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THE MITOCHONDRIAL GENOME OF KINETOPLASTID PROTOZOA: Genomic Organization, Transcription, Replication, and Evolution

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INTRODUCTION

The mitochondrial or kinetoplast DNA of the kinetoplastid protozoa is one of the most unusual DNA structures in nature. The purpose of this review is to bring this interesting system to the attention of microbiologists and others

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interested in mitochondrial genetic systems and to demonstrate both the extent of our knowledge and the gaps in our understanding. I stress only those studies published in the last two to three years, since comprehensive reviews of previous work have been published (22, 87, 89, 99).

Kinetoplastid protozoa belong to the order Kinetoplastida and comprise monogenetic species in the genera *Crithidia*, *Leptomonas*, *Blastocrithidia*, and *Herpetomonas* and digenetic species in the genera *Leishmania*, *Trypanosoma*, *Phytomonas*, and *Endotrypanum*. These lower eukaryotic cells are known in the literature as "trypanosomatid protozoa," "kinetoplastid protozoa," "hemoflagellate protozoa," or simply "trypanosomes," although the latter is in the strict sense a genus name. All of these species are parasitic; the monogenetic species are parasites of invertebrates such as *Diptera* and *Hemiptera*, and the digenetic are parasites of both invertebrates and vertebrates such as mammals and lizards. The kinetoplastid protozoa appear to be among the most highly diverged eukaryotic cells known. Perhaps as a consequence of their long separation from the main eukaryotic lineages, they possess many unique biological properties not found in higher organisms: a subplasma membrane exoskeleton of microtubules surrounding the entire cell; a posttranscriptional addition of a strongly conserved 35-nucleotide miniexon sequence at the 5' end of most if not all cytosol mRNAs; a single mitochondrion containing a network of thousands of catenated minicircle DNA molecules and a smaller number of catenated maxicircle molecules; and a highly plastic karyotype, as visualized by orthogonal field gel electrophoresis of chromosomal molecules ranging from 50 kb to approximately 2000 kb. The digenetic species have, in addition, biphasic life cycles that involve substantial changes in morphology and physiology. Such charges include, for example, the complete repression of mitochondrial biosynthetic activity in the bloodstream form of the African *brucei* group trypanosome followed by derepression in the insect form, and the ability of the amastigote forms of mammalian *Leishmania* to withstand lysosomal attack within the intracellular environment of the macrophage (87, 89).

Kinetoplast DNA represents the sole mitochondrial DNA of the kinetoplastid protozoa. It consists of two molecular species, minicircles and maxicircles, catenated together into a single giant network of DNA situated adjacent to the basal body of the flagellum. There are approximately 5×10^3–10^4 minicircles and 20–50 maxicircles per network. In most species there is more than one class of minicircle sequence present in the same cell. The different sequence classes, however, share a strongly conserved region representing approximately 10% of the minicircle length. Maxicircle DNA is apparently homogeneous within a single organism and contains mitochondrial structural genes homologous to those found in other organisms as well as several still unidentified open reading frames.

KINETOPLAST MINICIRCLE DNA

Is There a Gene Product?

There is only one result in the literature that supports the presence of a minicircle-specific transcript. In 1979, Fouts & Wolstenholme (29) reported the existence of a 240-nucleotide transcript from the 2500-nt minicircle DNA of *Crithidia acanthocephali*. This result, however, has never been confirmed with gel technology, and there are several references to negative results in probing Northern RNA blots with minicircle probes in the *Leishmania tarentolae*, *Crithidia luciliae*, *Phytomonas davidi*, and *Trypanosoma brucei* systems (39, 41, 89, 99, 100). A small RNA transcript, however, would probably not be detected by the Northern blotting procedure owing to lack of binding to the filter, and an unstable transcript might not be apparent in steady-state RNA. Shlomai & Zadok (84) approached this problem by cloning random minicircle fragments from *Crithidia fasciculata* in bacterial expression vectors and using rabbits to elicit antisera that reacted specifically with *Crithidia* antigens. Their conclusion that kDNA minicircles contain long open reading frames (ORFs) that are transcribed and translated is probably premature for several reasons. First, nonspecific cross-reaction of polyclonal antisera raised against bacterial fusion proteins with parasite proteins could affect the results. Secondly, the published sequence of the predominant minicircle sequence class in *C. fasciculata* does not contain long ORFs (101). Finally, the short ORFs covering the conserved regions derived from the sequences of minicircles from *T. brucei* (15, 48), *Trypanosoma lewisi* (78), *Trypanosoma cruzi* (63), and *L. tarentolae* (51) show no significant similarity on the amino acid level. A final judgment on this important question must await sequencing of the cloned putative minicircle fragments and the putative minicircle transcript, identification of the minicircle ORF, and characterization of the cellular antigens that react with the antiserum.

Sequence Organization in Different Species

The size of kDNA minicircles varies from 465 bp in *Trypanosoma vivax* (10) to 2515 bp in *C. fasciculata* (7, 101). All kDNA minicircles analyzed appear to have the same type of genomic organization, i.e. a small conserved region and a larger variable region. "Conserved" in this sense implies sequence similarity within the different minicircle sequence classes within a single species or strain. The simplest type of organization, found in minicircles of *T. brucei*, *Trypanosoma equiperdum*, and *L. tarentolae*, is a single conserved region. The 1-kb *T. brucei* minicircles contain a 130-bp conserved region (15, 48), which is quite similar to that found in *T. equiperdum* (3). The three 870-bp *L. tarentolae* minicircles sequenced contain a 160–270-bp conserved region (51). Two conserved regions of 173 and 177 bp situated 180° apart are

found in the 2.5-kb minicircles of *C. fasciculata* (101). There are two direct repeats of 95 bp in the 1-kb minicircles of *T. lewisi* (78). Four conserved regions of 118 bp are found in the 1.4-kb *T. cruzi* minicircle, situated at 90° intervals (58, 63, 68).

Oligomer sequences within the conserved regions are also conserved among kinetoplastid species. Kidane et al (51) first recognized a dodecamer present in *L. tarentolae*, *T. brucei*, *T. equiperdum*, and *T. cruzi*, and Ponzi et al (78) discovered a pentadecamer present in *T. lewisi*, *T. brucei*, and *T. equiperdum*. The dodecamer sequence, GGGGTTGGTGTA, is also present in the major minicircle class of *C. fasciculata* strain Cf-C1 (101). This sequence has been termed the "universal" minicircle sequence (74), although adoption of this term may be premature, since sequences from several kinetoplastid genera are not yet available. Ntambi & Englund (74) showed that this sequence represents an origin of replication of the minicircle.

The sequence similarities of the conserved regions from the minicircles of the six species examined correspond with the presumed evolutionary affinities of the species. For example, as stated above, the *T. brucei* and *T. equiperdum* conserved sequences are almost identical, although the other minicircle sequences differ. The *T. cruzi* conserved sequences are more closely related to the *T. lewisi* sequences than to the *T. brucei* sequences and are least closely related to the *L. tarentolae* sequences (63). *T. equiperdum* is thought to be a recent genetic variant of *T. brucei* that has lost the tsetse host. *T. cruzi* and *T. lewisi* are both classified, in terms of developmental localization in the insect host, as the more primitive stecorarian trypanosomes. *T. brucei*, in contrast, is a salivarian trypanosome. These sequence homologies indicate that evolutionary constraints on sequence changes within the conserved region of the minicircle are greater than constraints on changes within the variable regions, as expected from the functional importance of an origin of replication. The origin of changes in the variable region is uncertain, but is probably a combination of a high rate of nucleotide substitutions and segmental rearrangements, possibly involving recombination between minicircles. Segmental rearrangements have been indicated by an electron-microscope heteroduplex analysis of *Crithidia* minicircles (42) and by a partial sequence analysis of a minor minicircle class from *L. tarentolae* kDNA (72). In addition, a high rate (approximately 0.3%) of single nucleotide changes was observed in multiple clones of the same sequence class from *C. fasciculata* (7, 101).

