CHAPTER 4

Hammerhead Ribozyme Crystal Structures and Catalysis

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4.1 Introduction

After the discovery of RNase P[1] (Chapter 9) and the Group I[2] (Chapter 10) intron ribozymes, both of which are comparatively large and complex catalytic RNAs, the identification of the hammerhead ribozyme offered hope that the phenomenon of RNA catalysis might be best understood within the framework of a smaller, more tractable RNA that catalyzed a simple phosphodiester isomerization reaction. Indeed, the first ribozyme crystal structures were in fact those of hammerhead ribozymes, but they seemed to create more questions than compelling explanations for RNA catalysis. The twelve years subsequent to the publication of these structures saw only increasing discord; the crystal structure analyses seemed hopelessly irreconcilable with a growing corpus of biochemical evidence. Meanwhile, crystal structures for many of the other ribozymes, including the Group I intron and RNase P, started to appear. Finally, a new crystal structure of the hammerhead ribozyme emerged, 20 years after the hammerhead's discovery. This structure included a set of distal tertiary contacts whose importance was largely unrecognized until 2003, but whose incorporation increased catalytic prowess by a factor of $\sim$1000. The new crystal structure reveals that this remarkable rate enhancement is a direct consequence of localized yet dramatic active site conformational changes that are stabilized by a comparatively distant set of tertiary interactions. The new structure appears to reconcile twenty years of discord while offering some new insights into RNA structure and catalysis, as well as the foibles of experimental interpretation. The hammerhead ribozyme has indeed taught us much about RNA catalysis, despite (or, more likely, because of) major deviations from our seemingly best-devised lesson plans.

4.2 A Catalytic RNA Prototype

The discovery that RNA can be an enzyme[1,2] created the fundamental question of how RNA enzymes work. Before this discovery, it was generally assumed that proteins were the only biopolymers that had sufficient complexity and chemical heterogeneity to catalyze biochemical reactions. Clearly, RNA can adopt sufficiently complex tertiary structures that make catalysis possible. But how does the three-dimensional (3D) structure of an RNA endow it with catalytic activity? What structural and functional principles are unique to RNA enzymes (or ribozymes), and what principles are so fundamental that they are shared with protein enzymes?

By understanding how ribozymes work, we might also learn more about how life originated. RNA may have been the original self-replicating pre-biotic molecule, according to the "RNA World" hypothesis,[3] potentially catalyzing its own replication. Understanding the fundamental principles of ribozyme catalysis therefore may also give us new insights into the origin of life itself. The answer to the question of how ribozymes work also has practical consequences, as RNA enzymes are particularly well-suited for design as targeted therapeutics for various diseases (for a recent review, see ref. 4).

The hammerhead ribozyme has been thought of as a "prototype" ribozyme in the same sense that lysozyme and serine proteases have been thought of as "prototype" conventional protein enzymes, and for that reason the hammerhead ribozyme has attracted intense experimental scrutiny. The hammerhead ribozyme is a comparatively simple and well-studied ribozyme that in principle should be capable of revealing the secrets of its catalytic potential, if we are able to pose the right questions and carry out useful and informative experiments. Much attention has been focused upon this particular ribozyme with the hope that if its catalytic properties become well-understood our grasp of the phenomenon of RNA catalysis in general will become more comprehensive so that generalizations may appear that are applicable to the larger ribozymes, to RNA splicing and peptidyl transfer, and perhaps even beyond to a unified understanding of RNA and protein enzymology.

4.3 A Small Ribozyme

Hammerhead RNAs are small self-cleaving RNAs that have a conserved motif found in several of the viroids and satellite RNAs associated with plant RNA viruses[5–8] and other species[9–11] and that replicate *via* a rolling circle mechanism. The minimal hammerhead sequence that is catalytically active consists of three base-paired stems flanking a central core of 15 conserved (mostly invariant) nucleotides, as depicted in Figure 4.1.[8,12,13] The conserved central bases, with few exceptions, are essential for ribozyme's catalytic activity.

The hammerhead ribozyme is arguably the best-characterized ribozyme. Its small size, thoroughly-investigated cleavage chemistry, known crystal structure, and its biological relevance make the hammerhead ribozyme particularly

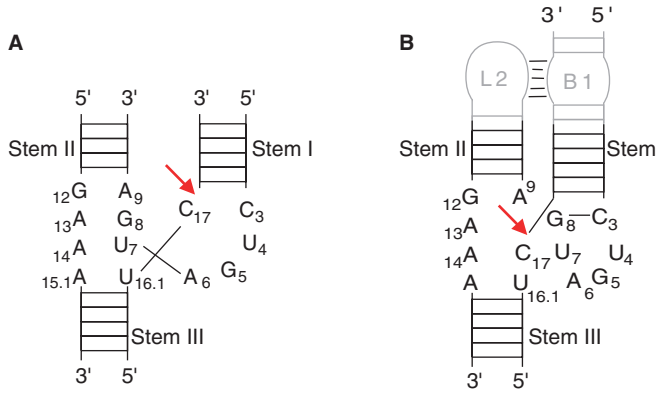

Figure 4.1 Schematic diagrams of the secondary structures of two hammerhead ribozyme constructs. The minimal hammerhead (*A*) consists of a conserved, mostly invariant core region that contains the cleavage site, as well as three flanking A-form RNA helices whose sequence is only restricted by the need to maintain base-pairing. The full-length hammerhead (*B*) contains additional nucleotides as depicted in grey. Although the sequences of the stem II loop (L2) and stem I bulge (B1) are not restricted in any obvious way that is apparent from the RNA sequence, the tertiary interaction that forms between these two regions is critically important and enhances catalysis by approximately three orders of magnitude.

