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RNase P: interface of the RNA and protein worlds

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Ribonuclease P (RNase P) is an endonuclease involved in processing tRNA. It contains both RNA and protein subunits and occurs in all three domains of life: namely, Archaea, Bacteria and Eukarya. The RNase P RNA subunits from bacteria and some archaea are catalytically active *in vitro*, whereas those from eukaryotes and most archaea require protein subunits for activity. RNase P has been characterized biochemically and genetically in several systems, and detailed structural information is emerging for both RNA and protein subunits from phylogenetically diverse organisms. *In vitro* reconstitution of activity is providing insight into the role of proteins in the RNase P holoenzyme. Together, these findings are beginning to impart an understanding of the coevolution of the RNA and protein worlds.

RNase P: a model ribozyme and ribonucleoprotein

To function, tRNAs must undergo several processing reactions including the removal of extra sequences at both the 5′ and 3′ ends and the modification of specific bases [1–3]. One such processing event is the endonucleolytic cleavage of the precursor sequence from the 5′ end of a primary tRNA transcript to form the mature 5′ end. This reaction is catalyzed by the ribonuclease P (RNase P) enzyme (Figure 1), a ribonucleoprotein that contains an RNA subunit plus one or more associated protein subunits (for a possible exception, see Ref. [4]).

Homologs of RNase P RNA subunits occur ubiquitously, indicating that a primordial version of the contemporary RNase P RNA was present in the earliest proto-life, even before the differentiation of the phylogenetic domains Bacteria, Eukarya and Archaea. RNase P RNA has been touted as a vestige of an early RNA world owing to this ancient heritage [5]. In conditions of high salt, the RNase P RNA from bacteria and some archaea can catalyze the processing reaction *in vitro* independently of proteins, proving that the RNA from these organisms is the catalytic subunit. Both RNA and protein subunits are, however, essential for viability *in vivo*.

Here we describe progress in our knowledge of the evolutionary variation in the structure of the RNA and protein subunits of RNase P. We also review recent results on the reconstitution of RNase P from its RNA and protein subunits. These advances in our understanding of RNase P provide insight into the interface between the RNA and protein worlds.

The universal subunit: RNase P RNA

In bacteria, the RNase P holoenzyme consists of one RNA of typically 350–400 nucleotides and one small basic protein of ~14 kDa (Figure 2). Although both subunits are essential *in vivo*, conditions of increased ionic strength activate catalysis by the RNA subunit alone *in vitro* [6]. Bacterial RNase P RNA is an RNA enzyme or 'ribozyme'. High concentrations of salt are thought to be required for screening electrostatic repulsion in the RNA, which otherwise distorts the active structure. The protein must fulfill this role *in vivo*.

RNase P is a metalloenzyme that requires divalent ions, preferably Mg^{2+}, for specific folding of the RNA and its catalytic mechanism [7]. In addition to precursor tRNAs (pre-tRNAs), other reported substrates of RNase P include signal recognition particle RNA, transfer-messenger RNA, phage regulatory RNAs, bacterial operon RNAs and riboswitches [8] (reviewed in Ref. [9]). RNase P recognizes the TΨC stem-loop and acceptor stem that are found in all tRNAs [10] (Figure 1), and other RNase P substrates might contain similar structural features that mimic tRNAs. The diversity of RNase P substrates and the ability of RNase P RNA to perform catalysis in the absence of protein have made determination of the structure of this enzyme an obvious scientific goal.

Comparative analyses: conserved core of the RNA

Perspective on the structure of RNase P RNA has accumulated incrementally, beginning with the evolutionary approach of phylogenetic comparative sequence analysis [11]. RNA sequences typically form helical elements of secondary structure by base-pairing, and other interactions then order these helices to form the functional tertiary structure. In a phylogenetic comparative analysis, an alignment of sequences that are homologous (of common ancestry) is scrutinized to identify bases that systematically co-vary in some way – usually to maintain complementarity – and thereby to identify potentially base-paired helical elements of secondary structure [12].

