# CHAPTER 2

# Unique Characteristics of the Kinetoplast DNA Replication Machinery Provide Potential Drug Targets in Trypanosomatids

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## Reevaluating the Kinetoplast as a Potential Target for Anti-Trypanosomal Drugs

Kinetoplast DNA (kDNA) is a remarkable DNA structure found in the single mitohondrion of flagellated protozoa of the order Kinetoplastida. In various parasitic species of the family Trypanosomatidae, it consists of 5,000-10,000 duplex DNA minicircles (0.5-10 kb) and 25-50 maxicircles (20-40 kb), which are linked topologically into a two dimensional DNA network. Maxicircles encode for typical mitochondrial proteins and ribosomal RNA, whereas minicircles encode for guide RNA (gRNA) molecules that function in the editing of maxicircles' mRNA transcripts. The replication of kDNA includes the duplication of free detached minicircles and catenated maxicircles, and the generation of two progeny kDNA networks. It is catalyzed by an enzymatic machinery, consisting of kDNA replication proteins that are located at defined sites flanking the kDNA disk in the mitochondrial matrix (for recent reviews on kDNA see refs. 1-8).

The unusual structural features of kDNA and its mode of replication, make this system an attractive target for anti-trypanosomal and anti-leishmanial drugs. However, in evaluating the potential promise held in the development of drugs against mitochondrial targets in trypanosomatids, one has to consider the observations that dyskinetoplastic (Dk) bloodstream forms of trypanosomes survive and retain their infectivity, despite the substantial loss of their mitochondrial genome (recently reviewed in ref. 9). Survival of Dk strains has led to the notion that kDNA and mitochondrial functions are dispensable for certain stages of the life cycle of trypanosomatids. This view has been challenged by Schnaufer et al,[10] who demonstrated that knock-down of RNA ligase in bloodstream forms of *Trypanosoma brucei*, was lethal to the parasite. Furthermore, in a recent report[11] they have demonstrated that silencing the expression of the α-subunit of mitochondrial $F_1$-ATP synthase complex, was lethal to bloodstream stage *Trypanosoma brucei*, as well as to the dyskinetoplastic species *Trypanaosoma evansi*. Schnaufer et al have suggested[9] that the lethality resulting from the loss of kDNA, or the lack of expression of its encoded genes, could be due to several possible reasons. One possibility is that several kDNA genes may have an essential role in the bloodstream stage of the parasite. In accord with this notion is the case of silencing the $F_1$-ATP synthase, where the lethal effect has

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apparently resulted from the collapse of the mitochondrial membrane potential.[11] As mitochondrial division and cytokinesis are highly coordinated, it is also possible that cell lethality results from the requirement for kDNA in order to conduct a normal process of cell division, which is mediated through specific checkpoints linking the cell cycle to kDNA segregation.[12-14] It is also possible that the lack of kDNA results in triggering of a series of events that lead to programmed cell death.[15,16]

These observations, suggesting that mitochondrial functions are not dispensable in bloodstream parasites, raise interest in mitochondrial targets for the development of drugs against pathogenic trypanosomatids. The following chapter describes recent advances in the study of kDNA replication, emphasizing the unique features of this system.

## The kDNA Network and Its Monomeric Components

One of the most extensively studied kDNA networks is that of the species *Crithidia fasciculata*. The kDNA network (approximately 10 by 15 μm in dimensions[17]) is condensed in the mitochondrial matrix into a disk-like structure of about 1 by 0.35 μm.[18] Several histone-like proteins are involved in the structural organization of the condensed network.[19-25] The kinetoplast was shown by biochemical and molecular studies to be physically attached to the basal body.[3,14,26,27]

The *C. fasciculata* kDNA network consists of ~5,000 minicircles of 2.5 kb and ~25 maxicircles of 37 kb. Minicircles in the network are relaxed and singly interlocked to each other[28,29] forming a two dimensional DNA network. Maxicircles form independent topological catenanes,[30] that are threaded into the minicircles network and are embedded in different patterns within kDNA networks in the various trypanosomatid species, to form 'network within a network'.[30,31]

