REVIEW ARTICLE
Cytoskeleton, November 2012 69:931–941 (doi: 10.1002/cm.21074)

Cytokinesis in Trypanosomes

Helen Farr* and Keith Gull*

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom

Received 7 August 2012; Accepted 6 September 2012
Monitoring Editor: Joseph Sanger

Cytokinesis is a crucial step in the cell division cycle whereby the cell membrane and underlying cortex is remodelled and drawn together to create two new daughter cells. While in many eukaryotic systems this process is accomplished by an actomyosin contractile ring, the protozoan parasite *Trypanosoma brucei* displays an unusual mechanism for cytokinesis, with an increased reliance on microtubules. There are a number of crucial preparatory steps involving the replication and segregation of organelles that must be undertaken in order for cytokinesis to occur. In this review, we will discuss the cellular architecture of the trypanosome and its importance within cytokinesis, and the recent progress in understanding the regulatory systems involved. Recent advances in three-dimensional imaging techniques have improved our understanding of the mechanisms driving cytokinesis and are likely to yield further insights in the future. © 2012 Wiley Periodicals, Inc

Key Words: cytokinesis, microtubules, cell cycle, trypanosoma

Cytokinesis Compared

Cytokinesis is a crucial step in the cell division cycle whereby the cell membrane and underlying cortex is remodelled and drawn together to create two new daughter cells. In animal cells, this process is accomplished by an actomyosin contractile ring (reviewed in [Levayer and Lecuit, 2012]). Multiple pathways with varying degrees of redundancy bring about the formation and constriction of the contractile ring. After the replicated chromosomes have segregated in anaphase, regulatory and structural proteins start to assemble in the middle of the cell, using the spindle microtubules as a scaffold. At this time, the chromosome passenger complex (CPC) moves from the chromosomes and onto the microtubules. The CPC contains the kinases Aurora B and Polo, which act on the centralspindlin complex, containing MKLP kinesin and RhoGAP, phosphorylating and thereby activating these proteins [Mishima et al., 2002; Mishima and Glotzer, 2003]. These regulatory proteins in turn locally activate RhoA, a GTPase, which allows it to interact with the plasma membrane [Piekny et al., 2005]. Once activated, RhoA acts on effector proteins Rho kinase and Citron kinase which bring together filamentous actin (F-actin) and nonmuscle myosin II to form the actomysin contractile ring. Other proteins also localise to the contractile ring such as anillin, which recruits septins, GTP-interacting proteins that form filaments around the ring. During contraction of the ring, the microtubules of the spindle become compacted into a bundle in a bulge between the dividing cells called the midbody. To bring about abscission, membrane is deposited and fused to the existing membrane by the endosomal sorting complexes required for transport (ESCRT) machinery.

The trypanosome cytokinesis process contrasts to the above in that it is much more focused on the microtubule, rather than the actomyosin, portion of the cytoskeleton. However, before discussion of the process, it is useful to set cytokinesis into a more general cell cycle context and the variations between eukaryotic cells.

Cytokinesis follows a series of organelle duplications and segregation events that occur at particular points in the cell cycle after the major G1/S phase transition initiation point. These duplication and segregation events involve both cytoplasmic organelles and nucleus and occur in a particular temporal order. Perhaps, the most important cytoplasmic complex that has it own duplication, segregation and maturation cycle is the centriole/centrosome. The centriole/centrosome cycle and its regulation has been described in increasing complexity over the past few years (reviewed in [Mardin and Schiebel, 2012; Pelletier and Yamashita, 2012]). Cells often exhibit three types of microtubule organising centre (MTOC)—cytoplasmic, spindle and cilium/flagellum. The mammalian centrosome is an example of MTOC organisation where all of these

*Address correspondence to: Helen Farr or Keith Gull, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom. E-mail: helen.farr@path.ox.ac.uk or keith.gull@path.ox.ac.uk

Published online 1 October 2012 in Wiley Online Library (wileyonlinelibrary.com).

functions are physically grouped together into a distinct cellular singularity. The key features of mammalian centrosome regulation and modulation of MTOC activity can be seen in other organisms such as budding yeast whose MTOC is located in the nuclear envelope and can nucleate both cytoplasmic and spindle microtubules. However, in protists such as the trypanosome, rather different levels of complexity are seen and the many MTOCs of the cell are usually dispersed and exhibit possibilities of distinct individual regulation within the cell cycle [Gull, 2005]. Thus, the eukaryotic cell cycle integrates the duplication and segregation of cytoplasmic organelles and centres, particularly the MTOCs, with the replication and segregation of chromosomes in the nucleus to arrive in late G2 where the main event of cytokinesis must be completed. However, cytokinesis has been influenced and presaged by the preceding nuclear and cytoplasmic events. The cytokinesis process in trypanosomes illustrates this concept in an organism whose shape and form is influenced heavily by its microtubule cytoskeleton and an accompanying set of filaments that interact both with the membranes of organelles and the surface.

Trypanosome Cellular Architecture

The protozoan parasite *Trypansoma brucei* causes devastating disease in both humans and animals in Sub-Saharan Africa. Laboratory work focused on understanding the basis of its pathogenicity has developed molecular and genomic approaches that mean that the organism is also one of the most understood and experimentally tractable protists. A member of the kinetoplastid family, its deep-branching status within the eukaryotes is often reflected in extreme versions of eukaryotic cell biological processes [Stevens and Gibson, 1999; Daniels et al., 2010; Echeverry et al., 2012]. Two forms of the parasite are grown routinely in the laboratory—the procyclic form characteristic of the Tsetse fly gut and the mammalian bloodstream form. Both types have a trypomastigote cell form but vary somewhat in their biochemistry and cell biology. The procyclic cell is the most studied in vitro in cell biological terms and much of this review will deal with that form.

Cytokinesis in *T. brucei* does not involve a centripetal contraction but an apparent movement of the furrow longitudinally through the cell between two defined points. Actin appears to play no major part in the process since RNA interference (RNAi) mutants have major abnormalities in endocytosis in bloodstream forms of the parasite but the procyclic form is able to proliferate [Shi et al., 2000; Garcia-Salcedo et al., 2004]. However, RNAi is a knockdown of protein level rather than a complete ablation and therefore it is possible that a low level of actin protein may remain and be sufficient for cytokinesis. *T. brucei* possesses a number of single copy organelles which must be duplicated and segregated accurately in order for a successful cytokinesis to occur. Unlike many animal cells where the main centrosomal MTOC for the mitotic spindle contains a centriole at the core of the pericentriolar material, in trypanosomes the centriole functions as a basal body nucleating a flagellum throughout the entire cell cycle The nucleus divides via an intranuclear spindle and thus the basal bodies are never located at the spindle poles nor indeed connect to the intranuclear spindle. Nevertheless, the duplication of the basal body, one of the earliest markers in the cell cycle, is essential for the development of several structures with a contributing role in cytokinesis.

T. brucei cells have an elongated shape conferred on them by a single layer of connected microtubules called the subpellicular microtubule corset, which underlies the entire cell membrane. The one exception is the area where the flagellum exits the cell from a specialised region termed the flagellar pocket. The single flagellum characteristic of the G1 cell is maintained throughout the cell cycle and originates close to the posterior end of the cell. The flagellum is attached along the length of the cell body wrapping around the long axis with a left-handed helix (Fig. 1).

