Biochimica et Biophysica Acta, 521 (1978) 407—411

BBA Report

BBA 91477

RNA FROM THE INSECT TRYPANOSOME *CRITHIDIA LUCILIAE* CONTAINS TRANSCRIPTS OF THE MAXI-CIRCLE AND NOT OF THE MINI-CIRCLE COMPONENT OF KINETOPLAST DNA

J.H.J. HOEIJMAKERS and P. BORST

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, P.O. Box 60.000, 1005 GA Amsterdam (The Netherlands)

(Received August 8th, 1978)

Summary

We have hybridized total cellular RNA of *Crithidia luciliae* with the kinetoplast DNA of this organism. To allow the discrimination of DNA from mini-circles (2300 base pairs) and maxi-circles (33 000 base pairs), kinetoplast DNA was digested with restriction endonucleases and the fragments were separated by electrophoresis through an agarose gel and transferred to nitrocellulose filters by blotting. No mini-circle transcripts were found under conditions where maxi-circle fragments showed extensive and specific hybridization. Since maxi-circle sequences are present at less than 1% of the concentration of mini-circle sequences, we conclude that mini-circles may not be transcribed at all. Predominant hybridization with the maxi-circle fragments is obtained with a segment of only 2300—2500 base pairs. The possibility that this segment codes for unusually small mitochondrial ribosomal RNAs is discussed.

The kinetoplast DNA (kDNA) of trypanosomes consists of a large network of catenated circles, found in the single mitochondrion of these unicellular flagellates. Analysis of the networks from the insect trypanosome *Crithidia luciliae* with restriction endonucleases and electron microscopy has revealed the presence of two structural components: mini-circles ($1.5 \cdot 10^6$ daltons) make up about 95% of the network, while maxi-circles ($22 \cdot 10^6$ daltons) form the remainder [1]. Analogous results have been obtained with kDNA from other trypanosomes [2—4].

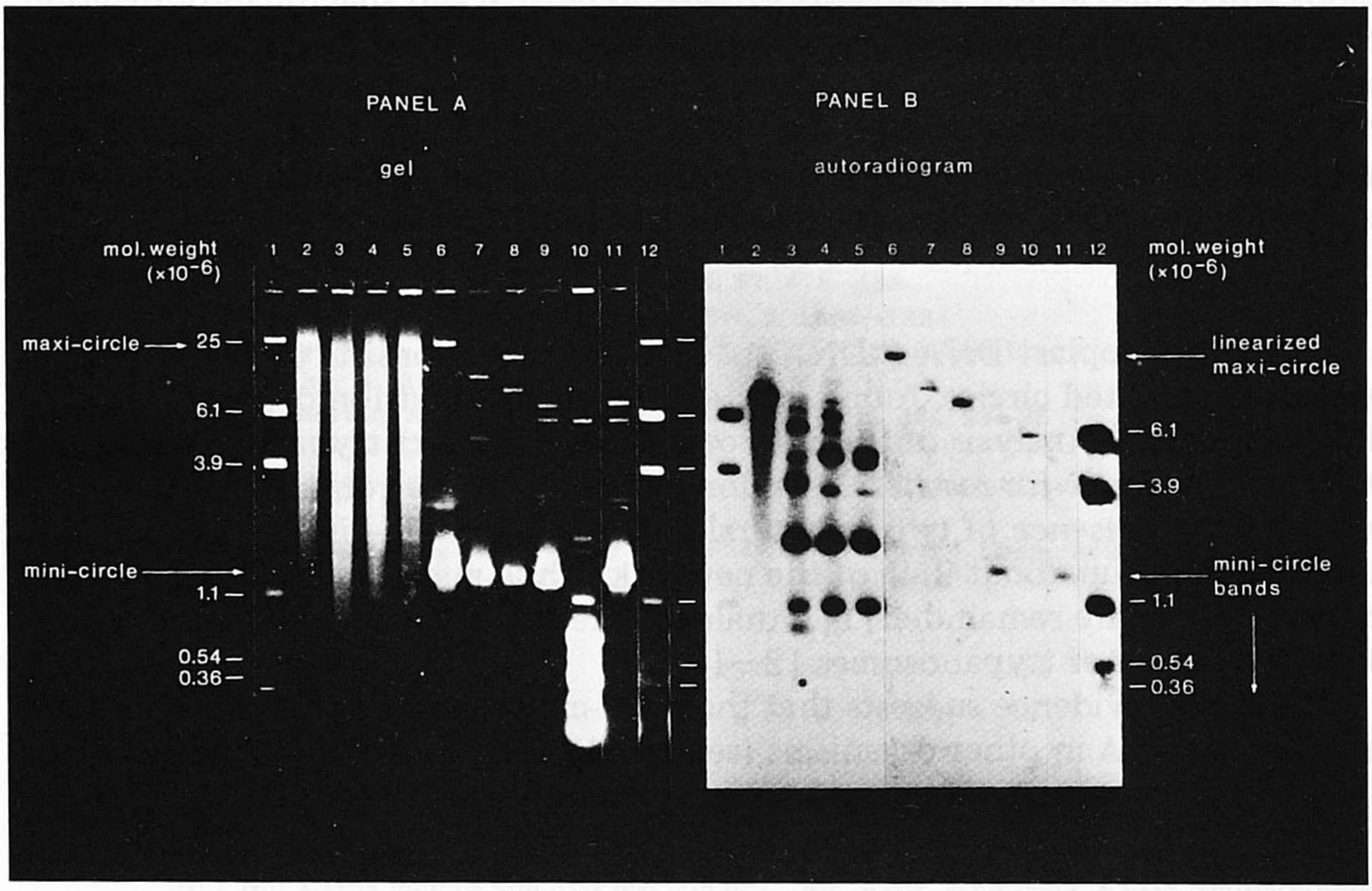
Indirect evidence suggests that the maxi-circles are the trypanosome equivalent of mtDNA in other organisms (see ref. 4). They resemble other mtDNAs in size and complexity; their sequence evolution is restricted; they are absent

