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PERSPECTIVE

A major leap toward the tertiary structure of large RNAs

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RNA is more than just a DNA with an additional 2′ hydroxyl group. Two specific features characterize this wonderful molecule: its capacity to form stable and complex tertiary structures, and its ability to catalyze chemical reactions, similar to an enzyme. Group I self-splicing introns, the first RNAs shown to combine both properties, have been at the heart of a biological revolution (Cech, 1990). Understanding the molecular details behind the structure and catalytic mechanism of this molecule at the atomic level has been a challenge for the past years. The recent crystal structure determination of the P4-P6 domain of the *Tetrahymena thermophila* intron represents a major leap in our understanding of the folding of large RNAs (Cate et al., 1996a, 1996b).

For more than 20 years, our knowledge of RNA molecules at the atomic level was based on the crystal structures of transfer RNAs (Kim et al., 1974; Robertus et al., 1974) and a few additional small oligonucleotides, either alone, as double-stranded helices, or as single-stranded RNAs complexed with proteins (Dock-Brégeon et al., 1988). Until the early 1970s, nucleic acid structures were limited to two-dimensional helices of various forms. With tRNA, we entered the three-dimensional world; this "honorary protein," as it was then termed, provided the initial building blocks (stem, loop) and the first visualisation of long-distance tertiary interactions. More recently, the crystal structure of the hammerhead ribozymes allowed us not only to visualize another medium-size RNA molecule, but also for the first time to get the catalytic mechanism of an RNA enzyme (Pley et al., 1994; Scott et al., 1995). At this point, the rules governing the structural assembly of the oligonucleotides were close enough to those of the tRNA structure.

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The difficulty in crystallizing large RNAs stimulated alternative approaches toward the structure determination. The most successful one was modeling based on three types of experimental information. The first innovative contribution was the use of phylogenetic data, which revealed covariation of bases and long-range interactions between predicted secondary structures. The resulting distance limitations impose useful constraints in the modeling process, much as NOE-derived distances do in NMR-determined structure. A more limited, but sometimes very efficient, source of information was the use of spectroscopic methods, such as transfer of fluorescence (FRET), which provides additional soft distance constraints. A third avenue constituted the analysis of crosslinking and chemical protections toward various reagents; these data, when properly interpreted, yield a large amount of structural information and correlate very well with the two previous approaches. Several significant contributions are based on these methods: the model of the group I introns core domain (Michel & Westhof, 1990), the modeling of *Escherichia coli* RNase P (Harris et al., 1994), and that of the hammerhead ribozyme (Tuschl et al., 1994).

The beauty of crystallography, however, is that it provides a direct visualization of the object under investigation. When the resolution of the diffraction data permits, the crystal structure makes available a wealth of information unbeatable by any other experimental approach. The resulting data go far beyond the rational organization of experimental results usually included in a good model; indeed, they often reveal new folds and/or unexpected mechanisms of molecular recognition as designed by nature. What is fascinating about the present work on group I introns is not only the results, but also the rationale behind the experimental investigation. The concept of building blocks having been accepted, the next step was to realize that large RNAs could form independent domains of smaller size (about two times the tRNA molecule), much as

large proteins fold into small globular domains that can be isolated. Solution studies, including crosslinking and chemical protections, were essential to characterize these domains. The analogy with the structural investigation of large proteins can go even further. The complete intron containing the functional catalytic center being too large a leap, the study was pursued stepwise with the isolation and crystallization of the two domains that together form the catalytic intron. The first domain from which good diffracting crystals were obtained was the domain P4-P6 (Doudna et al., 1993). A more classical skillful crystallographic analysis led to the high-quality electron density map, which could be interpreted in the beautiful model shown in Figure 1.

Most of the interesting features revealed by this crystal structure have been reported extensively in two *Science* papers, as well as many short reviews published or to come. A thorough analysis of these results goes beyond the scope of this note. I will only mention few remarkable features, such as the adenosine-rich corkscrew bridging the minor grove of the P4 helix and the 3-helix junction P5a, P5b, and P5c, or the GAAA tetraloop locked to its tetraloop receptor in helix P6. A new motif responsible for mediating intra- and intermolecular interactions was discovered: adenosine platforms, whose distinctive chemical modification signature make them detectable in other RNA structures. The location of metal ions (Cate & Doudna, 1996), as well as the analysis of intermolecular interactions