The cytoskeletal structures have been extensively studied, initially using thin section electron microscopy [Vickerman, 1969; Vickerman and Preston, 1970; Vickerman, 1985] and then via whole-mount negatively stained cytoskeletons [Sherwin and Gull, 1989a]. These detailed and informative micrographs provided much of the details of structures involved in cytokinesis and the likely mechanism by which it occurs. Information about the timing of cell cycle stages was obtained from observations and measurements on asynchronous populations of cells [Woodward and Gull, 1990]. The *T. brucei* cell cycle displays the unusual feature of having periodic S phases for both the nuclear and mitochondrial DNA [Woodward and Gull, 1990; Siegel et al., 2008]. The huge mitochondrial DNA complex termed the kinetoplast is replicated and segregated prior to the onset of mitosis, giving rise to G2 cells with two kinetoplasts and one nucleus (2K1N). The nucleus divides subsequently via an intranuclear spindle that forms from a rather unstructured nuclear matrix located MTOC.

Overview of the Early Main Events

At an early stage of the cell cycle, around the time of entry into S phase, the probasal body matures and elongates, docking with the membrane of the flagellar pocket and two new probasal bodies are formed. The basal body/probasal body complexes then separate as the cell begins to construct and extend a new flagellum. In procyclic form cells, the tip of this new flagellum is attached to the side of the old flagellum by the flagella connector, a multi-layered transmembrane mobile junction. To date,

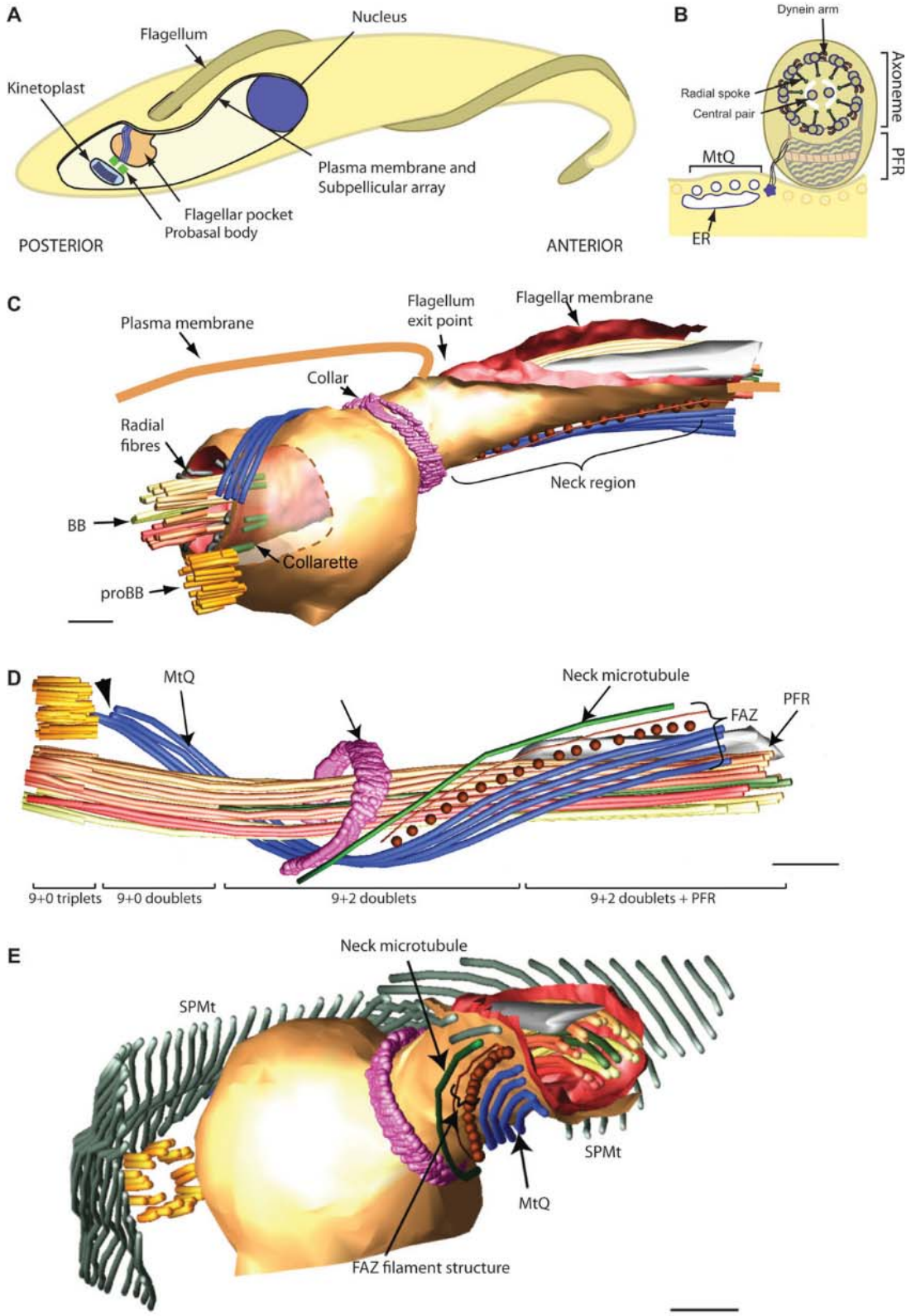

Fig. 1. Cell architecture of *T. brucei*. (A) Cartoon representing the cell architecture of *T. brucei*, showing the flagellum associated with the plasma membrane and emerging from a specialised region termed the flagellar pocket. The proximal end of the flagellum is associated via the basal body with a probasal body and with the kinetoplast (mitochondrial DNA-containing structure). (**B**) Cartoon illustrating a cross section of the *T. brucei* flagellum showing the links to the cell body via the FAZ. (**C**) Model of tomogram showing major membrane and cytoskeletal features. The flagellar pocket is bound by the neck region and collar defining the exit boundary, and the radial fibres and collarette defining the flagellum entry boundary. BB, basal body. (**D**) Model of tomogram with all membrane structures excluded. Note the left-handed helical pattern of the microtubule quartet (MtQ, origin indicated by arrowhead), the position of the collar relative to the axoneme and the origin points of the FAZ and paraflagellar rod (PFR). (**E**) Model of tomogram (viewed from anterior end of cell) showing the structure of the FAZ filament and MtQ around the neck region membrane. These structures then join the subpellicular microtubule array (SPMt). Cartoons designed by Sylvain Lacomble, tomogram models from Lacomble et al. [2009] Scale bars: 200 nm.

no flagella connector structure has been identified in bloodstream form cells—yet the tip of new flagellum is also in close proximity to the old flagellum and follows the path of the old flagellum as it grows. The flagella connector moves along the old flagellum coordinately with the growth of the new flagellum, thus providing a templated path which the new flagellum follows in a helical manner around the cell. Therefore, here as in perhaps other areas of the microtubule cytoskeleton in trypanosomes is evidence of cytotactic inheritance [Sonneborn, 1964; Beisson and Sonneborn, 1965; Moreira-Leite et al., 2001]. At a fixed point about 0.6 of the distance along the old flagellum, immediately anterior to the nucleus, the flagella connector reaches a stop point and travels no further. Detailed measurements have shown that the new flagellum continues to extend and the basal bodies move further apart. The kinetoplast is attached to the basal bodies by a complex filamentous structure, the tripartite attachment complex (TAC) [Ogbadoyi et al., 2003]. The TAC links the kinetoplast mitochondrial DNA across the mitochondrial membranes to the proximal end of the basal bodies. Robinson and Gull [1991] were able to show that segregation of the basal bodies was responsible for segregation of the kinetoplasts in a microtubule dependent manner. The mitotic spindle straddles the line that the old flagellum transects across the cell body such that after anaphase each daughter nucleus ends up in a position that facilitates the segregation of a kinetoplast/basal body and nucleus cohort to each daughter cell. Measurements of cell size and inter-organelle distances made by Robinson et al. [1995] suggested that the increase in cell body length and the increase in interbasal body and kinetoplast distance appear to occur at a similar rate, suggesting perhaps some level of shared control between these two events.

