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Organization of mini-exon and 5S rRNA genes in the kinetoplastid *Trypanoplasma borreli*

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Mini-exon gene repeats from *Trypanoplasma borreli*, which belongs to the Cryptobiidae family of the Bodonina suborder of the Kinetoplastida, were isolated by PCR amplification and cloning. The presence of kinetoplastid-like mini-exon genes in *T. borreli* is consistent with the taxonomic status of this organism as a kinetoplastid protozoan. Two families of repeats were found: 597 nt (T1) and 794 nt (T2), each of which encodes an approximately 95-nt medRNA transcript. The T1 repeats also contain a complete 5S rRNA gene on the complementary strand. The T2 repeats contain a defective copy of a 5S gene, in which the 5′ portion is absent. The intergenic regions between the 5′-ends of the mini-exon genes and the 5S rRNA genes in the T1 and T2 repeats are highly diverged. All or most mini-exon genes and 5S genes are located within either the T1 or the T2 repeats. The T1 repeats were localized to a megabase-size chromosome, while the T2 repeats were localized within at least 4 large chromosomes.

Key words: Mini-exon; medRNA; 5S rRNA; Kinetoplastida; Cryptobiidae; *Trypanoplasma borreli*; *Leishmania donovani*

Introduction

Trans-splicing of mRNA has been shown to exist in trypanosomatid protozoa [1], nematode [2] and trematode [3] worms and *Euglena gracilis* [4]. The process involves the joining of the exon portion of the mini-exon gene transcript (medRNA) with the exon of a precursor mRNA. In trypanosomatids, the exon is 39 nt long and highly conserved. In *E. gracilis*, the exon (spliced leader) is 24 nt. In the metazoans, the spliced leaders are 35 nt and 22 nt long and show no apparent sequence identity with the trypanosomatid counterpart. Mini-exon genes of trypanosomatids are organized in families of direct tandem repeats that are present in 100–300 copies per haploid genome. In 3 species of *Leishmania* [5–7], *Crithidia fasciculata* [8], *Leptomonas seymouri* [9], *Trypanosoma brucei* [10], *Trypanosoma congolense* [11] and *Trypanosoma cruzi* [12], the mini-exon gene repeats are unlinked to any other genes. However, the mini-exon genes are linked to 5S rRNA genes in *Trypanosoma rangeli* [13,14], *Trypanosoma vivax* [15], *Herpetomonas samuelpessoai* [16], *Bodo caudatus* [17], and *E. gracilis* [18]. A similar tandem linkage of the mini-exon and the 5S genes was also

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Note: Nucleotide sequence data reported in this paper have been submitted to the GenBank™ data base with the accession numbers L08172 (T2 repeat) and L08173 (T1 repeat).

Abbreviations: Mb, megabase pairs; nt, nucleotides; kDNA, kinetoplast DNA; PCR, polymerase chain reaction; medRNA, mini-exon derived RNA.

reported for nematodes, with the major difference being that in *Caenorhabditis elegans*, these genes are transcribed in opposite directions [19].

In this paper we show the presence of trypanosomatid-like mini-exon genes in *Trypanoplasma borreli*, which belongs to the Crytobiidae family of the Bodonina suborder of the Kinetoplastida. We show that the mini-exon and 5S rRNA genes are linked in this organism and that there is a second family of mini-exon repeats which contain a defective 5S gene. We speculate that the second gene family can represent an intermediate evolutionary situation that eventually could lead to a loss of the gene linkage.

Materials and Methods

Strain origin and growth conditions. The strain of *T. borreli* Pg-JH was isolated from a leech, *Piscicola geometra*, in Southern Bohemia [20]. The cells are cultivated in Difco liver infusion-tryptose medium (LIT) supplemented with 10% fetal bovine serum and 10 mg ml^{-1} hemin with slow agitation at 15°C. The stationary phase of growth with a density 6–9 $\times$ 10^6 cells ml^{-1} occurs after 6–7 days. The mid-log phase doubling time is approximately 50 h.

Oligonucleotides. Oligonucleotides were synthesized by standard phosphoramidite methods on an ABS 392 synthesizer and purified by thin-layer chromotography. The following oligonucleotides were used in this study: (1) BB-237: (GGGAAGCTT)CTGTACTATATTGGTA (mini-exon gene 5′ amplification primer; ref. 14) correspond to positions 25–39 of the exon and 40–41 of the intron; bracketed nucleotides correspond to a linker added to facilitate cloning. (2) BB-234: (GGGAATTC)AATATAGTACAGAAACTG (mini-exon gene 3′ amplification primer; ref. 14) corresponds to the inverse-complement of positions 21–39 of the exon; bracketed nucleotides correspond to a linker added to facilitate cloning. (3) S-624: CTTTCACTTTTTCACGGCGTTTACA (T2 specific probe) corresponds to positions 258–282, located in a region which does not share any similarity with T1. (4) S-632: GACCTCTTATCACAACAATCTGCTT (T1 specific probe) corresponds to positions 293–317, located in the intergenic region with no similarity to T2. (5) S-633: CCCAAGTCATCACTGACCTCAGTAC (5S rRNA antisense probe) corresponds to positions 154–178, includes stretches of sequence shared by the two repeats. (6) S-844: TACAGAAACTGTGACTTTTATAGCG (*T. borreli* antisense mini-exon-specific probe) corresponds to positions 31–7 of the exon.

