Characteristics of Nucleotide Sequences Flanking the trans-Spliced Leader SL1 Exon in Dirofilaria immitis, Brugia malayi, and Brugia pahangi

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ABSTRACT. Nucleotide sequences surrounding the trans-spliced leader SL1 exon in the 5S rRNA gene spacer regions of Dirofilaria immitis, Brugia malayi, and Brugia pahangi were determined after PCR amplification, aligned with the genus Onchocerca for comparison, and used for the prediction of secondary structures. The nucleotide sequence of this region in B. pahangi was first shown in the present study. Hypothetical secondary structures of the spacer region suggested that the SL1 transcript is capable to form a stable stem-loop structure which may render transposition of the SL1 sequence to mRNA molecules. A homologous sequence to Sm-binding site was assigned on a hypothetical secondary structure. The nucleotide sequence of this region in B. pahangi is first described in this study.

A spliced leader sequence at the 5′ end of a mRNA was first demonstrated in African trypanosomes [2]. This trypanosome leader sequence is derived from an RNA transcript of a repetitive DNA element, and transferred onto the 5′ ends of all mRNA [16]. This machinery has been called “trans-splicing” which is common in a variety of eukaryotes during the maturation of mRNA molecules [1], and may be a regulatory mechanism for gene expression. Similar to trypanosomes, a 22-nucleotide spliced leader SL1, 5′-GGUUUAAUACCCAAGUUGAG-3′, which has no homology to the trypanosome 39-nucleotide leader sequence has been shown at the 5′ end of 10 to 15% of mRNAs in the free-living nematode Caenorhabditis elegans [6]. The C. elegans SL1 sequence is encoded within the spacer region of the repetitive DNA element containing the 5SrRNA gene cluster and transcribed from the complementary strand to an RNA of about 100 nucleotides by RNA polymerase II during early embryonic development, just like U1 snRNA molecules in vertebrates. Linkage between the 5S rRNA gene and the SL1 RNA exon within a repetitive DNA element has also been found in other nematodes such as Onchocerca volvulus [17], Dirofilaria immitis [17], Brugia malayi [13], and Ascaris lumbricoides [14]. Although the 5S rRNA and SL1 loci in C. elegans are on complementary strands of the repetitive DNA element, the two loci for 5SrRNA and SL1 are on the same strand in the parasitic nematodes, O. volvulus, D. immitis, B. malayi, and A. lumbricoides. In the present paper we show the nucleotide sequences of the spacer region including the SL1 exon downstream of the 5S rRNA gene in filarial nematodes, D. immitis, B. malayi, and B. pahangi which showed a closer relationship based on antigen cross-reactivity as revealed by monoclonal antibodies [10], and predict their secondary structures. The nucleotide sequence of this region in B. pahangi is first described in this study.

Of the four adult worms of D. immitis, two (male and female, each) were from Japan, which are referred to as “Japanese adult worms”, and two (male and female, each) were from the U.S.A. which are referred to as “American adult worms”, hereafter. Japanese adult worms in the heart were collected from naturally infected dogs at necropsy [11]. American adult worms were collected from naturally infected dogs. To determine the prevalence of D. immitis infection in dogs, these dogs were killed by euthanasic procedure and immediately provided from a dog pound of Wake County, Raleigh, North Carolina, U.S.A. Two Brugia species, B. malayi and B. pahangi, have been maintained in the second author’s laboratory. Exact source of the B. malayi strain has been described elsewhere [9]. All adult worms were identified by morphological features. DNA of D. immitis was isolated from a single adult worm, and DNA of Brugia was extracted from frozen specimens of adult worms by the hydroxyapatite batch elution technique [3]. Parasite DNAs were subjected to PCR to amplify the 5S rRNA gene and its 3′-flanking spacer region by using a pair of primers based on genomic sequences of high homology between Dirofilaria and Onchocerca [17]. The PCR primers F1 (5′-GTC TAC GAC CAT ACC ACG TT-3′) and R1 (5′-CAG GAC GTT CCA AAA TTT-3′) were custom-made by Takara Shuzo Co., Ltd. (Kyoto). The PCR amplification was carried out with 50-µl reaction mixtures containing 5 µl of DNA solution, 5 µl of 10X buffer, 1.25 U of Taq DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn.), dNTPs to a final concentration of 0.2 mM each, 0.25 µl of each pair of primers F1 and R1 (40 pmol/µl each), and water to a final volume of 50 µl. The cycle was repeated 30 times with denaturation at 94°C for 30 sec, annealing at 56°C for 120 sec, and extension at 72°C for 120 sec. The PCR products were extracted from gels after electrophoresis, and subjected to direct sequencing three times on each strand.
by the method of Sanger et al. [12] without cloning. Nucleotide sequences of the two species of the genus *Onchocerca* were obtained from the DNA databases. Accession numbers for *O. cervicalis* and *O. volvulus* are U13678 and U09024, respectively. The nucleotide sequences first reported in this paper will appear in the DDBJ, EMBL, GSDB and NCBI nucleotide sequence databases under the following accession numbers: D87036, D87037, D87038, D87039, D87040, and D87041.

