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Graduate Studies

CURRENT MOLECULAR REVIEW OF THE CELL DIVISION CYCLE WITH AN EMPHASIS ON THE UNIQUE KINETOPLAST IN TRYPANOSOMA BRUCEI AND THE MITOCHONDRIA OF ITS MAMMALIAN HOST

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Requirements for the Degree of Master of Science in Biology

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CURRENT MOLECULAR REVIEW OF THE CELL DIVISION CYCLE WITH AN EMPHASIS ON THE UNIQUE KINETOPLAST IN TRYPANOSOMA BRUCEI AND THE MITOCHONDRIA OF ITS MAMMALIAN HOST

By Craig Herman

We recommend acceptance of this project report in partial fulfillment of the candidate's requirements for the degree of Masters of Science in Biology

The candidate has met all of the project completion requirements.

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ABSTRACT

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Trypanosoma brucei, a unicellular protozoan parasite, is the causative agent of Human African Trypanosomiasis in Sub-Saharan Africa, and is spread by the bite of a tsetse fly. Treatments for this disease are outdated, injection based, and may be life threatening. Thus, it is imperative to develop safer and orally administered drugs. The most effective way to treat this disease would be to exploit molecular differences between the parasite and its human host. A unique aspect of this parasite is the mitochondrial kinetoplast DNA, which differs greatly from DNA found in the mitochondria of human cells. This paper will review key mitochondrial differences and similarities between *T. brucei* and its human host with respect to the composition of this organelle, and how it replicates and divides with respect to mitosis and cytokinesis.

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INTRODUCTION

The causative agent of Human African Trypanosomiasis (HAT) is *Trypanosoma brucei* (two subspecies in particular: *T. b. gambiense* and *T. b. rhodesiense*) [1]. This unicellular protozoan parasite is a socio-economic burden in rural areas of sub-Saharan Africa, and its distribution mimics that of its vector, the tsetse fly (ssp. *Glossina palpalis* and *G. morsitans*). Roughly 70% of all HAT infections occur in the Democratic Republic of Congo, and it is currently estimated that 37 countries contain an at risk population. As of 2010, the World Health Organization estimated *T. brucei* newly infects 30,000 individuals annually, and 60 million more are believed to be at risk of infection [2]. This disease is estimated to result in 1.7 million Disability Adjusted Life Years, the loss of healthy life years by premature mortality and disability and is the third most significant parasitic disease after schistosomiasis and malaria [3]. This disease is so prevalent and devastating to the developing countries of Africa that an estimated $1.5 billion is lost in agricultural revenues alone each year. In addition, HAT has now surpassed HIV/AIDS as the biggest killer in Angola, Congo, and Southern Sudan. This disease obviously has lasting human and economic effects, and consequently it is imperative that efforts are focused at preventing and controlling the spread of HAT [4].

Following *T. brucei* infection prognosis is poor without treatment. Death commonly occurs within months to a few years depending on the parasitic sub- species responsible for the infection. For instance, *T. b. gambiense*, prevalent in central and

western Africa, causes a chronic progression of symptoms and death commonly occurs within roughly three years if proper treatment is not obtained. In contrast, *T. b. rhodesiense*, prevalent in eastern and southern Africa, causes an acute infection with death occurring within a few months post infection. Both sub species, however, provoke a similar two-stage host response. In the first haemolymphatic stage, this unicellular parasite resides and multiplies within the blood and lymph of its human host, producing wide-ranging symptoms such as fever, headaches, and joint pain. The neurological and psychiatric symptoms manifest at the onset of the second stage when the parasite crosses the blood-brain barrier. Here the patients' circadian rhythm becomes deregulated, which leads to fragmented sleep patterns; for this reason HAT is commonly referred to as African sleeping sickness [1,2].

Currently, four drugs are offered for the treatment of African sleeping sickness, with a fifth expected soon. None of these drugs are easily administrable, and all require numerous intramuscular injections that can span many weeks of treatment. Pentamidine (Sanofi-Aventis) and Suramin (Bayer), are both used for the treatment of hemolymphatic first stage HAT, and are considered to be toxic and outdated.. Pentamidine was discovered in the 1940s and Suramin in the 1920s [5]. Melarsoprol and Elfornithine, both discovered over 50 years ago, are stage two treatment options but the treatments are also extremely toxic. In contrast to the treatment options for first stage HAT, Melarsoprol and Elfornithine are subspecies dependent treatment options. Melarsoprol is the only form of treatment for HAT caused by *T. b. rhodesiense* infection, but it may also be used with a *T. b. gambiense* infection. Melarsoprol, the current primary stage two chemotherapeutic drug used to treat *T. b. gambiense* infections, is expected to be progressively replaced by

the more expensive combination therapy of Nifurtimox and Elfornithine as this treatment has reduced *Trypanosoma brucei* resistance, relapse incidence, and mortality rates [6]. Nonetheless, less toxic and orally introduced treatments are essential for future successful HAT therapies.

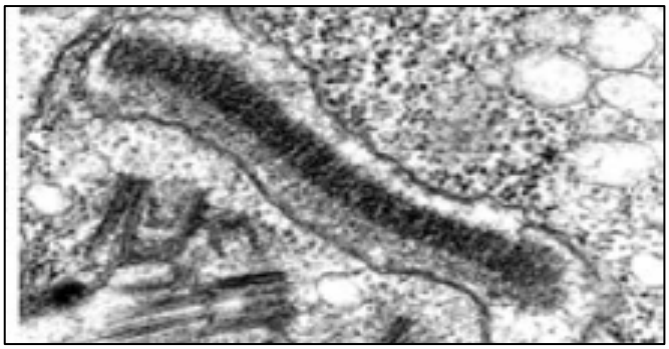

Figure 1. Cross-section of the kinetoplast disk of *Trypanosoma brucei*. The elongated kinetoplast is in the late stage of replication, and the dense

Synthetic heterocyclic diamidine pro-drugs, such as furamidine, offer less toxic potential trypanocidal HAT treatments and target the unique mitochondrial kinetoplast DNA (kDNA) of trypanosomes (**Figure 1**, ref. 7). To better understand the potential of such pro-drugs, a brief explanation of the kinetoplast and its contents is necessary. This structure contains the circular mitochondrial DNA of the cell, and it is organized as a single network of thousands of catenated mini-circles and tens of maxi-circles that appear as chainmail when viewed with an electron microscope (**Figure 2**, ref. 8) Mini-circles are roughly 1-kilobase in size, and their larger counterparts are generally estimated to be about 23-kilobases. Maxi-circles encode usual mitochondrial gene products, such as

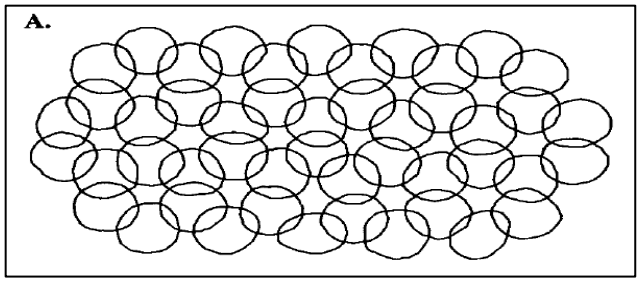

Figure 2. Interlinked chain-mail kDNA located within kinetoplast of *T. brucei*

rRNAs and proteins involved in mitochondrial energy transduction [9]. A unique feature of these products is that most must receive posttranscriptional modifications. This process, known as RNA editing, requires the assistance of an enormous and heterogeneous population of guide RNAs (gRNAs). The mini-circles encode gRNAs that execute the RNA editing ability of inserting or deleting uridine residues within maxi-circle transcripts. This renders them capable of performing their mitochondrial responsibilities. Due to the extensive editing required to generate functional maxi-circle transcripts, an expansive and stable cohort of mini-circles must be present to supply the essential gRNAs. For this reason, it is hypothesized that these circular DNA molecules are organized into a single network to ensure random segregation, and consequently the possible loss of required mini-circles, does not occur. More importantly, despite the fact mini-circles are heterogeneous sequences, all contain regions of innately bent DNA structures, which occur when four to six consecutive adenine or thymine ("A-tracts") nucleotide bases are repeated in helical periodicity [10]. The mechanism of heterocyclic diamidine pro-drug action is currently thought to bind to the minor groove of the innately bent DNA double helix. This binding is thought to induce a bend in the helix that inhibits mini-circle replication and its subsequent reinsertion within the kinetoplast DNA network [9]. Consequently, 24 – 48 hours after diamidine exposure, the kinetoplast disappears and the trypanosomes perish. For that reason many believe heterocyclic diamidines will be the future primary treatment of HAT. As it stands currently, these pro-drugs have a long way to go before they would be a favorable treatment option, because these compounds have low oral bioavailability due to low p*Ka* values and, the mechanism of action is still unidentified. The p*Ka* value, known as the acid dissociation constant, is the quantitative

measure of the strength of an acid in a solution. Drugs with low p*Ka* values have a greater tendency to dissociate a proton in a solution. As a result, the lower the p*Ka* value of a drug, the higher the likelihood it would be classified as a strong acid [11]. Therefore, discovering an easily administrable and less toxic drug for the treatment of HAT is still germane. The importance of this discovery cannot be understated; it demonstrates the innately bent mini-circle DNA of the kinetoplast, a structure absent from DNA located in the mitochondria and nucleus of its human host, is a great potential drug target. For that reason, and because of the importance of the dissimilarities between *Trypanosma brucei* and humans, this paper will act as a review on the parallels and differences between the two in regards to the cell-division cycle with an emphasis on each of their mitochondria.

T. brucei **Scientific Classification and Divergence from Other Eukaryotes**

Trypanosoma brucei is an early diverging eukaryote, and belongs to a group of organisms that are thought to be near the root of the eukaryotic tree. This unicellular eukaryotic microorganism is classified in the Excavate super-group of eukaryotes, in the phylum Euglenoza, and the Kinetoplastid class. Through 18S small subunit rRNA sequence analysis, the *T. brucei* clade is thought to have originated from African ancestors, co-evolved with tsetse flies, and through transmission parasitized early mammals. These ancestors then became isolated when Africa split from the other continents, and consequently this dates the parasites origins to the mid-Cretaceous period. *Trypanosoma brucei* utilizes a single flagellum, and consequently is characterized as a Trypanosomatid [13].

Kinetoplast DNA and the Mini-circle Bent-DNA Periodicity

Mini-circle nucleotide sequences are known to be heterogeneous both amongst species and within the mitochondrion of a single trypanosome. These sequences only share the intrinsically bent helical structure and a 100 to 200 base pair conserved sequence. This conserved sequence contains the origins of replication, the universal mini-circle sequence (UMS, the initiation site for leading-strand synthesis), and the hexamer (the start site for lagging strand/Okazaki fragment synthesis). This conserved region is adjacent to the innately bent DNA of unknown functionality [14]. Although every mini-circle contains this conserved inherently bent DNA configuration, it is not closely conserved at the nucleotide sequence level.

In 1984, Ntambi et al. [15] was able to show that Kinetoplast mini-circles of *T. brucei* contain naturally curved sequences of bent DNA (**Figure 3**). This sequence dependent phenomenon was observed by slower than normal migration of fragmented mini-circle DNA in polyacrylamide gels [15]. The bent DNA helical arrangement causes slower migration through an electrophoretic gel due to its variant shape compared to un-bent DNA. Lazy gel migration is not only dependent on the bent DNA structure and

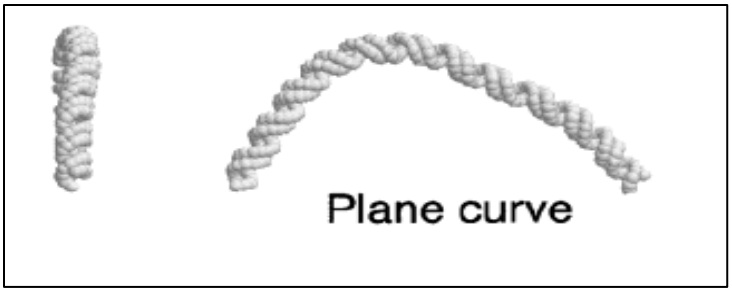

Figure 3. Plane curve of minicircle DNA. Two-dimensional illustration of the intrinsic bend shown to exist within the mini-circles of kDNA [17]. Adapted from Ohyama et al. (2001).

the pore size, but the size of the DNA fragment and the location of the DNA bend is also known to have an effect. It is now known four-to-six consecutive adenine or thymine base pairs ("AnTn, $n \geq 2$"), in a repeated periodicity, can alter the local and global structure of a DNA double helix. If these runs occur in helical repeats (10 or 11 base pairs) then the adenine or thymine bases will localize and interact on a particular side of the double helix, which would result in the macroscopic DNA curvature [10]. Although the precise molecular interactions are not yet understood, in a poly dA • dT-tract, this localization is assumed to allow the formation of a three-centered hydrogen bond between the N6 of an adenine and the O4 of two nearby thymine nucleotide bases. It should be noted that this dense helical confirmation is different from the natural canonical B-DNA arrangement, because the bases are highly twisted, and the minor DNA groove is much tighter and becomes progressively narrower with every 5' to 3' run. This minor groove narrowing was verified in 1994 by Carrera et al when they demonstrated an established bent sequence of DNA was less sensitive to hydroxyl radical and DNase I cleavage [16].