DNA isolation. Cells were lysed with 2% sarcosyl, 0.5 mg ml^{-1} pronase at 65°C for 1 h in SET buffer (10 mM Tris-HCl, pH 8.0/ 150 mM NaCl/ 100 mM EDTA). The lysate was extracted once with phenol/chloroform. High-molecular weight DNA was precipitated with 2 vols. of ethanol, dissolved in TE buffer, re-extracted with phenol/chloroform and precipitated with ethanol.

Preparation of chromosomal DNA and pulse-field gel electrophoresis. Agarose blocks with embedded cells were prepared as described [21]. Contour-clamped homogeneous electric field (CHEF) gels were used to separate the chromosomes. Gel concentration, voltage and switch time values are described in the figure legends.

Southern blotting and hybridization conditions. Transfer of high-molecular weight DNA from chromosome gels onto Magna NT filters (Micron Separations Inc.) was performed by capillary blotting with prior partial depurination in 0.25 N HCl. Oligonucleotide probes were end-labeled by T4 polynucleotide kinase to a specific activity of 1–3 $\times$ 10^8 cpm mg^{-1}. Filters were hybridized in 6 $\times$ SSC/ 5 $\times$ Denhardt's solution at 37°C and washed in tetramethylammonium chloride as described previously [22,23].

DNA amplification by PCR, cloning and

sequencing. These procedures were performed as described in references 14 and 24. The sequence of the 0.6-kb T1 fragment was determined by sequencing from both ends of the cloned DNA. The 0.8-kb T2 insert was initially sequenced from the ends, and then oligonucleotide primers were used to complete the sequence.

Northern blot analysis. Total cell RNA was isolated by sarcosyl lysis, proteinase K digestion, phenol extraction and ethanol precipitation. To resolve low-molecular weight species, 10 μg total RNA was electrophoresed through polyacrylamide-urea gel and electro-blotted onto nylon membranes (Nytran; Schleicher and Schuell) according to the manufacturer's instructions. To resolve high-molecular weight species, 10 μg total RNA was electrophoresed through 1.2% agarose gel containing formaldehyde and transferred to Nylon membrane by capillary action.

Computer analysis. DNA sequence analysis was performed on a VAX 4000 computer (UCLA Life Science Computing Facility) using the University of Wisconsin Genetics Computer Group programs, and on an IBM PC using the PC/GENE package (IntelliGenetics).

Results

T. borreli contains 2 types of repeats with mini-exon and 5S rRNA genes. The PCR approach to clone the mini-exon genes is based on the high DNA sequence conservation of the mini-exon from other kinetoplastids and the tandem repeat organization of these genes [17]. After PCR amplification from *T. borreli* genomic DNA using the two partially overlapping primers, BB-237 and BB-234, 4 products were obtained: 2 prominent bands of 600 bp and 800 bp, and 2 faint bands of 1200 bp and 1600 bp (designated T1, T2, T3 and T4, respectively). The T1, T2 and T3 products were cloned and subjected to DNA sequence analysis.

Alignment of the T1 and T2 DNA sequences is presented in Fig. 1. The 39-nt exon of the T1 and T2 repeats was identified by comparison with the mini-exon gene repeats from *B. caudatus* and other kinetoplastids. Both repeats contained identical intron regions of 57 nt followed by a T-rich block, that has been found downstream of almost all mini-exon genes. The T1 repeat contains a complete 5S rRNA gene, which is transcribed in the opposite orientation to the mini-exon gene, at positions 252–133. The intergenic region between the two genes and the 3′-end of the 5S rRNA gene are well conserved between the T1 and T2 repeats. However, the sequence of the 5S rRNA gene in the T2 repeat diverges in the middle of the gene with the subsequent loss of the 5′-end of the gene. This sequence divergence results in the loss of the essential internal transcriptional control element, Box A, suggesting that the T2 5S rRNA sequence represents a pseudogene.

Conservation of the intergenic region upstream of the mini-exon genes in T1 and T2 repeats extends for about 200 nt. This region of similarity is, however, interrupted by a 25-bp deletion in the the T1 repeat. The deletion is flanked by 8-bp direct repeats (CCCAAAAA). Similar deletions flanked by short direct repeats have also been found in the intergenic region of the *L. donovani* mini-exon gene (O. Fernandes and D.A.C., unpublished), suggesting that homologous recombination between the repeats may have caused the deletion. Partial sequencing of T3 showed that it represents a dimer of T1. It is likely that T4 is a dimer of T2, but this was not confirmed by sequence analysis.