The comparative analysis of several hundred RNA sequences has resulted in refined secondary structure

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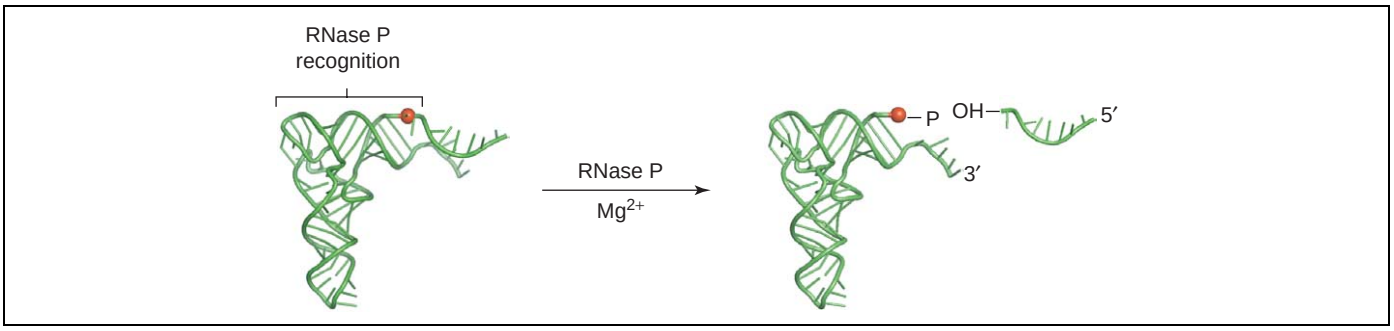

Figure 1. The RNase P reaction. In the presence of divalent cations (Mg^{2+}), ribonuclease P cleaves the 5′ leader sequence of all precursor tRNAs, producing a mature 5′-phosphate on the end of the tRNA and a hydroxyl group on the 3′ end of the leader sequence. The red sphere indicates the cleavage site.

models of the RNase P RNAs (Figure 3). The bacterial RNase P RNAs fall into two distinct classes of secondary structure: ancestral-type or A-type, such as *Escherichia coli* RNase P RNA; and *Bacillus*-type or B-type, such as *Bacillus subtilis* RNase P RNA [13] (Figure 3). In most cases, the eukaryal RNA is shorter than the bacterial RNA and seems to be more rudimentary: it typically lacks structural elements that are known to stabilize global structure in bacterial RNA. The archaeal RNA tends to be closer in size to that found in bacteria and usually contains extra helices as compared with the eukaryal RNA.

Allowing for some length variation, the helical elements P1–P4 and P7–P11 occur at the same relative positions in all RNase P RNAs. Five distinct, universally conserved regions (CRI–CRV) are located in comparable positions in the secondary structures [14]. Portions of CRI and CRV pair to form helix P4, one of the most conserved substructures in all RNase P RNAs. CRII and CRIII are located in a universally conserved loop between helices P10 or P11 and P12. These features occur in all RNase P RNAs and constitute the structural core of the RNA. This minimum structural core must have been present in the ancestral RNase P RNA (Figure 3). An artificial, simplified RNA that contains only the core of the bacterial RNase P RNA is catalytically active *in vitro*, albeit far less so than the wild-type RNA because of structural instability [15,16]. Thus, in wild-type bacterial RNase P RNAs, structurally stabilizing helices are attached to the conserved core and dock elsewhere, forming a brace to stabilize structure important for function [17,18]. Examples of stabilizing helices in the bacterial RNase P RNAs include P6, P14 and P18 for A-type RNAs and P5.1 and P15.1 for B-type RNAs [19] (Figure 3).

