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Review

DNA segregation in mitochondria and beyond: insights from the trypanosomal tripartite attachment complex

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The tripartite attachment complex (TAC) of the single mitochondrion of trypanosomes allows precise segregation of its single nucleoid mitochondrial genome during cytokinesis. It couples the segregation of the duplicated mitochondrial genome to the segregation of the basal bodies of the flagella. Here, we provide a model of the molecular architecture of the TAC that explains how its eight essential subunits connect the basal body, across the mitochondrial membranes, with the mitochondrial genome. We also discuss how the TAC subunits are imported into the mitochondrion and how they assemble to form a new TAC. Finally, we present a comparative analysis of the trypanosomal TAC with open and closed mitotic spindles, which reveals conserved concepts between these diverse DNA segregation systems.

Highlights

The single unit nature of the mitochondrial genome (kinetoplast DNA; kDNA) of trypanosomes requires precise mechanisms that guarantee the segregation of the duplicated kDNA during cytokinesis.

Segregation of the duplicated kDNA during cytokinesis depends on the tripartite attachment complex (TAC), which physically links the basal body (BB) of the flagellum with the kDNA.

Much progress has been made in identifying the molecular core subunits of the TAC and how they interact with each other, allowing formulation of a model of TAC architecture that explains how the TAC connects the BB across the mitochondrial membranes with the kDNA.

Recent studies highlight how individual TAC subunits are imported into mitochondria, sorted to, and integrated into, the TAC, illustrating the central role of the mitochondrial outer membrane TAC module in the assembly process.

A comparative analysis between the TAC and the mitotic spindle reveals conserved features and concepts shared between these DNA segregation complexes.

Mitochondrial genome segregation in *Trypanosoma brucei*

Distribution of newly replicated genomes to daughter cells is critical for all organisms. In eukaryotes, segregation of the nuclear genome is mediated by the **mitotic spindle** (see Glossary), a large self-organizing structure that is based on microtubules (MTs). Eukaryotes also have mitochondria, which are of bacterial evolutionary origin and, therefore, have their own genomes. Thus, in addition to the nuclear chromosomes, eukaryotes must segregate mitochondria and their genomes to daughter cells.

In contrast to most other eukaryotes, the parasitic protozoan *T. brucei* and its relatives have only a large single mitochondrion, which forms a tubular network and contains a single **nucleoid** genome, the highly condensed disc-shaped kinetoplast DNA (kDNA) (see Figure I in Box 1) [1]. Therefore, the trypanosomal mitochondrion requires a highly precise genome segregation system, which guarantees that, during cytokinesis, each daughter cell receives one kDNA disc only. In this review, we focus on this system, termed the 'TAC', a fascinating structure exclusively found in **Kinetoplastea**. The TAC hardwires the **basal body (BB)** of the single *T. brucei* flagellum, across the two mitochondrial membranes, with the kDNA [2,3]. Thus, it couples segregation of the replicated kDNA to segregation of the BBs [4,5]. It is an exceptionally stable structure, given that the kDNA was observed to remain connected with the BB in a purified flagellar fraction despite the harsh isolation procedure to which it was subjected [6].

The TAC is not well known outside the field of molecular parasitology, although it could serve as a paradigm for DNA segregation complexes and be used to elucidate common features of such systems. Here, we discuss recent advances in the understanding of the molecular composition and architecture of the TAC. Furthermore, we propose a model of how the TAC subunits are imported into mitochondria and integrated into the TAC. Finally, we present a comparative analysis showing that, although the TAC is specific for trypanosomes and their relatives, it recapitulates some features of the well-studied mitotic spindles of other eukaryotes.

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Box 1. Organization and replication of kDNA

The single mitochondrion of *Trypanosoma brucei* contains a single nucleoid mitochondrial genome, termed kDNA, which can easily be detected under a microscope using DNA dyes, such as DAPI. The highly unusual kDNA comprises a network of two genetic elements, the maxi- and the minicircles, which are topologically interlocked forming a disc-like structure that is connected to the cytosolic BB via the TAC (Figure I), the subject of this review [2–4,12]. The 23 kb maxicircle is present in ~25 copies and homologous to the mitochondrial genome of other eukaryotes. It encodes two mitochondrial rRNAs, 12 subunits of the oxidative phosphorylation complexes, two ribosomal proteins, and four proteins of unknown function [18,73]. Twelve of these proteins are encoded by cryptogenes, meaning that their primary transcripts must be converted into translatable mRNA by an RNA-editing process consisting of multiple uridine insertions and/or deletions [13–15]. In addition, the kDNA network contains ~5000 minicircles, which are 1 kb in length and heterogeneous in sequence. Minicircles comprise more than 90% of the kDNA mass and encode the guide RNAs that mediate RNA editing [74]. Intriguingly, unlike most other mitochondrial genomes, the kDNA does not encode any tRNAs, indicating that all mitochondrial tRNAs are imported from the cytosol [75].

Determining how such an intricate network of two intercatenated genetic elements replicates is a formidable problem. Thus far, more than 25 different proteins have been identified that contribute to this process [3,12,25]. kDNA replication in *T. brucei* is initiated shortly before nuclear S-phase by the topoisomerase-mediated release of minicircles into the **kinetoflagellar zone (KFZ)** [76,77]. Replication of the free minicircles then progresses unidirectional through θ-intermediates. Next, each daughter minicircle moves to two diametrically opposed regions at the kDNA disc called **antipodal sites**, where primer removal and gap repair takes place. The minicircles are then reattached to the kDNA disc, which gradually increases in size [77–79]. More recently, a modified model of minicircle replication has been proposed [12]. Maxicircle replication occurs likely unidirectionally, as well as through θ-intermediates within the kDNA network [80]. After replication is completed, the kDNA adopts a bilobed shape, with the maxicircles bridging the two daughter discs. Further into the segregation process, a thin filament forms, termed the nabelschnur, which connects the two kDNA discs [27]. Finally, the maxicircles are released from the region by a postulated topoisomerase activity and the nabelschnur dissolves, allowing complete segregation of the two kDNA discs.

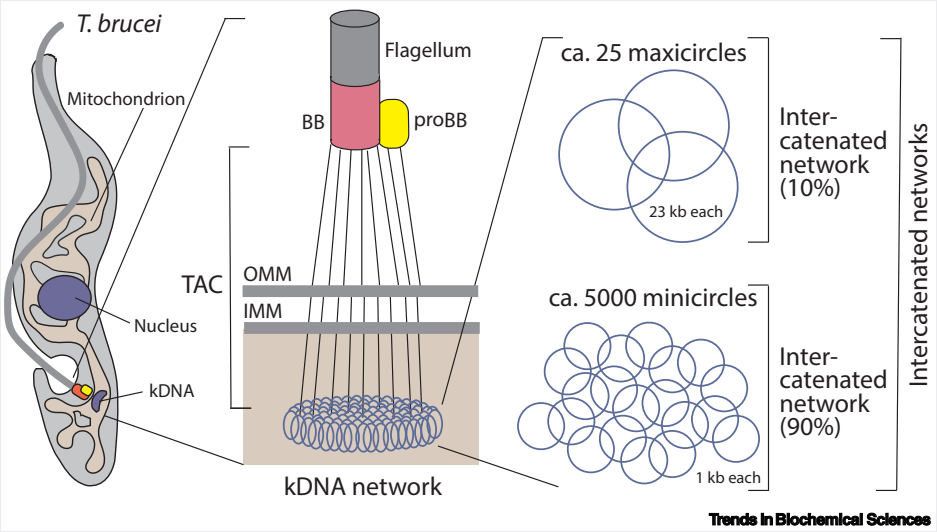

Figure I. Overview of the tripartite attachment complex (TAC) and the organization of the kinetoplast DNA (kDNA). Left: *Trypanosoma brucei* cell with the single network-like mitochondrion. Middle: enlarged region containing the TAC linking the basal body (BB) and the proBB to the disc-shaped kDNA network. Right: the kDNA disc comprises intercatenated maxicircles and intercatenated minicircles. Both networks are intercatenated with each other. Abbreviations: IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane.