Linkage between mini-exon and 5S rRNA genes. Genomic blots of *T. borreli* DNA were probed with mini-exon- and 5S rRNA-specific oligonucleotides (Fig. 2). From the DNA sequences of the T1 and T2 PCR products, the restriction enzymes, *Ava*II, *Bgl*II and *Sca*I, should cleave the T1 repeat once, while the T2 repeat should be cleaved once with *Bgl*II and not cleaved with *Ava*II and *Sca*I. Figs. 2A and B represent the results of probing the blots with oligonucleotides S-632

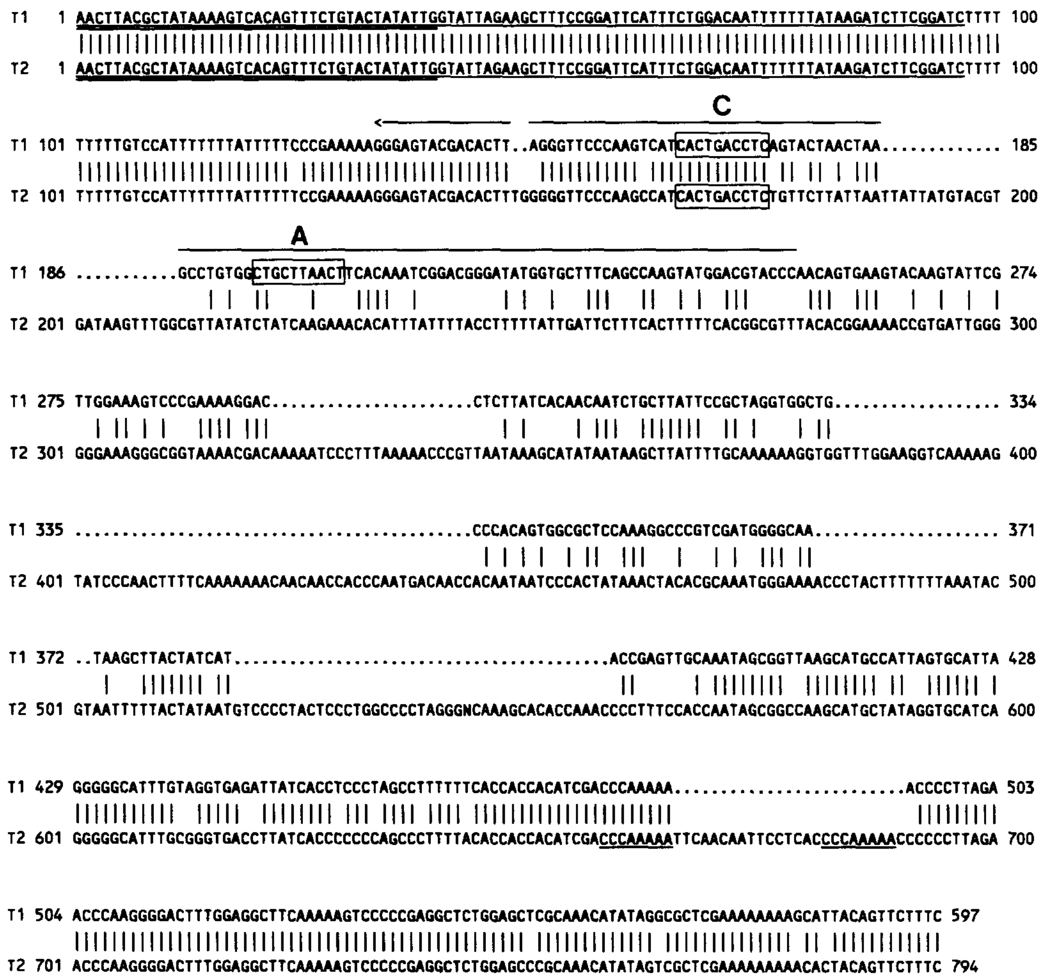

Fig. 1. Nucleotide sequences of mini-exon gene repeats, T1 and T2, from *T. borreli*. Alignment of T1 and T2 sequences, using UWGCG GAP program (gap weight, 5.0, and length weight, 0.3). Nucleotide 1 is the first nucleotide of the exon. Gaps are indicated by dots. The exon portion of the mini-exon gene in both repeat sequences is double-underlined, and the intron portion is single-underlined. The 5S rRNA gene in the T1 repeat is shown by a long arrow above the sequence. Two 8-bp direct repeats, which were possibly involved in generation of the 25-bp deletion seen in T1, are underlined. Box A and C consensus sequences are boxed in T1. The GenBank™ data base accession number for the T1 sequence is L08173, and for the T2 sequence is L08172.

