Hammering out the shape of a ribozyme

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One of the important scientific breakthroughs of the past decade was the discovery that certain RNA molecules have inherent catalytic capacities. These RNAs, or ribozymes, seemed likely to have defined three-dimensional conformations analogous to the structures required for the enzymatic activity of proteins. Since the only RNA structures known at atomic resolution were transfer RNAs and simple duplexes, however, the detailed architecture of an RNA active site was a mystery. Thus, great excitement has been generated by the recently reported crystal structure of a hammerhead ribozyme by David McKay and coworkers at Stanford University [1].

The hammerhead motif is a self-cleaving RNA structure that occurs within the genomes of various plant viroids and virusoids and probably facilitates rolling-circle replication of these infectious agents [2]. The secondary structure of the RNA consists of three base-paired stems extending from a conserved central core region, a layout which crudely resembles the head of a hammer (Fig. 1) [3]. Cleavage occurs through the attack of the 2′-hydroxyl of a specific nucleotide within the core on its adjacent phosphodiester bond to produce 2′,3′-cyclic phosphate and 5′-hydroxyl termini. Normally a single-turnover catalyst, the hammerhead has been engineered to be a multiple turnover enzyme by separating the strand containing the cleavage site from the rest of the core [4] (Fig. 1). This design facilitated crystallization by allowing substitution of the substrate strand with an all-DNA strand which binds the ribozyme but is not cleaved.

The crystal structure reveals the three-dimensional fold of the hammerhead RNA to resemble a wishbone, with Stems I and II forming the arms and Stem III and the core at the base (Fig. 2) [1]. While the three stems are all A-form helices, the structure of the central core region is created by non-canonical pairings of the phylogenetically conserved nucleotide bases. Despite the defined fold of the RNA, this is clearly a flexible molecule, as revealed by the existence of three distinct RNA molecules within each asymmetric unit of the crystal. The three molecules, while similar in overall structure, have varying conformations of the wishbone arms (Stems I and II). Stems II and III are essentially coaxially stacked, with stacked purine bases of the core in between. The highly conserved sequence CUGA between Stems I and II forms a tight turn within the core that is strikingly similar to the uridine turn first observed in the anticodon and pseudouridine loops of phenylalanine transfer RNA [1,5]. It is reassuring to note that the crystal structure is consistent with a recently published solution study of similar hammerhead sequences. Fritz Eckstein and coworkers [6] used fluorescence energy transfer to determine the relative distances between pairs of fluorescently labeled helices in the hammerhead RNA. The model they proposed on the basis of the derived constraints agrees well with the crystal structure.

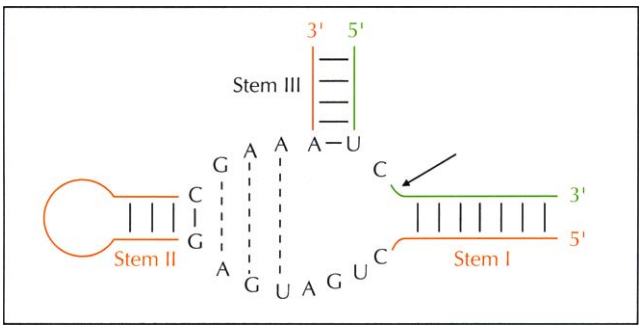

Fig. 1. Secondary structure of the hammerhead motif. The substrate strand is shown in green, the ribozyme strand in red. The cleavage site is indicated by an arrow.

One important question about RNA structure and function concerns the role of divalent metal ions in folding and catalysis. In the hammerhead and other ribozymes, divalent metals such as magnesium or manganese are required for activity and probably serve both structural and catalytic roles. Since Pley *et al.* [1] could not see magnesium ions in the native hammerhead structure, manganese or cadmium ions were soaked into the crystals and their positions located by their unique X-ray scattering properties. This analysis revealed a neatly constructed pocket for divalent metal binding formed by two adjacent G-A base pairs in the core. The hydrogen bonds between the guanine and adenine bases in the G-A pairs are similar to those observed in solution studies of an RNA duplex containing two G-A pairs [7]. The metal ion fits in between the N-7 of one of the guanine bases and its neighboring phosphate, consistent with results from previous thiophosphate interference and nucleotide substitution experiments. Interestingly, the same sequence of two consecutive G-A mismatches has been found near the active sites of a lead-dependent self-cleaving ribozyme and of ribozymes that catalyze RNA ligation [8,9]. Thus, this motif may turn out to be a common way of positioning divalent metals within an RNA structure. Since the observed metal ion is not accessible to the cleavage site in the hammerhead RNA, however, it probably has a structural rather than a direct catalytic function. Thus, the positions of metal ions needed for catalysis remain to be found, particularly in light of the biochemical evidence for an essential divalent metal ion bound to one of the oxygens of the scissile phosphodiester bond [10]. Perhaps such catalytic metals will be apparent when a substrate more similar to the natural RNA substrate strand is used, or with different crystallization conditions.