The heterogeneity of the variable region varies from species to species. The most extreme case is *T. brucei*, which has at least 200–300 different minicircle sequence classes within the same cell (98). The frequency of the different sequence classes is also variable. However, frequency has only been measured quantitatively for one cloned minicircle of *T. brucei*, which appeared in 500 copies per cell (100), and for five minicircles of *L. tarentolae*, which

comprised 50% of the total minicircle DNA. Of these five minicircles, one class (pKSR1/pLt19) represented 26% of the total population (51). An additional five minicircle sequence classes were detected by partial sequence analysis of cloned fragments (72). The kDNA from the Borst laboratory strain of *C. fasciculata* was estimated to contain 13 different minicircle sequence classes by summation of restriction-fragment gel bands (40, 42, 56). In the case of the Cf-C1 strain of *C. fasciculata*, however, over 90% of the kDNA minicircles consist of a single sequence class, which exhibits approximately 0.3% nucleotide variation in different clones (7, 101). This strain variation was unexpected, since Camargo et al (13) had observed no differences in the kDNA restriction profiles of three *C. fasciculata* strains from different labs. The variation between the Borst strain and the Cf-C1 strain has been confirmed by a side-by-side gel comparison (L. Simpson, unpublished results). It should be noted that visual estimates of frequencies of minicircle classes can be highly inaccurate owing to nonlinear staining of overloaded bands. Furthermore, it should be remembered that kinetic complexity values of kDNA minicircles from the Borst strain of *C. fasciculata* and two other *Crithidia* species are only 1–2.5 times minicircle unit size (28, 42, 56).

The highly complex minicircles from the African trypanosomes appear to possess a sequence adjacent to the conserved region that maintains a characteristic purine versus pyrimidine strand bias and terminates with a series of oligo (A) tracts (48). In addition there is an 18-bp sequence that occurs as 3–4 pairs of inverted repeats throughout the variable region. These inverted repeats flank 102–110-bp segments that show slight sequence conservation among different minicircle classes. Jasmer & Stuart (49) have suggested that these transposonlike sequences represent a mechanism for the development of minicircle sequence diversity in this group of cells. However, such sequences are absent in minicircle DNA from *L. tarentolae*, *T. lewisi*, and *T. cruzi*, and therefore could not represent a general mechanism for generation of sequence diversity. The oligo (A) tracts are a constant feature of minicircle sequences from all species so far examined.

In addition to the *C. fasciculata* Cf-C1 strain, two other kinetoplastid species, *T. evansi* (24, 31) and *T. equiperdum* (3, 80), possess apparently homogeneous kDNA minicircle populations within a single cell. These species probably represent genetic variants of *T. brucei* that have lost the ability to develop in the insect host and are transmitted contaminatively or venereally. Maxicircle DNA is absent from *T. evansi* (24) and modified in *T. equiperdum* (31). Minicircle DNA from one strain of *T. equiperdum* has been sequenced, and minicircles from six strains of *T. evansi* have been compared by restriction enzyme analysis. Two types of minicircle sequences were found in the six *T. evansi* strains; this suggests that *T. evansi* had at least two separate origins from the presumed ancestral *T. brucei* stock. The level of sequence homogeneity in *T. evansi* and *T. equiperdum* is unknown; sequence

analysis of multiple homologous clones is necessary to determine the actual nucleotide sequence variation. The reason for minicircle sequence homogeneity in these two species is unknown, but the minicircle sequence heterogeneity in most species appears to be correlated with the presence of a functional maxicircle. Borst et al (9) have suggested that the recently discovered genetic recombination that occurs in the insect phase of the *T. brucei* life cycle is responsible for creating minicircle sequence heterogeneity, but direct evidence is lacking.

Sequence Heterogeneity as a Diagnostic and Epidemiological Tool

The existence of multiple classes of minicircle sequences within a single cell is mirrored by the existence of different sequence classes in different species and even in different strains of the same species. This diversity implies a rapid rate of sequence change in nature. The rate of minicircle sequence change varies among the different genera. The extensive minicircle sequence complexity within the *T. brucei* group in fact renders this molecule unusable as a marker to distinguish different stocks or strains. However, lesser minicircle complexity renders the sequences of pathogenic *Leishmania* and *T. cruzi* ideal for this purpose. In addition, the high copy number of the minicircle DNA within the network provides an amplified target for visualization of bands in a gel from few cells and for hybridization techniques.

In the case of *T. cruzi*, different stocks exhibited characteristic minicircle restriction fragment profiles in acrylamide gels (69). These patterns were stable over a 2-yr period of continuous culture and did not change during the parasite life cycle. In addition, Southern hybridization analysis showed that differences in minicircle restriction profiles represent differences in sequences. Morel et al (69) proposed the term "schizodeme" to describe subpopulations of *T. cruzi* (or any species of kinetoplastid protozoa) that possess similar minicircle restriction profiles in acrylamide gel electrophoresis. Since similar restriction profiles imply similarities in minicircle DNA sequences, this term is also applicable to subpopulations that exhibit cross-hybridization with kDNA probes as shown by dot blot hybridization. Schizodeme analysis of *T. cruzi* has proved to be a sensitive and reliable method for distinguishing strains and stocks (16, 30, 71).

Schizodeme analysis has been used to show that different strains of *T. cruzi* can simultaneously coexist in the vertebrate host and that the inoculation schedule and the method of reisolation affect the recovery of individual strains (16). In one experiment, mice were doubly infected with the F and Y strains of *T. cruzi*. The parasites were recovered from the blood either directly into culture or through passage in irradiated mice and were identified by schizodeme analysis. The researchers found that both the timing and the method of parasite isolation act as selective factors.

Labeled kDNA and cloned minicircles have also been used as probes in dot blot hybridization experiments with *T. cruzi* strains (32, 62, 83). In addition, oligonucleotide probes synthesized from the known sequence of a cloned *T. cruzi* CL strain minicircle have been used to distinguish different stocks and strains in dot blot experiments with intact cells sported onto filters (70). Oligonucleotides from different minicircle regions have different specificities: A sequence from the conserved region was specific for the *T. cruzi* species (i.e. versus *Leishmania*), and sequences from the variable region were specific for homologous *T. cruzi* stocks.

In the case of the pathogenic *Leishmania*, schizodeme analysis by comparison of acrylamide gel profiles and direct Southern and dot blot hybridization using kDNA or intact cells probed with labeled kDNA or cloned minicircle DNA have been used with success (1, 37, 44, 45, 50, 60, 61, 97). A clinically useful variant of the dot blot method is the touch blot method; fragments of infected lesions are briefly touched to nitrocellulose filters, leaving parasite amastigotes attached to the filter (103). Owing to the high copy number of kDNA minicircles, a hybridization signal can be seen with as few as 100 cells. This method was used to distinguish *Leishmania brasiliensis* subspecies from *Leishmania mexicana* subspecies and from *Leishmania tropica*. Schizodeme analysis has also been used to distinguish *Leishmania major* from *L. tropica* (50, 97). A cloned kDNA minicircle has been used as a sensitive and specific diagnostic probe for Kenyan visceral leishmaniasis (76).