Nucleotide sequences including the 5S rRNA gene and spliced leader SL1 exon of the five species were aligned by the Clustal method of Higgins et al. [5] using the DNASIS software (Hitachi Software Engineering Co., Yokohama). The 5S rRNA gene was well conserved among the five species, *D. immitis*, *B. malayi*, *B. pahangi*, *O. cervicalis* and *O. volvulus* (Fig. 1), suggesting their close genetic relatedness. On the other hand, the spacer region was highly variable in both sequence and length among these species. We found a small difference in numbers of tandem repetition of the ATT sequence in the spacer region among the adult worms of *D. immitis*, though their biological significance remains unclear. We did not succeed in amplifying the same region of *Litomosoides carinii* and *Acanthocheilonema viteae* by PCR (data not shown).

Although the primary and secondary structures of the rRNA molecules are well documented, little is known about the secondary structures of the 5S rRNA gene and spliced leader SL1 exon of the five species were aligned by the Clustal method of Higgins et al. [5] using the DNASIS software (Hitachi Software Engineering Co., Yokohama).

![Fig. 1. Nucleotide sequence alignment of the PCR products. The nucleotide sequence numbers are given from a consensus alignment. Nucleotide numbers from 1 to 120 represent the 5S rRNA gene. Numerals 1 and 2 after *D. immitis* represent Japanese female and male worms, respectively, and numerals 3 and 4 represent American male and female worms, respectively. Nucleotides that are identical in two out of three sequences are shown as inverted characters. Dashes indicate spaces between adjacent nucleotides introduced for maximum alignment. Tandem repetition of ATT sequence is underlined.](image-url)
those of the spacer regions. The spliced leader SL1 exon was shown to be completely conserved among the five species examined, suggesting that the SL1 sequence is an indispensable element in filarial nematodes. The role of SL1 sequence at the 5' end of mRNA molecules is currently unknown, although it has been suggested that it contributes to cleavage of polycistronic RNAs from tandem genes to generate monomeric RNA units [7] and functions as a CAP structure to those mRNAs [8].

The dinucleotide GT was found at 3' end of the 22-nucleotide SL1 exon, raising the possibility that the SL1 sequences could be competent to donate the SL1 leader sequence. The secondary structures of the spacer region predicted according to the algorithm of Zuker and Stiegler [18] indicated that the SL1 sequence contributes to form a stable stem-loop structure after transcription. Computer-assisted potential secondary structures of the SL1 and Sm-binding regions in five species were remarkably similar (Fig. 2). No significant difference was apparent between Japanese and American adult worms of *D. immitis*, and between male and female worms, though it was of interest to find any parameter to distinguish the *D. immitis* worms in terms of biological features. The SL1 sequence in a stem-loop structure may render transcription of SL1 sequence and thus explain rationally the trans-splicing event of this particular sequence. The spacer region also contains a consensus nucleotide sequence, 5'-AAUUUUGG-3', identical to the binding site for Sm, an antigen associated with small nuclear ribonucleoprotein particles in *C. elegans* [4, 15].

In conclusion, we showed the SL1 is capable to form a stable stem-loop structure and an Sm-binding site is located on a bulge loop, which are well conserved in *D. immitis, B. malayi,* and *B. pahangi,* though other parts of the spacer region are variable in sequence and length. No difference, except for tandem repetition of ATT, was found in *D. immitis* irrespective of sex or location of isolation. No difference was also found in different species of the genus *Brugia.* The 22-nucleotide SL1 sequence is invariably adjacent to the dinucleotide GT characteristic of potential splice donor sites. The functional significance of the SL1 exon must await more extensive examination of the spacer regions downstream of the 5S rRNA genes.

REFERENCES