Human mitochondrial DNA (mtDNA)

The human host cells of trypanosomes also contain mitochondrial DNA, and so it is important this DNA is not damaged while using drugs that attack the parasitic mtDNA. Consequently, it is paramount that researchers understand both biological systems when attempting to create a drug for the treatment of HAT.

The human mtDNA is a circular-double stranded piece of DNA consisting of 16,569 base pairs in size that encodes a limited number of genes (**Figure 4**). Human mtDNA encodes 13 protein subunits that are involved in oxidative phosphorylation

within the electron transport chain, 2 rRNA subunits (12S and 16S), and 22 t-RNA coding genes. Most of these genes are encoded on the H-strand of the mtDNA. The distinction between the H-strand and the light (L-strand) arises due to the different proportions of heavier nucleotide bases on the two strands of the DNA. For instance the H-strand will contain a higher concentration of purines (adenine and guanine), and because of complementary base pairing the L-strand then consists of a higher proportion of pyrimidine nucleotides (cytosine and thymidine). A single human cell can contain several thousand copies of mtDNA, and a mitochondrion can contain several entire copies of this small circular genome. The genetic information of the mtDNA is highly condensed and consequently very little of it is considered to be non-coding. Practically the entire non-coding DNA is concentrated within a single 1-kb region of the circular DNA known as the non-coding region (NCR). The NCR contains the promoters and the origin of heavy strand replication (H-strand) [18].

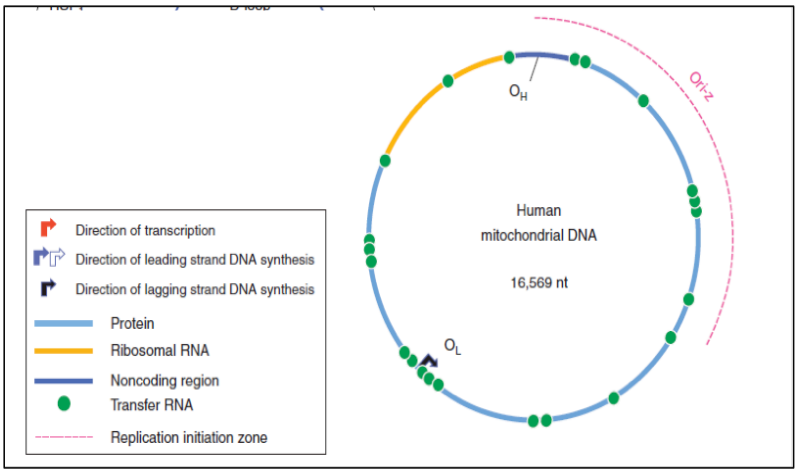

Figure 4. The human mitochondrial genome. The coding region (light blue) of the mtDNA greatly exceeds that of the non-coding region (dark blue) [18].

The mitochondria are the metabolic powerhouses that provide the cell with the energy required to perform even the most basic of processes [19]. Therefore, it should

come as no surprise that aberrant regulation of, or defects in, this organelle can lead to severe health complications. Genetic defects in the mtDNA nucleotide sequence or in genes that code for mitochondrial proteins have been shown to cause specific diseases Several human disease have defects in mtDNA replication. Like nuclear DNA replication, mtDNA requires the actions of a DNA polymerase and numerous replicative factors. However, unlike nuclear DNA replication, only one polymerase, DNA polymerase γ, is found within the mitochondria of humans, and consequently this single enzyme bears the burden of replication and repair functions. mtDNA replication requires the cooperative actions of DNA polymerase γ, mitochondrial single-stranded DNA binding proteins (mtSSB), and the mtDNA helicase known as Twinkle. The DNA polymerase γ catalytic subunit is encoded within POLG, and its accessory 140 kDa dimeric subunit is encoded within POLG2. This catalytic subunit contains the 3'-5' exonuclease and lyase activity, and the accessory dimer is required for DNA binding and processing [20]. Mutations within either of these two genes, but generally within that encoding the catalytic subunit, have been determined to be causative in neurodegenerative diseases such as Alpers-Huttenlocher syndrome (AHS), childhood myocerebrohepatopathy, and autosomal dominant progressive external ophthalmoplegia (adPEO), autosomal, recessive progressive external ophthalmoplegia (arPEO), to name a few [20]. These PEO phenotypes are characterized by mtDNA depletion and/or accumulation of mtDNA point mutations and deletions. Individuals with PEO generally show symptoms between 20 and 40 years of age, and these symptoms can include weakening of the eye muscle, proximal muscle weakness, and even exercise intolerance [20]. The last symptom should come as no surprise since skeletal muscles of PEO patients generally present with decreased

respiratory enzyme activity. Alpers syndrome, on the other hand, is a severe heritable disease that affects children within the first few years of life. This devastating disease causes progressive cerebral deterioration that results in seizures, blindness, deafness, liver failure and ultimately death. Biopsies portrayed patients with a phenotype of up to 30% less mtDNA content, and absolute loss of DNA polymerase γ function [20]. Biochemical analysis showed DNA pol γ retained only 4% of its intrinsic activity compared to that of its wild type counterpart.

The final mitochondrial related disease that will be referenced is one that focuses on defects in mitochondrial nucleotide metabolism. As with nuclear DNA replication, mtDNA replication requires the availability of deoxyribonucleotides (dNTPs) so as to incorporate the appropriate base into the growing DNA strands during replication. These dNTPs are actively transported from the cytosol into these membrane bound organelles, or obtain dNTPs through the pyrimidine salvage pathways by the actions of two mitochondrial kinases, thymidine kinase 2 (TK2) and deoxyguanosine kinase (DGUOK). In cells that are not actively proliferating, the mitochondria completely depend on the two kinases to replenish the mitochondrial dNTP pools. Mutations in the genes that code for these two kinases can lead to several diseases that present with mtDNA depletion syndromes, or a decrease in its mtDNA content as seen in Alpers syndrome [20].

Trypanosoma brucei **cell architecture**

Trypanosma brucei is a single-celled organism, with numerous interesting organelles. This parasite has a unique life cycle that allows it to adapt to changing environmental conditions. For instance, through antigenic differentiation it is able to evade the immune system of its human host after it is transmitted via the bite of a tsetse

fly. The overall structure and organization of T. brucei will be explained in this section, but it is first important to expand upon the life cycle of this organism (**Figure 5**). This parasite takes on numerous morphologies, and undergoes dramatic changes in proliferation throughout its life cycle [3]. Initially it survives in a short-stumpy procyclic trypomastigote form in the midgut of tsetse fly after it is ingested via a blood meal. In this first stage, the cell cycle is arrested and the cells do not proliferate. The parasite will then migrate to the salivary glands of the fly where it first transforms into the epimastigote form before developing into the metacyclic trypomastigote which can then infect a human during the next blood meal event. Upon intradermal transmission to humans, the long-slender metacyclic form of the parasite survives and replicates at the site of infection. Then these tryposmastigotes move via the lymphatics to the lymph nodes, and eventually the bloodstream. In this bloodstream stage, proliferation, via binary fission, occurs at a rapid pace. The parasite undergoes differentiation and will attempt evade the human host via a process known as antigenic

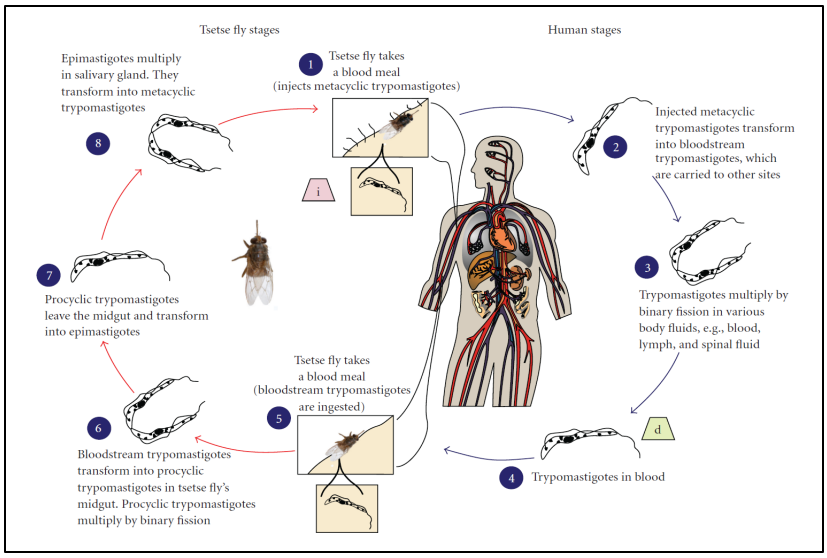

Figure 5. Life cycle stages of *Trypanosoma brucei*. Each stage of this parasites life cycle, from its procyclic/espimastigote forms within the tsetse fly to its

variation. With roughly 10^7 copies of variable surface glycoproteins (VSGs) expressed that protrude outward from the cell membrane. These antigens are capable of inducing an antibody response upon immune cell recognition. During ascending proliferation phase, a majority of the cells express a homotype of VSGs. Thus a host immune response is initiated that will aim to deplete the body of this particular homotype. Among the homotype-expressing cells, a few cells will begin to express a different VSG called a heterotype. Consequently, those few heterotypes will survive and reproduce, which will then initiate a new host response. Thus, a constant battle between the parasite and the human immune system is begun. Although these alternate morphological arrangements of the parasite exist, this review will focus on that of the long-slender bloodstream forms imply because most is known about this life stage.

The asymmetric long-slender form of *T. brucei* contains a single flagellum and numerous required organelles (**Figure 6)**. The nucleus surrounds the 11 large chromosomes (1-6 Megabases), 3-5 intermediate chromosomes (200 to 500 kilobases), and roughly 100 mini chromosome (50-100 kilboses) pairs [21]. The large chromosomes contain most of the protein-coding genes, while the smaller chromosomes appear to encode numerous genes involved in the process of antigenic variation. Other non-conventional features include the single kinetoplast and the glycosome.

The flagellum exits from the invaginated flagellar pocket (FP) and maintains attachment along the cellular membrane for most of its length. This region is referred to as the flagellum attachment zone (FAZ). The anterior distal tip of the flagellum is not attached and therefore freely rotates which then drags the cell behind. The hydrodynamic drag created from the mechanical movement of the flagellar tip culminates into the

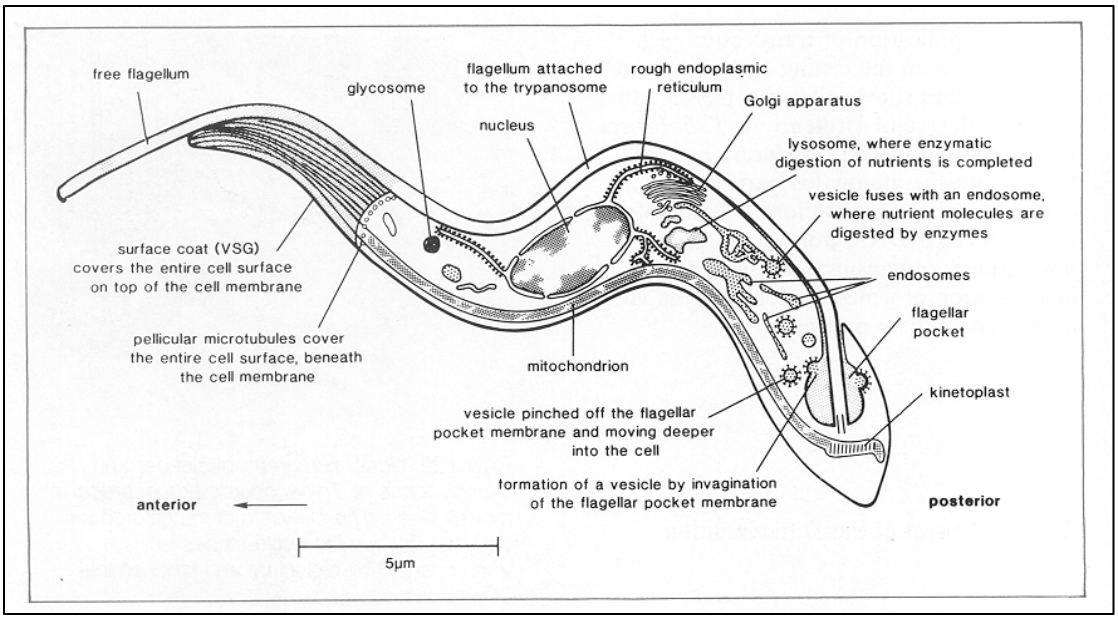

Figure 6. Schematic diagram of *Trypanosoma brucei* in its bloodstream form. Illustrates its major organelles and cellular structures. The mitochondrion is the unique kinetoplast that contains the interlocked mini-circles and maxi-circles [22].

rotation of the entire cell. This unique manner of mobility, relative to other flagellates, is required for the viability of the long-slender bloodstream form of *T brucei*. The flagellum is also required for attachment to host surfaces, and the rotation of the entire cell within blood and lymphatic systems is believed to maximize the probing capability of the flagellum as it is able to interact three-dimensionally with its environment. Finally, the flagellum is also required for cellular division, but this will be explained in greater detail in a later section.