well-suited for biochemical and biophysical investigations into the fundamental nature of RNA catalysis. Despite the extensive structural and biochemical characterization of the hammerhead ribozyme (reviewed in refs. 14–16) many important questions have remained concerning how this RNA molecule's structure enables it to have catalytic activity. Our understanding of the relationship between the structure of the hammerhead RNA and its catalytic activity has enjoyed a particularly tumultuous history.

4.4 Chemistry of Phosphodiester Bond Isomerization

The cleavage reaction is a phosphodiester isomerization reaction that is initiated by abstraction of the 2′-hydroxyl proton from the 2′-oxygen, which then becomes the attacking nucleophile in an "in-line" or S_N2(P)-like reaction (Figure 4.2),[17–19]although it is not known whether this proton is removed before or during the chemical step of the hammerhead cleavage reaction. (The cleavage reaction is technically not bimolecular, but behaves in the same way a genuine S_N2(P) reaction does; it undergoes inversion of configuration subsequent to forming an associative transition-state consisting of a pentacoordinated oxyphosphorane.) The attacking and leaving group oxygens will both occupy the two axial positions in the trigonal bipyramidal transition-state structure as is required for an S_N2-like reaction mechanism.

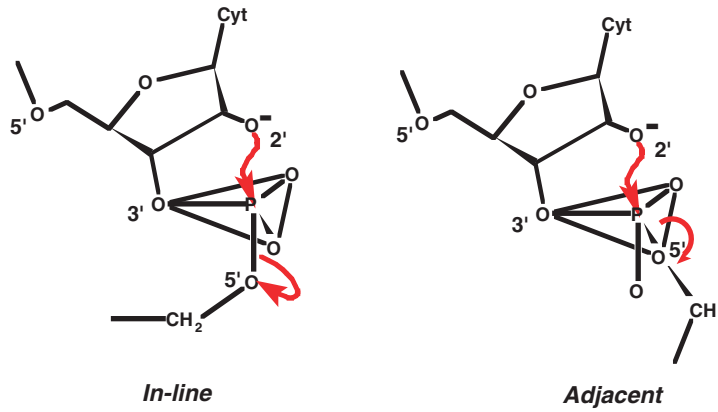

Figure 4.2 Different phosphate backbone conformations required for an "in-line" *vs.* an "adjacent" cleavage mechanism.

The 5′-product, as a result of this cleavage reaction mechanism, possesses a 2′,3′-cyclic phosphate terminus, and the 3′-product possesses a 5′-OH terminus,[20–21] as with non-enzymatic alkaline cleavage of RNA. The reaction is therefore, in principle, reversible, as the scissile phosphate remains a phosphodiester, and may thus act as a substrate for hammerhead RNA-mediated ligation without a requirement for ATP or a similar exogenous energy source. The hammerhead ribozyme-catalyzed reaction, unlike the formally identical non-enzymatic alkaline cleavage of RNA, is a highly sequence-specific cleavage reaction with a typical turnover rate of approximately one molecule of substrate per molecule of enzyme per minute at pH 7.5 in 10 mM Mg^{2+} (so-called "standard reaction conditions" for the minimal hammerhead RNA sequence), depending upon the sequence of the particular hammerhead ribozyme construct measured. This represents an approximately 10 000-fold rate enhancement over the non-enzymatic cleavage of RNA.

4.5 Hammerhead Ribozyme Structure Nailed Down

The first hammerhead ribozyme 3D structure was published in 1994 by Pley, Flaherty and McKay.[22] This structure consisted of a 34 nucleotide hammerhead enzyme strand consisting of unmodified RNA, and a 13 nucleotide hammerhead substrate-analogue composed of DNA that functions as a competitive inhibitor of the ribozyme, preventing cleavage due to the lack of a nucleophile at the active site (Figure 4.3a and b). It was the first RNA whose structural complexity approached that of tRNA published in the 20 years subsequent to the elucidation of the yeast phenylalanine tRNA structures and, more importantly, it was the first structure of a catalytic RNA. This breakthrough was celebrated in an accompanying *News and Views* highlight in *Nature*, authored by Thomas Cech and Olke Uhlenbeck, entitled

