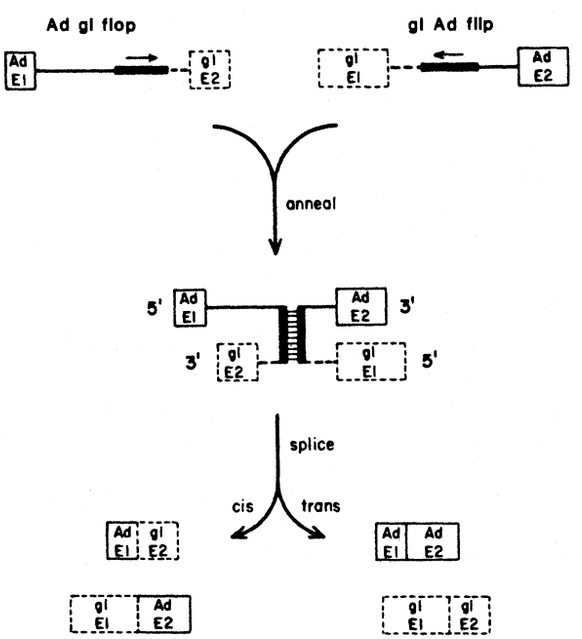
Making mRNA from a Pair of Precursors

Most protein-coding genes of eukaryotes and some prokaryotes contain intervening sequences, or introns, which are removed in the formation of mature messenger RNA (mRNA). The mechanism by which the introns are removed and the coding regions, or exons, are accurately spliced together in the correct order remains obscure, although great strides have been made since the development 2 years ago of in vitro splicing systems (*1*). One thing that had seemed clear, however, is that for the most part the splicing process seems to produce a single mature messenger molecule from one precursor molecule (pre-mRNA); in other words, it appeared to be an intramolecular reaction. It therefore comes as something of a surprise to learn that under appropriate experimental conditions the splicing reaction can readily link together exons from two separate precursor molecules (*2*).

Phillip Sharp and his colleagues Richard Padgett and Maria Konarska, of the Massachusetts Institute of Technology, constructed two artificial messenger precursors, one of which contained the L1 exon of adenovirus and the other the L2 exon. Under optimum experimental conditions, the two exons were spliced together at about 15 percent of the rate expected from a single, intact precursor. In an independent set of experiments, David Solnick, of Yale University, made chimeric precursors, one of which comprised the adenovirus exon adE1 linked via an intron to a globin exon glE2, while the other was the reciprocal, having adE2 linked to glE1. Intermolecular, or *trans*, splicing in this case proceeded at a rate comparable to intramolecular, or *cis*, splicing. In both the MIT and Yale studies, the efficiency of *trans* splicing depended greatly on the pair of precursor molecules being bound together through base-pairing within the introns, which the researchers achieved by the insertion of suitable complementary sequences (see diagram). Sharp and his colleagues also report *trans* splicing in the apparent absence of base-pairing between the two precursors, albeit at an extremely low level.

It has been known for some time that there is no inherent barrier preventing the splicing together of normally unassociated exons. For instance, Sharp and his colleagues showed that a chimeric pre-mRNA, made from exons from SV40 and mouse globin, could be spliced normally. The shuffling of exons in the formation of immunoglobulins is a natural experiment of the same sort, albeit less exotic.



Chimeric mRNA precursors

Chimeric precursors contain complementary sequences in the intron (straight arrows). Following annealing and incubation in a splicing system, four spliced products are possible. The trans *product Ad/Ad was slightly favored over the* cis *product gl/Ad; the other two were made in small amounts.* [*Source:* Cell ***42****, 157 (1985)*]

What, then, is there to prevent exons on different precursors being accurately spliced, providing they could be brought together? The answer, it seems, is—Nothing.

But, is there any evidence to suggest that such *trans* splicing might occur in vivo? Yes, there is. Every mRNA in the trypanosome, a blood parasite, has a short (35 nucleotide) sequence at its 5′ end that is not encoded in the gene that produces the message. One possible explanation is that the 35-nucleotide addition is the result of *trans* splicing, although this remains to be proved. Other examples are elusive as yet, perhaps because they don't exist or because, being unexpected, no one has looked hard enough. Both the MIT and Yale groups point out the potential flexibility in mRNA production that *trans* splicing would endow upon a cell.

If natural *trans* splicing requires the formation of duplexes, as in these experiments, then there is no shortage of potential candidates. A healthy number of genes are known in which some introns contain repetitive elements, which could, theoretically, bring two pre-mRNAs together through base-pairing. But, as both research groups comment, base-pairing may not be necessary for the close alignment of two pre-mRNAs; mutual binding of a polypeptide, perhaps as part of a splicing complex, could do the trick.

In any case, the demonstration of experimental *trans* splicing does focus attention on the way in which opposite ends of the same intron are brought together during the natural splicing process. One idea has been that the splicing machinery "reads" the sequence as the pre-mRNA threads through it, picking up at the beginning and end consensus sequences. This base-scanning idea has been slowly losing popularity for a number of reasons, and the fact that the splicing machinery can apparently circumvent a substantial encumbrance of secondary structure, as demonstrated here, adds further doubt to the scheme. Solnick suggests that scanning may instead be along the phosphodiester backbone or along an RNA-protein complex, which might be formed as part of a large particle. Such scanning would probably be able to jump over the base of a stem that is formed when complementary sequences form a duplex, says Solnick.

—ROGER LEWIN

References

1. R. Lewin, *Science* **228**, 977 (1985); *ibid.* **225**, 301 (1984).
2. D. Solnick, *Cell* **42**, 157 (1985); M. M. Konarska *et.al.*, *ibid* **42**, 165 (1985).