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Research paper

A functional difference between native and horizontally acquired genes in bdelloid rotifers

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ABSTRACT

The form of RNA processing known as SL *trans*-splicing involves the transfer of a short conserved sequence, the spliced leader (SL), from a noncoding SL RNA to the 5′ ends of mRNA molecules. SL *trans*-splicing occurs in several animal taxa, including bdelloid rotifers (Rotifera, Bdelloidea). One striking feature of these aquatic microinvertebrates is the large proportion of foreign genes, i.e. those acquired by horizontal gene transfer from other organisms, in their genomes. However, whether such foreign genes behave similarly to native genes has not been tested in bdelloids or any other animal. We therefore used a combination of experimental and computational methods to examine whether transcripts of foreign genes in bdelloids were SL *trans*-spliced, like their native counterparts. We found that many foreign transcripts contain SLs, use similar splice acceptor sequences to native genes, and are able to undergo alternative *trans*-splicing. However, a significantly lower proportion of foreign mRNAs contains SL sequences than native transcripts. This demonstrates a novel functional difference between foreign and native genes in bdelloids and suggests that SL *trans*-splicing is not essential for the expression of foreign genes, but is acquired during their domestication.

1. Introduction

When an organism acquires a gene by horizontal gene transfer (HGT) from another species (Boto, 2010; Syvanen, 2012), particularly where that species is evolutionarily very distant, it is unlikely that the transcription machinery of the recipient will initially express the incoming DNA appropriately. Accordingly, a newly acquired 'foreign' gene might not benefit its new host and its sequence will decay or be lost. This problem seems especially acute for HGT between major phylogenetic groups, such as prokaryotes and metazoans. Nevertheless, despite this anticipated difficulty, examples of HGT from micro-organisms to animals are known (reviewed in Keeling and Palmer, 2008; Hotopp, 2011; Boto, 2014). For instance, sequences from the bacterial endosymbiont *Wolbachia* are present in the genomes of their nematode or arthropod hosts (Kondo et al., 2002; Hotopp et al., 2007), and fungal genes for carotenoid biosynthesis have been transferred to the pea aphid genome (Moran and Jarvik, 2010). In the latter case, carotenoid synthesis results in a red pigmentation that is proposed to confer a selective advantage.

Assessment of how foreign gene expression compares to that of what we term native or indigenous genes (i.e. those resulting from purely vertical descent) is impeded by the relatively low frequency of HGT in Metazoa as, in most animals, generally fewer than 1% genes in the genome result from HGT (Crisp et al., 2015). This is not the case, however, for bdelloid rotifers (Bdelloidea, Rotifera), a group of ubiquitous freshwater microinvertebrates in which HGT contributes significantly to genome composition, with approximately 8–14% transcripts estimated to be of foreign origin (Gladyshev et al., 2008; Boschetti et al., 2011, 2012, 2013; Flot et al., 2013, Eyres et al., 2015). Such high levels of HGT are probably partly attributable to the bdelloid's desiccation tolerance (Tunnacliffe and Lapinski, 2003): desiccation leads to leaky membranes (Crowe et al., 1984, 1992) and DNA breaks (Mattimore and Battista, 1996; Neumann et al., 2009), which could facilitate the uptake and incorporation of foreign DNA (Mattimore and Battista, 1996; Neumann et al., 2009; Hespeels et al., 2014).

One signature of bdelloid rotifer transcripts is a short, conserved spliced leader (SL) sequence at the 5′ end of a proportion of mRNAs (Pouchkina-Stantcheva and Tunnacliffe, 2005). In a process known as SL *trans*-splicing, the leader sequence is transferred from a separate, small SL-containing RNA after removal of the 'outron' at the 5′ end of

Abbreviations: SL, spliced leader; HGT, horizontal gene transfer; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; GSP, gene-specific primer.

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the pre-mRNA (Hastings, 2005; Lasda and Blumenthal, 2011). Thus, SL *trans*-splicing is analogous to the removal of introns from pre-mRNA by *cis*-splicing, which occurs in all eukaryotes (Wahl et al., 2009; Braunschweig et al., 2013), but instead takes place between different RNA molecules. Other organisms that perform SL *trans*-splicing on at least a proportion of their transcripts include the protists Euglenozoa (Murphy et al., 1986; Sutton and Boothroyd, 1986; Tessier et al., 1991) and Dinoflagellata (Zhang et al., 2007; Bachvaroff and Place, 2008); basal metazoans like some Porifera (Douris et al., 2010), Ctenophora (Derelle et al., 2010; Douris et al., 2010) and Cnidaria (Stover and Steele, 2001; Derelle et al., 2010); protostome metazoans such as chaetognaths (Marletaz et al., 2008; Marletaz and Le Parco, 2008), flatworms (Rajkovic et al., 1990; Davis et al., 1994) and nematodes (Krause and Hirsh, 1987); and some chordates including tunicates (Vandenberghe et al., 2001; Yuasa et al., 2002; Ganot et al., 2004; Satou et al., 2006). There is little conservation of SL sequences, even among closely related species (Davis, 1997; Nilsen, 2001), and therefore a particular SL sequence (or sequences) is characteristic of a species or taxonomic group. Although its function is still unclear, SL *trans*-splicing has been proposed to be involved in processing, regulation or translation of mRNA (Lasda and Blumenthal, 2011), but also in growth recovery in *Caenorhabiditis elegans* (Zaslaver et al., 2011) and in nutrient-dependent translational control in the chordate *Oikopleura dioica* (Danks et al., 2015).

