Kinetoplast DNA in Trypanosomid Flagellates

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I. Introduction 120
II. Minicircle DNA: Structure and Complexity 120
A. *Trypanosoma brucei* 121
B. *Trypanosoma gambiense* 127
C. *Trypanosoma equiperdum* 127
D. *Trypanosoma evansi* 129
E. *Trypanosoma mega* 129
F. *Trypanosoma lewisi* 129
G. *Trypanosoma cruzi* 130
H. *Crithidia* Species 130
I. *Leishmania tarentolae* 133
J. *Phytomonas davidi* 141
III. Evolution of Minicircle Sequence Heterogeneity 141
IV. Transcription of Minicircle DNA 148
V. Replication of Minicircle Kinetoplast DNA 149
VI. Maxicircle DNA: Isolation, Cloning, and Restriction Mapping 150
A. *Trypanosoma brucei* 151
B. *Trypanosoma evansi* 154
C. *Trypanosoma equiperdum* 154
D. *Trypanosoma mega* 155
E. *Trypanosoma cruzi* 155
F. *Leishmania tarentolae* 155
G. *Crithidia* Species 157
H. *Herpetomonas samuelpessoa* 160
VII. Replication of Maxicircle DNA 160
A. Replication by Rolling-Circle Model 160
B. Autonomous Replication Sequences in Maxicircle DNA 160
VIII. Transcription of Maxicircle DNA 161
A. *Trypanosoma brucei* 161
B. *Leishmania tarentolae* 163
IX. Kinetoplast Ribosomes 166
A. Isolation of Ribosomes 166
B. DNA Sequences of 9 and 12 S RNA Genes 166
C. Secondary Structures of 9 and 12 S RNAs 167
X. Genomic Organization of Maxicircle DNA 167
A. Maxicircle Structural Genes 167
B. Evolution of Maxicircle DNA: Structural and Ribosomal Genes 172
C. Evolution of Maxicircle DNA: Divergent Region 172

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XI. Unusual Kinetoplast DNAs 173
XII. Conclusions 174
References 176

I. Introduction

This review will emphasize recent developments in the application of recombinant DNA technology to problems of the structure, replication, and transcription of the unusual mitochondrial DNA in the kinetoplastid protozoa known as kinetoplast DNA. Kinetoplast DNA will be discussed in terms of its two molecular components, minicircles and maxicircles, and species-dependent variations will be emphasized.

II. Minicircle DNA: Structure and Complexity

The minicircle component of the kinetoplast DNA is the most unusual aspect of this mitochondrial genetic system. Circular DNA molecules are not uncommon in nature nor in other mitochondrial genetic systems, but nowhere in nature does one find thousands of small circles catenated together into a single giant network of DNA such as found in the kinetoplastid protozoa. The function of minicircle DNA is still a mystery, but much has been learned recently about the structure and replication of this DNA in several kinetoplastid species: *Trypanosoma brucei*, *Trypanosoma equiperdum*, *Leishmania tarentolae*, *Crithidia fasciculata*, and *Crithidia luciliae*. In general minicircle DNA from all these species has the following general characteristics: (1) circles exist catenated with each other, although there is a small percentage of unattached circles that may have a functional significance; (2) within any one species the circles are of fairly uniform size although the size varies from species to species; (3) there is a species-dependent variable amount of sequence heterogeneity among the minicircles from any one clonal population; (4) the sequence heterogeneity is expressed on the level of the individual minicircle as a variable region and a constant region and sequence changes among the minicircle population in a kinetoplastid species occur rapidly in nature; and (5) there is no apparent sequence homology between the minicircles of a given species and the maxicircle DNA. In addition to these general common properties of minicircle DNA, there are many differences between the various kinetoplastid species, and these differences may prove

to have phylogenetic significance. Therefore, I will discuss results from each species in succession.

A. *Trypanosoma brucei*

From the initial optical reassociation studies of Steinert *et al.* (1976) it was realized that the kDNA of *T. brucei* was perhaps the most complex in terms of sequence heterogeneity and the most rapidly changing in terms of evolution. The DNA reassociated with a single copy complexity of 300 times the size of the minicircle, and no hybridization between different *T. brucei* strains was observed. The high complexity of kDNA from *T. brucei* was confirmed by the S_1 C_0t analysis of Donelson *et al.* (1979) in which [^{3}H]kDNA renatured with a single copy $C_0t_{1/2}$ 316 times the $C_0t_{1/2}$ when a cloned 1.1-kb minicircle (pkT51) was the excess driver (Fig. 1). However, there was a minor component (15%) of the kDNA which renatured at a rate suggestive of repetitive sequences. Renaturation of a cloned minicircle, pkT51, in the presence of excess DNA from two other cloned minicircles, pkT3 and pkT204, showed that approximately 25% of the sequence of the pkT51 insert is homologous to sequences in both the pkT3 and pkT204 inserts (Fig. 1). Stuart (1979) confirmed that there are approximately 300 different minicircle sequence classes in *T. brucei* 164 kDNA and showed that the minicircle classes differ in relative abundance (Stuart and Gelvin, 1982). For example, a *Hin*dIII cut minicircle cloned into the *Hin*dIII site of pBR322 renatured at a rate implying the existence of 500 copies per network, whereas *Bam*HI cleaved uncloned minicircles renatured at a rate implying the existence of 60 copies per network (Fig. 2).

