

Opinion

Kinetoplast Division Factors in a Trypanosome[☆]

Kojo Mensa-Wilmot ^{1,2,*} Benjamin Hoffman,^{1,2} Justin Wiedeman,^{1,2} Catherine Sullenberger,¹ and Amrita Sharma^{1,2}

Inheritance of the single mitochondrial nucleoid (kinetoplast) in the trypanosome requires numerous proteins, many of whose precise roles are unclear. By considering kinetoplast DNA (kDNA) as a template for cleavage into two equal-size networks, we predicted sets of mutant kinetoplasts associated with defects in each of the five steps in the kinetoplast cycle. Comparison of these kinetoplasts with those obtained after gene knockdowns enabled assignment of proteins to five classes – kDNA synthesis, site of scission selection, scission, separation, and partitioning. These studies highlight how analysis of mutant kinetoplast phenotypes may be used to predict functional categories of proteins involved in the biogenesis of kinetoplasts.

Kinetoplast Biogenesis and Inheritance Cycle

The single-cell eukaryote *Trypanosoma brucei* causes human African trypanosomiasis (HAT) and the cattle disease nagana in regions of sub-Saharan Africa. *T. brucei* is spread to vertebrates through the bite of an infected tsetse fly which harbors insect-stage (procyclic) trypanosomes. The mitochondrial genome of *T. brucei* is comprised of circular double-stranded DNAs (minicircles and maxicircles) catenated into a disk-like network in a single **kinetoplast** (see [Glossary](#)). Loss of **kinetoplast DNA** (kDNA) disrupts mitochondrial functions in bloodstream *T. brucei* and interferes with development in the tsetse fly, breaking vector-to-mammal transmission that is needed to spread disease [1,2]. Bloodstream trypanosomes may lose kDNA, becoming dyskinetoplastic, in which case their long-term survival is possible only if they acquire a mutation in the γ -subunit of the ATP synthase [3]. Naturally occurring dyskinetoplastic *Trypanosoma equiperdum* and *Trypanosoma evansi* strains are known (reviewed in [1]). Trypanosome **basal bodies** are ‘modified’ centrioles found near kinetoplasts. The region between basal bodies and the kinetoplast constitutes a **tripartite attachment complex** (TAC) [4]. We term proteins that associate with the TAC ‘TAC-associated proteins (TACAPs)’ (reviewed in [5]).

‘Segregation’ is widely used in the field to describe aspects of the kinetoplast cycle. Given our current state of knowledge about proteins involved in biogenesis and inheritance of kinetoplasts, the meaning of the word ‘segregation’ is ambiguous, at best. Originally used to describe scission of kDNA networks, inferred from examination of electron micrographs [6], ‘segregation’ has also been used to describe movement and/or duplication of kinetoplasts [4,7].

Biogenesis of kinetoplasts is coordinated with the cell cycle. Trypanosomes in G1 have a single kinetoplast (K) and one nucleus (N) (1K1N, [Figure 1](#)). kDNA is synthesized in S phase (1K₁1N, [Figure 1](#)) [8,9]. Two kinetoplasts per cell are observed in G2 in 2K1N trypanosomes [10]. Kinetic analysis using pre-S phase-enriched trypanosomes indicates that duplication of kinetoplasts

Highlights

Kinetoplasts (mitochondrial genome nucleoids) are important in bloodstream trypanosomes for the establishment of mitochondrial membrane potential.

Many proteins involved in segregation of kinetoplasts have been identified.

A region between a kinetoplast and basal bodies is described as a tripartite attachment complex (TAC).

A set of TAC-associated proteins (TACAPs) has been proposed as the machinery for kinetoplast segregation.

Subcomplexes of TACAPs that form *in vivo* have been described.

Several proteins that do not associate with TAC are involved in the maintenance of the kinetoplast.

New kinetoplast-associated proteins have been identified.

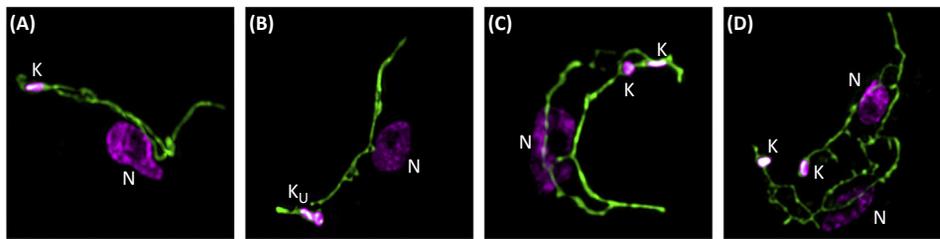
We are approaching an exciting period in the field when a molecular understanding of how all aspects of kinetoplast biogenesis are executed seems achievable.

¹Department of Cellular Biology, 724 Biological Sciences Building, University of Georgia, Athens, Georgia 30602, USA
²Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia 30602, USA

[☆]To my extraordinary mentor Professor Roger McMacken (Johns Hopkins Bloomberg School of Public Health), who patiently converted me into a scientist, and taught me unforgettable principles of DNA Replication Mechanisms.

*Correspondence: mensawil@uga.edu (K. Mensa-Wilmot).





