Molecular Biology Reports **22:** 115–123, 1996.

Analysis of the tertiary structure of bacterial RNase P RNA

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Key words: ribonuclease, ribozyme, RNase P RNA, RNA structure, tRNA

Abstract

The ubiquitous occurrence of ribonuclease P (RNase P) as a ribonucleoprotein and the catalytic properties of bacterial RNase P RNAs indicate that RNA fulfills an ancient and important role in the function of this enzyme. This review focuses on efforts to determine the structure of the bacterial RNase P RNA ribozyme. Phylogenetic comparative analysis of a library of bacterial RNase P RNA sequences has resulted in a well-developed secondary structure model and allowed identification of some elements of tertiary structure. The native structure has been redesigned by circular permutation to facilitate intra- and inter-molecular crosslinking experiments in order to gain further structural information. The crosslinking constraints, together with the constraints provided by comparative analyses, have been incorporated into a first-order model of the structure of the ribozyme-substrate complex. The developing structural perspective allows the design of self-cleaving pre-tRNA-RNase P RNA conjugates which are useful tools for additional structure-probing experiments.

Abbreviations: cpRNA – circularly permuted RNA.

Introduction

Ribonuclease P (RNase P) is a ubiquitous enzyme that catalyzes an essential step in tRNA maturation. In the presence of required divalent metal ion cofactors (optimally magnesium) RNase P catalyzes the hydrolysis of a specific phosphodiester bond in pre-tRNA to generate the 5′ end of mature tRNA. Analysis of RNase P enzymes from representatives of all three phylogenetic domains, Bacteria, Archaea, and Eucarya, has revealed that all contain an essential RNA component. In Bacteria the RNase P holoenzyme is composed of single protein (ca. 120 amino acids) and RNA (ca. 400 nucleotides) components. Under *in vitro* reaction conditions of high ionic strength, bacterial RNase P RNAs retain the substrate-binding and catalytic properties of the holoenzyme, and so are one class of large ribozymes [1,2]. The widespread occurrence of RNase P as a ribonucleoprotein and the catalytic nature of the bacterial RNase P RNAs suggests an ancient and important role for RNA in the function of this enzyme. A detailed understanding of the function of RNase P RNA requires perspective on its three-dimensional structure and the roles of specific functional groups in substrate binding and catalysis.

A limited number of methods are available for determining the structures of large RNAs. Detailed structures of tRNAs [3,4] and most recently the hammerhead ribozyme [5,6] have been determined by X- ray crystallography. Attempts to crystallize and solve the structures of large RNAs have proven largely unsuccessful, however. Recent developments in NMR spectroscopy have helped elucidate the structures of several small RNA motifs [e.g. 7,8], but the redun-

dancy of the nucleotide subunits limits the usefulness of NMR to analysis of RNAs of less than about fifty nucleotides. Phylogenetic comparative analyses have been used to derive the secondary structures of several RNAs, and global tertiary structure models of 16S, 5S and Group I intron RNAs based on phylogenetic and other experimental data, have been proposed [9–13]. Although limited in resolution, such models provide useful frameworks for further experimentation and interpretation of structural data. A detailed model of the secondary structure of bacterial RNase P RNA is now available. With that model as a base, experimentally derived structural constraints together with new structure modeling techniques are helping to refine our perspective on the structure of the bacterial RNase P ribozyme.

The phylogenetic-comparative approach

Phylogenetic-comparative sequence analysis is the most useful method for determining *in vivo* RNA secondary structure and some elements of tertiary structure [14]. In this method, RNA sequences of common ancestry are compared and structure is inferred based on changes, or 'covariations', that occur in concert between sequences. For example, paired bases in helices covary to maintain complementarity. The secondary structure of bacterial RNase P RNA is now well defined by comparison of over 80 different sequences (Figure 1) [15–17]. The nomenclature used in describing the secondary structure is modeled after that of group I introns [18] as described [16]. Helices are numbered as they occur 5′ to 3′, preceded by the designation P (paired; e.g. P3 is the third helix from the 5′ end). The loops of individual helices are numbered as the helix with which they are associated, with the prefix L (loop; e.g. L3 is the loop on P3). Sequences that occur between helices are numbered with the helices they connect, with the prefix J (joining; J3/4 is the sequence linking P3 and P4).

