Full Length Research Paper

Identification and characterization of an mRNA trans-splicing leader in *Schistosoma japonicum*

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RNA trans-splicing occurs in a wide range of eukaryotes, from protozoa to chordates. Here, the study present the discovery of a spliced-leader (SL) RNA in the zoonotic trematode, *Schistosoma japonicum*. The 36-nucleotide SL in *S. japonicum* was derived from a 90-nucleotide non-polyadenylated RNA transcript encoded by 55 copies of SL genes dispersed throughout the genome. Differential transcription patterns of the SL gene among the parasite developmental stages were observed, indicating that trans-splicing may be a mechanism of post-transcriptional gene regulation in *S. japonicum*. Further, the distribution of SL genes in the genomes of *S. mansoni* and *S. japonicum* has been found to be very different, though the two parasites have been believed to be genetically close. These data open up a novel avenue for study on parasite biology linked to schistosomiasis, a disease affecting more than 200 million people world-wide.

Key words: *Schistosoma mansoni*, *S. japonicum*, spliced leader RNA, trans-splicing, gene regulation.

INTRODUCTION

RNA trans-splicing, a post-transcriptional process initially observed in the kinetoplastid protozoan *Trypanosoma brucei (*Campbell et al., 1984; Kooter et al., 1984; Milhausen et al., 1984), is an evolutionarily ancient mode of gene expression and regulation (Blaxter and Liu 1996). During an RNA trans-splicing process, a short RNA transcript of approximately 90 nucleotides (nt), derived from genes scattered in the genome, is further processed into a small RNA fragment, called splicing leader (SL), which is ligated to the 5′ end of an mRNA transcript. RNA trans-splicing has been found in organisms from single cell eukaryotes to humans. In trypanosomes, genes are transcribed polycistronically and all mature mRNAs are generated through a trans-splicing process, where an SL sequence is spliced and added to the 5′ end of each mRNA (Campbell et al., 1984; Kooter et al., 1984; Milhausen et al., 1984). In other organisms, such as nematodes, only a portion of mRNAs is trans-spliced. In

Caenorhabditis elegans (Krause and Hirsh, 1987), there are two SL RNA species which have distinct sequence characteristics. SL1, a 22-nt spliced leader derived from a 100-nt SL RNA, is found in 70% of mRNAs. SL1 homologues have been identified in most nematodes except *Trichinella spiralis*, suggesting that it may have arisen from a common ancestor of most modern nematodes. The *C. elegans* genome has 110 copies of the SL1 gene arranged in tandem with the 5S rRNA genes; whereas, there are only 18 copies of the SL2 gene which are dispersed throughout the genome. The sequence of SL2 differs from that of SL1 and is only found in about 15% of trans-spliced mRNA templates. SL1 and SL2 homologues have also been identified in platyhelminthes. For instance, in *Schistosoma mediterranea*, 42-base spliced leader were derived from either a 107-nt transcript (SL1 type) or a 106-nt transcript (SL2 type) (Zayas et al., 2005). The basal nematode *T. spiralis* possesses a putative divergent trans-splicing mechanism. This organism does not have SL1 or SL2 homologues, but rather it contains 19 genes encoding 15 distinct SLs, indicating that *T. spiralis* evolved its trans-splicing mechanism along an independent path (Pettitt et

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al., 2008). To date, only SL1-related trans-spliced RNAs have been found in *S. mansoni,* which contains approximately 61 copies arranged in a tandem repeat in the genome (Rajkovic et al., 1990).

This study, report the identification of a spliced leader sequence in *S. japonicum*, a zoonotic parasite that infects millions of people and animals in Asian countries. The spliced leader sequence (36-nt) of *S. japonicum* is derived from a 90-nt RNA which is encoded by a gene of 55 copies scattered throughout the genome. SL RNA is differentially transcribed during the developmental stages of the parasite.