Cloned minicircle fragments from visceral *Leishmania* species exhibited different taxonomic specificities in hybridization assays (2, 61). One cloned fragment could distinguish visceral and cutaneous species. Another cloned fragment was specific for the strain from which it was derived. Similar results were obtained with cloned minicircle fragments from *Leishmania chagasi*. These results imply either an intermolecular heterogeneity in minicircle sequence specificity, an intramolecular heterogeneity, or both. The intramolecular sequence heterogeneity is clearly due to the presence of both conserved and variable regions. Rogers & Wirth (81) recently used hybridization specificity with minicircle DNA from the same species or group to show the existence of a gradient of sequence conservation within the conserved region of the *Leishmania* minicircle. Based on sequence analysis of two cloned *L. mexicana amazonensis* minicircles, the boundary between the conserved and strain-specific variable region appears to be sharp; this implies the existence of a mechanism that maintains sequence conservation of the conserved region while allowing the variable region to diverge rapidly (D. Wirth, personal communication). The discovery of a gradient of sequence conservation within the conserved region is important, since it indicates that kDNA hybridization can be a precise as well as sensitive method for diagnosis and classification down to the group and strain levels for the leishmanial diseases and probably for other kinetoplastid-caused diseases.

Analysis of Conformational Bend in Minicircle DNA

Cloned or native kDNA minicircles and minicircle fragments from most kinetoplastid species exhibit much slower electrophoretic migration in acrylamide gels than in agarose gels. This was first shown for minicircle DNA from *L. tarentolae* (14, 51, 88, 92) and was also found to be true for minicircle DNA from *T. brucei*, *T. equiperdum*, *Herpetomonas muscarum*, and *C. fasciculata* (54, 64, 79), but not for that from *T. cruzi* (63, 69). Such abnormal migration behavior in a cloned minicircle fragment from *L. tarentolae* [which was originally thought to represent a 490-nucleotide fragment and which was later shown by resequencing (66) to be identical to a 410-nt Sau3A fragment of the KSR1/LT19 minicircle (51)] was ascribed (65) to the existence of a conformational sequence-dependent bend in the DNA molecule. The abnormal migration could be eliminated by cutting the minicircle fragment within the putative bend region (51), which was localized at one edge of the region that was conserved among three cloned and sequenced minicircles from *L. tarentolae*. The precise location of the bend was determined by a gel electrophoretic analysis of circularly permuted minicircle DNA fragments to be within a regular repeat of the sequence element $CA_{5-6}T$ with 10-bp periodicity (105) (nucleotide 20 of the KSR1 sequence). A second adjacent bend region (nucleotides 78–137 of the KSR1 sequence) was located by analysis of the gel migration behavior of restriction fragments (20). This region also contains runs of oligo (dA) with 10-bp periodicity.

Two mechanisms have been proposed to account for this conformational bend. In the first model it is assumed that DNA remains in the B conformation and that the dinucleotide ApA produces a wedge by a combination of tilt and roll (102), or that a stereochemical clash of consecutive purine residues on opposite strands deforms the helix (34–36). In the second model it is assumed that the oligo (dA) tracts confer a non-B conformation on the DNA strand and that bending occurs at junctions between regions of B-DNA and oligo (dA) tracts (57, 59). High salt, increased temperature, and binding of distamycin all decrease the bend, as measured by electrophoretic migration in acrylamide (20, 105). There is some evidence against the ApA wedge model (36), but it is not yet possible to decide between the purine clash model and the junction bend model on the basis of existing evidence.

The most striking bend is found in the *C. fasciculata* minicircle, which has 16 successive oligo (dA) tracts at one point in the circle (54, 79, 101). This bent segment can be easily visualized in the electron microscope as 200–300-bp loops within the larger molecule and as looped 300-bp linear fragments (33). It is significant that the single bend in the 2.5-kb *C. fasciculata* minicircle is well separated from the two conserved regions (79) and is not at one edge of the conserved region as in *L. tarentolae* (51) and *T. brucei* (15). This appears to eliminate the possibility that the bend is mechanistically

involved in DNA replication. Also, *T. cruzi* minicircles do not contain a bend and replicate quite well.

Muhich & Simpson (72) recently reported a single major cleavage of multiple minicircle sequences from *L. tarentolae* kDNA by mung bean nuclease in the presence of formamide. No specific sequence was common to all minicircle cleavage sites, but the main region of nuclease cleavage was approximately 350 bp from the unique SmaI site in the conserved region. The authors suggested that the nuclease recognized an unusual structural feature of the DNA molecule such as the static bend.

The function of the minicircle bend is unknown, as is the function of the minicircle itself. Perhaps the bend has a role in DNA packaging into the network and formation of the highly organized kDNA nucleoid, or in the targeting of newly replicated networks to a catenation site on the network.

The existence of a sequence-dependent bend in the kDNA minicircle is but one example of local polymorphism of DNA structure. In some cases such polymorphisms are apparently involved with DNA function. One example is sequence-induced DNA curvature at the origins of replication of bacteriophage λ (106) and simian virus 40 (SV40) (82) and at a yeast autonomously replicating sequence (96). Another is protein-induced curvature caused by the binding of catabolite gene–activating protein to its recognition sequence near the promotor of the *Escherichia coli* lac operon (106).

Replication of Minicircle DNA

The decatenation-recatenation model for the replication of kDNA minicircles (21) was based on the early observations that newly replicated kDNA was localized in two loci at the edge of the network: this DNA was chased into a peripheral, widening ring of open circular minicircle DNA in the network, which only became covalently closed after the S phase (86, 94). This model has been substantiated by direct analysis of replicating minicircle intermediates in vivo in *C. fasciculata* and *T. equiperdum* (52, 53, 74, 75) and in an isolated *C. fasciculata* organelle system (6, 8). The minicircle initiates replication with random detachment of the covalently closed circle from the network by a putative topoisomerase II activity, followed by an apparent Cairns-type replication. The newly synthesized heavy strand consists of small oligonucleotides. The light strand, in the case of *T. equiperdum*, contains a single gap of 10 nucleotides with a 5' attached ribonucleotide overlapping the "universal" dodecamer sequence (75). In the case of *C. fasciculata*, the light strand has two gaps overlapping the two "universal" dodecamer sequences (6, 8). The gapped minicircles are then recatenated to the edge of the network. Only after the S phase is completed are all discontinuities filled in and nicks ligated.

KINETOPLAST MAXICIRCLE DNA

Mitochondrial Ribosomal RNA Genes

The 9S and 12S RNAs are the major stable RNA species in purified kinetoplast fractions from several kinetoplastid species (91). They represent the smallest rRNAs yet identified. In spite of radically different sizes and primary sequences as compared to known rRNAs, models of secondary structure can be constructed for the 9S RNA and for portions of the 12S RNA (17, 19, 23, 95). These models conform well to portions of the *E. coli* 16S and 23S rRNA models (12, 104). These models can be verified and extended by the evolutionary method of Woese et al (104), in which compensatory nucleotide substitutions are observed in helical regions of sequences from different kinetoplastid species that preserve base pairing. By comparing the sequences of the 9S and 12S RNAs from *L. tarentolae* and *T. brucei*, several helical regions in the models were verified (17, 19). Similar partial models of secondary structure were constructed from a comparison of 9S and 12S sequences from *T. brucei* and *C. fasciculata* (95). Verification and extension of these models will depend on determination of additional 9S and 12S RNA sequences from other kinetoplastid species and analysis of the alignments.

