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Short Communication

Polykinetoplast DNA structure in *Dimastigella trypaniformis* and *Dimastigella mimosa* (Kinetoplastida)☆

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The mitochondrial DNA (kinetoplast [k]DNA) of kinetoplastid flagellates is unique in its complexity, abundance and structure. In *Crithidia fasciculata*, the kDNA is a single network composed of about 5000 minicircles and 25 maxicircles [1]. Maxicircles encode conventional mitochondrial proteins, the transcripts of which are edited via a uridine insertion/deletion process (reviewed in Ref. [2]). The kDNA has been analyzed in detail in trypanosomes, leishmanias, phytomonads and crithidias, all members of the obligatory parasitic suborder Trypanosomatina. The evolutionary history of this unusual structure can be better understood by studying the predecessors of trypanosomatids that belong, according to the morphology [3] and rRNA-based [4] systems, to the suborder Bodonina. Work thus far has shown the kDNA of three bodonids to lack the characteristic kDNA network structure of trypanosomatids. In the kDNA of the fish pathogen *Trypanoplasma borreli*, classical minicircles are absent [5,6], and the minicircle-like sequences are concatenated into ~200-kb long molecules [7]. In *Cryptobia helicis* and *Bodo saltans*, minicircles are present as circular, supercoiled and relaxed monomers, respectively [8,9]. In *B. saltans*, two guide RNA genes of opposite polarity reside on the minicircles along with a putative bent helix sequence and a conserved region [10]. In DAPI-stained cells and electron micrographs, the kDNA of the bodonid *Dimastigella trypaniformis* appeared to be located in multiple distinct nucleoids in the mitochondrial lumen [11,12]. This type of kDNA was coined "polykinetoplast" (poly-kDNA) [12,13], but no information about its structure was provided. In this work, the structure of poly-kDNA is described in the parasitic *D. trypaniformis* and free-living *D mimosa*.

D. trypaniformis (the Ulm strain) and *D. mimosa* isolated from the intestine of a termite in Germany and a sewage plant in Russia, respectively, were cultivated as described elsewhere [4]. After partial removal of the feeder bacteria by differential centrifugation, total DNA was extracted ensuring vortexing and shearing were avoided [8].

When the undigested *D. trypaniformis* DNA was loaded on an ethidium bromide (EtBr)-stained agarose gel, two prominent bands migrating at 1.1 and 1.45 kb were visible and only relatively low amount of DNA remained in the slot (Fig. 1A, lane a). The same picture was obtained with the *D. mimosa* DNA with bands migrating at 0.9 and 1.3 kb (Fig. 1A, lane c). Large amounts of DNA in the compression zone represent the chromosomal DNA of the flagellates and the contaminating bacteria. Digestion of the *D. mimosa* DNA with *Sac*I resulted in the disappearance of the light band while the heavier band slightly shifted its mobility to 1.25 kb (Fig. 1A, lane d). This result indicates that the 1.3 kb band contains the open circle (OC) form and the 1.25 kb band represents the *Sac*I-linearized minicircles.

☆ *Note:* Nucleotide sequence data reported in this paper have been submitted to the GenBank™ database with the accession number AF257462.

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Two of these minicircles were cloned and sequenced (Fig. 1C and see below), and one (DM1) was labeled by random priming and used as a probe in Southern hybridizations of both DNAs (Fig. 1A and B). The fast migrating band in the *D. mimosa* DNA either represented another minicircle class of a different size or a supercoiled species of the 1.25-kb-long linears.

In order to test the topological status, we subjected the undigested *D. mimosa* DNA to electrophoresis in an agarose gel poststained with EtBr after the run. The 1.3 kb band was present in the same amount as in the undigested DNA run in the presence of EtBr while the 0.9 kb band was missing, proving that supercoiling was artificially induced by EtBr (Fig. 1B, lane c). In the EtBr-poststained gels with the *D. trypaniformis* DNA hybridized with the labeled 1.45 kb gel-isolated minicircles of the same species (data not shown) or the DM1 probe, a weak signal migrating at 1.1 kb was consistently present (Fig. 1B, lane a). We did not further explore whether it represented a small fraction of naturally supercoiled 1.45 kb minicircles or a rare class of small-sized cross-hybridizing minicircles. In both species, very long exposures also revealed a low-intensity band (less than 1% of the total minicircle signal) migrating at about 3.0 kb that most likely represented dimers composed of two interlocked OC molecules, while no hybridization signal was present in the slot (Fig. 1A and B).