Abbreviations: kDNA, kinetoplast DNA; SSC, 150 mM NaCl/15 mM sodium citrate (pH 7.0).

from some trypanosome species that have lost the ability to make functional mitochondria; no other DNA than kDNA has been found in trypanosome mitochondria. The function of mini-circles on the other hand is unknown. Their sequence micro-heterogeneity and rapid sequence evolution [5] are hard to reconcile with a coding function and various other unorthodox functions have been considered [4].

To get more information on the possible function of kDNA we have analysed *C. luciliae* for the presence of maxi-circle and mini-circle transcripts. To avoid the possible loss of transcripts during cell fractionation, we have used total cellular RNA, directly isolated from a lysate by the hot phenol extraction procedure described by Penman [6]. After removal of DNA by extensive treatment with deoxyribonuclease (Worthington, ribonuclease free), the RNA was degraded with alkali to a size of about 500 nucleotides, phosphorylated in the presence of [γ-^{32}P]ATP and polynucleotide kinase [7] (from phage T_4-infected *Escherichia coli*) to a specific activity of $20 \cdot 10^6$ cpm/μg and hybridized to *Crithidia* DNAs. To distinguish hybridization with maxi-circle, mini-circle and nuclear DNA sequences, the DNAs were digested with various restriction endonucleases as described [1,8] and the fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose filters by the Southern [9] blotting procedure. RNA hybridized to the DNA, immobilised on the filters, was detected by autoradiography.

The results of one of these hybridization experiments is presented in Fig. 1. The photograph of ethidium-stained gels in panel A shows the characteristic (cf. ref. 1) faint maxi-circle bands in the upper half of the gel (lanes 6—11) and the prominent mini-circle bands in the lower half. After a short exposure, the corresponding autoradiograms in panel B show only one labelled maxi-circle band (lanes 6—11) in each digest. After long exposure more maxi-circle