However, it is still unclear how much this kinetoplast segregation is orchestrated by events such as either the connector stop point, or an intrinsic motor function for basal body rotation/separation, or the duplication and extension of the specialised microtubule quartet or other rootlets nucleated near the basal bodies or finally, by the process of incursion of new microtubules into the corset between the two flagellar pockets. It has been proposed [Absalon et al., 2007] that the stop point provides a model whereby "pressure applied by movements of the growing new flagellum on the flagella connector leads to a reacting force that in turn contributes to migration of the basal body at the proximal end of the flagellum." This appears highly unlikely to be the case. The geometry and anatomy of the cell at this life cycle stage do not easily lend itself to this explanation. Many points could be made, but suffice to say if true, this explanation would require a discrete longitudinal, coordinated movement of the complex connections between the flagellum and the cell body, as well as structures within the cell body such as the flagellar pocket. Moreover, some variants of the possible model would necessitate a series of discrete release and reattachments of the many macula adherens transmembrane junctions that hold the new flagellum in place alongside the cell body during the process of kinetoplast segregation. In fact, it may well not be necessary to consider this kinetoplast segregation as a movement of the posterior basal body in such a limited linear manner. Rather, we know that this basal body complex undergoes an early rotation around the old basal body/flagellum as the new flagellum elongates. There is a definite posterior relocation during this process that continues to ensure full kinetoplast segregation. However, the factors that could contribute to this complicated two step (at least) process are many and diverse. The part played by subpellicular microtubule array remodeling in this process has been underplayed and could be the single most important target for the antimicrotubule drug sensitivity of basal body and kinetoplast separation as shown by our laboratory some time ago [Robinson and Gull, 1991]. If the model of Absalon et al. [2007] was indeed to be all that was required, then it must be able to explain a number of RNAi phenotypes such as kinetoplast segregation in the absence of flagellar pocket morphogenesis in the BILBO1 mutant [Bonhivers et al., 2008] or in the absence of a new flagellum in the IFT20 or DHC1b mutants [Absalon et al., 2007].

The microtubules of the subpellicular corset are aligned with their plus ends at the posterior of the cell; thus, it is mostly at this end of the cell that the cytoskeleton extends by adding to the length of the microtubules [Sherwin and Gull, 1989b; Robinson et al., 1995]. Studies of the subpellicular microtubules using a monoclonal antibody recognising newly polymerised tyrosinated alpha-tubulin have shown that during the course of the cell cycle new microtubules are inserted among the existing microtubules [Sherwin and Gull, 1989b]. In this way, the cell volume is able to increase while the inter-microtubule distance remains constant.

The flagellum of *T. brucei* procyclic cells is attached along almost the whole of its length to the cell body by a structure termed the Flagellum Attachment Zone (FAZ). This structure, which spans the membranes of both the cell body and the flagellum, consists of a quartet of specialised microtubules, along with a long FAZ filament and macula adherens junctions [Vickerman, 1969; Robinson et al., 1995]. Recently, novel structures have been defined in the intermembrane space defining points of connection between the membranes of the flagellum and cell body [Hoog et al., 2012]. The four microtubules underlying the cell body membrane are in close proximity with the endoplasmic reticulum, originate at the basal bodies and have been shown to have a polarity opposite that of the rest of the subpellicular microtubules. A new FAZ filament is extended at the same time as the new flagellum in

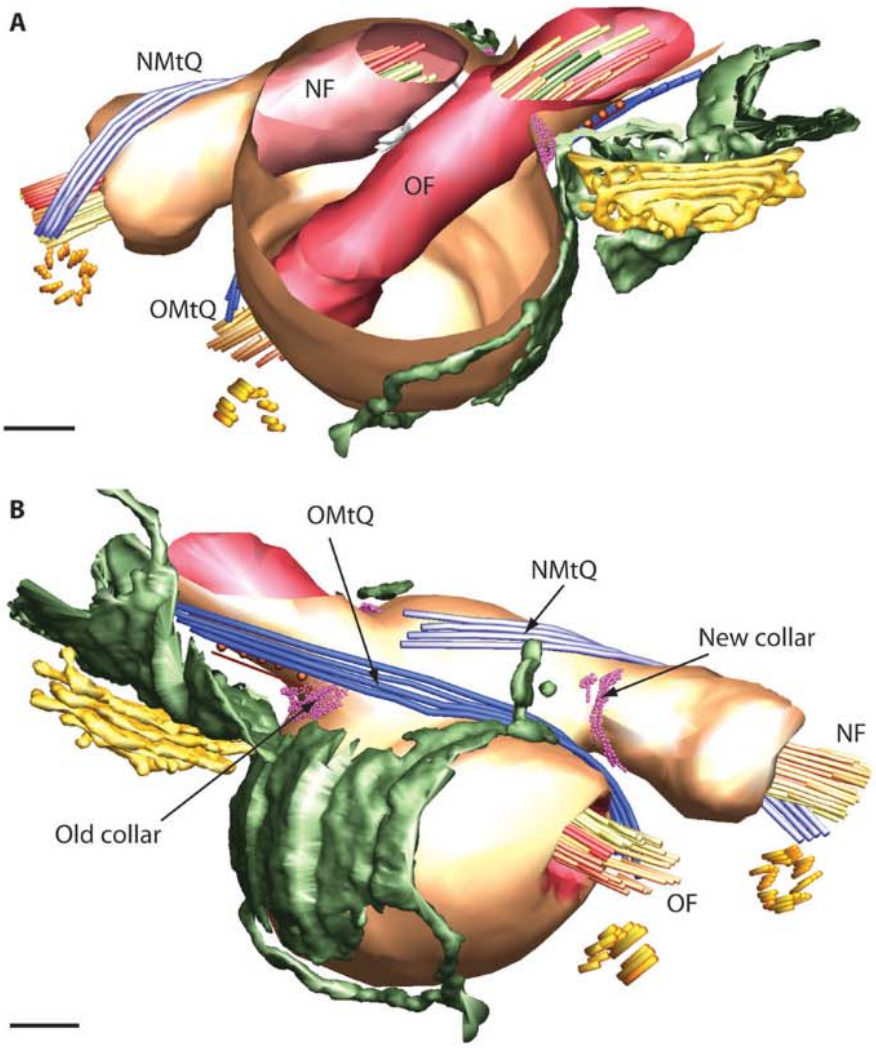

Fig. 2. Formation of the new flagellar pocket. Two views of a model of a tomogram illustrating the morphogenesis of the new flagellar pocket as it forms. (**A**) A nascent pocket forms around the new flagellum (NF), along with a new microtubule quartet (NMtQ). (**B**) Position of the old and new microtubule quartets and the old and new collars. Reproduced from Lacomble et al. [2010] Scale bars: 200 nm.