Trends in Parasitology

Figure 1. Kinetoplast Duplication Inside Mitochondria. Mitochondria of bloodstream trypanosomes were labeled with mitotracker (green) [46], and DNA in the nucleus (N) or kinetoplast (K) labeled with DAPI (4,6-diamino-2-phenylindole). Images were captured with a fluorescence microscope. Panels (A) 1K1N, (B) 1K_U1N trypanosome, (C) 2K1N trypanosome, (D) 2K2N trypanosome. K_U has replicated kDNA but the kinetoplast is undivided.

peaks 1 h after termination of kDNA synthesis [11]. The period between termination of kDNA synthesis and division of kinetoplasts may be termed kinetoplast G2 (G2^K). Nuclei of 2K1N trypanosomes divide to generate 2K2N cells that produce two 1K1N cells after cytokinesis [12]. Restriction of kinetoplast duplication to G2 indicates that a kinetoplast is 'licensed' for scission or separation at this stage of the cell cycle.

A long-term goal of the field is to provide a molecular description of the kinetoplast cycle (Figure 2) [11,13–18]. That objective calls for assignment of proteins to roles at specific steps of the kinetoplast cycle. In this context, we suggest that investigators try to avoid using the term 'segregation' because of the ambiguity in its meaning. We offer terminology that the field may consider using to describe the major steps in the duplication cycle of kinetoplasts. Further, we provide guidelines for analysis of mutant kinetoplasts from gene knockdowns that allows placement of proteins into one of five steps in the kinetoplast cycle (Figure 2).

"Synthesis of kDNA" refers to incorporation of nucleotides into the mitochondrial DNA network (Figure 1). **Scission** involves **cleavage** of kDNA into two networks. **Separation** denotes movement of cleaved kinetoplasts away from each other inside a single mitochondrion (Figure 1). **Partitioning** is the sorting of kinetoplasts into daughter trypanosomes (Figure 2). Minimally, the kinetoplast cycle has five steps (Figure 2): (1) replication of kDNA; (2) selection of a site for scission of kDNA; (3) scission of kDNA; (4) separation of cleaved kinetoplasts; and (5) partitioning of kinetoplasts into two trypanosomes.

Five Classes of Mutants for Kinetoplast Biogenesis and Inheritance

On the basis of the steps involved in the kinetoplast cycle (Figure 2), protein functions may be categorized as follows. First, identify and quantitate all trypanosome cell types produced within three cell division cycles after knockdown of a gene. Second, compare unusual cell types to those predicted after loss of genes at different steps of the kinetoplast cycle (Figure 2). Third, assign a gene to the class with the closest set of predicted abnormal trypanosomes (Table 1).

Class I – kDNA Synthesis Mutants

Due to failure to synthesize a full component of mitochondrial DNA, the kDNA content of a mutant is less than normal at the end of kinetoplast S phase. This smaller kDNA intermediate is cleaved into two, and each kinetoplast of the immediate product (2K_S1N) has less kDNA than normal kinetoplasts. In contrast, nuclei in the cells have normal content (N) compared to control cells (Figure 3A).

Glossary

Basal body: microtubule-organizing center for the flagellum axoneme. Movements of basal bodies are associated with division of kinetoplasts. Some proteins that are found between basal bodies and kinetoplasts (i.e., TAC-associated proteins) are important in aspects of the kinetoplast biogenesis cycle.

Kinetoplast: mitochondrial nucleoid consisting of kDNA and associated proteins. During trypanosome proliferation, a second copy of the kinetoplast is detected by light microscopy in the G2 stage of the cell cycle. At cytokinesis, two kinetoplasts are sorted, one into each of the two daughter trypanosomes.

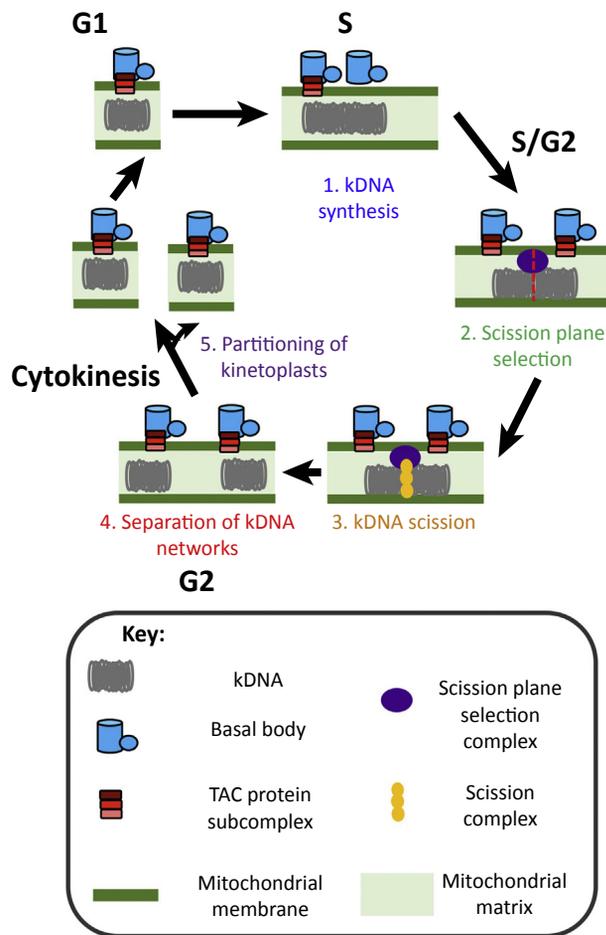
Kinetoplast DNA (kDNA): a mitochondrial DNA network in trypanosomes. It is composed of interlocked (i.e., catenated) circular minicircles and maxicircles. Maxicircles encode the mitochondrial genome. Many genes encoded by maxicircles are incomplete until edited at the RNA level to incorporate (or remove) nucleotides. Information (guide RNAs) for editing maxicircle transcripts are found predominantly in minicircles. Edited pre-mRNAs can be translated to proteins by ribosomes.

