaptin complexes which, by phylogenetic analysis, are homologues of metazoan AP1, AP3 and AP4 (Fig. 3). After extensive searches, it appears that there is no apparent homologue for AP2 in *T. brucei*, the adaptin complex central to clathrin-mediated receptor endocytosis in higher eukaryotes and yeast. If this observation is confirmed, it highlights an extremely unusual feature because AP2 complexes are present in nematodes, yeasts, insects and *Leishmania*, and clearly suggests that an unusual mechanism for endocytosis must operate in the African trypanosome. The trunk domains of kinetoplastid adaptins are highly conserved and retain consensus sequences for numerous protein interactions [16]. Some of the clathrin-interaction motifs are also conserved in the kinetoplastid β-adaptins. However, they lack the canonical clathrin-binding box motif in the hinge domain of mammalian β1, β2, and β3 adaptins, but so do the *Saccharomyces cerevisiae* β-subunits and the mammalian β4 subunit. The small adaptin subunits, μ and σ, can also be recovered from the databases, but the data are at present too fragmentary for detailed analysis.

In bloodstream forms, TbCLH expression is upregulated at least tenfold compared with procyclics. TbCLH is distributed to numerous vesicular and tubular structures throughout the posterior end of the cell [13]. These results have been extended by cryo-immunogold electron microscopy, which confirms that TbCLH is localized to the collecting tubules associated with the flagellar pocket and also to the *trans*-Golgi network. Upregulation of TbCLH expression in the bloodstream form correlates with elevated rates of endocytosis and recycling present in bloodstream forms. In procyclics, clathrin is restricted to structures close to the flagellar pocket, consistent with a role in endocytosis. A lack of Golgi localization in procyclics is also indicative of a decreased role for clathrin-dependent post-Golgi transport in this stage. Mechanistically, the reduction in TbCLH expression in procyclics is consistent with the reduced endocytic activity in this stage compared with the bloodstream form.

The trypanosome β1 adaptin subunit is predominantly localized to the Golgi and is not developmentally regulated. In higher eukaryotes, dynamin, a large molecular weight GTPase, is recruited to the constriction at the neck of coated pits, where it assists with vesicle fission [17]. Only a single dynamin-like protein (TbDLP) is present in ~90% of the current *T. brucei* genome sequence. The majority of TbDLP protein is located on the internal membranes and not at the flagellar pocket (G.W. Morgan and M.C. Field, unpublished). This is similar to the localization of the three dynamin-like proteins identified in *S. cerevisiae*, for which current data indicate no role in endocytosis, and a less prominent role for clathrin in endocytosis than in the secretory pathway [18].

There are two important questions that emerge. First, does the apparent absence of TbDLP from the flagellar pocket and the lack of a clear AP2 homologue indicate that clathrin-mediated endocytosis has a minor role in uptake? Second, because the majority of protein endocytosed from the flagellar pocket is GPI-anchored and thus cannot interact directly with adaptins, does endocytosis of GPI-anchored proteins in trypanosomes occur by a clathrin-independent mechanism, by clathrin-mediated endocytosis assisted by additional membrane carriers, or is the system nonselective, and operates in the absence of a concentration step? Direct determination of the roles of TbCLH and other proteins directly involved in endocytosis in *T. brucei* remains to be achieved, and is clearly an important goal.

The machinery of endocytosis has not been described in *Leishmania* at a molecular level. However, coated pits and coated vesicles at the flagellar pocket of promastigotes have been seen in electron microscopy studies [17]. These structures are reminiscent of clathrin-coated transport intermediates, whereas recent work indicates that clathrin and adaptor components are expressed and polarized in the anterior end of the cell in *Leishmania major*, in an analogous fashion to *T. brucei* (P.W. Denny, unpublished).

Coatamer I

A second major protein vesicle coat system, coatamer I (COP I), has also been described in trypanosomes [20]. COP I is involved primarily in *intra*-Golgi transport and retrograde trafficking from the Golgi to the ER in higher eukaryotes, but an additional role for these factors in phagocytosis and recycling is also emerging in yeast. In particular, COP I function is partly controlled by small GTPases of the ADP-ribosylation factor family (ARF) and, ARF I in particular, which has been implicated in recycling and phagocytosis [21]. In *T. brucei*, all the COP I subunits are now identified, and they form a complex with biochemical properties similar to the COP I complex from higher eukaryotes. Hence, it is possible that the trypanosome COP I complex is also involved in some aspect of endocytosis [20].