The major conclusion from the preceding rRNA analyses is that in spite of a high degree of primary sequence divergence and the absence of several regions that are conserved in most other rRNAs, there is a striking conservation of many of the sequences and structures that are implicated in the biosynthetic function of the ribosome, e.g. the peptidyl transferase region in the 12S RNA. To explain the small size of these rRNAs, we have speculated that some of the functions of rRNA have been taken over by proteins and that those that remain are crucial for basic translational functions (19). Thus, an analysis of these unusually small rRNAs may prove to be of general significance in terms of understanding the structure-function relationships of rRNA.

Mitochondrial Structural Genes and Open Reading Frames

The basic organization of the maxicircle genome in all kinetoplastid species examined consists of an actively transcribed informational region containing the rRNA genes and structural genes and a nontranscribed divergent or variable region containing a variety of repeated sequences of unknown function. The complete nucleotide sequences of the informational regions and portions of the divergent regions of the maxicircle DNA from *L. tarentolae* and *T. brucei* and of portions of the maxicircle DNA from *C. fasciculata* are known (4, 18, 25, 38, 46, 77). Simpson et al (90) and de la Cruz et al (84) have compared the maxicircle genomes of *L. tarentolae* and *T. brucei* in detail. They identified specific ORFs as structural genes homologous to those in other organisms by measuring the statistical significance of amino acid

alignments and by analyzing the similarity of hydropathic profiles of the translated amino acid sequences. In *L. tarentolae* the following structural genes were identified: cytochrome oxidase subunits I, II, and III; cytochrome *b;* and NAD dehydrogenase subunits 1, 4, and 5. In addition, several ORFs are present that most likely represent protein coding sequences. The criteria used to identify a maxicircle ORF as a functional gene are (*a*) identification of the translated amino acid sequence as a known protein; (*b*) transcription; (*c*) a T:A ratio of approximately 2 on the coding strand; (*d*) conservation of amino acid sequence between *L. tarentolae* and *T. brucei;* and (*e*) conservation of a characteristic codon bias profile between the two species (18, 90).

Appropriate translation initiation codons could not be identified for several of the maxicircle genes (CYb, ND1). Cross-species alignments of translated amino acid sequences of maxicircle genes have aided in assignment of putative initiation codons for COI, COII, ND4, ND5, MURF1, and MURF2. Leucine may represent an alternative initiation codon in the maxicircle genome. Clearly, however, amino acid sequences of the protein products are required for definitive identification of initiation codons of maxicircle genes. In this regard, an antiserum directed against a carboxy-terminal peptide of the putative COII gene product has recently been generated (J. Shaw & L. Simpson, unpublished results). This antiserum detected a polypeptide of the predicted molecular weight in Western blot analysis of a mitochondrial lysate from *L. tarentolae*. Amino acid sequence analysis of this and other kinetoplast gene products should establish unequivocally the functional nature of the kinetoplast transcription-translation system, confirm the specific mitochondrial genetic code employed by this species, and perhaps allow the determination of the specific translation initiation codons used.

Comparison of the Maxicircle Genomes from Two Kinetoplastid Species

The informational portions of the maxicircle genomes of *T. brucei* and *L. tarentolae* are colinear in terms of gene localization and gene polarity except for two regions, one between the 9S RNA gene and the CYb gene and one 3' of the CYb gene (73, 90). The COIII gene and the ORF3–4 and ORF12 genes are unique to *L. tarentolae*. MURF1 and MURF2 represent two unidentified genes that are conserved between these two species.

The absence of the COIII gene in the *T. brucei* maxicircle genome is unprecedented and is due either to a nuclear localization of the COIII gene or to the absence of this subunit from the holoenzyme in this species.

Short regions of DNA sequence homology are present within the nonhomologous regions, but their significance is not understood. In addition, several short GC-rich regions that do not show gene characteristics are nevertheless transcribed, and the pattern of intergenic guanine versus cytosine

strand bias is strongly conserved between *T. brucei* and *L. tarentolae* in the absence of nucleotide sequence conservation. Short transcripts off the C-rich strand are found in steady-state RNA (47, 90). The function of these regions is unknown, but it may involve tRNA synthesis, RNA processing, or regulation of transcription.

The COII Frameshift

The COII gene presents a special problem in that there is a −1 frameshift in the DNA sequence that is conserved between *T. brucei* and *L. tarentolae* (18, 38, 77). RNA sequencing of the *T. brucei* COII mRNA has indicated that there is an insertion of several uridines in this region of the transcript; this insertion overcomes the frameshift and allows translation of the complete COII protein (5). This represents a novel posttranscriptional activity, which must be studied for confirmation and for understanding of the mechanism involved. There is one other gene, the MURF2 gene in *L. tarentolae*, which may exhibit the same phenomenon: the DNA sequence gives rise to two overlapping ORFs, yet there is a single transcript, and both ORFs are homologous to a single MURF2 ORF in *T. brucei*.

Divergence of Maxicircle Genes from Mitochondrial Genes of Other Organisms

All identified maxicircle genes have diverged significantly from homologous genes from other organisms. As discussed above, the rRNA genes have diverged so far from rRNA genes of other eukaryotic cells that structural analysis was necessary for definitive identification. The structural genes have diverged to great but differing extents. The most conserved gene is COI, and the least conserved is COIII. In all cases, however, the extent of sequence similarity is less between the kinetoplastid gene and the homologous gene from fungal or animal mitochondrial genomes than between the same genes from fungal and animal lines. The sequence similarity was determined by calculating both the statistical significance of amino acid alignments and the absolute amino acid mismatches.

The Divergent Region of the Maxicircle

The divergent or variable region was first detected in a comparison of maxicircle DNAs from several *T. brucei* strains; this region of the molecule showed up to a 1-kb size variation between strains. In comparisons of the maxicircle DNAs of *L. tarentolae* and *T. brucei*, this region showed no cross-hybridization. A blot hybridization comparison of maxicircle DNAs from *Crithidia oncopelti*, *Crithidia luciliae*, *Leptomonas pessoai*, and *Leptomonas gymnodactyli* allowed Maslov et al (67) to construct a general model of the structural organization of maxicircles; the molecule is represented by a

17-kb conservative region common to all kinetoplastids and a divergent region showing both length and sequence variation. Several fragments of the divergent region from *L. tarentolae* and also from *T. brucei* have been cloned and sequenced. Fragments from both species contain tandem repeats of varying length which constitute appropriate substrates for the presumed insertion/deletion and rearrangement events that produce sequence change within this region of the maxicircle. Similar conclusions were reached by a previous electron microscope heteroduplex analysis of maxicircle DNA from *T. brucei* (11). The function of the divergent region is unknown, but a putative origin of replication of the leading strand was roughly localized to a site within the divergent region of the *C. fasciculata* maxicircle. In addition, several fragments of the divergent region of *L. tarentolae* and *C. oncopelti* were found to have autonomous replicating sequence activity in yeast (43, 67).