Sequence comparisons identify a subset of helices, termed core helices (P1–11 and P15), that are present in all bacterial RNase P RNAs (Figure 1B) and so are likely to be important in the function of the ribozyme. In addition, there are regions of the secondary structure which in different organisms often contain non-conserved structural elements. Most of these variably present helices are subject to substantial variation in sequence-length, indicating that they are likely to be located on the periphery of the common core tertiary structure. Experimental deletion of variably present helices generally results in some destabilization of enzyme structure, but not misfolding of the residual RNAs since they retain significant enzymatic activity. Examples of such dispensable elements include P16/P17/P6, P12, P13/P14 and P18, each of which is not present in some RNase P RNAs and which can be individually deleted from the *Escherichia coli* version of the RNA without significant effect on catalytic function [16,19]. Deletion of any one of these elements results, however, in an enzyme which is thermally less stable than the native RNA and requires increased levels of monovalent ion for optimal catalytic activity *in vitro*. The picture that is emerging from structural studies (see below) is that these variably present elements act as stabilizing struts that are anchored at several sites and serve to reinforce the core structure of the RNA.

Sequence comparisons also identify phylogenetically conserved nucleotides, which presumably are particularly important for structure or function. Nucleotides that are invariant among all known bacterial RNase P RNAs are distributed throughout the core of the secondary structure model (Figure 1B). A subset of these nucleotides can be found in homologous positions in the archaeal type of the RNA [20]. A still- smaller subset of homologous nucleotides may occur in eucaryal RNAs as well, although it is not yet possible to rigorously align the eucaryal and bacterial RNase P RNA sequences [21]. It is imagined that the active site of the bacterial ribozyme is composed of several conserved functional groups located in separate regions of the RNA chain and brought together in the three-dimensional structure. Generally, deletion or mutation of many of the conserved nucleotides individually has only marginal effects on the catalytic activity of the ribozyme [22–25]. As with deletions of variably present helical elements, most effects of mutations in conserved sequences can be suppressed by elevated concentrations of monovalent ions. In fact, every nucleotide in RNase P RNA can be deleted individually without completely abolishing enzymatic activity [26]. In some portions of the molecule, however, for instance sequences in or adjacent to helix P4, deletions resulting in gapped molecules reduce enzyme activity by several orders of magnitude. Determining the identity and arrangement of functional groups in the active site of the enzyme remain important goals.

Interactions of tertiary structure are more difficult to identify by comparative analysis than base-pairs which comprise secondary structure. Bases involved in tertiary structure are generally more conserved and path-