Non-clathrin-mediated endocytosis

Alternative mechanisms of endocytosis, independent of clathrin, are an important feature of the endocytic systems of eukaryotes. The best characterized of these is the caveolae/lipid raft system. Caveolae, flask-like structures present as invaginations of the plasma membrane, were originally described by George Palade over 50 years ago, and are most probably the one morphological presentation of biochemically defined membrane microdomains. Plasma membrane microdomains are enriched in glycosphingolipids and sterols, and also contain a selected population of plasma membrane proteins, in particular GPI-anchored proteins on the external face and acylated cytoplasmic proteins on the internal face [22]. Caveolae or raft-mediated endocytic routes are distinct from clathrin-mediated pathways through the earliest phases of endocytosis, but the paths meet comparatively rapidly, probably at or close to the early endosome [23]. A pronounced emphasis among trypanosomatids for GPI-linkage of cell surface macromolecules means that a major endocytic route for surface proteins could be raft-related. In mammalian cells, lipid rafts exist as microdomains of ~100 nm diameter on the cell surface, but these structures become larger due to clustering when receptors or surface molecules are crosslinked.

Lipid rafts are present in trypanosomatids. In *T. brucei*, a proportion of the variant surface glycoprotein (VSG) is present within a detergent-resistant membrane, and BARP is in the raft environment [5,24]. It should be noted that the dimensions of BARP clusters are larger than the 100 nm typical of a mammalian microdomain.

In *L. major*, most gp63 and a proportion of glycosylinositolphospholipids are raft associated [24]. *Leishmania* rafts are also depleted of phosphatidylinositol and enriched in inositolphosphoceramide, similar to rafts from higher eukaryotes. Some invaginations budding from the flagellar pocket are also suggestive of caveolae. The *T. brucei* genome database currently has no detectable caveolin-related sequences, but caveolin is not necessary for raft function because some mammalian cell lines, such as the Jurkat T-cell line, have no caveolin but use rafts as signalling platforms [25].

In bloodstream forms of *T. brucei,* the concept of a raft as a specialized and distinct microdomain is at first difficult to reconcile with the uniform high density of VSG on the external face of the plasma membrane. A raft-like microdomain is, however, fully consistent with the strong punctuate distribution of BARP over the cell surface [5]. Possibly, VSG is distributed evenly both within and outside of rafts marked by BARP, with a function that is currently undefined.

RAB GTPases

RABs are membrane-bound small GTPases involved in vesicle targeting and docking, and are excellent experimental markers for subcellular compartments. In *T. brucei*, a total of five TbRABs have been identified and localized within the endocytic pathway TbRAB7 is associated with the lysosome and pre-lysosome (M. Boshart and M. Engstler, pers. commun.), whereas the remainders (TbRAB4, TbRAB5A, TbRAB5B and TbRAB11) are components of the early endosome and recycling arms of the endocytic pathway. Homologues of TbRAB7 and TbRAB11 have been described in *Trypanosoma cruzi* [26,27] and *L. major* [42]. Two TbRAB5 isoforms are present in *T. brucei* – TbRAB5A and TbRAB5B. In procyclic forms, these two proteins co-localize,

but they have distinct locations in the bloodstream stage [7,10,12]. The increased complexity in the early endosome system of bloodstream forms possibly reflects a greater number of endosomal pathways. Consistent with this model is the observation that TbRAB5A co-localizes with both endocytosed antibody–VSG complexes and transferrin, but not with ISG_{100}, whereas TbRAB5B co-localizes with ISG_{100}, but not VSG–antibody complexes or transferrin.

Division into two pathways potentially allows endocytosis of certain proteins (e.g. VSG) to take place without interference by a turnover of other molecules. The signal for targeting to TbRAB5A or TbRAB5B compartments might be the mode of membrane attachment in that the two molecules co-localized with TbRAB5A are GPI-linked, whereas ISG_{100}, a transmembrane protein, co-localizes with TbRAB5B. This hypothesis is supported by data from a study in *T. brucei*, where a 65-kDa transmembrane protein was shown to internalize to compartments distinct from VSG following binding of cognate antibody [29], but this remains to be tested rigorously.

