Alternate histories of cytokinesis: lessons from the trypanosomatids

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ABSTRACT Popular culture has recently produced several "alternate histories" that describe worlds where key historical events had different outcomes. Beyond entertainment, asking "could this have happened a different way?" and "what would the consequences be?" are valuable approaches for exploring molecular mechanisms in many areas of research, including cell biology. Analogous to alternate histories, studying how the evolutionary trajectories of related organisms have been selected to provide a range of outcomes can tell us about the plasticity and potential contained within the genome of the ancestral cell. Among eukaryotes, a group of model organisms has been employed with great success to identify a core, conserved framework of proteins that segregate the duplicated cellular organelles into two daughter cells during cell division, a process known as cytokinesis. However, these organisms provide relatively sparse sampling across the broad evolutionary distances that exist, which has limited our understanding of the true potential of the ancestral eukaryotic toolkit. Recent work on the trypanosomatids, a group of eukaryotic parasites, exemplifies alternate historical routes for cytokinesis that illustrate the range of eukaryotic diversity, especially among unicellular organisms.

INTRODUCTION

Over the past 50 years, eukaryotes such as yeasts, mammals, nematodes, and insects have been used to identify a core, conserved set of regulatory and structural elements that are essential for cell division. These include cyclins and cyclin-dependent kinases to control entry into and exit from the cell cycle, molecular motors that organize microtubules for DNA segregation, and a cytokinetic mechanism that relies on an actomyosin ring (Hartwell et al., 1970; Schroeder, 1972; Enos and Morris, 1990; Pfarr et al., 1990). While studying these organisms has provided invaluable insights, the

organisms all fall within a single eukaryotic group known as Opisthokonta (Burki et al., 2020). Genomic sequencing of more disparate eukaryotes has shown that genes thought to be essential for cytokinesis may not be present outside of Opisthokonta. Nonmuscle myosin II, which is thought to provide most of the constrictive force during cytokinesis in Opisthokonta, is not well conserved outside of this supergroup, arguing that cytokinesis may be a more mechanistically diverse process than previously thought (Richards and Cavalier-Smith, 2005; Foth et al., 2006). Most of the undersampled eukaryotes are unicellular organisms generally referred to as protists, which are likely to represent the bulk of eukaryotic diversity.

While genomes are invaluable for phylogenetic and evolutionary study, a molecular understanding of gene function still requires direct interrogation using biochemistry and cell biology approaches, especially in the case of highly divergent organisms that contain many genes whose function cannot be inferred from bioinformatics. Even then, inferences made based on homology can also lead to a false sense of understanding if the function of the gene has been repurposed in a divergent organism. Our understanding of gene function is greatly outstripped by our ability to generate genome sequences and may remain impossible for organisms that cannot be cultivated in the laboratory. Therefore, leveraging organisms that

are evolutionarily distant from current model systems but can be manipulated in the laboratory is vital for understanding eukaryotic diversity.

Parasites represent a significant portion of available genomes from unicellular eukaryotes due to their importance to the health of humans and/or agriculturally important animals. Among the sequenced parasites are the trypanosomatids, which cause human diseases including Kala-azar, human African trypanosomiasis (HAT), and Chagas disease (Berriman *et al.*, 2005; El-Sayed *et al.*, 2005; Ivens *et al.*, 2005). *Trypanosoma brucei*, which causes HAT, is among the best studied laboratory-tractable parasites. *T. brucei* has a dixenous life cycle that includes the tsetse fly as a vector and definitive host and mammals as intermediate hosts (Vickerman, 1985). The two main proliferative forms of the parasite (in insects, procyclics; in mammals, bloodstream forms) present in each host have been the focus of considerable work. The morphology of these parasites has been extensively studied by light and electron microscopy (Sherwin and Gull, 1989a; Wheeler *et al.*, 2013; Hughes *et al.*, 2017). Along with several high-quality genomes of closely related strains, *T. brucei* has many properties that make it amenable to study in the lab. These include inducible, inheritable RNA interference (RNAi), a high rate of homologous recombination for straightforward tagging of endogenous gene loci, and in vitro cultivation methods that allow access to sufficient material for biochemistry and interaction mapping (Cunningham, 1977; Wirtz *et al.*, 1999; Shi *et al.*, 2000; Wickstead, 2003). These methods have been leveraged to perform whole-genome RNAi screens that have identified a core set of essential genes, mNeonGreen tagging and localization of more than 90% of open reading frames, and mapping of interactions between the many novel components within the genome (Alsford *et al.*, 2011; Obado *et al.*, 2016; Dean *et al.*, 2017). These traits make *T. brucei* an excellent divergent model system for studying unique adaptations in core processes such as cell division.