Structural analyses: architecture of the RNA

The first information on the global packing of the RNA helices was obtained by using spatial constraints provided by intramolecular crosslinking [20,21]. In these studies, photoaffinity crosslinking agents were attached at homologous sites in the *E. coli* and *B. subtilis* RNAs and irradiated with ultraviolet light to form crosslinks with other parts of the same RNA molecule. The nearly identical crosslink patterns obtained between A- and B-type RNAs indicated that these RNAs fold into a similar 3D shape despite structural differences. However, the resulting tertiary structure models, which were based on only the

Bacteria		Eukarya				Archaea					
Eco		*Sce*		*Hsa*		*Pfu*		*Pho*		*Mth*	
RNA	(121)	RNA	(118)	RNA	(109)	RNA	(106)	RNA	(106)	RNA	(94)
RnpA	(13.8)										
		Pop5	(19.6)	hPop5	(18.8)	PF1378	(13.8)	PH1481*	(14.0)	MTH687	(14.6)
		Rpp1	(32.2)	Rpp30	(29.3)	PF1914	(24.5)	PH1877	(24.7)	MTH688	(27.7)
		Rpr2	(16.3)	Rpp21*	(17.6)	PF1613	(14.3)	PH1601*	(14.6)	MTH1618	(17.0)
		Pop4	(32.9)	Rpp29*	(25.4)	PF1816	(15.0)	PH1771*	(15.1)	MTH11	(10.7)
		Pop1	(100.5)	hPop1	(114.7)						
		Pop3	(22.6)	Rpp38	(31.8)						
		Pop7	(15.8)	Rpp20	(15.7)						
		Pop6	(18.2)								
		Pop8	(15.5)								
				Rpp40	(34.6)						
				Rpp25	(20.6)						
				Rpp14	(13.7)						

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Figure 2. Comparison of the composition and reconstitution requirements of the RNase P holoenzyme highlights evolutionary history. Homologous components of the RNase P holoenzyme from *Escherichia coli* (*Eco*), *Saccharomyces cerevisiae* (*Sce*), *Homo sapiens* (*Hsa*), *Pyrococcus furiosus* (*Pfu*), *Pyrococcus horikoshii* (*Pho*), *Methanothermobacter thermoautotrophicus* (*Mth*) are aligned horizontally. The molecular mass (in kDa) of each subunit is given in parentheses. The universal RNA subunit, which is essential both *in vivo* and *in vitro*, is highlighted by a red box. This RNA subunit is sufficient for *in vitro* activity in bacteria and some archaea. The composition of the *E. coli* holoenzyme, a single RNA subunit plus the small basic protein RnpA, is highlighted by a blue box. The minimal holoenzyme composition shared before the divergence of the Archaea and Eukarya – namely, the RNA subunit and at least four of the proteins – is highlighted by a green box. Asterisks indicate the minimal protein subunits required, along with the RNA subunit, for the *in vitro* reconstitution of human [55] and *P. horikoshii* [57] RNase P activity.