Maxicircles, the trypanosomal equivalent of mitochondrial genomes in other eukaryotic cells, are approximately identical in size within a given species, but vary in size (19-39 kb) in different trypanosomatids.[32,33] They consist of a conserved coding region and a nontranscribed variable region. Maxicircles genome encodes typical mitochondrial products, such as ribosomal RNA and protein subunits of the respiratory chain,[34] but not for mitochondrial tRNAs, which are encoded by nuclear genes.[35,36] Their transcripts undergo a remarkable process of post-transcriptional editing that includes insertions and deletions of uridine residues, to create functional ORFs (recently reviewed in refs. 7,8,37).

Whereas it has long been known that maxicircles' genome contains typical mitochondrial genes, the function of kDNA minicircles, the major constituent of the network, has remained a puzzle for many years. Currently, their only known genetic function is to encode guide RNA (gRNA) molecules that provide the specificity for RNA editing of maxicircles' transcripts.[38] Studies, suggesting that kDNA minicircles may encode for other RNA and protein products in various trypanosomatids, have also been reported.[39-42] Minicircles within the network of a given trypanosomatid species are virtually identical in size, but are heterogeneous in their nucleotide sequence, in an apparent correlation with the extent of RNA editing in the various trypanosomatid species.[33,43-45] Despite this substantial heterogeneity, they all contain conserved regions of 100-200 bp, whose location and copy number vary in different species.[46-52] These regions contain a common sequence motif[46] that consists of three short conserved sequence blocks (CSBs) that are present in the same order and spacing in all species studied. A 12-mer sequence (CSB-3), known as the universal minicircle sequence (UMS), and a 10-mer sequence (CSB-1), were proposed to contain the replication initiation sites for the minicircle light (L) and heavy (H) strands, respectively.[53-59] Minicircles in most trypanosomatid species contain an additional common structural motif of a region forming a local bend in the DNA double helix,[60-62] whose biological function is yet unknown.

## Unique Characteristics of the kDNA Replication System

The unusual topology of the kDNA network and its unique mode of replication, division and segregation, pose several major challenges to the trypanosomatid cell. These are addressed

by a replication machinery that carries out a replication scheme with no precedent in any other replication system studied. Some of the individual kDNA replication proteins, such as DNA polymerases, ligases and topoisomerases are similar in function and structure, to various degrees, to the respective enzymes in other replication systems. Others, such as the proposed minicircle origin binding protein UMSBP (see below), are unique in structure and function to the kDNA replication machinery. However, the unique features of kDNA and of the process of its replication, provide potential targets for the development of anti-trypanosomal and anti-leishmanial drugs. The general outlines of the kDNA replication scheme and several unique features of the kDNA replication machinery are discussed in the following paragraphs.

Unlike the replication of mammalian mitochondrial DNA that takes place throughout the entire cell cycle (for review see refs. 63-66), replication of kDNA networks occurs during a discrete S-phase of the cell cycle.[67] Kinetoplast S phase initiates immediately before that of the nuclear S phase, but it is considerably shorter and the kinetoplast segregation is completed before the onset of mitosis.[67-70] kDNA replication includes the duplication of minicircles and maxicircles and the division of the replicated network into two progeny networks that subsequently segregate into the two daughter cells.