and S-624, which are specific for the intergenic regions of T1 and T2, respectively. A single band at 0.6 kb in each lane (Fig. 2A) corresponds to a T1 unit repeat. Oligomeric forms of T1 which are seen in lane 3 are probably due to an incomplete digestion with *Sca*I. As seen in Fig. 2B, the T2 fragments form a distinct set of bands, with a 0.8-kb monomer generated only by *Bgl*II. The mini-exon-specific probe, BB-234, (Fig. 2C) and the 5S rRNA-specific probe, S-633, (Fig. 2D) hybridize with the combined set of fragments corresponding to both T1 and T2 repeats. We conclude from these data that most, if not all, genes for mini-exon and 5S rRNA are present in a linked form within the T1 and T2 repeats. Overexposure of the Fig. 2D autoradiogram showed, however, that several 5S rRNA sequences occur elsewhere in the genome (data not shown).

Chromosomal localization of mini-exon and 5S

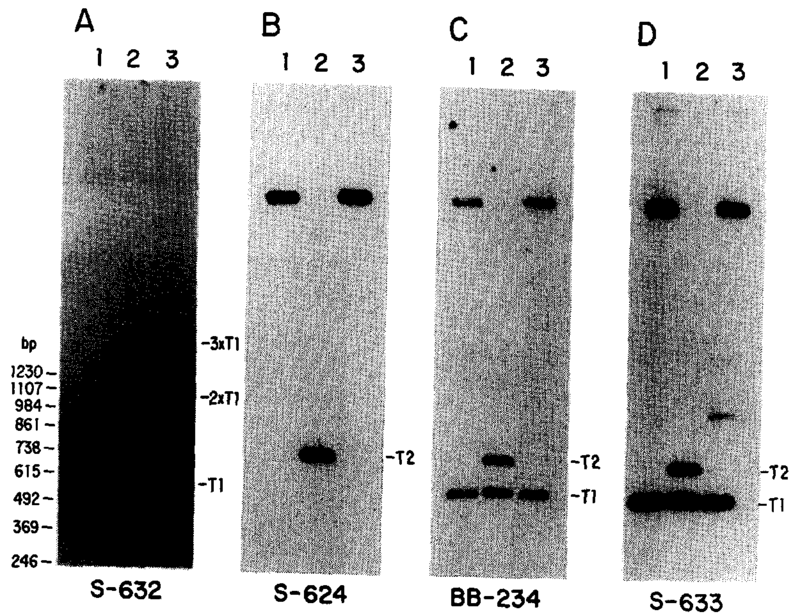

Fig. 2. Southern blot analysis of the T1 and T2 repeats in *T. borreli*. Total cell DNA (2.5 μg) was digested with *Ava*II (lane 1), *Bgl*II (lane 2) and *Sca*I (lane 3). Digests were separated in 1% agarose, blotted onto a nylon filter, and probed with the 5'-labeled oligonucleotides: (A) T1-intergenic region-specific probe, S-632; (B) T2-intergenic region-specific probe, S-624; (C) mini-exon gene-specific probe, BB-234; (D) 5S rRNA-specific probe, S-633.

genes. CHEF electrophoresis of *T. borreli* DNA revealed a pattern of several intense chromosome bands larger than 0.8 Mb, as well as a 0.25-Mb mini-chromosome band (Fig. 3A and B). A number of faint chromosome bands can also be seen. The observed intensity differences could be due either to differences in chromosomal copy number or to the possible presence of polymorphic genotypes in the uncloned isolate used.

As evidenced by hybridization of the intergenic probe, S-632, with a chromosome blot, the T1 repeats are localized on a 2-Mb chromosome band (Fig. 3C). Hybridization with the intergenic probe, S-624, showed that some T2 repeats are located on the same chromosome, while most reside on 3 smaller chromosomes – 1.8, 1.3 and 0.9 Mb (Fig. 3D) in size. As expected, the mini-exon gene-specific probe, BB-234, and the 5S rRNA-specific probe, S-633, hybridized with the same set of chromosomes (Figs. 3E and 3F). However, 2 additional bands with sizes of 1.2 and 1.1 Mb are seen which do not hybridize with either T1 or T2 intergenic region-specific probes. We suggest that T1 and/or T2 repeats with minimally diverged intergenic sequences may reside on these two chromosomes.

In *Leishmania donovani*, the mini-exon gene probe hybridizes to a 0.32-Mb chromosome and the 5S rRNA probe hybridizes to 0.8-Mb and 0.6-Mb chromosomes (Figs. 3E and 3F, respectively), which agrees with the previously reported [6] unlinked state for these genes in this species.

