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Review

Structure and function of regulatory RNA elements: Ribozymes that regulate gene expression

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ABSTRACT

Since their discovery in the 1980s, it has gradually become apparent that there are several functional classes of naturally occurring ribozymes. These include ribozymes that mediate RNA splicing (the Group I and Group II introns, and possibly the RNA components of the spliceosome), RNA processing ribozymes (RNase P, which cleaves precursor tRNAs and other structural RNA precursors), the peptidyl transferase center of the ribosome, and small, self-cleaving genomic ribozymes (including the hammerhead, hairpin, HDV and VS ribozymes). The most recently discovered functional class of ribozymes include those that are embedded in the untranslated regions of mature mRNAs that regulate the gene's translational expression. These include the prokaryotic glmS ribozyme, a bacterial riboswitch, and a variant of the hammerhead ribozyme, which has been found embedded in mammalian mRNAs. With the discovery of a mammalian riboswitch ribozyme, the question of how an embedded hammerhead ribozyme's switching mechanism works becomes a compelling question. Recent structural results suggest several possibilities.

1. Background and context

1.1. Discovery of ribozymes

RNA catalytic activity in the absence of proteins was first observed in the context of Group I intron self-splicing [1], and the first example of a true RNA enzyme (or ribozyme) that exhibits multiple turnover was the RNA subunit of RNase P, which hydrolyzes the 5′-end of precursor tRNAs [2]. Subsequently, a number of smaller and chemically similar self-cleaving genomic RNAs were discovered [3–5]. The structures of the prokaryotic ribosome [6–11] confirmed an earlier suggestion that the peptidyl transferase center was comprised entirely of RNA [12]; no ribosomal protein approaches closer than 18 Å to the active site. Subsequent to the discovery of bacterial riboswitches [13], a prokaryotic ribozyme riboswitch has been identified in the form of a new ribozyme (glmS) [14], and a familiar ribozyme (the genomic hammerhead ribozyme that mediates rolling-circle replication in RNA virus-like genomes) has been identified, as a discontinuous sequence, embedded in mammalian mRNAs [15], a realm wherein riboswitches have not yet been identified.

1.2. Chemistry of ribozyme catalysis

With the exception of the peptidyl transferase, all of the naturally occurring ribozymes employ simple variations of acid/base catalyzed phosphodiester chemistry, and all but the hydrolytic RNase P catalyze a phosphodiester isomerization reaction that breaks or joins the RNA backbone [16]. Each ribozyme, however, has a completely unique sequence, tertiary structure, and detailed catalytic strategy, sharing in common only the general principles of acid/base catalysis [17]. Several of the small, self-cleaving genomic RNAs catalyze a chemically identical site-specific self-cleavage reaction in which the 2′-oxygen attacks an adjacent phosphate, producing products with 5′-OH and 2′,3′-cyclic phosphate termini; the reaction is the same as the first step catalyzed by RNase A (Fig. 1). As with RNase A, most of these ribozymes (the exception is the HDV ribozyme) do not require the presence of a divalent metal ion for catalysis [18]. The glmS riboswitch ribozyme also catalyzes the same self-cleavage reaction.

1.3. Comparison to a protein enzyme: RNase A

RNase A provides a convenient and familiar reference point with respect to protein mechanistic enzymology for understanding ribozyme acid/base catalysis [19,20]. Two invariant histidines in the active site of RNase A function as a general base and a general acid. In the first step of the RNase A cleavage reaction, an unprotonated histidine (His 12) abstracts a proton from the 2′-OH of the substrate RNA at the cleavage site, thus generating the nucleophile that attacks the adjacent phosphate, and a protonated histidine (His 119) donates a proton to the 5′-oxygen leaving group, which begins to accumulate an unstable negative charge as its bond to the scissile phosphorus atom breaks. A bond between the 2′-oxygen, the attacking

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nucleophile, and the phosphorus forms at the same time the scissile bond breaks, as the reaction passes through a pentacoordinated oxyphosphorane transition state in which the attacking nucleophile and leaving group oxygen occupy the two opposite, axial positions. The products are two strands of RNA; one with a 5′-OH terminus and the other with a 2′,3′-cyclic phosphodiester (The second step of the RNase A reaction involves hydrolysis of the cyclic phosphate).

Each of the small self-cleaving ribozymes employs a unique catalytic strategy to perform acid/base catalysis, but the chemical mechanism of the cleavage reaction is the same as the fist step of the RNase A reaction, and the pentacoordinated transition-state structure appears to be essentially the same. Obviously, ribozymes lack histidines in their active sites, but various RNA functional groups appear to play analogous roles as general acids and bases [21].

In the case of the hairpin ribozyme, a deprotonated guanosine appears to be the general base that plays the analogous role to the deprotonated histidine in RNase A, and a (protonated) adenosine appears to be the general acid [22]. In the case of the hammerhead ribozyme, a deprotonated guanosine also appears to be the general base, but instead of adenosine, the 2′-OH of a conserved nucleotide appears to function as a general acid (Fig. 1) [23]. In the case of the glmS ribozyme, guanosine again appears to be the base, whereas the acid catalyst is not the RNA itself, but rather the sugar, GlcN6P, the small molecule riboswitch effector, that binds specifically to the active site of the ribozyme and activates it [24,25].

