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RESEARCH BRIEF

# *Trypanosoma avium:* Novel Features of the Kinetoplast Structure

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*Index Descriptors and Abbreviations:* kinetoplast DNA; minicircles; ultrastructure; *Trypanosoma*; kDNA, kinetoplast DNA; kb, kilobase pair.

A unique feature of the mitochondrial genome of kinetoplastid flagellates is its composition of maxicircles, bearing classical mitochondrial genes, and minicircles, coding for guide RNAs that provide information for editing of the (encrypted) maxicircle transcripts (reviewed by Arts and Benne 1996; Alfonso *et al.* 1997). Thus far, three different types of organization of this unusual mitochondrial DNA [termed kinetoplast (k) DNA] have been described. The early branching biflagellate members of the suborder Bodonina, most of which are free-living species, contain abundant noncatenated minicircles that are dispersed throughout the mitochondrial lumen (Lukeš *et al.* 1998) or located in a single large DNA bundle (Blom *et al.* 2000). In the obligatory parasitic members of the suborder Trypanosomatina the kDNA forms a huge planar network, located close to the kinetosome of the flagellum. In a model trypanosomatid, *Crithidia fasciculata*, the kDNA disk consists of about 5000 non-supercoiled, mutually interlocked minicircles and about 25 maxicircles (Shapiro and Englund 1995).

One of the paradigms attempting to explain the complex structure of the network in Trypanosomatina holds that all minicircles are aligned in parallel to the axis of the disk. For *Trypanosoma equiperdum* it was suggested that a double tier of minicircles is compacted in the kinetoplast disk (Silver *et al.* 1986), while for *Trypanosoma cruzi* and several other trypanosomatids a single tier structure was proposed (Delain and Riou 1969; Simpson 1972; Ferguson *et al.* 1992). In order to test this important paradigm we have used a subset of *Trypanosoma* species possessing widely diversified minicircles. We conclude that the kinetoplast ultrastructure is determined by the minicircle size, formed by a single minicircle tier, and has novel interesting species-specific features.

*Trypanosoma carassii* (strain PF-FR) and *Trypanosoma boissoni*, evolutionarily closely related species (Lukeš *et al.* 1997), were isolated from the blood of a European freshwater perch and an African marine ray, respectively (Jirků *et al.* 1995). *Trypanosoma avium* strains A1412 and A493 were isolated from the bone marrow of a raven and a blackbird, respectively (Yurchenko *et al.* 1999). Cultivation and kDNA isolation were performed as described elsewhere (Jirků *et al.* 1995). For transmission electron microscopy cells were washed repeatedly in a phosphate-buffered saline, fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer at 4 °C overnight, and processed as described (Lukeš 1992). Thin sections were examined in a Philips 420 electron microscope, where selected images were captured with a CCD BIOscan camera, and analyzed using the GATAN Digital Micrograph software.

The isolated kDNA networks were subjected to restriction analysis with enzymes that cut only once in most minicircles and thus release the linearized minicircles, visible as a band upon agarose gel electrophoresis. In Fig. 1, widely different sizes of minicircles ranging from 1.6 kb in *T. carassii* PF-FR (lane 1) to 10 kb in *T. avium* A1412 (lane 4) are shown. Previous analyses of the kDNA networks of these trypanosomes demonstrated that the minicircles are part of a large catenated network and that their vast majority belongs to a single-size class (Jirků *et al.* 1995; Yurchenko *et al.* 1999).

For each *Trypanosoma* spp. in the stationary phase, the thickness and the diameter of kinetoplasts were measured if they met the following criteria: (i) most of the DNA fibers visible in a section spanned

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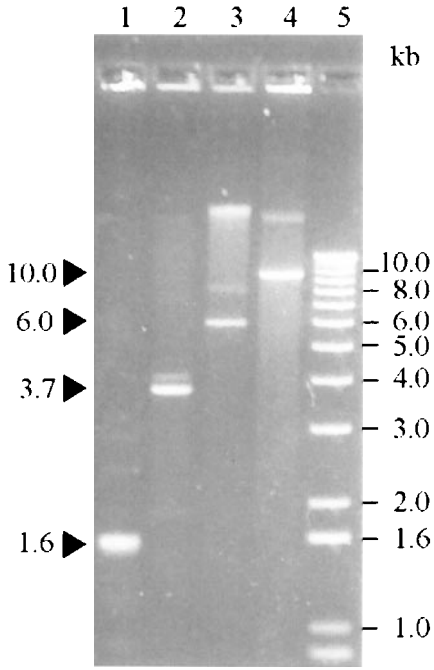


**FIG. 1.** Agarose gel electrophoresis of the kDNAs of *Trypanosoma* spp. *T. carassii* kDNA digested with *Hin*dIII (lane 1); *T. boissoni* kDNA digested with *Sac*I (lane 2); *T. avium* A493 kDNA digested with *Eco*RI (lane 3); *T. avium* A1412 kDNA digested with *Sac*I (lane 4); DNA ladder (lane 5). The sizes of linearized minicircles are indicated by arrowheads. Since only some minicircles were linearized by the restriction enzymes used, part of the kDNA networks remained in the slot.