TRYPANOSOMA BRUCEI MORPHOLOGY AND CELL DIVISION

T. brucei has an elongated, bore-like shape that is optimized for motility in crowded and viscous environments within its hosts (Bargul *et al.*, 2016). The parasite is shaped by a layer of highly cross-linked microtubules arranged in a helical pattern. These microtubules are known as the subpellicular microtubule array (SPA) and lie directly under the plasma membrane (Vickerman, 1962; Sinclair and de Graffenried, 2019). The SPA microtubules are all polarized so that the plus ends are directed toward the cell posterior (Robinson *et al.*, 1995). A single flagellum is nucleated by a basal body that is docked to an invagination of the plasma membrane known as the flagellar pocket (FP), which is present near the posterior end of the cell (Field and Carrington, 2009). The basal body consists of a mature basal body and an adjacent probasal body. The mature basal body is attached via a transmembrane complex to the mitochondrial DNA aggregate, which is called the kinetoplast (Robinson and Gull, 1991; Ogbadoyi *et al.*, 2003). The extracellular portion of the flagellum that emerges from the FP is adhered to the cell surface by a series of desmosome-like structures that are collectively known as the flagellum attachment zone (FAZ) filament, which includes unique domains within the cell body and the flagellar membrane (Vickerman, 1969; Sunter and Gull, 2016). A set of four microtubules known as the microtubule quartet (MtQ) nucleates between the pro- and mature-basal body, wraps around one side of the cytosolic face of the FP, and then becomes part of the SPA. The MtQ is situated directly on the right side of the FAZ filament if the cell body is observed from the anterior end toward the posterior (Taylor and Godfrey, 1969;

Lacomble *et al.*, 2009). The MtQ microtubules likely have the inverse polarity of those comprising the SPA, with their plus ends directed toward the cell anterior end (Robinson *et al.*, 1995). The MtQ is connected to the FAZ filament and is considered part of the FAZ (Sunter and Gull, 2016) (Figure 1Ai). The junctions between the MtQ–SPA, MtQ–FAZ filament, and FAZ filament–SPA represent three unique connections between cytoskeletal elements (Sunter and Gull, 2016).

T. brucei cells remain polarized throughout cell division and must duplicate several single-copy organelles (including the Golgi apparatus, mitochondrion, flagellum, and FAZ) and then place them at specific locations within the cell body so that they can be partitioned into the two daughter cells (Wheeler *et al.*, 2019). All the single-copy organelles are directly or indirectly connected to the flagellum, which argues that controlling the timing and location of flagellar inheritance may function as an organizer for organelle inheritance in *T. brucei* (Sunter and Gull, 2016). Cell division begins with the nucleation of a new MtQ, which is quickly followed by the maturation of the probasal body in a process that docks it with the FP membrane and nucleates a new flagellum (Lacomble *et al.*, 2010). In the procyclic form of the parasite, the tip of the new flagellum is attached to the old by the flagella connector (FC), which extends along the length of the old flagellum as the new flagellum elongates (Moreira-Leite *et al.*, 2001). Two new probasal bodies are then produced, followed by a rotation around the axis of the old flagellum that places a new flagellum at the posterior of the cell (Lacomble *et al.*, 2010). This process leads to the duplication of the FP, which segregates the two flagella. Once the new flagellum emerges from the FP, it is attached laterally to the cell surface by a growing new FAZ, which extends slightly behind the new flagellum. The tip of the new FAZ appears to contact the old FAZ in an arrangement that mirrors the connection between the old and new flagella by the FC. The extension of the new flagellum and FAZ occurs in two distinct stages. At first, the tip of the new flagellum moves along the old flagellum as it extends until the length ratio between the new and old flagella reaches ~0.6 (Davidge *et al.*, 2006). At this point, the FC appears to stop moving, which locks the tip of the new flagellum in place. Subsequent new flagellum growth is accommodated by separation of the basal bodies and extension of the cell posterior. This process also leads to segregation of the kinetoplast. Karyokinesis occurs after kinetoplast segregation and uses a closed mitosis organized by spindle pole body–like structures within the nuclear membrane (Woodward *et al.*, 1990; Ogbadoyi *et al.*, 2000).