MATERIALS AND METHODS

Parasites

Adult *S. mansoni* parasites were isolated from infected hamsters and kept in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH of 7.4) at -20°C before DNA purification. Adult worms and eggs of *S. japonicum* were isolated from infected rabbits at 7 weeks post-infection. Male and female adult worms were manually separated under a light microscope. Lung and hepatic schistosomula were isolated from infected mice at day 3 and 14 post-infection. Cercariae were harvested from an intermediate host, the snail *Oncomelania hupensis*. Adult worms and hepatic schistosomula were stored in RNAlater Solution (Invitrogen, CA, USA). Cercariae and eggs were stored in liquid nitrogen before RNA purification.

RNA and gDNA isolation

Total RNA of *S. japonicum* (eggs, cercariae, schistosomula, and adult worms) was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. Genomic DNA of adult worms (*S. japonicum* and *S. mansoni*) was extracted with DNeasy Blood and Tissue Kit (QIAGEN, CA, USA) according to the manufacturer's protocol.

Bioinformatic analyses

The genomic sequence and predicted coding sequences (CDS) of *S. japonicum* were downloaded from the Chinese National Human Genome Center at Shanghai (CHGC). The sequence of *S. mansoni* spliced leader RNA was used in a BLAST search of the *S. japonicum* genome with default parameters. The target sequences were extracted from the *S. japonicum* genome sequence and a multiple alignment was produced using the program ClustalW 1.84.

Secondary structure predictions of the SL RNA was performed using MFOLD Version 2.38 using the default folding conditions (1 M NaCl, 37°C), and with the constraint that the Sm-binding site (5´-GCUUUCUUUGC) required to be a single strand. To generate graphical output, the mfold predictions were imported into RnaViz9 (Version 2.0) and a graphical display of the output was created using Boxshade 3.21.5 (Zuker, 2003).

For identification of trans-spliced mRNA transcripts, the *S. japonicum* SL RNA sequence verified by 5´and 3´RACE was used to blast against the EST database (SjTPdb) of *S. japonicum*. The ETSs with or without a spliced leader were named SL-ESTs and NonSL-ESTs, respectively. For functional categorization of the SL-ETSs, SL sequence was removed from the SL-ESTs (named SL_Cuted-ESTs), and the SL_Cuted-ESTs were assembled into unique transcripts using CAP3 with default parameters. Unique trans-spliced transcripts and predicted CDSs were annotated in the Clusters of Orthologous Groups of proteins (COG) database of NCBI using Blastx and Blastp respectively, with the cutoff value of E<= 1e-10. Targeted sequences with significant matches were classified into different functional categories of COG using a perl scripts.

SL RNA sequence identification in *S. japonicum* by rapid amplification of cDNA ends

Rapid amplification of cDNA ends (RACE) was performed using the FirstChoice RLM-RACE kit (Ambion, TX, USA). Total RNA was first treated with the DNA-free™ KIT (Ambion) to eliminate DNA contamination. For 5´RACE, gene-specific primers were designed from *S. japonicum* genomic DNA using the predicted SL RNA sequence. To control amplification specificity, two reverse primers 5´-GGGCTGGCAAAGAAAGCTTCGATTC-3´ and 5´-GAAAGCTTCGATTCTTGGTCGACGG-3´ were used for the outer and inner amplification reactions, respectively. For 3´RACE, poly(A) tails were added to 10 µg total RNA using yeast poly(A) polymerase (Ambion). The first strand cDNA was synthesized from the polyadenylated RNA. The gene-specific forward primer used for 3´RACE was 5´-CGTCACGGTTTTACTCTTGTGATTTG-3´

Analysis of SL expression in different developmental stages and gene copy number determination

For determination of transcription variation of SL gene during parasite development by real-time RT-PCR, the cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) and used as the template for amplification with the primer pair 5´-CGTCACGGTTTTACTCTTGTGAT-3´ and 5´-GCTGGCAAAGAAAGCTTCGATT-3´. Similarly, a primer pair of 5´-CATGGTAGACAACGAAGCTATTTATGA-3´ and 5´-GATTAGTGTAGGTTGGACGCTCTATG-3´ was used to amplify the α-tubulin transcript for normalization of the amount of mRNA. PCR reactions were set up by combining 0.4 nM final concentrations of each primer pair, the appropriate amount of cDNA, 12.5 µl of Power SYBR Green PCR Master Mix (ABI) and in a final volume of 25 µl with DEPC-treated water.