continually from one side of the kinetoplast to the other, proving that the kinetoplast disk was cut parallel to its axis and (ii) a single flagellar kinetosome was seen, indicating that the section was cut through the central region of the kinetoplast disk and that the cell had not been dividing. The measurements revealed a tight correlation between the disk thickness and the size of minicircles (Table I). Interspecific differences in the thickness and diameter of kinetoplasts in *Trypanosoma* spp. are very prominent. The classical disk-shaped structure, known from the model trypanosomatids such as *T. cruzi, T. brucei, Leishmania tarentolae*, and *C. fasciculata* (Shapiro and Englund 1995), was observed only in *T. carassii* and *T. boissoni*. In contrast, kinetoplasts of the *T. avium* strains analyzed here show a rather cylindrical shape. Moreover, its growing thickness is at the expense of the kinetoplasts' diameter (Fig. 2 and Table I), suggesting that the longer the minicircles, the lower their number in the network. In all species analyzed, the DNA strands were packed in parallel to the axis of the disk (Figs. 2A–2D). The position of minicircles perpendicular to the plane of the network seems to be a conserved feature as well as their catenation.

The correlation between the minicircle size and the kinetoplast thickness clearly favors a single minicircle tier arrangement in the flagellates studied. If a 1-kb-long DNA fragment is 0.317 $\mu$m long (Coggins 1987), then the 1.6- and 3.7-kb minicircles are approximately 0.5 and 1.1 $\mu$m in length, respectively. These values are consistent with the measured mean thickness of *T. carassii* (0.23 $\mu$m) (Fig. 2A) and *T. boissoni* (0.53 $\mu$m) disks (Figs. 2B and 2E), which is half the circumference of their respective minicircles. For the *T. avium* strain 493, which has 6-kb minicircles, the measured thickness of the kinetoplast cylinders was 0.77 $\mu$m, while for the strain A1412, with its 10-kb minicircles, it was 1.19 $\mu$m (Table I). However, the theoretical thickness calculated from the lengths of minicircles of these strains was 0.95 and 1.58 $\mu$m, these values being 18 and 25%, respectively, higher than the measured thickness. The proposed structure of the kinetoplast in bird trypanosomes (see below) that is reflected by its ultrastructural features may provide for the above discrepancy.

Interestingly, in sectioned cells the sizes of kDNA minicircles corresponded with the values calculated for the deproteinized DNA of the same size. However, several proteins are abundantly present in the kinetoplasts that bind specifically to minicircles (Xu and Ray 1993). These kinetoplast-associated proteins resemble histone H1 (Xu *et al.* 1996; Hines and Ray 1997), which is known to be located at the linker region between nucleosomes (Lewin 1994). Our measurements indicate that binding of DNA by these proteins may not, as in the case of histone H1, induce any curvature of the bound minicircular DNA.

While the kDNA seems to be packed at a uniform density in the two aquatic species (*T. carassii* and *T. boissoni*), in *T. avium* strains A493 and A1412 the kinetoplast cylinders always display three and seven regularly spaced electron-dense stripes, respectively (Figs. 2C and 2D; arrowheads). From electron micrographs and measurements we conclude that in these kinetoplasts the DNA fibers of a large minicircle are regularly crossed (Figs. 2C and 2D; insets). A similar model with minicircles containing a single crossing, thus shaped as a number 8, has been proposed for *T. cruzi* (Delain and Riou 1969) but not for other trypanosomatids. Such an arrangement would be compatible with the absence of supercoiling in trypanosomatid minicircles (Rauch *et al.* 1993) through the alternation of left-handed and right-handed crossings. The lack of minicircle supercoiling in the above-mentioned species was confirmed using agarose gel electrophoresis and electron microscopy (results not shown; Jirků *et al.* 1995; Yurchenko *et al.* 1999). These crossings would represent a novel way of packaging large minicircles within the kinetoplast of *T. avium*. Proteins associated with the kinetoplast disk (Tzfati *et al.* 1992; Xu *et al.* 1996) may be involved in such a formation. For topoisomerase II, which participates in the kDNA replication (Melendy *et al.* 1988; Ferguson *et al.* 1992), it must be a challenge to catenate such minicircles into a network in an organized manner. This task may be facilitated by regular crossings, kept together by the kinetoplast-associated proteins. We can only speculate whether the bizarre structures rarely present in analyzed samples (Fig. 2F) represent some stage of kinetoplast replication. The lack of condensation manifested by loose DNA fibers (arrow in Fig. 2F) in

TABLE I

Size Measurements of *Trypanosoma* spp. Kinetoplasts

| Species | Thickness | Diameter | *n* | Minicircle size (kb) |
|---|---|---|---|---|
| *T. carassii* | 0.233 ± 0.026 | 1.172 ± 0.307 | 46 | 1.6 |
| *T. boissoni* | 0.532 ± 0.062 | 0.901 ± 0.252 | 40 | 3.7 |
| *T. avium* (A493) | 0.773 ± 0.057 | 0.539 ± 0.104 | 49 | 6.0 |
| *T. avium* (A1412) | 1.195 ± 0.099 | 0.606 ± 0.119 | 44 | 10.0 |

*Note.* Values (in $\mu$m) are means ± standard deviation; *n*, number of kinetoplasts measured.