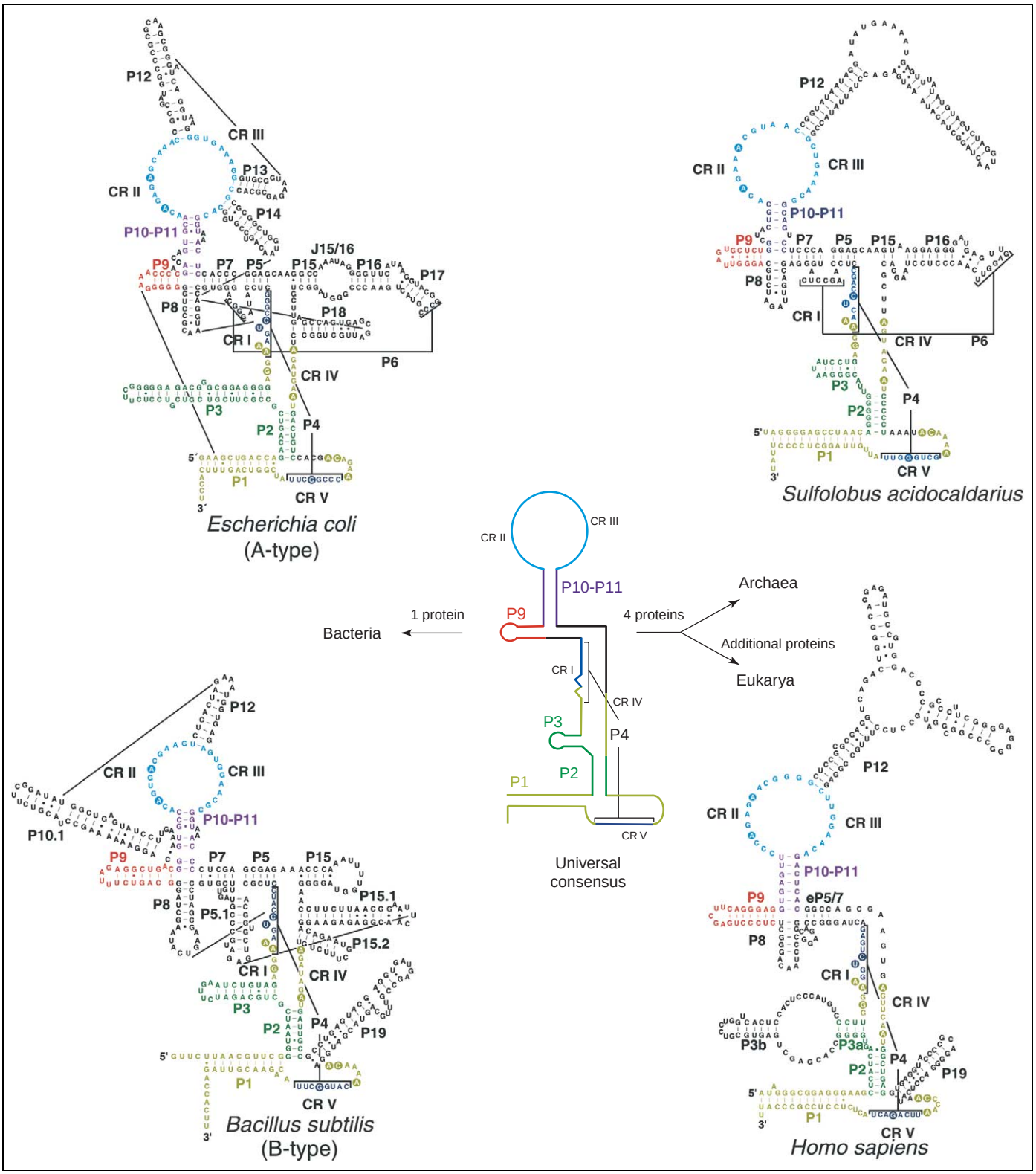

Figure 3. Varieties of RNase P: commonalities in diversity. Shown are representative secondary structures of RNase P RNA from the three domains of life, Bacteria (A- and B-type), Archaea and Eukarya, in addition to the universal minimum consensus structure. Only structural elements that are conserved in all known RNase P RNAs are included in the universal minimum consensus structure. These structures are thought to have been present in the primordial RNA. The secondary structures are colored to highlight homologous structural elements found in representative RNAs. Structural elements that are not found in all RNase P RNAs are shown in black. Arrows indicate the path of evolution according to rRNA phylogenetic analysis, consistent with the changes seen in RNase P RNA and proteins. Structural elements in the RNAs are labeled, 5′ to 3′, according to the definitions for the bacterial RNA: 'P' for paired region, 'L' for loop and 'J' for joining region. Base pairs represented by dots indicate a non-canonical base interaction. Long-range tertiary interactions (e.g. base-pairings in helix P4) are shown as brackets and/or lines. The five conserved regions (CRI–V), are noted and the universally conserved nucleotides among all three domains are highlighted. RNase P RNA sequences and secondary structures are available from the RNase P Database (http://www.mbio.ncsu.edu/RNaseP) [69]. The universally conserved adenine that is shown as unpaired in P4 on the basis of the *B. stearothermophilus* RNase P RNA structure (Figure 4) is likely to be unpaired in all RNase P RNAs owing to its homology.

comparative analysis and crosslinks, provided no details on the interhelical sequences that contain many highly conserved nucleotides. In the *E. coli* RNA, for example, interhelical regions constitute approximately one-fifth of the nucleotide sequence. Only a high-resolution crystal structure of the RNA would be able to model these sequence elements, which are expected to be crucial to both structure and function.