For determination of SL copy number in the genome of *S. japonicum*, the primer pair 5´-CGTCACGGTTTTACTCTTGTGAT-3´ and 5´-GCTGGCAAAGAAAGCTTCGATT-3´ was used to detect the SL sequence. A primer pair of 5´-GCTACGCAAAAGACCGTTGAT-3´ and 5´-CGCCCCACGACCATCTC-3´ was used to amplify the GAPDH gene (single copy) for normalization of the amount of gDNA. Similarly, the primer pair 5´-CCGTGTCGGTTTCGCTAAA-3´ and 5´-GGGCTATATACTTTGGTGTGGTGAT-3´ was used to amplify the aspartic proteinase gene (single copy) for calibration of SL copy number. For calculation of SL copy number in the genome of *S. mansoni*, the primer pair 5´-ATACTGCTGCTTGTGCCATCA-3´ and 5´-CCTTTGCTCTCGACATCTTGAA-3´ was used to amplify the GAPDH gene and the primer pair 5´-GACGGCTTGTCCTGATGGA-3´ and 5´-CCGGCGATCATCGAAGTG-3´ was used to amplify the aspartic proteinase gene, while the primers used to detect the SL sequence of *S. mansoni* were the same as those for *S. japonicum*.

PCR reactions were set up by combining 0.5 nM final concentration of each primer pair, 10 ng gDNA, 12.5 µl of Power SYBR Green PCR Master Mix (Applied Biosystems) and in a final volume of 25 µl with DEPC-treated water. Amplification reaction was performed using the ABI PRISM 7300 sequence detection system (Applied Biosystems). Relative expression was calculated using the SDS 1.4 software (Applied Biosystems). PCR efficiency

Figure 1. Alignments of *S. mansoni* SL RNA and homologous sequences identified in various contigs of *S. japonicum* genomic sequence. SmSL is the SL RNA of *S. mansoni*.

for the amplicons was calculated using a recently described method (Ruijter et al., 2009).

Southern and Northern blot analyses

Southern blot assays were performed to investigate genomic distribution of SL genes in the genome of *S. mansoni* and *S. japonicum*. Genomic DNA (1 µg) of *S. mansoni* and *S. japonicum* was digested with 0.5 unit of Sal I and resolved in 0.8% agarose gel, denatured in 0.5 M NaOH solution and transferred to Hybond-N membranes. For northern blot assays, 10 µg of total RNA of *S. japonicum* was separated in 2% formaldehyde/agarose gels and 15% denatured polyacrylamide gels, respectively and transferred to Hybond-N membranes using standard procedures. The oligonucleotide probe used in Southern and Northern blots was 5´-CCATGCAACAAATCACAAGAGTAAAACCG-3'. End-labeling of the oligonucleotide (1 pmol) was carried out with a T4 polynucleotide kinase and γ^{32}P ATP (6000 Ci.m mol-1; 10 mCi.ml-1). Prehybridization and hybridization was carried out in 2x SSC (saline-sodium citrate) with 1% SDS and 100 mg/ml sheared, denatured salmon sperm DNA at 60 °C. Post-hybridization washes were in 0.2 × SSC, 0.2% SDS at temperatures ranging from 40 - 60 °C. The images were recorded using a FLA-5000 system (Fuji, Japan) with Aida Image Analyser software.

RESULTS

Identification of a SL gene in the genome of *S. japonicum*

A BLAST search of the *S. japonicum* whole-genome shotgun reads performed with SL sequence derived from *S. mansoni* revealed eight distinct genomic contigs that contain potential SL sequences (Figure 1). The sequences of SL genes from *S. mansoni* and *S. japonicum* are nearly identical. Final confirmation of sequence and structure of the SL in *S. japonicum* was achieved by sequence alignment with the SL genes of *S. mansoni* and other metazoans (Figures 2 and 3).

