A *Trypanosoma brucei* minicircle encodes the same gRNAs as do minicircles of *T.equiperdum* ATCC 30019 and *T.evansi* type-A minicircles

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We report the sequence of a minicircle from *T.brucei* IsTaR 1 that is related to the type-A minicircles of some *T.evansi* and *T.equiperdum* ATCC 30019 (1) but which is not found in *T.brucei* 18E2 (2). It has ~65% identity to the *T.equiperdum* minicircle and retains the conserved sequence (CS), periodic A tracts spanning the adjacent sequence (AS)/CS junction that is associated with the DNA bend, 18 bp repeats that flank and thus define gRNA gene casettes (3, 4), and the same gRNA genes are in the same order as type-A minicircles (Figure 1). The other sequences have a pattern of divergence previously observed (3, 4) such that most of the sequence variation is restricted to the intercassette (IC) region, the non-gRNA coding region of the cassettes and the AS. The variations among gRNA genes are primarily G/A transitions that probably do not affect gRNA function since both can pair with U in mRNA. A nucleotide insertion in cassette 2 and deletion in cassette 3 of two *T.evansi* strains (2, 5) would affect the ability of gRNA to duplex with *T.brucei* mRNA. The gRNA from cassette 3 has been demonstrated in *T.brucei* IsTaR 1 and all three gRNAs have been demonstrated in *T.equiperdum* ATCC 30019 but they are unstudied in *T.evansi*.

The retention of a related minicircle in *T.brucei*, *T.equiperdum* ATCC 30019 and some *T.evansi* indicates their divergence from a common ancestor and that the kDNA deletions were secondary to this divergence. The *T.evansi* with type-B minicircles and the Pasteur strain of *T.equiperdum* with a third type of minicircle must have had a different evolutionary history. We cannot discriminate between divergence of all these trypanosomes from a common immediate ancestor by progressive loss of minicircle classes or independent origins.

Conservation of gRNA gene sequences within flanking sequence divergence, except for the 18 bp repeats, suggests that most type-A minicircle sequence evolution preceded divergence of *T.equiperdum* ATCC 30019 and type-A containing *T.evansi* from their common *T.brucei*-like ancestor. Selection for gRNA sequence conservation in *T.equiperdum* and *T.evansi* which lack maxicircle mRNA genes and mitochondrial protein synthesis seems unlikely. The absence of selective pressure imposed by the insect life cycle stages which require mitochondrial gene expression may have allowed the minicircles to become essentially homogeneous. Their generation by recombination appears unlikely given the related minicircle in *T.brucei*. We propose, therefore, that *T.equiperdum* ATCC 30019 and *T.evansi* with type-A minicircle are derived from *T.brucei*-group trypanosomes by sequence loss after minicircle sequence divergence.

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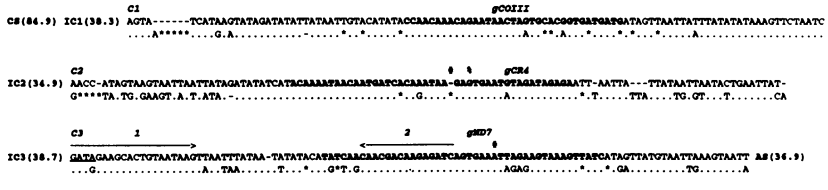

Figure 1. Comparison of the *T.brucei* minicircle (top) with type-A minicircles of *T.equiperdum* and *T.evansi* (bottom). The gRNA gene cassette sequences (C1–3) are shown without their flanking 18 bp repeats with the gRNA coding sequences in bold and labelled according to their cognate mRNA. The locations of the 160 bp CS, the 81, 43 and 87 bp IC regions 1, 2 and 3, respectively, and the 198 bp AS regions are diagrammed with percent homology between species shown in parenthesis. The minicircle was cloned as two overlapping fragments generated by PCR using primers 1 and 2 (arrows) with CS primers. Nucleotide identities are indicated by (.), substitutions by (*) and (–) indicates gapping for alignment. The (#) indicates nucleotide insertions or deletions which alter gRNA/mRNA duplex and (%) indicates G in *T.equiperdum* gRNA (6)where the DNA sequence predicts a C. Sequence accession numbers: M14763 (*T.equiperdum*), M34848 and M57459–62 (*T.evansi*).

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