Kinetoplast scission factor (KSF): a protein required for cleavage of kinetoplast DNA networks, generating two daughter kDNA networks, each of which retains catenated minicircles and maxicircles.

Partitioning: the distribution of cleaved kinetoplasts into two daughter trypanosomes during cytokinesis. Scission of a double-size kinetoplast with replicated kDNA is required ahead of partitioning the nucleoid.

Scission (cleavage) of kinetoplast: endonuclease cleavage of a double-size (i.e., replicated) kDNA network into two networks of approximately equal size. Daughter kDNAs retain catenated minicircles and maxicircles but they are not substrates for further scission reactions.

Separation: movement of cleaved kinetoplasts away from each other inside a single mitochondrion.



Trends in Parasitology

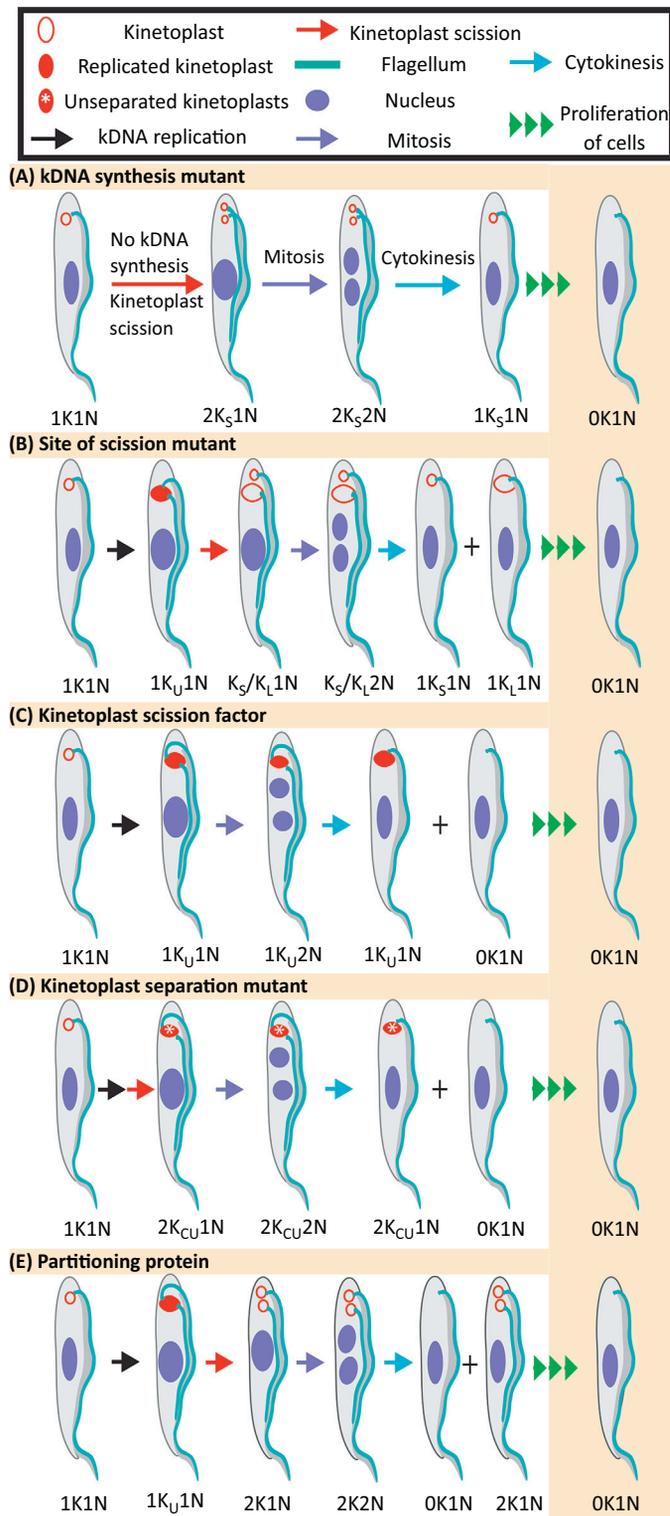
Tripartite attachment complex (TAC): a region of the trypanosome between the kinetoplast and basal body. Some proteins that associate with the TAC affect kinetoplast inheritance. However, not all TAC-associated proteins are important for kinetoplast scission and/or partitioning.

Figure 2. Five Steps in Kinetoplast Biogenesis and Inheritance. Major steps in the kinetoplast cycle are depicted: (1) kDNA synthesis, (2) scission site selection, (3) scission (cleavage), (4) separation of cleaved kDNAs, and (5) partitioning (at cytokinesis).