The SPA must be enlarged to accommodate the duplicating organelles and to provide a sufficient number of microtubules to form two arrays during cytokinesis. New tubulin dimers are constantly added to the plus ends of the SPA microtubules at the cell posterior (Sherwin *et al.*, 1987). Just after the initiation of new flagellum growth, heavily tyrosinated tubulin is detectable throughout the SPA at the microtubule ends facing the cell anterior, indicating growth from their minus ends (Sherwin and Gull, 1989b). Tyrosinated tubulin represents newly made microtubules, as once incorporated into the microtubule lattice, the tyrosine is removed. Whether this represents extension of extant microtubules or the insertion of new microtubules into the array is currently unclear. Either scenario likely represents invasion between existing microtubules, necessitating the breakage of existing intermicrotubule cross-links and subsequent formation of new cross-links to anchor the invading microtubules within the array. Tyrosination on the minus ends declines around the time of mitosis. A new set of short microtubules then appears in the middle of the cell body in postmitotic cells. Both ends of these microtubules are heavily tyrosinated, suggesting
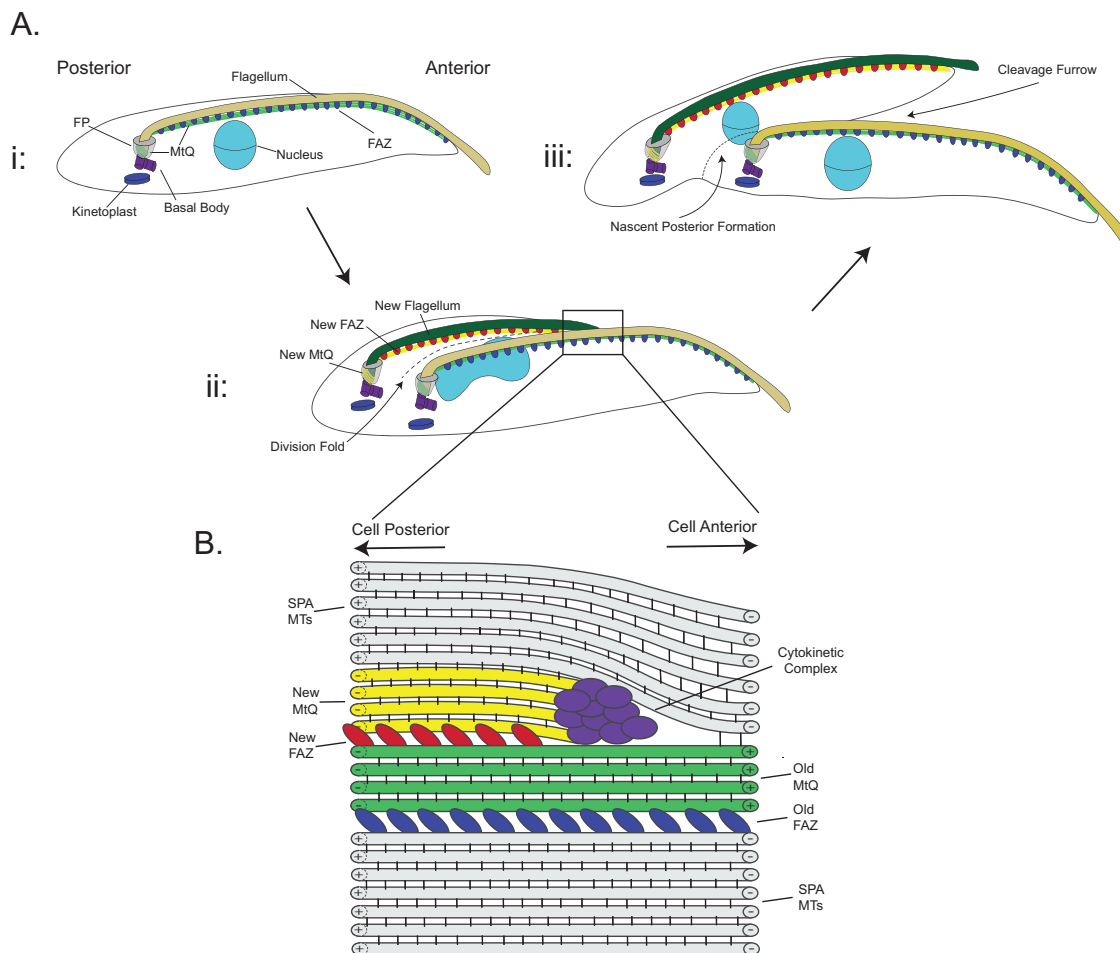
FIGURE 1: (A) (i) Schematic of a *T. brucei* cell with relevant organelles depicted. (ii) *T. brucei* cell undergoing cell division. The new flagellum and FAZ extend in contact with the existing structures. Dotted line bisecting the cell longitudinally denotes the forming division fold and location of microtubule insertion into the SPA. (iii) *T. brucei* cell during cleavage furrow ingression. Dotted line denotes the formation of the nascent posterior end of the daughter cell. (B) Schematic of region denoted by box in Aii. The new FAZ filament and MtQ are inserted between the old MtQ and existing SPA microtubules. Cytokinetic complex transits along the growing MtQ, likely breaking intermicrotubule cross-links between old MtQ and SPA microtubules. MTs: microtubules.

growth from both termini (Sherwin and Gull, 1989b). These short microtubules may nucleate from the sides of neighboring microtubules within the array. More recent studies have shown that the part of the array present between the new and old FAZ is a primary location for the addition of new microtubules to the array, which would increase the distance between the two FAZ as cell division proceeds (Wheeler *et al.*, 2013; Sheriff *et al.*, 2014). As the SPA enlarges, the array microtubules along the dorsal side of the midzone of the cell are drawn inward to generate a fold along the long axis of the cell that roughly bisects it into two equal volumes that will make up the daughter cells (Figures 1Aii and 2A). Drawing the dorsal side of the array close to the ventral side likely serves to facilitate reorganizing the microtubules during cytokinesis.

