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Catalytic Properties of RNA

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<title>RNA, Catalytic Properties of</title>

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<para>Enzymes are hallmarks of living systems, catalyzing the vast majority of chemical transformations in all life forms with high precision and efficiency, accelerating chemical reactions by many orders of magnitude at physiological conditions. This remarkable catalytic property was initially assigned only to proteins and the terms *protein* and *enzyme* were often used interchangeably; however, with the discovery of the genetic code and structured ribonucleic acids (RNAs) such as transfer RNAs (tRNAs) and ribosomal RNAs, a hypothesis that RNA played a central role in the origin of today's biosphere was put forward. This "RNA World" hypothesis stipulates that some or perhaps all chemical transformations in the early biosphere were catalyzed by RNAs, which could serve as both information carriers and structured macromolecules capable of enzymatic catalysis (Gilbert 1986).</para>

<para>Early arguments for the plausibility of catalytic RNAs came from the discovery of the genetic code, which describes how genetic information is transcribed from DNA into messenger RNA (mRNA) and then translated into proteins (Crick 1968). RNA is central to this process, not only in form of the informational molecule, mRNA, but also as the adaptor (tRNA) matching each codon to the right amino acid, thus translating the DNA into a protein sequence. Ribosomesmacromolecular machines responsible for both mRNA decoding and peptide bond formation during protein synthesisare mostly made up of RNA. This observation suggested that RNA may be directly involved in peptide synthesis, solving a chicken-and-egg problem for the origin of proteins. One other observation suggested an intimate link between RNA and catalysis: Many co-factorssmall molecules that aid enzymes in catalysisare derivatives of ribonucleosides, supporting early adaptation of nucleic acids for chemical transformations.</para>

<para>While the proposal of an RNA catalyst arose from the discoveries of the genetic code and the ribosomes in the 1950s and 1960s, it took until 1982 for the first catalytic RNAs to be discovered, and until 1992 for the first experimental evidence that ribosomal RNA is responsible for polypeptide synthesis.</para>

<h1>The First Ribozymes</h1>

<para>In the 1970s, messenger RNAs of eukaryotes were shown to be spliced out of much longer precursors, removing introns (typically, non-coding sequences interspersed among the

protein-coding sequences (exons) from the original transcripts. While studying a specific intron in a ribosomal RNA of a unicellular ciliate *Tetrahymena thermophila*, the American biochemist Thomas Cech (1947–) and co-workers discovered that the precursor RNA could be purified from the nuclear extract and the intron was still spliced out. This surprising activity was first thought to be a result of contamination with a protein that co-purified with the RNA, but regardless of how stringent the purification was, the RNA retained its self-splicing activity. This initial finding suggested that the intron could splice itself out of the precursor RNA without the assistance of a protein and the reaction must involve two steps: cleavage of the intron from the upstream exon, utilizing a molecule of guanosine in the process, and ligation of this exon to the downstream exon, liberating the intron from the precursor. Except for the need for a molecule of guanosine, this process was analogous to splicing reactions described in other eukaryotic systems, which, however, required an enzyme complex called spliceosome (itself consisting of both RNA and proteins).

These findings suggested that the intron was enzyme-like in that it catalyzed two chemical steps, greatly accelerating the reactions over the background rate, while maintaining superb specificity for the site of splicing. It also largely prevented side reactions, particularly hydrolysis of the reaction intermediates. Moreover, when the RNA was prepared using a bacterial RNA polymerase enzyme in a laboratory and purified under denaturing conditions, thus precluding contamination by any protein from *Tetrahymena*, the intron retained its self-splicing activity. Because of these properties, the molecule was termed a ribozyme (for *ribonucleic acid enzyme*) (Kruger et al., 1982).