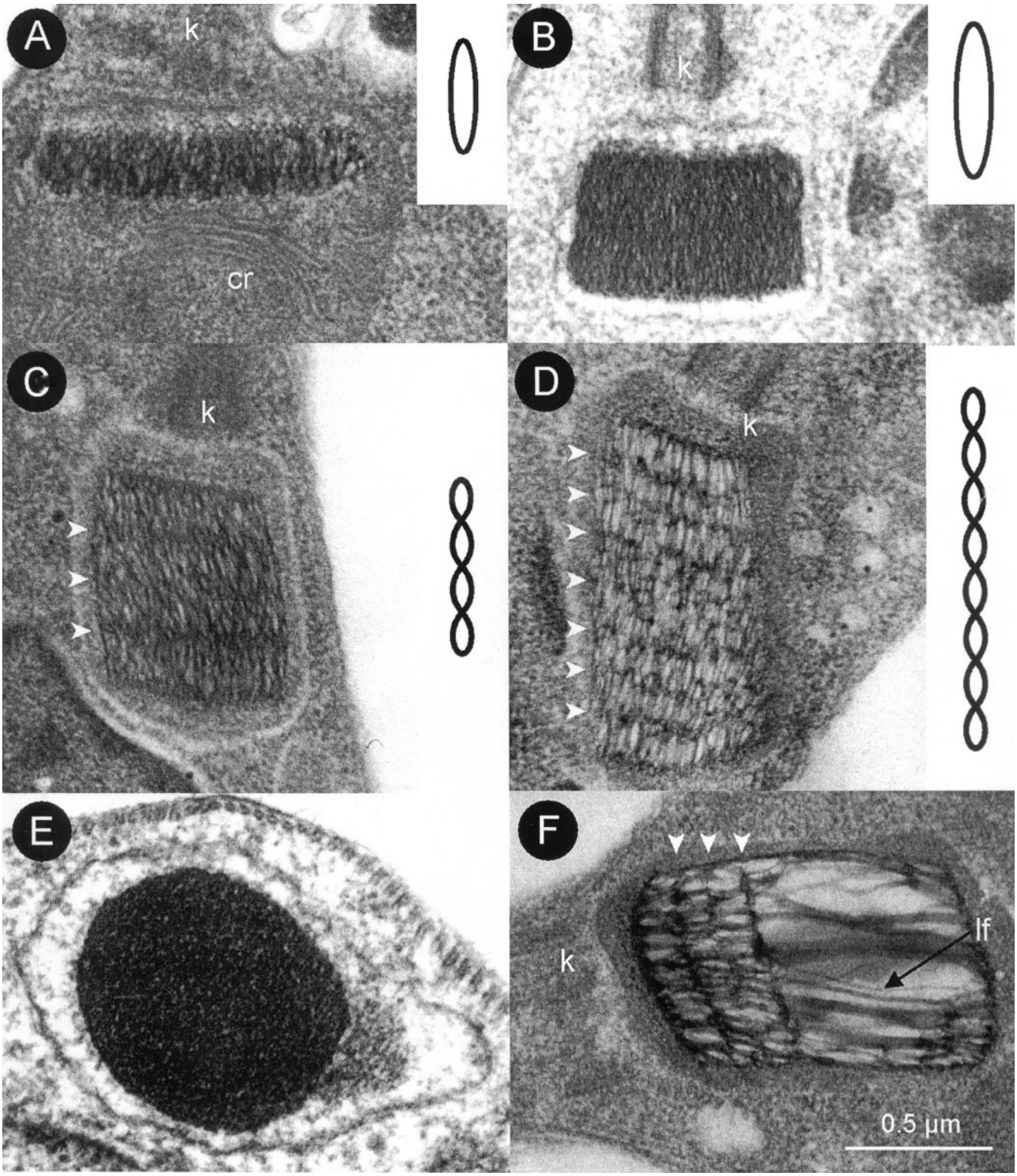


**FIG. 2.** Transmission electron microscopy of kinetoplasts in fixed cells. *T. carassii* (A); *T. boissoni* (B and E); *T. avium* A493 (C); *T. avium* A1412 (D and F). Schematic drawings of individual minicircles are shown to the right of the micrographs. A cross-sectioned kinetoplast (E) and a kinetoplast with loose DNA fibers (F) are shown; k, kinetosome of the flagellum; cr, mitochondrial cristae; lf, loose DNA fiber; arrowheads, electron-dense stripes. Scale bar, 0.5 $\mu$m; all micrographs are to the same scale.

the lumen of these kinetoplasts indicates that during the division the crossings may be released. Due to its unusual size the kinetoplast of *T. avium* A1412 may become a useful model for further analysis of this fascinating structure.

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