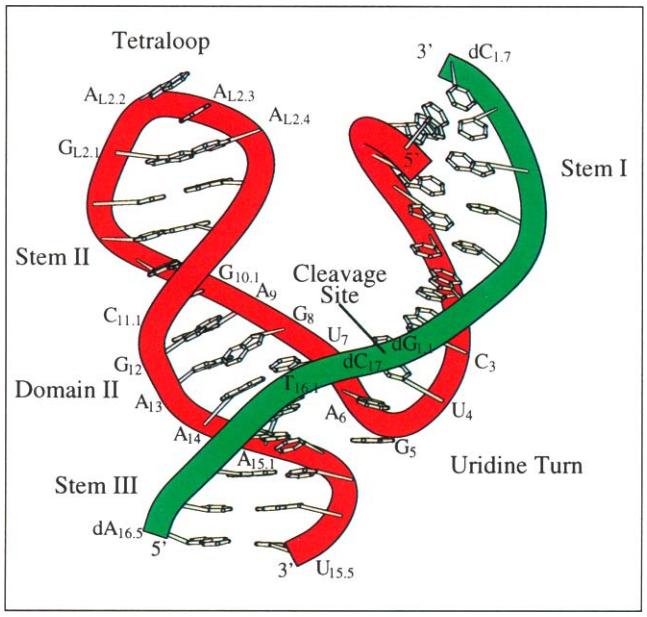

Fig. 2. In three dimensions the hammerhead ribozyme is shaped like a wishbone. The ribozyme strand is shown in red, and the substituted 'substrate' DNA strand in green. (Reprinted with permission from [1].)

A second question about RNA tertiary structure concerns the role of 2′-hydroxyl groups in stabilizing three-dimensional conformation. It makes intuitive sense that these functional groups would provide a unique means of tethering large RNA structures by virtue of their hydrogen-bonding capacity and accessibility. The hammerhead structure provides examples of 2′-hydroxyl contacts both within and between molecules. Within the core, several 2′-hydroxyl group interactions play an important role in molding the active structure of the ribozyme, consistent with previous mutagenesis studies [1]. A network of contacts is also observed between adjacent molecules packed in the crystal lattice. Hairpin loops of the sequence GNRA (where N is any nucleotide and R is G or A) have a defined structure that lends itself to docking into the minor groove of RNA helices [11], and it is just such an interaction that is observed between the GAAA loop of Stem II and the minor groove of Stem II of another molecule in the crystal lattice [12]. Strikingly, the majority of hydrogen bonds between the loop and the target helix involve 2′-hydroxyl groups. These interactions correlate with the proposed specificity of GNRA loops for sites on RNA duplexes observed by Michel and Westhof [13]. Since GNRA and other stable 'tetraloop' sequences occur frequently in ribozymes and ribosomal RNAs, this mode of loop–helix docking may be a common tertiary structural motif in large structured RNAs.

An exciting application of this structural information involves the design of hammerhead ribozymes for therapeutic purposes. Research on this front has focused on engineering physiologically stable hammerhead sequences to cleave target RNAs *in vivo* [14]. The hammerhead structure now provides a framework for testing nuclease-resistant derivatives of the RNA and for optimizing the ribozyme for multiple-turnover reactions [15].

Solving the hammerhead crystal structure has not immediately revealed all of the details of the catalytic mechanism, of course. In addition to the requirement for a metal ion at the cleavage site, biochemical experiments suggest that strand scission occurs via an 'in-line' attack by the 2′-hydroxyl at the cleavage site. In the crystal structure, however, the nucleotide at the cleavage site is not positioned correctly for an in-line mechanism. This may result from the absence of the 2′-hydroxyl in the DNA substrate analog, and indeed, Pley *et al.* [1] propose the possibility of a conformational change which would reposition the nucleotide at the cleavage site appropriately. A related question regards the flexibility of the structure. Is this lack of rigidity a general feature of RNA, perhaps facilitating conformational changes during catalysis and upon binding to proteins [16]? While these and other questions about ribozyme structure and function remain to be answered, the hammerhead crystal structure greatly advances current understanding of RNA architecture and will facilitate further biochemical and crystallographic experiments.

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