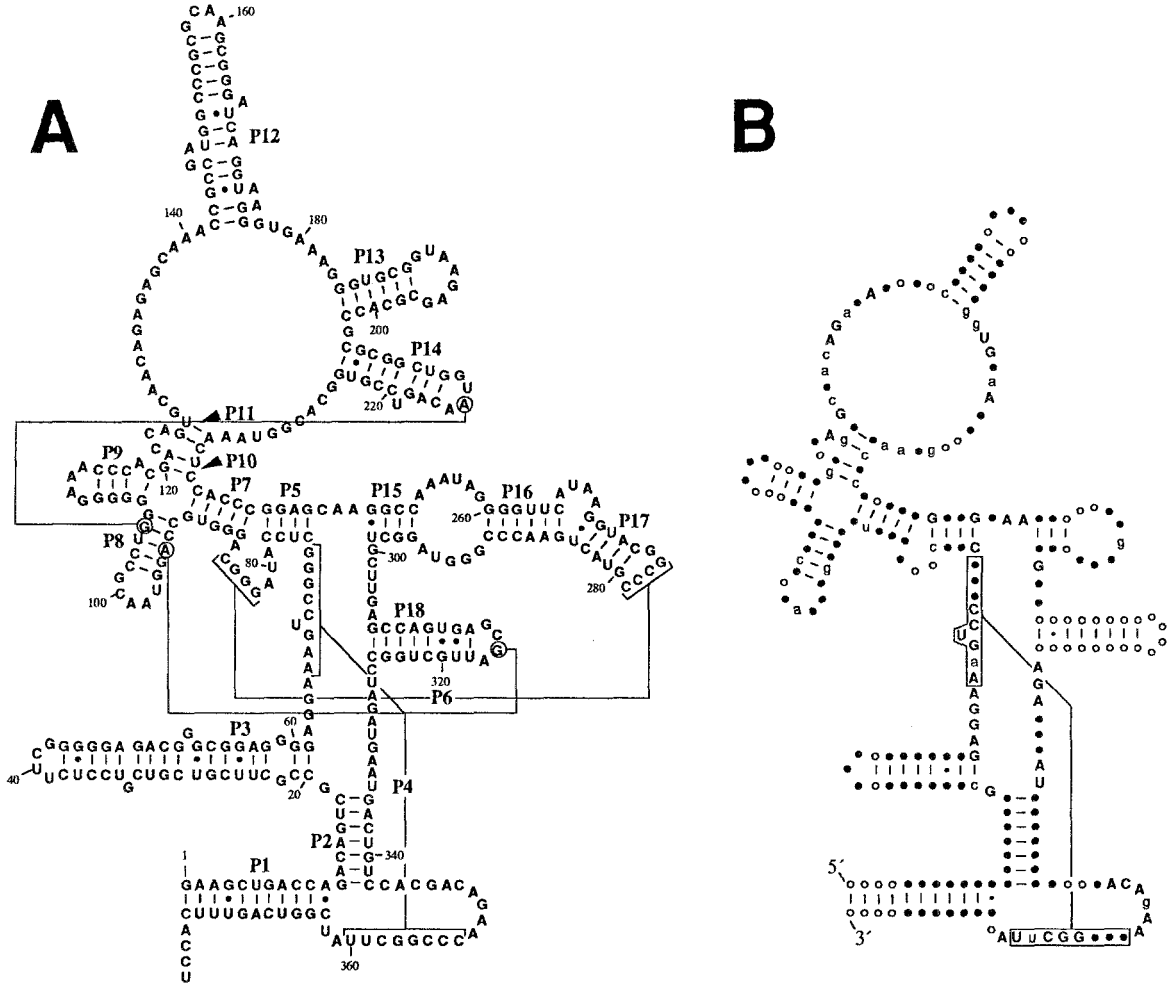

Figure 1. Bacterial RNase P RNA secondary structure. (B) Secondary structure of *E. coli* RNase P RNA determined by phylogenetic comparative analysis. The helix nomenclature is that developed for group I introns [18] as described for use with RNase P RNA [16]. Base pairings in P4 and P6 are shown as brackets and lines. Nucleotides involved in base-triple interactions are circled and connected by lines. Two base-triple interactions are indicated by circled nucleotides connected by lines. (B) Phylogenetic minimum bacterial consensus RNase P RNA. Base pairs in P4 are shown as brackets and lines. Only the structural elements present in all known bacterial RNase P RNAs are included. Universally conserved nucleotides are in upper case letters; those which are at least 80% conserved are shown in lowercase. Nucleotides not conserved in identity, but present in all bacterial RNase P RNAs are indicated by filled circles; those which are present in at least 80% of sequences are indicated by open circles.

ways leading to covariation are potentially more complex. Thus, accumulation of large sequence collections is required to identify elements of tertiary structure by comparative approaches. Recently, PCR amplification of DNAs from mixed, naturally occurring microbial populations, using oligonucleotide primers complementary to highly conserved RNase P RNA sequences, was used to obtain 52 novel RNase P RNA genes [17]. Comparative analysis of the expanded sequence collection confirmed and refined the secondary structure model based on sequences from cultivated organisms, and also provided evidence for some elements of tertiary structure. Two of the interactions involve the docking of GNRA tetraloops into helices by triple base pair interactions (Figure 1A). The purine and conserved adenosine in the GNRA tetra loops formed by L14 and L18 are proposed to interact with base pairs in P8. Although this kind of structural motif appears to be common in large RNA structure [12,27,28], RNase P RNA is so far unique in that it contains two such interactions occurring at adjacent base pairs in a helix. These long-range interactions provide important con-

straints for modeling of global structure, but even in light of the well-developed secondary structure model are insufficient to specify a general conformation for the ribozyme.

Circularly permuted RNAs as photoaffinity probes of tertiary structure