Cytokinesis is initiated once the new:old flagellum length ratio reaches ~0.8 (Wheeler *et al.*, 2013). Furrow ingression starts from the anterior tip of the cell and follows the helical path traced out by the subpellicular microtubules toward their plus ends at the cell posterior. The FC severs during the last stages of furrow ingression, which breaks the link between the two flagella and allows the tip of the new flagellum to move freely. The posterior end of the array, which has elongated as the basal bodies segregate, is remodeled to

produce a new posterior end (Figure 1Aiii). This arrangement produces daughters that are chimeras of old and new cytoskeletal structures, with one daughter inheriting the new flagellum, old cell posterior, and new cell anterior, and the other daughter inheriting the old flagellum, new cell posterior, and old cell anterior. The new cell anterior and posterior have distinct shapes from the older structures and are remodeled before the daughter cells undergo subsequent divisions (Abeywickrema *et al.*, 2019). The new flagellum is also shorter than the old even after the completion of cytokinesis, so it must be extended as well (Farr and Gull, 2009).

IDENTIFICATION OF T. BRUCEI CYTOKINETIC PROTEINS

The divergent mechanism of *T. brucei* cytokinesis is reflected by the absence or repurposing of cytokinetic proteins that are conserved in Opisthokonta. Among the conserved genes is Polo-like kinase (PLK), which appears to have two homologues in *T. brucei*, one of which has been extensively characterized (Kumar and Wang, 2006; Hammarton *et al.*, 2007; de Graffenried *et al.*, 2008). In other eukaryotes, PLKs play important roles during multiple stages of cell division including centriole duplication, spindle pole assembly, and cleavage furrow formation (Zitouni *et al.*, 2014). In *T. brucei*, TbPLK
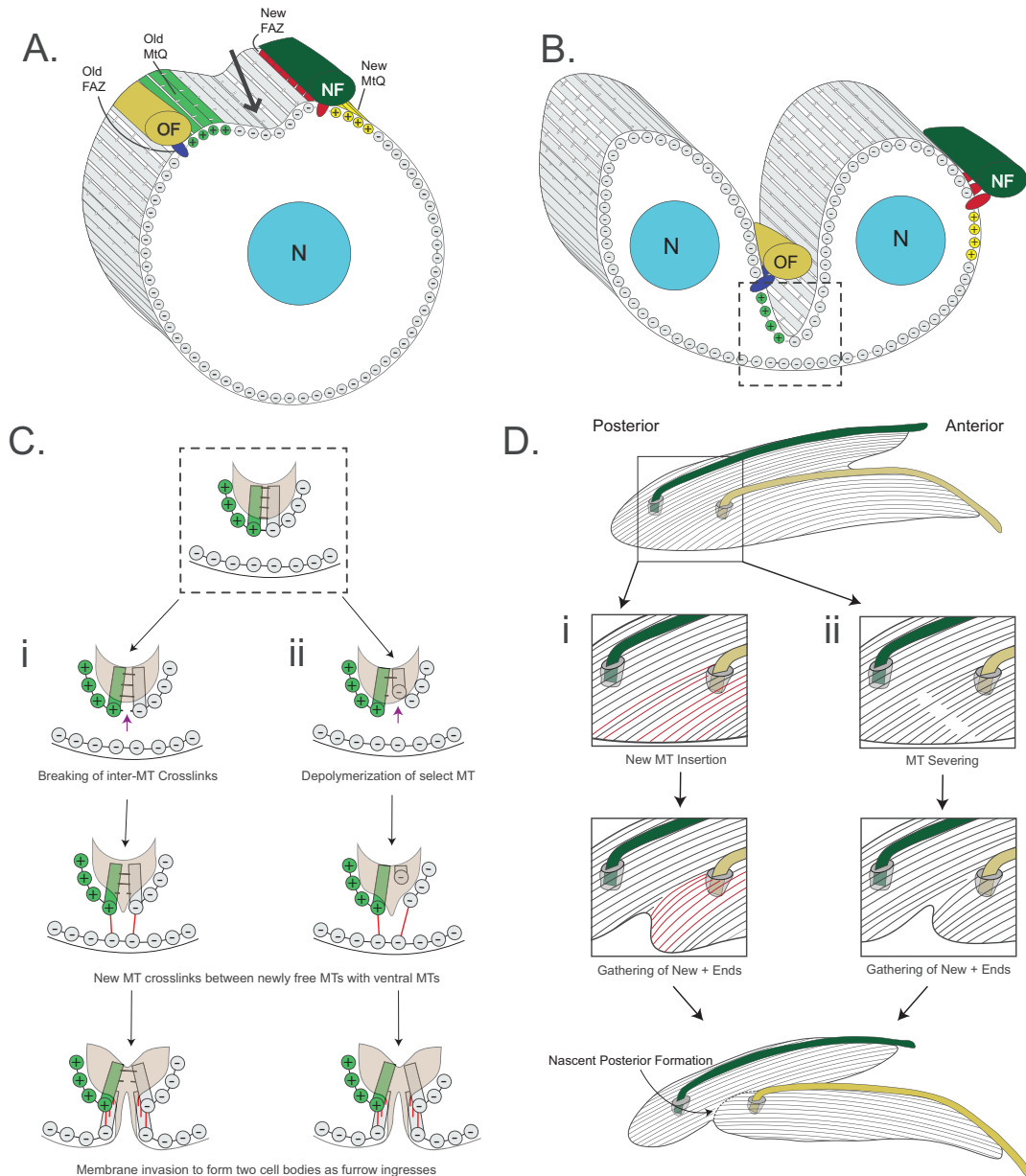
FIGURE 2: Proposed models of cleavage furrow ingression and posterior end remodeling. (A–C) View from anterior to posterior portraying major cytoskeletal structures. + and – denote microtubule polarity within the SPA. (A) Cross-section of a cell before cytokinesis initiation. The cell has nucleated a new flagellum, FAZ, and MtQ. New microtubules are inserted between the two flagella. A division fold, denoted by the arrow, has begun to form. (B) Cross-section slice of a cell just before cleavage furrow ingression. The division fold has progressed to nearly bisect the cell, with the old MtQ positioned near the bottom of the division fold. (C) Schematics of the two models proposed to fully segregate the array. Region is blowup of region denoted by box in B. Only the last microtubule of old MtQ and adjacent SPA microtubule are depicted in detail for simplicity. (i) Intermicrotubule cross-links between old MtQ and adjacent SPA microtubule are broken, progressing from anterior to posterior (purple arrow). Membrane invades this space as new intermicrotubule cross-links form between the freed microtubules and the ventral microtubules in the array. The intermicrotubule cross-link between the ventral SPA microtubules are broken, and the plasma membrane invades the space to fully segregate the two cell bodies. (ii) Intermicrotubule cross-links are broken between microtubules adjacent to the old MtQ, and these microtubules are then selectively depolymerized. As the microtubule is depolymerized as the cytokinetic complex moves toward the posterior, the dorsal plasma membrane invades the space to begin to segregate the cell bodies. New intermicrotubule cross-links are formed between the ventral SPA microtubules and newly free dorsal array microtubules. The intermicrotubule cross-links between the ventral SPA microtubules are broken, and the plasma membrane invades the space to fully segregate the two cell bodies. (D) Depiction of a cell with a partially ingressed furrow. Box denotes location of insets in D, i and ii. (i) New microtubules are nucleated and inserted between existing array microtubules at the location where the new posterior will form. These new microtubule's plus ends are gathered to form the nascent posterior end of the new cell. (ii) Select microtubules within the array are severed to produce new microtubule plus ends that are then gathered to for the nascent posterior end of the new cell. N = Nucleus, OF = Old Flagellum, NF = New Flagellum.