Table 1. Sets of Unusual Kinetoplast Combinations Predicted for Classes of Mutants^a

Category	Stage in the kinetoplast cycle	Expected set of abnormal trypanosomes
Class I	kDNA synthesis	1K _S 1N, 2K _S 1N, 2K _S 2N, 0K1N
Class II	Selection of scission site	K _L /K _S 1N, K _L /K _S 2N, 1K _L 1N, 1K _S 1N, 0K1N
Class III	Scission of kDNA network	1K _L 1N, 1K _L 2N, 0K1N. Electron microscopy shows one uncleaved kDNA per kinetoplast
Class IV	Separation of cleaved kDNA networks	1K _L 1N, 1K _L 2N, 0K1N. Two cleaved kDNAs per kinetoplast, detected in electron microscopy analysis
Class V	Partitioning of kinetoplasts into new cells	2K1N, 0K1N

^aDifferent types of kinetoplast and the expected sets appearing together are presented for mutants belonging to classes I through V. Kinetoplast designations are as follows: K, kDNA containing one genome's worth of minicircles and maxicircles; K_S, kinetoplast contains less than one genome equivalent of kDNA; K_L, kinetoplast contains greater than one genome equivalent of kDNA; K_L, kDNA has two genomes amount of kDNA.



Trends in Parasitology

(See figure legend on the bottom of the next page.)

Thus, the early unusual products of this class of mutants are $2K_S1N$ and $2K_S2N$. Should these cells proliferate, loss of kDNA continues until the nucleoid is no longer detectable and the trypanosomes are dyskinetoplastic ($0K1N$, Figure 3A) [19]. Class I mutants are exemplified by Pol1B [20], p38 [21], MIRF172 [22], TbKAP6 [23], mitochondrial Topo II [19], and Tb927.2.6100 [17].

Class II – Site of Scission Selection Mutants

During trypanosome proliferation, kinetoplasts are divided into two equal-size networks before partitioning into two cells (Figure 2). Thus, it stands to reason that trypanosomes have molecular machinery to recognize correct sites of scission on kinetoplasts. Erroneous selection of the site of scission would lead to cleavage of kDNA into two kinetoplasts of unequal size: one large (K_L) and the other small (K_S) in the same cell (K_L/K_S1N , i.e., asymmetric kinetoplast division [24], Figure 3B). After mitosis, K_L/K_S1N cells produce K_L/K_S2N trypanosomes. Thus, the abnormal early products of mutants in scission site selection are kinetoplasts that are either oversized (K_L) or undersized (K_S) (specifically, K_L/K_S2N , K_L/K_S1N , $1K_LN$, and $1K_S1N$, Figure 3B). Sustained proliferation of these early cell types will produce dyskinetoplastic ($0K1N$) trypanosomes (Figure 3). Examples of class II genes are TACAPs p166 [25], p197 (in procyclic *T. brucei*) [26], TAC65 [27], and TAC60 [28]. Acyl carrier protein (ACP) [29] is also a class II protein.

Class III – Kinetoplast Scission Mutants

Scission of a kinetoplast entails the conversion of one double-size kDNA into two networks of equivalent size. From kinetic analysis of chemically synchronized trypanosomes, and the use of molecular markers [30], the earliest time when two kinetoplasts can be detected in a population of trypanosomes is G2 [11]. Should scission of a kinetoplast fail, the immediate product is a $1K_U1N$ trypanosome (Figure 3C) in which the kinetoplast contains two equivalents of kDNA. After mitosis, $1K_U1N$ yields $1K_U2N$ trypanosomes since failed kinetoplast division does not prevent mitosis (Figure 4). Documentation of this ‘failed kinetoplast division phenotype’ is compelling when $1K_U2N$ comprises 5% or more of the total trypanosome population. If $1K_U2N$ trypanosomes divide,

Figure 3. Predicted Sets of Kinetoplasts in Mutant Trypanosomes following Failure of Specified Steps of the Kinetoplast Cycle. Kinetoplast DNA (kDNA) (hollow red circle) was considered as a substrate for synthesis to produce a double-sized kinetoplast (K_U). Cleavage of a kinetoplast precedes mitosis, and normally produces two equal-sized kDNAs and one nucleus ($2K1N$) in one trypanosome. Mitosis of $2K1N$ trypanosomes results in $2K2N$ cells that can go through cytokinesis and generate $1K1N$ cells. Early abnormal kinetoplasts predicted for each class of mutant in the kinetoplast cycle is illustrated. In all cases, sustained proliferation of early mutant trypanosomes leads to an increased fraction of dyskinetoplastic ($0K1N$) cells, but the path to this end result, as revealed by the panel of abnormal kinetoplasts that accompanies each mutation, varies. (A) Class I, kDNA synthesis mutants. After loss of a protein that is essential for synthesis of kDNA, the total amount of DNA per kinetoplast decreases. Hence, after scission of that kDNA, the progeny has less than one kinetoplast’s equivalent (i.e., K_S) whereas the nucleus has a regular amount of DNA, making the cell a $2K_S1N$ trypanosome. After mitosis, a $2K_S2N$ trypanosome emerges which can go through cytokinesis. Following multiple rounds of division, the trypanosome gradually loses all of its kDNA and becomes dyskinetoplastic (i.e., $0K1N$). (B) Class II, site of scission selection. After synthesis of kDNA in a normal cell, a double-size uncleaved kinetoplast (K_U) is the product in a $1K_U1N$ cell: the kDNA is cleaved normally into two equal-sized progeny before mitosis. Faulty choice of the site of scission resulting from knockdown of a factor that is important for identification of the scission site will cause cleavage of kDNA into two networks of unequal size (i.e., asymmetric scission of the kinetoplast). The larger kinetoplast (K_L) and the smaller kDNA (K_S) can be found in premitotic ($1K_S/K_L1N$) cells or in postmitotic K_S/K_L1N and K_S/K_L2N trypanosomes. Repeated asymmetric kinetoplast scission and proliferation of progeny eventually leads to a population of trypanosomes with a significant fraction of dyskinetoplastics ($0K1N$). (C) Class III, kinetoplast scission factors (KSFs). Cleavage of a double-size kinetoplast ($1K_U1N$) into two networks is essential for inheritance of kinetoplasts. After knockdown of genes encoding KSFs, $1K_U1N$ remains uncleaved in premitotic trypanosomes. However, failure of kDNA scission does not foil mitosis, so nuclear division results in $1K_U2N$ trypanosomes that may accumulate in the population. Cytokinesis of $1K_U2N$ cells produces $0K1N$ (dyskinetoplastic) and $1K_U1N$ cells that, after proliferation, increase the fraction of dyskinetoplastic trypanosomes. (D) Class IV, separation of cleaved kinetoplasts. After scission of kinetoplasts the kDNA networks are detected by light microscopy as two entities when they move apart. Thus, in mutants where the separation of kinetoplasts does not take place, only one kDNA will be detectable by light microscopy, although scission of kinetoplasts has occurred producing cleaved but unseparated kinetoplasts (K_{CU}). Kinetoplasts in class IV mutant $1K2N$ and $1K1N$ cells are distinguishable from those of class III (KSF) mutants only in electron microscopy studies; they will reveal cleaved but adjacent kinetoplasts for separation factors, but show uncleaved kDNA in mutants of KSFs. (E) Class V, partitioning of kinetoplasts. During cytokinesis, two kinetoplasts from mitotic $2K2N$ trypanosomes are sorted into two new progeny, each of which has a $1K1N$ organelle content. If partitioning of the two kinetoplasts fails, the progeny trypanosomes are $2K1N$ and $0K1N$. Thus, detection of $0K1N$ cells in the absence of $1K_U2N$ early after gene knockdown is diagnostic of partition mutants.