A model that provided the basic concepts for understanding the replication of kDNA networks[71,72] had been proposed by Paul Englund almost three decades ago (reviewed in ref. 73) and has since been updated and refined. According to this model, minicircles are not replicated while attached to the network. Instead, covalently closed minicircles are released from the network, prior to their replication, through the action of a type II DNA topoisomerase, and replicate as free DNA circles.[72] The resulting progeny minicircles, which are nicked and gapped, reattach to the network, by the action of another DNA topoisomerase II.[74] Following topological remodeling of the network (see below) and the repair of gaps and nicks in the newly-replicated minicircles, the network splits and subsequently segregates during cell division into the two daughter cells.

kDNA replication proteins were localized to defined sites in the mitochondrial matrix, flanking the kDNA disk. Several of these proteins are clustered at these sites during S-phase, in correlation with the progress in the cell cycle and the process of kDNA replication.[75-78] Fluorescence microscopy studies have localized replication proteins to three distinct regions within the mitochondrial matrix (Fig. 1): (i) at the kineto-flagellar zone (KFZ), which is located between the kDNA disk and the flagellar basal body; (ii) at two antipodal sites flanking the kDNA disk; and (iii) throughout the entire network. It is speculated that proteins that are clustered at overlapping location in the mitochondrial matrix are likely to interact with each other during minicircles replication, to form functional complexes that catalyze related replicative activities. KFZ has been proposed as the site where replication of free minicircles occurs (Fig. 1). It contains (i) the universal minicircle sequence binding protein (UMSBP)[76] (Fig. 2), proposed to function as the origin binding protein;[76,79-84] (ii) DNA primase, that can catalyze the synthesis of RNA primers, is localized close at the two faces the kDNA disk;[78,85] (iii) DNA polymerases Pol IB and Pol IC, shown by RNAi analysis to be required for kDNA replication;[86] and (iv) the kinetoplast associated protein 1 (KAP1), a histone-like protein, which surrounds the kDNA disk, overlapping with DNA primase.[85] Historically, replication proteins clusters were first detected at two sites flanking the kDNA disk (Fig. 1), 180° apart on its circumference.[74] The partial repair of newly-replicated minicircles, as well as their reattachment to the network, occurs at these sites. These sites contain (i) DNA topoisomerase II[74] that catalyzes the topological interconversions of free minicircle and catenane networks[87] and has recently been shown, by RNAi analysis, to be essential for the post-replication reattachment of minicircles;[88] (ii) DNA polymerase β[18] (Fig. 2A), whose catalytic properties, including its dRP-lyase activity,[89-92] suggest a function in the gap-filling of newly replicated minicircles; (iii) a structure-specific endonuclease 1 (SSE1),[77] whose proposed function in primer-excision is supported by its catalytic properties,[92,93] as well as by a recent RNAi analysis;[94] and (iv) the recently discovered DNA ligase kβ,[95,96] whose involvement in the sealing of nicks in the

