RNA Catalysis Gives Fresh Perspective on the Origin of Life

The old chicken-and-egg problem of the origin of life is illuminated in unexpected ways by recent results on the splicing of RNA precursors

UNTIL very recently RNA was generally considered something of a country cousin in the world of molecular biology, a passive conveyor of information stored in the more glamorous DNA. The demonstration within the past couple of years that in two clear instances RNA can have catalytic activity has, however, begun to change its image. And new data just reported make it obvious that these were not mere isolated curiosities, that RNA catalysis might be quite widespread in certain systems, and that RNA has the capacity to replicate itself in the absence of protein enzymes.

For those who like to speculate about the beginnings of life, these new results strongly support the hypothesis that RNA, not DNA, could have been the start of it all.

The new data all come from the realm of RNA splicing, that is the removal of intervening sequences, or introns, from the precursors of various RNA molecules, including transfer RNA, messenger RNA, and ribosomal RNA. One set of data, reported in last week's *Science*, demonstrates that a self-spliced intron from *Tetrahymena thermophila* has the capacity to work as a true biosynthetic enzyme in vitro, specifically in building new RNA molecules based on a template. And the second set of data, published in several papers in the current issue of *Cell*, shows that the ability of introns to self-splice—that is, to edit themselves out of the precursor molecule in the absence of proteins—is more widespread than suspected.

RNA splicing has been the focus of a tremendous research effort in the 9 years since the phenomenon was discovered. Progress was slow initially, mainly because no one could crack the problem of getting good in vitro systems. When that was eventually done, the picture began to clear, showing, apparently, several discrete categories of the phenomenon.

First was the splicing of transfer RNA precursors, the enzyme mechanics of which are now largely known. The second category, first described from fungal mitochondria, is distinguished by a series of conserved sequences within the intron, which are important in folding the molecule during splicing. These are known as group I introns. Third is a set of introns, again first described from fungal mitochondria, that have different conserved sequences and are known as group II introns. Last are introns in RNA precursors formed in nuclei.

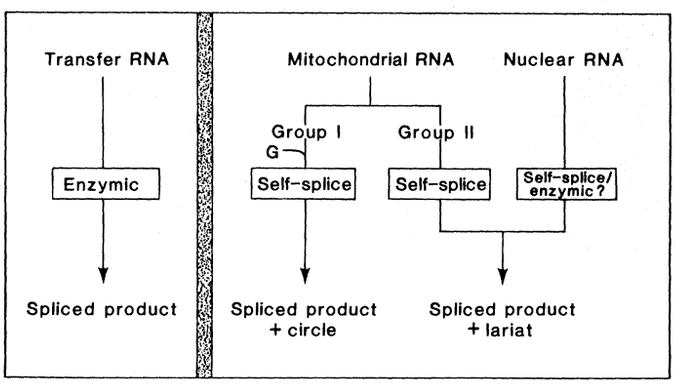
Although these last three categories are somewhat arbitrary, with more and more examples appearing that cross the lines, they appeared to represent three different splicing mechanisms, probably reflecting three different evolutionary origins. The new data show, however, that they are closely related to one another, probably with a single origin.

Self-splicing was first discovered 3 years ago in a group I intron, specifically in a ribosomal RNA precursor from *Tetrahymena*. Thomas Cech and his colleagues at the University of Colorado showed that the 414-nucleotide-long intron snipped itself out of the precursor, and spliced the two loose ends to form the mature molecule. The two-step process occurred in the absence both of proteins and an external source of energy, such as ATP, but required a guanosine molecule to initiate it. The subsequent discovery of similar self-splicing in other group I introns, many of which release the excised intron as a circle, confirms that the process was not simply a molecular fossil.

Work on splicing in nuclear RNA precursors, meanwhile, shows that excision of introns involves the formation, not of a circle, but of a lariat. And splicing in this system seems to require the presence of several protein molecules and some small RNA's, known as U1 and U2 RNA. The discovery seemed to emphasize the difference between splicing in nuclear RNA and in group I introns, where circle formation is common and protein is not absolutely required. Group II introns, like group I introns, also appeared to be removed as circles.

It turns out, however, that the group II introns represent a "missing link" between group I introns and those in nuclear RNA precursors, as Cech recently described it. Not only do group II introns self-splice (like group I), but they are also excised as lariats (like nuclear introns), not circles. The conserved sequences at the splice sites of group II and nuclear introns are also rather similar. These results, reported by Craig Peebles, Phillip Perlman, and their colleagues at the University of Pittsburgh and Ohio State University, and by A. Van der Veen and his colleagues in Amsterdam, demonstrate that self-splicing is even more widespread than known previously. Moreover, they raise the tantalizing possibility that splicing in nuclear introns might also be catalyzed by RNA.

It is possible, for instance, that nuclear introns contain a catalytic center, just as group I and group II introns do. Or the catalysis might be carried out by the small RNA's—U1 and U2—that are necessary for splicing. The numerous protein molecules that also appear to be required for splicing in this system might be performing a structural role, ensuring that the introns fold in the correct way for correct cleavage and ligation. Splicing in group I and II introns is



Intron types
Splicing in transfer RNA introns is distinctly different from those in group I, group II, and nuclear introns.

enhanced by the presence of protein in vivo, presumably for this same reason.

The emerging commonalities between these three different types of intron are strengthened by the further discovery, reported by A. C. Arnberg and colleagues in the same issue of *Cell*, that a group I intron in a ribosomal RNA precursor in yeast mitochondria is excised as a lariat, not a circle. Chances are, therefore, that all these introns have a rather close evolutionary relationship.