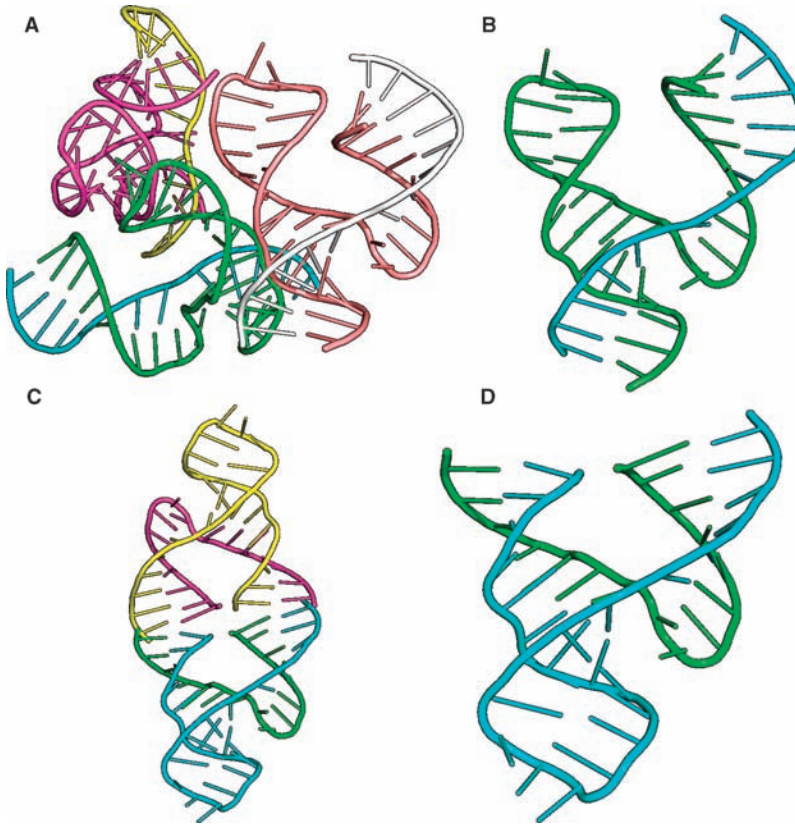

Figure 4.3 Three crystallographically independent hammerhead ribozymes (*A*) occupied the asymmetric unit in the first hammerhead crystal structure; one of these is displayed in what has become the conventional orientation (*B*) such that the RNA enzyme strand (green) and DNA substrate-analogue (cyan) are clearly visible. Two crystallographically independent all-RNA hammerheads occupied the asymmetric unit (*C*) of the second hammerhead ribozyme crystal structure. One of these (*D*) is chosen and displayed to facilitate comparison with (*b*). Here, the shorter strand is (nominally) the enzyme strand and the longer strand (cyan) is the substrate, where a 2′-OMe nucleotide occupies the cleavage site. A subsequent crystal form having but one molecule per asymmetric unit (not shown) is otherwise identical, despite the absence of the 2′-OMe modification.

"Hammerhead nailed down".[23] Subsequent publication of two all-RNA hammerhead ribozyme crystal structures, the first with a 2′-OMe replacing the nucleophile at the active site,[24] and the second with an unmodified nucleotide at the active site,[25] revealed the same 3D structure of the invariant core nucleotides in the ribozyme well within experimental error (Figure 4.3c and d), despite significant differences in the sequence within the nonessential regions, DNA *vs.* RNA substrates, presence *vs.* absence of divalent metal ions and active

nucleophile, and crystal packing schemes. In essence, the spatial positioning of the invariant region of the hammerhead ribozyme appeared to be immune to a series of potential perturbations in at least six crystallographically independent molecules, further corroborating, it seemed, the initial assessment that the structure had indeed been "nailed down" in 1994.

4.6 Catalysis in the Crystal

The hammerhead ribozyme sequence that contained all unmodified RNA, including the active-site nucleophile, was catalytically active both in solution and in the crystal.[25,26] The crystals of the active hammerhead ribozyme thus enabled us to test for cleavage activity in the crystalline state. We initiated the cleavage reaction by flooding the crystal with divalent cations while raising the pH above the apparent kinetic pK_a using a soaking solution buffered at pH 8.5.[25–27] In conditions with a Mg^{2+} concentration of 50 mM and pH 8.5, the self-cleavage rate in the crystal is approximately 0.4 molecules min^{-1}. Under similar conditions in solution, this same hammerhead ribozyme construct, a sequence that was optimized for purposes of growing crystals rather than for catalytic prowess, cleaves at a rate of approximately 0.08 molecules min^{-1}, permitting us to suggest that the crystal lattice did more to aid in the proper folding of the ribozyme than it did to inhibit the hammerhead ribozyme's cleavage activity. Moreover, the extent of cleavage of the substrate in the crystal was almost complete.[26]

Although this collection of minimal hammerhead ribozyme crystal structures provided rationalizations for several of the previously reported experimental observations, many important problems remained unresolved (see, for example, ref. 14). One of the most important and immediately recognized[22] problems was that the scissile phosphate in all of the structures just described was observed to be in a conformation that is completely incompatible[28] with an "in-line" mechanism. Hence the need to bring the scissile phosphate into a conformation amenable to an in-line attack can be (and in fact was) taken as *prima facie* evidence for a required conformational change prior to bond cleavage.