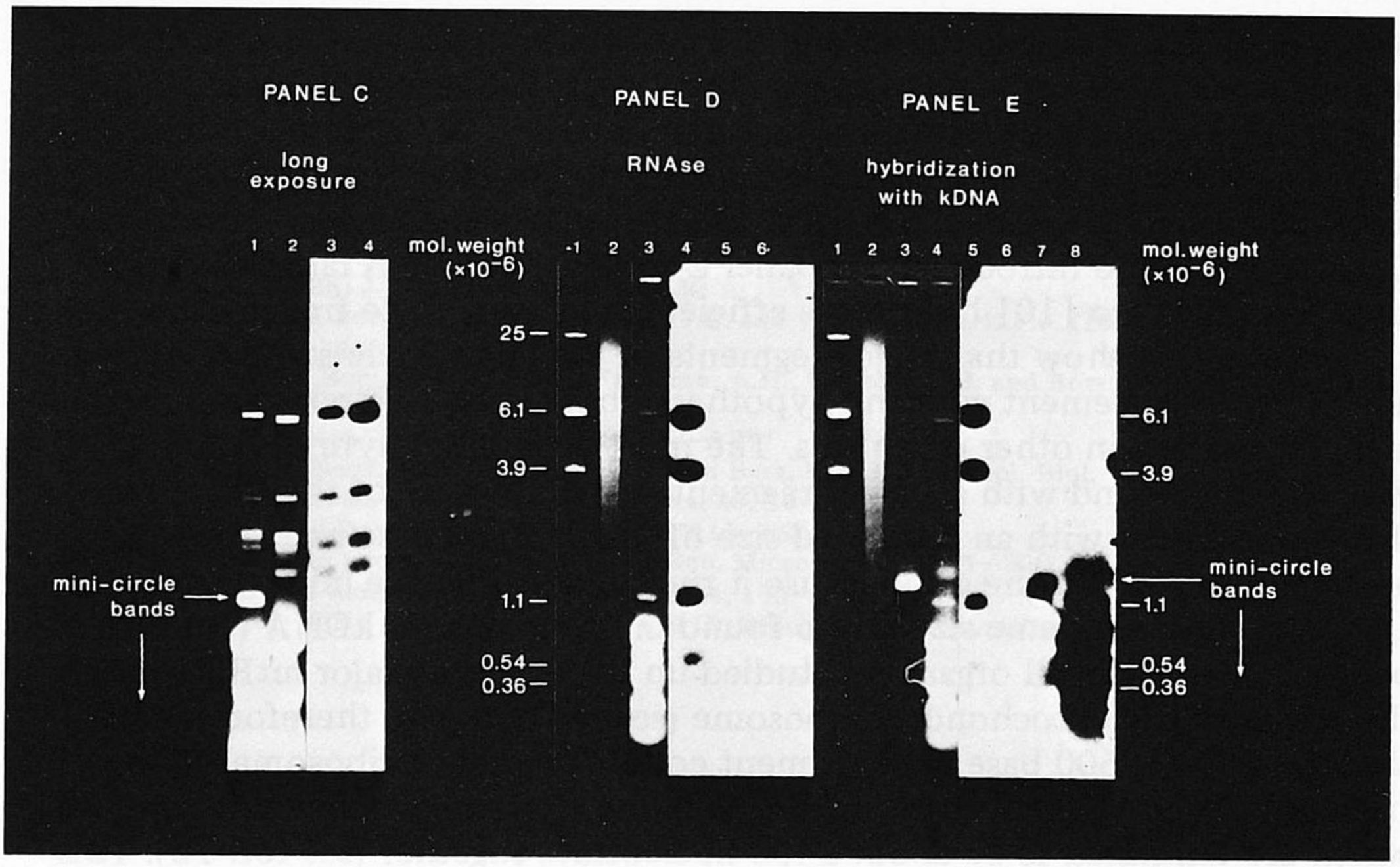


Fig. 1. Hybridization of *Crithidia* RNA with restriction fragments of *Crithidia* DNA. Nuclear DNA (1 μg) and kDNA (2.5 μg unless stated otherwise) of *C. luciliae* and *C. fasciculata* were digested with various restriction endonucleases and the fragments separated by electrophoresis on a horizontal 0.5% agarose gel [3]. After denaturation in situ and blotting of the DNA to a nitrocellulose filter by the method of Southern [9], the DNA on the filter was allowed to hybridize with ^{32}P-labelled total cell RNA (1.5 μg) of *C. luciliae* for 2 days, at 56—60° C in 2 × SSC/0.1% sodium dodecyl sulphate and 0.2% ficoll/0.2% polyvinylpyrrolidone/0.2% bovine serum albumin and 25 μg yeast RNA per ml. Denatured phage φ 29 DNA, labelled with ^{32}P by nick translation [10] was added to the hybridization mix to label the marker bands. After hybridization, filters were washed three times in 2 × SSC and 0.1% sodium dodecyl sulphate at 56—60° C for 15 min and four times in 2 × SSC at room temperature. Panel A, Photograph of a gel stained with ethidium bromide. Lanes 1 and 12 contain molecular weight markers: phage T_7 DNA, phage φ 29 DNA and φ 29 DNA after digestion with endonuclease EcoRI [8]. Lanes 2—4 contain nuclear DNAs of *C. luciliae* digested with BamHI, BamHI + HindIII and HindIII, respectively. Lane 5 contains nuclear DNA of *C. fasciculata* digested with HindIII. Lanes 6—10 contain kDNA of *C. luciliae* digested with endonucleases XbaI, HindIII, SalI, EcoRI and HapII, respectively. Lane 11 contains kDNA of *C. fasciculata* treated with EcoRI. Panel B, Autoradiogram of the DNAs in panel A after transfer to a nitrocellulose filter and hybridization with ^{32}P-labelled *Crithidia* RNA. The numbers of the lanes correspond to those in panel A. Panel C, Lanes 1 and 2 show a photograph of a gel stained with ethidium bromide containing kDNA (3 μg) of *C. luciliae* (lane 1) and *C. fasciculata* (lane 2) digested with endonuclease HapII. Lanes 3 and 4 show the corresponding