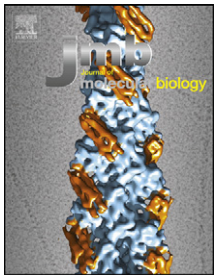

Theme and Variation in tRNA 5′ End Processing Enzymes: Comparative Analysis of Protein *versus* Ribonucleoprotein RNase P

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DOI of original article: http://dx.doi.org/10.1016/j.jmb.2015.11.025
http://dx.doi.org/10.1016/j.jmb.2015.12.001
Edited by A. Pyle

Establishing unifying principles of enzyme function requires testing the generality of structure and mechanism in many representative experimental systems. The differences between analogous enzymes are also important because they reveal instances of specialization and important structure–function relationships. Phosphoryl transfer is catalyzed by enzymes composed of both RNA (ribozymes) and protein and they provide systems for understanding theme and variation in biological catalysis. Exploration of similarities and differences between RNA-based and protein-based catalysts has contributed to illuminating fundamental mechanisms including metal ion and acid/base catalytic modes and coupling binding energy to catalysis [1–3].

The tRNA processing endonuclease ribonuclease P has been a useful system for exploring mechanisms of RNA enzymes because it occurs widely in biology as a ribonucleoprotein (RNP) with a catalytic RNA subunit [4–7]. The RNP RNase P version of the enzyme is found in organisms in all three domains of life; however, in some eukaryotes, the RNP RNase P has been entirely replaced or replaced in one or more cellular compartments with a protein-only RNase P (PRORP) [8–11]. The precise functional equivalence between RNP RNase P and PRORP despite such obvious difference in structure and ancestry makes them an excellent context in which to consider fundamental principles of enzyme function and to gain a deeper understanding of the role of RNase P in the RNA metabolism. New insight into the similarities and differences between different classes of PRORP enzymes reported by Karasik *et al.* (2016–in this issue) [12] represents an important step forward in allowing informative comparisons to be made.

Ribonuclease P (EC 3.1.26.5) catalyzes site-specific RNA phosphodiester bond hydrolysis to generate the mature tRNA 5′ end from precursor tRNA (ptRNA) substrates (Fig. 1) [4–7]. Both RNP RNase P and PRORP are multiple substrate enzymes that must process many tRNA precursors (e.g., over 80 different ptRNA in *Escherichia coli*). Based primarily on studies of bacterial RNP RNase P, it is known that the RNA subunit (P RNA) is composed of two domains, the *catalytic domain* that contains the active site and binds the base of the acceptor stem and the *specificity domain* that interacts with the D stem–loop [13,14]. In contrast to the high conservation of the catalytic RNA subunit, the number and the sequences of the protein subunits of RNP RNase P are variable [6]. There are four or greater protein subunits in archaebacterial enzymes and ca 10 in eukaryotic RNP RNase P. The function of the single small protein subunit of bacterial RNP RNase P is best characterized and it is known to contribute to specificity by contacting proximal 5′ leader sequences [15].

PRORP is a multidomain protein that functions as ribonuclease P. PRORP enzymes are composed of pentatricopeptide repeat (PPR) motifs, a central linker region and a metallonuclease domain [10,12,16,17]. PPR motifs are modular RNA-binding domains and contribute to tRNA binding specificity in PRORP [18]. The metallonuclease domain contains conserved acidic amino acids that bind divalent metal ions functioning as cofactors for catalyzing phosphodiester bond hydrolysis [10,17,19,20]. Recent structures of PRORP1, MRPP3 and now PRORP2 reveal the overall folding of these domains and their relative orientations (Fig. 1). The structure of PRORP1 from *Arabidopsis* is V shaped with the metallonuclease domain and PPR forms the ends connected by a zinc-binding domain in the middle [17]. The MRPP3 structure is similar [10] and Karasik *et al.* a report a comparable architecture for PRORP2.