Protein	Localization	Putative function
TbPLK	Basal body, tip of new FAZ	Kinase: formation of new FAZ and cytokinesis
TbAUK1	Mitotic spindle, tip of the new FAZ	Kinase: initiation of cytokinesis
TbCPC1	Nucleus, tip of the new FAZ	Necessary for TbAUK1 localization
TbCPC2	Nucleus, tip of the new FAZ	Necessary for TbAUK1 localization
TOEFAZ1	Tip of the new FAZ	Scaffold to recruit cytokinetic proteins
KPP1	Tip of the new FAZ	Phosphatase:TbPLK and TOEFAZ1 recruitment to new FAZ tip
FRW1	Tip of the new FAZ, cleavage furrow	Cytokinetic initiation in bloodstream form
KLIF	Tip of the new FAZ, cleavage furrow	Kinesin: formation of the new cell posterior
PAVE1	Posterior and ventral edge of SPA	Formation of the new cell posterior

TABLE 1: Proteins discussed in this Perspective.

is essential specifically for cytokinesis and flagellar inheritance. TbPLK does not enter the nucleus at any point of the *T. brucei* cell cycle, but instead is initially present at the basal body as the probasal body matures, then the flagellar pocket region, and subsequently the tip of the extending FAZ (Table 1 contains the localization and function of the proteins described in the following sections). Consistent with this localization pattern, TbPLK depletion or inhibition has no effect on nuclear duplication but blocks the segregation of the basal bodies and assembly of a new FAZ, leading to detachment of the new flagellum and failed cytokinesis. An analogue-sensitive inhibition approach of TbPLK in synchronized cells subsequently showed that kinase activity at late stages of the cell cycle is dispensable for cytokinesis, which suggests that TbPLK plays an essential role at an early stage of the process, perhaps in assembling the new FAZ (Lozano-Núñez *et al.*, 2013). Many of the conventional binding partners of TbPLK such as Cdc25 appear to be absent in *T. brucei*, which suggests that repurposing of the kinase has primarily occurred by the acquisition of novel binding partners (Szöör, 2010).