Subsequent experiments carried out over the rest of the 1980s showed that these self-splicing introns occur in many species, including bacteria and bacteriophages, and an unrelated family of self-splicing introns with domains resembling the eukaryotic spliceosome was also discovered. Biochemical analyses showed that these self-splicing introns fold into specific shapes organized around conserved structural and sequence elements, and that they can be converted into multiple-turnover catalysts capable of polymerizing an RNA from shorter building blocks. Extensive analysis of the catalytic mechanism of these ribozymes showed that they use three divalent metal ions to catalyze the two transesterification steps (Shan et al., 2001). The ribozymes have also been modified to act on other substrates, such as DNA strands, and other types of esters. Biophysical studies revealed that Mg^{2+} was required for both folding of the RNAs and catalysis, and that the correct structure was required for the formation of a catalytically-competent ribozyme, mirroring the requirement of protein enzymes to fold into correct structures to form active molecules. Thus in both ribozymes and protein enzymes, the polymer (RNA or polypeptide) sequence dictates the secondary and tertiary structure that forms the active site where catalysis occurs (Figure 1).

In 2004 and 2005, the first crystal structures of intact self-splicing introns revealed compact RNA structures, in which buried active sites contained divalent metal ions poised for

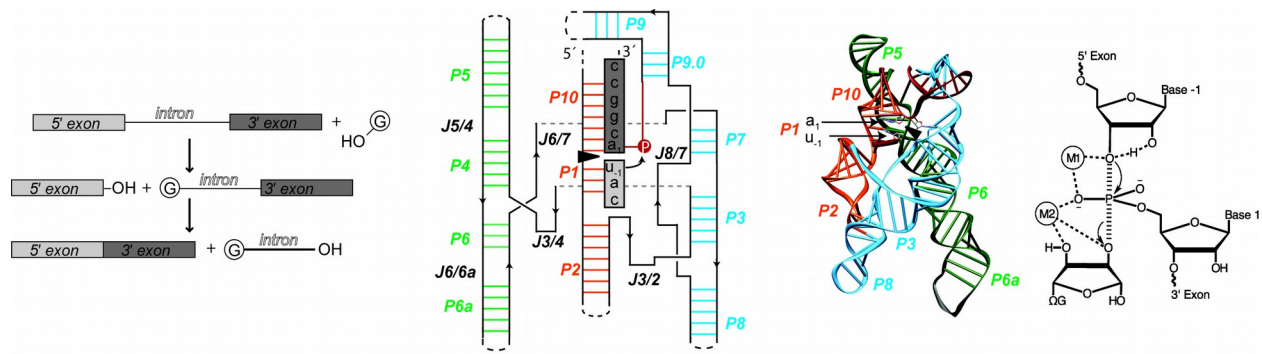


Figure 1. Group I self-splicing introns. A) Overall mechanism of splicing 5' and 3' exons. **B)** Secondary and **C)** tertiary structures of a group I intron with exons shown in grey. **D)** Stabilization of the transition state by divalent metal ions.

catalysis, as they would appear in a protein enzyme, validating the biochemical data and fully establishing these ribozymes as enzymes (Figure 1) (Adams et al., 2004; Golden et al., 2005).

Spliceosome.

Because of the central role of the spliceosome in the information transfer from DNA to proteins in eukaryotes, much effort has gone into elucidating its mechanism of action. The aforementioned similarity between the second type (group II) self-splicing introns and the spliceosome further motivated testing the hypothesis that the spliceosomal RNA is responsible for the catalysis. The first evidence confirming this hypothesis with purified components showing that splicing of pre-messenger RNA can be achieved using just the RNA components came in 2001 (Valadkhan and Manley 2001). In 2013, a study showed that the catalytic metal ions in the group II intron and in U6 spliceosomal RNA match each other, definitively establishing that the active site of the spliceosome is formed by RNA (Fica et al., 2013).

Ribonuclease P.

At the time of the discovery of self-splicing introns, the Canadian-American molecular biologist Sidney Altman (1939-) was studying ribonuclease P (RNase P), a protein-RNA complex responsible for processing several types of cellular RNAs, including tRNAs. RNase P is found in all cellular life, catalyzes the site-specific hydrolysis of the pre-tRNA, requires Mg^{2+} for its activity, and can process multiple substrates, making it a true multi-turnover enzyme. Like Cech, Altman and co-workers discovered that preparations of RNase P lacking their protein components retained their ability to catalyze the phosphoryl transfer reaction (Guerrier-Takada et al., 1983). The proteins associated with the RNase P RNA aid catalysis by lowering the magnesium required for tRNA cleavage, thereby enhancing folding of the RNase P RNA, and increasing the turnover number of the enzyme. The early 2000s brought increasingly more detailed structures of the enzyme, culminating in 2010 in a crystal structure of the entire RNase P in complex with tRNA (Reiter et al., 2010). Two active-site metals were found and their location suggested that one interacts with the non-bridging scissile phosphate oxygens, activating a hydroxide to preform an SN_2 nucleophilic substitution, whereas