X-ray crystallographic structures of bacterial RNase P RNA emerged initially from analyses of fragments of the RNA [22,23] and more recently from analyses of full-length A- and B-type RNase P RNA from *Thermotoga maritima* [24] and *Bacillus stearothermophilus* [25], respectively. These are the largest free RNA structures that have been solved crystallographically. Both structures contribute to our overall view of the RNase P RNA architecture. For example, a region of the RNA that was disordered in the crystals of *B. stearothermophilus* RNase P RNA could be modeled in the structure of *T. maritima* RNA, whereas the higher resolution of the B-type structure provided more detailed structural information pertinent to the proposed active site.

The crystal structure of the full-length RNA subunit (338 nucleotides) from *T. maritima* was solved to 3.85 Å. At this level of resolution, the interhelical regions could not be modeled accurately, but the general structure of the whole RNA could be inferred and was in agreement with the overall *B. stearothermophilus* structure. Long-range interactions that stabilize the global structure of the molecule were found to be consistent with those predicted by comparative analysis and biochemical data [18,26,27] (Figure 3). As mentioned, a portion of the RNA in the crystals of the *B. stearothermophilus* RNase P RNA was disordered; however, 298 of the 417 nucleotides could be modeled at 3.3 Å resolution (Figure 4). Importantly, all of the elements required for catalysis are included in this model.

As can be seen in the *B. stearothermophilus* structure, CRI, CRIV and CRV are drawn together to form the catalytic core of the enzyme. The positions of the core helical elements are stabilized by long- and short-range interactions, some of which were detected by comparative analysis [19]. For example, the loops of P5.1 and P15.1 interact with each other and the loop of P8 docks into the minor groove of P4. These interactions plus several similar ones stabilize the overall folding of the RNA. This buttressing of the tertiary structure by long-range interactions is an 'RNA solution' to the thermodynamic problem of global molecular stability. This method of stabilization differs from the predominant theme of protein folding – internal hydrophobic interactions.

The crystal structures of the RNase P RNAs offer no specific information on how the pre-tRNA substrate and protein might interact with the RNA. They do, however, provide a framework for correlating a wealth of biochemical data and thereby for approximating such interactions. For example, photoaffinity crosslinking has been used to map the active site of bacterial RNase P RNA [28]. In this approach, a photoaffinity agent was attached to the 5′-phosphate of mature tRNA; this phosphate is the product of the RNase P cleavage reaction and thus is expected to occupy the active site. This modified tRNA was then crosslinked to RNase P RNA. The crosslinked sites were mapped to phylogenetically conserved sequences in the RNA that, in principle, are located near the active site (Figure 4a,b). These structural results have been bolstered by biochemical studies that located particular functional groups in the RNA (including specific purine N-7, guanosine N-2, 2′-ribose hydroxyl groups and non-bridging phosphates) that impair enzyme function when modified. These functional groups are found in interhelical regions surrounding the active site, as indicated by crosslinking to the 5′ end of tRNA (reviewed in Ref. [9]). These results, in addition to other crosslinking data, have facilitated modeling of the tRNA bound to the crystal structure of the bacterial RNase P RNA [24,25] (Figure 4c,d).

RNase P activity: requirement for structural stability?

Comparative analysis shows that the eukaryal RNase P RNA is a homolog of the archaeal and bacterial versions and contains most of the elements shown to be essential for activity in the bacterial RNAs [29,30] (Figure 3). However, the eukaryal RNase P RNAs that have been tested so far are inactive *in vitro*. The typical eukaryal RNase P RNA is approximately two-thirds of the sequence length of the typical bacterial RNase P RNA and lacks key stabilizing helices found in bacterial RNAs (Figure 3). Another chief difference between eukaryal and bacterial RNase P RNAs is the presence, in most eukaryal RNAs, of a bulge-loop in helix P3. This structure is required for nucleolar localization of the human RNase P RNA [31].

The archaeal RNAs tend to be similar in size to the bacterial RNAs and they lack, or have seemingly different versions of, structurally stabilizing helices similar to those found in bacterial RNAs. Although most archaeal RNase P RNAs that have been tested are catalytically inactive without their protein subunits, a low level of activity has been detected for some RNAs under *in vitro* conditions of extraordinarily high concentrations of monovalent and divalent ions (well above those required for bacterial RNase P RNAs) [32]. This 'RNA-alone' activity seems to correlate with the presence of helical elements that are likely to contribute to structural stability.