Transcription of Maxicircle Genes

The conserved region of the maxicircle is actively transcribed, yielding high steady-state levels of the 9S and 12S rRNAs and lower levels of polyadenylated mRNAs and smaller G-rich RNAs of unknown significance (47). Transcripts for six *L. tarentolae* structural genes and several ORFs have been identified, and the locations of the 5' ends have been determined by primer runnoff (85). The distance from the 5' end to the putative translation initiation codon (where determined) varies from 20 to 64 nucleotides. This arrangement differs from that in the human mitochondrion and is similar to that in the yeast mitochondrion, where there are multiple promotors and substantial 5' untranslated sequences. Transcription occurs from one strand for all genes but ND1, MURF1, and COI. Single transcripts cover the overlapping ORF3 and ORF4 and the overlapping ORF5 and ORF6 (=MURF2). The number of promotors is unknown. In some cases transcripts of higher molecular weight can be seen in Northern blots; these transcripts may be precursors. A preliminary capping experiment indicated the presence of separate promotors for the 9S and 12S rRNA genes and three to four additional promotors for the structural genes, but these must be confirmed by direct sequence analysis of the capped species (85). In the case of *T. brucei*, low-abundance high–molecular weight putative precursor bands were seen in Northern blots for several of the maxicircle genes (25).

The abundance of the steady-state transcripts from the *L. tarentolae* maxicircle genes showed some variation, which implies either separate transcriptional control or processing. For example, the 1800-nucleotide COI and the 1200-nucleotide CYb transcripts were much more abundant than the 1000-nucleotide MURF2 transcript (85, 93).

Regulation of Maxicircle Transcription During the Life Cycle of Trypanosoma

Extensive Northern blot analysis of maxicircle transcription in *T. brucei* has indicated the presence of double transcripts differing in size by 150–200 bp for all potential protein-coding genes except ND5 (25–27, 46). The size difference is not due to polyadenylation, as both transcripts occur in poly (A)+ RNA (25, 27, 46). In *L. tarentolae*, single RNA species are associated with ND4, ND5, ORF3–4, COI, and MURF2, whereas the COII, COIII, and CYb genes appear to hybridize to at least two transcripts each (85).

The life cycle of *T. brucei* involves dramatic changes in mitochondrial morphology and physiology. The long, slender bloodstream form (LS-BF) in the mammalian bloodstream lacks mitochondrial cytochromes and contains an empty mitochondrial tubule without cristae. The short, stumpy bloodstream form (SS-BF) is fixed the in G_0 phase and possesses some Krebs cycle enzymes and a mitochondrial NADH diaphorase activity. These forms differentiate into the procyclic trypomastigote form (PF) in the insect midgut or in culture. Established procyclic forms contain a complete functional phosphorylating cytochrome chain. The regulation of these biosynthetic changes is complex, involving both nuclear and mitochondrial genes. Modulation of several maxicircle gene transcripts occurs in the different stages of the life cycle. The transition from the LS-BF to the SS-BF appears to be an important developmental step, since the steady-state level of the 9S and 12S mitochondrial ribosomal RNAs increases approximately 30-fold and the originally undetectable levels of the CYb, COI, and COII transcripts increase to levels approaching those in PF cells (68a). On the other hand, Feagin, Jasmer, et al (25–27, 46) have reported that the larger of the two transcripts for the COI, COII, and CYb genes is more abundant in PF cells than in LS-BF cells by 2–9 fold, whereas the smaller transcript is more abundant in SS-BF than in either LS-BF or PF cells. These quantitative differences may reflect parasite strain differences or differences in the purity of the LS-BF cell population.

Regulation appears to be complex; it involves changes in the steady-state levels of one of the two transcripts for each gene as well as changes in the degree of polyadenylation. Usually the bloodstream form transcripts show less polyadenylation than the procyclic form transcripts (25–27, 46). The single ND5 transcript is unusual in that it is either more abundant in BF than in PF cells (25–27, 46) or has the same steady-state levels in both (68a).

One factor involved in the generation of multiple transcripts of the CYb gene in *T. brucei* is an insertion of 36 uridines at the 5' end of the mRNA which are not encoded in the DNA sequence (26a). This is analogous to the insertion of four uridines in the *T. brucei* maxicircle COII transcript, which overcomes a translational frameshift in the DNA sequence (5). The mech-

anism for this is unknown. It is clear that extensive transcriptional modulation occurs at the mitochondrial level during the life cycle of the African trypanosomes.

CONCLUSIONS

I have reviewed the current status of our understanding of the unusual mitochondrial genome of the parasitic kinetoplastid protozoa and have pointed out the utility of this genome as a model system for the study of several basic biological problems. The very existence of such an unusual biological phenomenon as the kinetoplast DNA is of great inherent interest. I trust that further study of these organisms will remove some of the mystery and add to our understanding of the selective pressures involved in the evolution of mitochondrial genomes in general and of this mitochondrial genome in particular.

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Literature Cited

1. Arnot, D., Barker, D. 1981. Biochemical identification of cutaneous leishmanias by analysis of kinetoplast DNA. II. Sequence homologies in *Leishmania* kinetoplast DNA. *Mol. Biochem. Parasitol.* 3:47–56
2. Barker, D., Gibson, L., Kennedy, W., Nasser, A., Williams, R. 1986. The potential of using recombinant DNA species-specific probes for the identification of tropical *Leishmania*. *Parasitology* 91:8139–74
3. Barrois, M., Riou, G., Galibert, F. 1982. Complete nucleotide sequence of minicircle kinetoplast DNA from *Trypanosoma equiperdum*. *Proc. Natl. Acad. Sci. USA* 78:3323–27
4. Benne, R., DeVries, B., Van den Burg, J., Klaver, B. 1983. The nucleotide sequence of a segment of *Trypanosoma brucei* mitochondrial maxi-circle DNA that contains the gene for apocytochrome *b* and some unusual unassigned reading frames. *Nucleic Acids Res.* 11:6925–41
5. Benne, R., Van den Burg, J., Brakenhoff, J., Sloof, P., Van Boom, J., Tromp, M. 1986. Major transcript of the frameshifted *coxII* gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. *Cell* 46:819–26
6. Birkenmeyer, L., Ray, D. 1986. Replication of kinetoplast DNA in isolated kinetoplasts from *Crithidia fasciculata*. *J. Biol. Chem.* 261:2362–68
7. Birkenmeyer, L., Sugisaki, H., Ray, D. S. 1985. The majority of minicircle DNA in *Crithidia fasciculata* strain CF-C1 is of a single class with nearly homogeneous DNA sequence. *Nucleic Acids Res.* 13:7107–18
8. Birkenmeyer, L., Sugisaki, H., Ray, D. 1987. Structural characterizations of site-specific discontinuities associated with replication origins of minicircle DNA from *Crithidia fasciculata*. *J. Biol. Chem.* 262:2384–92
9. Borst, P., Fase-Fowler, F., Gibson, W. 1987. Kinetoplast DNA of *Trypanosoma*

