

FOREWORD

Twenty-Five Years of Ribozymes

Twenty-five years have passed since the discovery of the first ribozyme! It hardly seems possible. In 1982, when we announced that the pre-ribosomal RNA intron from the ciliated protozoan *Tetrahymena* was a self-splicing RNA with enzyme-like properties, it required a bit of daring to speak of catalytic RNA. And we really stuck our necks out when we coined the word “ribozyme” to describe the universe of ribonucleic acid molecules with enzyme-like activities, because at that time this universe consisted of just the one example.¹ Now, the field is so well-established that several books, including this one, have been dedicated to the topic. And high school and college textbooks have included sections on ribozymes for so long that the students think they’ve been known “forever”.

Of course, had there been just the one example, the ribozyme would have remained a curiosity, not a field. The first addition to the list was RNase P, an endonuclease that produces the mature 5′ end of transfer RNAs in all organisms.^{2,3} This was a key addition, especially because the RNA acted naturally as a true enzyme, processing a limitless number of substrate RNAs without being altered in the process. It later turned out that ribozymes including the self-splicing *Tetrahymena* intron, self-splicing group II introns, and the self-cleaving hammerhead and hairpin ribozymes could be easily engineered to be trans-acting, *i.e.*, to cleave exogenous substrates with multiple turnover.^{4–8} Thus, the distinction between a self-acting ribozyme and a true enzyme remains biologically significant, but the distinction is not so important when considering the chemical and mechanistic features of transition state stabilization in the active site.

At the same time that these boutique catalytic RNAs were being studied, Harry Noller was steadfastly claiming that the central ribonucleoprotein particle of life, the ribosome, was an RNA machine.^{9,10} His elegant biochemical experiments and the compelling evolutionary argument for an ancestral RNA-based protein synthetic apparatus^{11–13} convinced many of us, but it was nevertheless thrilling to see directly the RNA forming the peptidyltransferase center¹⁴ and binding a transition-state analog.¹⁵ Not only is the ribosome the most important ribozyme, but it also serves as a rich model to understand the contributions of protein components of RNP enzymes.

Many details of the chemistry, biology, and structure of ribozymes have by now been revealed, thanks to the intense international effort documented in this volume. Thus, it is with some apprehension that I look back on what we initially predicted about RNA catalysis in 1982. One prediction of general interest concerned nuclear pre-mRNA splicing, which was still two years away from being recapitulated *in vitro*.^{16,17} We suggested that “while mRNA precursors are unlikely to be self-splicing, it remains possible that they undergo such a reaction when complexed with small nuclear RNPs. If the RNA moiety of the small nuclear RNP bound a nucleotide cofactor or participated in catalysis in any way, it would be a ribozyme.”¹ The finding that group II introns underwent self-splicing by the same lariat-formation reaction as pre-mRNA introns provided support for this contention,^{18–20} which is now widely accepted but not yet definitive.²¹

Another conjecture was that “the autoexcision of the IVS may be reversible,” the reverse reaction consisting of “integration of the IVS into another RNA molecule” that might then provide a retrotransposition pathway “for introduction of an IVS into a gene that was formerly contiguous.”¹ [IVS, intervening sequence, is synonymous with “intron.”] It was later possible to demonstrate such activity for group I and group II introns.^{22,23} Even more exciting was the finding that group II introns directly insert themselves into double-stranded DNA, a reaction that may provide a new tool for *in vivo* mutagenesis and potentially for gene therapy.^{24,25}

The last general prediction was that “single-stranded DNA might, under some conditions, be self-splicing. Such an event might take place when the DNA strands were separated for replication, and, if it could be regulated, might provide a mechanism for DNA rearrangements during cellular differentiation.”¹ In fact, *in vitro* selection was later used to identify catalytic single-stranded DNAs with RNA-cleavage activity, dubbed “DNAzymes” or “deoxyribozymes”.²⁶ However, the speculation about a biological role for DNA catalysis has yet to be substantiated.

Finally, we made specific proposals that the IVS RNA would have several enzyme-like properties.¹

- “It lowers the activation energy for specific bond cleavage and formation events.” This was a safe prediction, because it was just restating our observations in chemical terms! However, in this first paper we completely missed the role of metal ion catalysis, which subsequently became a significant research area.^{27–30}
- “Its activity depends on a precise structure.” After an immense effort, the crystal structure of a domain of the *Tetrahymena* ribozyme was solved,³¹ and then an entire active ribozyme at modest resolution,³² and finally three different group I introns at higher resolution.^{33–35}
- “It has a specific binding site for the guanosine cofactor.” When we provided indirect evidence for direct ligand-binding by the *Tetrahymena* ribozyme;³⁶ the idea was met with disbelief by those who thought that only proteins could have such ability. Yet the site was eventually

pin-pointed within the RNA³⁷ and visualized in the various crystal structures, and, with the discovery of riboswitches, the concept has been found to be central to a long-undiscovered mode of regulation of gene expression.³⁸

- “The RNA forms an active site cleft or hole that can exclude water, thereby preventing hydrolysis after each cleavage step.” A concave active site in fact existed,³² and the exclusion of water by RNA active sites has been best described for the case of the ribosome.³⁹

In retrospect, it is amusing to see how these proposals, which seemed rather bold in 1982, seem so obvious today.

On a more personal note, the study of RNA catalysis has been great fun because it has brought me into contact with such great people. The list of contributors to this volume, for example, includes several former postdoctoral fellows and collaborators, and the rest are all “science friends”. Students who are still deciding on a career may not yet appreciate how the life of a scientist is not at all a solitary pursuit, but involves entry into a community of scholars who share common goals, collaborate, critique each others’ work, and generally enjoy each others’ company.

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