The numbers and distribution of the SL genes of both *S. japonicum* and *S. mansoni* were investigated by real time quantitative PCR and Southern blot. A total of 55 SL RNA gene copies were found in the *S. japonicum* genome, while *S. mansoni* has 61 copies (Figure 4A). The distribution of SL genes in the two schistosomal genomes was found to be different. SL genes are randomly dispersed in the genome of *S. japonicum,* whereas they are concentrated in tandem repeats in *S. mansoni*. This was confirmed by incomplete digestion of the genomic DNA of the two parasites with Sal I restriction enzyme. Since there is only one recognition site in the SL sequence, the hybridization patterns of the digested DNA were obviously different. In the genome of *S. mansoni*, the SL gene is separated by a 600 bp sequence in tandem. Thus the bands of *S. mansoni* DNA hybridized by the probe were evenly distributed (600, 1200, 1800, 2400 and 3000 bp) (Figure 4B). But the hybridization pattern with the digested DNA of *S. japonicum* was irregular (Figure 4B), confirming the finding from the unassembled genome sequences.

Characterization of SL RNA of *S. japonicum*

The 90-nt transcript of *S. japonicum* SL RNA was cloned by 5' and 3' RACE, and the sequence was found to be consistent with the genomic SL sequence identified in the BLAST search. Just like SL RNA of *S. mansoni*, *S. japonicum* SL RNA is predicted to contain only one stem-loop structure preceded by a typical spliceosome (Sm)-binding sequence (Figure 3). The sequences of the 5' end region, potential splice donor junction and putative Sm-binding site of *S. japonicum* SL RNA were either similar or identical to that of other flatworms (Figure 2). The predicted SL leader of *S. japonicum* is 36 nt in length with an AUG at the 3´end.

Transcription of SL RNA in *S. japonicum*

The transcription of SL RNA in different developmental

A

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S. japonicum       1  AACCGTC-ACGGTTTTAC----TCTTGTGATTTG-TTGCATGGTAAGAACCG-------T
Echinococcus       1  ACCGTTA-ATCGGTCCTT---ACCTTGCAGTTT---TGTATGGTCAGTATCGATGCAGCT
Fasciola           1  AACCTTA-ACGGTTCTCT---GCCCTGTATATTA-GTGCATGGTAAGAATCG-----TT
Haematoloechus     1  -ACCTAT-ACGGTTCTCT---GCCGTGTAT--CA-GTGCATGGTAAGAATCGA-----GTT
Notoplana          1  GGTCTCA-AAAATTTGGT---GTTTATTGCAATAATTGCATGGTAAGCATCAA------A
S. mansoni         1  AACCGTC-ACGGTTTTAC----TCTTGTGATTTG-TTGCATGGTAAGAACCG-------T
SmedSL1            1  GCCGTTAGACGGTCTTATCGAAATCTATATAAATCTTATATGGTACGGACCG-------T
SmedSL2            1  GCCGTTAGACGGTCTTATCGAAATCTATATAAAATTATATGGTGAGGACCG-------T
Stephanostomum     1  -ACCTAT-ACGGTTCTCT---GCCGTGTATATTA-GTGCATGGTAAGAATCGA----ATT
Stylochus          1  GGTCTCA-AAAATTTGGT---GTTTATTGCAATAATTGCATGGTAAGCATCAA------A