T. brucei: a model for mitochondrial biology

Life is characterized by its vast diversity, yet most experimental research in eukaryotic biochemistry and cell biology has focused on a limited number of model systems, such as yeast and mammals, which are part of the **eukaryotic supergroup** of the opisthokonts [7]. However, such a myopic view of eukaryotic diversity hardly scratches the surface of the true

breadth of molecular complexity in this domain [8]. *T. brucei* is a member of the eukaryotic supergroup Discoba [7]. In addition to its clinical importance as the causative agent of **human sleeping sickness** and of **nagana** in cattle [9], it offers a unique opportunity to expand our knowledge of eukaryotic biology beyond the confines of the opisthokonts. *T. brucei* can be readily studied through molecular genetics, biochemical, and cell biological approaches, making it an ideal experimental model system. Over the past decades, its mitochondrion has emerged arguably as the most extensively studied of such organelles outside of the opisthokonts [10,11].

The trypanosomal mitochondrion has many unique features, including the structure of its genome [3,12] (see Figure I in Box 1), the RNA-editing process [13–15], atypical mitochondrial ribosomes [16–18], and a noncanonical protein import system [19]. However, upon closer examination, it becomes clear that these features are better characterized as extreme rather than unique. For instance, mitochondrial RNAs in many species undergo some degree of editing [20], although not to the same level as in *T. brucei*. Similarly, mitochondrial ribosomes in most eukaryotes have short rRNAs [21–23], but none are as short as those found in trypanosomes. In addition, while active sorting mechanisms likely contribute to mitochondrial genome segregation in mammalian mitochondria [24], they do not rely on a permanent tether to BBs, as in the trypanosomal TAC, the subject of this review.

Nuclear and mitochondrial cell cycles

As first recognized in the pioneering study of Robinson *et al.*, rather than separately ensuring the inheritance of the single-copy BB and kDNA, trypanosomes physically connect the two structures via the TAC, guaranteeing that successful segregation of the BBs leads to simultaneous segregation of the replicated kDNA [6]. Coupling of BB segregation to kDNA segregation via the TAC can only work if kDNA replication and cytokinesis are tightly regulated. This is indeed what is observed: there is a periodic mitochondrial S phase, and replication and segregation of the kDNA are precisely coordinated with the nuclear cell cycle [25] (see Figure I in Box 2).

Composition and organization of the TAC

Morphological composition of the TAC

Before its biochemical characterization, the TAC had been analyzed by transmission electron microscopy (TEM). These studies revealed three morphologically distinct TAC regions [26] (Figure 1). The first comprises the exclusion zone filaments (EZFs), which connect the proximal end of the BB to the surface of the outer mitochondrial membrane (OMM), bridging a distance of ~270 nm [26]. The EZFs are tightly associated with the differentiated membranes (DMs), which includes the OMM domain as well as the closely opposed region of the inner mitochondrial membrane (IMM). The OMM and IMM remain intact in the DM region in detergent-extracted cytoskeletal fractions, as evidenced by TEM, suggesting a lipid composition different to other membrane regions [26]. Finally, in the mitochondrial matrix, the unilateral filaments (ULFs) bridge a distance of ~100 nm, connecting the kDNA disc to the IMM. The ULFs comprise two subregions, outer and inner ULFs, of roughly equal size. The inner ULFs are enriched for basic proteins, some of which may interact with the kDNA, whereas no such enrichment is seen in the outer ULFs [27].

The core subunits of the TAC

To be considered as a TAC core subunit, a protein must meet the following three criteria: (i) its removal should result in a growth arrest accompanied by kDNA loss and over-replication of the kDNA disc in the few cells that have retained the kDNA in both procyclic and bloodstream form

Glossary

γ-tubulin complex: nucleates MTs from various MT-organizing centers or from MT surfaces.
Acentriolar centrosome: MT-organizing centers that lack centrioles (e.g., nuclear membrane-embedded spindle pole body in closed mitosis in yeast).
Antipodal sites: two minicircle replication centers at the opposing ends of the kDNA disc in *Trypanosoma brucei*.
Atypical translocase of the outer membrane (ATOM): main OMM protein translocase in *T. brucei*.
Basal body (BB): modified centriole; forms the base of the flagellum.
Centriolar centrosome: MT-organizing center in open mitosis, comprising two centrioles and pericentriolar material (e.g., most animal cells).
Centriole: MT-based structure in the centrosome, related to the BB.
Closed mitosis: mitosis without disassembly of the nuclear membranes (e.g., yeast and *T. brucei*).
Eukaryotic supergroup: highest taxonomic level of eukaryotes.
Human sleeping sickness: also called human African trypanosomiasis; a parasitic disease caused by protozoans of the genus *Trypanosoma*. It is transmitted to humans via bites by infected tsetse flies.
Kinetochore: large protein assemblies that connect nuclear chromosomes to microtubules during mitosis.
Kinetoflagellar zone (KFZ): region between the kDNA and the mitochondrial IMM; it contains the unilateral filaments.
Kinetoplastea: class of flagellated protists characterized by kinetoplast DNA that belongs to the phylum Euglenozoa.
L262P bloodstream form trypanosome: engineered bloodstream form cell line, which can grow in the complete absence of kDNA due to a single L262 to P compensatory mutation in the nuclear-encoded F_1 γ-ATP synthase subunit.
Mitotic spindle: structure comprising centrosomes and microtubules that forms during cell division and separates duplicated chromosomes.
Nagana: parasitic disease occurring mainly in cattle and horses. It is transmitted by several species of the protozoan *Trypanosoma vivax* bites by infected tsetse flies.
Nucleoid: regions in a prokaryote or in an organelle that contain the genetic material and associated proteins. In contrast to mammalian mitochondria,

Box 2. *Trypanosoma brucei* has coordinated nuclear and mitochondrial cell cycles

Unlike most other eukaryotes, which have many mitochondria with multiple genomes each, *T. brucei* and its relatives have a single mitochondrion with a single nucleoid genome, the kDNA. Consequently, replication and segregation of kDNA must be precisely coordinated with the nuclear cell cycle (Figure I) [1,4]. The use of DNA staining dyes, such as DAPI, allows the microscopic detection of both the nucleus and the kDNA. Therefore, this has been used as an easy way to monitor the trypanosomal cell cycle. Another important cell cycle marker is whether the cell has one or two flagella and how long the new flagellum is [25]. All BBs and proBBs remain attached to a TAC throughout the cell cycle, indicating that the formation of a new TAC must begin immediately after the formation of the new proBB [49].

The G1 *T. brucei* cell has a single nucleus and a single kDNA (1K1N configuration) as well as a single flagellum (Figure IA). In the next cell cycle stage, the proBB matures into a new BB and the new flagellum begins to grow. Simultaneously, the mitochondrial and nuclear S-phases are initiated (Figure IB). In the next step, the new flagellum continues to grow, protrudes from the flagellar pocket, and its BB changes sides by rotating around the BB of the old flagellum (Figure IC) [33,34] (this step may be specific for the procyclic stage of *T. brucei* and may not occur in all Kinetoplastea). In addition, the kDNA network continues to expand and simultaneously the two antipodal sites can be detected. In the next stage, the nuclear spindle is formed (*T. brucei* has a closed mitosis [81]). The new flagellum elongates further and simultaneously mediates the initial segregation of the BBs (Figure ID). As a result, the nearly completely replicated kDNA network adopts a dumbbell shape. Finally, a thin maxicircle-containing thread, the nabelschnur, is formed as the final link between the two kDNA networks [27]. After the nabelschnur is resolved, the cell has a 2K1N configuration. In the next step, the BBs with the attached kDNA networks are further segregated (Figure IE) and cytokinesis is initiated at the anterior part of the cell, which now has a 2K2N configuration. Cytokinesis then progresses to the anterior part until the final division plane intersects between the two kDNA networks [82,83]. At the same time, the single mitochondrion is divided into two in a process requiring TbDLP, the only dynamin-like protein of *T. brucei* [84].