preparation for cell division, with growth of the FAZ lagging slightly behind growth of the flagellum [Kohl et al., 1999]. The FAZ plays an extremely important role in determining the site of initiation of the cytokinesis furrow [Robinson et al., 1995]. The maturation of the probasal body to a basal body and extension of a new flagellum is one of the first events in the preparation for a new round of cytokinesis. Previous ultrastructural studies have shown that the probasal body is always positioned on the anterior side of the mature basal body. The new flagellum, however, is always located on the posterior side of the old flagellum. The question of how and when the two basal bodies change position has only recently been answered with electron tomography. Lacomble et al. [2010] reconstructed the whole basal body/flagellar pocket area of trypanosomes at a number of critical stages in the cell cycle. When the probasal body matures and elongates, it docks with the existing flagellar pocket, while still located anterior to the basal body extending the old flagellum. There then occurs a rotational movement of the newly matured basal body around the old basal body in an anticlockwise direction when viewed from the posterior of the cell. This rotation appears to contribute to the formation of the new flagellar pocket, which can be seen to develop from the side of the existing flagellar pocket (Fig. 2).

Lacomble et al. [2010] also revealed that the nucleation of the new microtubule quartet precedes probasal body extension or new probasal body formation. This probably establishes early cytoskeletal contact with the flagellar pocket membrane facilitating orientation and docking of the transition zone and axoneme after maturation and extension of the probasal body. This is also likely to influence the inheritance of the left-handed helical pattern that will subsequently be consolidated via the connector,

flagellar growth and directionality of FAZ filament assembly. The basal body and microtubule quartet remain together as the rotational movement occurs. The old microtubule quartet could act as a cytoskeletal rib, over which the flagellar pocket membrane is drawn, allowing the new flagellar pocket to be structurally defined as a consequence of the rotary movement. Thus, the direction and polarity of this set of rootlet microtubules is therefore crucial to morphogenesis. The tomography studies also illustrated the lateral movement of the new and old basal bodies that segregates the mitochondrial genome [Robinson and Gull, 1991]. More recently, Gluenz et al. [2011] have provided an integrated view of the kinetoplast duplication cycle in *T. brucei* based on three-dimensional reconstructions from serial-section electron micrographs. This structural model was enhanced by analyses of the replication process of DNA maxi- and minicircles of the kinetoplast mitochondrial DNA which showed that the kinetoplast S phase occurs concurrently with the repositioning of the new basal body from the anterior to the posterior side of the old flagellum.

In summary, duplication and segregation of the microtubule cytoskeleton in preparation for cytokinesis involves three types of inheritance pattern. Daughter cells inherit the subpellicular array of microtubules and basal bodies in a semi-conservative manner whilst the flagellum, microtubule quartet, and FAZ filament are inherited in a conservative manner. Finally, the flagellum connector and perhaps other areas are structures that provide inheritance via a cytotactic influence. After cell division, there is, therefore, not a 'new' cell and an 'old' cell *per se*, but rather a chimeric series with inheritance and maturation patterns extending over a number of cell cycles. It is this complexity that is resolved at cytokinesis in the trypanosome. The single copy organelles represented in a highly organised and reproducible pattern make this phenomenon relatively apparent in the trypanosome. It is likely that elements of this biology are present in other apparently more plastic eukaryotic cells.

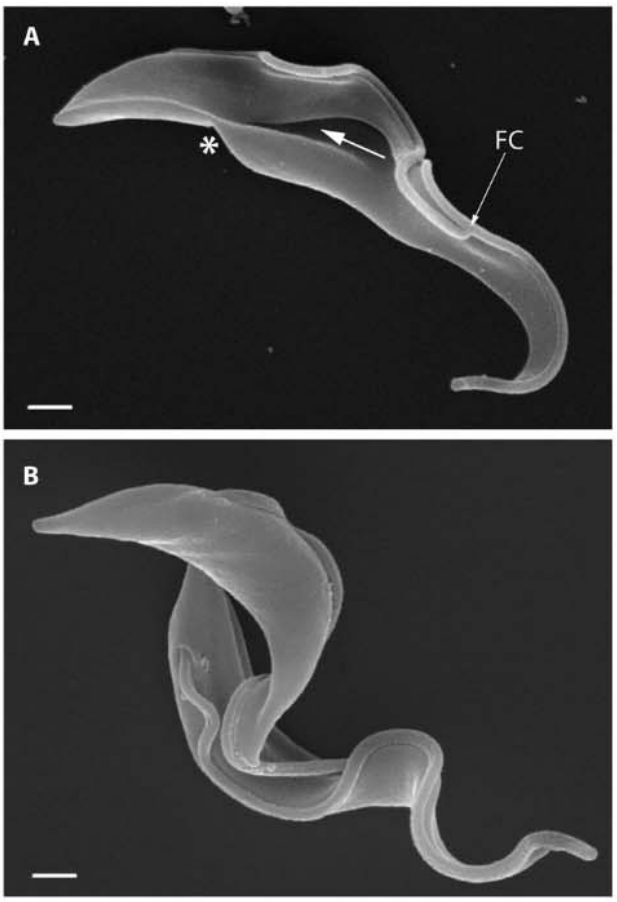

Fig. 3. Morphogenesis of the cytokinesis furrow. Scanning electron micrographs showing the cytokinesis furrow in *T. brucei*. (**A**) Cell in early cytokinesis. The new flagellum is attached to the side of the old flagellum by the flagella connector (FC) and a characteristic fold has developed longitudinally along the cell (white arrow). The forming new posterior end is indicated by an asterisk. (**B**) Cell in late cytokinesis, immediately prior to abscission. The furrow has separated the two daughter cells which remain attached at the posterior end and by the flagella connector.