S. japonicum      48  CGACCAA------GAATCGAA-GCTTTCTTTGCCAGCCCTGCA--------AAGGGCT--
Echinococcus      54  GAGGCTGTGCCTACGAGCTGACCCAGTATTTGGCTGGTCCTT--------CGAGGGCC--
Fasciola          50  GGACCATC-----GGTCCAAACCCATTATTTGGCTAGCCTCCATTCTG--CAGAGGCTAA
Haematoloechus    49  CGACTCACATCGTTGGTCGAATAGATTATTTGGCTAGCCTCCACTCTGGTCGGGGGCTA-
Notoplana         51  -GATCCA------TGTGATCGTCGAGTCTTTGACAGGCGGC---------AAGGCCTAT
S. mansoni        48  CGACCAA------GAATCGAA-GTTTTCTTTGGCAGCCCTACA--------CACGGGG--
SmedSL1           54  TATCCAACA----TTAGTTGG-TTAATTTTTGACAGTCACTTGAATC-ACAAGTGACTAT
SmedSL2           54  TTGCCAGCA----TTAGTTGG-GTAATTTTTGACAGTAGCTTGCAT--GCAAGTGACTAT
Stephanostomum    51  CGACCTA------TGGTCGAATAAATTCTTTGGCTAGCCTCT-------TCGGGGGCTAA
Stylochus         51  TGATCCAG-----TGTGATCGTCGAGTCTTTGACAGGCCGC---------GAGGCCTAT
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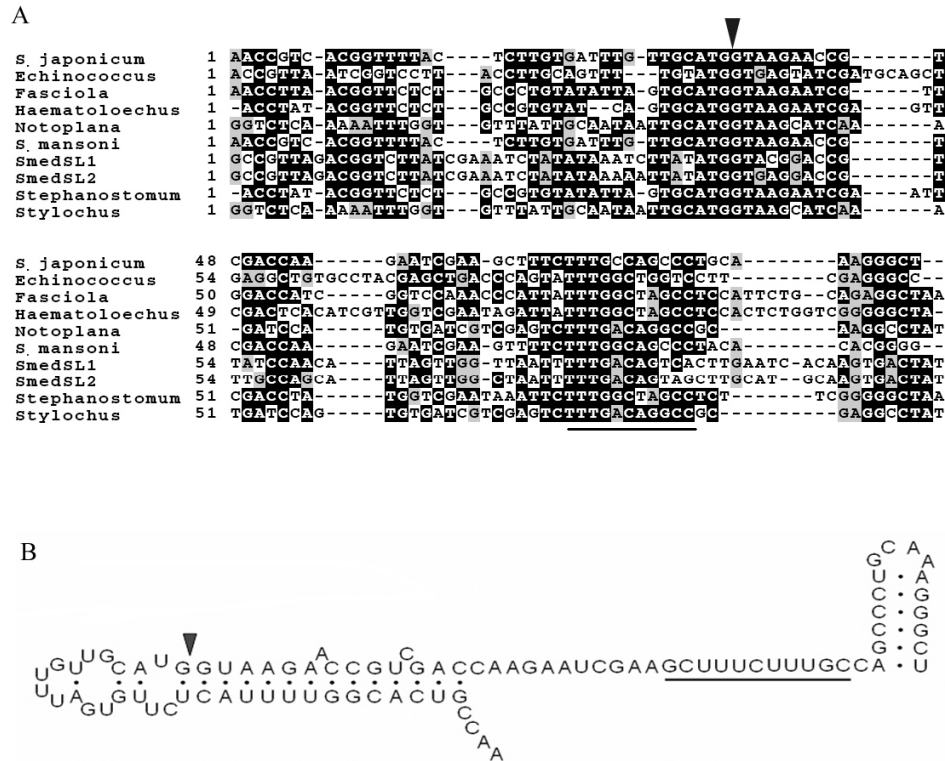
B

Figure 2. Sequence characteristics of S. japonicum SL RNA A. Alignment of SL RNA sequences of S. japonicum with that of other flatworms. The conserved splicing site is indicated with an arrowhead, and the Sm-binding domain is underlined. B. Predicted secondary structure of S. japonicum SL RNA.

stages of *S. japonicum* was investigated by Northern blot and real time RT-PCR with RNA purified from cercariae, 3 and 14 day schistosomula, male and female worms and hepatic eggs. Northern blots of total RNA from adult parasites separated on 15% denatured polyacrylamide gels revealed two bands of 90 and 30-nt (Figure 5A) which represented the full-length transcript and SL leader sequence, respectively. Further, Northern blot analysis of total RNA separated in a denatured agarose gel, detected a prominent band of around 90-nt as well as other transcripts ranging in size from 0.15 to 5 kb (Figure 5B). The expression of SL RNA was found predominantly in two developmental stages, egg and cercariae, while female worms had least SL RNA (Figure 5B). No significant expression difference between male and schistosomulum (3 and 14 d) was observed.