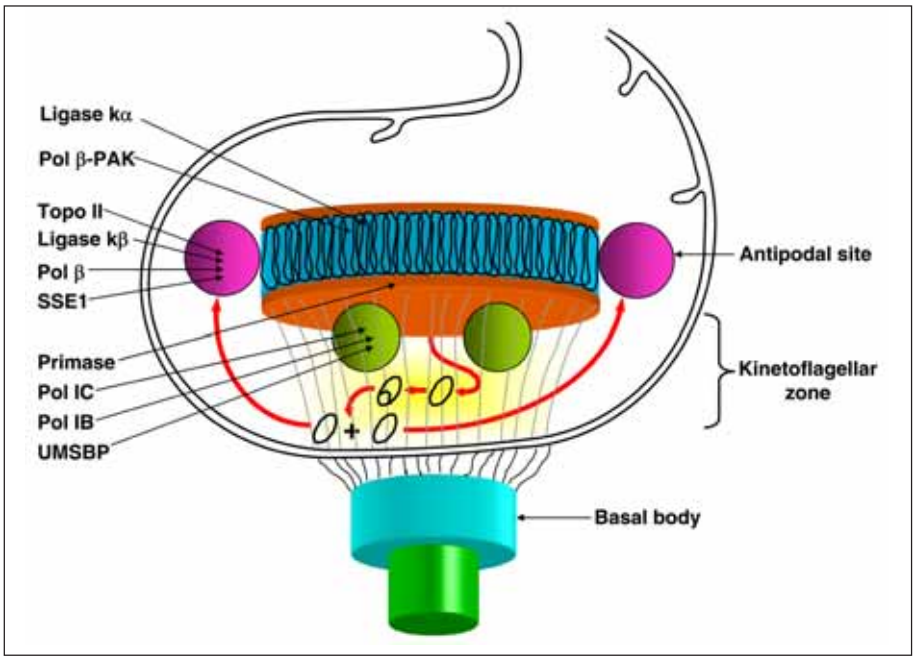


Figure 1. Intramitochondrial location of kDNA replication proteins, and kDNA-replication model. The scheme is based on studies of both *Crithidia fasciculata* and *Trypanosoma brucei*. The kDNA disk, organized with minicircles stretched parallel to its axis, is surrounded by replication proteins. Covalently closed minicircles are released from the network into the KFZ, in which they initiate replication as θ structures [this process probably involves UMSBP, primase, DNA polymerases (Pols) IB and IC, and other proteins]. The progeny free minicircles then migrate to the antipodal sites at which the next stages of replication occur (primer removal by SSE1, gap filling by DNA polymerase β and the sealing of most of the nicks by DNA ligase kβ). The minicircles (still containing at least one nick or gap) are then linked to the network periphery by topoisomerase II (Topo II). DNA polymerase β-PAK and DNA ligase kα are probably involved in the repair of the remaining minicircle gaps when replication is completed. The figure shows the filament system linking the kDNA to the flagellar basal body. Reprinted from: Liu B et al. Fellowship of the rings: The replication of kinetoplast DNA. Trends Parasitol 2005; 21(8):363-9; ©2005 with permission from Elsevier.[4]

newly-replicated minicircles was supported by its coimmunoprecipitation with the mitochondrial DNA polymerase β.[96] Finally, the proteins dispersed throughout the entire kDNA disk, are enzymes involved in the final repair of gapped and nicked progeny minicircles (Fig. 1), and histone-like proteins (KAPs) that are most probably involved in the condensation of the network in the mitochondrial matrix. These include (i) DNA polymerase β-pak,[97] which was suggested to function during the late stage of gap-filling of the reattached minicircles;[97] (ii) the recently discovered DNA ligase kα, whose essential role in the repair of reattached kDNA minicircles was demonstrated recently by RNAi analysis;[96] and (iii) The histone-like proteins KAP2, KAP3 and KAP4.[24]

Another unique feature of the kDNA replication machinery is the mechanisms it utilizes to overcome the major topological challenges in the course of the network replication. Forming a giant topological catenane, which consists of several thousands DNA circles and yet remains confined to a defined space within the mitochondrial matrix, kDNA has to go through dynamic changes in the network topology, termed "remodeling" of the network.[28,98] Prior to

replication, each minicircle in the network is interlocked to the average of three other minicircles, yielding a valence of 3. At the end of S-phase, the replicated network contains twice the number of minicircles in the same surface area. As a result, the density of the network increases and its valence is now 6. During G2 phase a remarkable process of topological remodeling of the network occurs, in which the network size increases and its topological valence returns to its prereplication value of 3. This process is followed by the completion of the final steps in the gap-filling and sealing of the topologically linked newly-replicated minicircles.[17]

The subsequent stage of scission of the covalently-sealed, double-size network, demonstrates another remarkable aspect of the kDNA replication machinery. Based on the catenane nature of the network it is presumed that a type II DNA topoisomerase is involved in this process. Since division of the double-size network is almost symmetrical, yielding two daughter networks of approximately the same size,[31,99-101] scission of the network has to be a highly precise process. Performance of such a highly accurate scission has to be tightly controlled by a mechanism that directs the action of the operating topoisomerase to unlink the correct pairs of interlocked minicircles along the virtual line that divides the network into two equal daughter networks. At present, neither the mechanism used for the scission, nor the mode of its regulation is known.