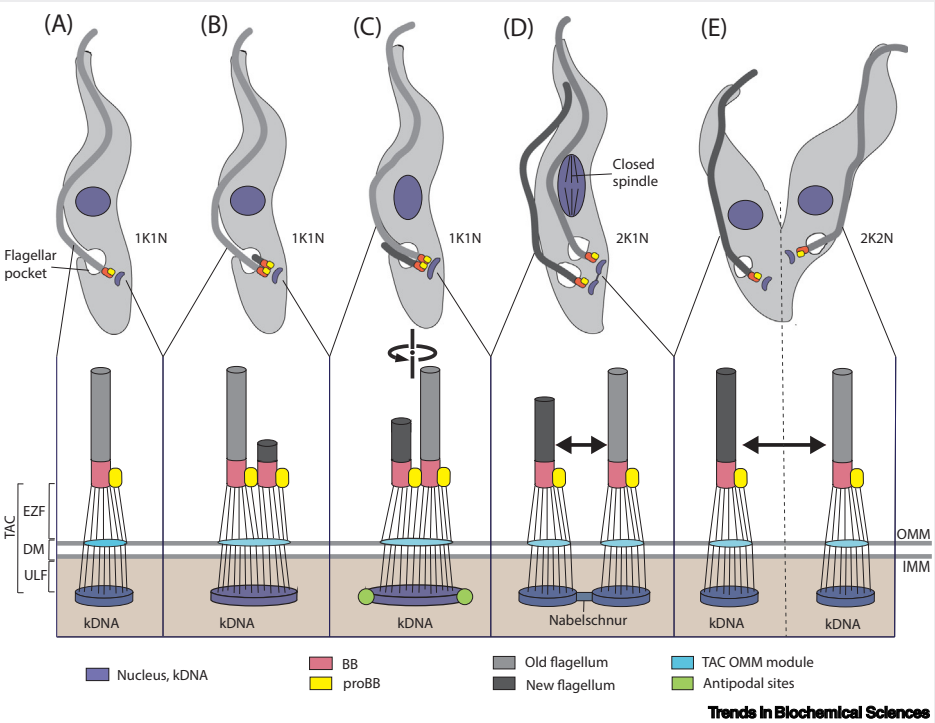

Figure I. Cell cycle stages in procyclic *Trypanosoma brucei*. Top panel: Morphological changes in an insect-stage procyclic *T. brucei* cell during the cell cycle. (A–E) depict different cell cycle stages [25]. The configuration regarding the number of kinetoplast DNA (kDNA) networks (K) and nuclei (N) is indicated for each stage. Bottom panel: schematic of an enlargement of the kDNA region of the cell depicted on top. The morphologically defined tripartite attachment complex (TAC) regions are indicated on the left. The TAC filaments are indicated as lines. Broken line in (E) depicts the plane of cytokinesis. Abbreviations: BB, basal body; DM, differentiated membranes; EZF, exclusion zone filaments; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; ULF, unilateral filaments.

which each have multiple nucleoids, the trypanosomal mitochondrion has only one, the kDNA.

Open mitosis: mitosis with disassembly of the nuclear membranes (e.g., in animals).

Semi-open or semi-closed mitosis: mitosis with partial disassembly of the nuclear membranes.

Sorting and assembly machinery (SAM): OMM protein insertase specific for β-barrel proteins; inserts its substrates from the intermembrane space side into the OMM.

Spindle pole body (SPB): membrane-embedded MT-organizing center in closed mitosis that lacks centrioles.

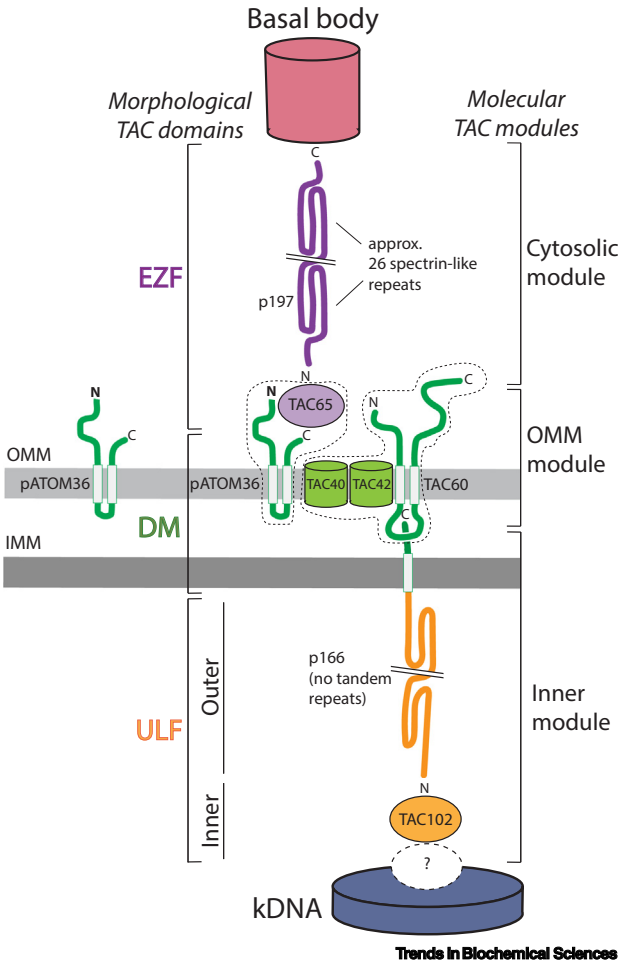

Figure 1. Proposed molecular arrangement of the eight core tripartite attachment complex (TAC) subunits within the TAC. The three morphological TAC domains, exclusion zone filaments (EZFs), differentiated membranes (DMs), and unilateral filaments (ULFs) are indicated on the left in colors that match those of the corresponding TAC subunits. Note, p166 has two colors because it belongs to both the DM and the ULF domains. The right side shows the newly proposed molecular TAC modules: the cytosolic, outer mitochondrial membrane (OMM), and inner modules, which do not precisely coincide with the morphological domains. The known interactions between the eight core TAC subunits are sufficient to form a continuous TAC filament reaching from the basal body to a region close to the kinetoplast DNA (kDNA). The recently proposed protein complex mediating anchoring of the TAC to the kDNA [47] is indicated by a question mark. The dual localization of pATOM36 reflects its dual function as an OMM protein biogenesis factor (left) and a core TAC subunit (right). The exact number of transmembrane domains (TMDs) of pATOM36 is unknown; depending on the prediction tool, it varies between two and five. The OMM module of the TAC comprises the two subcomplexes pATOM36/TAC65 and TAC40/TAC42/TAC60 (indicated by broken lines); however, how exactly they interact is not known. p197 and p166 both have a predicted central coiled-coil region, but only p197 has tandem repeats.

trypanosomes [5]; (ii) it should be dispensable for growth of the engineered trypanosome cell line that can survive without kDNA (**L262P bloodstream form**) [28]; and (iii) it should localize to the TAC region in both intact cells and isolated flagella [5]. There are some caveats to using these criteria, given that TAC subunits may have an additional function in BB maintenance or kDNA replication and, therefore, behave differently.

In recent years, eight TAC subunits have been identified and characterized, seven of which meet the criteria for core TAC subunits mentioned earlier [28]. One core TAC subunit has a dual function unrelated to the kDNA inheritance and, therefore, is also essential in the L262P bloodstream form [29]. For most of these proteins, their precise localization, how they align within the TAC structure, and with which other TAC subunits they interact with are known (Table 1). This allows, for the first time, to provide a detailed model of the molecular architecture of the TAC, explaining how it connects the cytosolic BB, across the two mitochondrial membranes, with the mitochondrial matrix-localized kDNA (Figure 1). The fact that these eight core subunits are sufficient to form a continuous tether from the BB to the kDNA disc region makes it possible that they represent the full complement of essential TAC subunits. Here, we discuss the arrangement of these subunits within the TAC, starting from the BB.

