Recent insights into the structure and function of the ribonucleoprotein enzyme ribonuclease P

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In bacteria, the tRNA-processing endonuclease ribonuclease P is composed of a large (~400 nucleotide) catalytic RNA and a smaller (~100 amino acid) protein subunit that is essential for substrate recognition. Current biochemical and biophysical investigations are providing fresh insights into the modular architecture of the ribozyme, the mechanisms of substrate specificity and the role of essential metal ions in catalysis. Together with recent high-resolution structures of portions of the ribozyme, these findings are beginning to reveal how the functions of RNA and protein are coordinated in this ribonucleoprotein enzyme.

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Current Opinion in Structural Biology 2003, **13**:325–333

This review comes from a themed issue on
Nucleic acids
Edited by Carl C Correll and David MJ Lilley

0959-440X/03/$ – see front matter

DOI 10.1016/S0959-440X(03)00069-1

Abbreviations

NAIM	nucleotide analog interference mapping
pre-tRNA	precursor tRNA
RNase P	ribonuclease P

Introduction

Because of its highly conserved structure and essential biological function, the ribonucleoprotein enzyme ribonuclease P (RNase P) provides an attractive experimental arena for understanding the molecular basis of how RNAs interact with each other, with essential divalent metal ions and with specific proteins. Additionally, because RNase P functions as a phosphodiesterase and a multiple turnover enzyme, comparisons with protein enzymes can illuminate fundamental features of enzyme catalysis [1••]. Several recent reviews of the composition and functional properties of RNase P enzymes from different organisms are available [1••,2–6]. This review is intended to provide a concise extension of these more comprehensive reviews, focusing on recent advances in our understanding of the structural basis of substrate recognition and catalysis by the archetypical bacterial RNase P enzyme.

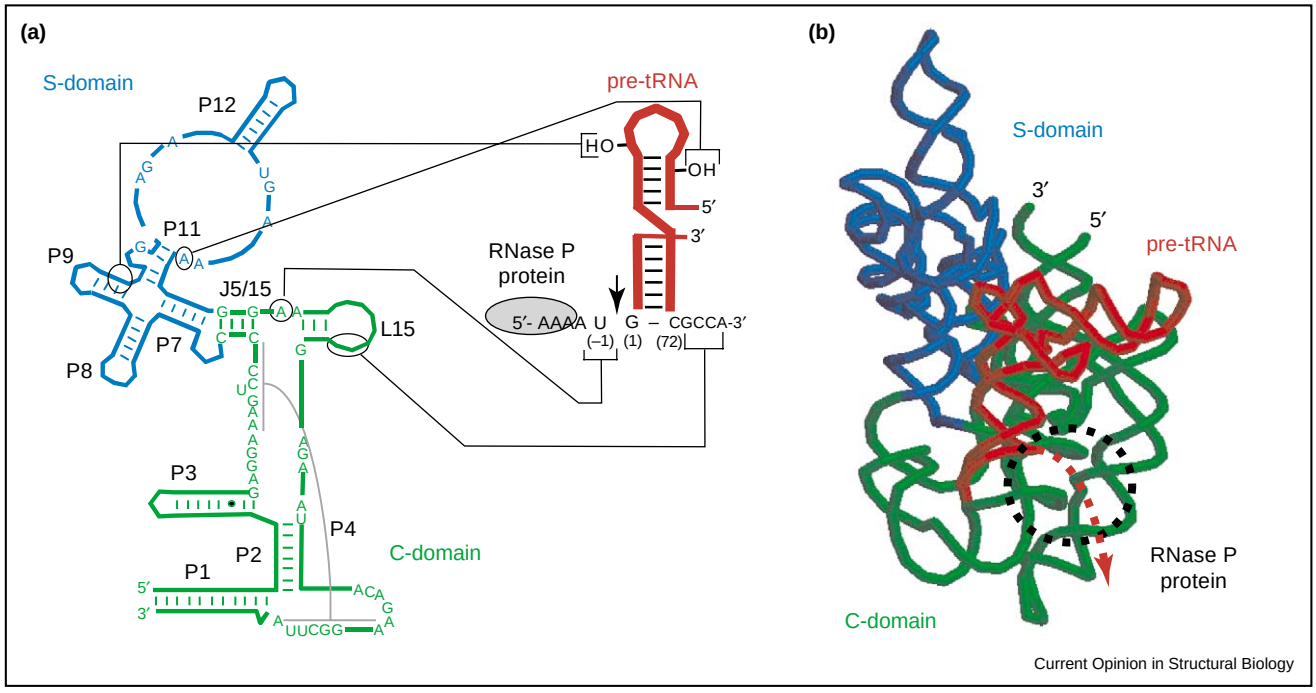
The physiological function of RNase P is to catalyze the hydrolysis of a specific phosphodiester bond in RNA precursors to generate the mature 5′ ends of tRNAs and other precursor RNAs. Flexibility in substrate recognition is inherent to the function of RNase P and the complete complement of RNA substrates for this class of enzymes is not yet known. All RNase P enzymes characterized to date possess an essential and highly conserved RNA subunit that contains the active site of the enzyme [7,8], and at least one essential protein subunit. The composition of the bacterial enzyme is the simplest, containing a single ~400-nucleotide RNA and a small ~100 amino acid protein subunit. Archaeal and eukaryotic enzymes are more complex, with multiple distinct proteins in addition to the conserved RNA subunit [5,9]. Although the single protein subunit of bacterial RNase P contributes only about 8% of the mass of the holoenzyme, it plays an essential role in substrate recognition [10–12].

Recent studies of bacterial RNase P have revealed remarkable new features of its structure and function, including a better understanding of the modular architecture of the enzyme–substrate complex and glimpses of specific active site interactions. Importantly, the structure of a large ribozyme domain involved in substrate binding has recently been determined. Also, an increasingly detailed description of interactions with the divalent metal ions that are essential for catalytic function is emerging from analysis of atomic substitutions in the catalytic core of the ribozyme. Finally, recent work has provided a better understanding of the contribution of the essential RNase P protein to substrate binding and catalysis, as well as the location of the protein subunit in the enzyme–substrate complex. This new information raises intriguing questions about how the RNA and protein subunits collaborate to achieve specificity and optimal rate enhancement.