Finally, the segregation of the divided network during cytokinesis is yet another unique feature of the kDNA replication machinery. The molecular connections between the kinetoplast and the basal body has been demonstrated by biochemical and molecular studies, as well as electron microscopy.[14,102,103] It was found that segregation of the basal body drives the separation of the replicated kDNA progeny network, through a microtubules-mediated process,[14,104] resulting in their segregation into the two daughter cells. Segregation of the network was found to be highly coordinated with the process of cytokinesis.[12-14]

## Replication of Free kDNA Minicircles and Catenated Maxicircles

Early studies have suggested that replication of the minicircle light (L) strand is continuous, while that of its heavy (H) strand is discontinuous and proceeds through the synthesis of short Okazaki fragments. The conserved sequences at CSB-3 (UMS) and CSB-1 were implicated in minicircle replication initiation, as the functional replication origins for the synthesis of the L and H strands, respectively. Replication of free minicircles initiates by the synthesis of an RNA primer at the conserved UMS site on the H-strand template. The primer is elongated continuously and unidirectionally by a replicative DNA polymerase, displacing the parental L-strand. Subsequently, the discontinuous synthesis of the H-strand is initiated, at the CSB-1 region, and proceeds unidirectionally, using the displaced parental L-strand as a template.[53-59] The mechanism used for priming the synthesis of the minicircle H-strand is yet unknown.

Advances over the past twenty years in the characterization of kDNA replication proteins, their intramitochondrial localization and their role in kDNA replication, have shaped our current view of the process of minicircle replication. According to the refined replication model (Fig. 1), minicircle replication begins by the vectorial release of covalently sealed prereplicated minicircles to the KFZ.[105] This region, which accommodates UMSBP (Fig. 2) and DNA primase, as well as the DNA polymerases Pol IB and Pol IC (Fig. 1), also contains minicircle replication intermediates during S phase.[105] It has been suggested that assembly of a minicircle replication-initiation complex takes place at the KFZ, triggering the priming of the minicircle's leading (L) strand synthesis, and the assembly of the replication fork (reviewed in refs. 3,4). It has been further speculated that synthesis of the minicircle leading and lagging strands, as well as segregation of the daughter minicircles, occur in the KFZ, and that the progeny minicircles migrate from the KFZ to the antipodal reattachment sites. Free minicircle replication intermediates have been detected at these two sites during S-phase (Fig. 2B).[4,18,78] The mechanism that controls the migration of the newly replicated kDNA minicircles to the antipodal sites is yet unknown. While at these sites, prior to their catenation onto the network by the type II DNA topoisomerase,[74,87] the newly replicated minicircles are partially repaired, by the excision of the