the second metal is thought to stabilize the transition state and promote proton donation to the 3' scissile oxygen. Like the group I and II self-splicing introns, the RNase P active site is organized to position the substrates and the catalytic metals to facilitate catalysis, making these ribozymes true metalloenzymes.

Cech and Altman's experiments demonstrating that RNAs form catalytically-competent macromolecules radically changed our view of the chemical roles of biological polymers. Their discoveries blurred the boundary between protein-only and RNA-only functions and support the hypothesis that the current biosphere might have been preceded by one dominated by RNA molecules acting as both information carriers and catalysts. For their discovery of catalytic RNAs, Cech and Altman received the 1989 Nobel Prize in Chemistry.

Self-Cleaving Ribozymes.

In 1986, the first example of an RNA catalyzing self-scission (nucleophilic attack of a 2' hydroxyl on the adjacent phosphate) was discovered in a plant viroid (Prody et al., 1986). Within the next two years, two more classes of these ribozymes were found, including one in a human pathogen, hepatitis delta virus (HDV), and by 2014 two more were described. These self-cleaving ribozymes have distinct secondary structures, ranging from a simple three-helix junction of the hammerhead ribozyme to a double pseudoknot of the HDV ribozymes. The ribozymes often employ nucleobases for general acid or base catalysis, accelerating the reactions by promoting proton transfer during the transesterification. In the case of hairpin ribozyme (Figure 2), the Japanese-Mexican-American biophysicist and structural biologist Adrian Ferré-D'Amaré (1966-) and coworkers determined its structure with an active-site vanadate, an analog of the bipyramidal transition state (Rupert et al., 2002). The transition state analog interacted with the ribozyme through more hydrogen bonds than either the precursor or the product, suggesting that the ribozyme is a classical Pauling enzyme, catalyzing the reaction by stabilizing the transition state.

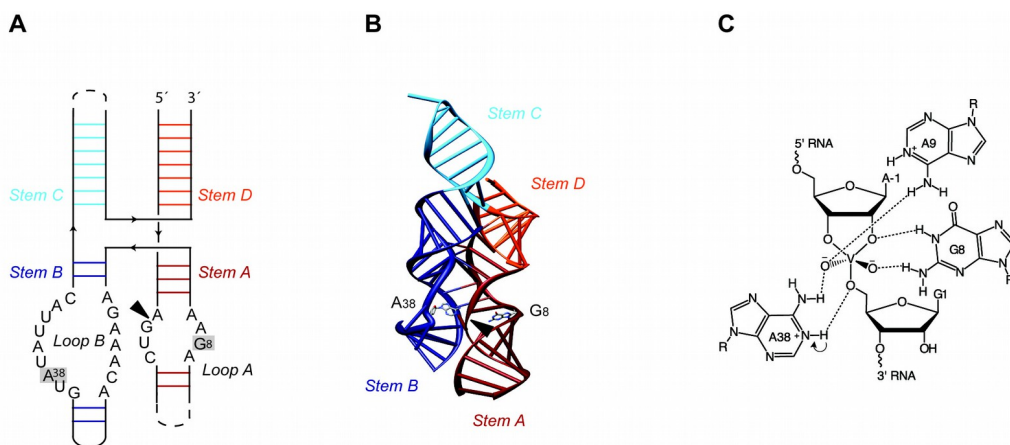


Figure 2. Hairpin self-cleaving ribozyme. **A)** Secondary and **B)** tertiary structures of the four-helix ribozyme with catalytic residues A38 and G8 indicated. Black arrowheads show the scissile position. **C)** Hydrogen bonding interactions between the ribozyme and vanadate, analog of the bipyramidal transition state of self-scission.