Identification of trans-spliced mRNAs

Messenger RNAs that are trans-spliced were identified by Blast searches of the *S. japonica* EST database with SL sequence. Among 84499 ESTs analyzed, 678 ESTs of 342 mRNA transcripts were identified as containing a trans-spliced leader. Among the proteins encoded b the

342 trans-spliced mRNAs, 65 (E<1e-10) could be functionally classified based on characterized biological functions. Proteins encoded by trans-spliced mRNAs were predominantly related to signalling, metabolism, and information storage and regulation.

DISCUSSION

This report, provide evidence of RNA trans-splicing mechanism in *S. japonicum*. The sequence of the SL gene of *S. japonicum* possesses consensus SL motifs, with a conserved splicing site and a splicesome-binding site. Genes of SL RNA in *S. japonicum* and *S. mansoni* are nearly identical in sequence and have similar copy numbers in the two parasites. The only major difference between the two parasites is the distribution patterns of SL genes in the genome: SL genes are arrayed in tandem in *S. mansoni* as in most organisms, but are dispersed throughout the genome in *S. japonicum*. Thus, the genome organization and the post-transcriptional gene regulation mechanisms of the two schistosomal parasites may have followed separated evolutionary pathways.

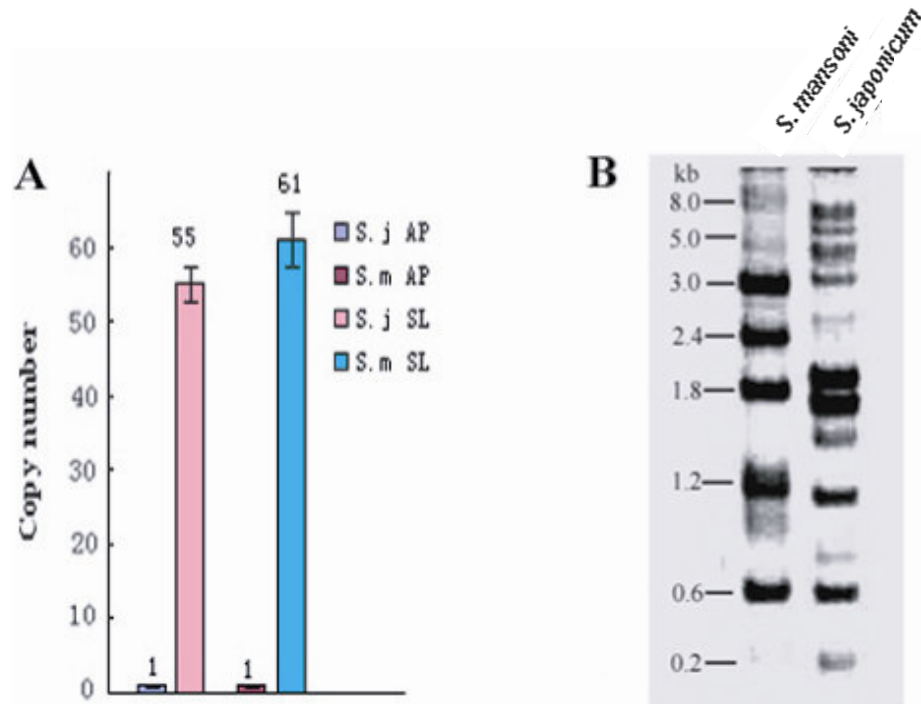The function of RNA trans-splicing in metazoans