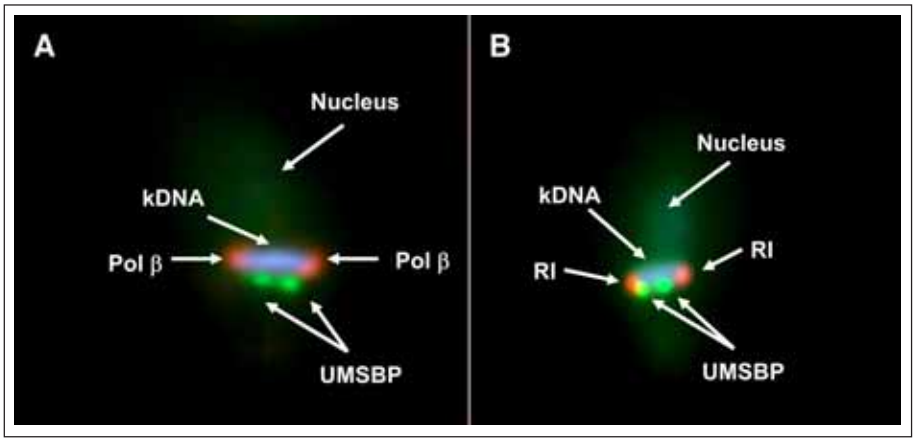


Figure 2. The intra-mitochondrial localization of UMSBP, DNA polymerase β and centers of minicircles replication intermediates (RI). Fluorescence microscopy, demonstrating the localization of replication proteins and minicircle replication intermediates (RI) in the mitochondrial matrix, surrounding the kDNA disk. A) Localization of DNA polymerase β (pol β) and UMSBP: An overlay presentation of DAPI staining (blue) of the kDNA disk, and immunostaining of pol β (red), showing its localization at the two antipodal sites, and of UMSBP (green), at the kineto-flagellar zone (KFZ). B) Antipodal localization of minicircle replication intermediates (RI): An overlay presentation of DAPI staining (blue), Alexa-dUTP fluorescence (red), and UMSBP (green). Minicircle replication intermediates (RI), which are gapped, were selectively labeled in situ by incorporation Alexa-dUTP using terminal deoxynucleotididyl transferase.[76] Reproduced from: Abu-Elneel K et al. Intramitochondrial localization of universal minicircle sequence-binding protein, a trypanosomatid protein that binds kinetoplast minicircle replication origins. J Cell Biol 2001; 153(4): 725-34; by copyright permission of The Rockefeler University Press.[76] A color version of this figure is available online at www.Eurekah.com.

remaining primers, filling of the gaps and sealing of nicks, through the action of SSE1,[77,93] DNA polymerase β (Fig. 2A),[18,90] and ligase kβ.[95,96] Complete repair of discontinuities in the newly replicated minicircles, a process that is carried out in other replication systems during DNA synthesis, is delayed here until all minicircles have been duplicated and reattached to the network. It has been suggested that the final repair of gaps and nicks in the minicircle, which precedes the division of the network, involves the action of DNA polymerase β-PAK[97] and ligase kα.[96] The reason for the delayed repair of newly replicated minicircles is yet unknown. It has been proposed that the presence of one or more nicks and gaps in the reattached minicircles may serve in a 'book-keeping' mechanism, marking replicated minicircles to insure the replication of each minicircle molecule only once per generation.[106,107]

Much less is known about the replication of kDNA maxicircles, which occurs during S-phase, concurrently with the replication of minicircles. Unlike minicircles, that replicate as free detached DNA circles, maxicircles replicate while attached to the network.[108,109] Maxicircles replication initiates from a replication origin, located in their noncoding (variable) region and proceeds unidirectionally through theta (θ) structure intermediates.[108] Involvement of RNA polymerase in maxicircles replication was also reported.[110]

## Regulation of kDNA Replication

Considerable progress has been made in recent years in our understanding of the enzymatic machinery that catalyzes the assembly of kDNA networks in trypanosomatids. Nevertheless, our understanding of the mechanisms that regulate kDNA replication in the cell remained

poor. The following paragraphs outline several aspects of the control of kDNA replication, including the activation of the replication origins, regulation of the origin binding protein action, and the control of expression of kDNA replication proteins.

Kinetoplast DNA replicates in the trypanosomatid cell during a discrete S phase. The kinetoplast S phase ($S_k$) and kDNA segregation precedes the nuclear S phase ($S_n$) and mitosis.[13,67] Kinetoplast segregation is presumably well-coordinated with mitosis.[14] However, studies on the mechanism of cell cycle control[111-117] indicated an apparent uncoupling between the kinetoplast and the nuclear cycle. As the kDNA network replicates at a discrete S-phase, the thousands of origins present in its minicircles and maxicircles have to be regulated by a mechanism, which allows the activation of these origins only during the S phase of the cell cycle. The licensing of chromosomal origins in eukaryotes involves the action of MCM proteins (recently reviewed in refs. 118,119). MCM proteins encoding genes are also present in the trypanosomatids genomes, yet the mechanism that functions in the licensing of kDNA replication origins is unknown.