However, assuming a *T. brucei* network mass of 4.2×10^9 Da that is composed of 15% maxicircle DNA of 22-kb molecules and 85% minicircle DNA of 1.1-kb molecules, one can calculate that there are approximately 45 maxicircles and 5500 minicircles (Stuart and Gelvin, 1980). Furthermore, assuming that there are about 300 different minicircle sequence classes, this would imply about 18 copies of each sequence class if the classes are of equal size. The finding (Stuart and Gelvin, 1980) that one cloned minicircle is present in 500 copies implies that there is a wide divergence in frequency of the different sequence classes in *T. brucei*.

The sequence heterogeneity of *T. brucei* minicircle DNA shown by C_0t analysis is evidenced also by restriction enzyme analysis on gels (Fig. 3). Digestion of *T. brucei* kDNA with most restriction enzymes yields low release of unit-length linearized minicircles with no smaller bands (Borst *et al.*, 1980a, 1981a; Brunel *et al.*, 1980a; Fairlamb *et al.*, 1978; Simpson and Simpson, 1980). Most of the DNA remains catenated in the network

that does not enter the gel. This low release of mainly once-cut minicircles by most restriction enzymes is due both to the presence of many minicircle sequence classes and to the high percentage of AT in the minicircle DNA of *T. brucei*. However, digestion with *Taq*I, *Alu*I, or *Mbo*I does release most of the minicircles from the network and produce fragments of less than unit length, implying that sites for these enzymes are present more than once in conserved regions of the minicircles. The apparently single bands of unit-length minicircle DNA released by digestion with enzymes such as *Eco*RI, *Pst*I, *Hpa*II, *Hae*III, *Hin*dIII, and *Hha*I were

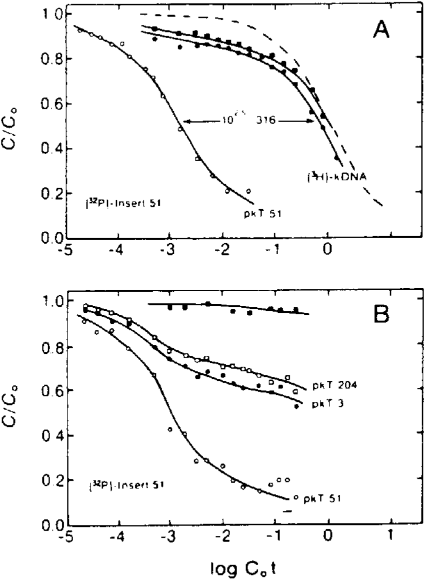

FIG. 1. Renaturation kinetics of the *Trypanosoma brucei* kDNA insert of pkT51 which had been labeled *in vitro* with ^{32}P. (A) The renaturation of ^{32}P-labeled pkT51 insert DNA in the presence of an excess of pkT51 DNA (open circles) or an excess of total network kDNA (solid circles). The network kDNA was labeled with ^{3}H *in vitro* prior to shearing so that its renaturation could also be monitored (solid squares). The broken line is a model curve generated by the standard renaturation equation when it is assumed that a single-copy DNA sequence has a log $C_0t_{1/2} = 0.15$. (B) The renaturation of ^{32}P-labeled pkT51 insert DNA (open circles), pkT3 DNA (solid circles), pkT204 DNA (open squares), or pBR322 DNA (solid squares). The initial concentration, C_0, of the excess unlabeled driver DNAs refers to the concentration of only the inserted kDNA sequence when a pkT recombinant plasmid is the driver DNA. In all of the experiments C_0 was chosen so that the final time point was taken 11 hours after the start of the reaction. About 4000 ^{32}P cpm were sampled at each time point. The pBR322 sequence does not drive the renaturation of the kDNA insert 51 within the time span of the renaturation experiments (solid squares). Reprinted from Donelson *et al.* (1979) with permission.