autoradiogram of the filter after long exposure (3 weeks). Panel D, Lanes 1—3 show the ethidium bromide-stained gel with molecular weight markers in lane 1 (as in panel A), a HindIII digest of nuclear DNA of *C. luciliae* in lane 2 and a HapII digest of kDNA of *C. luciliae* in lane 3. Lanes 4—6 show the corresponding autoradiogram of the hybridized filter. Before hybridization (conditions as in panel A) the labelled RNA was treated with pancreatic ribonuclease (50 μg/ml) for 1 h at 37° C. Panel E, Lanes 1—4 show the stained gel with molecular weight markers in lane 1, nuclear DNA of *C. luciliae* after digestion with endonuclease HindIII in lane 2, and kDNA of *C. luciliae* treated with EcoRI (lane 3) and HapII (lane 4). Lanes 5—8 show the corresponding autoradiogram of the filter, after hybridization with denatured kDNA labelled with ^{32}P by nick translation [10] mixed with ^{32}P-labelled phage φ 29 DNA, but without labelled RNA added.

bands are labelled, e.g. four bands in the HapII digest, comprising >50% of the maxi-circle sequence (panel C, lanes 1 and 3). Even at the longest exposure time the mini-circle bands are not labelled. Similar results were obtained with digests of kDNA from *C. fasciculata* (panels A and B, lane 11 and panel C, lanes 2 and 4).

Hybridization to maxi-circle bands is not due to DNA contamination, because it is completely removed by ribonuclease under conditions that leave DNA-DNA hybrids intact (panel D). The specific hybridization to different

bands in different digests shows that it is not attributable to non-specific binding of nuclear transcripts or to contamination of kDNA with nuclear DNA. This is confirmed by the hybridization with nuclear DNA digests in lanes 2—5 of panels A and B, which give entirely different patterns. The absence of hybridization of the mini-circle bands is not due to poor transfer of smaller DNA-fragments to nitrocellulose: panel E shows that kDNA labelled by nick translation in vitro [10] hybridizes efficiently to mini-circle fragments.