The chromosomal passenger complex (CPC) plays important roles in chromosomal attachment to microtubules in the mitotic spindle and in regulating the actomyosin contractile ring in Opisthokonta. In *T. brucei*, the CPC lacks its conventional localization module (survivin, borealin, and INCEP) but the signaling module component Aurora B, known as TbAUK1, is present (Tu *et al.*, 2006). TbAUK1 is essential for mitotic spindle formation and chromosome segregation, which is consistent with Aurora B function in other systems. However, following chromosome alignment on the metaphase plate, TbAUK1 exits the nucleus and localizes to the tip of the new FAZ, where it plays an essential role in the initiation of cytokinesis. The proteins TbCPC1 and TbCPC2 were identified as interacting partners of TbAUK1 and lack any homology to the CPC components found in Opisthokonta. TbCPC1 and TbCPC2 initially localize to the nucleus and are found on the spindle midzone during anaphase before translocating along with TbAUK1 to the tip of the new FAZ. Depletion of either CPC1 or CPC2 inhibits TbAUK1 translocation to the FAZ, leading to cytokinetic defects (Li *et al.*, 2008).

Over the past few years, a series of proximity-dependent biotin identification (BioID), yeast two hybrid, and phosphoproteomic screens have been performed in an attempt to identify novel *T. brucei* cytokinetic regulators (Hu *et al.*, 2015; McAllaster *et al.*, 2015; Hilton *et al.*, 2018; Benz and Urbaniak, 2019). Detailed analyses of the identified proteins have recently been published elsewhere, so this discussion will be limited to a small subset (Hammarton, 2019; Wheeler *et al.*, 2019). The first proteomic screen was performed to identify TbPLK interactors and substrates. Among the identified

proteins was TOEFAZ1 (tip of the extending FAZ protein 1), also called cytokinesis initiation factor 1 (CIF1), which has been shown to be a TbPLK interactor and substrate (McAllaster *et al.*, 2015; Zhou *et al.*, 2016). TOEFAZ1 localizes to the tip of the new FAZ once it is assembled during cell division. TOEFAZ1 depletion causes defects in cytokinesis, primarily by misplacement of the cleavage furrow, which leads to cells with aberrant DNA content that cannot undergo productive cell divisions. TbPLK is also absent from the tip of the new FAZ when TOEFAZ1 is depleted, although the importance of TbPLK activity late in the cell cycle is unclear. TOEFAZ1 is necessary for recruitment of a number of proteins to the cytokinetic complex, making it likely that TOEFAZ1 functions as a scaffold (Hilton *et al.*, 2018; Kurasawa *et al.*, 2018; Zhou *et al.*, 2018; Hu *et al.*, 2019). The localization of TbPLK and TbAUK1 to this structure suggests that phosphoregulation is occurring, which is consistent with the high degree of phosphorylation found on TOEFAZ1, especially in a central intrinsically disordered domain (Urbaniak *et al.*, 2013). A phosphatase called kinetoplastid-specific protein phosphatase 1 (KPP1) localizes to the tip of the extending FAZ similarly to TOEFAZ1. Depletion of KPP1 showed that this phosphatase is essential for recruiting TOEFAZ1 and TbPLK to the extending FAZ tip, suggesting that KPP1-mediated dephosphorylation may remove phosphosites that antagonize recruitment of essential components of the cytokinetic complex (Hilton *et al.*, 2018; Zhou *et al.*, 2018).

A specific cohort of proteins localize to the tip of the growing FAZ at the latest stages of its extension and are then present on the furrow during ingression. FRW1 (Furrow 1) is essential for cytokinesis initiation in the bloodstream form of the parasite, but not the procyclic form (Zhou *et al.*, 2018; Zhang *et al.*, 2019). An orphan kinesin identified in the TOEFAZ1 BioID screen localizes to the tip of the new FAZ just before cytokinetic initiation and then tracks along the cleavage furrow. Depletion of this kinesin, called KLIF (kinesin localized to the ingressing furrow), in procyclics results in cells that can initiate cleavage furrow ingression but are unable to complete the process. Ingression stalls near the point where the new cell posterior begins to taper to form the new cell posterior, suggesting that KLIF is essential for the formation of this structure (Hilton *et al.*, 2018). The discovery of these proteins and initial description of their depletion phenotypes argue that there is a sequential recruitment of proteins to the cytokinetic complex during new FAZ extension and cleavage furrow ingression. This change in complex composition likely reflects changes in the activities necessary to ingress the furrow. There appear to be at least three distinct phases of the cytokinetic complex: the first being transit along the tip of the new FAZ toward the cell anterior, followed by triggering of furrow ingression

and movement of the complex back towards the posterior, and ending with construction of the new cell posterior.

POTENTIAL MECHANISMS FOR T. BRUCEI CYTOKINESIS
The available morphological data and recent discovery of novel cytokinetic proteins make it possible to propose potential models for how this process occurs in *T. brucei* and highlight outstanding questions in the field.