Because there is little information in any eukaryote on whether foreign genes behave like native genes, we asked in this study whether foreign genes in bdelloid rotifers share with their native counterparts the functional characteristic of SL *trans*-splicing. We show that, indeed, transcripts arising from foreign genes can be *trans*-spliced in all five bdelloid species tested, but that this occurs in a significantly smaller proportion than in native mRNAs. This represents a novel functional difference between foreign and native genes in animals.

2. Materials and methods

2.1. RNA extraction and spliced leader-polymerase chain reaction (SL-PCR)

The bdelloid rotifer *Adineta ricciae* was grown and harvested as described previously (Boschetti et al., 2011; Szydlowski et al., 2015). Total RNA was isolated (NucleoSpin RNA II, Macherey-Nagel) from harvested rotifers and RNA purity and concentration measured with a NanoDrop spectrophotometer. We synthesized cDNA using either oligo-(dT) or random primers and SuperScript II reverse transcriptase (Invitrogen) for SL-PCR. An SL-specific forward primer (SL primer; GCTTATTACAACTTACCAAGAG) was used with gene-specific reverse primers (GSPs) for PCR (Q5 Hot Start High-Fidelity DNA Polymerase, New England Biolabs). Products were analysed by electrophoresis on 1% agarose gels, then cloned (using a Zero Blunt TOPO PCR Cloning Kit, Invitrogen) and dideoxy sequenced by EUROFINS (www.eurofins.com) or a facility at the Department of Biochemistry, University of Cambridge. Control experiments using both forward and reverse GSPs (primer sequences available on request) were performed to confirm the presence of each transcript in the mRNA population.

2.2. Rapid amplification of cDNA ends (RACE)

The 5′ ends of mRNAs were amplified according to the manufacturer's guidelines by RACE (Frohman et al., 2008) using a GeneRacer Kit with a SuperScript III module (both Invitrogen) and a reverse GSP specifically designed for each transcript. HeLa RNA and primers supplied with the kit were used as positive control. Q5 Hot Start High-Fidelity DNA Polymerase (New England BioLabs) was used to amplify RACE-ready cDNA.

2.3. Transcriptome analysis for A. ricciae and four Rotaria species

The splice leader sequence was aligned with blastn (Altschul et al., 1990; Camacho et al., 2009) to the transcriptomes of *A. ricciae* and four *Rotaria* species (Eyres et al., 2015). A transcript was considered to have a SL sequence if there was an alignment with a minimum length of 13 bp (AACTTACCAAGAG, the 3′ end of the bdelloid SL; Pouchkina-Stantcheva and Tunnacliffe, 2005) that begins within 10 bp of the start of the transcript. The transcripts were aligned to genes predicted from the draft *A. ricciae* genome using blastn (transcripts were considered to have originated from a gene if their alignment had an e-value ≤ $1\ e^{-10}$) to give the number/proportion of genes producing a transcript that is *trans*-spliced. The level of "foreignness" of a given transcript was determined using the HGT index (h_U), which we calculated as the difference in bitscores between best non-metazoan and best metazoan matches in blast alignments, according to Boschetti et al. (2012).

2.4. Statistics

Chi-square tests were performed using the online tool at http://in-silico.net/tools/statistics/chi2test.

3. Results

3.1. Foreign gene transcripts are SL trans-spliced in bdelloid rotifers

To begin to address possible functional differences between native and foreign genes and to determine whether and to what extent foreign gene expression involves SL *trans*-splicing, we examined the 5′ ends of transcripts from the bdelloid rotifer *Adineta ricciae* and four other bdelloid species. We initially randomly selected approximately equal numbers of native and foreign sequences from an *A. ricciae* transcriptome library (Boschetti et al., 2012) and experimentally tested for the presence of the bdelloid SL in corresponding mRNAs. First, we carried out SL-PCR on either oligo(dT)-primed or random-primed cDNA using a forward primer corresponding to the SL sequence and a gene-specific reverse primer, as previously described (Pouchkina-Stantcheva and Tunnacliffe, 2005); a second PCR for each target sequence using both forward and (the same) reverse gene-specific primers acted as an expression control. A

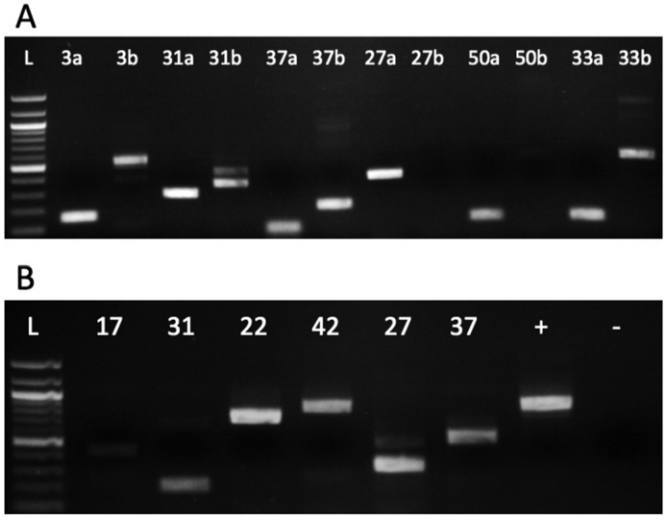

Fig. 1. PCR amplification of *A. ricciae* mRNA 5′-ends by (A) SL-PCR and (B) 5′-RACE. See Tables 1 and 2 for codes and accession numbers. (A) L: 100 bp ladder (New England BioLabs); for each sequence, lane 'a' represents the gene-specific PCR expression control, while lane 'b' shows the SL-PCR experiment. (B) L: 100 bp ladder; +: 900-bp positive control (manufacturer-supplied kit control; HeLa sequence); −: no template (negative control).