1K_U1N and 0K1N (dyskinetoplastic) cells are the earliest products (Figure 3C). Extended proliferation of these initial cells will produce more dyskinetoplastic trypanosomes (Figure 3C).

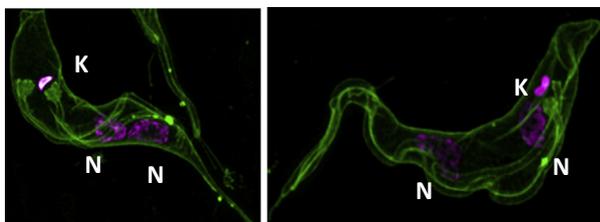
Examples of gene knockdowns that led to accumulation of 1K2N are TbBBP46, TbCEP57, KMP-11, TbUMSBP1/TbUMSBP2, TbCK1.2, and GCP3 (γ -tubulin subunit) [31–35]. Electron microscopy of K_U is predicted to show one undivided kDNA (longer than unit size) as observed after knockdown of UMSBP1/2 [32]. Genes that perturb flagellum biogenesis (e.g., produce detached or shortened flagella on 1K2N trypanosomes) are excluded from our list of potential class III proteins, to limit digression into studies of proteins with multiple functions in *T. brucei*. We recognize that ‘asymmetric cytokinesis’ of normal 2K2N cells can produce 1K2N and 1K0N (anucleate trypanosomes) [36,37]. However, these kinetoplasts have normal amounts of kDNA; they are not 1K_U and can be easily distinguished from 1K_U by quantitation of kDNA content [18,28,38].

Class III genes encode **kinetoplast scission factors** (KSFs), a knockdown of which causes accumulation of 1K_U2N trypanosomes. After division of 1K_U2N cells, a new population of 1K_U1N and dyskinetoplastic trypanosomes are produced. However, kDNA synthesis, basal body duplication, and flagellum biogenesis will be normal in cells lacking KSFs (Figure 4). Class III genes may encode polypeptides that (i) regulate trypanosome entry into G2 of the cell cycle, or (ii) cleave kDNA into two. Thus, for a better understanding of KSF function we need to learn whether 1K_U2N trypanosomes obtained from knockdown of class III genes are in G2 phase or M phase of the cell cycle.

Finally, we considered a possibility that a protein could inhibit cleavage of kinetoplasts without accumulation of 1K_U2N cells if trypanosomes proliferated efficiently. Such a scenario is possible only if loss of the protein has three concurrent effects: (i) it blocks scission of a kinetoplast, (ii) it accelerates nuclear mitosis, and (iii) it promotes rapid cytokinesis, such that all traces of 1K_U2N are lost from the population. There is no genetic support for this model.

Class IV – Separation of Kinetoplast Mutants

After cleavage of a double-size kinetoplast network into two, daughter kDNAs are presumed to be next to each other initially. Separated kinetoplasts are observed in G2 (Figure 1) [11,30,39], implying that daughter kDNAs move apart in G2 of the cell cycle. Based on these facts, the initial products from mutation of a gene that is important for separation of kinetoplasts will include 1K_U1N and 1K_U2N that, after dividing, produce 1K_U1N and 0K1N cells (Figure 3D), similar to class III genes. However, although kinetoplasts of 1K_U2N in class IV knockdowns appear to have one kDNA network in fluorescence microscopy, electron microscopy analysis of the nucleoids will reveal two kDNA disks juxtaposed to each other. Currently, we have not identified any gene for



Trends in Parasitology

Figure 4. 1K_U2N Trypanosomes Obtained after Knockdown of a Kinetoplast Scission Factor (KSF). Bloodstream *Trypanosoma brucei* containing an RNAi construct was induced to knock down a KSF gene (TbCK1.2) [33]. After inducing knockdown of the gene with tetracycline, plasma membrane and flagella were stained with mCLING (green) [47], and kinetoplast (K) and nuclei (N) were detected with DAPI. Images were captured using a super-resolution microscope as described [47].

which convincing data is available for its classification as a class IV mutant. However, we suspect that some genes producing 1K2N trypanosomes when knocked down might belong to this class.