Global structure of the enzyme–substrate complex

The secondary structure and some elements of the tertiary structure of bacterial RNase P RNAs are now well defined by extensive phylogenetic comparative analyses [3,13]. As shown in Figure 1a, bacterial RNase P RNAs all share a minimal core of secondary structure elements, as well as conserved sequences clustered primarily in a characteristic multihelix junction [14,15]. Extensive chemical probing and deletion studies demonstrated that the catalytic bacterial RNA subunit is composed of two independently folding domains with distinct functions [16–19]. The specificity domain, or S-domain, encompasses conserved

Figure 1



Global structure of the RNase P enzyme–substrate complex. **(a)** Consensus secondary structure of bacterial RNase P RNA [3,13]. Individual helices are given the designation P, for paired, and numbered from the 5′ end of the RNA. Helix P4 is shown as gray bars connected by a line. Elements of non-Watson–Crick structure between helices are given the designation J, for joining, and numbered for the helices they connect. Structural elements referred to in the text are indicated. Blue and green lines indicate the path of the phosphodiester backbone; universally conserved nucleotide positions are designated by capital letters. The catalytic domain, or C-domain, is shown in green, whereas the specificity domain, or S-domain, is shown in blue. The stacked acceptor and T-stems of pre-tRNA that are recognized by RNase P are shown in red. Specific substrate elements recognized by RNase P are indicated by capital letters. The pre-tRNA cleavage site is indicated by an arrow. Known interactions between the ribozyme and substrate are shown by lines connecting the interacting positions in RNase P RNA and pre-tRNA. The interaction between the RNase P protein and the substrate 5′ leader sequence is indicated by a gray oval. **(b)** Global three-dimensional structure derived from manual computer modeling of the *E. coli* RNase P RNA–pre-tRNA complex [36,66,67]. Colors of the C-domain, the S-domain and pre-tRNA are the same as in (a). The probable location of the RNase P protein is indicated by a dashed black circle and the proposed path of the 5′ leader sequence is indicated by a dashed red line.

helices P7–P12 and contains a large region of non-Watson–Crick structure defined by J11/12 and J12/11. Photochemical cross-linking, mutagenesis and chemical interference studies demonstrated that this portion of the ribozyme contacts the T-stem and T-loop of the bound precursor tRNA (pre-tRNA) substrate [20–22], including contacts between conserved adenosines in P11 and 2′ OH groups in the T-stem [23,24]. The catalytic domain, or C-domain, contains a complex multihelix junction centered on the highly conserved helix P4 (Figure 1a). This domain includes the ribozyme active site, as demonstrated by interactions with the 3′ CCA sequence of pre-tRNA [21,25,26], by intermolecular cross-linking to photo-agents positioned adjacent to the substrate cleavage site [27–30] and, crucially, by the ability to cleave certain substrates in the absence of the S-domain [19].

The protein subunit represents a third ‘domain’ of the RNase P holoenzyme. Extensive comparative analysis of the reaction kinetics and binding thermodynamics of the RNA alone and the holoenzyme have established that the protein contributes to RNase P substrate binding by interacting with the pre-tRNA 5′ leader sequence [10,11]. Photocross-linking studies indicate that the 5′ leader sequence binds in a specific cleft on the surface of the protein [12]. Importantly, these studies provide evidence of the position and orientation of the leader sequence in the cleft, which places the protein in close proximity to the ribozyme active site within the C-domain.

Notably, the protein can form a specific complex with the C-domain alone, albeit at lower affinity compared to the entire ribozyme. This abbreviated enzyme is active, but unlike the native enzyme it cannot distinguish between small hairpin substrates and full pre-tRNA substrates due to a lack of interactions with the T-loop [31]. The ability of the C-domain–protein complex to recognize single-stranded and hairpin substrates supports the idea that, in this context, binding is dominated by protein interactions with the leader sequence. Together with the available

body of enzymological and structure-probing data [2], this new information has led to an overall functional picture of the enzyme–substrate complex in which the C-domain contains the active site and makes contacts at the pre-tRNA cleavage site, but otherwise has little substrate affinity or specificity on its own. Thus, interactions between the S-domain and the T-stem and T-loop, and between the protein and the 5′ leader sequence give rise to the observed structural specificity of the holoenzyme for pre-tRNA.

A fundamental feature of this perspective is that the protein binds primarily to RNase P RNA within the C-domain. However, chemical and enzymatic probing experiments show that the protein can have a remarkably large apparent footprint on the ribozyme that includes nucleotides within both domains [32–34]. However, interpretation of these data for the *Bacillus subtilis* enzyme is complex because the large apparent footprint may contain contributions from protein-mediated dimerization (see below). By contrast, recent experiments using hydroxyl radical reagents positioned on specific protein residues exclusively detected nucleotides in the catalytic core of RNase P RNA [35,36]. Based on these results and much of the previous data, the isolated protein structure has been docked onto a global model of the enzyme–substrate complex [36] (Figure 1b). The resultant model is consistent with a large body of the available data and is necessarily in harmony with the known functional role of the protein. Nonetheless, as exemplified by recent modification interference analysis [37], evidence that nucleotides in the S-domain are important for protein contacts cannot be discounted. The involvement of the S-domain in protein interactions is further indicated by site-directed photo-affinity cross-linking between specific protein residues and the ribozyme [38]. Thus, our understanding of the RNA–protein interface has advanced considerably, but current models are not consistent with all of the available biochemical data; consequently, defining the individual interactions and structure at this interface remains a crucial goal.