The *Sac*I-linearized *D. mimosa* minicircle (DM1) has a 1.25-kb-long highly AT-rich sequence that contains short stretches of adenines reminiscent of the bent helix motif typical for the trypanosomatid minicircles (Fig. 1C). We have found two regions showing similarity to the minicircle conserved blocks CSB I (8 out of 10 nt identical) and CSB II (7 out of 8 nt identical) that are invariably present in the minicircles of Trypanosomatina. A thorough homology search against mitochondrial transcripts (edited and pre-edited) available from various trypanosomatids and bodonids identified a 13-nt-long perfect match with part of the *Trypanosoma brucei* cytochrome oxidase subunit (cox) III edited mRNA (Fig. 1C). Surprisingly, another *Sac*I-lin-

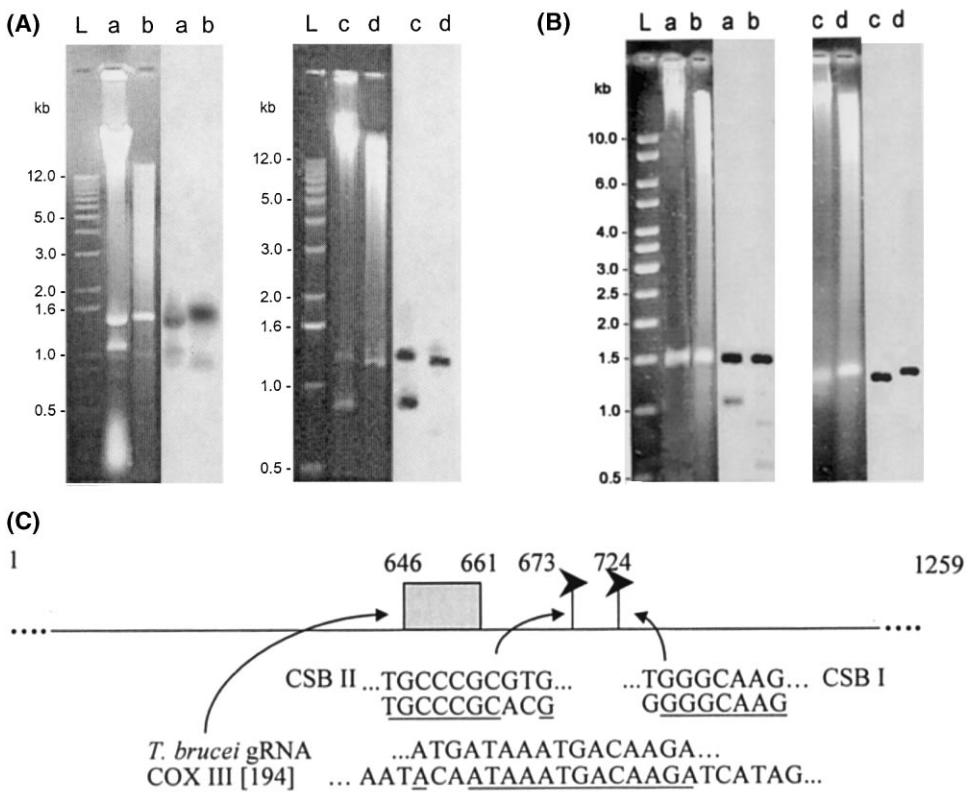

Fig. 1. (A) Agarose gel (0.75%) electrophoresis and Southern blots of *D. trypaniformis* undigested (lane a) and *Hind*III-digested DNA (lane b), and *D. mimosa* undigested (lane c) and *Sac*I-digested DNA (lane d) run in the presence of EtBr; 1 kb ladder (BRL) (lane L). Before blotting the slots were filled in by agarose and the gel was exposed to 600 kJ at 254 nm to nick the circles. The gel was blotted and probed with the random primed DM1 minicircle probe at hybridization temperatures 55°C (lanes a,b) or 65°C (lanes c,d) overnight. The membranes were washed three times for 30 min in 3 × SSC, 0.1% SDS at 55 or 65°C. (B) Agarose gel (0.75%) electrophoresis and Southern blots of *D. trypaniformis* undigested (lane a), and *Dra*I-digested DNA (lane b), and *D. mimosa* undigested (lane c), and *Sac*I-digested DNA (lane d), run in the absence of EtBr, and poststained with 1 μg ml^{-1} EtBr for 1 h; 1 kb ladder (BRL) (lane L). Blotting and hybridization of this gel was as described in Fig. 1A. (C) Map of the 1.25-kb long *D. mimosa* minicircle (DM1). The CSB I and CSB II motifs and the putative guide RNA gene are compared with their homologs in *T. brucei*.

earized *D. mimosa* minicircle sequenced in its entirety only differed from the DM1 sequence by a single nucleotide. The sequence analysis of the *D. mimosa* minicircles shows unusually high sequence homogeneity in this poly-kDNA component.

Numerous attempts to amplify a part of the maxicircle-located cox I and II genes using a set of oligonucleotides that allowed amplification of their homologs in other bodonids [9] failed. Hybridizations under various low-stringency conditions with the labeled cox I gene of *C. helicis*, which is related with the genus *Dimastigella* [4], did not show a specific pattern (data not shown).