Class V – Partitioning Mutants

Kinetoplasts in 2K2N trypanosomes are sorted into two 1K1N cells during cytokinesis. Mutations in genes encoding partitioning proteins or their regulatory factors will cause inappropriate sorting, producing 2K1N and 0K1N (dyskinetoplastic) trypanosomes as early abnormal trypanosomes (Figure 3E). After many rounds of proliferation, 0K1N trypanosomes become a major population in class V mutants. Genes whose products qualify as partitioning factors include TACAPs p197 [18], TAC102 (in bloodstream *T. brucei*) [18], and TAC40 [40]. Protease PNT1 [14] and α -KDE2 [41] are also partitioning proteins.

Although one could consider 2K0N and 0K2N (binucleate dyskinetoplastic) trypanosomes as products of severe defects in partitioning (Table 1), that decision is debatable. Knockdown of a protein involved in sorting of kinetoplasts should not affect mitosis. Therefore, 0K2N cells cannot be products of partitioning defects; they are products of asymmetric cytokinesis (aberrant placement of the cleavage furrow) of 2K2N cells [42], with 2K0N as the other abnormal product. One expects anucleate 2K0N cells to be short-lived and probably undetectable in an RNAi knockdown study because it takes days for data to be collected. The considerations above illustrate the importance of examining a full panel of early abnormal trypanosomes from gene knockdowns before concluding that dyskinetoplastic cells originated from improper partitioning of kinetoplasts (Table 1).

Concluding Remarks

In the last four years the field has seen excellent papers describing the involvement of proteins that localize between the kinetoplast and basal bodies (TACAPs) in inheritance of the nucleoid [14,18,22,28,40,43]. It seems likely that TACAPs execute their functions by forming subcomplexes instead of acting together as one massive macromolecule complex comprised of all proteins that localize to TAC. Proteins (e.g., KSFs) that are not found on TAC can have profound effects on kinetoplast scission and/or separation. Several potential KSFs are encoded in the genome, from evaluation of published literature [31–35]. An effort to integrate KSFs into models of the machinery for mitochondrial genome scission and sorting will be well received since TACAPs are not essential for scission of kinetoplasts.

Our phenotypic analysis of early products from ‘loss of function’ mutants allowed us to assign them to different steps in kinetoplast biogenesis and inheritance (Figure 5). This ordering of proteins enables investigators to develop new hypotheses on whether (or not) any subset of proteins function sequentially or interactively *in vivo*. Studies of possible interactions between TACAPs [18,27,28], as well as proteins that are not TAC-associated, will be bolstered by functional categorization of genes. For example, do the postulated partitioning proteins TAC40, p197, PNT1, TAC102, and α -KDE2 interact physically? Similarly, do site of scission selection factors p197, p166, ACP, TAC65, and TAC60 form a subcomplex in trypanosomes (Figure 5)? Since techniques for testing protein interactions *in vivo* are available it should not be long before we obtain answers to some of these questions (see Outstanding Questions). We anticipate that the initial protein categorization summarized in Figure 5 will be expanded with new information provided by other investigators. It is possible that the initial assignment of proteins to different classes of mutants (Table 1) will be revised as more comprehensive data become available. These developments will help the field to move closer to a more detailed molecular understanding of mitochondrial genome biogenesis and inheritance in the African trypanosome.

Outstanding Questions

What is the value of an initial assignment of genes to specific steps in the kinetoplast cycle through phenotypic analysis of mutant kinetoplasts?

How many kinetoplast scission factors are there, and what are their precise functions?

How are kinetoplasts attached to the machinery for scission or partition?

How are the functions of kinetoplast scission factors and TAC-associated proteins co-ordinated and/or integrated?

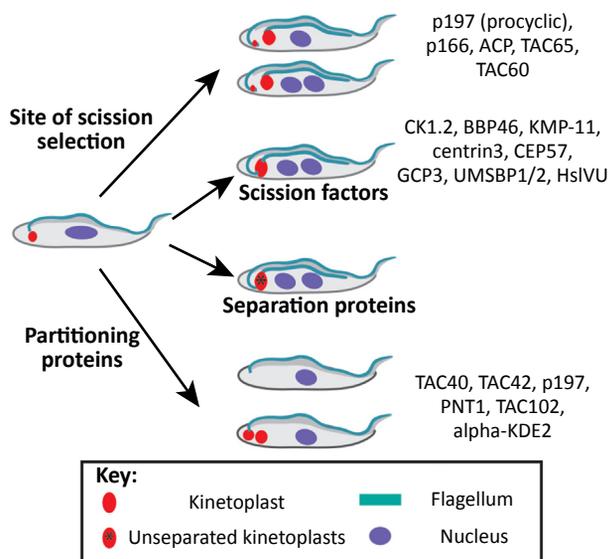


Figure 5. Distinction between Functions of Tripartite Attachment Complex (TAC)-associated Proteins and Kinetoplast Scission Factors (KSFs). Early abnormal trypanosomes reported after knockdown of several proteins that affected the kinetoplast cycle were analyzed as described (see Table 1 for summary). Genes were assigned to one of the five steps in kinetoplast biogenesis (see Figure 2), based on a comparison of known phenotypes of mutant trypanosomes with predicted aberrant kinetoplasts (Table 1 and Figure 3).