The FP is formed by an invagination in the plasma membrane, and is the location not only where the flagellum exists, but is also where all endocytosis and exocytosis occurs (i.e., the "feeding grove" common to all excavates) [23]. In addition, this organelle performs processes such as protein trafficking, cell signaling, and immune invasion. For instance, the FP contains the inaccessible invariant surface receptors that would otherwise

be recognized by the human host immune system if they protruded outward from the plasma membrane [24].

The cellular architecture of typical human cells

Human host cells and those of T brucei share many similar organelles and characteristics. Even though the overall composition may differ slightly between the two, they each contain a plasma membrane that interacts with the external environment [25].

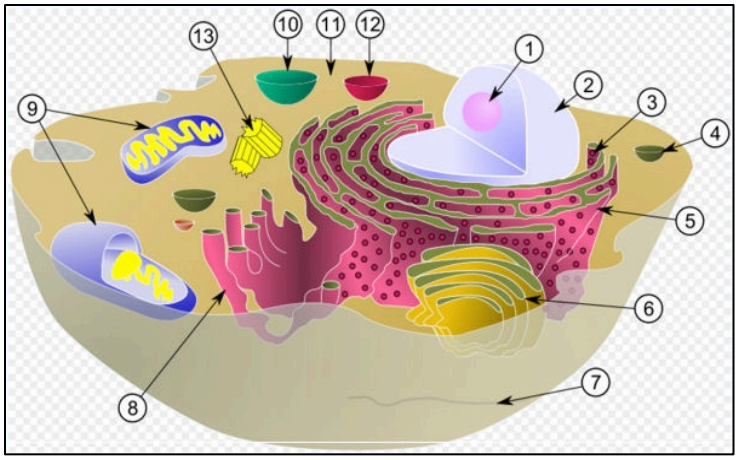

Figure 7. Diagram of a typical animal cell. All organelles numbered as follows: 1) Nucleolus, 2)Nucleus, 3) Ribosome, 4) Vesicle, 5) Rough endoplasmic reticulum, 6) Golgi apparatus, 7) Cytoskeleton, 8) Smooth endoplasmic reticulum, 9) Mitochondrion, 10) Vacuole, 11) Cytoplasm, 12) Lysosome, 13) Centriole. [26]

Internal membrane systems exist in both and include the nucleus, and the endomembrane system. Roughly 50-90% of the membrane is utilized within the endomembrane system, which consists of the endoplasmic reticulum (ER), the Golgi complex, endosomes, lysosomes, and transport vesicles (**Figure 7**). The nuclear membrane surrounds the 22 autosome and two sex chromosome pairs of the human cell, which all together accounts for roughly 3 billion nucleotide base pairs. The ER plays, in concert with the Golgi complex, a major

role in protein synthesis and processing, lipid metabolism, carbohydrate metabolism, and detoxification.

A major difference between other eukaryotic cells and that of *T brucei* is that most eukaryotic cells contain numerous mitochondria (upwards of a hundred) compared to the single copy kinetoplast that exists in *Trypanosoma brucei* [27]. The mitochondria itself is generally smaller than 1 micrometer in diameter. This organelle is referred to as the cells "powerhouse" because it generates most of the cells adenosine triphosphate (ATP), which is utilized by proteins as a source of chemical energy. In addition, this organelle is known to participate in signaling processes, apoptosis, and is also a regulator of the cell cycle. The compartments that carry out these specialized functions are the outer and inner membrane, the intermembrane space, and the cristae (**Figure 8**).

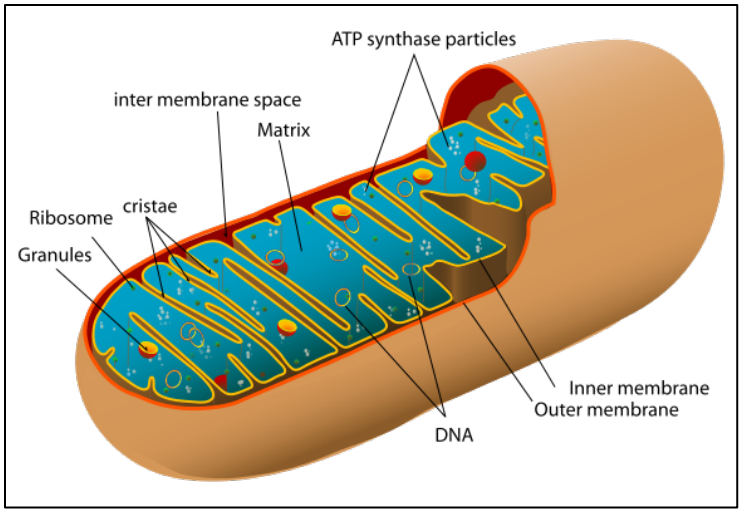

Figure 8. Components of the mitochondria. Illustration of single mitochondria outlining the major components and their locations within the organelle.

Both types of cells also require the presence of microtubules. *Trypanosoma brucei* contains a highly polarized microtubule cytoskeleton that defines the overall cellular shape, with the minus end posterior and the positive is anterior. In addition, exocytosis and endocytosis must exist in each cell to exchange materials within membrane-bound

components and the external environment. Even though some major differences may occur in the importance of each organelle within the context of each cell type, it is apparent that all of the aforementioned organelles play essential functions. Therefore, regardless the type of cell it is, cellular division must properly replicate and distribute the required organelles so as to ensure viable daughter cells.

BODY

Eukaryotic DNA Replication and Regulation

Although the cell cycle varies from organism to organism, the universal steps of the eukaryotic cell cycle consists of the mitotic phases (G1, S, G2, M) and cytokinesis. In addition, each cell must successfully duplicate and segregate all necessary cellular components (the mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and the microtubules along with the microfilaments; **Figure 9**). Clearly, cellular division is an intricate and complex task; therefore it is no surprise this process is regulated by numerous proteins within the cell-cycle control system [28]. This section will provide a brief overview of the cell cycle phases and how these steps are coordinated and regulated.

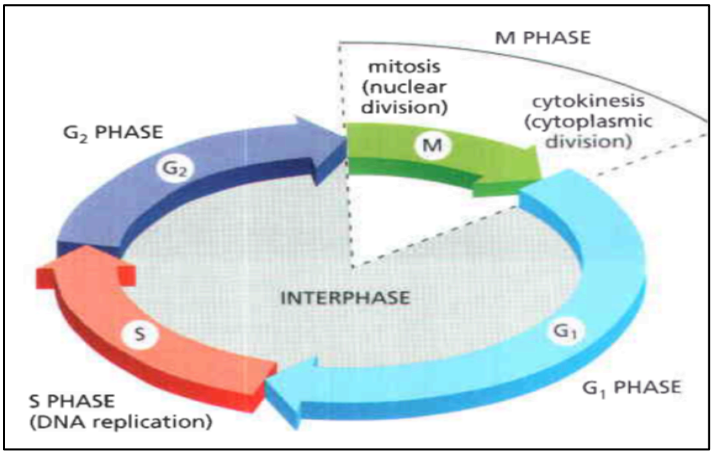

Figure 9. The cell cycle. The phases of the cell cycle include the two gap phases (G1, G2), DNA synthesis (S), and the M phase, which includes nuclear and

As previously mentioned, there are four phases of the mitotic cell cycle. In the second phase, known as S for DNA synthesis, the cell will duplicate its chromosomal DNA [25]. The synthesis phase is believed to occupy about half of the 24 hours it takes to develop a daughter cell. After DNA duplication has accurately occurred, chromosome segregation into two

daughter nuclei and cytoplasmic division, via cytokinesis, occurs in M phase. Mitosis is also broken down into five sub-phases (prophase, prometaphase, metaphase, anaphase, and telophase; **Figure 10**). The initial step of mitosis is prophase wherein the chromosomes, held tightly together via protein linkages, are compressed into dense rods called sister chromatids. At this juncture, the chromosomes are still held together, but the nuclear envelope dissembles and the sister chromosomes become attached to the mitotic spindle in prometaphase. In the third step of M phase, metaphase, each chromosomal copy, attached to opposing spindles, are aligned at the spindle equator and are primed for successful segregation to opposing poles once sister-chromatid adhesion is abolished in

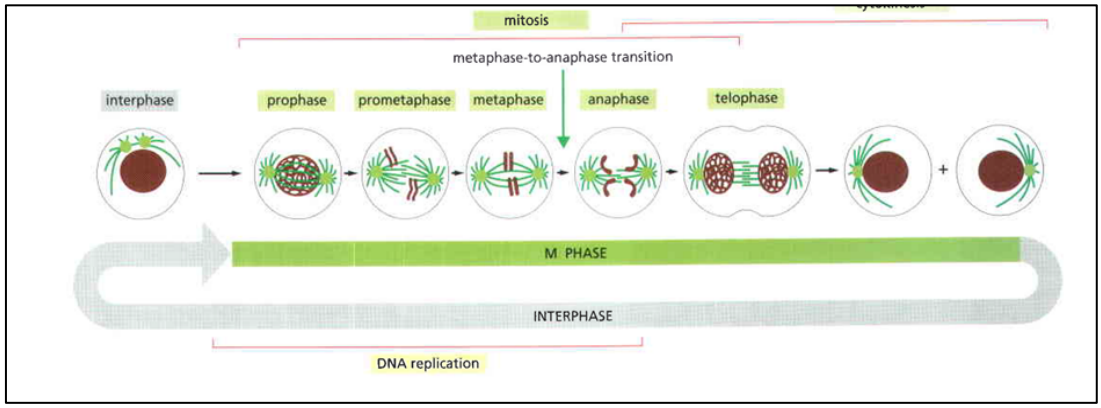

Figure 10. Phases of Mitosis. The five sub-phases of mitosis includes prophase, prometaphase, metaphase, anaphase and telophase, followed by cytokinesis. Interphase includes G1, S, and G2 [25].

anaphase. At this juncture the spindle is disassembled and the chromosomes are packaged into separate daughter cell nuclei in telophase. In cytokinesis the cytoplasm and organelles are divided, and this results in cell cycle completion and two genetically identical daughter cells.

The S phase and M phase are arguably the two most interesting, and therefore the most studied phases, but the most important may be the two gap phases. The first gap phase (G1) allows more time for cell growth between the synthesis and mitotic phases, monitors the environment thus ensuring conditions are optimal for growth. If conditions are ideal, the cell is permitted to proceed through a commitment point, known as the restriction point, and commit fully to S phase. Once the restriction point is passed, the cell will not abort DNA replication even if environmental conditions change, or replication signals are removed. Time allotment of G1 is solely dependent on the environmental conditions of the cell. If circumstances are not appropriate for synthesis, then initiation of DNA replication is postponed, and the cell may even enter a resting G0 state. This resting state can last for upwards of years before proliferation next occurs, if at all, prior to cell death.

Overall, the proteins and mechanisms involved in cell cycle control have been well conserved throughout evolution. So well conserved, in fact, that many of the proteins can function perfectly well if transferred from one eukaryote to another. The cell cycle control system triggers the major events of the cell

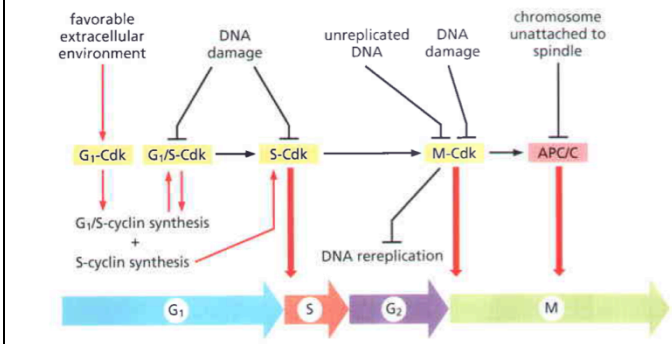

Figure 11. Cell Cycle Control. The generalized cell-cycle control system includes several cyclin-CDK complexes, and their interaction with inhibitory mechanisms that can block cell cycle progression if the cell is damaged or if the extracellular environment is not suitable to complete a full round of division [25].

cycle, and if a problem is detected these proteins can block the progression of the cell through cell cycle checkpoints (**Figure 11**; ref 29]. This control system utilizes proteins as binary biochemical switches that can block or allow the occurrence of a single event in an all-or-nothing manner. In most eukaryotic cells there exist three separate checkpoints that act as regulatory transitions from one phase of the cycle to another. The first, as touched on previously, is referred to as the restriction point, followed by the G2/M checkpoint, and the last is the metaphase-to-anaphase transition. The G2/M checkpoint ensures cells do not initiate mitosis before completion of DNA replication and they have a chance to repair damaged DNA . The metaphase-to-anaphase regulators then initiate and ensure proper chromosomal disjunction. Accurate chromosomal segregation is an absolute requirement for the successful generation of two healthy daughter cells.