4.7 Making Movies from Crystallographic Snapshots

The fact that the minimal hammerhead RNA sequence can cleave after being crystallized[25] presented the opportunity to capture various states, including pre-catalytic conformational changes, along the reaction pathway, using crystallographic freeze-trapping techniques.

Although conventional X-ray crystallography is essentially static and by necessity involves both a spatial and a temporal averaging of a macroscopic number of molecules and a time period of hours (the duration of data collection), time-resolved crystallographic experiments may be possible under the

right conditions. Two options for time-resolved experiments exist that permit one to obtain crystallographic snapshots during catalysis.

The Laue method uses polychromatic X-rays to enable collection of a fairly complete X-ray dataset in a matter of milliseconds or less, and has been used with success in the case of many protein enzymes that catalyze rapid reactions.[29,30]Laue experiments require ideal crystals that possess very low mosaicity, as well as a way of very rapidly initiating a reaction simultaneously throughout the crystal lattice. Once the reaction has been initiated, each enzyme–substrate complex evolves stochastically with respect to time. Intermediates can be observed only when the majority of molecules in a crystal occupy the same intermediate state at the same time. Lower-occupancy intermediates remain essentially unobservable.

When combined with flash-freezing (*e.g.*, immersion in liquid nitrogen) as a physical trapping method, conventional monochromatic X-ray data collection also offers the opportunity to conduct time-resolved experiments using a second approach. Instead of recording live snapshots, as with the Laue method, the reaction is simply initiated in the crystal, allowed to evolve, and the molecular contents of the crystal are immobilized by freeze-trapping prior to standard data collection.[31] Since most conventional X-ray data collection is already performed using crystals frozen at 100 K, this procedure in practice involves very little additional experimental modification, and possesses the added advantage of being much more tolerant of mosaicity and the other small crystal imperfections that often accompany reactivity in the crystal. The utility of this approach is confined to comparatively slowly reactive enzymes. Fortunately, the cleavage rate of the minimal hammerhead ribozyme not only is on the order of 1 min^{-1}, it can be further modulated as a function of pH and presence of divalent cations, making it an ideal candidate for the freeze-trapping approach.

To better understand the structural basis of the proposed conformational change required to activate the hammerhead ribozyme for catalysis, we performed monochromatic time-resolved crystallographic freeze-trapping studies with the aim of observing conformational intermediates preceding catalysis.[25,27,32,33] To trap the structure of a precatalytic structural intermediate, a hammerhead ribozyme having a "kinetic bottleneck" at the final or bond-breaking point on the reaction pathway was synthesized using a modified leaving-group, (Figure 4.4a). This modified hammerhead RNA was used to capture a conformational intermediate that began to approach an in-line conformation[27] that evolved subsequent to triggering the reaction (Figure 4.4b). Prior to triggering the reaction, the modified RNA possessed a structure indistinguishable from that of the initial-state structure of the unmodified RNA, an important control that was conducted and reported,[27] contrary to some claims that have been made.[34] Two other pre-catalytic conformational changes were also captured using complementary approaches that did not involve modification of the leaving group, and appeared consistent in that they made up a trajectory toward an in-line structure.[25,33] (In retrospect, this

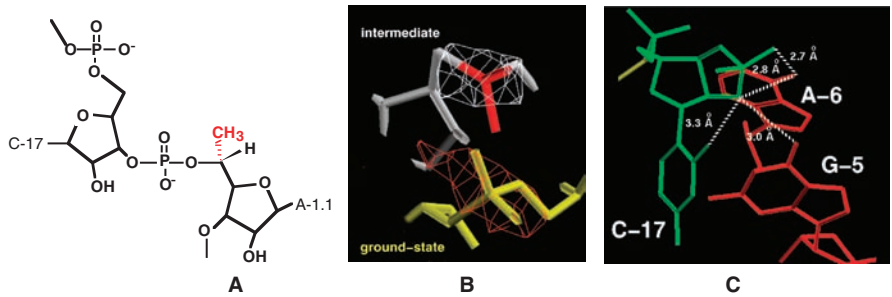

Figure 4.4 (*A*) Depiction of the talo-5′-methyl-ribose "kinetic bottleneck" modification of the hammerhead ribozyme leaving group. (*B*) Initial (yellow) and intermediate (gray) structures, with the scissile phosphate of the intermediate modeled into positive difference density (white), and the initial-state phosphate occupying negative difference density (red). (*C*) Structure of the cleavage product.

structure still would require significant additional but unobserved changes to reach the transition-state.)

