Gene 533 (2014) 156–167

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Molecular cloning and characterization of SL3: A stem cell-specific SL RNA from the planarian *Schmidtea mediterranea*

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ARTICLE INFO

Article history:
Accepted 26 September 2013
Available online 8 October 2013

Keywords:
Stem cells
SL RNA
Planarians
Regeneration
Trans-splicing

ABSTRACT

Spliced leader (SL) *trans*-splicing is a biological phenomenon, common among many metazoan taxa, consisting in the transfer of a short leader sequence from a small SL RNA to the 5′ end of a subset of pre-mRNAs. While knowledge of the biochemical mechanisms driving this process has accumulated over the years, the functional consequences of such post-transcriptional event at the organismal level remain unclear. In addition, the fact that functional analyses have been undertaken mainly in trypanosomes and nematodes leaves a somehow fragmented picture of the possible biological significance and evolution of SL *trans*-splicing in eukaryotes. Here, we analyzed the spatial expression of SL RNAs in the planarian flatworm *Schmidtea mediterranea*, with the goal of identifying novel developmental paradigms for the study of *trans*-splicing in metazoans. Besides the previously identified SL1 and SL2, *S. mediterranea* expresses a third SL RNA described here as SL3. While, SL1 and SL2 are collectively expressed in a broad range of planarian cell types, SL3 is highly enriched in a subset of the planarian stem cells engaged in regenerative responses. Our findings provide new opportunities to study how *trans*-splicing may regulate the phenotype of a cell.

1. Introduction

Spliced leader (SL) *trans*-splicing is an inter-molecular splicing reaction in which the mini-exon of a small SL RNA is joined to the 5′ end of pre-mRNA molecules (Lasda and Blumenthal, 2011) (Fig. 1A). This process was first discovered in the protozoan *Trypanosoma*, where the totality of transcripts shares a common 5′-terminal sequence of 35 nt (Boothroyd and Cross, 1982; Milhausen et al., 1984). Later, SL *trans*-splicing was found in animals such as nematodes and flatworms (Krause and Hirsh, 1987; Rajkovic et al., 1990), leading to the suggestion that the process could be peculiar to lower invertebrate phyla (Davis, 1996). However, increasing sampling of genomic data from diverse species has clearly shown that SL *trans*-splicing is found in all major metazoan lineages (Pre-bilateria, Lophotrocozoa, Ecdysozoa and Deuterostomes) (Douris et al., 2010; Lasda and Blumenthal, 2011).

Despite such wide representation in the animal kingdom, SL *trans*-splicing has been studied at genetic and biochemical levels only in trypanosomes and nematodes (Blumenthal, 2005; Liang et al., 2003). In fact, a remarkable amount of research has been done in these organisms on the fundamental role of SL *trans*-splicing in resolving poly-cistronic transcripts (Evans et al., 2001), the biochemical requirements for SL *trans*-splicing (Hannon et al., 1991), the purification of factors associating with the SL ribonucleoprotein (RNP) (Denker et al., 2002), and the identification of translation factors binding the trimethylguanosine cap at the 5′ of the SL (Fig. 1A) or mediating its methylation (Keiper et al., 2000; Liu et al., 2011; Takagi et al., 2007; Wallace et al., 2010). Yet, even with such systematic and rigorous molecular dissection, and with the exception of the role played by SL RNAs in operon processing, it is still unclear what the biological effect of adding an SL sequence to monocistronic transcripts in these organisms may be.

Given the current status of knowledge on this unresolved biological problem, we reasoned that the study of *trans*-splicing in a still understudied metazoan phylum may help inform our understanding of

Abbreviations: A, adenosine; ATP, adenosine triphosphate; b, brain; BAC, bacterial artificial chromosome; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; bp, base pair(s); C, cytidine; CB, chromatoid bodies; cDNA, DNA complementary to RNA; cNeoblast, clonogenic neoblast; d, deoxyribo; DAPI, 4′,6-diamidino-2-phenylindole; DIG, digoxigenin; DNP, dinitrophenol; EST, expressed sequence tag; FISH, fluorescent in situ hybridization; FITC, fluorescein isothiocyanate; g, gut; G, guanosine; IRR, irradiation; Kb, kilobase(s) or 1000 bp; MMG, 7-methylguanosine; mRNA, messenger RNA; NBT, Nitro Blue tetrazolium; nc, nerve chords; nt, nucleotide(s); OCT, Optimal Cutting Temperature compound; p, pharynx; PBS, phosphate buffered saline; PCR, polymerase chain reaction; pr, photoreceptors; R, purine; RACE, rapid amplification of cDNA ends; RNP, ribonucleoprotein; rNTP, ribonucleotide; rRNA, ribosomal RNA; RT-PCR, reverse transcriptase-PCR; S, in vitro transcribed standard; sDMA, symmetrical dimethylarginine; SDS, sodium dodecyl sulfate; SL, spliced leader; SL RNA, spliced leader RNA; SL RNP, spliced leader RNA ribonucleoprotein; Sm, Smith's antigen; *smedwi-1*, *Schmidtea mediterranea* piwi-like protein; snRNA, small nuclear RNA; SSC, 0.15 M NaCl/0.015 M Na_3·citrate pH 7.6; T, thymidine; TMG, 2,2,7-trimethylguanosine; U, uridine; UTR, untranslated region(s); WT, wild type.