Bacterial holoenzyme: one small protein

Although the bacterial RNase P RNA is catalytically active *in vitro* under conditions of high salt, the protein subunit is necessary *in vivo* and under physiological conditions of low ionic strength. Proposed roles of the protein subunit include stabilization of the catalytically active conformation of RNase P RNA [33], participation with the RNA to discriminate between substrate and product by directly binding to the 5′ precursor sequence [34–36], and mediation of holoenzyme dimerization [37]. These potential roles are not mutually exclusive and there is evidence to support each [38].

Structures of RNase P protein subunits from three phylogenetically distinct bacteria, *B. subtilis*, *Staphylococcus aureus* and *T. maritima* (representing both A- and B-type RNase P enzymes) have been determined by X-ray crystallography and NMR [34,39,40]. Although the

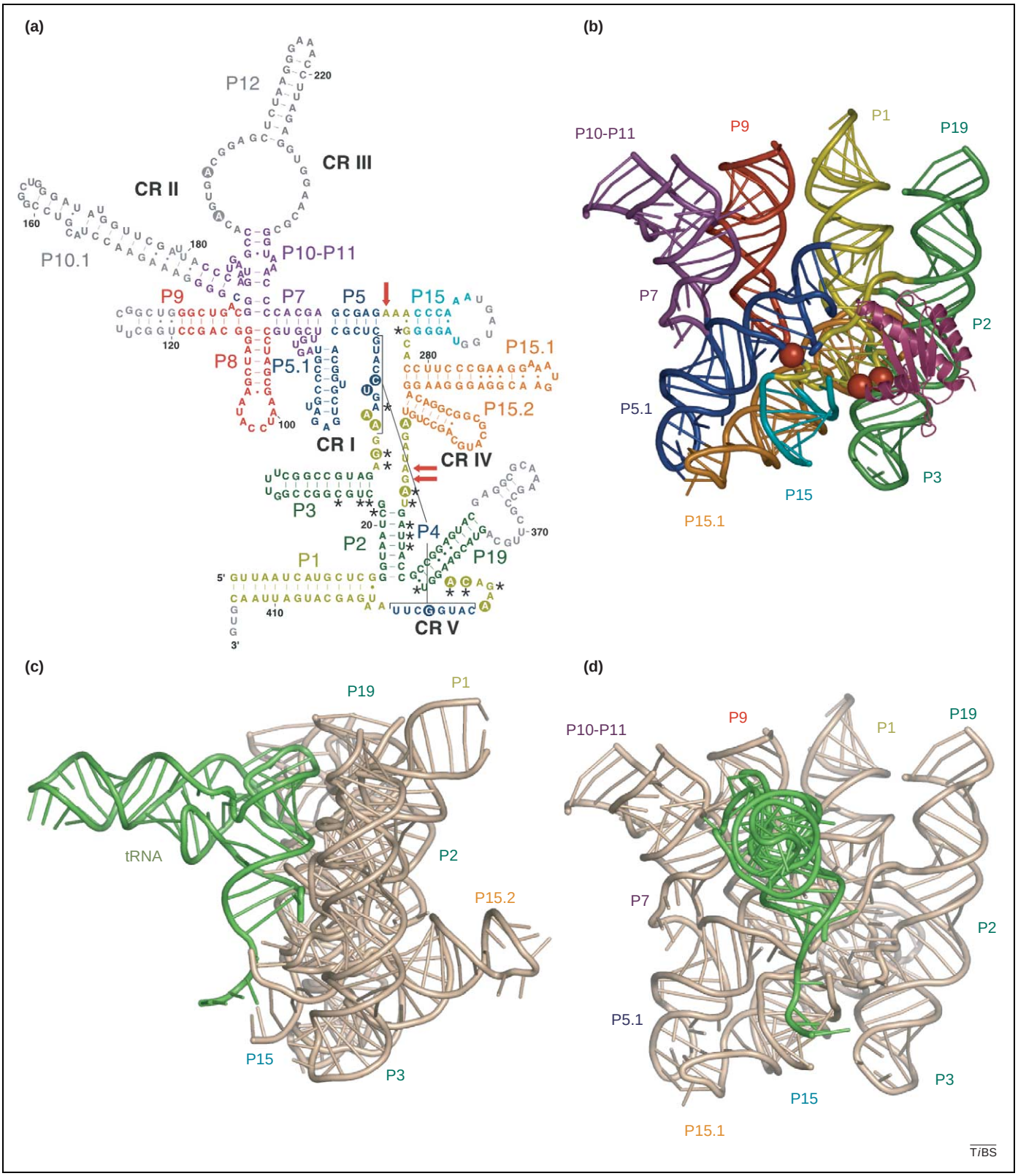

Figure 4. The structure of *B. stearothermophilus* RNase P. **(a)** Secondary structure of *B. stearothermophilus* RNase P RNA, colored as in Figure 3 to highlight structural elements in the ribbon representation of the structure [25]. Nucleotides in gray indicate parts of the structure that could not be determined owing to disorder in the crystal [25]. Red arrows indicate the sites of tRNA 5′ crosslinking inferred from experiments with the *B. subtilis* RNA [28]. Asterisks indicate nucleotides protected from cleavage by the protein [43]. Highlighted nucleotides are universally conserved. **(b)** Tertiary model of the bacterial holoenzyme. Shown is a ribbon representation of the *B. stearothermophilus* RNA and the *B. subtilis* protein subunit, which was positioned by using footprinting data [43]. The sites of tRNA 5′ crosslinking, indicated with arrows in (a), are shown as red spheres. **(c,d)** Respective side and front view reconstructions of *B. stearothermophilus* RNase P RNA in complex with tRNA [25]. In the side view reconstruction, the coaxial stack of P19, P2 and P3 is in the forefront. In the front view reconstruction, the anticodon loop of tRNA is in the forefront. Figure created with MacPyMol (DeLano Scientific; http://www.pymol.org).