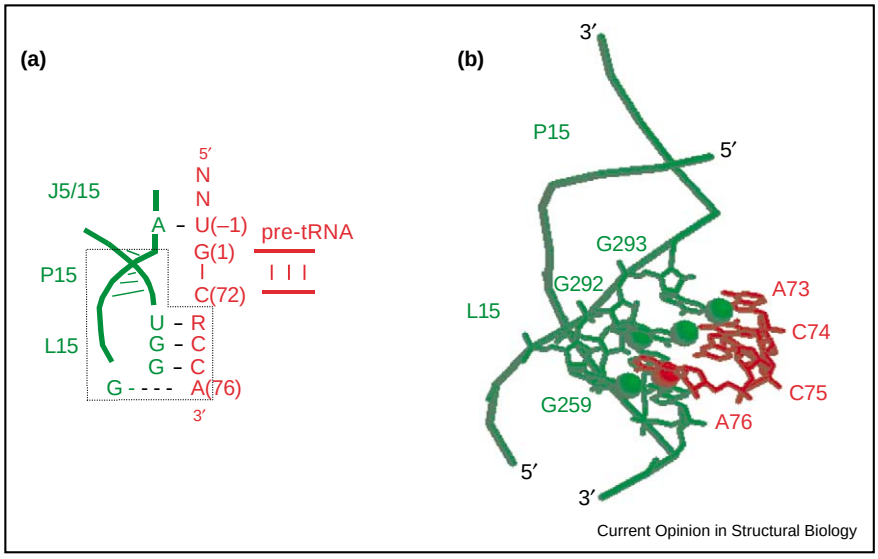
Extensive protein sequence comparisons identify characteristic conserved basic and planar hydrophobic residues postulated to play a role in RNA interactions. Individual mutations at these conserved positions have relatively little effect on cell viability in a genetic complementation system, although certain double mutations can be lethal, suggestive of redundant functions [39]. Intriguingly, biophysical analysis of RNase P protein folding shows that, like only a handful of other proteins, the structure of the RNase P protein is intrinsically unstable and requires the binding of small-molecule anions to fold [40••]. Such studies provide a new basis for thinking about the mechanism of holoenzyme assembly and raise the issue of how ion binding and protein dynamics influence this process.

An unanticipated development in ongoing analyses of the physical properties of the holoenzyme was the detection of dimers (containing two RNA and two protein subunits) of the *B. subtilis* RNase P holoenzyme by small-angle X-ray scattering and affinity retention [41•]. Dimerization is mediated by the protein subunit as the RNA alone is monomeric. Interestingly, binding of a single pre-tRNA substrate results in the formation of monomers; however, substrates containing two tRNAs result in enzyme–substrate complexes containing the dimeric form of the holoenzyme [42••]. The recent finding that the holoenzyme can process both single-stranded and hairpin substrates suggests that autolytic processing of RNase P RNA precursors might occur in the context of the dimer *in vivo* [43]. The demonstration that the *B. subtilis* enzyme can cleave synthetic precursors *in vitro* and that this activity displays a similar dependence on monovalent ion concentration to dimer formation supports this hypothesis [42••]. Do different oligomeric forms of the enzyme occur *in vivo*? If so, these authors suggest that these different forms could be related to the diversity of substrates processed by the enzyme or perhaps to processivity in the maturation of large tRNA operons.

Structural basis of substrate specificity

Currently, we know that the 3′ terminal RCCA sequence of tRNA pairs with nucleotides in L15 and that specific 2′ OH groups in the T-loop are contacted by conserved adenosines in P10/P11 of the S-domain; nonetheless, these interactions do not fully describe the enzyme–substrate interface [2]. Accordingly, significant effort has been directed at identifying nucleotides that contribute to substrate binding and a detailed chemical picture of individual functional groups involved in binding has been gained [44–47]. Collectively, these residues occur at conserved nucleotides also detected by intermolecular cross-linking; however, interpreting these data in terms of specific structure remains difficult. Recently, information from nucleotide analog interference mapping (NAIM), together with parallel biochemical analyses, is giving a clearer view of substrate C(74)C(75)A(76) interactions with ribozyme L15 nucleotides G259, G292 and G293 (*Escherichia coli* numbering), and their contribution to specificity and catalysis (Figure 2). The data are consistent with a structural model of the interaction [48] in which the C(75)•G292 pair is part of a base triple with A258 [46,49]. These data are also consistent with a proposed Hoogsteen face interaction between A(76) and the Watson–Crick face of G259. Thermodynamic and kinetic studies of the contributions of the C(74) and C(75) pairs illustrate that disruption of either affects catalysis as well as ground state binding; however, compensatory substrate mutations that restore the C(75) interaction do not entirely rescue the catalytic defect, consistent with the involvement of this pair within higher order structure [50].