Transcripts from the T1 repeats. To determine the size of the *T. borreli* medRNA we have analyzed low-molecular weight RNA by Northern blotting (Fig. 4A). Probing the filter with the complete cloned T1 repeat (lane 1) revealed a major band of hybridization at 125 nt, which corresponds to 5S rRNA by its size and abundance, and a second band of hybridization at about 95 nt. Hybridization of a similar filter with the 5S-specific oligonucleotide (lane 2) confirms the identity of the 125-nt band as the 5S rRNA. Because no other transcript is detected by the 5S probe, we conclude that the truncated 5S gene in the T2

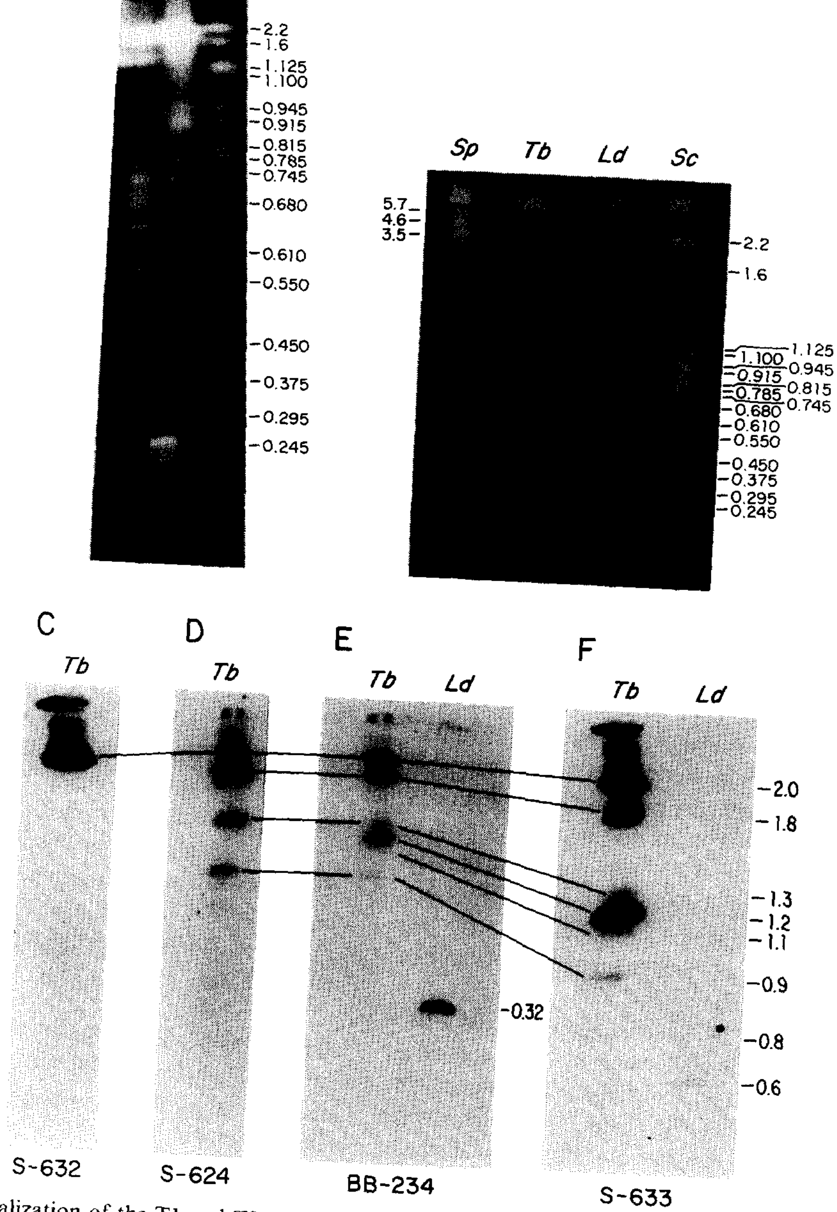

Fig. 3. Chromosomal localization of the T1 and T2 repeats. Lane designations are: Tb – *T. borreli*, Ld – *L. donovani*, Sc – *Saccharomyces cerevisiae*, Sp – *S. pombe*. CHEF configuration of electrodes with a 5 V cm^{-1} electric field gradient was used. Switch frequencies were as indicated below. Panels A and B: Ethidium bromide stained chromosome gels of *T. borreli*. (A) resolution of lower molecular weight chromosomes in 1.3% agarose with a switch time of 60 s for 21 h, 90 s for 8 h and 120 s for 16 h. Sizes of *S. cerevisiae* chromosomes are shown to the right of the panel. (B) resolution of higher molecular weight chromosomes in 1% agarose gel at 60 s switch time for 6 h, and 150 s for 24 h. Panels C to F: Hybridization analysis of chromosome gels. The blots in panels D and E originate from the gel shown on panel B. For the blots in panels C and F, a switch time of 60 s for 9 h and 150 s for 37 h was used. The blots were probed with labeled oligomers: (C) T1 intergenic region-specific probe, S-632; (D) T2 intergenic region-specific probe, S-624; (E) medRNA gene-specific probe, BB-234; (F) 5S rRNA specific probe, S-633. The positions of identical chromosomes are connected