Central proteins of the cell-control system are cyclin-dependent kinases (CDKs) [30]. CDK protein activity, within the cell, will rise and fall as a cell progresses through each phase of the cell cycle. Their primary activity within the cell, since they are kinases, is to phosphorylate and consequently alter the activity of downstream intracellular proteins. In turn these phosphorylated proteins may then regulate a particular event of the cell cycle. Such cycle changes in CDK concentrations are known to have a direct effect on chromosomal condensation, nuclear envelope breakdown, and even spindle assembly. These aptly named CDKs, in turn, are regulated by other proteins known as cyclins, and are as a result dependent upon cyclins for their activity. Cyclins appear not only to participate in the activation of CDK, but they also direct the interaction of CDKs with specific substrate target proteins. These cyclins alter the three dimensional structure of CDK, which allows a CDK-activating kinsase (CAK) to bind and phosphorylate a

particular amino acid within the activation site. This phosphorylation event deems CDK active. The Cyclin-CDK complex can also be inhibited by the kinase Wee1, which phosphorylates two CDK amino acids, thereby inhibiting its activity [31]. CDKs, however, generally rely on cyclin concentrations to adjust their activity. Interestingly, the levels of CDKs within a cell are generally constant, but cyclin-dependent kinase activities fluctuate with the concentration of cyclins when the two interact and form a cyclin-CDK complex. This complex triggers cell cycle events. The particular event that ensues coincides with the type of cyclin, which is present within the cyclin-CDK complex (25). There exist four classes of cyclins (G1, G1/S, S, and M cyclins) and they are named based on the stage of the cell cycle they interact with cyclin-dependent kinases. For instance, when S-cyclins interact with CDKs, and subsequent CAK activation occurs, the cell continues into the second step of interphase where duplication of chromosomal DNA ensues as the cyclin-CDK complex stimulates the expression of required genes

Mitochondrial Dynamics

The highly regulated processes of fusion (merging) and fission (splitting) of mitochondria within a eukaryotic cell influences the morphology of the mitochondria, ensures proper mitochondrial distribution within the cell, controls copy number, and it determines the genetic makeup of daughter cells [**Figure 12**; ref 33]. Sites of mitochondrial fission may also act as sites of fusion. A fusion or a fission occurrence can immediately be followed by its opposing event, thus the mitochondrial morphology and copy number are extremely dynamic [34]. Mitochondrial morphology and distribution responds to the specific energetic requirements of a specific cell type. One such example

is seen in sperm mitochondria. These mitochondria fuse at the base of a sperm flagellum to provide the high amount energy required for cellular movement.

Mitochondria production cannot occur *de novo*, and consequently a balance of opposing fusion and fission events must take place in addition to phospholipid biosynthesis and mtDNA replication to yield new mitochondria [32].Research suggests a single protein, Drp1 (Dynamin-related protein 1), is the master regulator of cytoplasmic mitochondrial fission [35, 36]. Drp1, an 80 kDa protein, polymerizes spirally around mitochondria.

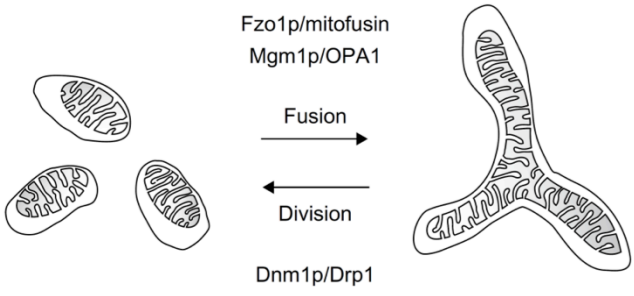

Figure 12. Mitochondrial dynamics. Mitochondrial copy number, morphology, and distribution are regulated by the rate of mitochondrial fusion and fission. An increased rate of division would result in a high number of small mitochondria, whereas an increased rate of fusion would result in larger, branched mitochondria. These processes are influenced by three dynamin GTPases, mitofusin, Mgm1p/OPA1, and Dnm1/Drp1 [32].

This protein contains an amino terminal GTPase domain, a middle "neck" domain, and a C-terminal GTPase effector "head" domain. The GTPase domain regulates Drp1 binding between opposing outer mitochondrial membranes. Following GTP hydrolysis the membranes constrict and this leads to membrane scission. This constriction ultimately

results in two genetically identical mitochondria. It is hypothesized Drp1 pinches off the outer membrane only after several molecules of itself interact to form filaments. This interaction results in a mechanical ring-like structure that utilizes GTP. Since Drp1 contains the intrinsic ability to split mitochondrial membranes, it should be of no surprise that it is highly regulated by other proteins.

One such regulatory protein is Fis1 [32,33,35]. RNA interference of Fis1 significantly decreases the concentration of Drp1 on the mitochondrial surface. The exact method of Drp1 action has not been elucidated, but research indicates Fis1 recruits Drp1 to the outer membrane where the dynamin related protein then anchors to the mitochondria via the actions of proteins that have not yet been isolated [36]. The association of Drp1 with Fis1 is not the only mammalian method of regulation. Drp1 can be post-transcriptionally modified via reversible methods of phosphorylation, ubiquitination, S-nitrosylation, and SUMOylation [35, 36]. These modifications not only alter the activity of the 80 kDa protein, but it can also change its intracellular localization and dynamics. For instance, Drp1 phosphorylation via Calcium/calmodulin-dependent protein kinase or ROCK1 increases its concentration on the surface of mitochondria, and thus increases the frequency at which fission occurs. In contrast, if cAMP-dependent protein kinase phosphorylates Drp1 at the same location, Drp1 activity is completely inhibited. Ubiquitination, mediated by E3 ligases (MARCH5, and Parkin) also regulates Drp1 activity [35, 36]. This method of regulation is not well understood, but it is apparent that Parkin ubiquitination of Drp1 targets it for proteasomal degradation. Degradation of Drp1, predictably, greatly diminishes the rate of fission.

Human mitochondria contain an outer and an inner membrane, and each of these membranes utilizes its own distinct fusion machinery. Interestingly, fusion of two separate, but comparable, membranes requires the activity of dynamin family proteins, such as mitofusins (Mfn1, Mfn2) for the outer and OPA1 for the inner mitochondrial membranes [36]. Mfn2, which crosses the outer membrane twice, contains two heptad repeat regions (HR1, HR2), that can increase the likelihood of fusion and mediate fusion by interacting with other Mfn proteins in a homotypic reaction [36]. This reaction requires the hydrolysis of GTP as the C-terminal coiled-coiled domains of mitofusins interact with those from other mitochondria. In a similar manner, OPA1, of the inner mitochondrial membrane, also interacts homotypically between the GTPase effector domains of OPA1 molecules from other mitochondria [35,36]. This contact mediates tethering and merging of the inner membranes. In mammals, OPA1 messenger RNAs are alternatively spliced, and this splicing generates 8 distinct isoforms of the protein [35]. It is thought these isoforms may play a role in cell-type specific regulation of OPA1, but the reason behind these alternative forms must be elucidated with further research. Moreover, researchers are currently searching for a protein that may regulate coordination between the two membranes, and the current hypothesis is that it may be a similar protein to that of Ugo1p [35]. This protein has been isolated in *Saccharomyces cerevisiae* and is known to form a complex and thus couple the yeast forms of mitofusin and OPA1. Membrane lipids, such as mitoPLD, are also important components in membrane fusion. mitoLPD converts cardiolipin, from the inner mitochondrial membrane, into phosphatidic acid, which is assumed to be transferred from the inner to the outer mitochondrial membrane at the fusion site [36]. As two mitochondria mechanically couple at the site of fusion,

fragmentation can arise when a cell lacks either one of the inner or outer mitochondrial membrane components. It is believed this coupling exists so the inner membrane proteins may rapidly interact with mtDNA immediately following fusion.

Mfn may also participate in fission when it binds to a specific domain within Drp1 [35,36]. The GTPase N-terminal domain of mitofusin, as well as its C-terminal tail, faces the cytosol In addition, Mitochondrial Fission Factor (Mff) acts as an anchor because it is C-terminally attached to the outer mitochondrial membrane and projects its N-terminal domain outward into the cytoplasm. Two N-terminal repeats of MFF bind with Drp1 and anchor it to the outer membrane. Mff does not appear to be the only protein required for the proper recruitment of Drp1 to the membrane. Mitochondrial dynamics proteins 49 and 51 are also anchored to the mitochondrial membrane, but these appear to do so via their N-terminal domains and therefore their C-terminal end protrudes outward [34]. MTP18, located within the inner mitochondrial membrane, and GDAP1, of the outer mitochondrial membrane, also participate in mitochondrial fission, but their actions are yet to be elucidated [37].

Mitochondrial DNA replication

It is believed mtDNA does not replicate in the conventional coupled leading and lagging- strand replication fork model seen in nuclear DNA. There are currently several eukaryotic mtDNA replication models. Only three, the asynchronous strand-displacement model (SDM), the strand-coupled bidirectional replication model, and the RNA incorporation throughout the lagging strand (RITOLS) model, will be examined in greater detail [18, 38, 39]. Human mtDNA, like that of T. brucei, is arranged in covalently closed circles of DNA. Almost all of the non-coding bases of human mtDNA

are concentrated within a single region of about one kilobase in length (**Figure 13**; 40). This NCR contains cis-elements essential for transcriptional control, and also contains two free 5' ends of DNA, 11 kilobases apart on separate strands, that are the putative replication origins. David Wolstenholme and colleagues first proposed the asynchronous strand displacement model in the 1960s. This laboratory generated electron micrographs of replicating rat mtDNA that were both fully duplex and others that contained single-stranded intermediates. These observations led to the hypothesis of a step-wise model of mtDNA replication.

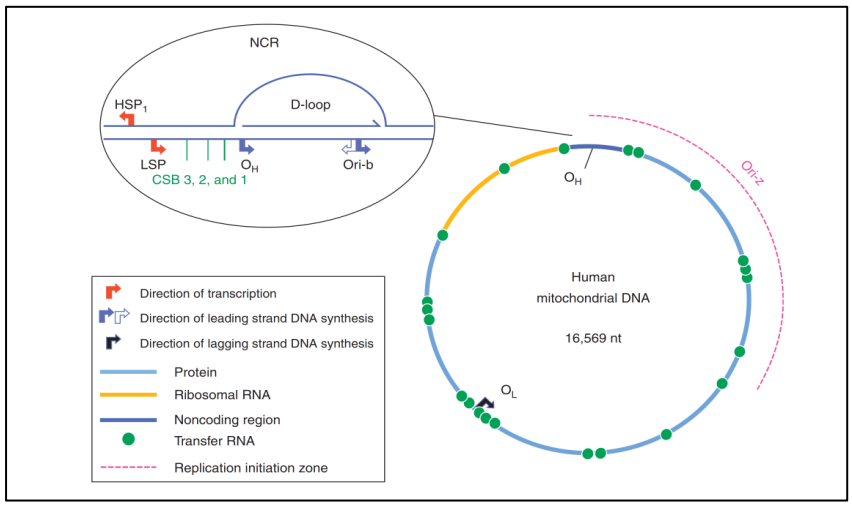

Figure 13. Human mitochondrial genome replication initiation sites. The current hypothesized mtDNA replication initiation sites. OH, OL, and Ori-B are major DNA replication initiation sites for the RITOLS replication model. In the conventional bidirectional replication model, replication can initiation anywhere within Ori-Z [18]

The strand displacement model suggests leading strand (5' to 3') synthesis initiates at a single point (OH) and unidirectionally proceeds around the circular molecule, while displacing the parental heavy strand (H-strand). This continues about two-thirds of the

way around the circular molecule before lagging strand synthesis begins at (OL) in the opposite direction.