Whereas the natural forms of these ribozymes have only been found to self-cleave and occasionally reverse the reaction (ligate), all of them have been redesigned to separate their active sites from the substrate strands, converting the ribozymes into multi-turnover enzymes through simple topological change of the RNA.

Ribozymes Evolved in the Laboratory, Aptazymes, and Riboswitch Ribozyme

In 1990, three molecular biologists—the Americans Larry Gold and Gerald Joyce (1956), and the Canadian-American Jack Szostak (1952)—each independently invented a method for laboratory selection and evolution of functional RNAs (Ellington and Szostak 1990; Robertson and Joyce 1990; Tuerk and Gold 1990). This method relies on isolation of ligand-binding (aptamers) or catalytic RNAs from highly diverse pools. The first ribozyme selected from a random sequence was an RNA ligase, which used a triphosphorylated terminus to attach itself to another RNA (Bartel and Szostak 1993). Mechanistically, this reaction mimics RNA polymerization. To test whether the ligase can be a model enzyme for RNA polymerization, possibly even self-replication, the ribozyme was first designed to act *in trans*, then evolved to polymerize a short RNA strand, and in 2011 further evolved to synthesize RNAs long enough to be ribozymes (Johnston et al., 2001; Wochner et al., 2011). In the first twenty years since the invention of *in vitro* selection, a large number of ribozymes catalyzing a broad range of chemical transformations were described, demonstrating that designer ribozymes can be readily evolved for many reactions.

Converting self-cleaving ribozymes into trans-cleaving enzymes by changing their topology led to proposals to use these catalytic RNAs in cutting target RNAs, especially disease-related mRNAs. While a ribozyme-based treatment has not been achieved yet, this idea has been used in bioanalytical applications and synthetic biology, particularly when the ribozymes were engineered to cleave their target RNAs only in the presence of a ligand. Such aptazymes are facile regulators of gene expression, allowing fine-tuning of synthetic metabolic pathways (Carothers et al., 2011).

The early 2000s brought another group of RNAs into the spotlight: metabolite-dependent regulatory elements called riboswitches. These RNAs typically interconvert between two conformations, one of which is stabilized by a co-factor, with strongly differential effects on gene expression. One of the riboswitches discovered by the American biochemist and molecular biologist Ronald Breaker in 2004 turned out to be a glucosamine-6-phosphate-dependent ribozyme (Winkler et al., 2004). Crystal structures of the ribozyme revealed that the metabolite binds the ribozyme in the active site, directly participating in catalysis in a manner reminiscent of co-factor-dependent protein enzymes. In this case, the ribozyme self-cleaves when the co-factor is bound, leading to degradation of the mRNA that harbors it.

The Ribosome is a Ribozyme.

In 1992, the American biochemist Harry Noller (1939) and co-workers demonstrated that ribosomes isolated from *Escherichia coli* or *Thermus aquaticus* treated to remove proteins retained peptidyl transferase activity, but when exposed to EDTA which removes divalent metal ions, or antibiotics that inhibit the active sites of ribosomes, the activity was lost (Noller et al., 1992). These results strongly suggested that the ribosomal RNA is the catalytic component responsible for synthesis of polypeptides. It took until 2000 when the American biophysicist and biochemist Peter Moore (1939), the American structural biologist and biochemist Thomas Steitz (1940), and their co-workers solved the crystal structure of the large ribosomal subunit, which contains the peptidyl transferase center, and found the active site devoid of proteins, thereby demonstrating that ribosomes are actually ribozymes (Nissen et al., 2000).

With the discovery that ribosomal RNA is the catalyst synthesizing proteins, and *in vitro* selection experiments yielding ever more sophisticated ribozymes, catalytic RNAs are now fully established enzymes that perhaps link us to the origin of our biosphere. Other than a self-replicating RNA, one type of ribozyme that remains to be found is a metabolic multi-turnover ribozyme. If such ribozymes exist and are remnants of ancient metabolic pathways from an RNA World, the case for RNA enzymes will grow even stronger. Perhaps the first metabolic ribozymes will link the production of activated nucleotides to RNA self-replication.

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