Figure 3. Distribution of SL genes in the genome of *S. mansoni* and *S. japonicum*. (A) Gene copy number determination in *S. mansoni* and *S. japonicum*. The copy number of SL genes in the two parasites was determined using qPCR with the single copy aspartic proteinase (AP) gene as a reference. Fifty five copies were identified in the genome of S. japonicum while 61 were identified in S. mansoni. (B) Distribution of SL gene in the genomes of *S. japonicum* and *S. mansoni*. Southern blot of Sal I-digested genomic DNA of *S. mansoni* and *S. japonicum* with SL probe shows that the SL gene in *S. mansoni* genome is arranged in tandem repeat with defined spacer sequence (around 600 bp) between two genes. While the SL genes in *S. japonicum* genome is randomly scattered.
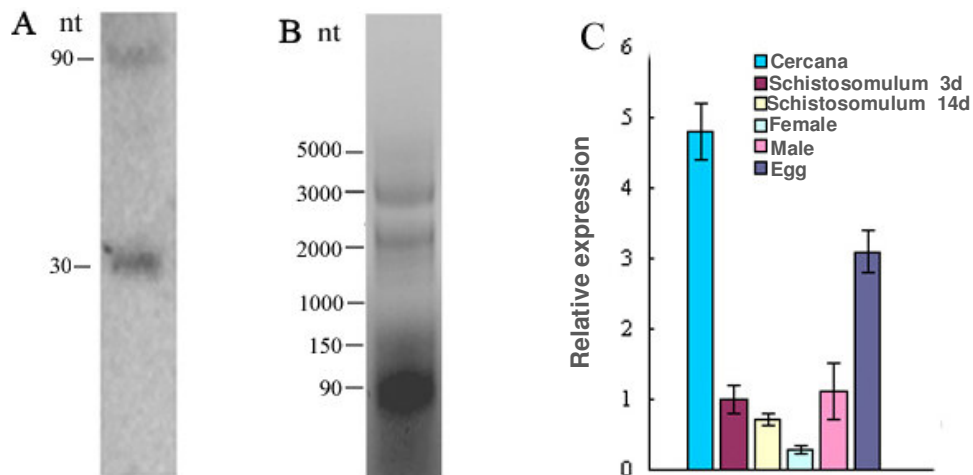
Figure 4. Transcription of SL RNA in *S. japonicum*. (A) and (B) Northern blot analyses on total RNA of *S. japonicum* separated in a 15% denatured polyacrylamide gel and a 2% formaldehyde-agarose gel with a radioactive-labelled SL probe. Two fragments of 90 and 30-nt in size were recognized in the RNA separated in the polyacrylamide gel and several bands ranging from 90 to 3 kb were visible in RNA separated in an agarose gel. (C) Relative expression levels of SL RNA in various developmental stages of *S. japonicum*. The SL. transcript is predominantly identified in cercariae and eggs.

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AACCGTCACGGTTTTACTCTTGTGATTTGTTGCATG GTAAGAACCGTCGACCAAGAATCGAAGCTTTCTTTGCCAGCCCTGCAAAGGGCTAA         SjSL-1
       CGGTTTTACTCTTGTGATTTGTTGCATG AAAATGGCAATTATAGCGATTCACGCCCGTCAAATCTTCGACAGCCGAGG Contig72    32  Enolase
     GAGGCGGTTTTACTCTTGTGATTTGTTGCATG AATGTCTTTGTTGTGCTCATTATCAAACGAGGTGCCTGAGCACCCAGTTG Contig59    12  GTP-binding-protein
     GGCGGTTTTACTCTTGTGATTTGTTGCATG AACAATGGAAACTATGCAAGATTTAGCGACTTCTGCTTTAAAAGATAATC Contig91    11  mitochondrial chaperone BCS1
      TTTTACTCTTGTGATTTGTTGCATG GGAGGAATATGATATCGCTATCAAAAAAGTGGCTATAGTTACAGGAGCAA Contig31    11  MGC84267 protein
      TTTTACTCTTGTGATTTGTTGCATG GATACAACGTTTATAAGCATCTTTTCACCGCTTCCAAGGATATTTAGTGG Contig116    8  phosphoglycerate kinase
     GTTTTACTCTTGTGATTTGTTGCATG AATGGCTCCGGGTGCTGCAAGCCGAACGGCACCTATATTTCAATTGGGAA Contig25     7  X-Pro dipeptidase
      TTTTACTCTTGTGATTTGTTGCATG CAACCTCGTGGACTTTATTACATGAAAAGAAATGAAGATGAAAGAGGACA Contig103    7  Cytosolic leucine-rich protein
        TGTGATTTGTTGCATG ACTCCAGATGATGAATCACATCCTAGTGTTACAGTTGAATTATTTGTTTC Contig108    7  Amyloid beta A4 precursor protein
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B