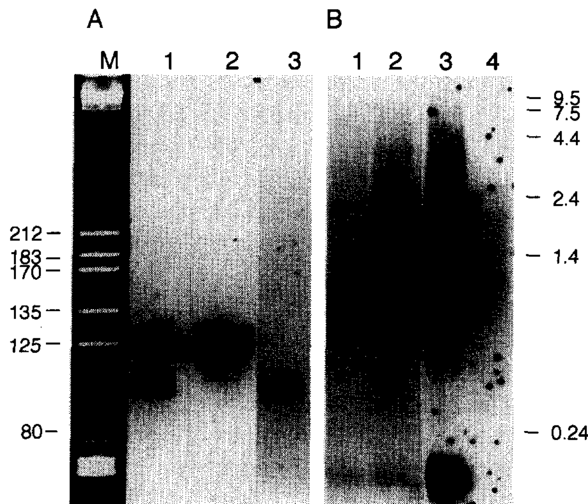

Fig. 4. Northern blot analysis of mini-exon gene transcription in *T. borreli*. (A) Total cell RNA was fractionated in a 7.5% polyacrylamide-urea gel and electroblotted onto a nylon filter. Blots correspondiing to parallel lanes were hybridized with the complete sequence of the cloned T1 repeat (lane 1), the 5S rRNA-specific oligonucleotide probe (lane 2) and the *T. borreli* mini-exon specific oligonucleotide probe (lane 3). Lane M is a similar lane that has been stained with ethidium bromide. The sizes of the low-molecular weight RNA species, which are given to the left of the panel, were determined by comparing with the low-molecular weight RNA from *T. brucei*. (B) High-molecular weight RNA from *T. borreli* (lanes 1 and 3) and *L. tarentolae* (lanes 2 and 4) was hybridized with either a generic mini-exon oligonucleotide probe (BB-234; lanes 1 and 2) or an oligonucleotide (S-844) specific for the *T. borreli* mini-exon. Dropout of the hybridization signal in the range 1.5–2.2 kb is most likely due to the mature ribosomal RNA species. Molecular size markers given to the right of the panel are those of the RNA marker ladder (BRL).

repeat is transcriptionally silent and thus confirms the earlier suggestion that it is a pseudogene. Hybridization of an additional filter with the *T. borrreli* mini-exon specific probe (lane 3) confirms the identity of the 95-nt transcript as the medRNA. In other kinetoplastids, the 3′ end of medRNA occurs just before the T-track, which may be associated with termination of transcription. Indeed, the juxtaposition of the 3′ ends of the mini-exon gene and the 5S rRNA gene in *T. borreli* provides evidence that the 36-bp T-rich region could function in termination of medRNA transcription.

To demonstrate that the *T. borreli* medRNA is trans-spliced, we have analyzed high-molecular weight RNA with mini-exon specific probes (Fig. 4B). Hybridization of the PCR amplification primer BB-234 to a Northern blot of *T. borreli* RNA (lane 1) or *L. tarentolae* RNA (lane 2) reveals a smear in the 0.5–7.5-kb range, indicating the presence of the mini-exon on multiple mRNAs in both organisms. To demonstrate that the smear represents specific rather than non-specific hybridization, we have reprobed the filter with the *T. borreli* mini-exon specific oligonucleotide S-844. This reveals a similar smear for the *T. borreli* RNA (lane 3) and no hybridization to the *L. tarentolae RNA* (lane 4) thus demonstrating the occurrence of *trans*-splicing in *T. borreli*.

Discussion

The existence of trypanosomatid-like mini-exon genes and a medRNA transcript, whose exon is trans-spliced onto cellular mRNAs, in *T. borreli* is consistent with the taxonomic status of this organism as a kinetoplastid protozoan. Previous evidence for this taxonomic designation included ultrastructure and life cycle data (reviewed in ref. 25) and the presence of glycosomes [26]. A phylogenetic analysis using 18S rRNA sequences from kinetoplastids, indicates that *T. borreli* branched off early from the trypanosomatids and clearly represents another major lineage within the Kinetoplastida (D.A.M. and L.S., unpublished results).

