Splintrons in *Giardia intestinalis*

**Spliceosomal introns in a split form**

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The divergent eukaryotic unicellular organism *Giardia intestinalis* is an intestinal parasite in humans and various animals. An analysis of a draft genome sequence suggested that *G. intestinalis* has a much simpler genome organization and gene repertoire than those of other model eukaryotic organisms (e.g., Arabidopsis and human). This general picture of the *G. intestinalis* genome seemingly agrees with the fact that only four spliceosomal (cis-spliced) introns have been identified in this organism to date. We have recently shown that *G. intestinalis* possesses a unique gene expression system incorporating spliceosome-mediated trans-splicing. Some protein-coding genes in *G. intestinalis* are split into multiple pieces in the genome and each gene fragment is independently transcribed. Two particular pre-mRNAs directly interact with each other by forming an intermolecular-stem structure and are then trans-spliced into a mature mRNA by spliceosomes. We believe that this trans-splicing secondarily arose from the system that excises canonical (cis-splicing) introns. Based on these findings, we suspect that similar phenomena—split genes and post-transcriptional assembly of their transcripts via trans-splicing—may be prevalent in more distinct eukaryotic lineages than previously known, particularly in organisms possessing “intron-poor” genomes.

Splintrons in *Giardia intestinalis* are initially generated by transcription, the elimination of introns and concurrent joining of coding regions (exons) by splicing is required to produce mature translatable mRNAs (Fig. 1, left). In eukaryotic systems, intron splicing is mediated by a large molecular machines called spliceosomes composed of ribonucleoprotein particles and many other components.1 Introns excised by spliceosomes (so-called spliceosomal introns) are exclusively found in eukaryotes, and thus spliceosomal introns are hallmarks of eukaryotic nuclear genome organization. Nevertheless, some eukaryotes possess very few spliceosomal introns in their genomes as compared to well known model organisms (e.g., humans and Arabidopsis).2 The divergent eukaryotic intestinal protistan parasite *Giardia intestinalis* is one of such intron-poor species.3 In the *Giardia* draft genome, the genes encoding many spliceosomal components have been identified, but only four spliceosomal introns have been reported so far.3,4 Thus, *Giardia*, as well as other intron-poor organisms, is a key organism to understand the evolutionary dynamics of spliceosomal introns and spliceosomes in eukaryotes.

Recently, we reported a novel spliceosome-mediated trans-splicing system in *Giardia*.5 In the *Giardia* genome, two distant loci that are transcribed as two distinct polyA+ pre-mRNAs encode the N- and C-terminal amino acid sequences of HSP90. More interestingly, the non-coding regions of these pre-mRNAs possess stretches of nucleotides that are complementary to each other and thus have the...
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They form intermolecular stem structures between the split intron pieces (i.e., non-coding regions in two pre-mRNAs). It is very surprising that such an extremely distinct trans-splicing system has independently emerged on the branches leading to *C. elegans* and *Giardia*. Perhaps we should be searching for splintrons in other newly-characterized eukaryotic genomes, particularly in those that are “intron-poor” as Blumenthal has recently suggested.10

Here, as well as in our original article, we stress the significance of splintrons for understanding the evolution of spliceosomal introns. We argue that splintrons are unlikely to represent the ancestral form of spliceosomal introns. The latter in eukaryotic (nuclear) genomes were most likely derived from group II introns11 that were introduced into eukaryotes with the mitochondrial symbiont.12 The vast majority of group II introns are cis-spliced and it seems likely that they evolved directly into the cis-spliced spliceosomal introns in the nucleus of a common ancestor of all extant eukaryotes. In this view, all trans-splicing cases including splintrons in Giardia and *C. elegans* are secondarily derived from a cis-splicing ancestor.

**Figure 1.** Depiction of intron splicing processes in *Giardia*. Left: Cis-splicing of a canonical spliceosomal intron. A single, continuous pre-mature mRNA (pre-mRNA) including introns is transcribed from a single locus in a genome. An arrow indicates the transcription initiation site at the 5’ upstream region of exon 1. Canonical introns are then cis-spliced by spliceosomes. Right: Trans-splicing of a splintron. Two poly-A+ pre-mRNAs including a “left splintron piece” and a “right splintron piece” are independently transcribed from two distant loci in the genome (two different transcription initiation sites are highlighted by arrows). As the pre-mRNA forms an intermolecular stem structure, the pre-mRNA complex can be recognized as the substrate for spliceosomes.
Finally, although many have suggested that Giardia is a “deep-branching” eukaryotic lineage, it is widely thought that this position is due to the long-branch attraction artefact in phylogenetic tree reconstruction. Therefore we should be cautious to treat the significance of Giardia splintron to intron evolution separately from the “deep-branching” status of Giardia inferred by potentially biased analyses.

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