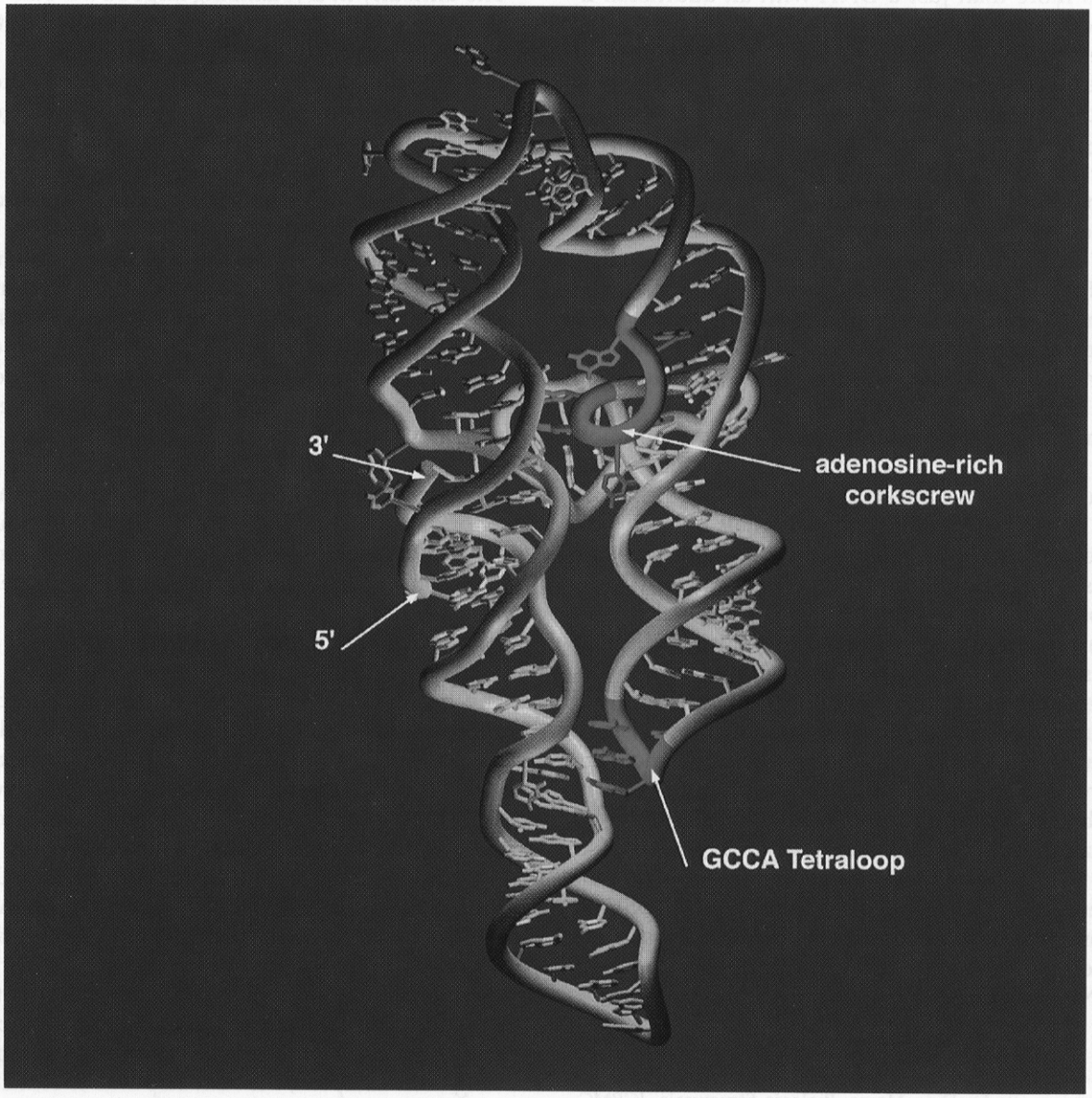

FIGURE 1. Three-dimensional model of P4-P6 domain of the group I intron drawn by L. Moulinier with the program Setor. Atomic coordinates were kindly provided by T. Cech.

through crystal packing, provide additional information for fans of RNA structures. An important task of correlating this with other known structures will also bring useful data. Nevertheless, the most exciting perspective will certainly be the structure determination of the second domain of the group I intron. The docking of these two structures that are known to be functional in solution should then enlighten our understanding of the catalytic mechanism—a long-awaited dream.

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