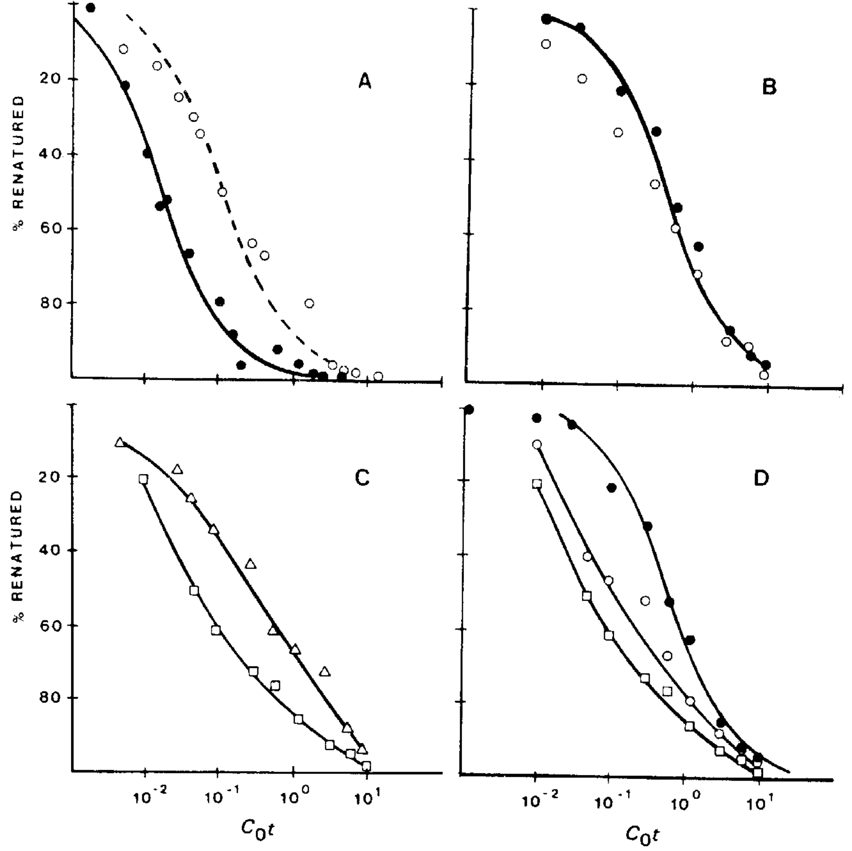

FIG. 2. Renaturation kinetics of nick-translated kDNA sequences of *T. brucei*. (A) kDNA and homologous driver (open circles) compared to lambda DNA standard (solid circle). Both lines are theoretical single component curves. (B) Maxicircle (open circles) and cloned maxicircle fragment (solid circles) driven by kDNA. (C) Cloned *Hin*dIII minicircle (open squares) and *Bam*HI minicircle (open triangles) sequences driven by kDNA. (D) Cloned *Hin*dIII minicircle (open squares), kDNA (open circles), and cloned maxicircle segment (solid circles) driven by kDNA. The curve through the cloned maxicircle segment points is a theoretical single component curve. Reprinted from Stuart and Gelvin (1980) with permission.

shown to be composed of 5–10 closely spaced bands by acrylamide gel electrophoresis (Simpson and Simpson, 1980) (Fig. 4), implying either a substantial minor length heterogeneity or the existence of gel mobility anomalies, as shown to exist in *L. tarentolae* minicircle DNA (Challberg and Englund, 1980; Simpson, 1979).

Analysis of cloned minicircle DNA has greatly assisted our understand-

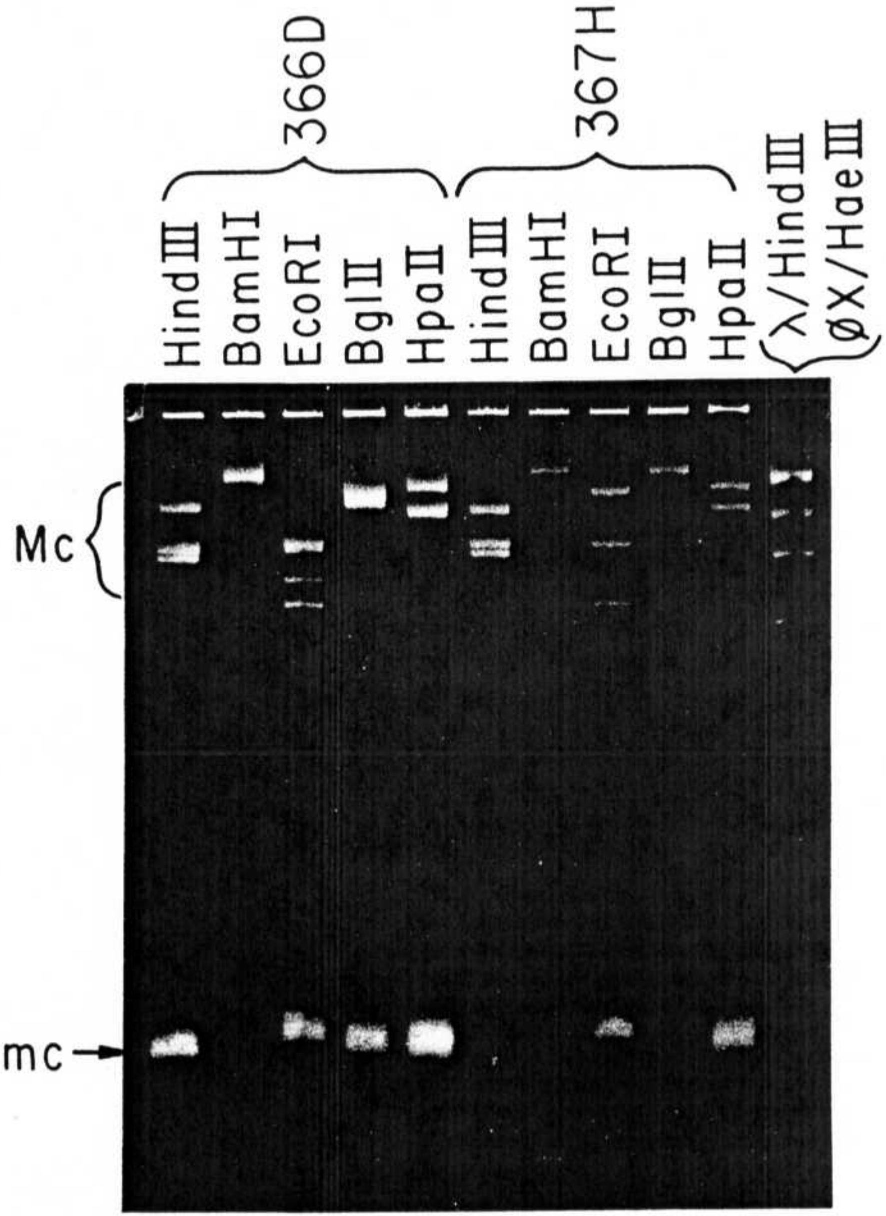

FIG. 3. Comparison of digestions of kDNA from two clonal strains (366D and 367H) of *T. brucei* procyclics in 0.8% agarose. The minicircle (mc) and maxicircle (Mc) regions are indicated. The molecular weight references are λ/*Hin*dIII and φXRF/*Hae*III fragments. Reprinted from Simpson and Simpson (1980) with permission.