This strand-displacement model was not challenged for several decades, and consequently was believed to be the primary method of mtDNA -replication. With the use of atomic force microscopy on mtDNA replication intermediates Brown et al., revealed there are several initiation sites on the lagging light strand [38]. It was not until a method of analysis able to resolve DNA molecules on the basis of mass and shape, two-dimensional neutral/neutral agarose gel electrophoresis (2DNAGE), was utilized that the aforementioned SDM was challenged. In 2000, Holt et al. utilized 2DNAGE in conjunction with a nucleic acid modifying enzyme that degraded single stranded DNA molecules to analyze mtDNA replication intermediates. Exploiting this method of investigation, Holt generated replicated intermediates that were inconsistent with SDM. These nuclease resistant intermediates were thus hypothesized to be the products of a coupled leading and lagging strand model of mtDNA synthesis, known as strand-coupled DNA synthesis. An issue arose wherein these intermediates obtained by Holt et al. were believed to be in a minority compared to the partially single-stranded products, and consequently the strand-displacement continued to be the assumed primary method of mtDNA replication.

In 2002, Yang et al. obtained unusually large and highly purified DNA particles in place of the aforementioned single stranded products that were manufactured from slow-moving arc DNA intermediates. At first these intermediates were believed to be consistent with those of SDM, but additional examination by Yasukawa et al. revealed intermediate masses consistent only with duplex DNA products [41]. These double

stranded DNA fragments were further scrutinized, and mtDNA was subject to endonuclease enzymatic digestion. The enzyme utilized, *BclI*, was chosen due to the fact it is one of the few endonucleases that could cut double-stranded and single-stranded DNA and thus could distinguish between SDM (absence of slow moving replication fork arcs) and strand-coupled DNA products (presence of slow moving replication fork arcs). *BclI* digested mtDNA revealed unexpected results. The observed novel arc products were of the same mass and shape of duplex DNA, but were not digested by the endonuclease on one branch, and thus the intermediates could not be explained by either replication model proposed to date. Since *BclI* was known to digest double-stranded DNA, and the products obtained were of the expected mass of duplex DNA, it was hypothesized the digestion was absent because RNA was present within the duplex. Consequently, mtDNA was subject to RNase H digestion, an enzyme proven to degrade RNA only if bound to DNA, ran on 2-D agarose gels, extracted, denatured, ran on 1-D and re-hybridized to reveal L-strand RNAs of 200-600 nucleotides in length. This experiment supported a new model of mtDNA replication RITOLS, wherein RNA is incorporated into the lagging strand of the circular mtDNA during replication.

The current hypothesis is that a lagging strand is initially complementarily bound to the displaced parental strand as preformed RNA, and then replaced by DNA via RNA polymerase or a novel primase in a maturation step of mtDNA replication (**Figure 14**; ref. 40). The mitochondrial transcription machinery uses the light strand promoter (LSP) to produce mRNA and tRNA molecules, but also the RNA primer required for OH leading-strand synthesis, Physiologically, the binding of transient lagging strand RNA to the displaced DNA strand does provide the added benefit of protecting the DNA strand from

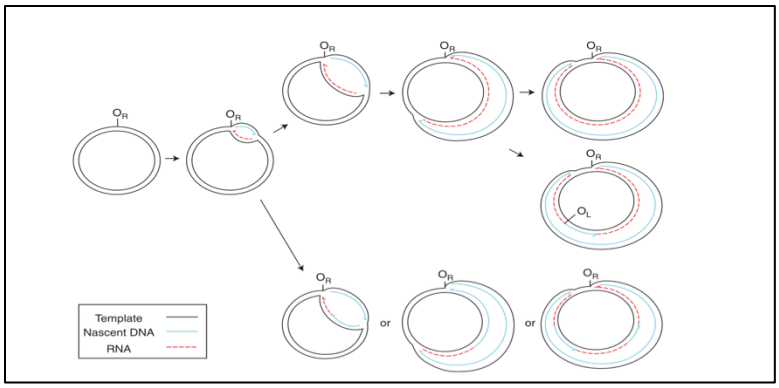

Figure 14. RITOLS method of mtDNA replication. Here, the replication origin is shown to initiate at a generalized OR site, but it in actuality it would initiate at OH or Ori-b. Leading strand

degradation, and it also could act as a RNA back-up as mtDNA Polymerase γ contains intrinsic reverse transcriptase abilities. Yasukawa et al. proposed the OL is the region of maturation initiation since data in chicken and mice indicated the lagging strand RNA, which was adjacent to the unidirectional origin, was the last to be replaced after completion of leading strand synthesis [41]. The 5' to 3' swapping of RNA for lagging strand DNA initiated at the final site of RNA synthesis of this lagging strand. Yasukawa also proposed the mitochondrial isoform of RNase H1 is a candidate processive protein of the maturation process simply because it is absolutely required for mammalian mtDNA maintenance. In addition, RNase H1 was shown to be processive by Gaidamkov et al. in 2005. Furthermore, biochemical evidence in mammalian mitochondria showed that RNA polymerase (POLRMT) is the primase for the lagging strand synthesis of the mtDNA molecule. Although this method of priming DNA synthesis in the absence of a true primase is relatively rare, it is not unprecedented. For example, *Escherichia coli* is known to contain an RNA polymerase that synthesizes a transcript that is used to prime replication of the single-stranded genome of the bacteriophage M13. Yasukawa showed POLRMT efficiently transcribes 400 nucleotide long regions of double stranded DNA,

but is only able to generate RNA fragments of 25-75 nucleotides long. These short RNA fragments were able to act as primers for DNA polymerase γ *in vitro*, and initiate DNA synthesis in the presence of mitochondrial single stranded binding protein (mtSSB). After further experimentation, Yasukawa demonstrated the combined action of DNA polymerase γ, mtSSB, and the TWINKLE helicase *in vitro* supported mtDNA leading strand synthesis on a double stranded template, and further addition of POLRMT to this scheme led to simultaneous leading- and lagging-strand synthesis [30, 40]. Although the source of the RNA and the mechanism of its incorporation into the lagging strand have not been discovered, it is logical to hypothesize the RNA may arise from a primase that incorporates an RNA primer into the duplex DNA. Alternatively, the RNA could be derived from preformed transcripts which are threaded onto the lagging strand template. It has been predicted, by 2DNAGE analysis, that at least 80% of the lagging strand may be composed of RNA. If RNA were to act as an mtDNA replication primer this percentage would certainly be much smaller [42]. It is important to note the RITOLS model shares commonalties with that of the SDM. For instance, both models predict a concomitantly synchronized model of leading and lagging-strand synthesis, define the NCR as the origin-containing region, and both mark OL as the initiation site of lagging strand synthesis. Consequently it appears as though each model of mtDNA replication is not mutually exclusive, and aspects of each may be correct. Future research is required and should be able to illuminate the actual mechanism of mtDNA replication in animals/eukaryotes.

Trypanosoma brucei nuclear DNA replication and regulation

Subcellular structures and organelles like (the kinetoplast (mitochondria), endoplasmic reticulum, Golgi apparatus, and flagellum) exist in single copies in G1 phase and are only duplicated at distinct times in the cell cycle [43, 44]. Trypanosoma brucei follows the typical eukaryotic DNA replication process and cell cycle regulatory scheme of G1/S/G2/M.

The genome of *T. brucei* encodes 10 cyclins and 11 Cdc2-related kinases (CRKs) [43, 44]. RNA interference experiments have shown CYC2 and CYC4 likely participate in the G1/S transition as cells that down-regulated these cyclins became arrested in G1. Research also points to CRK1 and CRK2 as the two Cdc2-related kinases that assist in the G1 to S progression through their interaction with CYC2 and/or CYC4. Again, this system mimics that of the animal cyclin/CDK system, but does appear to be more complicated than previously thought.

In G1, replication licensing of DNA occurs, wherein DNA replication origins become occupied by several proteins. Generalized eukaryotic licensing begins when DNA is bound by six related AAA+ ATPases, Orc1 though Orc6, which together form the origin recognition complex (ORC). Upon ORC binding, Cdc6 is recruited which then loads Cdt1 and the Mcm2-7 hexameric mini-chromosome maintenance complex. The two complexes, Cdc6, and Cdt1 together form the pre-replicative complex (pre-RC). DNA replication initiation is then free to occur and is triggered by S-phase CDK (S-CDK) and Dbf4-dependent kinase (DDK) or CDK7. S-phase cyclin dependent kinase then phosphorylates Sld2 and Sld3, which recruits Dpb11. Dpb11 binding initiates loading of the preloading complex (pre-LC) onto the origin. The pre-LC consists of Sld2, Dpb11,

the GINS complex (Sld5, Psf1,2, and3), and the leading-strand DNA polymerase ε. Dpb11 appears to act as a bridge between a Sld3-Cdc46 complex with Sld2, of the pre-LC, after it is recruited to the location via a Cdc45 mediated interaction with the Mcm2-7 complex. This Cdc45/Mcm2-7 complex is formed only after subunits of its N-terminal tails are phosphorylated by a Dbf4-Cdc7 complex, or CDK7. Since Cdc45 has been shown to interact with DNA Pol α-primase it is believed to be the protein that recruits the primase to the pre-LC. After the pre-LC has completely loaded onto the origin, Cdt1 is then phosphorylated and subsequently degraded by the 26S proteasome after phosphorylation. The CMG complex (Cdc45/Mcm2-7/GINS) and DNA polymerase remain at the origin, after Dpb11, Sld2, and Sld3 detach. The lingering proteins initiate DNA replication as it moves along the replication forks and unwinds the double-stranded DNA.

Trypanosomes contain at least five highly divergent ORC proteins (TbOrc1/Cdc6, TbOrc1b, TbOrc4, Tb3120, and Tb7980), but their importance is yet to be determined. It is known, however, that the CMG complex interacts *in vitro/in vivo* with TbOrc1/Cdc6 and Tborc1b via Mcm3. That being said it is unclear how the CMG complex is actually recruited to the replication origin as Cdc6 forms a component of the ORC with TbOrc1 and TbOrcb, and is thus unlikely to form a complex with Cdt1 as occurs in other eukaryotes. Another interesting quandary is that Mcm2-7 after DNA replication initiation does not appear to be exported out of the nucleus, as in yeast, but instead it has been shown to remain in the nucleus throughout the cell cycle. Cdc45, uniquely, is believed to be exported out of the nucleus after replication. Thus because of the highly divergent proteins and the apparent variation of protein location/activity in relation to that

of other eukaryotes it appears from early data that trypanosomes may have evolved their own mechanism of DNA synthesis initiation (**Table 1**).

Due to the apparent discrepancies in DNA initiation between *T. brucei* and other eukaryotes outlined thus far, Ziyin Li proposed a mechanism of *T. brucei* licensing (**Figure 15**). Licensing of the origin occurs in G1 of the cell cycle when one, or possibly even several, of the highly divergent ORC proteins bind the origin. The pre-replication complex is formed when Mcm2-7 binds the origin via a Mcm3-mediated interaction with TbOrc1/Cdc6 and TbOrc1b. Once the cell enters S phase, Cdc45 and the GINS complex are recruited by Mcm2-7 to the origin, which results in the complete formation of the CMG complex. At this juncture because the CMG complex contains DNA helicase activity, DNA replication is primed to initiate once DNA polymerase binds at this site. This entire complex moves along the

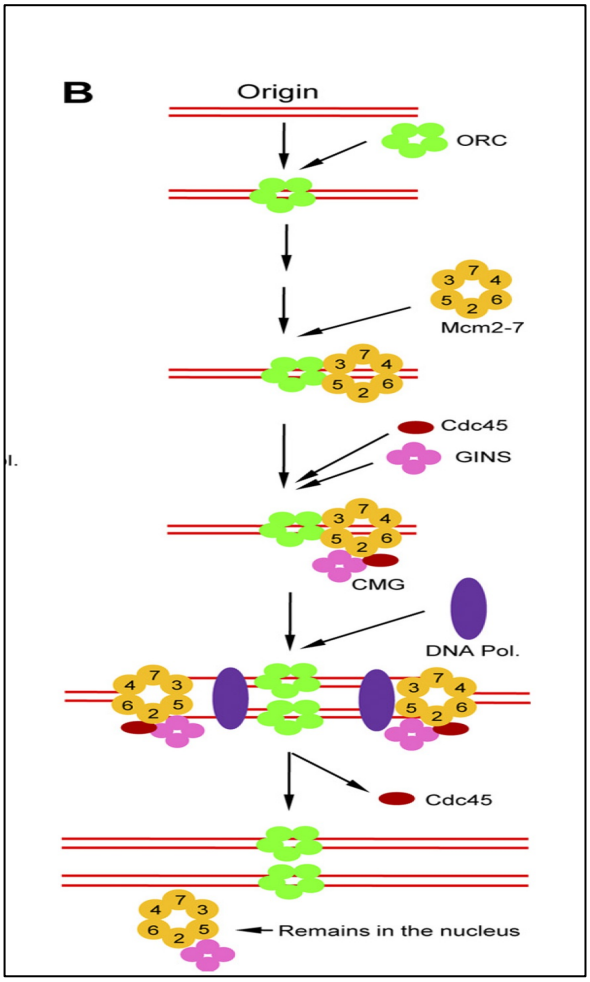

Figure 15. Proposed mechanism of *Trypanosoma brucei* DNA synthesis licensing and initiation. See text for details

DNA strand, through the replication forks, and synthesizes a nascent strand of DNA. At the conclusion of DNA replication, Cdc45 is excluded from the nucleus of *T. brucei* [44]. The interplay of other, unidentified homologous proteins identified in other eukaryotes (homologs of PCNA, TLK, Asf1, etc.) await detection and characterization.