The structure of the enzyme-product complex (Figure 4.4c) was also obtained.[32] In this structure, it is apparent that C-17 has rotated fully away from the CUGA uridine turn and occupies a site in which the newly generated 2′,3′-cyclic phosphate can potentially interact with the Watson–Crick pairing face of the invariant core residues G5 and A6. The crystals that permitted us to observe the product structures were actually mixtures of (at least) two partially occupied states, so we were reluctant to ascribe too much significance to any but the most well-defined structural changes.

When placed together in succession, the initial-state structure, three pre-catalytic conformational intermediate structures, and then the cleavage product, enabled construction of a five-frame "movie" that revealed a plausible reaction trajectory that we suggested might represent "at least a subset"[26,33] of the conformational movements required for catalysis.

4.8 An Ever-growing List of Concerns

Despite the observations of hammerhead ribozyme catalysis in a crystal in which the crystal lattice packing contacts by necessity confined the global positions of the distal termini of all three flanking helical stems,[25–27,32,33,35] many biochemical experiments designed to probe transition-state interactions and the chemistry of catalysis appeared to be irreconcilable with the crystal structures.

For example, the invariant core residues G5, G8, G12 and C3 in the minimal hammerhead ribozyme were each observed to be so fragile that changing even a single exocyclic functional group on any one of these nucleotides results in a dramatic reduction or abolition of catalytic activity,[14] yet few of these appeared to form hydrogen bonds involving the Watson–Crick faces of these nucleotide

bases in any of the minimal hammerhead structures, apart from a G-5 interaction in the product structure.

A particularly striking and only recently observed example consisted of G8 and G12, which were identified[36] as possible participants in acid–base catalysis (Chapter 3). Once it was demonstrated that the hammerhead RNA does not require divalent metal ions for catalysis,[37,38] it gradually became apparent that that the RNA itself, rather than passively bound divalent metal ions, must play a direct chemical role in any acid–base chemistry in the hammerhead ribozyme active site. It was, however, completely unclear how G12 and G8 could accomplish this, given the original structures of the minimal hammerhead ribozyme.

Other concerns included an NOE between U4 and U7 of the cleaved hammerhead ribozyme[39] that had also been observed during NMR characterization, which suggested that these nucleotide bases must approach one another closer than about 6 Å, although close approach of U7 to U4 did not appear to be possible from the crystal structure. Finally, as previously discussed, the attacking nucleophile in the original structures, the 2′-OH of C17, was not in a position amenable to in-line attack upon the adjacent scissile phosphate.[22]

Perhaps most worrisome were experiments that suggested the A-9 and scissile phosphates must come within about 4 Å of one anther in the transition-state, based upon double phosphorothioate substitution and soft metal ion rescue experiments;[40] the distance between these phosphates in the crystal structure was about 18 Å, with no clear mechanism for close approach if the stem II and stem I A-form helices were treated as rigid bodies. Taken together, these results appeared to suggest that a fairly large-scale conformational change must have take place to reach the transition-state within the minimal hammerhead ribozyme structure.

For these reasons, the two sets of experiments (biochemical *vs*. crystallographic) appeared not only to be at odds, but to be completely and hopelessly irreconcilable, generating a substantial amount of discord in the field.[34] No compelling evidence for dismissing either set of experimental results was ever made successfully, although many claims to the contrary[34,40,41] were made in favor of each.

4.9 Occam's Razor Can Slit Your Throat

The principle of parsimony, attributed to William of Occam, states that one should favor simple scientific explanations and hypotheses that make a minimum number of assumptions over more complicated alternatives. The "Occam's Razor" principle, in which one shaves away extraneous and irrelevant decorations to obtain the most parsimonious hypothesis, has also been extended to the physical investigation of nucleic acids and proteins: study the simplest macromolecular assemblies that possess the biological function of interest, and cut everything else off. This "reductionist paradigm" has been employed with such success in molecular biology that one can easily lose sight of the larger, physiological context.

When the hammerhead RNA was first discovered, it was observed in a ~370 nucleotide single-stranded genomic satellite RNA, most of which could be deleted while preserving the RNA's catalytic properties.[6–8] Eventually, it was found that about 13 core nucleotides and a minimal number of flanking helical nucleotides were all that was required for a respectable catalytic turnover rate of 1 to 10 min^{-1}, and this "minimal" hammerhead construct became the focus of almost all of the biochemical, biophysical and crystallographic investigations, as described earlier.

It thus came as a great surprise to most in the field when, in 2003, it was finally pointed out that for optimal activity, the hammerhead ribozyme requires the presence of sequences in stems I and II that interact to form tertiary contacts[42,43] (Figure 4.1b) that were removed in the process of shaving seemingly superfluous structures from the hammerhead ribozyme. Once the full ramifications of this revelation became apparent, *i.e.*, that the entire field had been studying the residual catalytic activity of an over-zealously truncated version of the full-length ribozyme, attention shifted away from the minimal constructs.[44] It also quickly became clear to us that a crystal structure of the full-length hammerhead ribozyme, in which these distal tertiary contacts were present, might be of considerable interest.