Electron microscopy inspection of the total *D. trypaniformis* DNA revealed the presence of very large linear molecules and small circular molecules. All 263 minicircles analyzed on randomly chosen sections of the grid were about 1.45-kb long and uniform in size, which corresponds with the size of minicircles estimated from the agarose gels. Only eight dimers were found to be composed solely of the OC molecules connected by a single interlock, while a network or larger catenanes were absent. The kDNA network of *C. fasciculata*, prepared as a control, was intact (data not shown). We assume that the interlocked circles represent replication intermediates rather than products of a network breakdown, since even after extensive destruction different small catenanes would testify of the presence of a large network [14].

Since phenol–chloroform extraction and ethanol precipitation can cause breaks in the DNA preparations, we have lysed about 3×10^8 cells of *D. trypaniformis* and loaded them onto a sucrose gradient performed according to Blom et al. [10]. As shown in Fig. 2A, almost all *D. trypaniformis* minicircles remained at the top of the gradient, a sedimentation expected for non-catenated circular molecules. As a control, the *C. fasciculata* lysate was run in parallel and screened with the *C. fasciculata* minicircle probe pDP312 [15]. A significant portion of the minicircles sedimented near the bottom of the gradient as a result of their presence in large networks (Fig. 2B).

As with all bodonids, except *T. borreli* [6], a drawback of the study of *Dimastigella* spp. is the abundant presence of feeder bacteria. Although underrepresented as a consequence of the contamination with bacterial DNA, prominent 1.45- and 1.3-kb minicircle bands were highly visible in the undigested DNAs of *D. trypaniformis* and *D. mimosa*, respectively. An additional fast migrating band appeared when the DNA was separated in the EtBr-containing gel, indicating that the intercalating agent induced supercoiling of minicircles that are present as relaxed OCs in *Dimastigella* kDNAs. The intercalation of EtBr induces supercoiling of OCs that do not contain nicks or gaps. Since, in the presence of EtBr, only a part of the minicircles was conversed into supercoils, we assume

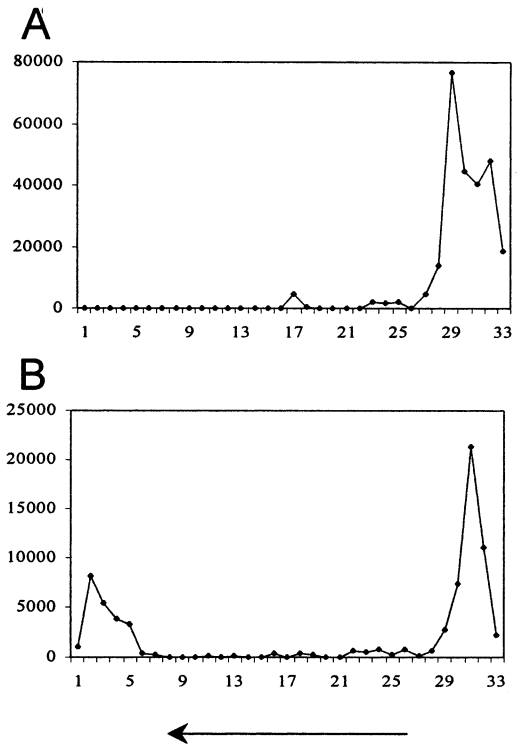

Fig. 2. Sucrose gradient centrifugation. The sedimentation of minicircles was followed by screening the dot blots of DNA extracted from gradient fractions with a random primed minicircle probe. Radioactive signal was quantified with phosphoimager Storm 860 and is indicated in ^{32}P cpm per 10 ml on the vertical axis. On the horizontal axis fractions 1 and 33 indicate the bottom and top of the gradient, respectively. (A) *D. trypaniformis* gradient hybridized with the DM1 minicircle probe; and (B) *C. fasciculata* gradient hybridized with the pDP312 minicircle probe. Arrow points to the bottom of the gradient.

that the remaining circular molecules contain nicks or gaps in vivo.

Several lines of evidence show that the poly-kDNA of the early-branching *Dimastigella* spp. [4] is composed of loose minicircles that occur predominantly in the form of OC molecules. A similar kDNA structure was recently described in *B. saltans* [10]. However, a principal difference between the kDNA structure of both bodonids is that the *B. saltans* minicircles, although non-catenated, are present in a single DNA bundle located in the anterior part of the mitochondrion close to the kinetosome of the two flagella [10]. Our ultrastructural analysis of D. *mimosa* and *D. trypaniformis* confirmed the distribution of the poly-kDNA in multiple loci spread throughout the mitochondrial lumen that was described previously [11] (data not shown). Similar distribution of minicircles is known to occur in the pan-kDNA of the bodonid *C. helicis*; however, the majority of minicircles are present as non-catenated supercoiled molecules [8]. Rauch et al. [16] speculated that the loss of supercoiling of kDNA minicircles was connected with the advantageous formation of a net-

work. With their non-catenated OC minicircles, both *Dimastigella* species may represent an intermediate stage in the formation of the kDNA network. Further analysis of the structure of the kDNA in bodonids may help us to better understand the evolution of the highly unusual mitochondrial DNA in Kinetoplastida.

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