Positioning the cytokinetic complex
While the location of the cleavage furrow in many of the previously studied model eukaryotes is selected by the plane of the mitotic spindle and positioning of the actomyosin ring, in *T. brucei* this process proceeds by the delivery of a set of proteins to a specific location within the polarized cell body, followed by triggering ingression of a unidirectional furrow once all the other organelles are duplicated and positioned. The lack of nonmuscle myosin II and the limited impact of actin depletion on cell division make it unlikely that an actomyosin ring is involved in *T. brucei* cytokinesis (García-Salcedo *et al.*, 2004). The presence of a persistent, highly ordered, and heavily cross-linked microtubule array in the parasite strongly suggests that any cytokinetic mechanism must prioritize the sorting, severing, and reorganization of microtubules as a means to complete cell division. This process requires the construction of a new organizational "landmark" to place the furrow and segregate the microtubules into two arrays that contain the correct complement of organelles. The growing new MtQ is likely to serve as this landmark due to the timing of its assembly and its location. Since it is part of the FAZ, the new MtQ microtubules are present at the location where furrow ingression initiates. MtQ biogenesis is indirectly linked to the assembly of a new flagellum, which provides the overall timing mechanism for cell division (Lacomble *et al.*, 2010). The MtQ also provides a set of microtubules with unique polarity compared with the rest of the SPA that may serve as a track for transport and distinct posttranslational modifications that likely aid in recruiting and moving the cytokinetic complex during cell division (Gallo *et al.*, 1988). As the cytokinetic complex moves toward the cell anterior, it is likely to be involved in remodeling the SPA microtubules to accommodate the invading new MtQ and the new FAZ filament.

Once furrow ingression is triggered, the cytokinetic complex moves back toward the cell posterior along an axis that allows all the replicated organelles to be correctly partitioned into the daughter cells. This axis appears to fall within the set of new SPA microtubules present between the old and new MtQ, which are part of the furrow fold. Localization of cytokinetic complex components such as KLIF during furrow ingression suggests that the complex employs the old MtQ as a track (Figure 1B). This would correctly position the cytokinetic complex so that it can access the newly invaded microtubules that are present near the bottom of the furrow fold (Figure 2B). The extension of the new MtQ likely functions to convey the cytokinetic complex to the correct location for initiating furrow ingression, which is then positioned on its route back toward the posterior end by the old MtQ.

Stages of cytokinesis
The phenotype of KLIF depletion suggests that furrow ingression has at least two distinct stages. The first stage involves ingression along a track dictated by the old MtQ and sorting microtubules into two SPAs. The second stage begins once the complex nears the FP and is responsible for gathering microtubule plus ends to form the new posterior. The first process appears to be KLIF independent as initial cleavage furrow ingression occurs in KLIF-depleted cells,

while the second stage appears to be KLIF dependent. Posterior end formation is reliant on the protein posterior and ventral edge protein 1 (PAVE1), which appears to be associated with the subpellicular microtubules present at the cell posterior throughout the cell cycle. Depletion of PAVE1 results in cells with truncated posterior ends that contain disorganized array microtubules, as well as cell division defects that appear to arise from an inability to complete cytokinesis (Hilton *et al.*, 2018). It is possible that PAVE1 plays a role along with KLIF in reorganizing the posterior portion of the SPA to produce and maintain a new posterior end, which is necessary for the completion of cytokinesis.

While interaction mapping has identified additional components of the *T. brucei* cytokinetic complex, most of these proteins lack identifiable domains and functions, which has limited progress on obtaining a mechanistic understanding of cytokinesis. Analysis of morphology data allows us to propose events that should occur, which can provide a framework for identifying the activities that must be present within the complex in order for these events to take place. From our view, there are two potential mechanisms for the first stage of furrow ingression: 1) The separation of neighboring microtubules by severing and reassembly of specific microtubule cross-links (Figure 2Ci) or 2) The selective depolymerization of one or more microtubules that are then replaced by new microtubules produced within the cell bodies of the daughter cells (Figure 2Cii). In the first case, the main microtubule remodeling activity within the furrow disrupts the intermicrotubule cross-links and pushes the microtubules apart, with the plasma membrane then being drawn into the gap. The increased curvature of the cell body along the furrow fold brings the upper and lower segments of the SPA into close proximity, which facilitates the formation of new microtubule cross-links (Figure 2Ci). In this case, the organized disruption and reassembly of microtubule cross-links is the primary activity of the cytokinetic complex, rather than microtubule severing. In the second case, microtubules are disassembled as the furrow ingresses (Figure 2Cii). This would then be followed by the incorporation of new microtubules into the array that are nucleated from within the two arrays. This model would require that the cytokinetic complex contain both microtubule-severing and microtubule-assembling activity, along with the manipulating cross-links. In support of this model, the severing enzyme katanin has been found at the ingressing furrow and was shown to be essential for cleavage furrow initiation (Casanova *et al.*, 2009; Benz *et al.*, 2012). In both cases, ingression of the plasma membrane would be controlled by the reorganization of the SPA microtubule–plasma membrane cross-links, although it is possible that additional membrane is deposited at the ingression site, or that local membrane lipid composition is altered to facilitate the novel curvature present. In support of local membrane remodeling, several components of the secretory pathway were TOEFAZ1 BioID hits (Hilton *et al.*, 2018).