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Genomic sequence
TTGTAATTCTGTTTGGCTTTATTCGGCTTTTGTTTATTGTTGTCTTGTTCCGTTTTCTATTCCATTTTTCTAACCTTTAG ATCACTTGACTGAATACCTTTCAAGAGCATCAGACACTTGGTCCTACATT
                         TTTTACTCTTGTGATTTGTTGCATG ATCACTTGACTGAATACCTTTCAAGAGCATCAGACACTTGGTCCTACATT MiA_13424 TS
            CTCATTACAAGAGTCGACGTACTTGTCCAGGATTTACGCT ATCACTTGACTGAATACCTTTCAAGAGCATCAGACACTTGGTCCTACATT FeA_03302 CS
      TTATTCGGCTTTTGTTTATTGTTGTCTTGTTCCGTTTTCTATTCCATTTTTCTAACCTTTAG ATCACTTGACTGAATACCTTTCAAGAGCATCAGACACTTGGTCCTACATT HeS_15718 NS
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Figure 5. Dominant trans-spliced mRNA transcripts indentified in the ETS database. (A) Top eight expressed trans-spliced transcripts. Contig numbers of the trans-spliced transcripts, the number of ESTs found and the names of the encoded proteins are listed to the right of the sequences. SL sequence is underlined and the putative AUG sites are highlighted in blue. (B) Alignment of EST and corresponding genomic sequence. TS: trans-spliced by SL leader; CS: cis-spliced; NS: not spliced; SL sequences are underlined

remains an interesting question. Unlike trypanosome parasites, only a portion of mRNA transcripts in metazoan organisms are trans-spliced. Based on available data derived from investigations in different biological systems, the significance of RNA trans-splicing can be categorized in three aspects. First, SL RNA contributes to mRNA stability. In *Ascaris*, the combination of SL and the trimethyl guanosine (TMG) cap facilitates mRNA translation and stability in a manner typical of a eukaryotic m7G-cap (Cheng et al., 2007). RNAs lacking the 5´ cap structure and 3´polyadenylation are more prone to chemical and enzymatic degradation than mature mRNAs (Blumenthal, 1995). Secondly, SL RNAs provide 5´ sequence elements essential for interaction with ribosomal machinery and the initiation of protein translation. This is believed to be achieved through the elimination of the 5´ end of the long untranslated region of pre-mRNAs or by donation of an AUG start codon. Studies have found that all trans-spliced mRNAs have a short 5´UTR (Bektesh et al., 1988; Blaxter and Liu, 1996). In *S. japonicum*, the function of SL RNA might be more related to the donation of a 5´UTR than to providing the AUG start codon, since most trans-spliced mRNA transcripts have an internal AUG codon after the splice site. Thus, the function of RNA trans-splicing in *S. japonicum* is probably more related to mRNA stability than to the initiation of translation. In *T. spiralis*, the mRNAs of a single gene can have different SLs (Pettitt et al., 2008). This observation has lead to speculation that SL genes in metazoan may be driven by tissue-specific promoters or be under developmental regulation. This study found that the SL genes of *S. japonicum* are differentially activated in the earlier developmental stages (Figure 5B). However, it is still not known whether the production of trans-spliced mRNAs is also predominant in these two stages.

Finally, schistosomiasis is one of the most prevalent helminth infections which causes tremendous morbidity and mortality, many biological aspects of the causative pathogen are, however, still unclear due to the complicated parasite life cycle. With the coming assembly of the whole genome sequences of both *S. mansoni* and *S. japonicum,* deep investigation in the relation between gene expression and pathogenesis as well as disease control can be pursued.

ACKNOWLEDGMENTS

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