Regulation of chromosome replication in cells and their subcellular organelles is mediated by interactions of origins of replication with their counterparts, the trans-acting proteins.[120] In kDNA minicircles, two short sequences were associated with the process of replication initiation, a dodecameric sequence GGGGTTGGTGTA (CSB-3, UMS) and a hexameric sequence ACGCCC (within the (CSB-1). Although the mode of activation of kDNA replication origins is unknown, recent studies on the regulation of UMSBP activity through redox signaling (see below), may shed light on the process of activation of minicircle replication initiation.

UMSBP binds specifically the two sequences conserved at the minicircle replication origins, the UMS dodecamer and a 14-mer sequence that contains the core hexamer. Based on its binding to the conserved origin sequences, UMSBP was suggested to play a role during minicircle replication initiation at the replication origin. However, its precise function has yet to be studied. The protein has been purified from *C. fasciculata* and its encoding gene and genomic locus were cloned and analyzed.[79-81,83,84,121] The 116 amino acids protein contains five tandemly arranged CCHC-type zinc-finger motifs. Immunofluorescence analyses demonstrated the dynamic nature of UMSBP localization within the kinetoplast, reaching its maximal level during S-phase, in correlation with the progress in kDNA replication.[76] Structure-function analyses[82] revealed that UMSBP oligomerizes in solution, but binds the origin sequence only in its monomeric form. Furthermore, these analyses indicated that zinc fingers that are involved in the binding of DNA differ from those mediating protein-protein interactions that lead to UMSBP dimerization. Both UMSBP binding to DNA and its dimerization are sensitive to redox potential. Oxidation of UMSBP results in the protein dimerization, mediated by its N-terminal domain, with a concomitant inhibition of its DNA binding activity. UMSBP reduction yields monomers that are active in the binding of DNA, through the protein C-terminal region. *C. fasciculata* tryparedoxins (*Cf*TXNI and II[122-125]) were shown to activate in vitro the binding of an oxidized UMSBP substrate to the DNA (Fig. 3). These results may imply that a cellular redox signaling mechanism may control the binding of UMSBP to the minicircle replication origin. Based on these observations, one may speculate that redox signaling may be involved in the triggering of replication initiation at the minicircle replication origin.

Cycling of the level of expression of kDNA replication proteins with the progress in cell cycle, may serve an important regulatory function during kDNA replication. Dan Ray and his colleagues have observed that the mRNA levels of several nuclear and kDNA replication genes in *C. fasciculata* cycle, as cells progress through the cell cycle,[126-131] reaching their maximal levels at the beginning of S phase, and then decline sharply as DNA synthesis is completed. They showed that cycling is controlled prior to mRNA maturation,[130] and that an octamer sequence [(C/A)AUAGAA(G/A)], located at either the 5' or the 3' untranslated regions (UTRs) of the mRNAs, was involved in the regulation mechanism. Two protein complexes, designated CSBP I and CSBP II, bind specifically to this sequence.[131] This phenomenon has been observed also in *Leishmania infantum*[132] and *Leishmania major*[133] and is presumably shared by other trypanosomatid species.