The G2 to M transition in humans appears to be primarily regulated by CDK1 interactions with A- and B-type cyclins [43, 44]. The key regulator of this switch is actually the CDK1-cylclin B complex. CDK1 is inactive in G2 due to its phosphorylation by Wee1 and Myt1, but Cdc25-mediated dephosphorylation fully activates CDK1. Polo-like kinase (PLK) not only activates Cdc25, but also prompts degradation of the CDK1 inhibitor, Wee1. The most critical step in the human G2 to M transition is the activation of PLK and Cdc25 by Aurora A kinase-mediated phosphorylation.

Present research has not been able to elucidate the details of the G2/M transition in *T. brucei,* but it is generally assumed it requires the actions of two CRKs (3,9) and at least three cyclins (CYC6, CYC2, and CYC8). CRK3 and CYC6, of *T. brucei*, also look as if they pair with other proteins just as their eukaryotic homologues. CYC6 has been shown to pair with CRK9 and this interaction has been shown to be essential for the G2/M progression. Also, the CYC2 interacts with CRK3 and activates this regulator protein. As in the similar case with the previously mentioned ORC proteins, the *T. brucei* genome has not been able to illuminate homologs of such proteins as Myt1 and Cdc25, and consequently it is believed these proteins are present, but highly divergent. Nonetheless, homologues of the Wee1 and Polo-like kinases have been identified using this method of genome analysis. Intriguingly this PLK homolog, TbPLK, is excluded

from the nucleus throughout the cell cycle, and does not appear to be mandatory for mitosis [44].

Once mitosis has initiated, all the organelles and DNA within the nucleus and kinetoplast must be split evenly between the two daughter cells. *T. brucei* undergoes a closed mitosis wherein the nuclear membrane remains intact through mitosis [45]. Just as in eukaryotes, this parasite utilizes microtubule organizing centers (MTOCs), but it does lack centrosomes. Consequently, the proteins required for centrosome-mediated spindle assembly, such as Aurora A kinase and kinesin-5 are absent from the genome of *T. brucei*. Even though a kinesin-5 homolog is absent, *T. brucei* does appear to contain two kinesins, TbKIN-A and TbKIN-B, which are hypothesized to participate in spindle assembly [43, 44].. These two kinesins have been shown to interact with TbAUK1 and TbKif13-1, a member of the kinesin-13 family of proteins where they concentrate in the nucleus as well as the spindle. The kinesin-13 protein contains microtubule depolymerase activity. RNAi of TbKif13-1 resulted in spindle assembly defects and complications in chromosomal segregation. Several other proteins are believed to play a role in chromosomal segregation, and are localized to the mitotic spindle, such as TbNOP86, but much is yet to be exposed on this subject matter. Not surprisingly then, those proteins involved in assembly of the bipolar spindle via chromatin-directed pathways, such as Aurora B kinase (TbAUK1) and MCAK, have been isolated and found to be required for spindle assembly. TbAUK1 forms a chromosomal passenger complex (CPC) with TbCPC1 and TbCPC2. These two CPC proteins are novel and do not share sequence homology with similar proteins, such as INCENP, Survivin, and Borealin, of other eukaryotes. The CPC of *T. brucei* displays dynamic localization from mitosis through

cytokinesis as it has been localized near the chromosomes, to the central spindle, to the anterior tip of the flagellum attachment zone at the time of the mitosis to cytokinesis transition. Near culmination of cytokinesis CPC is last seen along the cleavage furrow between the two separating daughter cells. These passenger proteins in animals form a single complex that act as substrate activators of Aurora B, in addition to INCENP and TLK, which localizes the protein to subcellular locations throughout the cell cycle. TLK was thought to be specific to multicellular animals, however, *T brucei* actually expresses two homologs of TLK, TbTLK1 and TbTLK2, but their actions have not yet been as extensively studied.

All the components of the APC/C E3 ubiquitin ligase components required for metaphase to anaphase progression are expressed within the genome of *T. brucei.* Only two of the components, APC1 and Cdc27, appear to be essential. Similar to other eukaryotes, APC/C in trypanosomes is believed to degrade Securin. Securin degradation results in the activation of Separase. Separase homologs as well as other components of the cohesion complex (SMC1, SMC3, SCC1, and SCC3) are all present within the genome, and are required for segregation of the chromosomes. Homologs of Securin have not been isolated, and are believed to exist as vastly divergent proteins with a similar function. RNA interference of the two subunits arrested the procyclic form in metaphase and that of the bloodstream form in anaphase, it would appear as though this complex is active at different times dependent upon *T. brucei's* life cycle stage. Other proteins/mechanisms involved in the metaphase to anaphase transition are yet to be determined.

The final step of cell division, the mitosis to cytokinesis transition arises after completion of the G2 to M transition. As has been the global situation thus far, the mechanisms underlying cytokinesis in eukaryotes and *T. brucei* are not well understood. However, scientists believe the core proteins and mechanisms of initiation and completion of cytokinesis have been well conserved throughout evolution [44]. That being said, in animals, the central spindle defines the cleavage plane, and the ensuing furrow is a contractile ring composed of actin and myosin II. Two cooperative proteins, Aurora B and Polo-like kinases, are concentrated on the central spindle and act as regulators of the centraspindlin complex. This complex is initially composed of MKLP1 and the Rho GTPase-activating protein [43,44], MgcRac-GAP. Ect2 is then recruited to the centraspindlin complex, where it activates the RhoA GTPase [44]. Activation of RhoA, in turn, results in the activation of the abovementioned contractile ring. The construction and activation of the contractile ring is important, as this complex is required to pinch off and separate a single large cytoplasm, which results in the establishment of two daughter cells [44].

Since cleavage plane development in *T. brucei* is determined by the position of the nascent flagellum and the FAZ, it is of no surprise the mechanism of division is vastly different from that of their human host. In this unicellular parasite, division is initiated at the anterior tip of the new FAZ and as a consequence the furrow ingresses from the anterior to the posterior end of the cell [46]. *T brucei* appears to lack a contractile ring. However, it is believed there is a comparable, but novel, structure that could perform similar duties. TbAUK1 appears to play a role in furrow ingression, and regulates the initiation of cytokinesis, furrow ingression, and abscission. In addition,

TbPLK is also believed to promote cytokinesis initiation. Both of these proteins have been localized to the active anterior tip of the new FAZ in trypanosomes. Their homologs have been localized to the central spindle in animals, which is why each of them are believed to play, an as of yet, unknown role in trypanosome cytokinesis. Because *T. brucei* appears to lack Aurora B and Polo-like kinase homologs it is assumed TbAUK1 and TbPLK must initiate cytokinesis via other pathways. Nevertheless, it is likely these two proteins initiate cytokinesis via the regulation of common protein factors at the FAZ anterior tip. This appears to currently be the extent of available knowledge regarding the proteins involved in trypanosome cytokinesis, but other studies have hinted at other possible players. For instance, RNAi of TbRHP, a RhoA-like small GTPase, and RhoGAP resulted in spindle formation defects and faulty cytokinesis. As a consequence, identification of the Rho-A protein and its partner suggests a Rho-A mediated cytokinesis pathway is present, likely downstream, of the aforesaid TbAUK1 and TbPLK proteins. In addition, it is hypothesized TbRHP interacts with TRACK during cytokinesis. TRACK is a known regulator of the initiation and progression of cytokinesis in trypanosomes. Keep in mind that regulation of initiation, progression, and completion of cytokinesis in animals involves upwards of 100 proteins, therefore it is likely much more is to be learned regarding this process in *Trypanosoma brucei.* With that being said, recently several proteins have been shown to be essential for cytokinesis in trypanosomes such as type III phosphatidylinositol 4-kinase, KMP-11, arginine methyltransferase, ARL2, Katanin and Spastin, CFB2, AIR9, and TbKIN-C/TbKIN-D. It is obvious much more research is required not only in regards to proteins involved in cytokinesis in animals and trypanosome, but in all phases of the cell cycle. There are certainly a lot of unanswered

questions, but the completion of *Trypanosoma brucei's* genome sequence, along with other advances in technology and methods, a lot has been learned regarding the cell cycle of this unicellular parasite within the past decade.

Kinetoplast DNA replication

The mtDNA of the *T. brucei* kinetoplast, kDNA, is copied in mitochondrial S phase as it doubles in size, and then subsequently splits in half as one copy is destined for each daughter cell [47]. Proper arrangement of the kDNA network prior to and during cytokinesis is paramount, and many proteins play a role in this phenomenon. This entire process is just now beginning to be elucidated. It is estimated kDNA replication may include upwards of 150 distinct proteins [48].

The initial step of kDNA replication is the release of each covalently closed mini-circle from the central kDNA network, followed by its assisted migration to the kinetoflagellar zone (KFZ). The mini-circles are elongated and catenated as they are attached to neighboring maxi and mini-circles (**Figure 16**). With the use of ethidium bromide time lapsed staining of the kinetoplast, a gradual decrease in the size of the central zone and an enlargement of the antipodal sites is seen. This migration progresses to eventually form an overall dumb bell shape as the mini-circles concentrate on the sides and the maxi-circles in the center. Once the mini-circles are inside the KFZ, unidirectional theta replication occurs via the actions of UMSBP, Pol1B, and several other proteins. Each mini-circle contains a UMS which is the binding site for the universal mini-circle binding protein (UMSBP). *T. brucei* contains two UMSBP proteins. These proteins are located within two distinct locations of the KFZ where mini-circle replication begins. The universal mini-circle binding protein will bind with the single-

stranded UMS of the template strand, or with the complementary strand of the hexamer. The hexamer is a sequence that is at the start of the first Okazaki fragment on the lagging strand of DNA. Although the function of UMSBP binding to the UMS is obvious, its function once it is bound to the hexamer site is has not been elucidated. Another protein hypothesized to participate in kDNA replication initiation is p38. Interestingly, p38 is an abundant protein that also binds to the template strand of the mini-circle replication origin, in addition to the complementary strand of the hexamer. Some have speculated p38, rather than UMSBP, is the origin recognition protein. However, studies have shown p38 concentrates at the antipodal sites, which is not where replication is believed to occur.

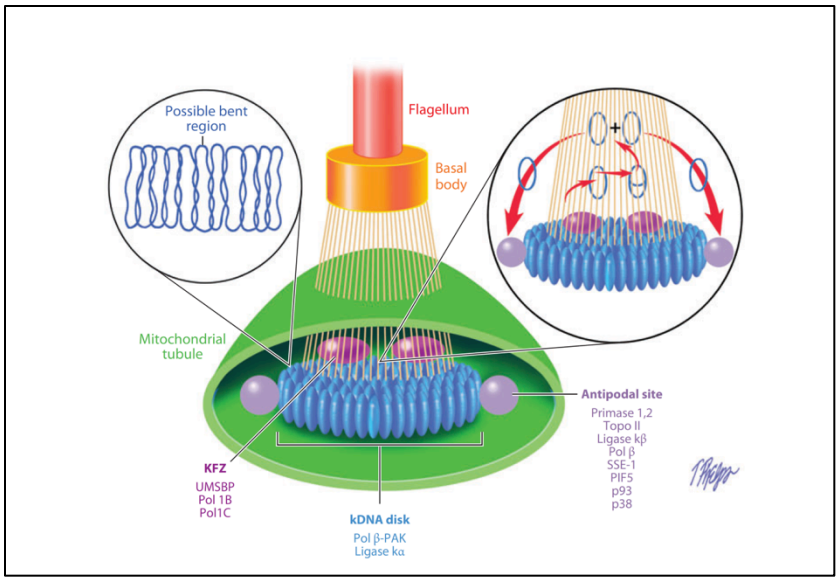

Figure 16. Significant kinetoplast replication sites and proteins. See text for additional information. The maxi-circles remain at the centralized disk. The mini-circles are freed from the disk and migrate to the KFZ. At the KFZ, theta replication occurs, and after replication the mini-circles relocate to the antipodal sites. Here, after cleavage of the thin maxi-circle threads, the two kDNA networks are ripe for segregation [48].