Formation of the new cell posterior
The FAZ can guide furrow ingression only as far as the FP because the FAZ terminates at this point of the cell body. Another mechanism must be employed at this point to guide the cytokinetic complex and to form the new cell posterior end. Once the furrow reaches the portion of the old MtQ that is adjacent to the old FP, the cytokinetic complex is tasked with gathering of a series of microtubule plus ends together and sorting them into a new posterior, which appears to proceed by the bundling of microtubule plus ends as a general mechanism. The new plus ends are likely created before the arrival of the cytokinetic complex, so the main function of the complex is to sort these new ends (Wheeler *et al.*, 2013). There

are two possibilities for how these new plus ends arise: the creation of new microtubules inserted within the duplicated array (Figure 2Di) or by severing microtubules at specific locations within the array to produce new plus ends at specific locations within the SPA (Figure 2Dii). The first mechanism requires selective construction of new microtubules at locations within the array suitable for producing a set of plus ends that are bundled, while the second mechanism requires microtubule severing at a specific point during furrow ingression, bundling of the new microtubule ends, and then filling in and repair of the microtubules present, most likely on the ventral side of the new flagellum daughter cell. Supporting this idea, RNAi depletion of spastin, a microtubule-severing enzyme, blocked abscission in bloodstream form cells, suggesting that microtubule severing is essential for the completion of cytokinesis (Benz *et al.*, 2012). The precise mechanism that gathers the individual plus ends among all the microtubules present in the SPA and organized to form the new posterior is not clear, although it requires the kinesin KLIF (Hilton *et al.*, 2018).

CONCLUSIONS AND FUTURE WORK

While there has been significant progress toward establishing the mechanisms that drive *T. brucei* cytokinesis, there are many areas that need further investigation. One of the primary issues is defining the trigger for initiating furrow ingression at the anterior end of the cell. While it is likely that phosphorylation plays an essential role in this process, the few kinases and phosphatases that localize to the tip of the new FAZ are either dispensable for the late stages of cytokinesis or exert their effects via upstream events such as blocking the assembly of the new FAZ or recruitment of TOEFAZ1. While the *T. brucei* kinome is under active study, establishing signaling networks has not progressed sufficiently to pinpoint likely candidates for triggering cytokinesis. Cataloguing of phosphosites on the novel cytokinetic proteins and mutagenic studies may provide a means of identifying key sites, but the number of known phosphosites on cytokinetic proteins such as TOEFAZ1 makes this prospect daunting (Urbaniak *et al.*, 2013; McAllaster *et al.*, 2015).

A second important question is the viability of a proposed alternate cytokinetic pathway that does not rely on TOEFAZ1 (Zhou *et al.*, 2016). It has been proposed that a TOEFAZ1-independent cytokinetic pathway exists in *T. brucei*, in which cytokinesis initiates from the posterior end of the cell instead of the anterior. This mechanism may occur at very low levels in cells lacking TOEFAZ1, but the process is so prolonged that many of the organelles, such as the flagellum and nucleus, undergo additional rounds of duplication before the completion of cytokinesis. While alternate cytokinetic pathways have been identified in other organisms, they usually occur in adherent cells with minimal polarity that can employ pulling forces in the absence of actomyosin contraction (Gerisch and Weber, 2000; Uyeda and Nagasaki, 2004; Rancati *et al.*, 2008; Choudhary *et al.*, 2013).

Finally, while we have primarily described cytokinesis in one form of procyclic *T. brucei*, there are other life cycle stages that differ in size, the degree of flagellar attachment, and the location of the FP. Several of the transitions from one life stage to another require unique cell divisions that produce very different progeny (Sharma *et al.*, 2008; Ooi *et al.*, 2016). Understanding how the core cytokinetic machinery is tuned to allow different cell divisions to occur will provide essential information about *T. brucei* biology and its cytoskeleton. The related trypanosomatids *T. cruzi* and *Leishmania* share essentially all of the identified cytokinetic components but have an even broader range of morphologies that merit investigation (Elias *et al.*, 2007; Sunter and Gull, 2017).

We have highlighted how the study of a single divergent eukaryote can provide fundamental insights into the plasticity and potential contained within eukaryotic cells. A better understanding of how other divergent organisms perform fundamental cellular processes will show how evolutionary niches, such as parasitism, can drive diversification of essential cellular pathways in manners that would be extremely difficult to predict. In cases such as cytokinesis, mechanisms that were thought to be highly conserved such as the actomyosin ring may end up being rare and divergent approaches when put into the broader context of how a more representative cohort of eukaryotes perform the same task. CRISPR editing and rapid genomic sequencing are now allowing groups to study novel organisms with interesting cell biology by simplifying the process of establishing protein localization and function. Recent work on marine protists has begun to identify tractable species among many understudied taxa, which will greatly improve our understanding of eukaryotic biology (Faktorová *et al.*, 2020). Further elucidation of the molecular mechanisms that drive unique adaptions within a broad range of organisms will be an essential addition to our understanding of the amazing variety hidden within the eukaryotic tree.

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