Figure 2

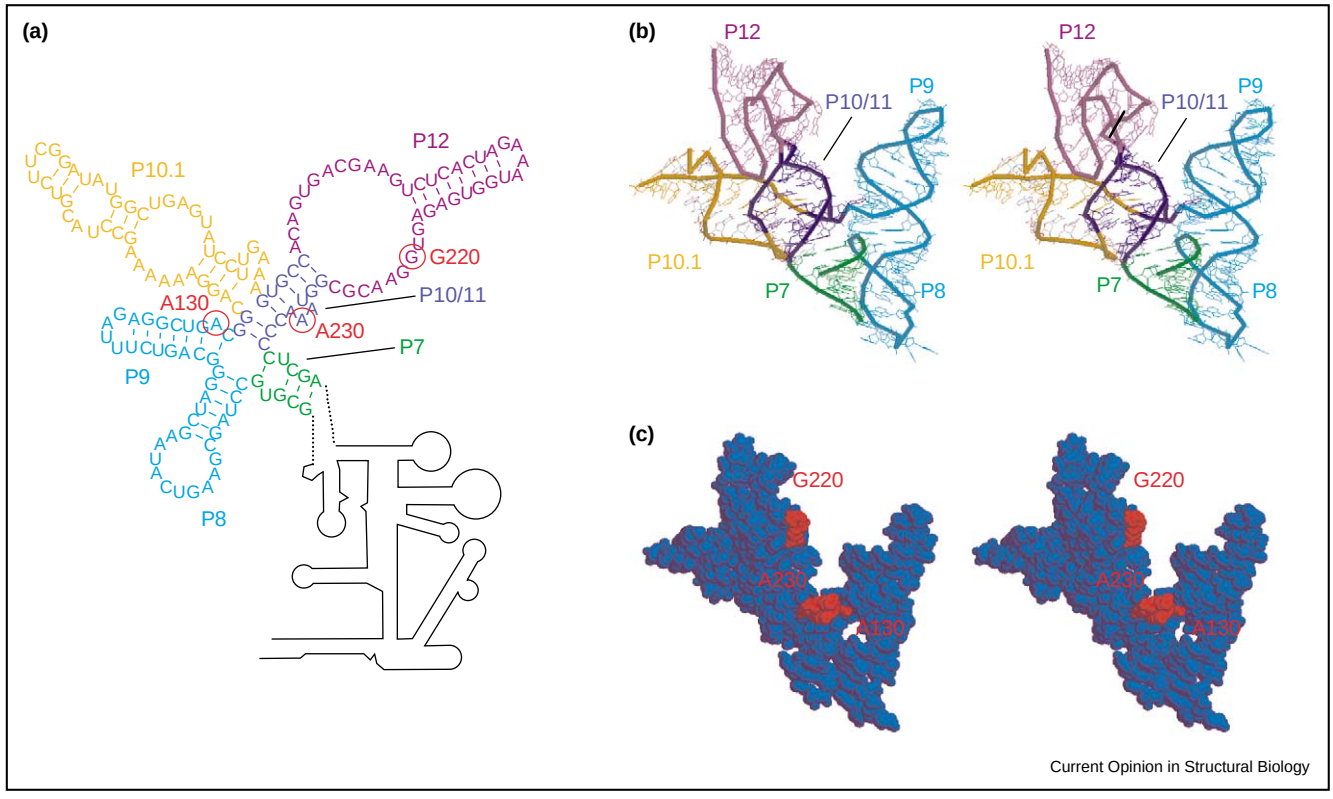


Contacts between the pre-tRNA cleavage site and the C-domain of RNase P RNA. **(a)** Diagram of active site pairing interactions between RNase P RNA and the pre-tRNA substrate. The base of the acceptor stem, the 5′ leader sequence and the 3′ RCCA sequence of pre-tRNA are shown in red. Helix P15 in RNase P RNA and adjoining structures involved in substrate interactions are indicated in green; such interactions include the pairing interaction between the 3′ end of pre-tRNA and L15, and between J5/15 and nucleotide N(−1) in the 5′ leader sequence. The portion of the structure depicted in the model in (b) is indicated by a dashed box. **(b)** Three-dimensional structure derived from computer modeling of the L15–RCCA interaction from the *E. coli* ribozyme [48]. The phosphodiester backbones of P15 and L15 are depicted in green; only the individual nucleotides involved in substrate contacts are shown. Functional groups identified by NAIM as important for binding are indicated by spheres [46,49].

In addition to the 3′ RCCA interaction, determinants at the substrate cleavage site include the G(1)•C(72) pair and positions N(−1) and N(−2) in the leader sequence; however, a complete understanding of specific interactions with these nucleotides is lacking. Recently, progress has been made in defining active site interactions with substrate functional groups at the cleavage site (Figure 2). Studies focusing on N(−1) show that this residue contributes to both specificity and catalytic efficiency, and that uridine is the optimal nucleotide base at this position [51,52]. NMR analysis of the orientation of N(−1) in model substrates shows that its conformation is dependent on base identity [53]. These data are consistent with the recent demonstration that compensatory mutations at N(−1) can suppress miscleavage engendered by mutations in the C-domain [52]. Together with previous cross-linking and chemical interference studies, these data demonstrate a direct pairing interaction between a conserved adenosine residue in J5/15 and the substrate N(−1) position, providing crucial insight into the detailed network of hydrogen bonds necessary for catalysis.

Although further biochemical studies will probably continue to reveal interactions that are important for specificity and other essential features of enzyme function, high-resolution structural information is required to truly understand the basis of specificity and catalysis. A major advance in this regard is provided by the recently reported structure of the S-domain of *B. subtilis* RNase P RNA determined by X-ray crystallography (Figure 3a) [54••]. The structure reveals the four-way helix junction containing helices P8–P11, as well as the large internal bulge formed by J11/12 and J12/11 (Figure 3b). The fold shows that the stacking arrangement and relative orientation of the P7–P11 junction are very close to that employed in previous low-resolution models. However, one of the most stunning features is the structure adopted by J11/12 and J12/11, for which very little concrete information had thus far been available. Intriguingly, J12/11 forms an internal loop against which an S-shaped J11/12 is packed. Conserved nucleotides in these elements form important contacts for this fold and thus a similar structure is anticipated to form in RNase P RNAs from other kingdoms. Additionally, the overall structure suggests ways in which phylogenetically variable elements found in other RNase P RNAs are oriented relative to the conserved core of this domain. Perhaps of most immediate importance is the finding that the structure brings together nucleotides known to be involved in substrate contacts that are distant in the primary and secondary structure (Figure 3c). These nucleotides lie within a cleft formed by the overall fold that presumably accommodates the T-loop and part of the T-stem. Clearly, with this molecular framework in hand, a more detailed understanding of the interface between ribozyme and substrate in this region should be achievable soon.

Figure 3



Crystallographic structure of the S-domain from *B. subtilis* RNase P RNA. **(a)** Secondary structure of the *B. subtilis* S-domain. Individual nucleotides involved in substrate contacts are indicated by red circles. The C-domain is shown as a black line. **(b)** Three-dimensional structure of the S-domain from X-ray crystallography. Individual helices are colored and designated as in (a). **(c)** Positions of S-domain residues involved in substrate binding. The S-domain structure is shown in CPK representation. Nucleotides indicated in the secondary structure in (a) are shown in red.