Cytokinesis and Cell Morphogenesis

The first external indication of cell cycle progression is a new flagellum that exits the flagellar pocket and whose tip is connected to the old flagellum (see Fig. 2A for detailed view) via a transmembrane mobile junction—the flagella connector. This connection is maintained as the new flagellum grows in an anterior direction and the two flagellar pockets segregate. The flagellar pocket of the new flagellum is always positioned posterior to that of the old flagellum; when viewed from the posterior end of the cell, the new flagellum is always positioned to the left of the old. The actual cytokinetic event in trypanosomes is initiated at a point near to the distal end of the new flagellum. In a cell where cytokinesis has initiated and progressed such as that in Fig. 3A there is a characteristic fold. This is an invagination of the cell body between the two flagella which allows the early definition of two nascent daughter cells. The fold does not extend all the way to the posterior end of the dividing cell but stops some way back from this. Eventually, there is fusion of both sides of the cell close to the fold's anterior end and this starts the process of resolution of the cytokinetic process. Figure 3A therefore illustrates a stage where the flagella connector is still present, connecting the end of the new flagellum to the side of the old. The cell still exhibits the posterior end of the original cell and a new posterior end is being morphed to accommodate the flagellar pocket associated with the old flagellum. The fold is placed asymmetrically along only a portion of the original cell but its position and resolution allow bisection of the cell yielding two

daughter cells. Resolution of the fold into a division cleft results in the appearance of a gap in the middle of the cell between the two nascent daughters. However, even at this stage both ends of the original cytokinetic cleft are anchored—at the posterior end by the emerging cell posterior ends and at the anterior since the distal tip of the new flagellum remains attached to the old via the flagella connector (Fig. 3B). The flagella connector then disconnects releasing the anterior ends of the daughter cells and then a final re-modelling and resolution of the new posterior end then facilitates final abscission.

Thus, the actual cytokinetic process in trypanosomes is much more spatially complex than a central closing of an actomyosin centripetal ring. There are a series of 3D morphological re-modellings such as extension of the existing posterior end coupled with morphing of a new posterior cell end. Actin seems to play no or only a minor role in these events which appear to be much more focused on the microtubule cytoskeleton in terms of rearranging the existing pellicular organisation at the same time as morphing new elements. The details of this subpellicular microtubule morphogenesis have not moved on much in 20 years since Sherwin and Gull demonstrated that new microtubules are inserted between old and visualised the post-assembly detyrosination cycle of alpha-tubulin. We know that most microtubules (all except the microtubule quartet) have their plus ends at the posterior end of the cell. Also, the inter-microtubule distance remains the same in the subpellicular array during cytokinesis, while the width of cell increases, such that new microtubules must be inserted into the array. Sherwin and Gull [1989b] showed that there appears to be a potential for fast plus end addition of tubulin and slower minus end addition and that some short uniformly labelled microtubules (i.e., containing a full complement of tyrosinated alpha-tubulin) are seen in the array during cytokinesis suggesting that these are the newly assembled microtubules. Thus, although we have some indication of the manner that microtubules are polymerised in the array we have no concept of how this is structured in 3D, how it is then restructured to form the fold and cleft and to congregate microtubules into the new posterior end. This level of understanding is now within our reach through improvements in 3D imaging at the electron microscope level.

Regulation

Since the discovery and application of RNAi in *T. brucei* [Bastin et al., 1998; Ngo et al., 1998; Bastin et al., 2000], this has become a useful tool with which to study function of a wide range of proteins within the two most commonly cultured life cycle stages, procyclic form and bloodstream form cells. A frequent observation in experiments of this type is that the same protein may have quite strikingly different effects in these two cell stages, the most usual conclusion being that the more severe phenotype is observed in bloodstream form cells. Whether this suggests RNAi is more efficient in that cell type, or a different regulation exists or rather that the procyclic cell is merely more robust in the organisation of its essential processes.

Cyclins and cyclin-dependent kinases are key proteins acting in the eukaryotic cell cycles. Several homologues of cyclins and cyclin-dependent kinase are conserved within trypanosomes. Cyclin-dependent kinases in *T. brucei* are termed cdc2-related kinases (CRKs) [Mottram and Smith, 1995]. To date, 11 cyclins and 12 CRKs have been identified however functions have not been ascribed to all of these. The G1/S transition has been shown to be regulated by a complex consisting of CYC2 and CRK1 [Tu and Wang, 2005; Gourguechon et al., 2007], while G2/M transition is controlled by complexes of CYC2 and CRK3 along with CYC6 [Van Hellemond et al., 2000; Hammarton et al., 2003; Gourguechon et al., 2007]. RNAi against the more recently identified cyclins 10 and 11, along with CRKs 7-12 showed that, with the exceptions of CRK9, these do not appear to be essential for growth in procyclic form cells [Gourguechon and Wang, 2009]. Knockdown of CRK9, however, leads to growth arrest of procyclic form cells after 4 days of induction, accompanied by an accumulation of cells in G2/M phase. The morphology of these cells reveals that they are arrested with one kinetoplast and one nucleus, but with two basal bodies. The interbasal body distance in CRK9 RNAi-induced cells was found to be less than in control cells suggesting that there are defects in basal body segregation and kinetoplast segregation which contribute to the block to mitosis [Gourguechon and Wang, 2009]. This highlights the importance of basal body segregation as an early step in the process, control and mechanism of cytokinesis. In vitro pull down assays showed an interaction between CRK9 and CYC6/cyclin B2, which has previously been shown to be important for the G2/M transition [Hammarton et al., 2003; Gourguechon and Wang, 2009].

Polo-like kinase (PLK) is a protein required for several roles connected to cytokinesis is most eukaryotes, including exit from mitosis [Hu et al., 2001] and entry into cytokinesis [Gruneberg and Nigg, 2003]. The homologue in yeast of PLK is Cdc5, which has been shown in *Saccharomyces cerevisiae* to localise to the nucleus, spindle pole body and bud neck [Shirayama et al., 1998; Snead et al., 2007]. Trypanosomes possess a single homologue of PLK [Graham et al., 1998], whose function is indeed required for cytokinesis, however its role appears to be more limited in comparison with the PLKs of other eukaryotes and it has been shown only to function in cytokinesis [Kumar and Wang, 2006]. Interestingly, it has been shown that TbPLK is able to complement Cdc5 in *S. cerevisiae* cells depleted of Cdc5 [Kumar and Wang, 2006] and furthermore, expression of YFP tagged Cdc5 in *T. brucei* shows

the same localisation as TbPLK [Sun and Wang, 2011]. The expression of TbPLK is cell cycle-regulated, with the protein first appearing in S phase and persisting through G2/M phase before disappearing during cytokinesis [Umeyama and Wang, 2008]. During its period of expression, TbPLK is first found associated at the basal bodies [Ikeda and de Graffenried, 2012], then moves to a point mid-way along the FAZ. In some studies, foci have also been observed at the anterior end of the cell, though as shown by Umeyama and Wang [2008] this appears to be found more commonly in cells where TbPLK is overexpressed. In studies using antibody raised against TbPLK [de Graffenried et al., 2008; Ikeda and de Graffenried, 2012], foci are observed at the anterior tip of the growing FAZ and it has also been reported to localise to the flagella connector [Ikeda and de Graffenried, 2012]. The localisation of TbPLK at the anterior tip of the new FAZ—the site at which cytokinesis initiates—and its disappearance following the initation of cytokinesis have prompted the suggestion that it is responsible for recruiting the CPC to the site of cytokinesis initiation [Umeyama and Wang, 2008]. In a recent study, a series of PLK mutants were characterised where motifs within PLK were deleted [Sun and Wang, 2011]. This showed that kinase function of TbPLK plays a role in blocking entry of TbPLK into the nucleus and revealed that two polo box domains are required for localisation to the FAZ [Sun and Wang, 2011].