Table 1. Features of core TAC subunits[a]

Name (mass, kDa)	Protein features	Molecular TAC module	Topology	Interactions with TAC subunits (experimental evidence)	Refs
p197 (~660)	Zn^{2+}-finger motif (N terminus) Central α-helical domain of ~26 coiled coil repeats (175 aa)	CYT module	N terminus: OMM C terminus: BB	TAC65 (CoIP) Unknown BB protein (C terminal deletion)	[30–32]
TAC65 (65)	Peripheral MOM protein	OMM module		p197 (CoIP) pATOM36 (recip. SILAC CoIP, BN PAGE)	[29,31]
pATOM36 (36)	2–5 TMDs GxxG and glycine zipper motifs	OMM module (also OMM outside TAC)	C terminus: CYT	TAC65 (recip. SILAC IP, BN PAGE) TAC60 and p197 (SILAC CoIP)	[29,37]
TAC60 (60)	2 TMDs	OMM module	N terminus: CYT C terminus: CYT	TAC40, TAC42 (recip. SILAC CoIP) p166 (CoIP in *Trypanosoma brucei* and yeast)	[36,42]
TAC40 (40)	Integral membrane β-barrel protein VDAC-like	OMM module		TAC60 (recip. SILAC CoIP)	[36,41]
TAC42 (42)	Integral membrane β-barrel protein	OMM module		TAC60 (recip. SILAC CoIP) TAC40 (SILAC CoIP)	[36]
p166 (166)	1 TMD (C terminus) Leu-zipper motif (N terminus) Large matrix-exposed coiled coil region	Inner module	N terminus: matrix TMD: IM C terminus: IMS	C terminus: TAC60 (SILAC CoIP) N terminus: TAC102 ($Y2H^e$ and CoIP)	[42,43,45]
TAC102 (102)	Matrix protein	Inner module		N terminus of p166 (Y2H and CoIP)	[45,46]

[a]Abbreviations: BN-PAGE, blue native polyacrylamide gel electrophoresis; CoIP, co-immunoprecipitation; CYT, cytosol; recip., reciprocal; SILAC, stable isotope labeling by amino acids in cell culture; TMD, transmembrane domain; Y2H, yeast two-hybrid system.

p197

p197 is the TAC subunit most proximal to the BB (Figure 1). Unlike its name suggests, its molecular weight is ~660 kDa, making it arguably the largest protein in *T. brucei* [30,31]. The numerous p197 molecules extending from the BB are oriented in parallel, with their C termini connecting to an as yet unknown BB protein at the base of the BB, while their N termini are anchored to the globular peripheral OMM protein TAC65 [31]. Therefore, p197 forms the EZFs, the length of which is highly variable, ranging from 230 to 625 nm [32]. This is due to its central α-helical portion comprising >90% of p197; it consists of ~26 spectrin-like repeats of 175 amino acids (aa) in length each, which can likely fold reversibly into a coiled-coil domain, explaining the large length variation of the EZFs [32]. This flexibility is in line with the function of p197 as a spacer that determines distance between the BB and the OMM.

p197 orthologs in other Kinetoplastea also have a central α-helical domain but lack repeats and generally are much shorter [31]. Moreover, a chimeric p197 in which the central repeat portion was replaced by the much shorter region of *Trypanosoma cruzi* p197 was not functional when expressed in *T. brucei*, even though it could still connect to both the BB and the OMM. The requirement of *T. brucei* for a longer, more flexible p197 than that found in *T. cruzi* could be explained by the fact that its cell cycle requires rotation of the new BB around the old BB before kDNA segregation [33,34] (see Figure IC in Box 2), a process that has not been observed in *T. cruzi* [35].

TAC65

TAC65 is a globular protein that is peripherally associated with the OMM [36]. It links the EZF p197 to the integral membrane protein, peripheral atypical translocase of the outer membrane 36 (pATOM36) [31] (Figure 1).

pATOM36

The integral OMM protein pATOM36 does not adhere to the strict definition of core TAC subunits as it is also essential in the engineered L262P bloodstream form trypanosome cell line [28]. This is because, in line with its dual localization to the TAC and the entire OMM, it has a dual function. First, it is an essential TAC subunit [29] (Figure 1). Second, it has a role in the biogenesis of a subset of OMM proteins [29,37]. For some of these proteins, pATOM36 is required for membrane insertion [38], while for the majority it mediates their integration into protein complexes [29]. Reciprocal complementation experiments in yeast and trypanosomes have demonstrated that with regard to its second function pATOM36 is a functional analog, but not a homolog, of the yeast mitochondrial import complex (MIM) [39] and, therefore, likely also of the recently characterized MTCH2 in mammals [40]. The C terminus of pATOM36 faces the cytosol and, depending on the prediction tool used, the protein has between two and five transmembrane domains (TMDs). Deletion of the C terminal 60 aa of pATOM36 abolishes its OMM protein biogenesis function but not its function as a TAC subunit [29]. Thus, in *T. brucei*, pATOM36 integrates biogenesis of OMM proteins with mitochondrial genome inheritance.

TAC40 and TAC42

TAC40 and TAC42 are β-barrel proteins of the OMM that form a subcomplex with TAC60 (see later) [36] (Figure 1). TAC40 belongs to a novel Kinetoplastea-specific subclass of the voltage-dependent anion-selective channel (VDAC)-like protein family [41], whereas TAC42 does not show homology to any other known β-barrel protein [36].

TAC60

The integral OMM protein TAC60 has two TMDs and both its N and C termini face the cytosol [36]. The 94 aa-long intermembrane space (IMS)-exposed loop serves as a binding site for the C terminus of p166 [42] (see later) (Figure 1).

p166

p166 is the only known TAC subunit integral to the IMM. It contains a single TMD adjacent to its 34 aa-long C terminus, which binds to TAC60 [42,43]. Thus, while most OMM–IMM contact sites are transient [44], TAC60 and p166 form one of the few permanent OMM–IMM contact sites known for any mitochondria [42]. The large matrix-exposed central region of p166 is predicted to comprise coiled-coil domains and localizes to the outer ULF region of the TAC (Figure 1). Analysis using the yeast-two hybrid system finally showed that the N terminal part of p166 interacts with TAC102 (see later) [45,46].

TAC102

The globular matrix protein TAC102 localizes to the inner ULF region very close to the kDNA [46]. In a recent publication, it was shown that TAC102 may interact with TbmtHMG44 and TbKAP68 [47]. In contrast to the core TAC subunits, RNAi-mediated ablation of the two proteins leads to a loss, but not to missegregation, of kDNA. TbmtHMG44 and TbKAP68 interact with each other, localize closer to the kDNA than TAC102, and their localization depends on both the TAC and the kDNA. TbKAP68 binds nonspecifically to DNA *in vitro* [47]. These results suggest that TbmtHMG44 and TbKAP68 are involved in the anchoring of the TAC to the kDNA.

A molecular view of TAC architecture

In conclusion, the TAC can be classified into three distinct molecular modules (Figure 1): (i) a cytosolic module consisting essentially only of p197 [31]; (ii) an OMM module containing the peripheral OMM protein TAC65 and the four integral OMM proteins pATOM36, TAC40, TAC42, and TAC60 [29,36]; and (iii) an inner module comprising p166 and TAC102 located in the IMM and the matrix, respectively [42,45,46]. The cytosolic module corresponds to the morphologically defined EZFs [26]. However, the OMM and the inner molecular modules do not

exactly coincide with the DM and ULF region defined by TEM, because the filaments in this region are composed of parallel oriented p166 molecules, the C termini of which extend across the IMM and directly bind to the OMM, while their N termini interact with TAC102 [42,45]. Thus, the unexpectedly complex composition of the OMM module may be explained by the fact that it serves as a platform with a defined but different number of anchoring points for two different types of filaments on either side of the OMM.

