# Uniformity amid diversity in RNase P

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The ribonucleoprotein RNase P, which catalyzes the $Mg^{2+}$-dependent removal of the 5′ leader sequence in all precursor tRNAs (ptRNAs), is remarkable for the diversity in its subunit composition (Fig. 1*A*). Although all RNase P holoenzymes have an essential RNA subunit, the number of protein subunits varies from 1 in Bacteria to at least 4 in Archaea and 9–10 in Eukarya (1–5). The finding that the bacterial RNase P RNA (RPR) subunit alone is catalytic under *in vitro* conditions of high ionic strength provided one of the first examples of a true cellular RNA enzyme (6). Despite a shared ancestry, as reflected by sequence/structure similarities especially in regions expected to comprise the catalytic core (Fig. 1 *B–E*), to date, only some archaeal RPRs were proven to be weakly catalytic, whereas all eukaryal RPRs examined were inactive in the absence of their cognate RNase P proteins (Rpps; refs. 1, 3, 7, and 8). Consistent with the RNA world hypothesis, these findings suggest that the primordial ribozyme activity observed in bacterial RPRs was somehow lost in many archaeal and all eukaryal RPRs with concomitant gains by their cognate Rpps that might have usurped the RPR's catalytic roles. However, such a postulate can now be laid to rest because in this issue of PNAS, Kikovska *et al.* (9) provide evidence that human RPR can catalyze processing of four different ptRNAs and a model substrate, albeit at rates much lower than that of bacterial RPR.

Why then has this eukaryal RPR activity eluded detection for nearly two decades? Two features in the study by Kikovska *et al.* (9) appear to have played a crucial role: the choice of a low pH assay buffer and the presence of a fortuitous mutation in human RPR. First, while balancing between conditions that favor maximal activity and those that minimize the inherent chemical instability of RNA during prolonged incubations at alkaline pH, emphasis on the latter has allowed for a more sensitive detection of cleavage products. The choice of an assay buffer at pH 6 is counterintuitive because the rate constant for the cleavage step increases with pH, consistent with an $S_N2$ reaction mechanism in which a metal-hydroxide nucleophile attacks the scissile phosphodiester linkage. Typically, an assay buffer at pH 7.5 or 8 is used unless the goal is to render cleavage the rate-limiting step. Second, an inadvertent deletion of C298 enhanced the activity of human RPR up to 5-fold with some substrates; the mechanistic basis for the increase is unclear.

Several observations validate the bona fide nature of the human RPR activity observed under single-turnover conditions. First, because RNase P cleavage generates 5′-phosphate and 3′-hydroxyl end groups (Fig. 1*A*), Kikovska *et al.* (9) ascertained that indeed a 5′-phosphate is present in the mature tRNA product. Second, human RPR was shown to accurately process four different ptRNAs and a model substrate illustrating that the cleavage observed was not a chance occurrence. Furthermore, the RPR from *Giardia lamblia*, a lower eukaryote, also processed the model substrate under conditions similar to those used for detecting human RPR activity (9). Finally, because it has been shown that the universally conserved P4 helix is proximal to the ptRNA cleavage site in bacterial RNase P and involved in binding functionally important metal ions (2), Kikovska *et al.* (9) deleted three nucleotides in the P4 helix of human RPR and demonstrated loss of activity.

**Eukaryal RNase P RNAs are weaker catalysts than their bacterial counterparts.**

Under identical assay conditions, the single-turnover rate constants ($k_{obs}$) for cleavage of the model substrate by *Escherichia coli*, human, and *G. lamblia* RPRs are 8, $2.6 \times 10^{-5}$, and $3.5 \times 10^{-6}$ $min^{-1}$, respectively, revealing that the eukaryal RPRs are weaker catalysts than their bacterial counterparts (9). Despite this large difference in activity, it is notable that the $k_{obs}$ value even for the bacterial RPR decreases to $10^{-3}$ $min^{-1}$ with certain atypical ptRNA substrates or when the RPR is modified to mimic the eukaryal RPR structure (9, 10). The rate enhancement afforded by human RPR can be accurately calculated only if the rate of spontaneous cleavage at the RNase P processing site (between the −1 and +1 positions in the ptRNA) is known. Because this information is unavailable, one must rely on the rate of uncatalyzed breakdown of an RNA phosphodiester linkage in other molecules (11) or at different positions in the ptRNA (9), which is only an approximation because the position, identity, and local structure in these models are different from the RNase P cleavage site. Such an approach nevertheless reveals that human RPR-catalyzed cleavage of ptRNA/model substrates enjoys a rate enhancement of at least $10^2$- to $10^3$-fold. Although this finding may not seem very impressive in light of some protein enzymes accomplishing rate accelerations of up to $10^{17}$-fold, any enhancement at all highlights the catalytic properties of human RPR. This observation underscores a common RNA-mediated catalytic mechanism in all RNase P holoenzymes, a premise supported by the RPRs from all three domains of life having ≈12 conserved nucleotides at nearly identical locations (Fig. 1), most of which are present in pared-down versions of bacterial/archaeal RPRs that retain activity.