The CPC in metazoa consists of Aurora B kinase and three non-enzymatic proteins, INCENP, the inner centromere protein, Survivin and Borealin. This is conserved from metazoa to budding yeast. However, the CPC of trypanosomes appears to be distinct, containing an Aurora kinase homologue but in conjunction with two novel proteins quite unlike Survivin and Borealin. These have been named CPC1 and CPC2 [Li et al., 2008a]. Two novel kinesin homologues, TbKIN-A and TbKIN-B, were identified which also associate with the CPC. These divergent kinesins were found to localise to the nucleus and spindle but do not translocalise with the CPC following mitosis [Li et al., 2008a].

Several constituents of the CPC have been characterised in *T. brucei*. An Aurora kinase homologue, AUK1, has been identified. During mitosis, AUK1 has been shown to localise to the chromosomes, but during telophase the protein then appears to move away from the chromosomes and takes up position at the site of the cytokinesis furrow, a localisation consistent with a function in the CPC. This movement has been studied in some depth; first AUK1 moves as a discrete focus from the nucleus to the dorsal side of the cell and anterior towards the distal tip of the new flagellum, then moves with the cytokinesis furrow towards the posterior of the cell [Li et al., 2008a,b]. Upon knockdown of AUK1, there is an increase in cells which have undergone mitosis but which do not have a cytokinesis furrow, suggesting a requirement of AUK1 for the initiation of cytokinesis [Li and Wang, 2006; Tu et al., 2006].

One of the substrates of AUK1 is Tousled-like kinase, TbTLK1 [Li et al., 2007]. TbTLK is located in the nucleus during metaphase and anaphase and is found in two discrete dots that correspond to the spindle poles. Knockdown of TbTLK1 by RNAi leads to a change in the localisation of the three components of the CPC, along with TbKIN-B. Instead of localising to the central spindle during anaphase as in control cells, these four proteins were found to be localised diffusely in the nucleus [Li et al., 2008b]. There appears to be a co-dependency of localisation: following knockdown of any of the three components of the CPC or TbKIN-B, TbTLK1 is no longer localised to the spindle poles [Li et al., 2008b].

The next stage of cytokinesis, furrow ingression, appears to require the presence of TbPLK, along with homologues of MOB1 and RACK1. MOB1 in trypanosomes, as in other organisms, is required for cytokinesis however, unlike in other organisms MOB1 does not appear to be required for exit from mitosis [Luca et al., 2001; Hammarton et al., 2005]. Upon ablation of MOB1 expression in procyclic form cells, cells were observed to be blocked in cytokinesis with the anterior ends of the cells divided but where the furrow appeared unable to complete division [Hammarton et al., 2005]. RACK1 is thought to be involved in multiple signalling pathways and has been shown to have and range of functions and localisations in different organisms [McCahill et al., 2002]. The homologue in *T. brucei*, known as TRACK, has also been ablated by RNAi giving rise to procyclic form cells in which cytokinesis initiates but is unable to complete [Rothberg et al., 2006]. A feature in this study is that cells blocked in cytokinesis in this way then undergo successive rounds of the cell cycle, resulting in cells with multiple cytokinesis furrows. RNAi against MOB1 and TRACK has been undertaken in bloodstream form cells, in both cases causing a rapid and severe onset of growth defects and failure of cytokinesis. Both TRACK and MOB1 have been shown to localise to the cytoplasm in trypanosomes at all cell cycle stages, and neither appears to localise to the cytokinesis furrow itself [Hammarton et al., 2005; Rothberg et al., 2006].

Several katanins have been identified as important for different stages of cytokinesis in *T. brucei* [Casanova et al., 2008; Benz et al., 2012]. These microtubule severing enzymes, from the AAA ATPase family, are involved in a range of microtubule re-modelling processes in eukaryotes. Katanin consists of two subunits, the p60 catalytic subunit and the p80 regulatory subunit. The *T. brucei* homologue of the p80 subunit, TbKAT80 appears to be involved in furrow ingression, since bloodstream form cells depleted of TbKAT80 by RNAi showed an increase in the proportion of the population with a cleavage furrow suggesting

cytokinesis was slowed or stalled during this phase [Benz et al., 2012]. TbKAT80 has been localised by adding a C-terminal GFP tag to one copy of the genome [Casanova et al., 2008]; the signal was found diffusely throughout the cytoplasm of the cell, however, these cells also exhibited defects in cytokinesis suggesting the recombinant protein was not fully functional. It is possible that the localisation of TbKAT80 may also have been affected by the GFP tag. In contrast to the knockdown of KAT80, upon knockdown of KAT60a, b, or c, multinucleate cells accumulate with a visible fold but without a cytokinesis furrow, suggesting that these proteins are required during early stages of cytokinesis [Benz et al., 2012].

Cytokinesis, the FAZ and Abscission

Given the importance of the attachment of the flagellum in cell division and morphology, it is perhaps surprising that only recently the first protein component of the FAZ has been identified, FAZ1 [Vaughan et al., 2008]. This large, repetitive protein was identified as the epitope recognised by a monoclonal antibody, which has previously be shown by immunogold electron microscopy to recognise the FAZ filament on the cell body side [Kohl et al., 1999]. Following knockdown of FAZ1 by RNAi, FAZ1 labelling is shown to be reduced and cells continue to build a new flagellum in each cell cycle. Defects in the structure of the FAZ were seen, however, with many flagella observed to be partially or fully detached and disorganisation of the FAZ filament at the ultrastructural level. Flagellum detachment has previously been shown to be lethal in *T. brucei* [Nozaki et al., 1996; Moreira-Leite et al., 2001; LaCount et al., 2002], and FAZ1 RNAi induced cells displayed growth and cytokinesis defects. While cells at early stages of the RNAi induction are still able to divide, the increasing presence of cells with one kinetoplast and two nuclei, and cells with one kinetoplast and no nucleus (termed zoids), indicates that the fidelity of cytokinesis is perturbed in these cells. We [Robinson et al., 1995] postulated that the FAZ filament system intimately associated with the subpellicular array of microtubules provides a structural correlate between the length and position of the new flagellum and the main growth of the cell body. Our interpretation from these first experimental results using drug perturbations was that the cytoplasmic FAZ marks the position and direction of the cleavage furrow. This conclusion has been strengthened by interpretation of phenotypes resulting from subsequent RNAi studies. Greater knowledge of the component proteins that localise to the FAZ (the transmembrane connections, FAZ filaments, the microtubule quartet, etc) would be enlightening in terms of understanding the dependency relationships that exist in constructing the FAZ and influencing cytokinesis.

Another microtubule severing enzyme, spastin has been shown to be required for abscission, the final stage of cytokinesis in bloodstream form cells [Benz et al., 2012], but not procyclic form cells [Casanova et al., 2008]. To date, this is the only protein that has been implicated in abscission, though it has been shown that motility plays an important role in the final division of the doublet cells of the bloodstream form particularly [Broadhead et al., 2006; Ralston et al., 2006]. Whilst such proteins as spastin are focused on the resolution of the dividing cytoskeleton, it is highly unlikely that the plasma membrane at the abscission site is resolved purely by tearing apart. We suggest that a specific mechanism exists for this separation of the membrane at cytokinesis. The most likely pathway for this is the ESCRT pathway [Leung et al., 2008; Caballe and Martin-Serrano, 2011]. It could be that the membrane separation is brought about by membrane re-modelling or may involve directed traffic of vesicles to the abscission site. Understanding this is an important target for future research made more interesting because if there is specialised vesicle addition to the site then this challenges the long-held belief that all membrane trafficking in trypanosomes is directed to the flagellar pocket.