It is perhaps appropriate that the *Tetrahymena* intron, which was the first to show self-splicing in vitro, should also be the first RNA molecule to show true biosynthetic activity. Once the intron has excised itself from the precursor, it nibbles off the first 19 nucleotides from its 5′ end, employing the same chemistry that effects the original cleavage and ligation. The reaction then stops. Cech and his colleagues decided to see if this foreshortened molecule still had catalytic activity, by adding a short RNA (five cytidylic acids joined together) to the reaction mixture.

Sure enough, the intron continued to perform the splicing-type reactions, which resulted in the shortening of some of the poly C molecules and the lengthening of others, some of which reached 30 nucleotides in length. The reaction involves the binding of the poly C substrate to a conserved sequence in the intron, which, in the splicing reaction, recognizes the 5′ splice site. This sequence acts as an internal template for the polymerization reaction. Cech and his colleagues speculate on the biosynthetic possibilities that might emerge if the sequence of the internal template is modified or is even separated from the catalytic molecule entirely.

Not only can one see how primordial RNA could have acted as a replicase, making copies of itself and other RNA species, in the complete absence of protein enzymes, but it is also tempting to consider the utility of suitably engineered RNA molecules in modern biotechnology. Cech points out that, although there are batteries of protein enzymes for manipulating DNA, there are very few for RNA. RNA enzymes may, therefore, fill this gap. ■ **ROGER LEWIN**

ADDITIONAL READING

C. L. Peebles *et al.*, "A self-splicing RNA excises an intron lariat," *Cell* **44**, 213 (1986).

A. Van der Veen *et al.*, "Excised group II introns in yeast mitochondria are lariats and can be formed by self-splicing in vitro," *ibid.*, p. 225.

A. C. Arnberg *et al.*, "Formation of lariats and circles in self-splicing of the precursor to the large ribosomal RNA of yeast mitochondria," *ibid.*, p. 235.

A. J. Zaug and T. R. Cech, "The intervening sequence RNA of *Tetrahymena* is an enzyme," *Science* **231**, 470 (1986).

Wisconsin Storage Ring Reaches 120 Milliamps

After long delays, the "1-GeV ring" now generates respectable VUV-XUV intensities, but continued funding is uncertain

EARLY last month, accelerator physicists at the University of Wisconsin's Synchrotron Radiation Center (SRC) in Stoughton for the first time were able to store and accelerate to full energy a beam current in excess of 100 milliamperes in its "1-GeV ring." The latest breakthrough caps a year of steady progress in commissioning the problem-plagued machine, once known as Aladdin and designed to be a high-brightness source of vacuum-ultraviolet and soft x-ray radiation for users throughout the United States.

While the recently achieved beam current can generate more than enough light to keep a flock of waiting users happy, the success may have come too late. Despite signs of progress evident last June, after years of delays and disturbed by studies concluding that even the expenditure of some $25 million for a proposed upgrade project could not ensure that the machine would meet its specs, the National Science Foundation pulled the plug on Aladdin. NSF continues to support the operation of Tantalus, an older, smaller, lower energy, but highly reliable VUV synchrotron source at SRC.

The university then took the bold step of spending its own money to operate Aladdin for a maximum of 9 months, beginning last October. The machine's performance had already improved enough by then to make it superior to Tantalus in light output and spectral range. Moreover, Tantalus was oversubscribed with about twice as many hours of beam time requested as could be accommodated. Since Aladdin is physically larger, it has room for more beam lines and consequently can service more experimenters. The hope was that the upward climb in light output would convince NSF or some other federal agency to fund the new machine as an alternative to the older one, even if the current remained well below the original design specification of several hundred milliamperes.

Last September, the university submitted a proposal requesting the switch to NSF, which is in the process of assembling a reviewing team to make a recommendation. According to SRC director David Huber, the proposal comes to about $3.5 million per year for 3 years, a little more than twice what it costs to operate Tantalus. During this period, six of the eight beam lines from the older machine would be moved over and several new ones added. Together with the ones that are in place now and taking data, these would make a total of 17 at the new facility.

One notable feature of the proposal is the total absence of the name Aladdin, now called simply the 1-GeV ring. As far as NSF is concerned, Aladdin was a project that aimed at an electron storage ring with a beam current of at least 500 milliamperes and did not succeed.

Particular concerns of the NSF reviewing team, according to Lewis Nosanow, director of NSF's materials research division, will include documentation of the need for the 1-GeV ring and assurance that no major extra expenditures are waiting in the wings. Although Nosanow mentioned no specific alternative, moving some experimenters into the rapidly saturating National Synchrotron Light Source at Brookhaven National Laboratory might relieve the pressure for a while. Then the third-generation Advanced Light Source proposed by the Lawrence Berkeley Laboratory, if approved, could be ready in about 5 years.

As for the 1-GeV ring, no formal decision to proceed can be expected from NSF until this May because the amount of money requested requires National Science Board concurrence. A negative decision would force Wisconsin to approach other agencies, such as the Departments of Energy or Defense, says Huber.

Until now, the main technical problem with Aladdin has been the inability to accumulate a large beam current. A small accelerator called a racetrack microtron was to boost pulses of electrons to 0.1 GeV and then squirt them into Aladdin, where, after enough pulses had been accumulated, the electrons were to be accelerated to the final operating energy. Although the first beam was stored in November 1981, by the fall of 1984 the largest current that could be accelerated was only 2.8 milliamperes. Not only