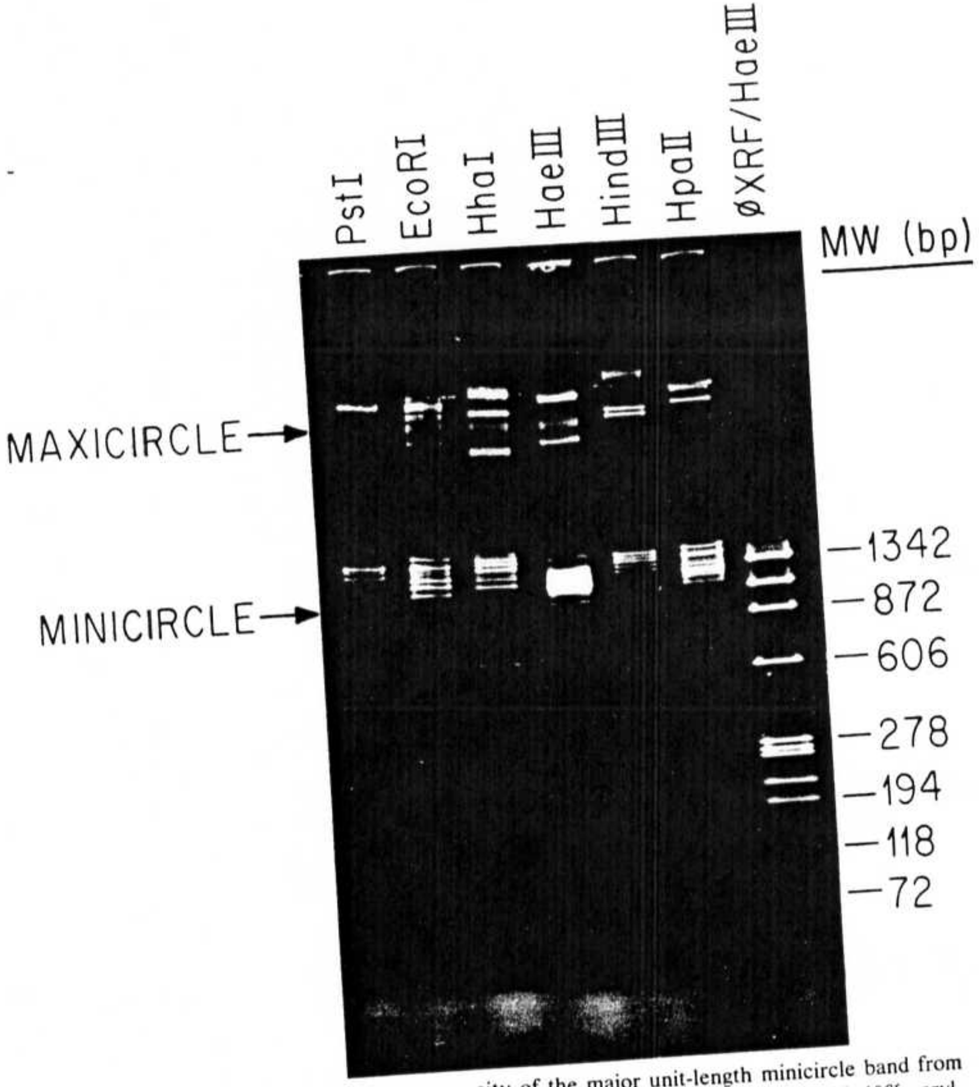

FIG. 4. Demonstration of heterogeneity of the major unit-length minicircle band from 366D procyclic *T. brucei* kDNA in an acrylamide gradient gel. Conditions: 3.5–10% acrylamide with a 3.0% stacking region. The molecular weight references are ϕXRF/*Hae*III fragments. Reprinted from Simpson and Simpson (1980) with permission.

ing of the observed sequence heterogeneity. Donelson *et al.* (1979) cloned minicircles from *T. brucei* clone 18E2 either by C-tailing minicircles released by digestion with *Hae*III, or with a mixture of *Hpa*II, *Hha*I, and *Alu*I and cloning into the *Pst*I site of pBR322, or by direct ligation of *Hin*dIII- or *Pst*I-released minicircles into the appropriate sites in pBR322. One minicircle, pkT3, was labeled *in vitro* and used as a probe in a Southern blot hybridization of kDNA digested with several enzymes. Hybridization was observed in all minicircle bands released by digestion with six enzymes. Similar results were obtained by colony hybridization, using three minicircle clones as probes. In general the probes hybridized strongly to themselves and weakly to all the other minicircle clones. Brunel *et al.* (1980a,b) cloned *Hin*dIII minicircles from *T. brucei* 427–60 in pBR322 and also *Eco*RI minicircles in λgtWES/λB (together with a maxicircle fragment, or with λB) and tested the cloned inserts for digestion with nine enzymes. The only sites found were single sites for *Eco*RI, *Hha*I, *Hae*III, and two to three sites for *Taq*I; all four of the clones studied contained different patterns of restriction sites. However, when the λ clones were spotted onto filters and probed with five of the pBR322 clones, some hybridization was observed with all clones, implying common sequences.

Simpson and Simpson (1980) cloned *Bam*HI-released minicircles from *T. brucei* 366D into pBR322 and tested 25 clones by colony hybridization using one, pTb7, as a probe. Four of the 25 clones showed strong hybridization, but all showed some hybridization, implying the existence of more than one semihomologous minicircle sequence class with a single *Bam*HI site.

Some heteroduplex studies of *T. brucei* minicircles have been reported, but the small size of these molecules precludes obtaining much information from this method (Brunel *et al.*, 1980a; Donelson *et al.*, 1979).