Cytokinesis in trypanosomes is now well mapped in its general architecture. However, we lack the detail of the events at the level of the individual microtubules and their regulation. All this is however tractable and enabled by the exquisite fidelity of the process. In a few years, we are likely to have a better understanding of the cytoskeletal rearrangements driving cytokinesis in trypanosomes than virtually any other eukaryotic cell.

Acknowledgments

The authors thank present members of Gull lab and some particular past members for useful conversations. They specifically thank Sylvain Lacomble for provision and assistance with figures, and Polly Hayes for SEM images for this review. Work in their laboratory is supported by the Wellcome Trust and BBSRC.

References

Absalon S, Kohl L, Branche C, Blisnick T, Toutirais G, Rusconi F, Cosson J, Bonhivers M, Robinson D, Bastin P. 2007. Basal body positioning is controlled by flagellum formation in Trypanosoma brucei. PLoS One 2(5):e437.

Bastin P, Sherwin T, Gull K. 1998. Paraflagellar rod is vital for trypanosome motility. Nature 391(6667):548.

Bastin P, Ellis K, Kohl L, Gull K. 2000. Flagellum ontogeny in trypanosomes studied via an inherited and regulated RNA interference system. J Cell Sci 113 (Pt 18):3321–3328.

Beisson J, Sonneborn TM. 1965. Cytoplasmic inheritance of the organization of the cell cortex in Paramecium Aurelia. Proc Natl Acad Sci USA 53:275–282.

Benz C, Clucas C, Mottram JC, Hammarton TC. 2012. Cytokinesis in bloodstream stage Trypanosoma brucei requires a family of katanins and spastin. PLoS One 7(1):e30367.

Bonhivers M, Nowacki S, Landrein N, Robinson DR. 2008. Biogenesis of the trypanosome endo-exocytotic organelle is cytoskeleton mediated. PLoS Biol 6(5):e105.

Broadhead R, Dawe HR, Farr H, Griffiths S, Hart SR, Portman N, Shaw MK, Ginger ML, Gaskell SJ, McKean PG, et al. 2006. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature 440(7081):224–227.

Caballe A, Martin-Serrano J. 2011. ESCRT machinery and cytokinesis: the road to daughter cell separation. Traffic 12(10): 1318–1326.

Casanova M, Portales P, Blaineau C, Crobu L, Bastien P, Pages M. 2008. Inhibition of active nuclear transport is an intrinsic trigger of programmed cell death in trypanosomatids. Cell Death Differ 15(12):1910–1920.

Daniels JP, Gull K, Wickstead B. 2010. Cell biology of the trypanosome genome. Microbiol Mol Biol Rev 74(4):552–569.

de Graffenried CL, Ho HH, Warren G. 2008. Polo-like kinase is required for Golgi and bilobe biogenesis in Trypanosoma brucei. J Cell Biol 181(3):431–438.

Echeverry MC, Bot C, Obado SO, Taylor MC, Kelly JM. 2012. Centromere-associated repeat arrays on Trypanosoma brucei chromosomes are much more extensive than predicted. BMC Genomics 13:29.

Garcia-Salcedo JA, Perez-Morga D, Gijon P, Dilbeck V, Pays E, Nolan DP. 2004. A differential role for actin during the life cycle of Trypanosoma brucei. Embo J 23(4):780–789.

Gluenz E, Povelones ML, Englund PT, Gull K. 2011. The kinetoplast duplication cycle in Trypanosoma brucei is orchestrated by cytoskeleton-mediated cell morphogenesis. Mol Cell Biol 31(5): 1012–1021.

Gourguechon S, Wang CC. 2009. CRK9 contributes to regulation of mitosis and cytokinesis in the procyclic form of Trypanosoma brucei. BMC Cell Biol 10:68.

Gourguechon S, Savich JM, Wang CC. 2007. The multiple roles of cyclin E1 in controlling cell cycle progression and cellular morphology of Trypanosoma brucei. J Mol Biol 368(4):939–950.

Graham TM, Tait A, Hide G. 1998. Characterisation of a polo-like protein kinase gene homologue from an evolutionary divergent eukaryote, Trypanosoma brucei. Gene 207(1):71–77.

Gruneberg U, Nigg EA. 2003. Regulation of cell division: stop the SIN! Trends Cell Biol 13(4):159–162.

Gull K, Briggs L, Vaughan S. 2005. Basal bodies and microtubule organization in pathogenic protozoa. In: Nigg EA, editor. Centrosomes in Development and Disease. Weinheim, FRG: Wiley-VCH.

Hammarton TC, Clark J, Douglas F, Boshart M, Mottram JC. 2003. Stage-specific differences in cell cycle control in Trypanosoma brucei revealed by RNA interference of a mitotic cyclin. J Biol Chem 278(25):22877–22886.

Hammarton TC, Lillico SG, Welburn SC, Mottram JC. 2005. Trypanosoma brucei MOB1 is required for accurate and efficient cytokinesis but not for exit from mitosis. Mol Microbiol 56(1): 104–116.

Hoog JL, Bouchet-Marquis C, McIntosh JR, Hoenger A, Gull K. 2012. Cryo-electron tomography and 3-D analysis of the intact flagellum in Trypanosoma brucei. J Struct Biol 178(2):189–198.

Hu F, Wang Y, Liu D, Li Y, Qin J, Elledge SJ. 2001. Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. Cell 107(5):655–665.

Ikeda KN, de Graffenried CL. 2012. Polo-like kinase is necessary for flagellum inheritance in Trypanosoma brucei. J Cell Sci 125(Pt 13):3173–3184.

Kohl L, Sherwin T, Gull K. 1999. Assembly of the paraflagellar rod and the flagellum attachment zone complex during the Trypanosoma brucei cell cycle. J Eukaryot Microbiol 46(2):105–109.

Kumar P, Wang CC. 2006. Dissociation of cytokinesis initiation from mitotic control in a eukaryote. Eukaryot Cell 5(1):92–102.

Lacomble S, Vaughan S, Gadelha C, Morphew MK, Shaw MK, McIntosh JR, Gull K. 2010. Basal body movements orchestrate membrane organelle division and cell morphogenesis in Trypanosoma brucei. J Cell Sci 123(Pt 17):2884–2891.

LaCount DJ, Barrett B, Donelson JE. 2002. Trypanosoma brucei FLA1 is required for flagellum attachment and cytokinesis. J Biol Chem 277(20):17580–17588.

Leung KF, Dacks JB, Field MC. 2008. Evolution of the multivesicular body ESCRT machinery; retention across the eukaryotic lineage. Traffic 9(10):1698–1716.

Levayer R, Lecuit T. Biomechanical regulation of contractility: spatial control and dynamics. 2012. Trends Cell Biol 22(2):61–81.

Li Z, Wang CC. 2006. Changing roles of aurora-B kinase in two life cycle stages of Trypanosoma brucei. Eukaryot Cell 5(7): 1026–1035.

