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RESEARCH HIGHLIGHT

Exo-endo *trans* splicing: a new way to link

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In addition to canonical *cis* splicing, which joins exons from a single pre-mRNA, various forms of *trans* splicing have been described, whereby two separate precursor transcripts are linked with each other. A new study by Hu *et al*. in *Cell Research* characterizes a novel and unusual splicing variation, called exo-endo *trans* splicing.

A recent study from Bo-Liang Li's group published in *Cell Research* [1] describes a novel type of *trans* splicing: an exogenous plasmid-derived RNA transcript is joined *in trans* with an endogenous mRNA, generating a chimeric mRNA that encodes a functional protein. Therefore, the authors have termed this unusual mRNA processing event exo-endo *trans* splicing. They describe and characterize in great detail this first example, in which a 56-kDa functional splice isoform of the acyl-CoA:cholesterol acyltransferase 1 (ACAT1) was produced, an enzyme making fatty-acid esters of cholesterol, which is physiologically important in cholesterol transport, metabolism and homeostasis.

Trans splicing was initially discovered in the protozoan parasite *Trypanosoma brucei*, in which a 39-nucleotide, non-coding "mini-exon" derived from the so-called Spliced Leader (SL) RNA is joined to the 5′ end of every protein-coding sequence [2, 3]. Thereby, *trans* splicing constitutes an essential step in gene expression in trypanosomes. This SL-type *trans* splicing also appears in a multitude of different organisms, including metazoans like Nematoda and Platyhelminthes, and mostly coexists together with the "normal" *cis* splicing (i.e., the accurate removal of non-coding intron sequences between the protein-coding exons of a pre-mRNA).

Even in *Drosophila* and mammals such as rats and humans, *trans* splicing has been shown to occur between independently transcribed pre-mRNAs [4-7]. In contrast to the canonical SL-type *trans* splicing, which is a prerequisite for generating all mature mRNAs in trypanosomes, different variations of *trans* splicing patterns have been described in higher eukaryotes (Figure 1): (1) intragenic *trans* splicing, in which two identical pre-mRNA molecules from the same gene are spliced together to generate an mRNA with duplicated exon sequences; (2) intergenic *trans* splicing, whereby an mRNA is generated from two pre-mRNAs that are derived from different genes, which can be located even on different chromosomes (interchromosomal *trans* splicing). A relatively well-characterized example for the latter case is the human *ACAT1* gene, as previously described by Bo-Liang Li and colleagues [8]; importantly, characterization of *trans* splicing has not only been performed at the mRNA level, as in many other studies, but also been extended to the protein level and the effects on biochemical activities. In this case, exons of the *ACAT1* mRNA are transcribed from two different chromosomes, namely 1 and 7, and the resulting 4.3-kb human *ACAT1* mRNA encodes two functional protein isoforms (50- and 56-kDa in size) with differential enzymatic activities [8].

Now, Hu *et al*. [1] continued their molecular analyses of *ACAT1 trans* splicing *en detail* by combining the characterization of both mRNA and protein products of *ACAT1*. They reveal a new form of *trans* splicing of the *ACAT1* mRNA: an antisense transcript derived from an exogenous ampicillin resistance gene (Amp^r) is joined with the endogenous *ACAT1* transcript, generating one of the ACAT protein isoforms. This 56-kDa isoform includes additional 46 amino acids at its amino-terminus, which is encoded by the antisense strand of Amp^r. By using mutational analysis, Hu *et al*. [1] demonstrate that the antisense transcript of Amp^r that is attached to the endogenous *ACAT1* mRNA by *trans* splicing provides the real start codon of the ACAT1 56-kDa isoform, which is different from the start codon previously proposed [9].

What is the significance of *trans* splicing of an antisense transcript in human cells? Surprisingly, Hu *et al*. [1] detected the DNA sequences of Amp^r with an upstream cryptic promoter and antisense transcripts of Amp^r in human blood cells and a diverse set of human cell lines by PCR, and also in a variety of higher eukaryotes by computational analyses, suggesting that such endo-exo *trans* splicing events may be more common than expected. Moreover, using both intact cells and an *in vitro* splicing system, Hu *et al*. [1] demonstrated that the exo-endo *trans* splicing can occur in different backgrounds of transfected constructs. Finally, they provide evidence based on mutational analysis that the exo-endo *trans* splicing utilizes the normal spliceosome machinery, as standard splice-site signals are required.

Previously the same group has demonstrated that the 4.3-kb ACAT1 mRNA is produced through interchromosomal *trans* splicing [8]. Now they

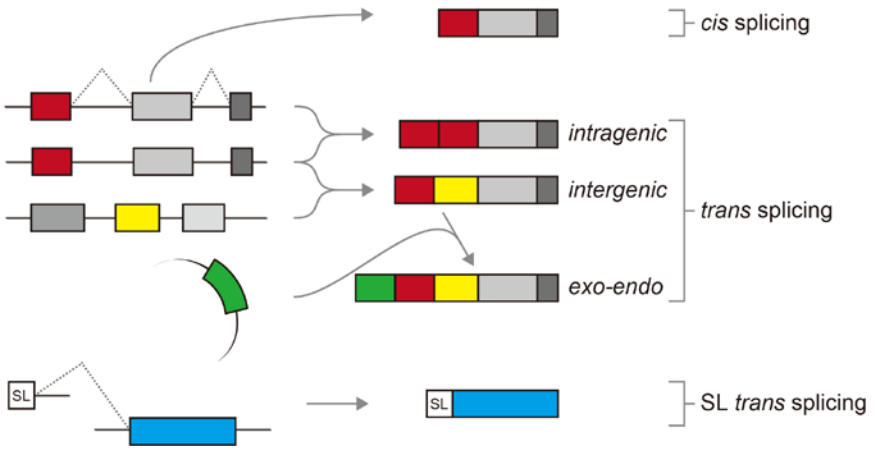

Figure 1 Alternative splicing modes in eukaryotes. Different types of pre-mRNA splicing in eukaryotes are schematically represented: canonical *cis* splicing, three types of *trans* splicing, and the SL-type *trans* splicing. Exons including the SL RNA-derived mini-exon (SL) are represented by boxes, and introns as lines.

show that the chimeric mRNA derived from chromosomes 1 and 7 is required for the exo-endo *trans* splicing event (Figure 1). This is the first report in the mammalian system, in which a functional protein is generated through *trans* splicing of an exogenous transcript and an endogenous cellular RNA. It remains to be investigated how common and widespread this phenomenon is in mammalian gene expression, but it may, in combination with the "normal" alternative splicing patterns, have the potential to further increase the proteome complexity. Moreover, in this special case, this phenomenon might help to explain how plasmid-derived DNA sequences, in particular antibiotic resistance genes, integrate into eukaryotic genomes and utilize the cellular mRNA-processing machinery to modulate protein function.

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