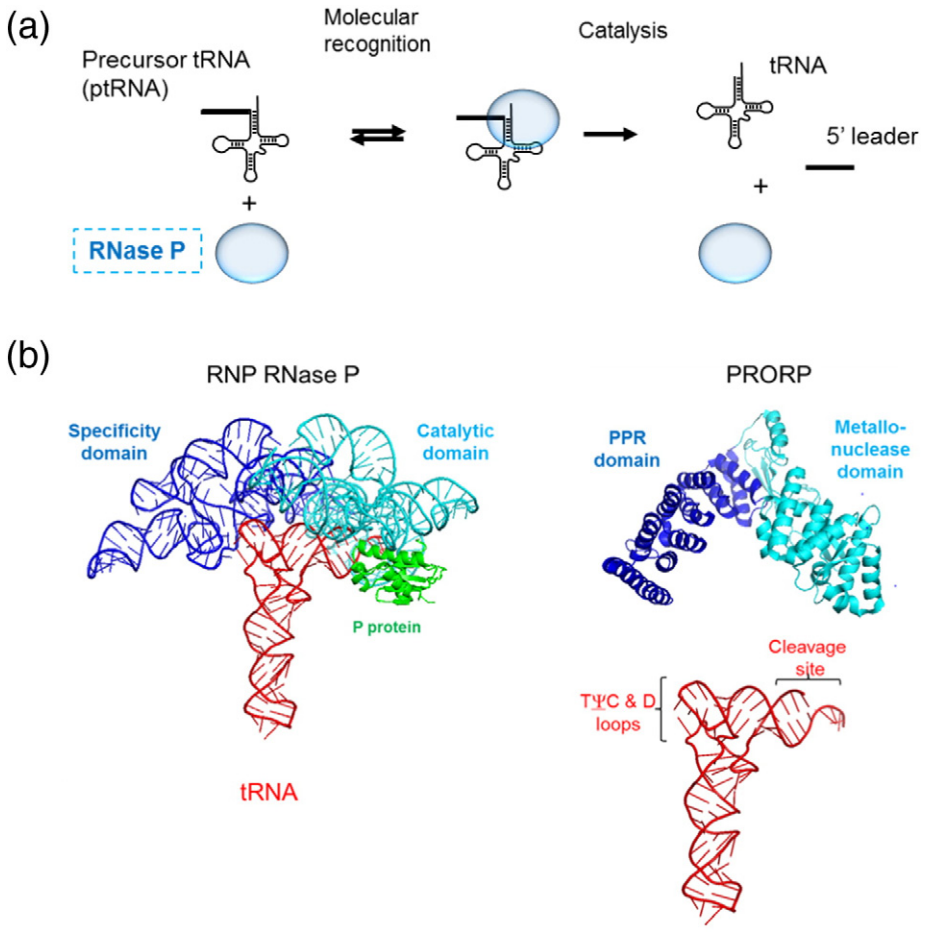

Fig. 1. (a) Ribonuclease P (RNase P) catalyzes maturation of ptRNA to generate the mature tRNA 5′ end. Both RNA-based and protein-based enzymes must accomplish molecular recognition and catalysis as indicated by the two-step mechanism. (b) Potential common theme in tRNA molecular recognition by RNP RNase P and PRORP. The domains that interact with the ptRNA proximal and distal to the cleavage site are colored light blue and dark blue, respectively.

However, the specific positions of the domains are different in the three structures. Also, in MRPP3, the loops connecting elements of secondary structure that form the proposed tRNA binding interface in the metallonuclease domain are disordered [10]. Conformational motion is often a direct contributor to enzyme function [21] and the apparent flexibility of PRORP enzymes is suggestive of functional importance. There is experimental evidence that RNP RNase P binds ptRNA in a two-step mechanism in which a conformational change is linked to catalysis [22,23]. However, the associated molecular motions and the enzyme–substrate interactions involved are not well understood. Simulations predict possible motions for PRORP2, and if true, they could be significant [12]. Direct tests of mechanistic proposals of the coupling of motion and catalytic function are needed in both RNA-based and protein-based RNase P enzymes.