Li Z, Gourguechon S, Wang CC. 2007. Tousled-like kinase in a microbial eukaryote regulates spindle assembly and S-phase progression by interacting with Aurora kinase and chromatin assembly factors. J Cell Sci 120(Pt 21):3883–3894.

Li Z, Lee JH, Chu F, Burlingame AL, Gunzl A, Wang CC. 2008a. Identification of a novel chromosomal passenger complex and its unique localization during cytokinesis in Trypanosoma brucei. PLoS One 3(6):e2354.

Li Z, Umeyama T, Wang CC. 2008b. The chromosomal passenger complex and a mitotic kinesin interact with the Tousled-like kinase in trypanosomes to regulate mitosis and cytokinesis. PLoS One 3(11):e3814.

Luca FC, Mody M, Kurischko C, Roof DM, Giddings TH, Winey M. 2001. Saccharomyces cerevisiae Mob1p is required for cytokinesis and mitotic exit. Mol Cell Biol 21(20):6972–6983.

Mardin BR, Schiebel E. 2012. Breaking the ties that bind: new advances in centrosome biology. J Cell Biol 197(1):11–18.

McCahill A, Warwicker J, Bolger GB, Houslay MD, Yarwood SJ. 2002. The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Mol Pharmacol 62(6):1261–1273.

Mishima M, Glotzer M. 2003. Cytokinesis: a logical GAP. Curr Biol 13(15):R589–R591.

Mishima M, Kaitna S, Glotzer M. 2002. Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. Dev Cell 2(1):41–54.

Moreira-Leite FF, Sherwin T, Kohl L, Gull K. 2001. A trypanosome structure involved in transmitting cytoplasmic information during cell division. Science 294(5542):610–612.

Mottram JC, Smith G. 1995. A family of trypanosome cdc2-related protein kinases. Gene 162(1):147–152.

Ngo H, Tschudi C, Gull K, Ullu E. 1998. Double-stranded RNA induces mRNA degradation in Trypanosoma brucei. Proc Natl Acad Sci USA 95(25):14687–14692.

Nozaki T, Haynes PA, Cross GA. 1996. Characterization of the Trypanosoma brucei homologue of a Trypanosoma cruzi flagellum-adhesion glycoprotein. Mol Biochem Parasitol 82(2):245–255.

Ogbadoyi EO, Robinson DR, Gull K. 2003. A high-order transmembrane structural linkage is responsible for mitochondrial genome positioning and segregation by flagellar basal bodies in trypanosomes. Mol Biol Cell 14(5):1769–1779.

Pelletier L, Yamashita YM. 2012. Centrosome asymmetry and inheritance during animal development. Curr Opin Cell Biol 24(4):541–546.

Piekny A, Werner M, Glotzer M. 2005. Cytokinesis: welcome to the Rho zone. Trends Cell Biol 15(12):651–658.

Ralston KS, Lerner AG, Diener DR, Hill KL. 2006. Flagellar motility contributes to cytokinesis in Trypanosoma brucei and is modulated by an evolutionarily conserved dynein regulatory system. Eukaryot Cell 5(4):696–711.

Robinson DR, Gull K. 1991. Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. Nature 352(6337):731–733.

Robinson DR, Sherwin T, Ploubidou A, Byard EH, Gull K. 1995. Microtubule polarity and dynamics in the control of organelle positioning, segregation, and cytokinesis in the trypanosome cell cycle. J Cell Biol 128(6):1163–1172.

Rothberg KG, Burdette DL, Pfannstiel J, Jetton N, Singh R, Ruben L. 2006. The RACK1 homologue from Trypanosoma brucei is required for the onset and progression of cytokinesis. J Biol Chem 281(14):9781–9790.

Sherwin T, Gull K. 1989a. The cell division cycle of Trypanosoma brucei brucei: timing of event markers and cytoskeletal modulations. Philos Trans R Soc Lond B Biol Sci 323(1218):573–588.

Sherwin T, Gull K. 1989b. Visualization of detyrosination along single microtubules reveals novel mechanisms of assembly during cytoskeletal duplication in trypanosomes. Cell 57(2):211–221.

Shi H, Djikeng A, Mark T, Wirtz E, Tschudi C, Ullu E. 2000. Genetic interference in Trypanosoma brucei by heritable and inducible double-stranded RNA. RNA 6(7):1069–1076.

Shirayama M, Zachariae W, Ciosk R, Nasmyth K. 1998. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae. Embo J 17(5):1336–1349.

Siegel TN, Hekstra DR, Cross GA. 2008. Analysis of the Trypanosoma brucei cell cycle by quantitative DAPI imaging. Mol Biochem Parasitol 160(2):171–174.

Snead JL, Sullivan M, Lowery DM, Cohen MS, Zhang C, Randle DH, Taunton J, Yaffe MB, Morgan DO, Shokat KM. 2007. A coupled chemical-genetic and bioinformatic approach to Polo-like kinase pathway exploration. Chem Biol 14(11):1261–1272.

Sonneborn TM. 1964. The determinants and evolution of life. The differentiation of cells. Proc Natl Acad Sci USA 51:915–929.

Stevens JR, Gibson WC. 1999. The evolution of pathogenic trypanosomes. Cad Saude Publica 15(4):673–684.

Sun L, Wang CC. 2011. The structural basis of localizing polo-like kinase to the flagellum attachment zone in Trypanosoma brucei. PLoS One 6(11):e27303.

Tu X, Wang CC. 2005. Coupling of posterior cytoskeletal morphogenesis to the G1/S transition in the Trypanosoma brucei cell cycle. Mol Biol Cell 16(1):97–105.

Tu X, Kumar P, Li Z, Wang CC. 2006. An aurora kinase homologue is involved in regulating both mitosis and cytokinesis in Trypanosoma brucei. J Biol Chem 281(14):9677–9687.

Umeyama T, Wang CC. 2008. Polo-like kinase is expressed in S/G2/M phase and associated with the flagellum attachment zone in both procyclic and bloodstream forms of Trypanosoma brucei. Eukaryot Cell 7(9):1582–1590.

Van Hellemond JJ, Neuville P, Schwarz RT, Matthews KR, Mottram JC. 2000. Isolation of Trypanosoma brucei CYC2 and CYC3 cyclin genes by rescue of a yeast G(1) cyclin mutant. Functional characterization of CYC2. J Biol Chem 275(12):8315–8323.

Vaughan S, Kohl L, Ngai I, Wheeler RJ, Gull K. 2008. A repetitive protein essential for the flagellum attachment zone filament structure and function in Trypanosoma brucei. Protist 159(1):127–136.

Vickerman K. 1969. On the surface coat and flagellar adhesion in trypanosomes. J Cell Sci 5(1):163–193.

Vickerman K. 1985. Developmental cycles and biology of pathogenic trypanosomes. Br Med Bull 41(2):105–114.

Vickerman K, Preston TM. 1970. Spindle microtubules in the dividing nuclei of trypanosomes. J Cell Sci 6(2):365–383.

Woodward R, Gull K. 1990. Timing of nuclear and kinetoplast DNA replication and early morphological events in the cell cycle of Trypanosoma brucei. J Cell Sci 95(Pt 1):49–57.