One method for obtaining additional information bearing on the tertiary structure of RNA chains is chemical crosslinking to establish proximal regions within or between molecules. The attachment of chemical reagents to functional groups on the interior of RNAs, although possible [29], is difficult to accomplish in an efficient and specific manner. On the other hand, the termini of RNAs are readily and specifically modified due to the presence of unique chemical groups. Circularly permuted RNAs (cpRNAs [30–32]) offer the ability to attach specific chemical reagents to nucleotides that are normally on the interior of RNAs with the specificity and efficiency of end modification. We have used circularly permuted tRNAs and RNase P RNAs to attach photoactivatable crosslinking agents at a variety of strategic sites in the ribozyme-substrate complex in order to investigate its global architecture [32–35].

The library of distance constraints obtained by chemical crosslinking, along with the established secondary structure, has been used to infer models of the ribozyme-substrate complex [34, 36]. The model by Westhof and Altman [36] treats the entire RNA. The model of Harris et al. [34], shown here (Figure 2A & B), is based on considerably more data than the other model, but does not include nucleotides 127–243 because the available data are insufficient to specify the structure without undue speculation. There are substantive differences in the two models, but some of the core helices are positioned similarly. One key difference involves the acceptor stem of the pre-tRNA substrate which is modeled as unfolded in the Westhof and Altman structure but retains the native tRNA structure in the model of Harris et al. [34]. Although the global model lacks atomic resolution, considerable of it can be extrapolated to the atomic level since helical elements are expected to adopt the A-form of helix. The global models position the core helices in specific ways relative to the substrate, pre-tRNA. Most notable in the model of Harris et al. [34] is the position of helix P4, which is conserved in both sequence and structure, and is located near the substrate phosphate in pre-tRNA (Figure 2A). This draws many of the conserved nucleotides in RNase P RNA into the proximity of the acceptor stem of the tRNA (Figure 2B), consistent with the expectation that the conserved sequences are involved in forming the active site of the ribozyme. In the Westhof and Altman model the nucleotides which make up the acceptor stem are more distant from the highly conserved P4 helix. Crosslinking experiments with tRNA modified with a photoagent at the 5′ terminus directly identify conserved nucleotides in J5/15 and J18/4 as being in close proximity to the substrate phosphate in the enzyme- substrate complex [33]. Intermolecular crosslinking using tRNA modified at position 53, at the TψC loop, indicates that conserved nucleotides in P9 are likely involved in making specific contacts with tRNA, consistent with the protection of a nucleotide in P9 (A118) from chemical modification in the presence of substrate [32,37]. Likewise, intermolecular crosslinking experiments with tRNA modified at positions 64 and 69 suggest that the J3/4 region is also involved in contacting tRNA at the base of the acceptor stem.