4.10 Structure of a Full-length Hammerhead Ribozyme

After several years of struggle, in 2006 we finally obtained a 2.2 Å resolution crystal structure of the full-length hammerhead ribozyme.[45] This new structure (Figure 4.5) appears to resolve the most worrisome of the previous discrepancies. In particular, C17 is now positioned for in-line attack, and the invariant residues C3, G5, G8 and G12 all appear involved in vital interactions relevant to catalysis. Moreover, the A9 and scissile phosphates are observed to be 4.3 Å apart, which is consistent with the idea that, when modified, these phosphates could bind a single thiophilic metal ion. The structure also reveals how two invariant residues, G-12 and G-8, are positioned within the active site – consistent with their previously proposed[36] role in acid–base catalysis. G12 is within hydrogen bonding distance to the 2′-O of C17, the nucleophile in the cleavage reaction, and the ribose of G8 hydrogen bonds to the leaving group 5′-O (Figure 4.6), while the nucleotide base of G8 forms a Watson–Crick pair with the invariant C3. This arrangement permits us to suggest that G12 is the general base in the cleavage reaction, and that G8 may function as the general acid, which is consistent with previous biochemical observations.[36] G5 hydrogen bonds to the furanose oxygen of C17, helping to position it for in-line attack. U4 and U7, as a consequence of the base-pair formation between G8 and C3, are now positioned such that an NOE between their bases is easily explained.

The crystal structure of the full-length hammerhead ribozyme thus clearly addresses all of the major concerns that appeared irreconcilable with the previous crystal structure.[46]

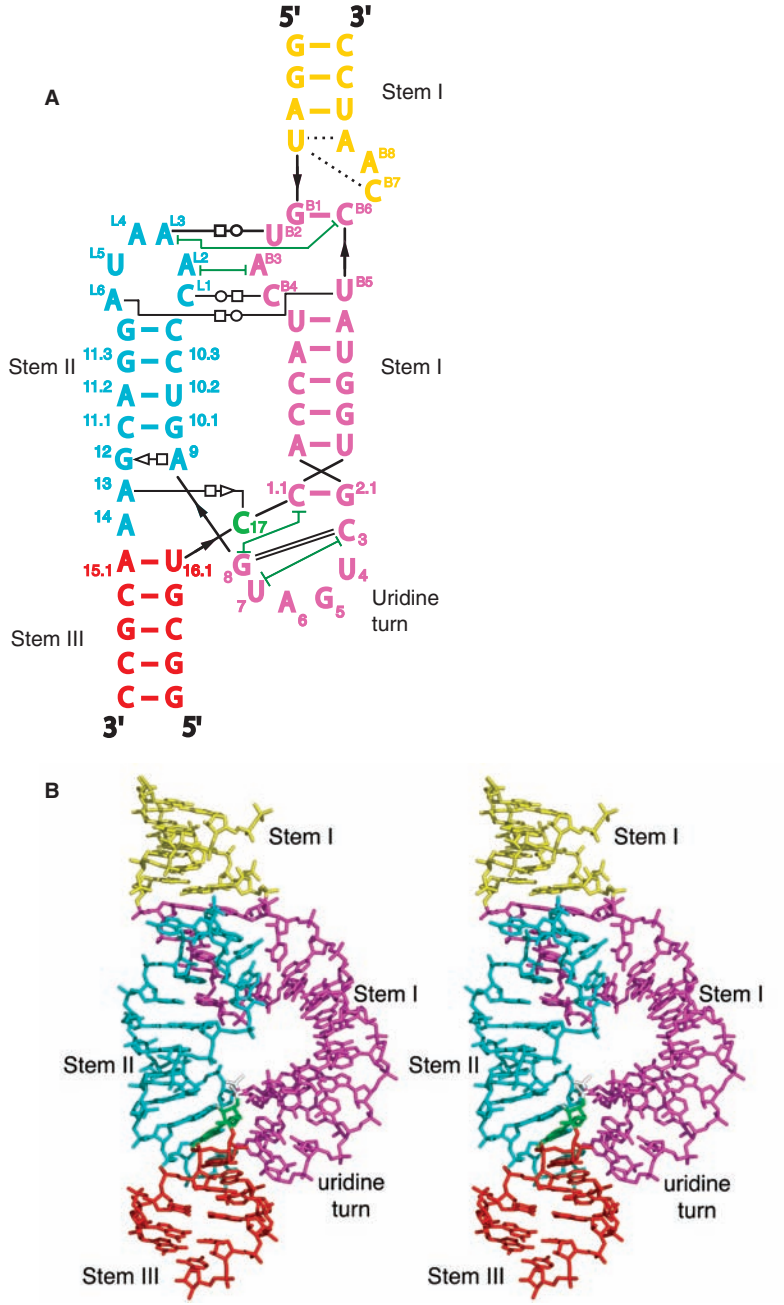

Figure 4.5 Secondary (*A*) and tertiary (*B*) structure of the full-length hammerhead ribozyme. The color-coding of the secondary structure corresponds to that of the tertiary structure. The cleavage-site nucleotide, C-17, is depicted in green in both figures.