A direct confirmation and extension of the hybridization results was reported by Chen and Donelson (1980) in a sequence analysis of two cloned minicircles from *T. brucei* (Fig. 5). pk51 was derived from a digest of kDNA with a mixture of *Hha*I, *Hpa*II, and *Alu*I, tailed with G and inserted into the *Pst*I site of pBR322. pk201 was released from kDNA by digestion with *Pst*I and ligated directly into the *Pst*I site of pBR322. Both molecules contained approximately 72% AT, as expected from the low buoyant density of the kDNA (ρ = 1.690 g/ml). The most striking aspect was a nearly perfect homology of 122 bp. There were also 12 other common regions containing 50% AT. pk201 contained a decanucleotide sequence repeated 5 times in tandem. The only region of dyad symmetry was an 11-bp sequence in pk51. There was a high frequency of termination codons, TAA, TGA, and TAG, in all reading frames in both mole-

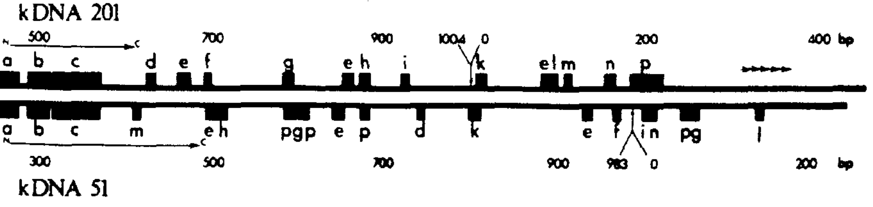

FIG. 5. Diagram showing the relative locations of perfect homologies between *T. brucei* cloned minicircle kDNAs 201 and 51 that are equal to or greater than 10 bp. The two circular kDNA sequences are represented by two lines aligned so that each begins with the first position of the 122-bp near-perfect homology. The other letters indicate the other smaller corresponding regions of homology. The numbers indicate nucleotide positions. The horizontal arrows with N at the left end and C at the right show the largest regions in the two kDNAs that could potentially code for a polypeptide. The five tandem arrows above the lines on the right indicate five repeats of a decanucleotide sequence in kDNA 201. Reprinted from Chen and Donelson (1980) with permission.

cules; the longest open reading frames were 52 amino acids in pk201 and 71 amino acids in pk51. Interestingly, these small open reading frames encompassed the 122-bp common region. The sequence data nicely confirmed the evidence from the C_0t analysis that the different minicircle classes had approximately 25% of their sequences in common, since summation of all the perfect homologies of 10 bp or greater gave a value of 27%.

B. *Trypanosoma gambiense*

Riou and Barrois (1981) found that the minicircles from the kDNA networks of *T. gambiense* were similar to those from *T. brucei* in terms of cleavage by restriction enzymes. The only enzymes that cleaved the majority of the network minicircles and produced fragments of less than unit length were *Taq*I, *Hin*fI, and *Xba*I. The patterns with these enzymes indicated extensive minicircle sequence heterogeneity. They also found that the minicircles hybridized somewhat with minicircle DNA from the Pasteur Institute strain of *T. equiperdum.*

C. *Trypanosoma equiperdum*

Unlike most other kinetoplastid species, the kDNA minicircles from *T. equiperdum* are homogeneous in base sequence. This was first shown by Riou and Barrois (1979) for a strain of *T. equiperdum* from the Pasteur Institute and by Frasch *et al.* (1980) for a strain from the ATCC (30019). However, these two strains differ signficantly in the maxicircle compo-

nents, indicating a possible molecular pleomorphism among strains of this species.

A unique circular restriction enzyme map for the uncloned minicircle from the Pasteur *T. equiperdum* strain was derived by Riou and Barrois (1979) and the complete nucleotide sequence was reported by Barrois *et al.* (1982). The molecule contained 73% AT, had a 6-fold tandem repeat of a 12-bp sequence, and had three small dyad symmetries of 9–11 bp distributed equidistantly from each other. In terms of possible codogenic function, the longest open reading frames were 18 and 22 amino acids (Fig. 6). A sequence of 130 bp was strikingly homologous to the 122-bp common sequence to *T. brucei* minicircles pkT51 and pkT201; it differed from pkT201 by 12 bp and from pkT51 by 15 bp. The remainder of the *T. equiperdum* molecule had no significant homology with the *T. brucei* sequences. This cross-species partial sequence conservation is intriguing in terms of the functional significance of the minicircle. Both Chen and Donelson (1980) and Barrois *et al.* (1982) speculated that the conserved

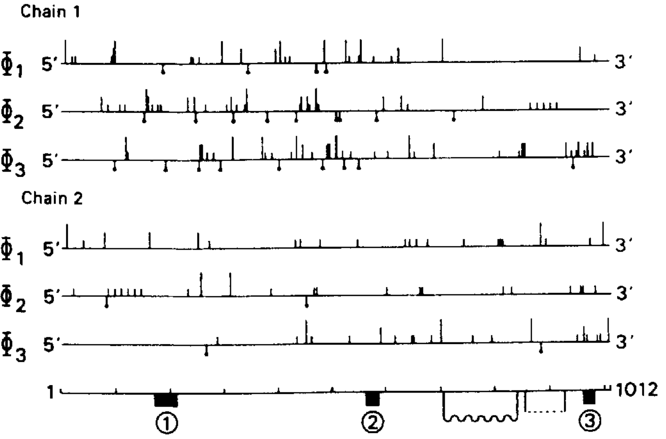

FIG. 6. Diagram showing the distribution of nonsense (vertical bars) and initiation (inverted bars with solid circles) codons in the six reading frames of *T. equiperdum* minicircle DNA. Three reading frames called ϕ_1, ϕ_2, and ϕ_3 were initiated from the 5′ end of each DNA strand. On chain 1, ϕ_1 is initiated by its first triplet AAT, ϕ_2 by ATC, and ϕ_3 by TCA. On chain 2, ϕ_1 is initiated by its first triplet ATT, ϕ_2 by TTC, and ϕ_3 by TCT. The length of the kDNA minicircle does not correspond to a multiple of three nucleotides. Therefore, going through *Hin*fI site, phase ϕ_1 is changed into phase ϕ_2. Vertical bars have different lengths, depending on the stop codon (small-sized vertical bar, TAA; medium-sized vertical bar, TAG; large-sized vertical bar, TGA). (Bottom) Schematic representation of the minicircle, with the locations of the three dyad symmetries (circled numbers 1, 2, and 3), the DNA region homologous to *T. brucei* minicircles (wavy line), and the six repeats of 12 bp each (dashed line). Reprinted from Barrois *et al.* (1982) with permission.