Trends in Parasitology

Concerning the systematic phenotypic analysis of gene knockdowns (Table 1, Figure 3), three issues are worth raising for discussion by the field. First, for a compelling quantitative description of the phenotypic observations, one should expect that a major diagnostic abnormal trypanosome cell type comprises at least 5% of the total population. Second, a chi-square test of the statistical significance of the differences in distribution of cell types before and after gene knockdown returns a P value $< 5 \times 10^{-3}$. The suggested higher threshold for the P value is influenced by the knowledge that most investigators use unsynchronized cells for these studies. Day-to-day variation of proportions for cell types in unsynchronized trypanosome populations can produce a distribution of cell types between the same control cells that gives P values close to 5×10^{-2} , a threshold that is frequently accepted for statistically significant differences. In addition, it will help if, in work with kinetoplast biogenesis and inheritance, the trypanosomes are analyzed at fixed times after RNAi induction, so that changes in phenotypes and morphology can be documented as early as possible. Third, recognizing the power of microscopy images to sway readers to a particular point of view (e.g., proposed pathway of events), we kindly request, for the sake of reproducibility of observations between laboratories, that images of abnormal cells should be presented in publications only if those cell types constitute at least 5% of the total population of trypanosomes.

Finally, how could TACAPs regulate scission site selection or partitioning of kinetoplasts when they lack DNA-binding domains (Figure 5)? The answer to the question may lie in three kinetoplast-binding proteins highlighted in our analysis, namely protease PNT1 [14] and universal minicircle-binding proteins UMSBP1 and UMSBP2 [32]. PNT1 is a partitioning protein (Figure 5), whereas UMSBP1 and UMSBP2, together, are KSFs (Figure 5) [32]. We hypothesize that PNT1 and UMSBP1/UMSBP2 have ‘moonlighting’ functions [44,45] as scaffolding proteins that anchor kinetoplasts to protein complexes needed for scission and partitioning of kDNA. Hence, two experimentally testable hypotheses from our phenotypic analyses of kinetoplasts and placement of genes in the kinetoplast cycle are: (i) PNT1 is a scaffold for a partitioning complex on kinetoplasts (Figure 5), and (ii) UMSBP1/UMSBP2 anchors kinetoplast scission complexes to kDNA (Figure 5). We look forward to reading answers to some of these questions in the near future.