RAB proteins, like other small GTPases, exist in active (GTP-bound) and inactive (GDP-bound) forms. Their function can be investigated by the ectopic expression of mutants locked in the active (QL) or inactive (SN) state. In procyclics, expression of $TbRAB5A^{QL}$ results in an increase in both fluid-phase endocytosis and uptake of human LDL to a rate similar to that of wild-type bloodstream forms. By contrast, expression of $TbRAB5A^{QL}$ in bloodstream forms does not increase LDL or fluid-phase endocytosis. Taken together, these data suggest that there is a rate-limiting step for endocytosis in procyclics that might be overcome by a quantitative increase in active RAB5, but that a distinct factor is responsible for restricting the endocytic rate. A secondary effect of $TbRAB5A^{QL}$ expression in procyclics is a ~20-fold increase in the number of both high- and low-affinity LDL-binding sites, suggesting that both classes of sites are due to the same protein. It is possible that the high-affinity class is clustered within the flagellar pocket, whereas the low-affinity class is dispersed on the flagellum [7,29].

The cell surface of *T. brucei* procyclics contains two families of repetitive proteins, termed procyclin, and are designated by the single amino acid code for the residues that make up the repeat region, EP and GPEET. Expression of the two gene families is regulated in a complex manner: EP appears during differentiation from the bloodstream form and is expressed continuously in the insect gut, whereas the GPEET family is normally only transiently expressed during and immediately after differentiation [30]. In culture, EP and GPEET have half-lifes of >100 h, indicating that the proteins are not shed at a significant rate. Both EP and GPEET are constantly internalized and recycled to the surface, albeit at a low rate (B.S. Hall *et al.,* unpublished). The relative amounts of EP and GPEET, and their subcellular distributions are altered by overexpression of TbRAB4. In higher eukaryotes, RAB4 regulates a recycling pathway; in *T. brucei*, TbRAB4 is also associated with early endosomes, suggesting a conserved function [10]. Hence, the protein trafficking system in trypanosomes has the potential to control surface protein display.

In mammalian cells, RAB11 is also involved in recycling, but in a distinct pathway from RAB4 [31]. TbRAB11 protein is more abundant in the bloodstream than in the procyclic form, and localizes close to, but distinct, from TbRAB5. TbRAB11 and TbRAB5 might be localized to distinct subdomains of the same endosomes [12,32]. Most interestingly, both endocytosed VSG and the transferrin receptor partially co-localize with TbRAB11, suggesting that this compartment might receive material from the TbRAB5A endosome, and is an intermediate stage *en route* back to the cell surface. Overall, the behaviour of TbRAB5A, TbRAB5B and TbRAB11 suggest the presence of a specialized recycling pathway in the bloodstream form through which VSG can be transported, and which might have a crucial role in defense against host antibodies

Sorting

Trypanosomes probably use cytoplasmic determinants as signals for targeting of transmembrane proteins [4], although direct interactions between such domains and adaptins have not been demonstrated. For the vast majority of the identified surface or endomembrane system proteins, such studies have not been performed. Sequence analysis of cytoplasmic domains of members of the ISG family does suggest that conventional signals are present, but this awaits rigorous demonstration.

Trypanosomes lack the mannose-6-phosphate lysosomal-targeting system, yet clearly direct a large cohort of proteins to this organelle, including cysteine proteases [33] and p67/CB-1 [34]. A possible identification of an alternative signal has emerged as a result of the use of tomato lectin, which binds polylactosamine (pNAL). Extensive analysis of the proteins to which the lectin binds suggests specificity for endosomal compartments [35]. Tomato lectin recognizes ISG_{100}, p67/CB-1 and transferrin receptor, all implicated as being present within the endosomal system. Recent data indicate that tomato lectin staining corresponds well with TbRAB5A localization, but not with TbRAB5B in bloodstream forms [7]. The simplest model for a role of the tomato lectin ligand in endocytosis is an analogous function to mannose-6-phosphate, and would be responsible for targeting several proteins into the endosomal system. This model implies the presence of an endogenous lectin, which recognizes the tomato lectin ligand and sorts proteins into the trypanosomal endosomal system. It also implies an *N*-glycan modification pathway able to discriminate between

lysosomal and non-lysosomal proteins. However, for some tomato lectin-reactive proteins, such a system appears unnecessary *sensu stricto* because, for example, p67/CB-1 is accurately targeted to the lysosome in procyclics, which do not contain proteins with complex class *N*-glycans.

Clearly, both the lipid rafts and lectin systems are provocative new developments in kinetoplastid endocytosis, but substantial further work is required to fully understand the importance and precise nature of these processes within the overall membrane transport systems of these organisms.