Our results show that major segments of the maxi-circle are transcribed and this is in agreement with the hypothesis that the maxi-circles are the equivalent of mtDNA in other organisms. The most prominent hybridization in each digest is found with a single fragment and in the case of the EcoRI digest, this is a fragment with an estimated size of 2300—2500 base pairs (not visible in the photograph of the gel, because it runs just behind the mini-circle band). (A fragment of the same size is also found in *C. fasciculata* kDNA (lanes 11 in panels A and B.) In all organisms studied up till now the major mtRNAs are the RNAs of the mitochondrial ribosome (see ref. 11). We, therefore, infer that the 2300—2500 base pairs segment codes for a set of ribosomal RNAs (rRNAs), which could even be smaller than the rRNAs of animal mitochondria, which are estimated to be about 2400 nucleotides together (see ref. 12). This inference fits the observation by Nichols and Cross [13] that the two largest and most prominent RNAs found in a mitochondrial (kinetoplast) fraction from *Crithidia* are only 14.6 and 11.4 S. The transcripts present at lower concentrations could be mitochondrial tRNAs and mRNAs. Specific hybridization to maxi-circle fragments of the 12 S and 9 S RNAs found in a kinetoplast fraction of another trypanosome, *Leishmania tarentolae*, has recently been reported by Simpson and Simpson [14].

Although negative experiments are usually not very persuasive, we consider our inability to find mini-circle transcripts significant. Mini-circle sequences are present at a concentration that is at least two orders of magnitude higher than the maxi-circle sequence. If mini-circle transcripts exist, one would expect them to show up under conditions where even the more infrequent maxi-circle transcripts hybridize. The base composition (43 mol percent G + C, ref. 15) of mini-circles is not unusual, under our hybridization conditions kDNA fragments hybridize preferentially with mini-circle fragments and we have not used any procedure in the purification of the RNA known to lead to differential losses. Of course, it is possible that mini-circles are only transcribed under unusual physiological conditions, not present in our log-phase cultures, or that the transcripts are too short for hybridization under our experimental conditions. The simplest interpretation, however, is that mini-circles do not have a coding function.

Acknowledgements

We thank Mr. B. Schoutsen and Mr. R.F. Evers for help in some of these experiments, Mrs. F. Fase-Fowler for providing phage ϕ 29 and phage T_7 DNAs and several restriction endonucleases and Mr. G.C. Grosveld, Mr. J.L. Bos and Dr. L.A. Grivell for supplying the endonucleases XbaI, SalI and HindIII, respectively.

References

1 Kleisen, C.M., Weislogel, P.O., Fonck, K. and Borst, P. (1976) Eur. J. Biochem. 64, 153—160
2 Borst, P., Fase-Fowler, F., Steinert, M. and Van Assel, S. (1977) Exp. Cell Res. 110, 167—173
3 Fairlamb, A.H., Weislogel, P.O., Hoeijmakers, J.H.J. and Borst, P. (1978) J. Cell Biol. 76, 293—309
4 Borst, P. and Hoeijmakers, J.H.J. (1978) Plasmid, in the press.
5 Hoeijmakers, J.H.J., Schoutsen, B., Fase-Fowler, F. and Borst, P. (1978) Parasitol., in the press
6 Penman, S. (1966) J. Mol. Biol. 17, 117—130
7 Goldbach, R.W., Borst, P., Bollen-De Boer, J.E. and Van Bruggen, E.F.J. (1978) Biochim. Biophys. Acta 521, 169—186
8 Weislogel, P.O., Hoeijmakers, J.H.J., Fairlamb, A.H., Kleisen, C.M. and Borst, P. (1977) Biochim. Biophys. Acta 478, 167—179
9 Southern, E.M. (1975) J. Mol. Biol. 98, 503—517
10 Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237—251
11 Borst, P. and Grivell, L.A. (1971) FEBS Lett. 13, 73—88
12 Klukas, C.K. and Dawid, I.B. (1976) Cell 9, 615—625
13 Nichols, J.M. and Cross, G.A.M. (1977) J. Gen. Microbiol. 99, 291—300
14 Simpson, L. and Simpson, A.M. (1978) Cell 14, 169—178
15 Kleisen, C.M., Borst, P. and Weijers, P.J. (1975) Biochim. Biophys. Acta 390, 155—167