# More Progress in Messenger RNA Splicing

This summer marks 8 years since eukaryotic genes were first discovered to be interrupted by noncoding sequences, known variously as intervening sequences or introns. The discovery raised two sets of questions. The first concerns the origin and function—if any—of introns, which, by its very nature, is a very difficult question to test and therefore remains somewhat in the realms of speculation, although significant insights are being made (*1*). The second focuses on the mechanics of removal of these sequences in the production of mature RNA molecules, and in principle should be experimentally more tractable. The immense effort directed at this second question has produced during the past 8 years some conventional biochemistry, some novel and surprising nucleic acid chemistry, and a great deal of frustration.

For instance, the removal of introns from transfer RNA (tRNA) molecules turns out to be the result of straightforward enzymology: the coding sequences, or exons, fold in such a way that nuclease and ligase enzymes can by turn snip out the intron and precisely join the exon ends. The big surprise in RNA processing was the discovery that in some classes of introns the excision and ligation process is catalyzed by the intron itself (*2*). In this case, which includes examples of ribosomal RNA (rRNA) and mitochondrial messenger RNA (mRNA) in certain "lower" organisms, the intron folds the molecule into a favorable configuration for bringing the exon ends together and catalyzes the concerted reaction.

The major frustration in RNA processing work has been in trying to understand how precursors of nuclear mRNA molecules are edited and spliced. Significant advances began to be achieved in this area only a year ago, following the development of until then elusive in vitro splicing systems. Two laboratories, at Harvard and the Massachusetts Institute of Technology, identified important intermediates in the splicing process (*3*). Now, three laboratories are independently beginning to home in on the machinery that carries out the pre-messenger splicing reaction. Data from one of them, that of John Abelson and Edward Brody, at the California Institute of Technology, are reported on page 963 of this issue.

Compared with the other two classes of RNA splicing, the processing of mRNA precursors appears to require a very large structure, which presumably contains several protein and RNA molecules. The splicing complex, which Abelson and Brody call a "spliceosome," sediments at 40*S* when prepared from yeast cells. Phillip Sharp and his colleagues at MIT identify a similar, but larger, complex from mammalian cells, which sediments at 60*S*. Michael Green and Albercht Bindereif, at Harvard, also working with a mammalian system, put the size somewhere between 40*S* and 60*S*. For comparison, a 30*S* ribosomal subunit contains 21 protein molecules and an RNA of some 1500 nucleotides.

Compared with introns in precursors of tRNA, rRNA and mitochondrial messengers, those in nuclear pre-mRNA can reach extravagent lengths: they range from 50 to 20,000 bases. And some genes contain as many as 50 or more introns. The task of the splicing machinery, therefore, is to excise the introns with absolute precision and to link the remaining exons in the correct order. The identification of putative spliceosomes does not as yet address this central problem. Indeed, aside from demonstrating that known splicing intermediates are associated with the complexes, none of the laboratories has yet definitively identified any other component. The nucleus does have something of a potpourri of ribonucleoprotein particles, some of which are large (the heterogenous ribonucleoprotein particles, hnRNP's) and some small (the small nuclear ribonucleoprotein particles, snRNP's), and either or both of which could be part of the spliceosome. It is a fairly good bet, however, that one of the components in the mammalian complex will be U1 RNA, which is one of the many ubiquitous small nuclear RNA's and has been shown to be intimately associated with the splicing of mRNA precursors. In addition, U1 RNA has been shown to bind to 5′ (but not 3′) splice sites. An equivalent RNA is likely to be involved pre-mRNA splicing in yeast.

How much of a discrete entity the spliceosome really is remains to be seen. So far there is no firm evidence to indicate whether the complex is preformed, and merely takes on a mRNA precursor when available, or comes together from a collection of subunits. Abelson and Brody suspect, however, that the spliceosome components associate and disassociate in functional cycles, with one of the splicing intermediates serving as a key signal.

Splicing proceeds in two steps, producing some surprising intermediates. The first involves the cleavage of the 5′ site, whereupon the intron folds back on itself to form a covalently bonded lariat structure. In the second the first exon comes together with the second, the two are ligated and the lariat lost. All three research groups find that mRNA precursor is associated with the putative spliceosome, only when splicing is functional: mutations that block splicing tend to block the association. Moreover, the products of the first and second splicing reactions are more or less tightly bound with the complex, with the exception of the mature mRNA, which appears to be rapidly released. Abelson and Brody speculate that the intron in lariat form holds the spliceosome together, and its debranching "functions as a signal to recycle the 'spliceosome' components for a new round of pre-mRNA splicing."

If the proposed existence of some kind of spliceosome withstands further scrutiny—and each laboratory acknowledges the possibility that it might be dealing with some kind of artifact—then the prospect of unraveling its biochemistry is little short of formidable. Identification of the individual components will of course be an important task and will include the search for enzymes that do the cut and paste jobs. It is possible of course that no such enzymes will be found, that the spliceosome represents a gigantic construction within which the ancient process of RNA catalysis will once again be discovered. Some insight into RNA catalysis in messenger processing is expected to emerge within the next few months.—**ROGER LEWIN**

**References**


1. W. Gilbert, *Science* **228**, 823 (1985).
2. T.R. Cech, *Cell* **34**, 713 (1983).
3. W. Keller, *ibid.* **39**, 423 (1984).