Crosslinking and chemical protection studies both identify the internal bulge between P15 and P16 as the binding site for the conserved tRNA 3′ terminal CCA sequence [35,37]. 3′ photoagent-modified tRNA crosslinks to this region and residues in the P15-P16 internal bulge are protected by the 3′ CCA sequence on the bound tRNA. Results with tRNAs lacking individual nucleotides of the 3′ CCA orient the interaction on the internal loop in RNase P RNA; the CCA sequence is parallel to nucleotides 254–259 and anti-parallel to nucleotides 290–295. Chemical and enzymatic susceptiblilty of residues in this internal bulge indicate that it may form an irregular helix. One or more of the terminal C residues likely interacts, perhaps by direct Watson-Crick base pairing [38], with exposed functional groups in one groove of the irregular helix formed by the P15-P16 internal bulge [Easterwood and Harvey pers. comm.].

Crosslinking analysis continues to provide important constraints on the position and orientation of the core helices and substantially refines the structural perspective [Harris, ME & Pace, NR, unpub.]. However, a more detailed picture of the architecture of the ribozyme requires information bearing on roles of specific functional groups in structure and catalysis.

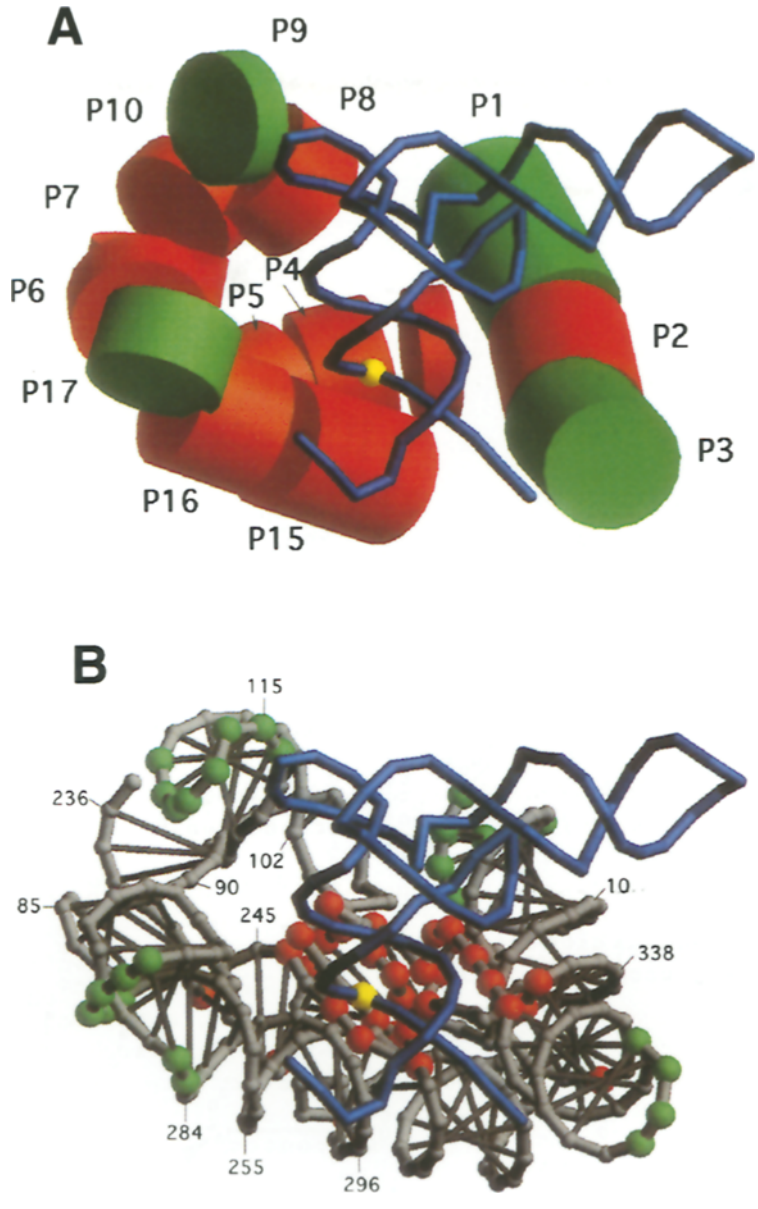

Figure 2. Three-dimensional model of *E. coli* RNase P RNA with bound pre-tRNA substrate from Harris et al., 1994 [34]. Sequences including P12 to 14 and P18 (Figure 1) were omitted from the model. (A) Helices which are invariant in length are shown as red cylinders. Helices which vary significantly in length are in green. The tRNA chain is in blue, with the substrate bond indicated in yellow. (B) The path of the phosphodiester chain in the model is shown. Nucleotide positions are indicated corresponding to Figure 1. Nucleotide bases that are invariant in identity are shown as red spheres. Nucleotides in regions which vary significantly in length are in green.

Design and use of self-cleaving ribozyme-substrate tethers

The intermolecular nature of the RNase P RNA cleavage reaction presents difficulties in the design of experiments to probe structure and catalytic function. RNase P RNA is the only naturally occurring RNA enzyme in the sense that it is not consumed or modified during catalysis. Kinetic and mutational analysis of RNase P RNA-mediated catalysis is made difficult due to the fact that multiple-turnover reaction rates are dominated by substrate binding and product release, rather than by the chemical step [39,40]. Also, powerful experimental strategies such as *in vitro* selection are not straightforwardly applicable as they require separation of active and inactive molecules from randomly modified or mutated RNA populations [41].