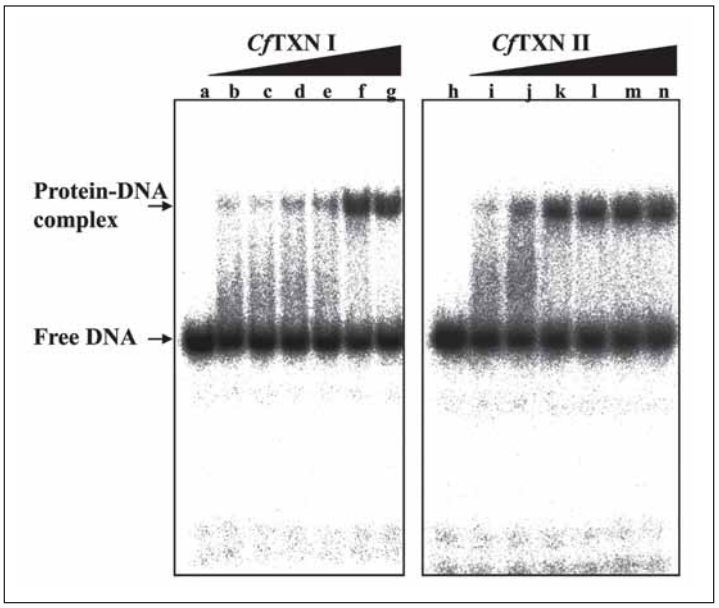


Figure 3. *C. fasciculata* tryparedoxins activate in vitro the binding of UMSBP to UMS DNA. A reactions, which leads from NADPH, through the action trypanothione reductase (TR), to the reduction of trypanothione disulfide ($TS_2$) to trypanothione ($T[SH]_2$) and subsequently to the reduction of tryparedoxin (TXN), was coupled in vitro to the binding reaction of UMSBP to DNA. The binding of preoxidized UMSBP to an oligonucleotide, representing the origin-associated universal minicircle sequence (UMS), was monitored. Generation of nucleoprotein complexes in the coupled reactions is demonstrated in the electrophoretic mobility shift analyses (EMSA). The two *C. fasciculata* tryparedoxins (*Cf*TXN I and *Cf*TXN $II^{122\text{-}125}$) were used. The tryparedoxin reaction included either increasing concentrations of *Cf*TXN I (lanes c-g: 0.01, 0.02, 0.04, 0.06, and 0.1 μM) or *Cf*TXN II (lanes j-n: 0.1, 0.25, 0.5, 0.75, 1 μM). Binding reactions, using 0.6 ng UMSBP that was preoxidized by diamide, were conducted in the presence of 12.5 fmol $^{32}$P-labelled UMS DNA. In lanes a and h: no UMSBP and tryparedoxin added; lanes b and i: no tryparedoxin added. Reproduced from: Onn I et al. Redox potential regulates binding of universal minicircle sequence binding protein at the kinetoplast DNA replication origin. Eukaryot Cell 2004; 3(2):277-87;[82] ©2004 with permission of American Society of Microbiology.

## Concluding Remarks

The significant advances achieved in recent years in the understanding of the enzymatic system that catalyzes the assembly of the kDNA network in trypanosomatids, was greatly enhanced by using the powerful combination of classical enzymology, coupled with molecular genetics, genomic and proteomic strategies. Nevertheless, several of the basic questions, concerning the structure, function, replication and segregation of kDNA remained unsolved. An intriguing problem is the functional advantage that led to the evolution of this unusual topological catenane. Other interesting mechanisms are the systems that control the accurate scission of the replicated network, the regulation of minicircles' segregation, the functional role of the clusters of replication proteins surrounding the kDNA disk, and the mechanism that 'licenses' kDNA origins to fire and initiate a new round of replication during S phase. Many other questions regarding the details of the replication scheme remained unanswered. Among these are the identity of the topoisomerase II that functions in the prereplication release of minicircles into the KFZ, the mechanism that directs the migration of newly replicated

minicircles from the KFZ to the two antipodal attachment sites, the functional rationale for the delayed repair of replicated minicircles, and the mechanism of replication of catenated maxicircles. These and other basic questions on the replication of kDNA will continue to intrigue investigators in this field in the coming years. However, the knowledge gained on the replication mechanisms, proteins, and intermediates, as well as the recent data suggesting that kDNA and mitochondrial functions are not dispensable in the parasites' life cycle, opens new possibilities for the selection of specific targets and the rational designing of drugs against pathogenic trypanosomatids.

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