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http://dx.doi.org/10.1016/j.gene.2013.09.101

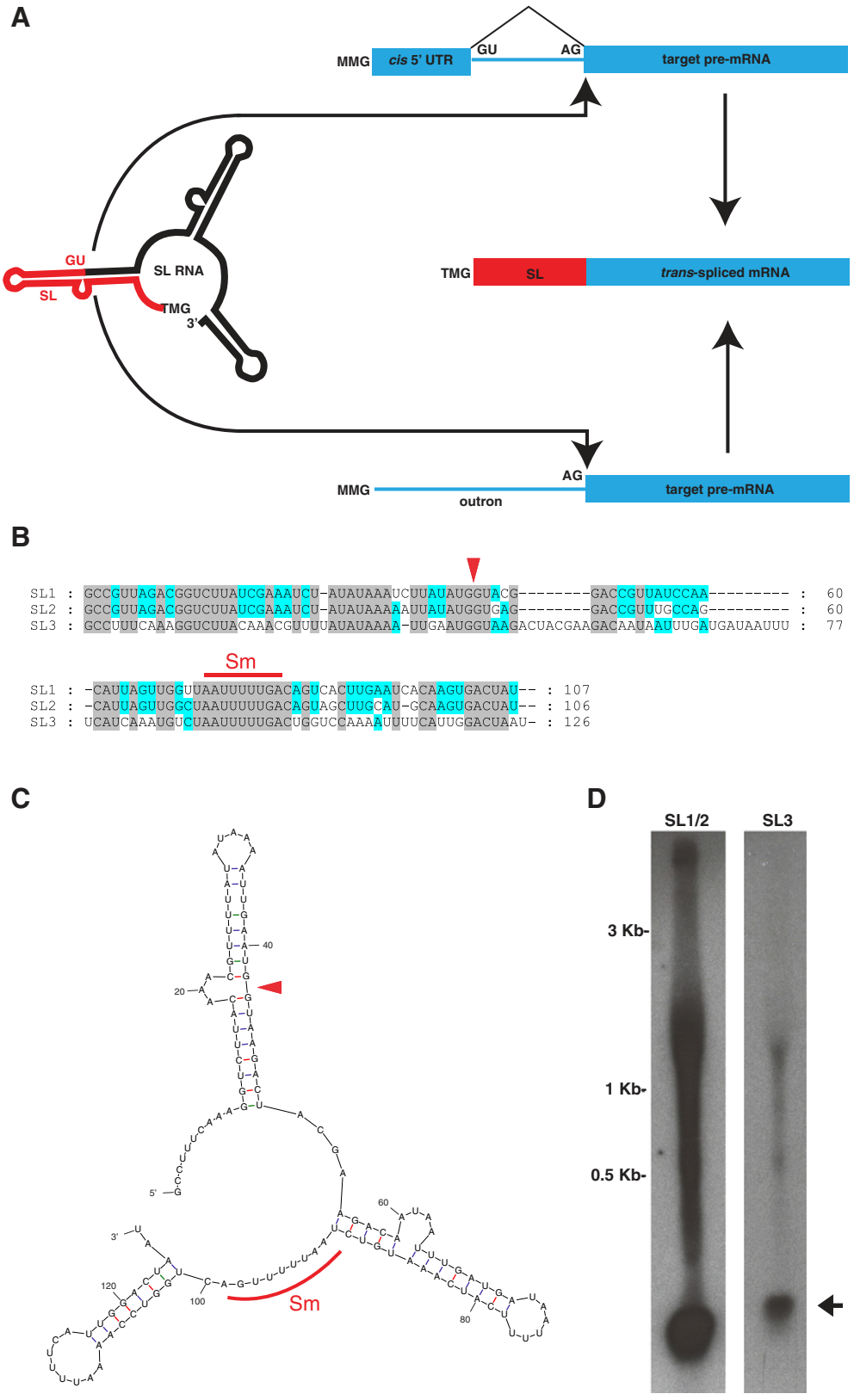

Fig. 1. Identification of the novel SL RNA SL3 in *S. mediterranea*. (A) Schematic representation of the SL *trans*-splicing reaction. The SL RNA is a small RNA containing a splicing donor site (GU) downstream of the spliced leader (in red). The donor site is engaged in a splicing reaction with the acceptor sites (AG) of target pre-mRNAs. In most of the metazoan examined to date the SL RNA is capped with a 2,2,7-trimethylguanosine (TMG) group. Consequently, mRNAs appended to the SL are also TMG-capped (Lasda and Blumenthal, 2011). Conversely, non *trans*-spliced transcripts possess a mono-methylated cap (MMG). In planarians, mRNAs competent for *trans*-splicing can also acquire a 5′ UTR in *cis* via splicing to an upstream exon or retention of an outron. (B) ClustalW alignment of the three known planarian SL RNAs. (C) MFOLD prediction of SL3 secondary structure. (D) Northern blot analysis of SL RNAs and *trans*-spliced mRNAs. The SL RNAs are indicated by the black arrow, while the rest of the signal corresponds to *trans*-spliced transcripts. (B–C) The spliced leader–intron boundary is indicated by the red arrowhead and the Sm protein binding site (consensus $RAU_{4-6}GR$) (Scofield and Lynch, 2008) is underlined in red.