What might account for the weak activity observed with human and some archaeal RPRs in the presence of high concentrations of monovalent and divalent cations (7, 9)? Is only a fraction of these RPRs able to adopt a catalytically active conformation in the absence of their Rpps, which might capture/stabilize these transient (functional) structures? High-resolution structures of bacterial RPRs reveal how long-range intramolecular RNA contacts serve as braces that precisely juxtapose the two independently folded substrate-specificity (S) and catalytic (C) domains (12, 13) and thereby facilitate their exquisite cooperation in substrate binding and catalysis. The lack of these tertiary structure-stabilizing struts might explain the decreased catalytic activity of archaeal/eukaryal RPRs, relative to bacterial RPRs, and their greater dependence on cognate Rpps for forming a functional structure. Indeed, eukaryal RPRs do

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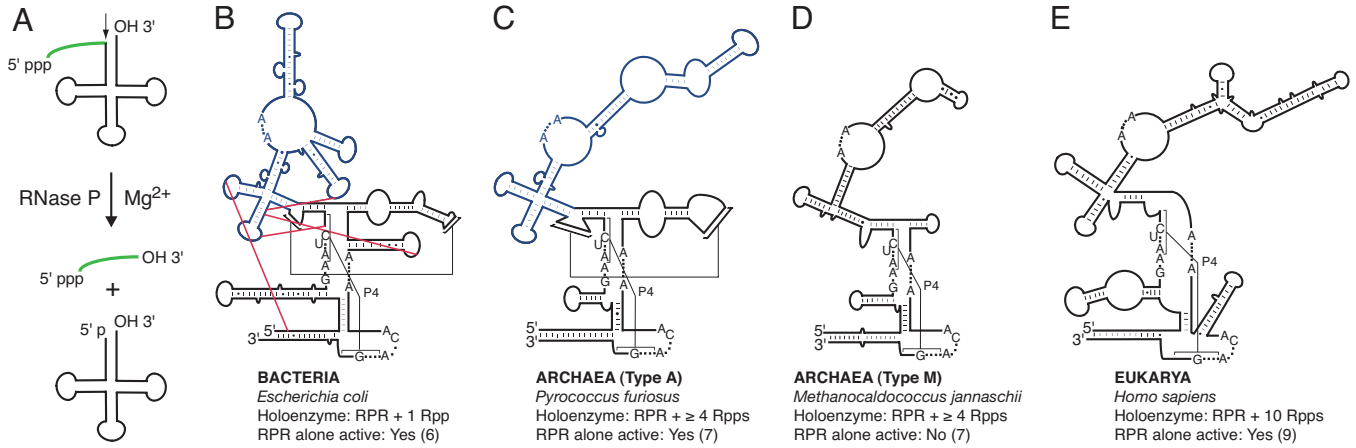


**Fig. 1.** Function and subunit composition of RNase P. (*A*) The reaction catalyzed by RNase P. (*B–E*) Universally conserved nucleotides are depicted in the secondary structures of representative RPRs from the three domains of life (17). Of the various structural elements, only the P4 helix is labeled. In *B* and *C*, the pared-down catalytic domains that are active in the presence of their cognate Rpps are shown in black. In bacterial RPR, blue indicates the substrate-specificity domain, and red lines indicate experimentally validated tertiary contacts. Archaeal RPRs are classified as types A (ancestral version; *C*) and M (those primarily from *Methanococci*; *D*); the latter have not been proven to be active in the absence of cognate Rpps (7).

seem more susceptible to thermal denaturation than bacterial RPRs (14).

Several immediately profitable directions merit consideration. First, reproducing the results of Kikovska *et al.* (9) with different archaeal/eukaryal RPRs will bolster the idea that the catalytic core really rests with the RPR during evolution of RNase P. The corollary is that Rpps facilitate RPR catalysis by fine-tuning/stabilizing the active site for productive positioning of the substrate and catalytically important metal ions. Second, having conditions under which the human RPR is weakly active will permit an evaluation of the functional roles of individual human Rpps, as demonstrated for archaeal RNase P (15, 16). Third, if eukaryal RPRs do form short-lived functional structures, cross-linking or RNA engineering could be attempted to trap these conformations and demonstrate that such RNAs could be weaned of their dependence on some Rpps. Last, the RNA subunit of eukaryal RNase MRP, an endoribonuclease involved in nuclear pre-rRNA processing and generation of RNA primers for mtDNA replication, should be examined for catalytic activity in the absence of its cognate proteins because RNase MRP RNA and RPR (*i*) share secondary structure similarities and even identity at positions suspected to be functionally important and (*ii*) share most of their protein cofactors; additional interest stems from mutations in human MRP RNA being associated with developmental disorders (3).

Despite the RPR being responsible for the cleavage step in all three domains of life, there is an almost inverse correlation in the catalytic efficiency of the different RPRs and their number of Rpps. In bacterial RNase P, although the RPR alone is folded into a stable structure that is highly active, the sole Rpp renders uniform the binding affinity and rate of cleavage of different ptRNAs by the RPR, making the RPR more efficient and versatile (10). A few bacterial RPR structural elements that are essential for substrate binding, catalysis, and global stability were either never acquired or lost during evolution of archaeal/eukaryal RPRs accounting for their lower stability/activity in the absence of cognate Rpps (4, 5, 14). Did the recruitment of multiple archaeal/eukaryal Rpps impose structural restrictions that limited the Rpp-independent catalytic capabilities of their respective RPRs? Perhaps this is the cost of tinkering with substrate specificity, subcellular targeting, and/or regulation of the archaeal/eukaryal RNase P holoenzymes. Could archaeal/eukaryal Rpps be distinguished by their structural (i.e., ability to replace the intramolecular RNA struts in bacterial RPRs) or catalytic (i.e., facilitate substrate binding and cleavage like bacterial Rpp) role? Answers to these questions will shed light on the evolution of an ancient ribonucleoprotein. For now, however, in the continuing saga to establish the catalytic roles of RNA moieties in large cellular ribonucleoproteins (e.g., ribosomes, spliceosomes, eukaryal RNase P), Kikovska *et al.* (9) have scored an important advance in demonstrating that the human RPR is catalytic in the absence of its 10 protein subunits.

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