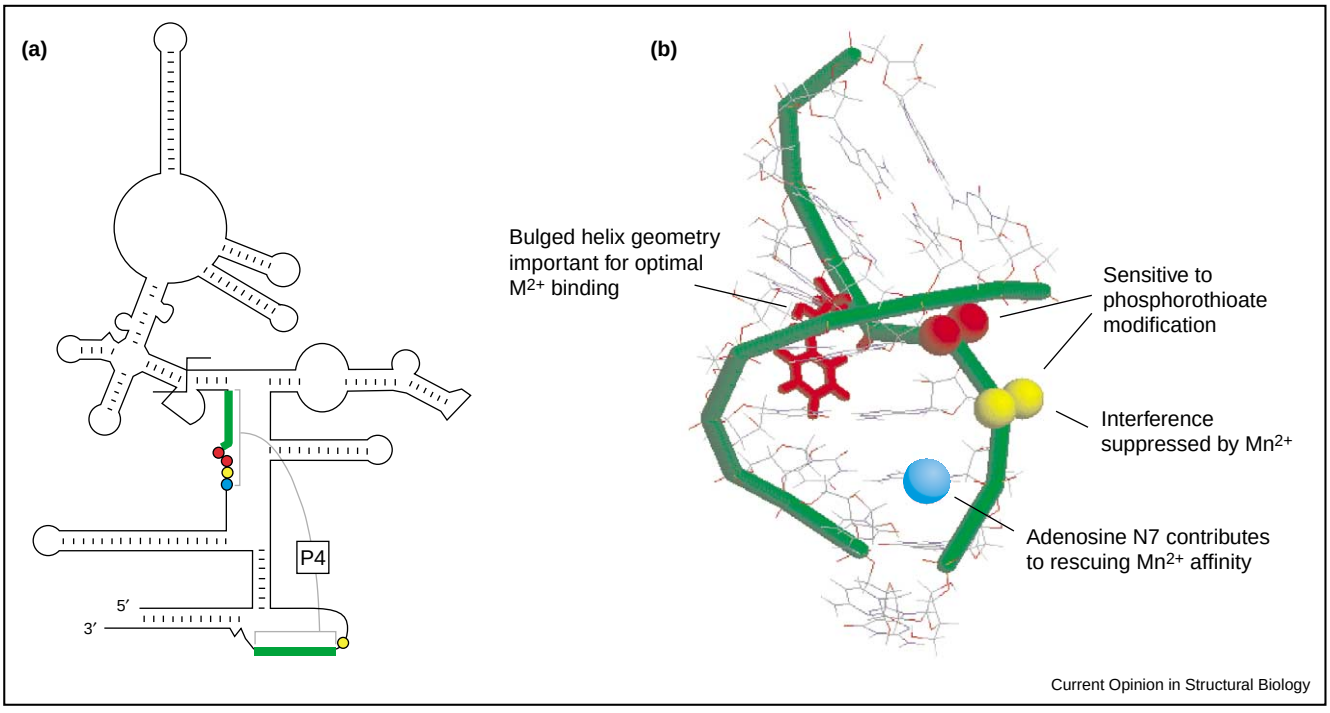
Understanding essential divalent metal ion interactions

One of the most important aspects of the function of the C-domain is interaction with the divalent metal ion cofactors that are essential for folding, substrate binding and catalysis. Based on protein phosphodiesterases and other large ribozymes, it is anticipated that the RNase P active site will contain a network of several metal ions. Brannvall and Kirsebom [55], in examining this concept, demonstrated that different mixtures of divalent metal ions can have profoundly different effects on cleavage specificity and catalytic rate, leading to the conclusion that catalysis involves the cooperation of metal ions bound at different sites. Using Pb(II) cleavage to monitor RNase P RNA conformation in the presence of different metal ions, these investigators showed that certain metal ions, such as Cd^{2+}, Cu^{2+} and Ni^{2+}, result in changes in the cleavage pattern and that different metal ions compete differently to interfere with Pb(II) cleavage [56]. Such data underscore the complex interdependence of different classes of bound metal ions and the importance of monitoring effects on RNA structure in mixed metal ion experiments.

Evidence that one or more of these classes of metal ions important for catalysis are coordinated with or influenced by the RCCA–L15 interaction comes from the observation that mutations in L15 result in different sensitivities to metal ion identity and that Mn^{2+} can result in miscleavage in substrates containing a N(−1)/N(73) pair [57]. Specific functional groups on the Hoogsteen face of N(73) appear to be important for these interactions. Thus, the overall picture that emerges is one in which the ground state configuration of the substrate can include an intramolecular interaction between N(−1) and N(73), which must be broken to form the intermolecular interactions between J5/15 and N(−1), and between L16/15 and N(73). Metal ion binding (or displacement) is likely to stabilize the final bound conformation.

Several lines of evidence indicate that a second, crucial site of metal ion binding within the C-domain occurs in the highly conserved multihelix junction characteristic of all RNase P RNAs. Initial interest was prompted by the observation that some phosphorothioate modifications of nonbridging oxygens in P4, which can disrupt metal ion coordination, resulted in significant (1000–10 000-fold)

Figure 4



Positions of metal ion interactions within the NMR structure of the universally conserved P4 helix. The locations of individual functional groups involved in divalent metal ion interactions, as determined by biochemical analysis of site-specific functional group modifications, are shown in the context of the three-dimensional structure of an isolated P4 helix. Phosphate oxygen (red and yellow) and purine N7 (blue) functional groups involved in metal contacts are shown as spheres. Yellow spheres represent phosphorothioate-sensitive positions that are rescued by Mn^{2+}, indicating sites of direct metal ion coordination. A conserved bulged uridine residue important for optimal metal ion binding is in red.

defects in ribozyme catalysis, some of which were rescued by Mn^{2+} [58,59] (Figure 4). Because Mn^{2+} can coordinate sulfur better than Mg^{2+}, the interpretation is that these residues serve as direct sites of metal ion coordination. In addition, second site functional group modifications within P4 and J3/4 affect the affinity and cooperativity of metal ion binding in P4, providing evidence of interactions with nucleotide base functional groups, as well as of additional metal ions bound within the P4 multihelix junction [60,61••] (Figure 4). A universally conserved structural feature of P4 is a bulged uridine adjacent to the site of metal ion coordination (Figure 4). Although this residue does not directly contribute functional groups for ribozyme function, the geometry of the bulge and its position within the helix facilitate divalent metal ion interactions important for catalysis [62]. A recent NMR structure of a model P4 helix demonstrates major groove binding of cobalt hexamine, a structural mimic of hydrated Mg^{2+} [63]. Changing the position of the bulged residue changes the position of the bound metal ion, suggesting that alterations of metal ion geometry are the basis of the catalytic defects seen upon mutation of the bulged residue in P4.