region may contain the origin of replication. *T. equiperdum* is apparently a recent derivative of *T. brucei* that has lost the ability to undergo cyclical transmission through the insect host [I^- (Opperdoes *et al.*, 1976)], and hence it is not entirely surprising that the *T. brucei* minicircle common region is conserved. It is of great interest, however, that minicircle sequence heterogeneity does not occur in *T. equiperdum*, and this may be related to defects in maxicircle DNA of *T. equiperdum*, that will be discussed below (Frasch *et al.*, 1980).

D. *Trypanosoma evansi*

This is another example of a species that contains kDNA minicircles of a single sequence class. In addition, the networks appear to lack maxicircle DNA (Fairlamb *et al.*, 1978). Frasch *et al.* (1980) reported that the *Mbo*I agarose gel restriction profiles of kDNA from *T. evansi* strains were very similar to that from *T. equiperdum* ATCC 30019 and suggested that there is a basic minicircle sequence characteristic of the *T. brucei* type trypanosomes, that is, obscured in *T. brucei* itself by the extensive sequence heterogeneity.

E. *Trypanosoma mega*

T. mega kDNA minicircles show extensive sequence heterogeneity by both restriction enzyme analysis and reassociation kinetics. Steinert and Van Assel (1980) have calculated that the network may contain approximately 70 different semihomologous sequence classes.

F. *Trypanosoma lewisi*

Two minicircles from *T. lewisi* bloodstream forms were cloned in M13 vectors and completely sequenced (Ponzi *et al.*, 1984). The molecules contain 1018 bp and show a conserved region of 95 bp that is repeated twice in the molecule. The conserved regions are oriented approximately symmetrically within the minicircle. A 15-mer sequence (5′-GGGGTTGGTGTAATA-3′) within the conserved region was found to be also present in the published *T. brucei* and *T. equiperdum* minicircle sequences. The longest polypeptides that could be encoded by the conserved regions of the two *T. lewisi* minicircles are 27 and 26 amino acids, and these amino acid sequences show no relation to the amino acid sequences derived from the *T. brucei* and *T. equiperdum* minicircle sequences (Ponzi *et al.*, 1984).

G. *Trypanosoma cruzi*

The first evidence on kDNA minicircle sequence heterogeneity came from studies on *T. cruzi* (Riou and Yot, 1975, 1977). The minicircle of *T. cruzi* kDNA is intermediate in size between *T. brucei* and *Crithidia* and exhibits a sequence heterogeneity that is extensive but less than that of *T. brucei* or *T. mega* minicircle DNA. The *T. cruzi* minicircle has the unique property of an intramolecular repetitive sequence (conserved region) that is repeated four times with some variation. The initial evidence for this was the fact that several restriction enzymes give major bands with similar mobilities corresponding to multiples of one quarter of the unit minicircle length (Leon *et al.*, 1980; Mattei *et al.*, 1977; Morel *et al.*, 1980; Riou and Gutteridge, 1978; Riou and Yot, 1975, 1977). The existence of a quarterly repetitive conserved sequence has been confirmed by direct sequence analysis of minicircle DNA cloned into the *Eco*RI site of pBR325 and M13mp7 (Van Heuverswyn *et al.*, 1982).

Saucier *et al.* (1981) reported that network kDNA from log phase *T. cruzi* culture forms has a low-density shoulder in CsCl when the kDNA was isolated without use of a proteolytic digestion step (Fig. 7). The kDNA from stationary phase cells lacked this low-density shoulder. They attributed this density shift to a bound protein since the shift was eliminated by pronase digestion of the DNA preparation and they suggested that the bound protein was involved in kDNA replication or in the organization of the kDNA nucleoid body *in situ*. Since 95% of the kDNA is minicircle DNA, it is likely that the bound protein is linked to minicircle DNA. It should be of great interest to extend these findings to other kDNA systems, to localize the position of the bound protein, and to determine the functional significance of this phenomenon.

H. *Crithidia* Species

kDNA from *C. fasciculata*, *C. luciliae*, and *C. acanthocephali* has been studied in detail. *Crithidia* spp. contain the largest kDNA minicircles (2.3 kb) in the kinetoplastid protozoa (Renger and Wolstenholme, 1972; Simpson *et al.*, 1974). The sequence heterogeneity of the minicircle DNA, however, is less than that found in *T. brucei*. Reassociation kinetic analysis has yielded values for the complexity of network DNA from *Crithidia* of 1–2.5 times the unit minicircle size (Fouts *et al.*, 1975; Hoeijmakers *et al.*, 1982b; Kleisen and Borst, 1975b), with no evidence for the presence of two or more components with different complexities (Kleisen and Borst, 1975b). In addition, the decrease in melting temperature of a self-annealed sonicated kDNA preparation from *C. luciliae* (Kleisen *et al.*,

