Nucleotide sequence of the spliced leader RNA gene from *Leishmania mexicana amazonensis*

Reuven Agami and Michal Shapira
Department of Membrane Research and Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel

Submitted February 26, 1992 EMBL accession no. X64317

Maturation of all mRNAs in trypanosomatid protozoan organisms occurs through the process of *trans*-splicing, which joins two exons: a 5′ capped 39 nt non translated mini-exon denoted also as the spliced leader (SL), with the exon that codes for the protein sequence; while each is derived from two distinct RNA molecules (1). *Trans*-splicing may serve as an important mode for control of gene expression and it was previously shown that exposure of *Trypanosoma brucei* procyclic cells to severe heat shock inhibited *trans*-splicing and processing of the $\alpha-\beta$ tubulin transcripts (2). To follow the effects of a physiological temperature switch on transcription of the SL RNA and on its related ribonucleoprotein particle in *L.mexicana amazonensis*, the gene that codes for the SL RNA was cloned and sequenced.

Genomic DNA of *L.mexicana amazonensis* was digested with RsaI, an enzyme that cuts once within the SL RNA gene of all trypanosomatids characterized to date, and hybridized with a 39 mer oligonucleotide derived from the *L.enriettii* mini-exon (3). The hybridization revealed a band of 0.3 kb, representing the genomic repeat unit which contains the SL RNA gene. The ladder of bands obtained by hybridization of partially digested DNA further supports this observation. The band sizes in the ladder grow in increments of 0.3 kb, which is the size of a single repeat unit (Figure 1). For cloning the SL RNA gene, genomic DNA was digested with RsaI, separated on an agarose gel and DNA sized 0.3 kb was electro-eluted and cloned into pBluescript. The resulting mini-library was screened with the oligomer described above.

The nucleotide sequence of the SL RNA gene and the flanking sequences that comprise the genomic repeat unit are presented in Figure 2, starting with the 39 nucleotides of the mini-exon. Computerized comparison with the similar molecule from *L. donovani* (4) and *L. enriettii* (3) shows complete conservation of the mini-exon sequence, whereas the intron portion of the transcript varies in 16 nucleotides among the species compared. A higher divergence is observed while comparing with the SL RNAs from *Trypanosoma brucei* (5) and *Leptomonas seymouri* (6). There is no conservation in the size of the DNA repeat unit among different trypanosomatids, nor is its size conserved among the different *Leishmania species*.

ACKNOWLEDGEMENTS

Supported by grants from the UNDP/World Bank/Special Program for Research and Training in Tropical Diseases, the Nathan Fund for Dermatological Research and the John and Catherine MacArthur Foundation. M.S. is incumbent of the Helena Rubinstein Career Development Chair.

REFERENCES

1. Borst,P. (1986) *Annu. Rev. Biochem.* **55**, 701–732.
2. Muhich,M.L. and Boothroyd,J.C. (1988) *Mol. Cell. Biol.* **8**, 3837–3846.
3. Miller,S.I. *et al.* (1986) *Nucleic Acids Res.* **14**, 7341–7360.
4. Wilson,K. *et al.* (1991) *Nucleic Acids Res.* **19**, 5787.
5. Milhausen,M. *et al.* (1984) *Cell* **38**, 721–729.
6. Bellofato,V. *et al.* (1988) *Nucleic Acids Res.* **16**, 7437–7456.

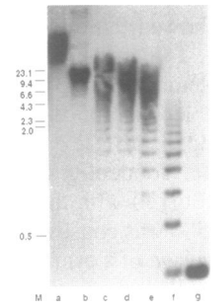

Figure 1. Southern blot of *L. mexicana amazonensis* genomic DNA fully and partially digested with RsaI, an enzyme which has a unique site within the repeat unit of the SL RNA gene and with TaqI, an enzyme which does not cut within this genomic repeat unit. Non digested DNA (a), DNA digested with TaqI (b), DNA digested with RsaI 0.6 U, 1.25 U, 2.5 U, 5 U and 10 U respectively (c–g). All digests were performed on 5 μg DNA for 10 min. Molecular weight standards (M) are λHindIII digest.

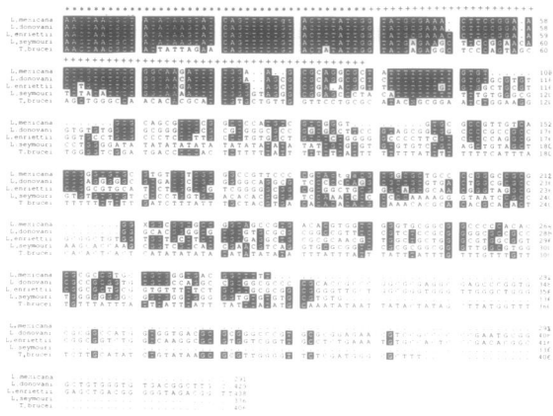

Figure 2. Nucleotide sequence of the genomic repeat unit from *L.mexicana amazonensis* that codes for the SL RNA transcript, compared to sequences from *L.enriettii*, *L.donovani*, *Leptomonas seymouri* and *Trypanosoma brucei*. The sequence begins with the mini-exon [nucleotides 1–39, marked with a star (*)] and is followed by the intron sequences [marked with a plus sign(+)]. Homologous sequences are presented within the black boxes.