References

- Schnauffer, A. *et al.* (2002) Natural and induced dyskinetoplastic trypanosomatids: how to live without mitochondrial DNA. *Int. J. Parasitol.* 32, 1071–1084
- Dewar, C.E. *et al.* (2018) Mitochondrial DNA is critical for longevity and metabolism of transmission stage *Trypanosoma brucei*. *PLoS Pathog.* 14, e1007195
- Dean, S. *et al.* (2013) Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 110, 14741–14746
- 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
- Schneider, A. and Ochsenreiter, T. (2018) Failure is not an option – mitochondrial genome segregation in trypanosomes. *J. Cell Sci.* 131, jcs221820
- Hoeijmakers, J.H.J. and Weijers, P.J. (1980) The segregation of kinetoplast DNA networks in *Trypanosoma brucei*. *Plasmid* 4, 97–116
- 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
- Woodward, R. and Gull, K. (1990) Timing of nuclear and kinetoplast DNA replication and early morphological events in the cell cycle of *Trypanosoma brucei*. *J. Cell Sci.* 95, 49–57
- Gluezn, E. *et al.* (2011) The kinetoplast duplication cycle in *Trypanosoma brucei* is orchestrated by cytoskeleton-mediated cell morphogenesis. *Mol. Cell Biol.* 31, 1012–1021
- Siegel, T.N. *et al.* (2008) Analysis of the *Trypanosoma brucei* cell cycle by quantitative DAPI imaging. *Mol. Biochem. Parasitol.* 160, 171–174
- Sullenberger, C. *et al.* (2017) AEE788 inhibits basal body assembly and blocks DNA replication in the African trypanosome. *Mol. Pharmacol.* 91, 482–498
- Sherwin, T. and Gull, K. (1989) The cell division cycle of *Trypanosoma brucei brucei*: timing of event markers and cytoskeletal modulations. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 323, 573–588
- Pena-Diaz, P. *et al.* (2017) A leucine aminopeptidase is involved in kinetoplast DNA segregation in *Trypanosoma brucei*. *PLoS Pathog.* 13, e1006310
- Grewal, J.S. *et al.* (2016) PNT1 is a C11 cysteine peptidase essential for replication of the trypanosome kinetoplast. *J. Biol. Chem.* 291, 9492–9500
- Wang, J. *et al.* (2014) TbKAP6, a mitochondrial HMG box-containing protein in *Trypanosoma brucei*, is the first trypanosomatid kinetoplast-associated protein essential for kinetoplast DNA replication and maintenance. *Eukaryot. Cell* 13, 919–932
- Povelones, M.L. (2014) Beyond replication: division and segregation of mitochondrial DNA in kinetoplastids. *Mol. Biochem. Parasitol.* 196, 53–60
- Beck, K. *et al.* (2013) *Trypanosoma brucei* Tb927.2.6100 is an essential protein associated with kinetoplast DNA. *Eukaryot. Cell* 12, 970–978
- 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
- Wang, Z. and Englund, P.T. (2001) RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. *EMBO J.* 20, 4674–4683
- Bruhn, D.F. *et al.* (2010) Mitochondrial DNA polymerase POLIB is essential for minicircle DNA replication in African trypanosomes. *Mol. Microbiol.* 75, 1414–1425
- Liu, B. *et al.* (2006) Role of p38 in replication of *Trypanosoma brucei* kinetoplast DNA. *Mol. Cell Biol.* 26, 5382–5393
- Amodeo, S. *et al.* (2018) Characterization of the novel mitochondrial genome replication factor MlRF172 in *Trypanosoma brucei*. *J. Cell Sci.* 131, jcs211730
- Wang, J. *et al.* (2014) TbKAP6, a mitochondrial HMG box-containing protein in *Trypanosoma brucei*, is the first trypanosomatid kinetoplast-associated protein essential for kinetoplast DNA replication and maintenance. *Eukaryot. Cell* 13, 919–932
- Wang, Z. *et al.* (2002) Asymmetrical division of the kinetoplast DNA network of the trypanosome. *EMBO J.* 21, 4998–5005
- Zhao, Z. *et al.* (2008) p166, a link between the trypanosome mitochondrial DNA and flagellum, mediates genome segregation. *EMBO J.* 27, 143–154
- 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
- Kaser, S. *et al.* (2016) Outer membrane protein functions as integrator of protein import and DNA inheritance in mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 113, E4467–E4475
- Kaser, S. *et al.* (2017) Biogenesis of the mitochondrial DNA inheritance machinery in the mitochondrial outer membrane of *Trypanosoma brucei*. *PLoS Pathog.* 13, e1006808
- Clayton, A.M. *et al.* (2011) Depletion of mitochondrial acyl carrier protein in bloodstream-form *Trypanosoma brucei* causes a kinetoplast segregation defect. *Eukaryot. Cell* 10, 286–292
- Benz, C. *et al.* (2017) Cell cycle synchronisation of *Trypanosoma brucei* by centrifugal counter-flow elutriation reveals the timing of nuclear and kinetoplast DNA replication. *Sci. Rep.* 7, 17599
- Dang, H.Q. *et al.* (2017) Proximity interactions among basal body components in *Trypanosoma brucei* identify novel regulators of basal body biogenesis and inheritance. *mBio* 8, e02120-16
- Milman, N. *et al.* (2007) Mitochondrial origin-binding protein UM5BP mediates DNA replication and segregation in trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19250–19255
- Jones, N.G. *et al.* (2014) Regulators of *Trypanosoma brucei* cell cycle progression and differentiation identified using a kinome-wide RNAi screen. *PLoS Pathog.* 10, e1003886
- 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
- Li, Z. and Wang, C.C. (2008) KMP-11, a basal body and flagellar protein, is required for cell division in *Trypanosoma brucei*. *Eukaryot. Cell* 7, 1941–1950
- Hu, H. *et al.* (2015) SAS-4 protein in *Trypanosoma brucei* controls life cycle transitions by modulating the length of the flagellum attachment zone filament. *J. Biol. Chem.* 290, 30453–30463
- Signorell, A. *et al.* (2009) Perturbation of phosphatidylethanolamine synthesis affects mitochondrial morphology and cell-cycle progression in procyclic-form *Trypanosoma brucei*. *Mol. Microbiol.* 72, 1068–1079
- Patel, G. *et al.* (2013) Kinase scaffold repurposing for neglected disease drug discovery: discovery of an efficacious, lapatinib-derived lead compound for trypanosomiasis. *J. Med. Chem.* 56, 3820–3832
- Janzen, C.J. *et al.* (2006) Selective di- or trimethylation of histone H3 lysine 76 by two DOT1 homologs is important for cell cycle regulation in *Trypanosoma brucei*. *Mol. Cell* 23, 497–507
- 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
- Sykes, S.E. and Hajduk, S.L. (2013) Dual functions of alpha-ketoglutarate dehydrogenase E2 in the Krebs cycle and mitochondrial DNA inheritance in *Trypanosoma brucei*. *Eukaryot. Cell* 12, 78–90

42. May, S.F. *et al.* (2012) The *Trypanosoma brucei* AIR9-like protein is cytoskeleton-associated and is required for nucleus positioning and accurate cleavage furrow placement. *Mol. Microbiol.* 84, 77–92
43. Trikin, R. *et al.* (2016) TAC102 is a novel component of the mitochondrial genome segregation machinery in trypanosomes. *PLoS Pathog.* 12, e1005586
44. Ginger, M.L. (2014) Protein moonlighting in parasitic protists. *Biochem. Soc. Trans.* 42, 1734–1739
45. Jeffery, C.J. (2018) Protein moonlighting: what is it, and why is it important? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372, 20160523
46. Vassella, E. *et al.* (1997) A mitochondrion-specific dye for multi-colour fluorescent imaging of *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 90, 381–385
47. Wiedeman, J. and Mensa-Wilmot, K. (2018) A fixable probe for visualizing flagella and plasma membranes of the African trypanosome. *PLoS One* 13, e0197541