We have identified 2 families of mini-exon gene repeats in *T. borreli*. An intact 5S rRNA gene is found within members of the T1 family of repeats, whereas the T2 family contains a defective 5S gene that lacks a 5′-end. Within each repeat, the mini-exon gene and 5S gene are located on opposite strands. The T1 repeats are located on a single 2-Mb chromosome, whereas the T2 repeats are additionally found on 3 other chromosomes. Two more chromosomes hybridize to mini-exon-specific and 5S rRNA-specific probes but not to T1- or T2-specific probes, suggesting the presence of additional variant loci. Because we found that

all mini-exon genes in *T. borreli* reside in the repeats of either T1 or T2 size (Fig. 2), we suggest that the variant loci could represent repeats with minor sequence heterogeneity in the intergenic regions. In this context, it is of interest that *E. gracilis* contains 2 equally sized subfamilies of the spliced leader (mini-exon) RNA genes which differ from each other by several point mutations in the intergenic region [18].

The cloned representatives of the T1 and T2 repeats demonstrate 2 genomic rearrangements relative to each other. One rearrangement, which is flanked by 8-bp direct repeats, is an apparent deletion of 25 bp in the T1 repeat. Because kinetoplastid protozoa possess active homologous recombination systems [27], we suggest that this is the most likely mechanism to explain the observed rearrangement. The other rearrangement, which has resulted in major sequence variation of the intergenic region and the loss of the 5′-end of the 5S rRNA gene, is noticeable because it caused the inactivation of the T2 5S rRNA gene.

It has been proposed that cells both select and maintain homogeneity of mutations with multicopy genes by a multifactorial process termed 'molecular drive' [28]. The stochastic component of molecular drive allows the cell to establish variant sequences within the genome. Genetic mechanisms that establish such variants include unequal chromosome exchange, gene conversion and gain or loss by transposition. All three mechanisms have been demonstrated to occur in kinetoplastid protozoa. We speculate that the original genomic organization is represented by the T1 repeat, and that the T2 repeat may have arisen by transposition mediated loss of the 5′ end of the 5S rRNA. It has been reported that the site-specific retroposons frequently interrupt the mini-exon gene repeats of several trypanosomatids [9,29]. Subsequent chromosomal translocations could then be postulated to separate the T2 repeat from the original locus and generate the two additional loci containing the T2 repeats. The directional component of molecular drive leads to homogenization within gene families and is the result of mechanisms such as duplicative transposition, biased gene conversion and slippage replication. The directional component could therefore establish and maintain homogenous tandem arrays of the T2 repeats. It remains to be determined whether the conservation of approximately 0.15 kb of intergenic region upstream of both mini-exon genes in T1 and T2 repeats reflects a functional constraint such as transcription initiation.

The linkage between mini-exon and 5S rRNA genes in some trypanosomatids and their separate genomic locations in others raises the question whether the linkage of the mini-exon and 5S rRNA genes is a primitive or derived character. The genomic linkage of mini-exon and 5S rRNA genes is found in 4 other kinetoplastid species, *B. caudatus, H. samuelpessoai, T. rangeli* and *T. vivax*, and also in the distantly related *E. gracilis*. Cladistic analysis of the mini-exon and 5S rRNA genes in the kinetoplastid-euglenoid group suggests that the linked stage represents a primitive feature. The unlinked state represents a derived character which has occurred independently multiple times in the Trypanosomatidae family. The existence in *T. borreli* of a family of T2 mini-exon repeats with defective 5S rRNA genes may represent an intermediate situation in the separation of the two genes.

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References

1 Agabian, N. (1990) *Trans*-splicing of nuclear pre-mRNAs. Cell 61, 1157–1160.
2 Nilsen, T.W. (1989) *Trans*-splicing in nematodes. Exp. Parasitol. 69, 413–416.
3 Rajkovic, A., Davis, R.E., Simonsen, J.N. and Rott-

man, F.M. (1990) A spliced leader is present on a subset of mRNAs from the human parasite *Schistosoma mansoni*. Proc. Natl. Acad. Sci. USA 87, 8879–8883.

4 Tessier, L.H., Keller, M., Chan, R.L., Fournier, R., Weil, J.H. and Imbault, P. (1991) Short leader sequences may be transferred from small RNAs to pre-mature mRNAs by *trans*-splicing in *Euglena*. EMBO J. 10, 2621–2626.

5 Agami, R. and Shapira, M. (1992) Nucleotide sequence of the spliced leader RNA gene from *Leishmania mexicana amazonensis*. Nucleic Acids Res. 20, 1804.

6 Wilson, K., Hanson, S., Landfear, S. and Ullman, B. (1991) Nucleotide sequence of the *Leishmania donovani* medRNA gene. Nucleic Acids Res. 19, 5787.

7 Miller, S., Landfear, S. and Wirth, D. (1986) Cloning and characterization of a *Leishmania* gene encoding a RNA spliced leader sequence. Nucleic Acids Res. 14, 7341–7360.

8 Köck, J. and Cornelissen, A.W.C.A. (1990) The 5S ribosomal RNA genes of *Crithidia fasciculata*. Mol. Biochem. Parasitol. 38, 295–298.