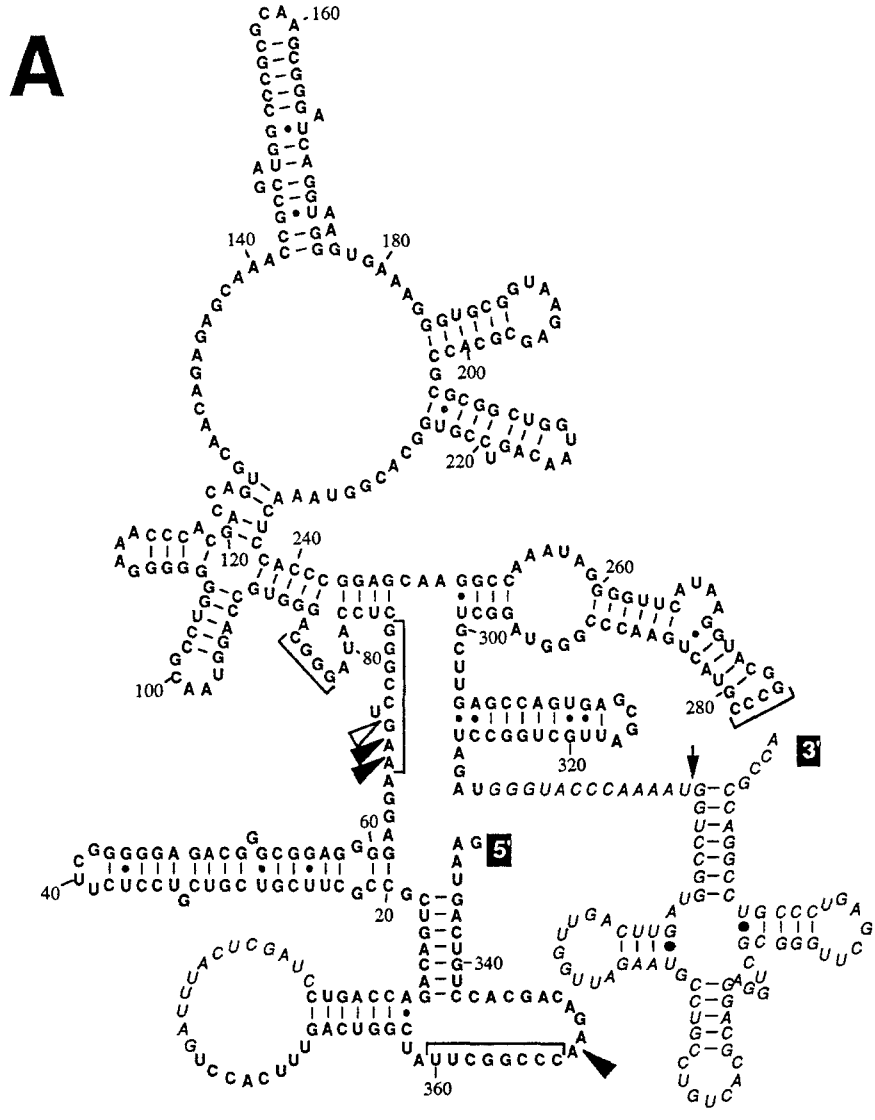

Figure 3. (A) Phosphate oxygens important for catalysis identified by modification-interference using a self-cleaving ribozyme-substrate conjugate. (a) Secondary structure of PT332 RNA. Native RNase P RNA sequences are shown in bold, tRNA and linker sequences are italicized. RNase P RNA sequences are numbered according to the native *E. coli* RNase P RNA sequence. Sites of phosphorothioate- sensitivity are shown by large arrowheads. A less sensitive, but significant, interference effect at U69 is indicated by an open arrowhead. The pre-tRNA cleavage site is indicated by a smaller arrow. (B) Position of phosphorothioate-sensitive sites in RNase P RNA relative to the tRNA substrate phosphate in a model of the ribozyme substrate complex. A portion of a low-resolution model of the RNase P RNA-pre-tRNA complex [34] is shown in crosseye stereo. The region of RNase P RNA included is shown on the secondary structure diagram at left. Only the tRNA acceptor stem is shown, for clarity. The position of the substrate phosphate in pre-tRNA is shown as a filled sphere. The positions of phosphates sensitive to phosphorothioate-substitution in RNase P RNA are shown as gray spheres. RNase P sequence is numbered according to Figure 1A.

Redesign of the RNA to generate a self-cleaving RNA containing both enzyme and substrate sequences circumvents these constraints. In order to accomplish this, we tethered tRNA sequences to cpRNase P RNAs at nucleotides implicated in substrate binding. In one configuration, pre-tRNA sequences were appended via the 5′ leader sequences to an RNase P RNA circularly permuted in J18/2. The resulting molecule , PT332 RNA, is shown in Figure 3A. In a second orientation, the tRNA 3′ terminal CCA sequence was attached

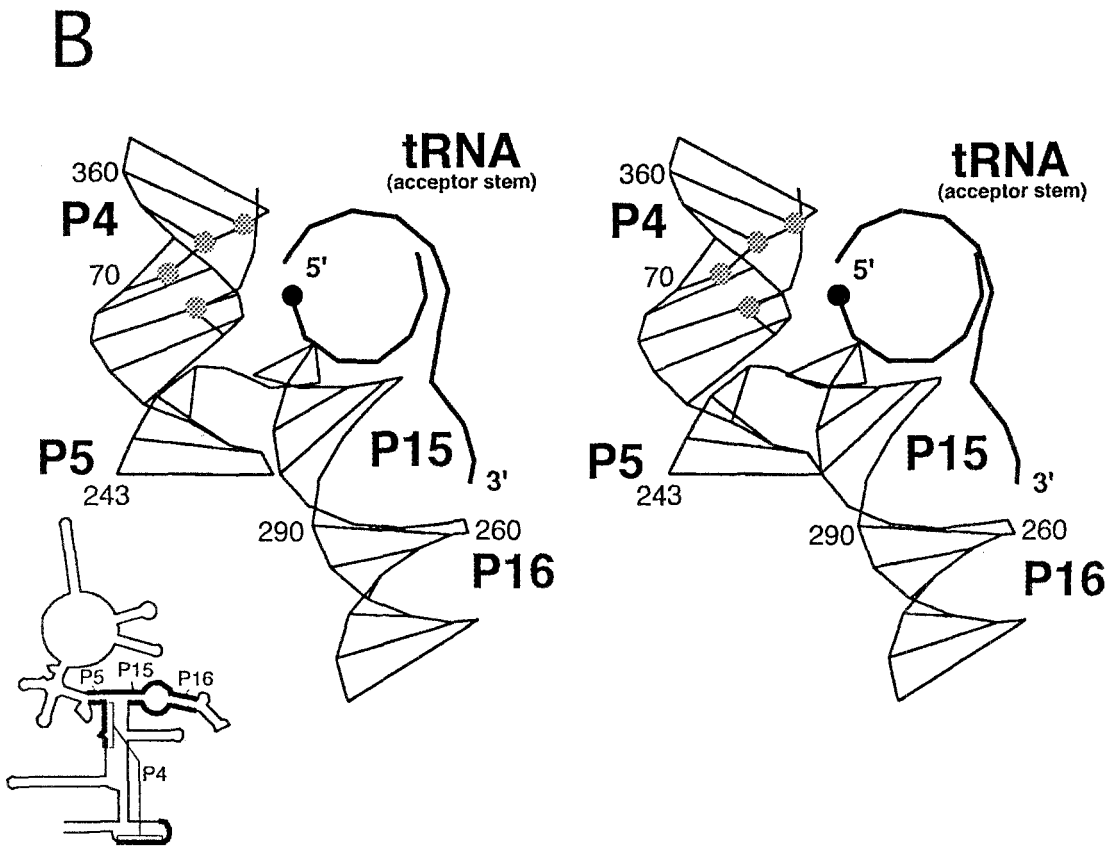

Figure 3. Continued.

to a cpRNase P whose novel termini were located in the internal bulge between P15 and P16. In both cases the sites of attachment were chosen based on complementary crosslinking and chemical protection data, which unambiguously identify the J18/2 region as being in proximity to the pre-tRNA 5′ leader sequence [33,34,37], and the P15-P16 internal bulge as the binding site for tRNA 3′ CCA [35,37].