A defining characteristic of biological catalysis is substrate specificity. For bacterial RNP RNase P, extensive structure probing, crosslinking, chemical protection and mutagenesis established the basic features of substrate recognition. Recent X-ray crystal structures of *Bacillus stearothermophilus* and *Thermotoga maritima* RNase P RNA [24,25] and the *T. maritima* RNase P holoenzyme [26] together with these data support a general model of the enzyme–substrate complex (Fig. 1b). In this model, the specificity domain binds the D stem–loop, while the catalytic domain interacts with the 3′-RCCA sequence and nucleotides flanking the ptRNA cleavage site. The P protein subunit interacts with proximal 5′ leader sequence nucleotides. The overall mechanism of PRORP recognition of tRNAs appears to parallel that of RNP RNase P enzymes [16,18]. Activity assays and footprinting experiments indicate that the anticodon stem–loop of ptRNA is dispensable, while the D and TΨC stem–loops are important for PRORP recognition. Mutational analyses of the substrate TΨC loop and amino acids within individual PPR motifs support a role for this domain in containing tRNA [27]. Using small-angle X-ray scattering, results are consistent with a model

in which the PPR motif interacts with the D and TΨC loops while the nuclease domain contacts the cleavage site [16,18].

This general perspective explains basic features of molecular recognition, but understanding how and why some substrates are preferred over others is an important current and future direction. Interestingly, Karasik *et al.* observe that *Arabidopsis* nuclear PRORP2 processes nuclear-encoded substrates up to 10-fold faster than a mitochondria-specific RNA precursor under single-turnover conditions [12]. Previously, it was shown that PRORP knockdown has unequal effects on the accumulation of different tRNAs [8]. Thus, substrate-specific differences in processing rates could be important for *in vivo* function. Koutmous and colleagues further show that PRORP2 preferentially binds ptRNAs with short 5′ leaders and 3′ trailers. These differences in functional substrate association are likely to be due to both direct contacts and differences in RNA structure in the free substrate ground state. RNA context is likely to exert a profound influence on RNA processing and this is an important and relatively unexplored aspect of RNA molecular recognition.

Transition-state stabilization is the second defining characteristic of biological catalysis. For RNase P enzymes, the active-site interactions that stabilize the transition state for phosphodiester hydrolysis have been most extensively investigated to date in bacterial RNP RNase P, although experimental evidence for specific catalytic modes remains sparse. A mechanism in which two active-site Mg^{2+} ions coordinate to the pro-Rp non-bridging phosphate oxygen of the reactive phosphoryl group is supported by metal ion concentration dependence of catalysis, effects phosphorothioate modification and thiophilic metal rescue experiments (Fig. 2a) (see Ref. [1] and references therein). Active-site ions are positioned in part by coordination to non-bridging phosphoryl oxygens in helix P4 of the catalytic