Although we have made progress in understanding the longitudinal architecture of the TAC from the basal body BB to the kDNA, we do not know whether the many filaments laterally interact with each other. Thus, we have yet to determine the stoichiometry of the eight core TAC subunits and to find out how the five subunits of the TAC OMM module interact with each other to potentially form a well-defined platform in the OMM.

TAC assembly

All TAC core subunits, except for the cytosolic p197 and TAC65, are targeted to or into the mitochondrion and, subsequently, in a separate step, integrated into the TAC. Mitochondrial targeting of TAC subunits is well understood (Figure 2, green boxes). It follows the expected routes using the **atypical translocase of the outer membrane (ATOM)** (for TAC60, TAC40, TAC42, p166, and TAC102) [36,42,46], the single trypanosomal protein translocase of the inner membrane (TIM) (for p166 and TAC102) [42,46], and the **sorting and assembly machinery (SAM)**

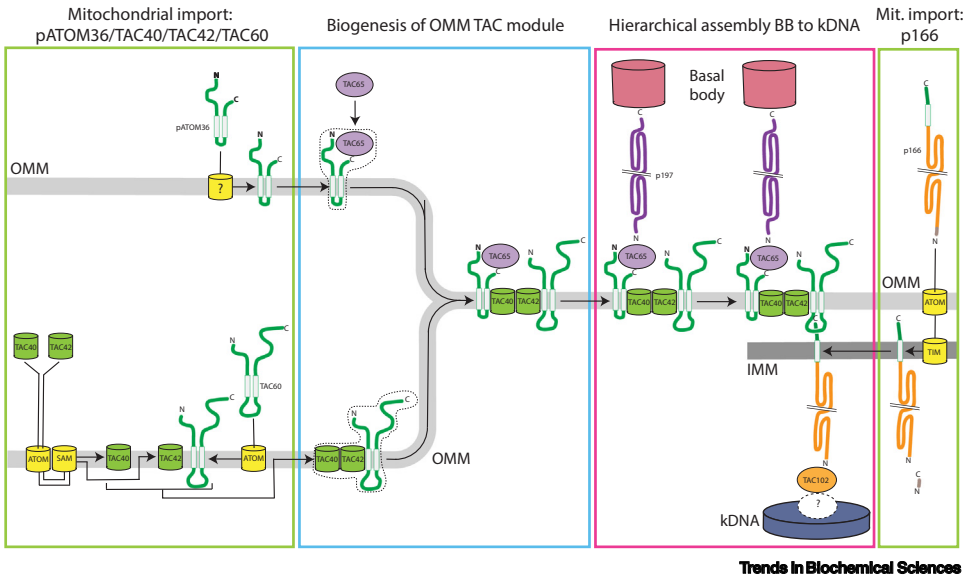

Figure 2. Model of tripartite attachment complex (TAC) assembly. Left green box: import of the outer mitochondrial membrane (OMM) proteins pATOM36, TAC40, TAC42, and TAC60. The mitochondrial protein import complexes atypical translocase of the outer membrane (ATOM) and sorting and assembly machinery (SAM) are indicated in yellow. Import of pATOM36 into the OMM is likely mediated by pre-existing pATOM36. Right green box: import of the mitochondrial inner membrane (IMM) protein p166. The mitochondrial protein import complexes ATOM and the translocase of the inner membrane (TIM) are indicated in yellow. p166 contains an N terminal presequence that is processed in the matrix. Azure box: biogenesis of the TAC OMM module. Left side: formation of the two preassembly complexes pATOM36/TAC65 and TAC40/TAC42/TAC60. Right side: the two preassembly complexes fuse. Magenta box: following the hierarchical assembly model, the basal body (BB) binds to p197, which subsequently interacts with TAC65 of the TAC OMM module. The imported IMM protein p166 diffuses laterally in the IMM until it binds to the intermembrane space-exposed loop of TAC60. Finally, TAC102 is added to the growing TAC and the complex binds to the kinetoplast DNA (kDNA). The color of the TAC subunits is the same as in Figure 1 in the main text. Abbreviation; Mit. Mitochondrion.

complexes (for TAC40 and TAC42) [36,41]; the exception is pATOM36, which is likely inserted into the OMM by pre-existing pATOM36 [29].

To explain how these subunits are integrated into the TAC, the hierarchical assembly model was proposed [48]. It posits that assembly of the TAC starts *de novo* at the base of the newly formed proBB and then extends across the two mitochondrial membranes to the kDNA (Figure 2, magenta box), and that TAC assembly is independent of the kDNA [48]. The best evidence for this model is that depletion of BB-proximal subunits results in the delocalization of all distal TAC subunits [48]. However, biogenesis of the OMM TAC module deviates somewhat from the strict hierarchical assembly model, reflecting its central function in connecting the cytosolic and the inner TAC modules. It involves the formation of two pre-assembly complexes in the OMM comprising: (i) pATOM36 and cytosolic TAC65; and (ii) TAC60, TAC42, and TAC40, respectively [36], which subsequently merge (Figure 2, cyan box). The large complex then diffuses laterally within the OMM until the cytosolically exposed TAC65 encounters and binds to the N terminus of p197, which is C-terminally linked to the base of the proBB [31,32,49]. Next, in agreement with the hierarchical assembly model, the IMS-localized p166 diffuses within the IMM until its C terminus binds to the IMS-exposed loop of TAC60 [42]. Finally, the matrix-localized N terminus of p166 binds to TAC102, which subsequently likely indirectly binds to the kDNA [45] (Figure 2, magenta box).

TAC and the mitotic spindle

Filamentous structures of different scale

The TAC and the mitotic spindle segregate kDNA and the nuclear chromosomes, respectively (Figure 3). Besides their analogous function, are there any other similarities between the two systems? At first glance, this appears unlikely, since their sizes differs by a factor of 10–200. The TAC

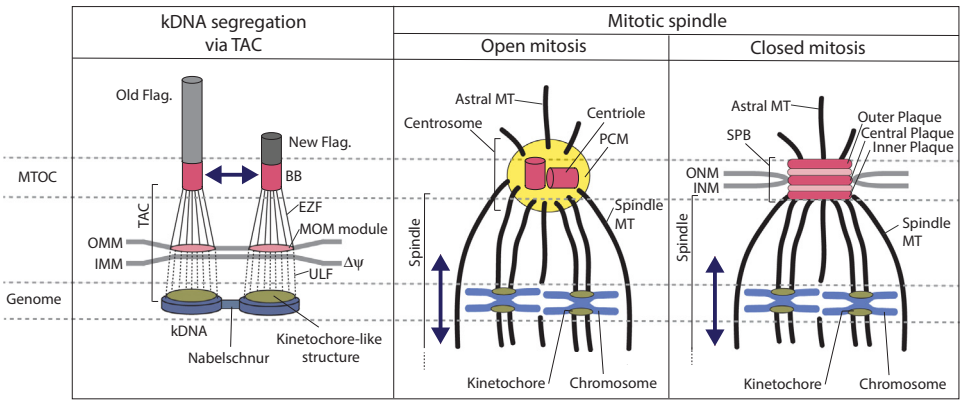

Figure 3. Comparison between the tripartite attachment complex (TAC) and the mitotic spindle. The TAC and the spindles in open and closed mitosis contain microtubule (MT)-organizing centers (MTOCs), namely the basal body (BB), the centriole-containing centrosome, and the spindle pole body (SPB), respectively. The MTOCs and the segregated genomes [kinetoplast DNA (kDNA) or nuclear chromosomes] of each system are highlighted at the top and bottom by the broken lines, respectively. The TAC OMM module and the SPB of closed mitosis are embedded in the outer and inner mitochondrial membranes (OMM and IMM) and the outer and inner nuclear membranes (ONM, INM), respectively. The IMM exhibits a membrane potential (ΔΨ). The BB and the centriole are highly conserved homologous structures. The SPB and the pericentriolar material (PCM) of the centrosome in open mitosis share conserved proteins. The TAC contains two different types of filament, exclusion zone filaments (EZFs; solid lines) and unilateral filaments (ULFs; broken lines), whereas all filaments in open and closed mitosis consist of MTs. The direction of genome segregation is indicated by the arrows. Some spindle MTs attach to the kinetochore of nuclear chromosomes; in the TAC, the ULFs are postulated to attach to a kinetochore-like structure at the kDNA. The images are not to scale, given that the TAC is much smaller than the mitotic spindles. Abbreviation: flag., flagellum.

spans a region of ~0.3–0.5 μm, whereas the length of mitotic spindles can be between 2 μm in *Saccharomyces cerevisiae* and 60 μm in the one-cell embryo of *Xenopus* [50,51]. Furthermore, while the TAC is a permanent structure, the spindle only forms during mitosis and its major components, the MTs, have a half-life of only 1–2 min [50].