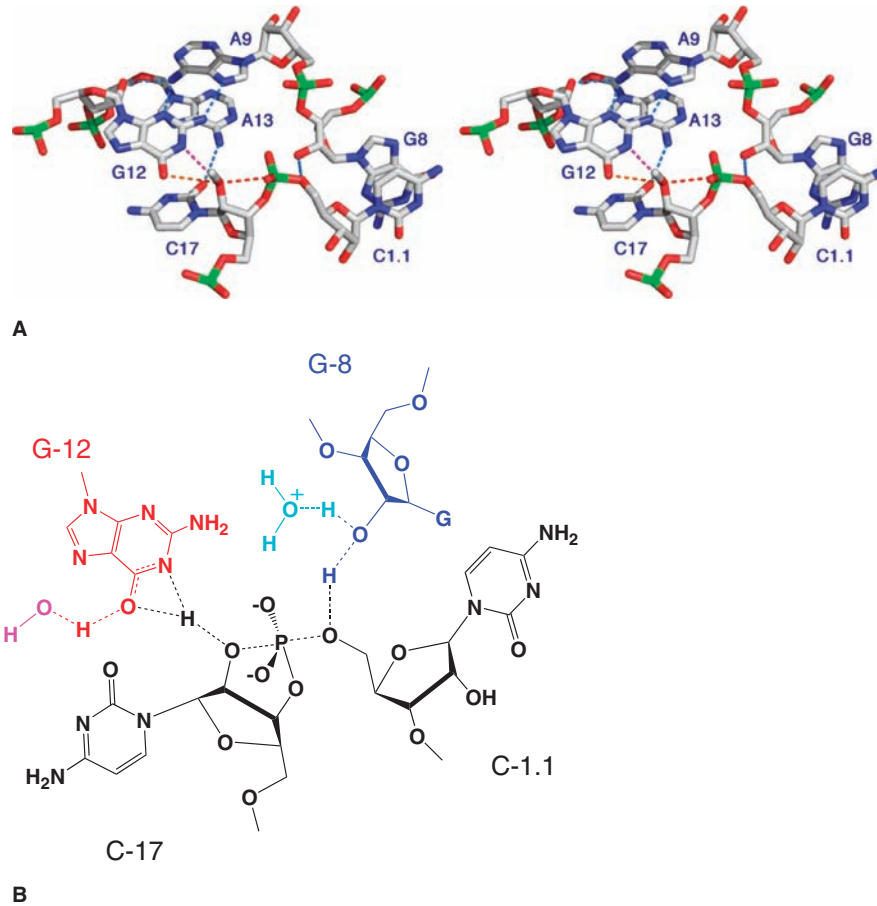

Figure 4.6 Wall-eyed stereo close-up view of the active site (*A*) of the full-length hammerhead ribozyme, and a corresponding mechanistic hypothesis (*B*) based upon the observed arrangement of nucleotides in (*A*).

4.11 Do the Minimal and Full-length Hammerhead Crystal Structures have Anything in Common?

Although the structure of the full-length hammerhead ribozyme, at first glance, appears to be radically different from the minimal hammerhead, both share some similarities in structure in both the global fold and in detail. The similarities are best seen by comparing the set of nucleotides both share in common. Specifically, if one compares the core residues and the first five base-pairs of stem I, as well as the shared residues of stems II and III, while omitting the capping loops, the similarities become most apparent. A side-by-side comparison of the folds of the minimal and full-length hammerheads is shown in Figure 4.7, each with a yellow substrate strand that includes the cleavage-site

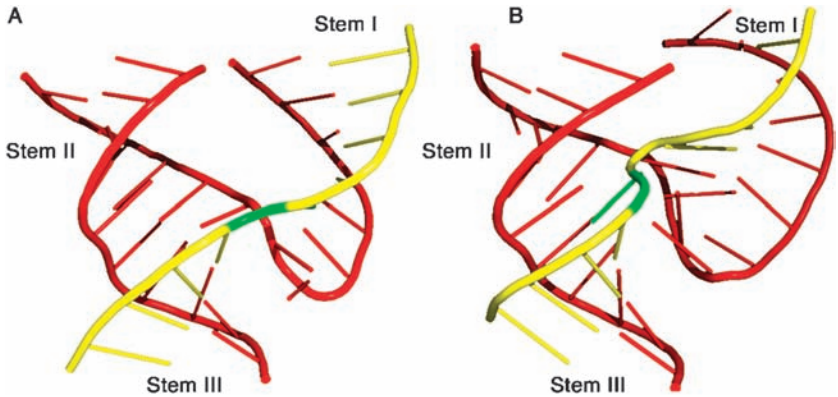

Figure 4.7 Backbone diagrams of the minimal (*A*) and full-length (*B*) hammerhead ribozyme core regions, showing the similarity of the local fold.

nucleotide highlighted in green. What is apparent from the comparison made in this manner, in which only the shared nucleotides are considered, is that the folds are strikingly similar, the largest difference being the kink in the substrate strand at the cleavage site that accompanies rearrangement of the active site nucleotides.