The lysosome

p67/CB1 was originally identified with a monoclonal antibody raised against fractions from *T. brucei* enriched for terminal β-galactosides [36]. It is a Type I membrane glycoprotein with a large N-terminal domain, a single transmembrane pass and a short 24-amino acid cytoplasmic domain [37]. The protein is an excellent marker for the lysosomal compartment in that it appears to be restricted to this structure at a steady state. p67 is extensively glycosylated, and the degree of glycosylation, the topology and the lysosomal localization suggest that p67 is possibly a LAMP paralogue. In mammalian lysosomes, LAMP glycoproteins constitute ~50% of the protein mass and are highly glycosylated [38]. p67 is expressed in both bloodstream and procyclic forms and, in both stages, the protein is cleaved into several distinct fragments; the role of this controlled proteolysis is not clear. The p67 forms in bloodstream stages are larger than in procyclics, due to stage-dependent differences in glycosylation. There is some evidence in bloodstream forms that lysosomal routing of p67 might occur via the flagellar pocket because a proportion of p67 molecules are accessible to surface-labelling reagents [37,39].

Conclusions

Many pathways are now known to be present within the trypanosome endocytic system, several of which have now been defined with molecular markers, but much remains to be discovered. New proteins are being described that appear to be trypanosome-specific components of the endosomal system, and insights from the genome projects are providing a new strategy for understanding these systems. The fundamental mechanisms of endocytosis appear to be conserved, albeit with some changes in emphasis reflecting the specialized requirements for parasitism. Excellent examples of these requirements include seclusion of some receptors within the flagellar pocket, the high rate of endocytosis in the bloodstream form of *T. brucei* and a possible GPI-specific endosomal pathway. A deeper understanding of the protein networks that control trypanosomatid endocytosis are likely to be forthcoming as the impact of the genome projects, coupled with postgenomic technologies such as RNA interference and proteomics, are fully appreciated and exploited. This past decade has been highly illuminating, and the next five years promise to be even more exciting.

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The comparative pathogenesis of neosporosis

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Since its first description in dogs in 1984, the protozoan parasite *Neospora caninum* has been found to infect a wide range of animals, worldwide. In cattle, *N. caninum* has particular significance as a cause of abortion in which persistence of infection in the mother, recrudescence of the parasite during pregnancy, and the vulnerability of the placenta and foetus to invasion are important features. This article discusses how *Neospora* invades cells, how it infects and causes disease in several animal species, and particularly how it has evolved a special niche in cattle and dogs that ensures its survival.

Neosporosis is the name given to a disease caused by the apicomplexan parasites *Neospora caninum* and *Neospora hughesi* [1], which are obligate intracellular protozoa closely related to *Toxoplasma gondii*. This review is almost solely concerned with *N. caninum*, which was first described in dogs in 1984 [2], then in calves with myeloencephalitis, and was subsequently isolated and named in 1988 [3,4]. The parasite infects a wide range of animals and can cause illness in several species, most significantly in cattle and dogs, although it does not appear to cause significant infection or disease in humans [5]. The current incidence of neosporosis in dogs is not known, and seroprevalence rates range from 0% in Kenya to 29% in Italy [6], but it is noteworthy that higher levels of seropositivity in farm dogs correlate with the presence of infection in cattle on the same premises [7]. Neosporosis is a serious cause of abortion in cattle, although subclinical infection is very much more common [5].

Life cycle and cell invasion

Neospora undergoes a life cycle involving three principle stages. First, oocysts are produced in the faeces of dogs, the definitive host, following ingestion of bradyzoites [8]. Second, bradyzoites, which multiply slowly, are found in tissue cysts in the central nervous system (CNS), both in the canine definitive host and in a wide range of intermediate hosts. They represent a persistent, quiescent infection, held in check by host immunity [9]. Third, tachyzoites, the rapidly multiplying stage, trigger lesion development by multiplying in and rupturing cells. In the absence of a host immune response, tachyzoites would continue to multiply, causing progressively more cell death until the host dies. However, by extrapolation from *T. gondii*, it is assumed that, with the onset of the host immune response and the presence of other physiological factors, tachyzoites differentiate into bradyzoites and a persistent tissue cyst infection is established [10]. The occurrence of cell destruction, and therefore disease, depends upon a balance between tachyzoites being able to penetrate and multiply in host cells and the ability of the host to inhibit parasite multiplication.

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