However, the TAC and the spindle both rely on filaments. In the spindle, these filaments are the MTs: dynamic, polar polymers with a fast (plus) and slow (minus) growing end that are composed of α- and β-tubulin. Many of the 40–1000 spindle MTs, depending on the system, extend from one of the two **centrosomes** to the **kinetochore** of the chromosomes [50,51]. Moreover, MTs can also nucleate at the kinetochores and within the spindle itself [50]. MT minus ends are enriched around the centrosome and the plus ends in spindle centers, explaining the functionally important bipolarity of the spindle. By contrast, the two types of numerous TAC filaments are much shorter and thinner compared with MTs. They consist of single molecules each of either p197 for EZFs or p166 (possibly together with TAC102) for ULFs [31,42,45], which are all oriented in parallel (Figure 1). However, at later stages of kDNA replication, two TACs emerge from the two BBs, illustrating that bipolarity is a feature shared between the TAC and the mitotic spindle.

Homologous organizer for TAC and spindles

There is a large range of mitotic architectures between different species and even between different cell types of the same organism. The main types of mitosis are termed '**open mitosis**', '**semi-open or semi-closed mitosis**', and '**closed mitosis**', depending to which extent the nuclear membranes remain intact during cell division [52].

The formation of most spindle MTs in open mitosis is controlled by **centriole-containing centrosomes** (e.g., most animal cells), which are surrounded by pericentriolar material. The centrosome in closed mitosis (e.g., in *S. cerevisiae* and *Schizosaccharomyces pombe*) lacks centrioles and comprises the membrane-embedded **spindle pole body (SPB)** [53]. Other types of **acentriolar centrosome** exist in many organisms (e.g., *Dictyostelium*) [52,54]. However, at the molecular level, **centriolar** and most acentriolar centrosomes share components, such as γ-tubulin, **γ-tubulin complex** components [55,56], calmodulin, and Spc110 (vertebrate ortholog of pericentrin) [52], indicating their common evolutionary ancestry.

The TAC is assembled using the BB as a platform [48]. Since the BB and the centriole are two highly conserved structures that can even be converted into each other in some organisms [57,58], we conclude that the TAC and the mitotic spindle are organized by evolutionarily closely related structures.

Driving forces and regulation

In mitosis, the engine that pulls the chromosomes apart in the spindle itself. The forces acting on the chromosomes involve different mechanisms, of which MT depolymerization and polymerization regulated by microtubule-associated proteins (MAP) appear to be major ones. Moreover, MAPs can crosslink antiparallel MTs in the spindle midzone, allowing motor proteins to induce MT sliding that pushes the centrosomes apart [50,59–61].

For the TAC, the force that pulls the replicated kDNAs apart is the one that segregates the BBs. It acts perpendicular relative to the TAC filaments, which are not actively involved in the process (Figure 3). The exact mechanism that segregates the BBs is not known. However, it is clear that the process is MT mediated [6] and that growth of the new flagellum contributes to it [62,63].

Moreover, the trypanosomal BB protein TbNRKC, a NIMA-related kinase (NRK), is involved in the regulation of BB segregation [64]. TbNRKC is related to the human centrosomally localized

NEK2, which is involved in the regulation of various mitotic events, including centrosome separation [65]. Similarly, the trypanosomal polo-like kinase (Plk) TbPlk is also involved in the regulation of BB segregation [66–68]. Plks are central regulators of mitosis in most eukaryotes (e.g., Plk1 and Plk4 in mammals and Cdc5/Plo1 in yeast) [52]. Surprisingly, however, TbPlk is not involved in nuclear mitosis in trypanosomes [66,67].

Thus, segregation of nuclear chromosomes in most eukaryotes and mitochondrial kDNA segregation in trypanosomes are both MT-mediated processes and are regulated, in part, by members of the same protein families, NRKs and Plks.

TAC OMM module and SBP

It has not escaped our notice that the TAC OMM module shows conceptual similarities to SPBs in closed mitosis. Both organize filaments that mediate DNA segregation and are embedded in a membranous structure. The TAC OMM module forms a platform that connects the EZFs to the ULFs. EZFs formed by p197 have been shown experimentally to function as spacers that determine the distance between the BB and OMM [31]. The SBP is associated with a large pore in the nuclear membranes, similar to the nuclear pore complex, and provides a platform for spindle MTs and astral MTs, which face into the nuclear lumen and into the cytosol, respectively [52]. Moreover, yeast Spc110, similar to p197 in the TAC [31], functions as a spacer determining the distance between the central and the inner plaque of the SBP [69]. However, while the TAC membrane module connects the *cis*- and *trans*-sides of the mitochondrial membranes, the SPB connects the cytosol with the nuclear lumen, two compartments that are topologically identical.

DNA capture

A crucial step in the assembly of both the TAC and the mitotic spindle is that their filaments need to be attached to structures on the kDNA or to the analogous kinetochores of the chromosomes. It is not known in detail how this works for TAC ULFs. However, assembly of the new TAC on the proBB adjacent to the mature BB [32,49], which is firmly connected to the old TAC, allows the new TAC filaments to be formed at exactly the correct site to extend across the mitochondrial membranes and bind to the replicating kDNA. By contrast, the mitotic spindle forms *de novo* once in each cell cycle; thus, capture of the kinetochore by spindle MTs is a more complicated process involving a stochastic search and capture mechanism that is mainly driven by the dynamic instability of spindle MTs [61].

Concluding remarks

The BB is the master organizer of the trypanosomal cell cycle: it controls the formation of the flagellum and orchestrates the architecture of the cell [25,33,70]. The single nucleoid nature of the kDNA requires a highly precise mitochondrial genome segregation system. This led to the evolution of the TAC, a tether that initiates at the BB and connects to the kDNA network. The TAC links two different worlds: the BB, a eukaryote-specific structure homologous to the centriole-containing centrosome [57], and the kDNA, which can be traced back to the genome of the bacterial ancestor of mitochondria. In addition, the TAC itself is of chimeric evolutionary origin, because two of its subunits, TAC40 and TAC42, are β-barrel proteins that were likely commandeered from the bacterial ancestor of mitochondria [36,41].

In this review, we have shown that the TAC can serve as a paradigm for a filament-based genome segregation system: while sharing few homologous features with the mitotic spindle, it recapitulates some of the same concepts using analogous components (Figure 3). In addition, the TAC serves as a rare example of a permanent contact site between the OMM and the IMM [71,72]. Finally, assembly of the complicated TAC at a single precisely defined site in the mitochondrion of *T. brucei* arguably represents the most extreme sorting event for any mitochondria known.

Outstanding questions

Are there further essential TAC core subunits yet to be discovered?

Which BB protein connects the C terminus of p197 to the base of the BB? Which kDNA-binding protein connects TAC102 to the kDNA?