1.4. The biological context of genomic RNA catalysis

The hammerhead ribozyme was the first of several catalytic RNAs to be discovered in the context of satellite RNA virus and virus-like genomes [3]. With the exception of the human hepatitis C virus, these were all discovered in plants [26]. The hammerhead ribozyme was first found in the sense strand of the satellite RNA of tobacco ringspot virus, which was known to replicate via a rolling-circle pathway. Arguably the simplest nucleic acid replicative strategy, the single-stranded, covalently closed circular satellite RNA serves as a substrate for a host RNA polymerase. As the polymerase circumnavigates the satellite RNA template for multiple processive cycles, it produces a linear concatomeric complementary copy of the circular RNA. The concatomer must be cleaved into monomeric fragments and then the ends of the monomers must ligate to form circular templates for the second half of replication [27].

The hammerhead ribozyme catalyzes the cleavage (and possibly ligation) reactions for the sense-strand RNA, and the subsequently identified hairpin ribozyme catalyzes cleavage and ligation of the complementary template. In addition to plant RNA viruses, active hammerhead sequences are occasionally found in satellite RNA transcripts of highly repetitive DNA sequences in organisms such as *Schistosoma*; the function of these satellite RNAs is unknown [28,29].

Because rolling-circle replication of genomic RNAs requires both an RNA cleavage and an RNA ligation event, it is quite understandable why a phosphodiester isomerization reaction might be preferred, in natural selection, over an RNA hydrolysis reaction. Formation of the 2′,3′-cyclic phosphate preserves the initial state of the scissile phosphate as a phosphodiester, and thus permits it to function as a substrate in a subsequent ligation reaction without the requirement for a high-energy cofactor such as ATP. Simple RNA hydrolysis would be unable to do that. It is therefore not particularly surprising that all of the various genomic ribozymes (including the hairpin, hammerhead, HDV and VS ribozymes) catalyze the same chemical reaction. The mechanisms of the switch between nuclease and ligase activities for the genomic ribozymes remains completely elusive, although structural analyses of the hammerhead ribozyme, described below, are beginning to offer some insights.

2. The hammerhead ribozyme

2.1. The structural basis for hammerhead ribozyme catalysis

Three-dimensional structures now exist for the hairpin, HDV and hammerhead ribozymes, each in both pre-cleavage and post-cleavage states [21,22,30–33]. Each of these ribozymes possesses a unique sequence and tertiary structure as well as a unique catalytic strategy for accelerating the same reaction; the one common theme is that all appear to use a form of acid/base catalysis as their primary mode of rate enhancement. For purposes of continuity, we will focus here on the hammerhead ribozyme.

Hammerhead RNA self-cleavage motifs consist of three A-form helices (called Stems I, II and III) flanking a junction comprised of 15 invariant or mostly conserved nucleotides that form the catalytic core (Fig. 2a) [34]. Two of the three helices (typically Stem II and Stem I) are capped by connecting loops, giving this self-cleaving RNA sequence a canonical secondary structure that resembles the shape of a hammer head. The natural full-length hammerhead ribozyme sequence typically contains an additional tertiary contact between Stems I and II, distant from the cleavage site (Fig. 2b), that greatly enhances the rate of catalysis by stabilizing formation of the active conformation of the cleavage site (Fig. 2c and d) [23,35,36].

The crystal structure of the full-length hammerhead ribozyme sequence derived from *Schistosoma mansoni* (Smα) was obtained in 2006 (2GOZ) [23]. Unlike previous structures of minimal hammerhead ribozyme sequences that lacked the distal tertiary contact, this structure reveals the active conformation of the cleavage site.

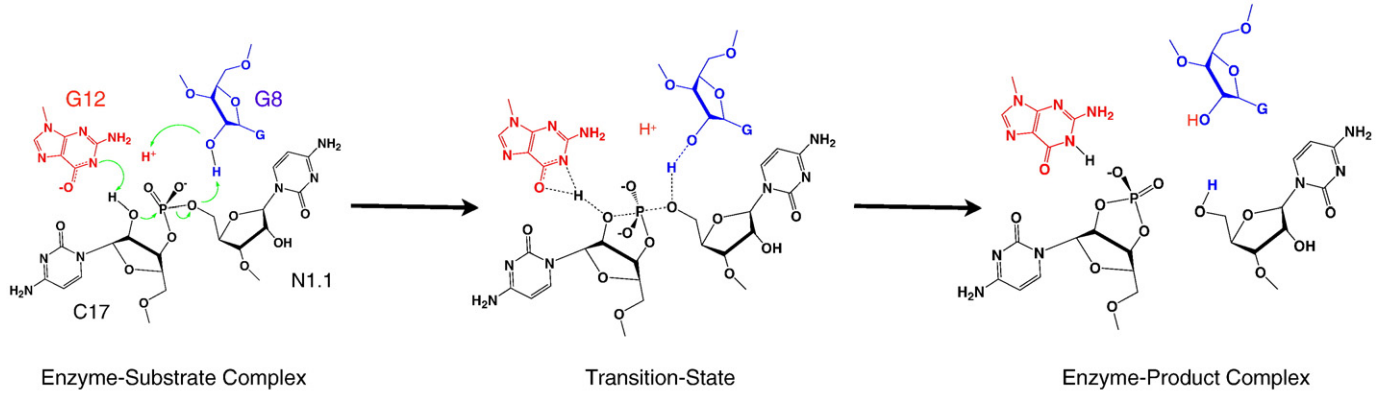

Fig. 1. Proposed mechanism for acid/base catalysis in the hammerhead ribozyme self-cleavage reaction. The enzyme–substrate complex is depicted schematically on the left, the putative transition-state structure in the center, and the enzyme–product complex is on the right.