evansi. *Mol. Biochem. Parasitol.* 23: 31–38
10. Borst, P., Fase-Fowler, F., Weijers, P., Barry, J., Tetley, L., Vickerman, K. 1985. Kinetoplast DNA from *Trypanosoma vivax* and *T. congolense*. *Mol. Biochem. Parasitol.* 15:129–42
11. Borst, P., van der Ploeg, M., van Hoek, J., Tas, J., James, J. 1982. On the DNA content and ploidy of trypanosomes. *Mol. Biochem. Parasitol.* 6:13–23
12. Brimacombe, R., Maly, P., Zwieb, C. 1983. The structure of ribosomal RNA and its organization relative to ribosomal protein. *Prog. Nucleic Acid Res. Mol. Biol.* 28:1–48
13. Camargo, E., Mattei, D., Barbieri, C., Morel, C. 1981. Electrophoretic analysis of endonuclease-generated fragments of kinetoplast DNA, of esterase isoenzymes and of surface proteins as aids for species identification of insect trypanosomatids. *J. Protozool.* 29:251–58
14. Challberg, S., Englund, P. 1980. Heterogeneity of minicircles in kinetoplast DNA of *Leishmania tarentolae*. *J. Mol. Biol.* 138:447–72
15. Chen, K., Donelson, J. 1980. The sequences of two kinetoplast DNA minicircles of *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA* 77:2445–49
16. Deane, M., Sousa, M., Pereira, N., Goncalves, A., Momen, H., Morel, C. 1984. *Trypanosoma cruzi:* Inoculation schedules and re-isolation methods select individual strains from doubly infected mice, as demonstrated by schizodeme and zymodeme analyses. *J. Protozool.* 31:276–80
17. de la Cruz, V., Lake, J., Simpson, A., Simpson, L. 1985. A minimal ribosomal RNA: Sequence and secondary structure of the 9S kinetoplast ribosomal RNA from *Leishmania tarentolae*. *Proc. Natl. Acad. Sci. USA* 82:1401–5
18. de la Cruz, V., Neckelmann, N., Simpson, L. 1984. Sequences of six structural genes and several open reading frames in the kinetoplast maxicircle DNA of *Leishmania tarentolae*. *J. Biol. Chem.* 259:15136–47
19. de la Cruz, V., Simpson, A., Lake, J., Simpson, L. 1985. Primary sequence and partial secondary structure of the 12S kinetoplast (mitochondrial) ribosomal RNA from *Leishmania tarentolae:* Conservation of peptidyltransferase structural elements. *Nucleic Acids Res.* 13:2337–56
20. Diekmann, S., Wang, J. 1985. On the sequence determinants and flexibility of the kinetoplast DNA fragment with abnormal gel electrophoretic mobilities. *J. Mol. Biol.* 186:1–11
21. Englund, P. 1979. Free minicircles of kinetoplast DNA in *Crithidia fasciculata*. *J. Biol. Chem.* 254:4895–900
22. Englund, P. 1981. Kinetoplast DNA. In *Biochemistry and Physiology of Protozoa*, ed. M. Levandowsky, S. Hutner, 4:333–81. New York: Academic. 2nd ed.
23. Eperon, I., Janssen, J., Hoeijmakers, J., Borst, P. 1983. The major transcripts of the kinetoplast DNA of *T. brucei* are very small ribosomal RNAs. *Nucleic Acids Res.* 11:105–25
24. Fairlamb, A., Weislogel, P., Hoeijmakers, J., Borst, P. 1978. Isolation and characterization of kinetoplast DNA from bloodstream form of *Trypanosoma brucei*. *J. Cell Biol.* 76:293–309
25. Feagin, J., Jasmer, D., Stuart, K. 1985. Apocytochrome *b* and other mitochondrial DNA sequences are differentially expressed during the life cycle of *Trypanosoma brucei*. *Nucleic Acids Res.* 13:4577–96
26. Feagin, J., Jasmer, D., Stuart, K. 1986. Differential mitochondrial gene expression between slender and stumpy bloodforms of *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 20:207–14
26a. Feagin, J., Jasmer, D., Stuart, K. 1987. Developmentally regulated addition of nucleotides within apocytochrome *b* transcripts in *Trypanosoma brucei*. *Cell* In press
27. Feagin, J., Stuart, K. 1985. Differential expression of mitochondrial genes between the life cycle stages of *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA* 82:3380–84
28. Fouts, D., Manning, J., Wolstenholme, D. 1975. Physicochemical properties of kinetoplast DNA from *Crithidia acanthocephali*, *Crithidia luciliae* and *Trypanosoma lewisi*. *J. Cell Biol.* 67:378–99
29. Fouts, D., Wolstenholme, D. 1979. Evidence for a partial transcript of the small circular component of kinetoplast DNA of *Crithidia acanthocephali*. *Nucleic Acids Res.* 6:3785–804
30. Frasch, A., Goijman, S., Cazzulo, J., Stoppani, A. 1981. Constant and variable regions in DNA minicircles from *Trypanosoma cruzi* and *Trypanosoma rangeli*: Application to species and stock differentiation. *Mol. Biochem. Parasitol.* 4:163–70
31. Frasch, A., Hajduk, S., Hoeijmakers, J., Borst, P., Brunel, F., Davison, J.