What is the stoichiometry of the TAC core subunits within the TAC?

How are the individual TAC subunits organized in the MOM TAC module?

How is TAC assembly regulated?

What is the mechanism of BB segregation?

Although we have learned much about the molecular architecture and assembly of the TAC, many questions remain (see Outstanding questions). Future research on the *T. brucei* TAC should focus on a detailed functional analysis of all of its subunits and a precise description of their assembly pathway. These studies should also be extended to other kinetoplastea to define which TAC features are universally conserved and which are more flexible between different species or life cycle stages. Finally, much insight could be gained from an *in situ* characterization of the TAC structure, preferably at high resolution and at different stages of the cell cycle. While this may have sounded like science fiction a few years ago, recent advances in cryo-electron microscopy suggest that it is an achievable goal.

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Declaration of interests

None declared by authors.

References

1. Tyler, K.M. *et al.* (2001) Anisomorphic cell division by African trypanosomes. *Protist* 152, 367–378
2. Cavalcanti, D.P. and de Souza, W. (2018) The kinetoplast of trypanosomatids: from early studies of electron microscopy to recent advances in atomic force microscopy. *Scanning* 2018, 9603051
3. Jensen, R.E. and Englund, P.T. (2012) Network news: the replication of kinetoplast DNA. *Annu. Rev. Microbiol.* 66, 473–491
4. Povelones, M.L. (2014) Beyond replication: division and segregation of mitochondrial DNA in kinetoplastids. *Mol. Biochem. Parasitol.* 196, 53–60
5. Schneider, A. and Ochsenreiter, T. (2018) Failure is not an option – mitochondrial genome segregation in trypanosomes. *J. Cell Sci.* 131, jcs221820
6. Robinson, D.R. and Gull, K. (1991) Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. *Nature* 352, 731–733
7. Burki, F. *et al.* (2019) The new tree of eukaryotes. *Trends Ecol. Evol.* 35, 43–55
8. Bolker, J. (2012) Model organisms: there's more to life than rats and flies. *Nature* 491, 31–33
9. Pays, E. *et al.* (2023) The pathogenesis of African trypanosomiasis. *Annu. Rev. Pathol.* 18, 19–45
10. Verner, Z. *et al.* (2015) Malleable mitochondrion of *Trypanosoma brucei*. *Int. Rev. Cell Mol. Biol.* 315, 73–151
11. Schneider, A. (2001) Unique aspects of mitochondrial biogenesis in trypanosomatids. *Int. J. Parasitol.* 31, 1403–1415
12. Amodeo, S. *et al.* (2022) Mitochondrial genome maintenance – the kinetoplast story. *FEMS Microbiol. Rev.* Published online November 30, 2022. https://doi.org/10.1093/femsre/fuac047
13. Maslov, D.A. (2019) Separating the wheat from the chaff: RNA editing and selection of translatable mRNA in trypanosome mitochondria. *Pathogens* 8, 105
14. Aphasizheva, I. and Aphasizhev, R. (2016) U-insertion/deletion mRNA-editing holoenzyme: definition in sight. *Trends Parasitol.* 32, 144–156
15. Read, L.K. *et al.* (2016) Trypanosome RNA editing: the complexity of getting U in and taking U out. *Wiley Interdiscip. Rev. RNA* 7, 33–51
16. Jaskolowski, M. *et al.* (2020) Structural insights into the mechanism of mitoribosomal large subunit biogenesis. *Mol. Cell* 79, 629–644
17. Saurer, M. *et al.* (2019) Mitoribosomal small subunit biogenesis in trypanosomes involves an extensive assembly machinery. *Science* 365, 1144–1149
18. Ramrath, D.J.F. *et al.* (2018) Evolutionary shift toward protein-based architecture in trypanosomal mitochondrial ribosomes. *Science* 362, eaau7735
19. Schneider, A. (2022) Evolution and diversification of mitochondrial protein import systems. *Curr. Opin. Cell Biol.* 75, 102077
20. Lukes, J. *et al.* (2021) RNA editing in mitochondria and plastids: weird and widespread. *Trends Genet.* 37, 99–102
21. Scaltsoyiannes, V. *et al.* (2022) Types and functions of mitoribosome-specific ribosomal proteins across eukaryotes. *Int. J. Mol. Sci.* 23, 3474
22. Greber, B.J. and Ban, N. (2016) Structure and function of the mitochondrial ribosome. *Annu. Rev. Biochem.* 85, 103–132
23. Feagin, J.E. (2000) Mitochondrial genome diversity in parasites. *Int. J. Parasitol.* 30, 371–390
24. Gustafsson, C.M. *et al.* (2016) Maintenance and expression of mammalian mitochondrial DNA. *Annu. Rev. Biochem.* 85, 133–160
25. Wheeler, R.J. *et al.* (2019) Coordination of the cell cycle in trypanosomes. *Annu. Rev. Microbiol.* 73, 133–154
26. Ogbadoyi, E.O. *et al.* (2003) A high-order trans-membrane structural linkage is responsible for mitochondrial genome positioning and segregation by flagellar basal bodies in trypanosomes. *Mol. Biol. Cell* 14, 1769–1779
27. Gluenz, E. *et al.* (2007) Structural asymmetry and discrete nucleic acid subdomains in the *Trypanosoma brucei* kinetoplast. *Mol. Microbiol.* 64, 1529–1539
28. Dean, S. *et al.* (2013) Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes. *Proc. Natl Acad. Sci. USA* 110, 14741–14746
29. Käser, S. *et al.* (2016) Outer membrane protein functions as integrator of protein import and DNA inheritance in mitochondria. *Proc. Natl Acad. Sci. USA* 113, E4467–E4475
30. Gheiratmand, L. *et al.* (2013) Biochemical characterization of the bi-lobe reveals a continuous structural network linking the bi-lobe to other single-copied organelles in *Trypanosoma brucei*. *J. Biol. Chem.* 288, 3489–3499
31. Aeschlimann, S. *et al.* (2022) Single p197 molecules of the mitochondrial genome segregation system of *Trypanosoma brucei* determine the distance between basal body and outer membrane. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2204294119
32. Bregy, I. *et al.* (2023) Cryo-electron tomography sheds light on the elastic nature of the Trypanosoma brucei tripartite attachment complex. *bioRxiv* Published online March 7, 2023. https://doi.org/10.1101/2023.03.06.531305
33. Vaughan, S. and Gull, K. (2015) Basal body structure and cell cycle-dependent biogenesis in *Trypanosoma brucei*. *Cilia* 5, 5
34. Lacomble, S. *et al.* (2009) Three-dimensional cellular architecture of the flagellar pocket and associated cytoskeleton in trypanosomes revealed by electron microscope tomography. *J. Cell Sci.* 122, 1081–1090