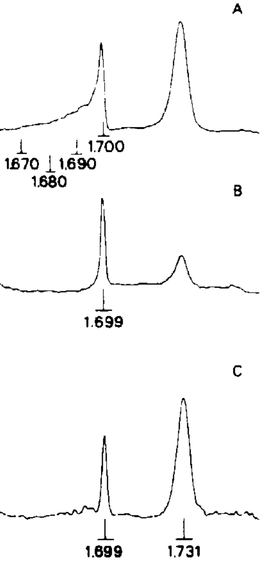

FIG. 7. Equilibrium density centrifugation in CsCl gradients of kDNA from *T. cruzi* and *Micrococcus luteus* DNA (ρ = 1.731 g/cm³). (A) Untreated sample extracted from trypanosomes grown for 5 days; (B) same sample dialyzed against SSC to remove CsCl and incubated with pronase (1 mg/ml for 2 hours at 37°C); (C) untreated sample extracted from trypanosomes grown for 9 days. Reprinted from Saucier *et al.* (1981) with permission.

1976a) was only 1°C, implying that only 2% of the base pairs were involved in the sequence heterogeneity.

A qualitative estimate of more than 13 semihomologous minicircle sequence classes has been made from analysis of restriction profiles in gels (Kleisen *et al.*, 1976a). An apparent minor length heterogeneity of unit-length linearized minicircles has also been reported (Kleisen *et al.*, 1976a), although it has not been shown that this length heterogeneity is real and is not due to the gel mobility anomalies first observed with *Leishmania* minicircle DNA.

Hoeijmakers *et al.* (1982b) reported the results of an informative electron microscopic heteroduplex analysis of denatured and annealed minicircles of *C. luciliae* that were released from the networks by *Hin*dIII digestion. In approximately 40% of the reannealed minicircles they found heteroduplex structures characteristic of sequence rearrangements. The majority of these rearrangements were interpreted as inversions and the remainder as translocations, insertions, and deletions. The rearrangements were not at random locations but were localized to four minicircle segments (Fig. 8). In addition, there was a region (65–95% of the molecule

FIG. 8. (A) Heteroduplex maps of 81 *C. luciliae* renatured minicircle molecules of classes $I^{a,b,c}$ and III. Ninety molecules of the heteroduplex categories $I^{a,b,c}$ and, in part, III (only molecules with apparent deletions replaced by inserts of different size) were traced and divided into groups on the basis of overlap of hetero- and homoduplex segments. Eighty-one molecules (90%) could be classified in the groups shown (a–k). Thin single line,

in the map in Fig. 8) that showed no rearrangements and could correspond to the "constant region" such as found in *T. brucei* minicircles. They concluded that "specific segmental rearrangements form the main basis of the minicircle sequence heterogeneity in *Crithidia*" (Hoeijmakers and Borst, 1982). In order to correlate these results with the previous report of complete renaturation of *Crithidia* kDNA and only a 2% base pair mismatch of the self-annealed DNA (Kleisen *et al.*, 1976a), it must be assumed that the minicircle fragments used in the renaturation analysis were smaller than the rearranged segments found in the electron microscope study. The rearrangement model for the generation of minicircle diversity implies the existence of extensive recombination between minicircles in the network. Some evidence for this was previously obtained by a density shift experiment using *C. acanthocephali* kDNA (Manning and Wolstenholme, 1976). In addition "fused dimer" type minicircle structures have been observed in electron micrographs of minicircle DNA from several kinetoplastid species (Simpson and da Silva, 1971), which are similar to structures involved in genetic recombination.

The nature of the minicircle sequence heterogeneity in *Crithidia* (Hoeijmakers and Borst, 1982) was tested by redigestion of specific bands with a second enzyme. By this method it was shown that the bands in the primary digests are largely homogeneous, implying again that the sequence heterogeneity is caused by rearrangements rather than by accumulation of point mutations.

I. *Leishmania tarentolae*

The minicircles in the *Leishmania* are the smallest reported to date. *L. tarentolae* minicircles are about 870 bp in size, show extensive sequence heterogeneity by restriction enzyme analysis, and renature with a $C_0t_{1/2}$

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homologous duplex region; parallel thin lines, single strands of a heteroduplex terminal segment; black box, internal heterologous region. The longest strand of a heteroduplex region is always shown above the base line, the smallest below. All molecules are normalized to a uniform length by putting the mean size of the upper and lower strands of each molecule (respectively the longest and shortest combination of strands) to a value of 100%. For absolute measurements the size of a *C. luciliae* minicircle was used (0.76 μm). The correction factor for dsDNA was determined from size measurements of circular ds minicircles (0.76 μm ± 0.03 SD) present in the same spreading. For ss regions the correction factor was based on the average size of ss circles and linears present in the spread preparation (0.72 μm ± 0.07 SD). (B) Histogram of heteroduplex regions of the groups of (A). Number of molecules versus percentage minicircle size. The designation of the histograms corresponds with that of the groups of (A). The two molecules constituting group h were not included here. The arrows labeled A, B, and C represent the three sites where the small heteroduplex areas are preferentially localized. Reprinted from Hoeijmakers *et al.* (1982b) with permission.