These 'active site-tethered' pre-tRNA-RNase P RNA conjugates undergo rapid, accurate and efficient self-cleavage *in vitro* [42]. In fact, the first-order reaction rates for self-cleavage of both conjugates were equivalent to the rate of the chemical step of the native intermolecular RNase P RNA cleavage reaction. The insensitivity of the reaction rate to dilution and competition by mature tRNA, added *in trans*, demonstrates the intramolecular nature of the cleavage reaction. In contrast to the 'active site-tethers', intermolecular cleavage predominates when pre-tRNA is linked to either the 5′ or 3′ terminus of native RNase P RNA [30,42,43]. Undoubtedly this is due to steric constraints, since the native 5′ and 3′ termini are relatively distant from the active site of the enzyme [34].

One application of these self-cleaving conjugates is in modification-interference experiments. In this approach, functional groups important for catalysis are identified by comparing the patterns of chemically modified residues in active and inactive molecules drawn from a randomly modified population [e.g. 44,45]. The tethered enzyme-substrate complexes proved ideal for such experiments since identification of active RNase P RNAs is readily accomplished via the self-cleavage reaction. Experiments involving phosphorothioate- substituted tRNAs have been used to identify phosphate oxygens in tRNA important for recognition by RNase P RNA [46]. In addition, a gel-mobility shift assay has been used to identify nucleotides in RNase P RNA where substitution of phosphate oxygens by sulfur disrupts substrate binding [47]. Substitution by sulfur at these sites is thought to inhibit binding of magnesium ions without signifi-

cantly altering secondary structure, but also potentially could perturb tertiary contacts [48].

By using the tethered RNase P RNA-tRNA conjugates for phosphorothioate modification-interference, we identified only four phosphate oxygens where substitution by sulfur dramatically reduced the catalytic rate (50–200 fold) [49]. All of the sites identified (A67, G68, U69 and A352) are in or adjacent to helix P4. In each case the phosphates identified as important for catalysis are between nucleotides that are universally conserved. The location of these phosphates in the current tertiary structure model is shown in Figure 3B. Although the resolution of the model is low (approximately 5–10É [34]), all of the sites are immediately adjacent to the cleavage site in pre-tRNA (Figure 3B). The large effect of substitution on cleavage rate, the location of sensitive positions in conserved sequence and structure, and the proximity to the substrate phosphate in the structure model are all consistent with a direct role of the implicated phosphates in RNase P RNA-mediated catalysis. Interference at one site (A67) was rescued in the presence of manganese suggesting a direct role for that phosphate in binding divalent metal ion required for catalysis. Defining the binding sites for divalent ions in RNase P RNA is crucial given the central role such ions play in RNA-mediated catalysis [50].

In order to infer a plausible, high-resolution structure for the ribozyme additional information bearing on the roles of specific nucleotides in structure and function is required. Given the importance of base-backbone interactions in tRNA and the hammerhead ribozyme structure, it is likely that some conserved nucleotides in RNase P RNA are involved in these kinds of interactions. The highly refined secondary structure and the developing, low-resolution structural perspective gained by crosslinking will help focus experimental approaches aimed at defining further specific elements of structure.

Acknowledgements

We thank all our colleagues for useful discussions and insights into RNase P structure and function. We thank Rob Siegel and Dan Frank for critical review of the manuscript. We thank Jim Brown for Figure 1. Research in the laboratory of N.R.P. is supported by grants from the NIH (GM34527) and DOE (92ER20088). M.E.H. is the recipient of an NIH postdoctoral fellowship (GM15979).