This observation only further clouds p38s actual function within *T. brucei*. In 2012 Jensen and Englund hypothesized p38 assists with the binding of DNA helicase and a topoisomerase, which results in helix unwinding. This was hypothesized because RNA interference of p38 resulted in kDNA loss and the accumulation of extremely under-wound DNA. In addition to UMBSP and p38, kDNA replication requires the assistance of at least seven DNA polymerases. As previously stated, mtDNA replication in eukaryotic mitochondria requires DNA polymerase γ. In contrast, this process in *T. brucei* does not utilize DNA polymerase γ. The polymerases required for viability in the kinetoplast, specifically in the KFZ, are Pol IB and Pol IC. Pol ID is also required for replication, but interestingly localizes to the antipodal sites during S phase. Additionally, a fourth DNA polymerase, DNA Pol IA, is believed to act as a repair enzyme and is distributed throughout the kinetoplast. The fifth and six DNA polymerases, located within the antipodal sites, are DNA polymerase β-like enzymes that, with DNA ligase Kβ, fill in the gaps at the end of kDNA mini-circle replication. Three mitochondrial topoisomerases (types IA, IB, and II) are encoded within *T. brucei's* genome. RNAi knockdown of topoisomerase II leads to a generalized loss of kDNA and an accumulation of gapped mini-circles. Thus it has been hypothesized that topoisomerase II plays a role in the reattachment of released and replicated mini-circles to the network. A reoccurring theme in *T. brucei* protein function is that multiple proteins may exist that perform a similar function, as shown previously with the seven DNA polymerases, three topoisomerases, and also by the fact that there are eight helicase proteins that are encoded within the genome of *T. brucei.* Of these eight helicases, only three (TbPIF1, TbPIF2, and TbIF8) are essential to cell viability. TbPIF1 plays an unknown role in mini-circle

replication, and TbPIF2 appears to perform a role in maxi-circle replication. Although TbPIF8 has been shown to be essential via RNAi, it is not believed to have helicase activity due to the fact it is considered to be highly divergent to its eukaryotic homologue. The last characterized helicase (TbPIF5) plays a role in Okazaki fragment primer removal. There have been two characterized primases in *T. brucei*, Primase 1 and Primase 2. Primases are the enzymes that synthesize RNA primers complementary to a strand of DNA due to the fact that DNA polymerases cannot initiate DNA strand synthesis *de novo*. Primase 1, which is a maxi-circle primase, and Primase 2 both localize to the antipodal sites. Thus it appears many proteins are speculated to play a role in kDNA replication. Their mechanisms of action should be examined further once their roles in replication are proved or disproved.

Upon completion of mini-circle replication, each sister mini-circle migrates to separate antipodal sites of the mitochondrial tubule. These sites do not change position relative to the kinetoplast at any point during replication/cytokinesis, and are always polar to one another. This migration is referred to as the polar mechanism of replication, and ensures each daughter cell receives a complete single copy of the kinetoplast DNA. Okazaki RNA primer removal does not occur simultaneously with strand elongation, but rather occurs at the antipodal sites with the actions of SSE-1 and PIF5. Due to the removal of the RNA primers, gaps exist within the DNA strands. These gaps are filled in by Pol β and the most of the nicks are then sealed by ligase kβ. Topoisomerase II then reattaches each mini-circle to the network periphery within the antipodal site. Each successfully replicated mini-circle should contain at least one nick within its nucleotide structure. It is believed the nick exists as a marker that the mini-circle has been

replicated, so the cell has a way to distinguish replicated from non-replicated, covalently closed, circles. This would ensure each circle is replicated only once per generation. Upon completion of replication and subsequent migration to the antipodal sites, the last nick of each mini-circle is sealed by ligase Pol β-Pak, DNA ligase kα, and it is assumed other proteins would play a role in this as well. After the mini-circles have all been replicated and distributed between the two antipodal sites, this then leaves the central zone of the tubule concentrated only with maxi-circles.

Similar to nuclear DNA replication, the last step of kDNA replication is the equal division of the newly replicated DNA into its respective organelle. Once the last mini-circle is replicated and transferred to the antipodal sites, the two networks are essentially only joined by a thin thread of DAPI stainable maxi-circles. It is presumed the maxi-circle thread is simply cleaved by topoisomerase II, and this allows the two networks to disjoin. Due to their location at separate antipodal sites, this cleavage results in two identical kDNA daughter networks.

Control of kDNA replication

Three control mechanisms currently exist to explain how kDNA replication is regulated during the cell cycle. Unlike other eukaryotes, kinetoplast DNA replication begins before nuclear S phase, and its division occurs before that of nuclear division. The first control mechanism, mRNA cycling, is primarily centered on the messenger RNA of proteins involved in kDNA replication (helicase, topoisomerase, etc.) These mRNAs concentrations fluctuate throughout the cell cycle. The highest mRNA concentrations exist just after the initiation of S phase, and decline in the last two stages of the cell cycle. Messenger RNA concentration regulation occurs because of a cis-acting octomeric

sequence present in the un-translated regions (UTRs) of the mRNA, which can be bound by their respective trans-acting protein. Upon protein kinase binding to the UTR, the mRNA becomes less stable. A second regulatory mechanism of kDNA replication requires the assistance of an ATP-dependent protease that is localized within the kinetoplast. This protease, named TbHslVU, is hypothesized to control kinetoplast DNA synthesis via the degradation of a kDNA replication positive regulator. RNA interference of this protease results in an accumulation of 20-fold more mini-circles, and 3-fold higher concentration of maxi-circles in the kinetoplast. The RNAi data shows that TbHslVU participates in kDNA segregation and not in kDNA replication. Other positive regulators affected by HslVU may be negatively regulated by the actions of this protease. The third and final control mechanism is the redox regulation of the universal mini-circle binding protein (UMSBP). This is the first known example wherein redox control plays a role in DNA synthesis. UMSBP is fully reduced when active. This protein contains several cysteine-containing zinc finger domains. Once oxidization occurs and the zinc-depleted form of UMSBP exists through the conversion –SH to S-S, this protein is no longer able to bind the UMS and initiate mini-circle replication. The reduced active form of UMSBP has been shown to fluctuate in a time-dependent manner in harmony with the stages of the cell cycle within Crithidia fasciclata, a close relative of T. brucei. Two S-phase peaks of activity were observed with the active form UMSBP. Thus, it appears one or several interesting forms of kDNA replication regulation may exist within T. brucei.

kDNA Segregation

With the use of electron micrographs scientists have been able to show an intimate connection between the kinetoplast DNA and the proximal end of the flagellum

of T. brucei. Most eukaryotes utilize an actomyosin contractile ring for cytokinesis. T. brucei, however, relies heavily on a microtubule cytoskeletal and filaments that interact with organelle membranes and the surface [49]. Since T. brucei procyclic mutants apparently proliferate normally, it appears RNAi of actin demonstrates it does not play a major role in the formation of the distinctive cleavage furrow along the longitudinal axis. Although the furrow completely exists along the axis, ingression occurs directionally from the anterior to the posterior end of the parasite. Trypanosomes utilize two basal bodies, after the initial duplication event, as chauffeurs of segregation. The FAZ, flagellar attachment zone, appears to provide the structural information required for cleavage furrow positioning. For instance, trypanosomes with defects in the FAZ are either unable to initiate cytokinesis or generate multinucleated or enucleated cells. The first major step in cytokinesis is the formation of the new flagellum that protrudes from the flagellar pocket. The new flagellum follows, in a helical manner around the cell, the actions of the old flagellum as it continues to grow outward. Because the flagella are attached to separate basal bodies, as the new flagellum extends it appears to pull the two basal bodies apart (**Figure 17)**.

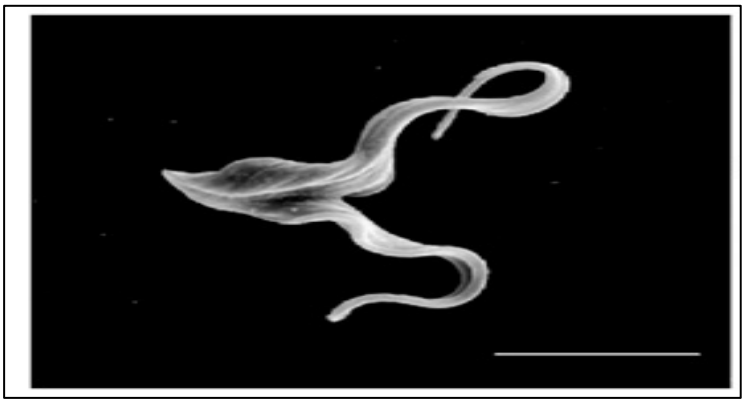

Figure 17. Cytokinesis in *Trypanosoma brucei*. Illustration of the new and old flagellum acting against each other during segregation [46]

Recent studies have revealed the tripartite attachment complex (TAC) links the kinetoplast to the basal bodies, and links the kDNA across the mitochondrial membranes to the proximal end of the basal bodies [47, 48]. This interaction exists because unilateral filaments running from one side of the basal body and through the kinetoplast membrane connect to the kDNA (**Figure 18**). Data then suggests the new basal body complex rotates around that of the old as the new flagellum continues to elongate, and stops once it has completed a 180° rotation [47]. This relocation ensures full kinetoplast segregation.

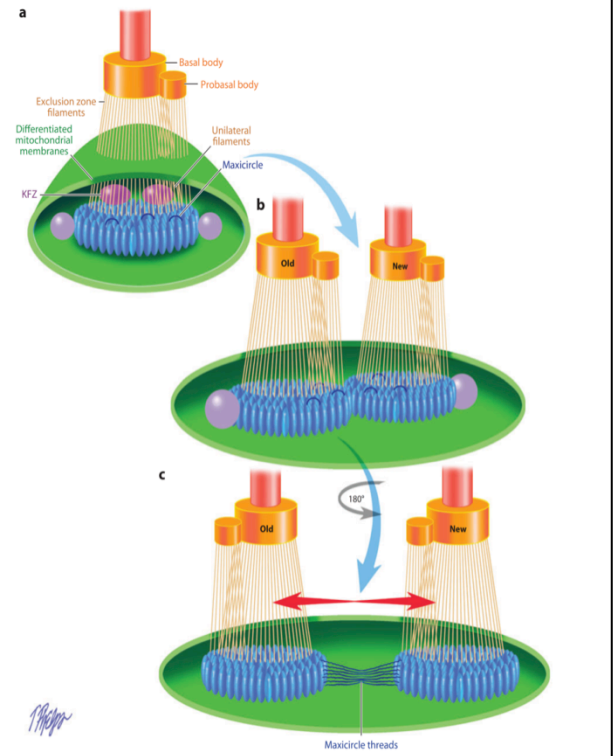

Figure 18. Mechanism of Tripartite attachment complex controlled kDNA segregation. A) The TAC is connecting to the kinetoplast to the basal body. B) The kinetoplast is undergoing replication and the TAC and Flagellum have duplicated. [47].

Until recently it was believed only morphological components such as the exclusion-zone filaments, mitochondrial membranes, and unilateral filaments of the TAC existed. However, a few molecular studies have shown there may also be three protein constituents [47]. The first of the three proteins is p166. This protein is thought to be a constituent of the TAC because RNAi causes cells to form oversized kDNA networks.

This is important as it suggests cells are capable of kDNA replication, but are not able to segregate and as a result one daughter cell is left without any kDNA while the other generally contains a network ten times its normal size. P166 has also been localized to the kinetoplast somewhere between the kDNA disk and the flagellar basal body. Mab22, a second possible protein component of TAC, was discovered using a collection of monoclonal antibodies to the kDNA-TAC complex, and its antigen localized to the exclusion-zone. The last TAC component, AEP-1, is the product of an alternatively edited mitochondrial mRNA of cytochrome oxidase subunit III (COXIII). This novel protein has been localized to the kinetoplast, and is believed to be distributed throughout this organelle. Immunofluorescence localized AEP-1 between the kDNA and the basal body, similar to that of p166. Also, similar to the RNAi product of p166, a truncated dominate-negative AEP-1 led to an increase in the concentration of cells that were deficient of a kDNA or those with an oversized kDNA network, and consequently it seems AEP-1 plays a crucial role in kDNA segregation.

CONCLUSION

The unicellular parasite, *Trypanosoma brucei*, is prevalent in rural foci of sub-Saharan Africa. Transmission of *Trypanosoma brucei* to its human hosts occurs after its vector, the tsetse fly, takes a blood meal. Without proper medical treatment, humans can suffer severe health complications from Human African Trypanosomiasis (HAT), which could ultimately result in death. Due to the fact HAT has surpassed HIV/AIDs as the biggest killer in several African countries, it should come as no surprise this parasite not only has lasting effects on human health, but also has severe negative economic complications on the region. To combat these ramifications research is essential to find healthy, inexpensive, and easily administrable alternative treatments for HAT, because the treatments currently available are extremely toxic and require several injections.