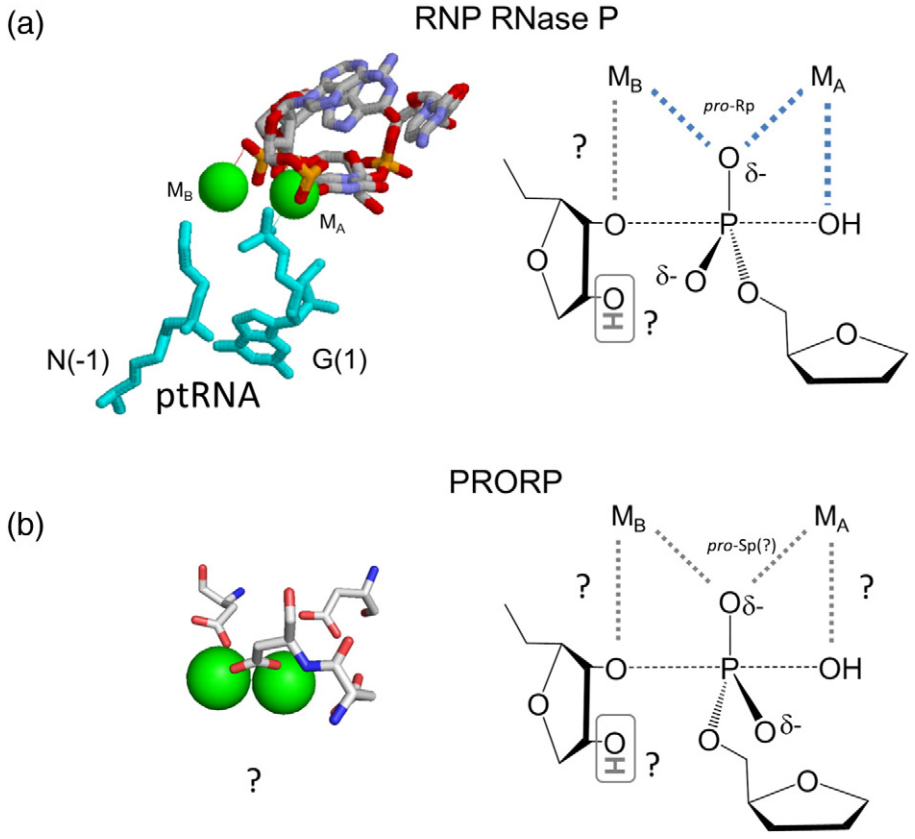

Fig. 2. (a) Proposed structure and mechanism of the bacterial RNP RNase P active site. Model for the position of active-site metal ions (green) from Reiter *et al.* The ptRNA is shown in the cleaved product state (cyan). A general two-metal-ion model involving direct coordination to nucleophile, leaving group and non-bridging oxygen is shown. Interaction for which supporting experimental evidence is available are shown in boldface. Evidence for leaving group stabilization is provided by thio effects although the precise mode is not clear. (b) Structure the PRORP active-site metal ion binding pocket in the metallonuclease domain. Conserved aspartic acid residues involved in metal in interactions are shown. Significant and, importantly, experimentally testable questions remain regarding parallels between the active sites of the two classes of enzyme.

domain. Similar to other metalloendonucleases, the pH dependence for RNP RNase P is consistent with base catalysis. Nucleophile ^{18}O kinetic isotope effects on RNP RNase P and solution hydrolysis reactions are consistent with equilibrium deprotonation and metal ion coordination of the nucleophile in the transition state. A second-ion active-site ion is proposed to stabilize the 3′O leaving group and could act either by inner sphere coordination or as general acid catalysis via a coordinated water molecule.

PRORP also requires divalent ions for catalysis and *Arabidopsis* PRORP1 is cooperatively dependent on Mg^{2+} concentration with a Hill coefficient of 2 [19]. This correlates with the observation of two divalent metal ions positioned by conserved aspartic acid residues in the metalloendonuclease domain (Fig. 2b). The functional importance of these residues is demonstrated by large decreases in activity resulting from their mutation [12]. PRORP enzymes are insensitive to Rp-phosphorothioate modification at the ptRNA cleavage site [20]. However, Rp and Sp coordination of active-site metal ions are observed in both RNA and protein enzymes [3]. Thus, at this point, it is attractive to speculate that both protein-based and RNA-based RNase P enzymes use two metal ions to catalyze phosphodiester bond hydrolysis and make similar interactions with the reactive phosphoryl group in the transition state. However, distinguishing between specific metal ion catalytic modes is difficult even when powerful experimental and computational tools have been applied (e.g., see Ref. [28]). Both experiment and computation will be needed to pin down the interactions made by active-site metal ions, and this will rely on clearer pictures of active sites of both RNP RNase P and PRORP. Regardless of whether or not strict convergence of structure and function turns out to be true, exploration of common themes and variation in PRORP and RNP RNase P will be broadly important for understanding enzyme mechanisms and their *in vivo* functions.

Available online 3 December 2015

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