The interpretation that P4 forms a binding site for catalytic metal ions is supported by the recent observation that phosphorothioate modifications at homologous positions in the *B. subtilis* ribozyme have similarly large effects on the cleavage rate constant. Importantly, Fierke and colleagues [64] show that such effects on catalysis occur in both the RNA alone and the holoenzyme reactions without disrupting ground state substrate binding. As with the *E. coli* ribozyme, Mn^{2+} rescue of specific interferences is observed; however, no rescue occurs in the context of the *B. subtilis* holoenzyme, suggesting that the protein directly influences ribozyme metal ion binding in P4. The intimate functional relationship between the RNA and protein subunits with respect to metal ion binding is powerfully underscored by recent transient kinetic and substrate binding studies demonstrating that the protein facilitates catalysis by increasing the affinities of metal ions bound in the *B. subtilis* RNase P RNA–pre-tRNA complex [65••]. This analysis is particularly noteworthy in that the investigators systematically evaluated the effect of protein on the affinity of the different functional classes of metal ions. They show that the protein does not change the number or apparent affinity of diffusely bound metal ions that are important for structure and stability, or of metal ions required for tRNA binding. Rather, binding of the protein to the ribozyme increases the affinity of metal ions that are important for catalysis, which the authors

suggest arises indirectly from the positioning of the 5′ leader and pre-tRNA. These studies raise several very intriguing questions concerning the interface between the RNA and protein subunits. Where are the affected metal ions bound within the ribozyme–substrate complex? Do protein functional groups contribute directly to metal ion coordination?

Conclusions

In sum, recent investigations of RNase P structure and function have revealed important new insight into the structural basis of specificity and catalysis. Additionally, they have raised important new questions, which illustrate that the structural and functional properties of this enzyme class will undoubtedly continue to challenge and surprise us. The global architecture of the enzyme–substrate complex is largely in hand, and specific interactions between enzyme domains and the substrate are being defined, including new active site interactions. Importantly, the recent crystal structure of the S-domain should provide a powerful new framework for understanding the architecture of the enzyme and for exploring the process of substrate recognition. Additionally, our insight into the contribution of the protein subunit to substrate binding and functional metal ion interactions continues to point to a close association between the protein and the ribozyme active site. Clearly, much can be learned from exploring how the RNA and protein subunits of RNase P collaborate to achieve substrate recognition, and the specific roles of RNA and protein residues in enzyme function. Such studies of the structure and function of the catalytic domain of RNase P RNA are providing a glimpse of the intricacies of divalent metal ion interactions in large, structurally complex RNAs. Therefore, we can now begin to see that these lines of investigation will ultimately converge to provide a fundamental understanding of the active site of this ribonucleoprotein enzyme.

Acknowledgements

We thank Frank Campbell, Mark Caprara, Adam Cassano and Nathan Zahler for critical reading and thoughtful comments on the manuscript. This work was supported by National Institutes of Health grant GM56742.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Kurz JC, Fierke CA: **Ribonuclease P: a ribonucleoprotein enzyme**. *Curr Opin Chem Biol* 2000, **4**:553-558.
A concise and articulate review of the basic issues that are important for understanding substrate binding and catalysis by bacterial RNase P, focusing on literature up to 2000. This review is of particular interest as it underscores the importance of understanding the properties of RNase P within the broad context of biological catalysis.

2. Christian EL, Zahler NH, Kaye NM, Harris ME: **Analysis of substrate recognition by the ribonucleoprotein endonuclease RNase P**. *Methods* 2002, **28**:307-322.

3. Hall TA, Brown JW: **The ribonuclease P family**. *Methods Enzymol* 2001, **341**:56-77.

4. Gopalan V, Vioque A, Altman S: **RNase P: variations and uses**. *J Biol Chem* 2002, **277**:6759-6762.

5. Xiao S, Scott F, Fierke CA, Engelke DR: **Eukaryotic ribonuclease P: a plurality of ribonucleoprotein enzymes**. *Annu Rev Biochem* 2002, **71**:165-189.

6. Jarrous N: **Human ribonuclease P: subunits, function, and intranuclear localization**. *RNA* 2002, **8**:1-7.

7. Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S: **The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme**. *Cell* 1983, **35**:849-857.

8. Pannucci JA, Haas ES, Hall TA, Harris JK, Brown JW: **RNase P RNAs from some Archaea are catalytically active**. *Proc Natl Acad Sci USA* 1999, **96**:7803-7808.

9. Hall TA, Brown JW: **Archaeal RNase P has multiple protein subunits homologous to eukaryotic nuclear RNase P proteins**. *RNA* 2002, **8**:296-306.

10. Crary SM, Niranjanakumari S, Fierke CA: **The protein component of *Bacillus subtilis* ribonuclease P increases catalytic efficiency by enhancing interactions with the 5′ leader sequence of pre-tRNAAsp**. *Biochemistry* 1998, **37**:9409-9416.

11. Kurz JC, Niranjanakumari S, Fierke CA: **Protein component of *Bacillus subtilis* RNase P specifically enhances the affinity for precursor-tRNAAsp**. *Biochemistry* 1998, **37**:2393-2400.

12. Niranjanakumari S, Stams T, Crary SM, Christianson DW, Fierke CA: **Protein component of the ribozyme ribonuclease P alters substrate recognition by directly contacting precursor tRNA**. *Proc Natl Acad Sci USA* 1998, **95**:15212-15217.

13. Brown JW: **The Ribonuclease P Database**. *Nucleic Acids Res* 1999, **27**:314.

14. Frank DN, Adamidi C, Ehringer MA, Pitulle C, Pace NR: **Phylogenetic-comparative analysis of the eukaryal ribonuclease P RNA**. *RNA* 2000, **6**:1895-1904.

15. Harris JK, Haas ES, Williams D, Frank DN, Brown JW: **New insight into RNase P RNA structure from comparative analysis of the archaeal RNA**. *RNA* 2001, **7**:220-232.

16. Pan T: **Higher order folding and domain analysis of the ribozyme from *Bacillus subtilis* ribonuclease P**. *Biochemistry* 1995, **34**:902-909.

17. Loria A, Pan T: **Domain structure of the ribozyme from eubacterial ribonuclease P**. *RNA* 1996, **2**:551-563.

18. Mobley EM, Pan T: **Design and isolation of ribozyme-substrate pairs using RNase P-based ribozymes containing altered substrate binding sites**. *Nucleic Acids Res* 1999, **27**:4298-4304.

19. Pan T, Jakacka M: **Multiple substrate binding sites in the ribozyme from *Bacillus subtilis* RNase P**. *EMBO J* 1996, **15**:2249-2255.