corresponding to a complexity between one and two times the minicircle length (Wesley and Simpson, 1973b). Reannealed open minicircles showed no decrease in the melting temperature and contained no regions of strand separation in the electron microscope (Wesley and Simpson, 1973a). Open circles did exhibit a multiphasic melting curve, however, that implied intramolecular sequence heterogeneity (Wesley and Simpson, 1973a). Intermolecular sequence heterogeneity is evidenced by restriction enzyme analysis (Challberg and Englund, 1980; Simpson, 1979) and also by cloning and sequencing of several sequence classes. The sequence heterogeneity of minicircle kDNA from *L. tarentolae* can best be visualized by high-resolution acrylamide gradient gel electrophoresis (Simpson, 1979) as shown in Fig. 9. The rich complexity of the banding pattern is almost obliterated by electrophoresis in agarose, but the major bands are visible (Figs. 10 and 11). Challberg and Englund (1980) concluded from analysis of minicircle fragments in agarose gels that there were three major sequence classes (Table I). Class I minicircles were linearized by *Hpa*II and represented 70% of the total DNA; class II minicircles were linearized by *Hin*dII and represented 15% of the DNA; and class III minicircles were linearized by *Hpa*II, could be separated from linearized class I by agarose gel electrophoresis, and represented 7% of the DNA. A remaining 4% of the DNA consisted of minor classes and

TABLE I
COMPONENTS OF *L. tarentolae* KINETOPLAST DNA NETWORKS[a]

Component	Approximate percentage of total kDNA	*Hpa*II fragments	Enzyme used to obtain full-length fragment
Class I minicircles	70	α	*Hpa*II
Class II minicircles	15		*Hin*dII
Class III minicircles	7	β	*Hpa*II
Other minicircles	4	—	—
Maxicircles	4	—	—

[a] To determine the percentage of kDNA in each component, a *Hpa*II digest of [^{3}H]kDNA was electrophoresed on a 1–4% agarose tube gel. The gel was stained with ethidium bromide to locate the bands and sliced, and the slices were counted. The percentages in each component were calculated from the total radioactivity recovered (about 75% of the original sample). Approximately 4% was found in three high-molecular-weight fragments presumed to be derived from maxicircles. Another 4% was spread throughout the gel and is assumed to correspond either to low-molecular-weight maxicircle fragments or to fragments from minor classes of minicircles. No corrections were made for possible variations in base composition of the different components. Reprinted from Challberg and Englund (1980) with permission.

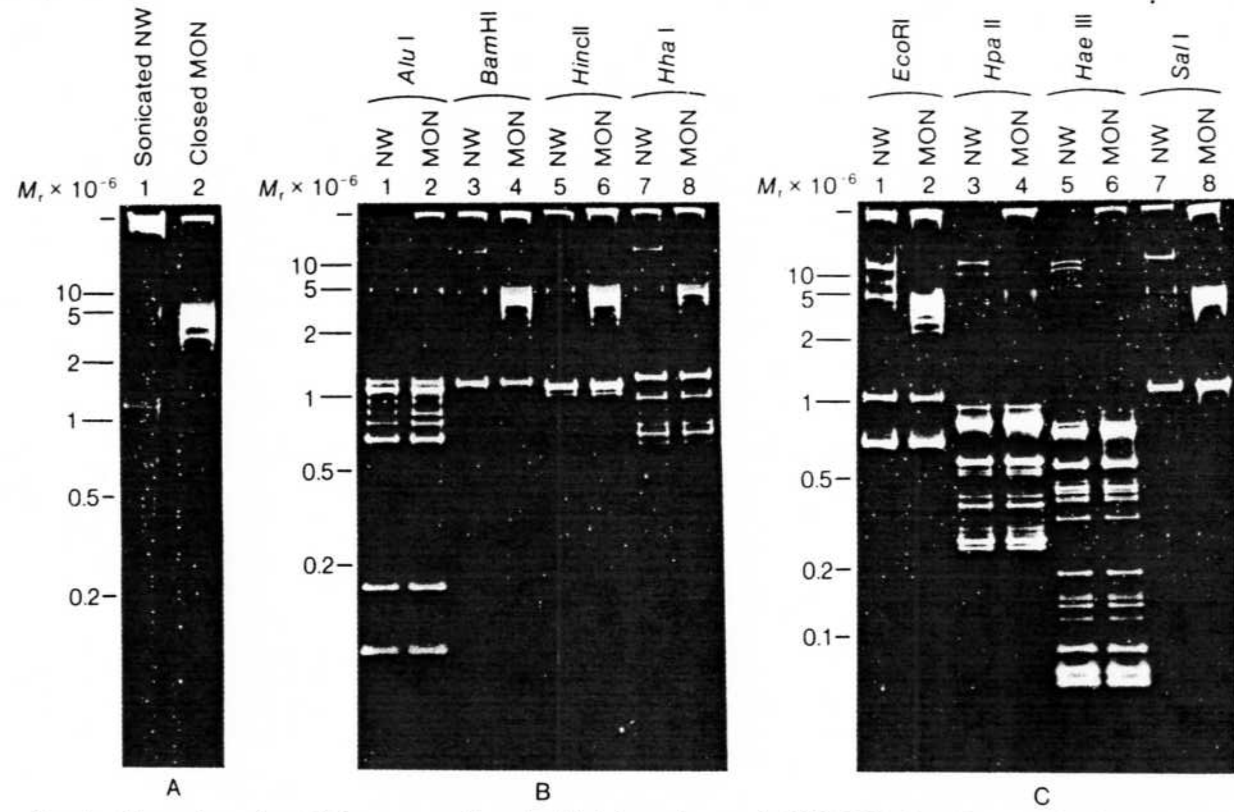

FIG. 9. Comparison of restriction enzyme-digested minicircles and network (NW) DNA from *L. tarentolae* by acrylamide gradient electrophoresis. (B and C) Closed monomeric minicircles (MON) and closed networks were digested with several restriction enzymes and electrophoresed on 3.5–10% acrylamide gradient gels with 3% stacking gels. (A) Mildly sonicated network DNA and undigested closed monomeric minicircles were electrophoresed. Several reference DNAs (not shown) were electrophoresed in each gel to obtain the molecular-weight scales. Reprinted from Simpson (1979) with permission.