The kinetoplast of *T. brucei* is unique from the human mitochondria. *T. brucei* contains a single copy of its mitochondria with kDNA arranged in as chainmail, whereas humans generally contain hundreds of dynamic copies of mitochondria. In order to find and develop drugs for the treatment of HAT it is necessary to locate key differences between *T. brucei* and humans so these differences may be exploited. This review provides an overview of such differences in not only the mitochondrial organelles of humans and *T. brucei*, but also in their cell-division cycles. Even though these two organisms are eukaryotes, their cellular architectures are vastly dissimilar, and it is now

apparent that in many instances *T. brucei* has evolved novel molecular pathways and machineries that perform otherwise analogous activities. It is also clear that much of what has been covered in this review is only as good as the information that is available to date. Our understanding of *T. brucei* is still quite poor. More is known about these processes in animal, Therefore, future research on humans and trypanosomes will need to focus on the basic mechanisms and molecular players of each of the topics covered within this review.

Table 1. Trypanosome and human cell cycle gene homologs

Cell Cycle stage regulated	Trypanosome gene	Human homolog	Function
G1/S Transition	CYC2	None	G1 cyclin
	CRK1	Unknown	G1 CDK
	CRK2	Unknown	G1 CDK
DNA Replication and S-Phase progression	Orc1/Cdc6	ORC1	Origin Recognition Complex
	Orc1b	None	Origin Recognition Complex
	Orc4	ORC4	Origin Recognition Complex
	Tb3120	None	Origin Recognition Complex
	Tb7980	None	Origin Recognition Complex
	Mcm2-7	Mcm2-7	CMG component
	Cdc45	Cdc45	CMG component
	GINS	GINS	CMG component
	TLK1	TLK1, TLK2	Toulsed-like kinase
	Asf1A/B	Asf1A, Asf1B	Histone H3 chaperone
G2/M transition	CYC6	Cyclin B	Mitotic cyclin
	CYC8	Cyclin B	Mitotic cyclin
	CRK3	Cdk1	Mitotic CDK
	CRK9	None	Mitotic CDK
Mitosis	AUK1	Aurora B	Chromosomal passengers
	KIN-A	None	Divergent kinesin
	KIN-B	None	Divergent kinesin
	Kif13-1	MCAK	Kinesin-13
	APC, Cdc27	APC1, Cdc27	Anaphase-promoting complex
	SMC3, SCC1	SMC3, SCC1	Cohesion
	Separase	Separase	Protease
	NOP86	None	Nucleolar protein
Cytokinesis	PLK	PLK1	Polo-like kinase
	MOB1	MOB1	Unknown
	PK50, PK53	NDR1	Nuclear DBF-2-related kinase
	TRACK	RACK1	Rho kinase
	RHP	RhoA	RhoA-like GTPase
	CFB2	None	F-box protein
	ARL2	Arl2	Small GTPase
	AIR9	None	Microtubule-associated protein
	KAT80, KAT60	P80, p60	Katanin
	SPA	Spastin	Spastin

REFERENCES

1. Malvy D., & Chappuis F. (2011). Sleeping sickness. Clin Microbiol Infect 17, 986-995.doi:10.1111/j.1469-0691.2011.03536.x.

2. Anonymous (2012, October). Trypanosomiasis, Human African (sleeping sickness). Who.int. Retrieved February 15, 2013.

3. Baral, T. N. (2009). Immunobiology of african trypanosomes: need of alternative interventions. *J Biomed Biotechnol* 2010, 1-24. doi:10.1155/2010/389153.

4. (2011). What is HAT disease? Bvgh.org. Retrieved February 15, 2013.

5. Bacchi, C. J. (2009). Chemotherapy of human african trypanosomiasis. interdiscip *Perspect Infect Dis* 2009, 1-5. doi:10.1155/2009/195040.

6. Burri, C. (2010). Chemotherapy against human african trypanosomiasis: Is there a road to success? *Parasitology* 137, 1987-1984. doi:10.1017/S0031182010001137

7. Wilson, D. W., Tanious, F. A, Mathis, A., Tevis, D., Hall, J. E., & Boykin, D. W. (2008). Antiparasitic compounds that target DNA. *Biochimie* 90, 999-1014.

8. Lukes, J., Guilbride, D., L., Votypka, J., Zikowa, A., Benne, R., Englund, P. T. (2002). Kinetoplast DNA network: evolution of an improbable structure. *Eukaryot Cell* 1, 495-502.

9. Liu, B., Liu, Y., Motyka S. A., Agbo, E. E. C., & Englund, P. (2005). Fellowship of the rings: the replication of kinetoplast DNA. *Trends Parasitol* 8, 363-369.doi:10.1016/j.pt.2005.06.008.

10. Haran, Tali, & Donald Crothers. (1989). Cooperativity in A-tract structure and bending properties of composite TnAn blocks. *Biochemistry* 28, 2763-2767.

11. Tro, Nivaldo J. (2008). *Introductory Chemistry Essentials*. 3rd ed. Upper Saddle River, NJ: Prentice Hall.

12. Torri, A. F., Carpenter, L. R., & Englund, P. T. (1996). Kinetoplast DNA replication DNA replication in eukaryotic cells. *Cold Spring Harb Perspect Biol* 40, 1029-1042.

13. Stevens, J. R., Noyes, H. A., Dover, G. A., & Gibson W. C. (1999). The ancient and divergent origins of the human pathogenic trypanosomes, trypanosoma brucei and t. cruzi. *Parasitology* 118, 107-116.

14. Hong, M. , & Simpson, L.. (2003). Genomic Organization of trypanosome brucei in kinetoplast DNA minicircles. *Protist*. 154, 265-279.

15. Ntambi, J., Marini, J., Bangs, J., Hajduk, S., & Jimenez, H. (1984). Presence of bent helix in fragments of kinetoplast DNA minicircles from several trypanosomatid species. *Mol Biochem Parasitol* 12, 273-786.

16. Carrera, P., Fernando, A.. (1994). Structural characterization of intrinsically curved AT-rich DNA sequences. *Nucleic Acids Res* 22, 3671-3689.

17. Ohyama, T. (2001). Intrinsic DNA bends: an organizer of local chromatin structure for transcription. *Bioessays* 23, 708-715.

18. Holt, I. J., & Reyes, A. (2012). Human mitochondrial DNA replication. *Cold Spring Harb Perspect Biol* 1, 4(12). pii: a012971. doi:10.1101/cshperspect.a012971.

19. Yamano, K., & Youle R. J. (2011). Coupling mitochondrial and cell division. *Nat Cell Biol* 9, 1026-1027. doi: 10.1038/ncb2334.

20. Copeland, W. C. (2012). Defects in mitochondrial DNA replication and human disease. *Crit Rev Biochem Mol Biol* 47, 64-74. doi:10.3109/10409238.2011.632763.

21. Matthews, A., Keith. R. (2005) The developmental cell biology of trypanosoma brucei. *J Cell Sci* 118, 283-290. doi:10.1242/jcs.01649.

22. Trypanosomiasis. www.ilri.org. Retrieved March 3, 2013.

23. Heddergott, N., Kruger, T., Babu, S. B., Wei, A., Stellamanns E., Uppaluri, S., Pfohl, T., Stark, H., 7& Engstler, M. (2012). Trypanosome motion represents an adaptation to the crowded environment of the vertebrate bloodstream. *PLOS Pathog* 8:e1003023. doi:10.1371/journal.ppat.1003023.

24. Field, M. C., & Carrington, M. (2009). The trypanosome flagellar pocket. *Nat Rev Microbiol* 7, 775-786. doi: 10.1038/nrmicro2221.

25. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2008). *Molecular Biology of the Cell* (pp. 79-109). 5th Ed. New York, NY: Garland Sciences.

26. Adapa Project. (2013, January). What are the main organelles in a eukaryotic cell? Adapaproject.org. Retrieved March 5, 2013.

27. Kasiviswanathan, R., Collings, R. L. T., & Coepland, W. C. (2011). The interface of transcription and DNA replication in the mitochondria. *Biochim Biophys Acta* 1819:970-978. doi:10.1016/j.bbagrm.2011.12.005

28. Imoto, Y., Yoshida, Y., Yagisawa, F., Kuroiwa, & H., Kuriowa. T. (2011). The cell cycle, including the mitotic cycle and organelle division cycles, as revealed by cytological observations. *J Electron Microsc (Tokyo)* 1,S117-1136. doi:10.1093/jmicro/dfr034.

29. Takeda, D. Y., & Dutta, A. (2005). DNA replication and progression through S phase. *Oncogene* 24, 2827-2843.

30. Pinheird, D., & Sunkel, C. (2012). Mechanisms of cell cycle control. *Canal* 9, 4-17.

31. Den Haese G. J.,Walworth N., Carr A. M., & Gould K. L. (1995). The Wee1 protein kinase regulates T14 phosphorylation of fission yeast Cdc2. *Mol Biol Cell* 6, 371–85. PMC 301198. PMID 7626804).

32. Tamura, Y., Lijima, M., & Sesaki, H. (2010). Mitochondrial dynamics: fusion and division. *Handbook of Cell Signaling* (pp. 2499- 2503). 2nd ed. Waltham, MA: Academic Press.

33. Margineantu, D. H., Cox, W. G., Sundell, L., Sherwood, S. W., Beechem, J. M., & Capaldi, R. A., (2002). Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion* 1, 425-435.

34. Elgass, K., Pakay, J., Ryan, M. T., & Palmer C. S. (2013). Recent advances into the understanding of mitochondrial fission. *Biochim Biophys Acta* 1833, 150-161. doi:10.1016/j.bbamcr.2012.05.002.

35. Benard, G., & Karbowski, M. (2009). Mitochondrial fusion and division: regulation and role in cell viability. *Semin Cell Dev Bio* 20, 365-374.

36. Cerveny, K. L., Tamaru, Y., Zhang, Z., Jensen R. E., & Sesaki, H. (2007). Regulation of Mitochondrial Fusion and Divison. *Trends Cell Biol* 17, 563-569. doi:10.1016/j.tcb.2007.08.006.

37. Lackner, L. L., & Nunnari, J. M. (2009). The molecular mechanism and cellular functions of mitochondrial division. *Biochim Biophys Acta*1792, 1138-1144. doi:10.1016/j.bbadis.2008.11.011.

38. Brown, T. A., Cecconi, C., & Tkachuk, A. N.(2005). Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. *Genes Dev* 19, 2466-2476.

39. Holt, I. J., (2009). Mitochondrial DNA replication and repair: all a flap. *Trends in Biochem Sci* 34, 358-365. doi:10.1016/j.tibs.2009.03.007.

40. Clayton, D. A. (1996). Mitochondrial DNA replication. replication in Eukaryotic Cells. *Cold Spring Harb Perspect Biol* 39, 1015-1027.

41. Yasukawa, T., Reyes, A., Cluett, T. J., Yang, M. Y., Bowmaker, M., Jacobs, H. T., Holt, I. J. (2006). Replication of vertebrate mitochondrial DNA entails transient ribonucleotide incorporation throughout the lagging strand. *EMBO J* 25:5358-5371.

42. Fust, J. M., Wanrooij, S., Jemt, E., Granycome, C. E., Cluett, T. J., Shi, Y. ,Atanassova, N., Holt, I. J., Gustafsson, C. M., & Falkenber, M. (2010). Mitochondrial RNA polymerase is needed for activation of the origin of light-strand DNA replication. *Mol Cell* 37, 67-78. doi:10.1016/j.molcel.2009.12.021.

43. Hammarton, T. C. (2007). Cell cycle regulation in trypanosoma brucei. *Mol Biochem Parasitol* 153, 1-8.

44. Ziyin, L. (2012). Regulation of the cell division cycle in trypanosoma brucei. *Eukaryt Cell* 11, 1180-1190. doi:10.1128/EC.00145-12.

45. Ersfeld, K., Melville, S. E., & Gull, K. (1999). Nuclear and genome organization of trypanosoma brucei. *Parasitol Today* 15, 58-63.

46. Hammarton, T. C., Monnerat, S., & Mottram, J. C. (2007). Cytokinesis in trypanosomatids. *Curr Opin Microbiol* 10, 520-527. doi:10.1016/j.mib.2007.10.005.

47. Mckean, P. G. (2003). Coordination of cell cycle and cytokinesis in trypanosoma brucei. *Curr Opin Microbiol* 6, 600-607.

48. Jensen, R. E., & Englund. P. T. (2012). Network news: The replication of kinetoplast DNA. *Annu Rev Microbiol* 66, 473-491. doi:10.1146/annurev-micro-092611-150057.

49. Farr, H., Gull, K. (2012). Cytokinesis in trypanosomes. *Cytoskeleton* 69, 931-941. doi: 10.1002/cm.21074.