20. Nolan JM, Burke DH, Pace NR: **Circularly permuted tRNAs as specific photoaffinity probes of ribonuclease P RNA structure**. *Science* 1993, **261**:762-765.

21. LaGrandeur TE, Huttenhofer A, Noller HF, Pace NR: **Phylogenetic comparative chemical footprint analysis of the interaction between ribonuclease P RNA and tRNA**. *EMBO J* 1994, **13**:3945-3952.

22. Knap AK, Wesolowski D, Altman S: **Protection from chemical modification of nucleotides in complexes of M1 RNA, the catalytic subunit of RNase P from *E. coli*, and tRNA precursors**. *Biochimie* 1990, **72**:779-790.

23. Pan T, Loria A, Zhong K: **Probing of tertiary interactions in RNA: 2′-hydroxyl-base contacts between the RNase P RNA and pre-tRNA**. *Proc Natl Acad Sci USA* 1995, **92**:12510-12514.

24. Loria A, Pan T: **Recognition of the T stem-loop of a pre-tRNA substrate by the ribozyme from *Bacillus subtilis* ribonuclease P**. *Biochemistry* 1997, **36**:6317-6325.

25. Kirsebom LA, Svard SG: **Base pairing between *Escherichia coli* RNase P RNA and its substrate**. *EMBO J* 1994, **13**:4870-4876.

26. Oh BK, Frank DN, Pace NR: **Participation of the 3′-CCA of tRNA in the binding of catalytic Mg^{2+} ions by ribonuclease P**. *Biochemistry* 1998, **37**:7277-7283.

27. Burgin AB, Pace NR: **Mapping the active site of ribonuclease P RNA using a substrate containing a photoaffinity agent**. *EMBO J* 1990, **9**:4111-4118.

28. Christian EL, McPheeters DS, Harris ME: **Identification of individual nucleotides in the bacterial ribonuclease P ribozyme adjacent to the pre-tRNA cleavage site by short-range photo-cross-linking**. *Biochemistry* 1998, **37**:17618-17628.

29. Christian EL, Harris ME: **The track of the pre-tRNA 5′ leader in the ribonuclease P ribozyme-substrate complex**. *Biochemistry* 1999, **38**:12629-12638.

30. Kufel J, Kirsebom LA: **Different cleavage sites are aligned differently in the active site of M1 RNA, the catalytic subunit of *Escherichia coli* RNase P**. *Proc Natl Acad Sci USA* 1996, **93**:6085-6090.

31. Loria A, Pan T: **Modular construction for function of a ribonucleoprotein enzyme: the catalytic domain of *Bacillus subtilis* RNase P complexed with *B. subtilis* RNase P protein**. *Nucleic Acids Res* 2001, **29**:1892-1897.

32. Vioque A, Arnez J, Altman S: **Protein-RNA interactions in the RNase P holoenzyme from *Escherichia coli***. *J Mol Biol* 1988, **202**:835-848.

33. Talbot SJ, Altman S: **Gel retardation analysis of the interaction between C5 protein and M1 RNA in the formation of the ribonuclease P holoenzyme from *Escherichia coli***. *Biochemistry* 1994, **33**:1399-1405.

34. Loria A, Niranjanakumari S, Fierke CA, Pan T: **Recognition of a pre-tRNA substrate by the *Bacillus subtilis* RNase P holoenzyme**. *Biochemistry* 1998, **37**:15466-15473.

35. Biswas R, Ledman DW, Fox RO, Altman S, Gopalan V: **Mapping RNA-protein interactions in ribonuclease P from *Escherichia coli* using disulfide-linked EDTA-Fe**. *J Mol Biol* 2000, **296**:19-31.

36. Tsai HY, Masquida B, Biswas R, Westhof E, Gopalan V: **Molecular modeling of the three-dimensional structure of the bacterial RNase P holoenzyme**. *J Mol Biol* 2003, **325**:661-675.

37. Rox C, Feltens R, Pfeiffer T, Hartmann RK: **Potential contact sites between the protein and RNA subunit in the *Bacillus subtilis* RNase P holoenzyme**. *J Mol Biol* 2002, **315**:551-560.

38. Sharkady SM, Nolan JM: **Bacterial ribonuclease P holoenzyme crosslinking analysis reveals protein interaction sites on the RNA subunit**. *Nucleic Acids Res* 2001, **29**:3848-3856.

39. Jovanovic M, Sanchez R, Altman S, Gopalan V: **Elucidation of structure-function relationships in the protein subunit of bacterial RNase P using a genetic complementation approach**. *Nucleic Acids Res* 2002, **30**:5065-5073.

40. •• Henkels CH, Kurz JC, Fierke CA, Oas TG: **Linked folding and anion binding of the *Bacillus subtilis* ribonuclease P protein**. *Biochemistry* 2001, **40**:2777-2789.

Initial biophysical analysis of RNase P protein structure and folding by CD and NMR reveals a structure that is intrinsically unfolded, requiring binding of anions to form the native structure. This new information impacts studies of ribonucleoprotein assembly and raises the issue of how conformational flexibility might influence function.

41. • Fang XW, Yang XJ, Littrell K, Niranjanakumari S, Thiyagarajan P, Fierke CA, Sosnick TR, Pan T: **The *Bacillus subtilis* RNase P holoenzyme contains two RNase P RNA and two RNase P protein subunits**. *RNA* 2001, **7**:233-241.

See annotation to [42••].

42. •• Barrera A, Fang X, Jacob J, Casey E, Thiyagarajan P, Pan T: **Dimeric and monomeric *Bacillus subtilis* RNase P holoenzyme in the absence and presence of pre-tRNA substrates**. *Biochemistry* 2002, **41**:12986-12994.

This paper, together with [41•], describes the unexpected formation of functional dimers of the *B. subtilis* holoenzyme. The authors characterize the sensitivity of dimer formation to substrate binding and provide support for the hypothesis that dimer formation contributes to the ability of the RNA subunit to undergo autolytic processing.

43. Loria A, Pan T: **The 3′ substrate determinants for the catalytic efficiency of the *Bacillus subtilis* RNase P holoenzyme suggest autolytic processing of the RNase P RNA *in vivo***. *RNA* 2000, **6**:1413-1422.