1980. The kDNA of *Trypanosoma equiperdum*. *Biochim. Biophys. Acta* 607:397–410
32. Frasch, A., Sanchez, D., Stoppani, A. 1984. Homogeneous and heterogeneous mini-circle subpopulations in *Trypanosoma cruzi* kinetoplast DNA. *Biochim. Biophys. Acta* 782:26–33
33. Griffith, J., Bleyman, M., Rauch, C., Kitchin, P., Englund, P. 1986. Visualization of the bent helix in kinetoplast DNA by electron microscopy. *Cell* 46:717–24
34. Hagerman, P. J. 1984. Evidence for the existence of stable curvature of DNA in solution. *Proc. Natl. Acad. Sci. USA* 81:4632–36
35. Hagerman, P. 1985. Sequence dependence of the curvature of DNA: A test of the phasing hypothesis. *Biochemistry* 24:7033–37
36. Hagerman, P. 1986. Sequence-directed curvature of DNA. *Nature* 321:449–50
37. Handman, E., Hocking, R., Mitchell, G., Spithill, T. 1983. Isolation and characterization of infective and non-infective clones of *Leishmania tropica*. *Mol. Biochem. Parasitol.* 7:111–26
38. Hensgens, A., Brakenhoff, J., De Vries, B., Sloof, P., Tromp, M., et al. 1984. The sequence of the gene for cytochrome *c* oxidase subunit I, a frameshift containing gene for cytochrome *c* oxidase subunit II and seven unassigned reading frames in *Trypanosoma brucei* mitochondrial maxicircle DNA. *Nucleic Acids Res.* 12.7327–44
39. Hoeijmakers, J., Borst, P. 1982. RNA from the insect trypanosome *Crithidia luciliae* contains transcripts of the maxicircle and not of the minicircle component of kinetoplast DNA. *Biochim. Biophys. Acta* 521:407–11
40. Hoeijmakers, J., Schoutsen, B., Borst, P. 1982. Kinetoplast DNA in the insect trypanosomes *Crithidia luciliae* and *Crithidia fasciculata*. I. Sequence evolution and transcription of the maxicircle. *Plasmid* 7:199–209
41. Hoeijmakers, J., Snijders, A., Janssen, J., Borst, P. 1981. Transcription of kinetoplast DNA in *Trypanosoma brucei* bloodstream and culture forms. *Plasmid* 5:329–50
42. Hoeijmakers, J., Weijers, P., Brakenhoff, C., Borst, P. 1982. Kinetoplast DNA in the insect trypanosomes *Crithidia luciliae* and *Crithidia fasciculata*. III. Heteroduplex analysis of the *Crithidia luciliae* minicircles. *Plasmid* 7:221–29
43. Hughes, D., Simpson, L., Kayne, P., Neckelmann, N. 1984. Autonomous replication sequences in the maxicircle kinetoplast DNA of *Leishmania tarentolae*. *Mol. Biochem. Parasitol.* 13:263–75
44. Jackson, P. 1987. Differentiation of *Leishmania* species and strains by analysis of restriction endonuclease-produced kinetoplast DNA fragments and DNA-DNA hybridization with 32P-kDNA from type isolates. In *Atomic Energy Applications in Parasitology, A Training Course*, ed. E. Hayunga, M. Steck. Vienna: Int. Atomic Energy Assoc.-Dep. Energy. In press
45. Jackson, P., Wolhieter, J., Jackson, J., Sayles, P., Diggs, C., Hockmeyer, W. 1984. Restriction endonuclease analysis of *Leishmania* kinetoplast DNA characterizes parasites responsible for visceral and cutaneous disease. *Am. J. Trop. Med. Hyg.* 33:808–19
46. Jasmer, D., Feagin, J., Payne, M., Stuart, K. 1985. Diverse patterns of expression of the cytochrome *c* oxidase subunit I gene and unassigned reading frames 4 and 5 during the life cycle of *Trypanosoma brucei*. *Mol. Cell. Biol.* 5:3041–47
47. Jasmer, D., Feagin, J., Payne, M., Stuart, K. 1987. Variation of G-rich mitochondrial transcripts among stocks of African trypanosomes. *Mol. Biochem. Parasitol.* 22:259–72
48. Jasmer, D., Stuart, K. 1986. Conservation of kinetoplastid minicircle characteristics without nucleotide sequence conservation. *Mol. Biochem. Parasitol.* 18:257–70
49. Jasmer, D., Stuart, K. 1986. Sequence organization in African trypanosome minicircles is defined by 18 base pair inverted repeats. *Mol. Biochem. Parasitol.* 18:321–32
50. Kennedy, W. P. 1984. Novel identification of differences in the kinetoplast DNA of *Leishmania* isolates by recombinant DNA techniques and in situ hybridization. *Mol. Biochem. Parasitol.* 12:313–25
51. Kidane, G., Hughes, D., Simpson, L. 1984. Sequence heterogeneity and anomalous electrophoretic mobility of kinetoplast minicircle DNA in *Leishmania tarentolae*. *Gene* 27:265–77
52. Kitchin, P., Klein, V., Englund, P. 1985. Intermediates in the replication of kinetoplast DNA minicircles. *J. Biol. Chem.* 260:3844–51
53. Kitchin, P., Klein, V., Fein, B., Englund, P. 1984. Gapped minicircles. A

novel replication intermediate of kinetoplast DNA. *J. Biol. Chem.* 24:15532–39
54. Kitchin, P., Klein, V., Ryan, K., Gann, K., Rauch, C., et al. 1986. A highly bent fragment of *Crithidia fasciculata* kinetoplast DNA. *J. Biol. Chem.* 261:11302–9
55. Kleisen, C. M., Borst, P. 1975. Are 50% of all cellular proteins synthesized on mitochondrial ribosomes in *Crithidia luciliae*? *Biochim. Biophys. Acta* 390:78–81
56. Kleisen, C., Borst, P., Weijers, P. 1976. The structure of kinetoplast DNA. I. The mini-circles of *Crithidia luciliae* are heterogeneous in base sequence. *Eur. J. Biochem.* 64:141–51
57. Koo, H., Wu, H., Crothers, D. 1986. DNA bending at adenine-thymine tracts. *Nature* 320:501–6
58. Leon, W., Frank, A., Hoeijmakers, J., Fase-Fowler, F., Borst, P., et al. 1980. Maxi-circles and mini-circles in kinetoplast DNA from *Trypanosoma cruzi*. *Biochim. Biophys. Acta* 607:221–31
59. Levene, S., Wu, H., Crothers, D. 1986. Bending and flexibility of kinetoplast DNA. *Biochemistry* 25:3988–95
60. Lopes, U., Momen, H., Grimaldi, G., Marzochi, M., Pacheco, R., Morel, C. 1984. Schizodeme and zymodeme characterization of *Leishmania* in the investigation of foci of visceral and cutaneous leishmaniasis. *J. Parasitol.* 70:89–98
61. Lopes, U., Wirth, D. 1986. Identification of visceral *Leishmania* species with cloned sequences of kinetoplast DNA. *Mol. Biochem. Parasitol.* 20:77–84
62. Macina, R., Sanchez, D., Affranchino, J., Engel, J., Frasch, A. 1985. Polymorphism within minicircle sequence classes in the kinetoplast DNA of *Trypanosoma cruzi* clones. *Mol. Biochem. Parasitol.* 16:61–74
63. Macina, R., Sanchez, D., Gluschankof, D., Burrone, O., Frasch, A. 1986. Sequence diversity in the kinetoplast DNA minicircles of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 21:25–32
64. Marini, J., Effron, P., Goodman, T., Singleton, C., Wells, R., et al. 1984. Physical characterization of a kinetoplast DNA fragment with unusual properties. *J. Biol. Chem.* 259:8974–79
65. Marini, J., Levene, S., Crothers, D., Englund, P. 1982. A bent helix in kinetoplast DNA. *Cold Spring Harbor Symp. Quant. Biol.* 47(Pt. 1):279–83
66. Marini, J., Levene, S., Crothers, D., Englund, P. 1983. Bent helical structure in kinetoplast DNA—Correction. *Proc. Natl. Acad. Sci. USA* 24:7678
67. Maslov, D., Kolesnikov, A., Zaitseva, G. 1984. Conservative and divergent base sequence regions in the maxicircle kinetoplast DNA of several trypanosomatid flagellates. *Mol. Biochem. Parasitol.* 12:351–64
68. Mattei, D., Goldenberg, S., Morel, C., Azevedo, H., Roitman, I. 1977. Biochemical strain characterization of *Trypanosoma cruzi* by restriction endonuclease cleavage of kinetoplast DNA. *FEBS Lett.* 74:264–68
68a. Michelotti, E., Hajduk, S. 1987. Developmental regulation of trypanosome mitochondrial DNA gene expression. *J. Biol. Chem.* 262:927–32
69. Morel, C., Chiari, E., Camargo, E., Mattei, D., Romanha, A., Simpson, L. 1980. Strains and clones of *Trypanosoma cruzi* can be characterized by restriction endonuclease fingerprinting of kinetoplast DNA minicircles. *Proc. Natl. Acad. Sci. USA* 77:6810–14
70. Morel, C., Goncalves, A., Simpson, L., Simpson, A. 1984. Recent advances in the development of DNA hybridization probes for the detection and characterization of *Trypanosoma cruzi*. *Mem. Inst. Oswaldo Cruz* 79:51–53 (Suppl.)
71. Morel, C., Simpson, L. 1980. Characterization of pathogenic trypanosomatidae by restriction endonuclease fingerprinting of kinetoplast DNA minicircles. *Am. J. Trop. Med. Hyg.* 29:1070–74 (Suppl.)
72. Muhich, M., Simpson, L. 1986 Specific cleavage of kinetoplast minicircle DNA from *Leishmania tarentolae* and identification of several additional minicircle sequence classes. *Nucleic Acids Res.* 14:5531–56
73. Muhich, M., Simpson, L., Simpson, A. 1983. Comparison of the maxicircle DNAs of *Leishmania tarentolae* and *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA* 80:4060–64
74. Ntambi, J., Englund, P. 1985. A gap at a unique location in newly replicated kinetoplast DNA minicircles from *Trypanosoma equiperdum*. *J. Biol. Chem.* 260:5574–79
75. Ntambi, J., Shapiro, T., Ryan, K., Englund, P. 1986. Ribonucleotides associated with a gap in the newly replicated kinetoplast DNA minicircles from *Trypanosoma equiperdum*. *J. Biol. Chem.* 261:11890–95
76. Oster, C., Jackson, P., Muigai, R., Wasunna, K., Rashid, J., et al. 1987. Diagnosis of Kenyan visceral leishmaniasis using a kinetoplast DNA probe. *East Afr. Med. J.* In press