The significance of this observation is that it explains why the hammerhead ribozyme cleavage reaction could take place in crystals of the minimal construct.[25] These crystals, being 78% solvent by volume, permit molecular motions to take place subject to constraints imposed by the crystal lattice contacts. The lattice contacts of the minimal hammerhead restrict the distal termini of stems I, II and III.[25]

4.12 How the Does the Minimal Hammerhead Work?

In solution, the simplest explanation (Occam's Razor is more safely applied to scientific hypotheses than to macromolecules) for all of the observed minimal hammerhead biochemistry (including the invariance of G5, G8, G12, C3 and the proximity of the A9 and scissile phosphates, as well as the 1000-fold slower cleavage rate of the minimal hammerhead) is that the active conformational state, which resembles the structure of the full-length hammerhead, occurs only transiently, such that only about 0.1% of the uncleaved molecules occupy this state at any given time. Thus for cleavage to occur, a transient conformational change must occur that deforms the structure observed in the minimal hammerhead crystals into that resembling the full-length hammerhead, in which the nucleotides critical for catalysis are correctly positioned.

This rearrangement can in fact also take place within the confines of the crystal lattice, because only the distal ends of the three helical stems are restricted in movement by the crystal contacts. Alternative hypotheses,

including the suggestion that the minimal hammerhead cleaves *via* a different pathway than that of the full-length hammerhead, and that the minimal hammerhead structure in solution is identical to the full-length hammerhead conformation observed in the crystal structure, have considerably less explanatory power. The first hypothesis cannot explain the requirement for the invariant residues, and the second hypothesis cannot account for the observed 1000-fold rate enhancement. Hence it seems most likely that in solution, the minimal hammerhead has nearly the same structure as it does in the crystal, and that in both cases the minimal ribozyme only occasionally visits the conformation that is stabilized and therefore dominates in the full-length hammerhead construct.

4.13 A Movie Sequel with a Happy Ending

In silico adiabatic morphing[47] of the minimal into the full-length hammerhead ribozyme structure is possible when the nucleotides shared in common by both hammerhead constructs are interpolated. The structure observed in the minimal hammerhead ribozyme can be continuously deformed *via* low energy-barrier torsion angle conformational changes into the structure observed in the full-length hammerhead. This process is best represented as a series of consecutive structures viewed as a movie (link to Quicktime movie: http://tinyurl.com/2vly4d). It is likely that the first (1994 and 1995), initial-state hammerhead ribozyme crystal structures, represent more or less accurately the dominant structure of the minimal hammerhead ribozyme in the crystal – consistent with the minimal hammerhead being 1000-fold less active in solution than the full-length hammerhead. The product, or cleaved state, of the minimal hammerhead in some ways resembles the full-length hammerhead to a greater extent than does the uncleaved minimal hammerhead structure. Specifically, in the cleaved structure,[32] the cleavage-site base, C-17, is observed to make contacts with G5 and A6 that are similar to those observed in the full-length structure, and the interactions with C3 are completely absent in both cases.

The more developed cleavage intermediates, in retrospect, appear to resemble a torsion angle conformational change of only about 1/3 of what is required to morph the structure from the minimal hammerhead to the full-length hammerhead active-site conformation. The crystallographic observations of various states along the cleavage reaction pathway thus appear in retrospect to be more incomplete than erroneous, in that they appear consistent with the first $\sim 1/3$ of the conformational change ultimately required to reach a structure similar to that of the full-length hammerhead. Missing by necessity from the set of snapshots was the low-occupancy transient conformation that is stabilized by the distal tertiary contacts in the full-length hammerhead ribozyme. In crystallographic experiments, one can only hope to resolve the dominant, high-occupancy, species in the crystal, so it is likely that the true pre-catalytic intermediate would never be observed crystallographically in the context of the minimal hammerhead construct.

4.14 Concluding Remarks

In summary, it appears that the actual experimental data obtained from the crystallographic analyses and the biochemical characterizations, which were performed on high-occupancy, near-ground-state and transient near-transition-state structures, respectively, were sound within the confines imposed by the minimal hammerhead structure. The mutually held interpretation that acceptance of one set of experimental results precluded acceptance of the other, however, was based on the flawed assumption that the two sets of observations were incommensurate and irreconcilable. In our case, the flawed assumption manifested itself most explicitly as the claim that unwinding and unpairing of helical elements was unlikely to take place.[41] In the other case, the flawed assumption manifested itself with the claim that any cleavage observed in the crystal must be due to an off-pathway artifact or experimental incompetence.[34,40] In retrospect, neither dismissal was justified, nor compelling. The resolution of the apparent paradox came with the structure of the full-length hammerhead, which reconciles and permits explanations of both sets of experimental results.

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Ribozymes and RNA Catalysis

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Chapter 4

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