44. Hardt WD, Warnecke JM, Hartmann RK: **Recent approaches to probe functional groups in ribonuclease P RNA by modification interference**. *Mol Biol Rep* 1995, **22**:161-169.

45. Hardt WD, Erdmann VA, Hartmann RK: **Rp-deoxy-phosphorothioate modification interference experiments identify 2′-OH groups in RNase P RNA that are crucial to tRNA binding**. *RNA* 1996, **2**:1189-1198.

46. Heide C, Pfeiffer T, Nolan JM, Hartmann RK: **Guanosine 2-NH2 groups of *Escherichia coli* RNase P RNA involved in intramolecular tertiary contacts and direct interactions with tRNA**. *RNA* 1999, **5**:102-116.

47. Siew D, Zahler NH, Cassano AG, Strobel SA, Harris ME: **Identification of adenosine functional groups involved in substrate binding by the ribonuclease P ribozyme**. *Biochemistry* 1999, **38**:1873-1883.

48. Easterwood TR, Harvey SC: **Ribonuclease P RNA: models of the 15/16 bulge from *Escherichia coli* and the P15 stem loop of *Bacillus subtilis***. *RNA* 1997, **3**:577-585.

49. Heide C, Feltens R, Hartmann RK: **Purine N7 groups that are crucial to the interaction of *Escherichia coli* RNAse P RNA with tRNA**. *RNA* 2001, **7**:958-968.

50. Busch S, Kirsebom LA, Notbohm H, Hartmann RK: **Differential role of the intermolecular base-pairs G292-C(75) and G293-C(74) in the reaction catalyzed by *Escherichia coli* RNase P RNA**. *J Mol Biol* 2000, **299**:941-951.

51. Brannvall M, Fredrik Pettersson BM, Kirsebom LA: **The residue immediately upstream of the RNase P cleavage site is a positive determinant**. *Biochimie* 2002, **84**:693-703.

52. Zahler NH, Christian EL, Harris ME: **Recognition of the pre-tRNA 5′ leader sequence by the active site of the RNase P ribozyme**. *RNA* 2003, in press.

53. Zuleeg T, Hansen A, Pfeiffer T, Schubel H, Kreutzer R, Hartmann RK, Limmer S: **Correlation between processing efficiency for ribonuclease P minimal substrates and conformation of the nucleotide -1 at the cleavage position**. *Biochemistry* 2001, **40**:3363-3369.

54. •• Krasilnikov AS, Yang X, Pan T, Mondragon A: **Crystal structure of the specificity domain of ribonuclease P**. *Nature* 2003, **421**:760-764.

The first description of the three-dimensional structure of a large portion of the *B. subtilis* RNase P RNA. In addition to the wealth of new information on RNA structure, we can now marvel at the remarkable structure formed by J11/12–J12/11, the clustering of functional groups involved in substrate binding and how phylogenetically variable elements are accommodated by the core domain structure.

55. Brannvall M, Kirsebom LA: **Metal ion cooperativity in ribozyme cleavage of RNA**. *Proc Natl Acad Sci USA* 2001, **98**:12943-12947.

56. Brannvall M, Mikkelsen NE, Kirsebom LA: **Monitoring the structure of *Escherichia coli* RNase P RNA in the presence of various divalent metal ions**. *Nucleic Acids Res* 2001, **29**:1426-1432.

57. Brannvall M, Pettersson BM, Kirsebom LA: **Importance of the +73/294 interaction in *Escherichia coli* RNase P RNA substrate complexes for cleavage and metal ion coordination**. *J Mol Biol* 2003, **325**:697-709.

58. Harris ME, Pace NR: **Identification of phosphates involved in catalysis by the ribozyme RNase P RNA**. *RNA* 1995, **1**:210-218.

59. Christian EL, Kaye NM, Harris ME: **Helix P4 is a divalent metal ion binding site in the conserved core of the ribonuclease P ribozyme**. *RNA* 2000, **6**:511-519.

60. Kaye NM, Christian EL, Harris ME: **NAIM and site-specific functional group modification analysis of RNase P RNA: magnesium dependent structure within the conserved P1-P4 multi-helix junction contributes to catalysis**. *Biochemistry* 2002, **41**:4533-4545.

61. Christian EL, Kaye NM, Harris ME: **Evidence for a polynuclear**
•• **metal ion binding site in the catalytic core of the ribonuclease P ribozyme**. *EMBO J* 2002, **21**:2253-2262.
Analysis of the effects of site-specific functional group modification provides evidence of a cluster of metal ion interactions in the universally conserved core of RNase P RNA.

62. Kaye NM, Zahler NH, Christian EL, Harris ME: **Conservation of helical structure contributes to functional metal ion interactions in the catalytic domain of ribonuclease pRNA**. *J Mol Biol* 2002, **324**:429-442.

63. Schmitz M, Tinoco I Jr: **Solution structure and metal-ion binding of the P4 element from bacterial RNase P RNA**. *RNA* 2000, **6**:1212-1225.

64. Crary SM, Kurz JC, Fierke CA: **Specific phosphorothioate substitutions probe the active site of *Bacillus subtilis* ribonuclease P**. *RNA* 2002, **8**:933-947.

65. Kurz JC, Fierke CA: **The affinity of magnesium binding sites in**
•• **the Bacillus subtilis RNase P x pre-tRNA complex is enhanced by the protein subunit**. *Biochemistry* 2002, **41**:9545-9558.
In an extension of the work described in [64], this paper systematically evaluates the effect of the protein subunit on the binding of different classes of metal ions important for RNase P RNA folding, substrate binding and catalysis. Evidence that the protein enhances the affinity of metal ions involved in catalysis is provided, underscoring the intimate relationship between the protein subunit and the active site of the catalytic RNA subunit.

66. Massire C, Jaeger L, Westhof E: **Derivation of the three-dimensional architecture of bacterial ribonuclease P RNAs from comparative sequence analysis**. *J Mol Biol* 1998, **279**:773-793.

67. Chen JL, Nolan JM, Harris ME, Pace NR: **Comparative photocross-linking analysis of the tertiary structures of *Escherichia coli* and *Bacillus subtilis* RNase P RNAs**. *EMBO J* 1998, **17**:1515-1525.