77. Payne, M., Rothwell, V., Jasmer, D., Feagin, J., Stuart, K. 1985. Identification of mitochondrial genes in *Trypanosoma brucei* and homology to cytochrome *c* oxidase II in two different reading frames. *Mol. Biochem. Parasitol.* 15:159–70
78. Ponzi, M., Birago, C., Battaglia, P. 1984. Two identical symmetrical regions in the minicircle structure of *Trypanosoma lewisi* kinetoplast DNA. *Mol. Biochem. Parasitol.* 13:111–19
79. Ray, D., Hines, J., Sugisaki, H., Sheline, C. 1986. kDNA minicircles of the major sequence class of *Crithidia fasciculata* contain a single region of bent helix widely separated from the two origins of replication. *Nucleic Acids Res.* 14:7953–65
80. Riou, G., Barrois, M. 1979. Restriction cleavage map of kinetoplast DNA minicircle from *Trypanosoma equiperdum*. *Biochem. Biophys. Res. Commun.* 90:405–9
81. Rogers, D., Wirth, D. 1987. Kinetoplast DNA minicircles: Regions of extensive sequence divergence. *Proc. Natl. Acad. Sci. USA* 84:565–69
82. Ryder, K., Silver, S., DeLucia, A., Fanning, E., Tegtmeyer, P. 1986. An altered DNA conformation in origin region I is a determinant for the binding of SV40 large T antigen. *Cell* 44:719–25
83. Sanchez, D., Madrid, R., Engel, J., Frasch, A. 1984. Rapid identification of *Trypanosoma cruzi* isolates by "dot blot" hybridization. *FEBS Lett.* 168:139–42
84. Shlomai, J., Zadok, A. 1984. Kinetoplast DNA minicircles of trypanosomatids encode for a protein product. *Nucleic Acids Res.* 12:8017–28
85. Simpson, A., Hughes, D., Simpson, L. 1985. Trypanosoma brucei: differentiation of in vitro–grown bloodstream trypomastigotes into procyclic forms. *J. Protozool.* 32:672–77
86. Simpson, A., Simpson, L. 1976. Pulse-labeling of kinetoplast DNA: Localization of two sites of synthesis within the networks and kinetics of labeling of closed minicircles. *J. Protozool.* 23: 583–87
87. Simpson, L. 1972. The kinetoplast of the hemoflagellates. *Int. Rev. Cytol.* 32:139–207
88. Simpson, L. 1979. Isolation of maxicircle component of kinetoplast DNA from hemoflagellate protozoa. *Proc. Natl. Acad. Sci. USA* 76:1585–1588
89. Simpson, L. 1986. Kinetoplast DNA in trypanosomid flagellates. *Int. Rev. Cytol.* 99:119–79
90. Simpson, L., Neckelmann, N., de la Cruz, V., Simpson, A., Feagin, J., et al. 1987. Comparison of the maxicircle (mitochondrial) genomes of *Leishmania tarentolae* and *Trypanosoma brucei* at the level of nucleotide sequence. *J. Biol. Chem.* 262:6182–96
91. Simpson, L., Simpson, A. 1978. Kinetoplast RNA of *Leishmania tarentolae*. *Cell* 14:169–78
92. Simpson, L., Simpson, A., Kidane, G., Livingston, L., Spithill, T. 1980. The kinetoplast DNA of the hemoflagellate protozoa. *Am. J. Trop. Med. Hyg.* 29:1053–63 (Suppl.)
93. Simpson, L., Simpson, A., Livingston, L. 1982. Transcription of the maxicircle kinetoplast DNA of *Leishmania tarentolae*. *Mol. Biochem. Parasitol.* 6:237–57
94. Simpson, L., Simpson, A., Wesley, R. 1974. Replication of the kinetoplast DNA of *Leishmania tarentolae* and *Crithidia fasciculata*. *Biochim. Biophys. Acta* 349:161–72
95. Sloof, P., Van den Burg, J., Voogd, A., Benne, R., Agostinelli, M., et al. 1985. Further characterization of the extremely small mitochondrial ribosomal RNAs from trypanosomes: a detailed comparison of the 9S and the 12S RNAs from *Crithidia fasciculata* and *Trypanosoma brucei* with rRNAs from other organisms. *Nucleic Acids Res.* 13:4171–90
96. Snyder, M., Buchman, A., Davis, R. 1986. Bent DNA at a yeast autonomously replicating sequence. *Nature* 324:87–89
97. Spithill, T., Grumont, R. 1984. Identification of species, strains and clones of *Leishmania* by characterization of kinetoplast DNA minicircles. *Mol. Biochem. Parasitol.* 12:217–36
98. Stuart, K. 1979. Kinetoplast DNA of *Trypanosoma brucei*: Physical map of the maxicircle. *Plasmid* 2:520–28
99. Stuart, K. 1983. Kinetoplast DNA, a mitochondrial DNA with a difference. *Mol. Biochem. Parasitol.* 9:93–104
100. Stuart, K., Gelvin, S. 1982. Localization of kinetoplast DNA maxicircle transcripts in bloodstream and procyclic form *Trypanosoma brucei*. *Mol. Cell. Biol.* 2:845–52
101. Sugisaki, H., Ray, D. 1987. Kinetoplast DNA minicircles of the insect trypanosomatid *Crithidia fasciculata* contain two nearly identical 173 bp and 177 bp sequences involved in DNA replication. *Mol. Biochem. Parasitol.* 23:253–64
102. Trifonov, E., Sussman, J. 1980. The pitch of chromatin DNA is reflected in

its nucleotide sequence. *Proc. Natl. Acad. Sci. USA* 77:3816–820

103. Wirth, D., Pratt, D. 1982. Rapid identification of *Leishmania* species by specific hybridization of kinetoplast DNA in cutaneous lesions. *Proc. Natl. Acad. Sci. USA* 79:6999–7003
104. Woese, C., Gutell, R., Gupta, R., Noller, H. 1983. Detailed analysis of the higher order structure of 16S-like ribosomal ribonucleic acids. *Microbiol. Rev.* 47:621–69
105. Wu, H., Crothers, D. 1984. The locus of sequence-directed and protein-induced DNA bending. *Nature* 308:509–13
106. Zahn, K., Blattner, F. R. 1985. Sequence-induced DNA curvature at the bacteriophage lambda origin of replication. *Nature* 317:451–53