35. Elias, M.C. *et al.* (2007) Morphological events during the *Trypanosoma cruzi* cell cycle. *Protist* 158, 147–157
36. Käser, S. *et al.* (2017) Biogenesis of the mitochondrial DNA inheritance machinery in the mitochondrial outer membrane of *Trypanosoma brucei*. *PLoS Pathog.* 13, e1006808
37. Pusnik, M. *et al.* (2012) An essential novel component of the non-canonical mitochondrial outer membrane protein import system of trypanosomatids. *Mol. Biol. Cell* 23, 3420–3428
38. Bruggisser, J. *et al.* (2017) Biogenesis of a mitochondrial outer membrane protein in *Trypanosoma brucei*: targeting signal and dependence on an unique biogenesis factor. *J. Biol. Chem.* 292, 3400–3410
39. Vitali, D.G. *et al.* (2018) Independent evolution of functionally exchangeable mitochondrial outer membrane import complexes. *eLife* 7, e34488
40. Guna, A. *et al.* (2022) MTCH2 is a mitochondrial outer membrane protein insertase. *Science* 378, 317–322
41. Schnarwiler, F. *et al.* (2014) Trypanosomal TAC40 constitutes a novel subclass of mitochondrial beta-barrel proteins specialized in mitochondrial genome inheritance. *Proc. Natl. Acad. Sci. U. S. A.* 111, 7624–7629
42. Schimanski, B. *et al.* (2022) p166 links membrane and intramitochondrial modules of the trypanosomal tripartite attachment complex. *PLoS Pathog.* 18, e1010207
43. Zhao, Z. *et al.* (2008) p166, a link between the trypanosome mitochondrial DNA and flagellum, mediates genome segregation. *EMBO J.* 27, 143–154
44. Horvath, S.E. *et al.* (2015) Role of membrane contact sites in protein import into mitochondria. *Protein Sci.* 24, 277–297
45. Baudouin, H.C.M. *et al.* (2020) A comparison of three approaches for the discovery of novel tripartite attachment complex proteins in *Trypanosoma brucei*. *PLoS Negl. Trop. Dis.* 14, e0008568
46. Trikin, R. *et al.* (2016) TAC102 is a novel component of the mitochondrial genome segregation machinery in trypanosomes. *PLoS Pathog.* 12, e1005586
47. Amodeo, S. *et al.* (2023) Characterization of two novel proteins involved in mitochondrial DNA anchoring in *Trypanosoma brucei*. *PLoS Pathog.* 19, e1011486
48. Hoffmann, A. *et al.* (2018) Molecular model of the mitochondrial genome segregation machinery in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U. S. A.* 115, E1809–E1818
49. Lai, D.H. *et al.* (2021) A specific basal body linker protein provides the connection function for basal body inheritance in trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2014040118
50. Petry, S. (2016) Mechanisms of mitotic spindle assembly. *Annu. Rev. Biochem.* 85, 659–683
51. Nannas, N.J. *et al.* (2014) Chromosomal attachments set length and microtubule number in the *Saccharomyces cerevisiae* mitotic spindle. *Mol. Biol. Cell* 25, 4034–4048
52. Ito, D. and Bettencourt-Dias, M. (2018) Centrosome remodelling in evolution. *Cells* 7, 71
53. Seybold, C. and Schiebel, E. (2013) Spindle pole bodies. *Curr. Biol.* 23, R858–R860
54. Graf, R. *et al.* (2021) The Dictyostelium centrosome. *Cells* 10, 2657
55. Zhou, Q. and Li, Z. (2015) gamma-Tubulin complex in *Trypanosoma brucei*: molecular composition, subunit interdependence and requirement for axonemal central pair protein assembly. *Mol. Microbiol.* 98, 667–680
56. Thawani, A. and Petry, S. (2021) Molecular insight into how gamma–TuRC makes microtubules. *J. Cell Sci.* 134, jcs245464
57. Carvalho-Santos, Z. *et al.* (2011) Evolution: tracing the origins of centrioles, cilia, and flagella. *J. Cell Biol.* 194, 165–175
58. Kobayashi, T. and Dynlacht, B.D. (2011) Regulating the transition from centriole to basal body. *J. Cell Biol.* 193, 435–444
59. Dumont, S. and Mitchison, T.J. (2009) Force and length in the mitotic spindle. *Curr. Biol.* 19, R749–R761
60. Bunning, A.R. and Gupta Jr., M.L. (2023) The importance of microtubule-dependent tension in accurate chromosome segregation. *Front. Cell Dev. Biol.* 11, 1096333
61. Elting, M.W. *et al.* (2018) The spindle: integrating architecture and mechanics across scales. *Trends Cell Biol.* 28, 896–910
62. Absalon, S. *et al.* (2007) Basal body positioning is controlled by flagellum formation in *Trypanosoma brucei*. *PLoS One* 2, e437
63. Kohl, L. *et al.* (2003) Novel roles for the flagellum in cell morphogenesis and cytokinesis of trypanosomes. *EMBO J.* 22, 5336–5346
64. Pradel, L.C. *et al.* (2006) NIMA-related kinase TbNRKC is involved in basal body separation in *Trypanosoma brucei*. *J. Cell Sci.* 119, 1852–1863
65. Fry, A.M. *et al.* (2017) Mitotic regulation by NEK kinase networks. *Front. Cell Dev. Biol.* 5, 102
66. Hu, H. *et al.* (2015) A novel basal body protein that is a polo-like kinase substrate is required for basal body segregation and flagellum adhesion in *Trypanosoma brucei*. *J. Biol. Chem.* 290, 25012–25022
67. Kurasawa, Y. *et al.* (2020) Polo-like kinase in trypanosomes: an odd member out of the Polo family. *Open Biol.* 10, 200189
68. Hammarton, T.C. *et al.* (2007) *Trypanosoma brucei* Polo-like kinase is essential for basal body duplication, kDNA segregation and cytokinesis. *Mol. Microbiol.* 65, 1229–1248
69. Kilmartin, J.V. *et al.* (1993) A spacer protein in the *Saccharomyces cerevisiae* spindle poly body whose transcript is cell cycle-regulated. *J. Cell Biol.* 123, 1175–1184
70. Gluenz, E. *et al.* (2011) The kinetoplast duplication cycle in *Trypanosoma brucei* is orchestrated by cytoskeleton-mediated cell morphogenesis. *Mol. Cell. Biol.* 31, 1012–1021
71. Tamura, Y. *et al.* (2019) Organelle contact zones as sites for lipid transfer. *J. Biochem.* 165, 115–123
72. Tamura, Y. *et al.* (2020) Lipid homeostasis in mitochondria. *Biol. Chem.* 401, 821–833
73. Hajduk, S. and Ochsenreiter, T. (2010) RNA editing in kinetoplastids. *RNA Biol.* 7, 229–236
74. Schneider, A. (2020) A short history of guide RNAs: The intricate path that led to the discovery of a basic biological concept. *EMBO Rep.* 21, e51918
75. Schneider, A. (2011) Mitochondrial tRNA import and its consequences for mitochondrial translation. *Annu. Rev. Biochem.* 80, 1033–1053
76. Drew, M.E. and Englund, P.T. (2001) Intramitochondrial location and dynamics of *Crithidia fasciculata* kinetoplast minicircle replication intermediates. *J. Cell Biol.* 153, 735–744
77. Melendy, T. *et al.* (1988) Localization of a type II DNA topoisomerase to two sites at the periphery of the kinetoplast DNA of *Crithidia fasciculata*. *Cell* 55, 1083–1088
78. Ryan, K.A. and Englund, P.T. (1989) Synthesis and processing of kinetoplast DNA minicircles in *Trypanosoma equiperdum*. *Mol. Cell. Biol.* 9, 3212–3217
79. Ryan, K.A. and Englund, P.T. (1989) Replication of kinetoplast DNA in *Trypanosoma equiperdum*. Minicircle H strand fragments which map at specific locations. *J. Biol. Chem.* 264, 823–830
80. Carpenter, L.R. and Englund, P.T. (1995) Kinetoplast maxicircle DNA replication in *Crithidia fasciculata* and *Trypanosoma brucei*. *Mol. Cell. Biol.* 15, 6794–6803
81. Ogbadoyi, E. *et al.* (2000) Architecture of the *Trypanosoma brucei* nucleus during interphase and mitosis. *Chromosoma* 108, 501–513
82. Campbell, P.C. and de Graffenried, C.L. (2020) Alternate histories of cytokinesis: lessons from the trypanosomatids. *Mol. Biol. Cell* 31, 2631–2639
83. Zhou, Q. *et al.* (2014) New insights into the molecular mechanisms of mitosis and cytokinesis in trypanosomes. *Int. Rev. Cell Mol. Biol.* 308, 127–166
84. Chanez, A.-L. *et al.* (2006) Ablation of the single dynamin of *T. brucei* blocks mitochondrial fission and endocytosis and leads to a precise cytokinesis arrest. *J. Cell Sci.* 119, 2968–2974