References

1. Cech TR (1993) In: RF Gesteland & JF Atkins (Eds) The RNA World (pp 241–264) Cold Spring Harbor Press
2. Pace NR & Brown JW (1995) J. Bacteriol. 177: 1919–1928
3. Kim S-H, Suddath FL, Quigley GL, McPherson A, Sussman JL, Wang AHJ, Seeman NC & Rich A (1974) Science 185: 435–440
4. Robertus JD, Ladner JE, Finch JR, Rhodes D, Brown RS, Clark BFC & Klug A (1974) Nature 250: 546–551
5. Pley HM, Flaherty KM & McKay DB (1994) Nature 372: 68–74
6. Scott WG, Finch JT & Klug A (1995) Cell 81: 991–1002
7. Heus H & Pardi A (1991) Science 253: 191–194
8. Wimberly B, Varani G and Tinoco I Jr, (1993) Biochem. 32: 1078–1087
9. Brimacombe R, Atmadja J, Stiege W & Schuler D (1988) J. Mol. Biol. 199: 115–136
10. Stern S, Weiser B & Noller HF (1988) J. Mol. Biol. 204: 448–481
11. Westhof E, Romby P, Romaniuk PJ, Ebel J-P, Ehresmann C & Ehresmann B (1989) J. Molec. Biol. 184: 417–431
12. Michel F & Westhof E (1990) J. Mol. Biol. 216: 585–610
13. Malhotra A & Harvey SC (1994) J. Mol. Biol. 240: 308–340
14. Woese CR & Pace NR (1993) In: RF Gesteland & JF Atkins (Eds) The RNA World (pp 91–117) Cold Spring Harbor Press
15. James BD, Olsen GJ, Liu JS & Pace NR (1988) Cell 52: 19–26
16. Haas ES, Brown JW, Pitulle C & Pace NR (1994) Proc. Natl. Acad. Sci. USA 91: 2527–2531
17. Brown JW, Nolan JM, Haas ES, Rubio MAT, Major F & Pace NR (1996) Proc. Natl. Acad. Sci. USA 93: 3001–3006
18. Burke JM, Belfort M, Cech TR, Davies RW, Schweyen RJ, Shub DA, Szostak JW & Tabak HF (1987) Nucl. Acids Res. 15: 7217–7221
19. Darr SC, Zito K, Smith D & Pace NR (1990) Biochemistry 31: 328–333
20. LaGrandeur TE, Darr SC, Haas ES & Pace NR (1993) J. Bacteriol. 175: 5043–5048
21. Tranguch AJ & Engelke DR (1993) J. Biol. Chem. 268: 14045–14055
22. Shiraishi H, & Shimura Y (1986). EMBO J. 7: 3673–3679
23. Baer MF, Reilly RM, McCorckle GM, Hai T-Y, Altman S, & RajBhandary L (1988) J. Biol. Chem. 263: 2344–2351
24. Kirsebom LA, & Svärd SG (1993) J. Mol. Biol. 231: 594–604
25. Schlegl J,. Hardt W-D, Erdmann VA, Hartman R (1994) EMBO J. 13: 4863–4869
26. Waugh DS & Pace NR (1992) FASEB J. 7: 188–195
27. Costa M & Michel F (1995) EMBO J. 14, 1276–1285
28. Tanner MA & Cech TR (1995) RNA 1: 349–350
29. Moore MJ & Sharp PA (1992) Science 256: 992–997
30. Waugh DS (1989) Ph.D. Thesis, Indiana University
31. Pan T, Gutell R and Uhlenbeck O (1991) Science 254: 1361–1364
32. Nolan JM, Burke DH & Pace NR (1993) Science 261: 762–765
33. Burgin AB & Pace NR (1990) EMBO J. 9: 4111–4118
34. Harris ME, Nolan JM, Malhotra A, Brown JW, Harvey SC & Pace NR (1994) EMBO J. 13: 3953–3963
35. Oh B-K & Pace NR (1994) Nucl. Acids Res. 22: 4087–4094
36. Altman S & Westhof E (1994) Proc. Natl. Acad. Sci. USA 91: 5133–5137
37. LaGrandeur TE, Hüttenhofer A, Noller HF & Pace NR (1994) EMBO J. 13: 3945–3952
38. Kirsebom LA & Svard SG (1994) EMBO J. 13: 4870–4876

39. Reich C, Gardiner KJ, Olsen GJ, Pace B, Marsh TL & Pace NR (1986) J. Biol. Chem. 261: 7888–7893
40. Tallsjö A & Kirsebom LA (1993) Nucl. Acids Res. 21: 51–57
41. Szostak JM & Ellington A (1993) In: RF Gesteland & JF Atkins (Eds) The RNA World (pp 511–533) Cold Spring Harbor Press
42. Frank DN, Harris ME & Pace NR (1994) Biochemistry 33: 10800–10808
43. Kikuchi Y, Sasaki-Tozawa N & Suzuki K (1993) Nucl. Acids Res. 20: 4685–4689
44. Siebenlist U & Gilbert W (1980). Proc. Natl. Acad. Sci. USA 77: 122–126
45. Conway L & Wickens M (1987) EMBO J. 6: 4177–4184
46. Guar RK & Krupp G (1993) Nucl. Acids Res. 21: 21–26
47. Hardt W-D, Warnecke JM, Erdmann VA & Hartmann RK (1995) EMBO J. 14: 2935–2944
48. Eckstein F (1985) Ann. Rev. Biochem. 54: 367–402
49. Harris ME & Pace NR (1995) RNA 1: 210–218
50. Smith D. (1995) In: J.A. Cowan (Ed) The Biological Chemistry of Magnesium (pp 111–135) VCH Publishers, Inc.