9 Bellofatto, V., Cooper, R., Cross, G.A. (1988) Discontinuous transcription in *Leptomonas seymouri*: presence of intact and interrupted mini-exon gene families. Nucleic Acids Res. 16, 7437–7456.

10 Lenardo, M.J., Dorfman, D.M., Reddy, L.V. and Donelson, J.E. (1985) Characterization of the *Trypanosoma brucei* 5S ribosomal RNA gene and transcript: the 5S rRNA is a spliced leader independent species. Gene 35, 131–141.

11 Cook, G.A. and Donelson, J.E. (1987) Mini-exon gene repeats of *Trypanosoma (Nannomonas) congolense* have internal repeats of 190 base pairs. Mol. Biochem. Parasitol. 25, 113–122.

12 Hernandez-Rivas, R., Martinez-Calvillo, S., Romero, M. and Hernandez, R. (1992) *Trypanosoma cruzi* 5S rRNA genes: molecular cloning, structure and chromosomal organization. FEMS Microbiol. Lett. 92, 63–68.

13 Aksoy, S,, Shay, G.L., Villanueva, M.S., Beard, C.B. and Richards, F.F. (1992) Spliced leader RNA sequences of *Trypanosoma rangeli* are organized within the 5S rRNA-encoding genes. Gene 113, 239–243.

14 Murthy, V.K., Dibbern, K.M. and Campbell, D.A. (1992) PCR amplification of mini-exon genes differentiates *Trypanosoma cruzi* from *Trypanosoma rangeli*. Mol. Cell. Probes 6, 237–243.

15 Roditi, I. (1992) *Trypanosoma vivax*: linkage of the mini-exon (spliced leader) and 5S ribosomal RNA genes. Nucleic Acids Res. 20, 1995.

16 Aksoy, S. (1992) Spliced leader RNA and 5S rRNA genes in *Herpetomonas* spp. are genetically linked. Nucleic Acids Res. 20, 913.

17 Campbell, D.A. (1992) *Bodo caudatus* medRNA and 5S rRNA genes: tandem arrangement and phylogenetic analysis. Biochem. Biophys. Res. Commun. 182, 1053–1058.

18 Keller, M., Tessier, L.H., Chan, R.L., Weil, J.H. and Imbault, P. (1992) In *Euglena*, spliced-leader RNA (SL-RNA) and 5S rRNA genes are tandemly repeated. Nucleic Acids Res. 20, 1711–1715.

19 Nelson, D.W. and Honda, B.M. (1989) Two highly conserved transcribed regions in the 5S DNA repeats of the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*. Gene 38, 245–252.

20 Peckova, H. and Lom, J. (1990) Growth, morphology and division of flagellates of the genus *Trypanoplasma* (Protozoa, Kinetoplastida) in vitro. Parasitol. Res. 76, 553–558.

21 Rovai, L., Tripp, C., Stuart, K. and Simpson, L. (1992) Recurrent polymorphisms in small chromosomes of *Leishmania tarentolae* after nutrient stress or subcloning. Mol. Biochem. Parasitol. 50, 115–126.

22 Wood, W., Gitschier, J., Lasky, L. and Lawn, R. (1985) Base composition-independent hybridization in tetramethylammonium chloride: A method for oligonucleotide screening of highly complex gene libraries. Proc. Natl. Acad. Sci. USA 82, 1585–1588.

23 Shaw, J., Feagin, J.E., Stuart, K. and Simpson, L. (1988) Editing of mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. Cell 53, 401–411.

24 Maslov, D.A., Sturm, N.R., Niner, B.M., Gruszynski, E.S., Peris, M. and Simpson, L. (1992) An intergenic G-rich region in *Leishmania tarentolae* kinetoplast maxicircle DNA is a pan-edited cryptogene encoding ribosomal protein S12. Mol. Cell. Biol. 12, 56–67.

25 Lom, J. (1979) Biology of the trypanosomes and trypanoplasms of fish. In: Biology of Kinetoplastida, Vol. 2 (Lumsden, W.H.R. and Evans, D.A., eds.), pp. 269–337. Academic Press, New York.

26 Opperdoes, F.R., Nohynkova, E., Van Schaftingen, E., Lambeir, A.-M., Veenhuis, M. and Van Roy, J. (1988) Demonstration of glycosomes (microbodies) in the Bodonid flagellate *Trypanoplasma borelli* (Protozoa, Kinetoplastida). Mol. Biochem. Parasitol. 30, 155–164.

27 Cruz, A. and Beverley, S.M. (1990) Gene replacement in parasitic protozoa. Nature 348, 171–173.

28 Dover, G.A. (1982) Molecular drive: a cohesive mode of species evolution. Nature 299, 111–117.

29 Aksoy, S. (1991) Site-specific retrotransposons of the trypanosomatid protozoa. Parasitol. Today 7, 281–285.