sequences of these proteins are not highly conserved (20–30% amino acid identity), the overall 3D shapes are remarkably similar. This structural similarity results in functional similarity: RNase P RNA and proteins from different organisms can be reconstituted *in vitro* to form active heterologous complexes [6]. The overall fold of the protein is an αβ sandwich, similar to that found in the ribonucleoprotein family. The protein has two experimentally supported RNA-binding sites: a highly unusual left-handed βαβ crossover, which contains the conserved Arg-Asn-Arg (RNR) motif; and a large central cleft proposed to bind several nucleotides of single-stranded RNA [34]. Mutation of amino acids in these binding sites results in defective pre-tRNA processing by the holoenzyme *in vitro* [41,42]. This information, coupled with the identification of nucleotides protected by the protein from chemical modification ('footprint' analysis), has provided the latest low-resolution view of the bacterial holoenzyme (Figure 4b). In this model, the protein binds at a site adjacent to the catalytic pocket of RNase P RNA and, according to biochemical data, has an effect on RNA conformation near the tRNA-binding site [43].

Eukaryal and archaeal holoenzymes: more proteins

The evolution of global stability in the archaeal and eukaryal RNase P holoenzymes has followed a path that is slightly different to that taken by the bacterial version. Whereas the bacterial RNA developed stability through an extensive RNA superstructure, the eukaryal and archaeal RNAs probably achieved stability through additional proteins.

RNase P proteins: homology, functions and interactions

Eukaryal RNase P holoenzymes have been purified from both *Saccharomyces cerevisiae* and *Homo sapiens*; they have a much greater protein content than the bacterial holoenzyme, containing at least nine and ten proteins, respectively [44,45]. Homologous sets of RNase P proteins from different organisms are summarized in Figure 2. Because the terminology for homologous proteins is not consistent across organisms, we use the *S. cerevisiae* protein names as a reference for the homologous protein from other organisms in the following discussion.

Only seven of the yeast proteins have identifiable homologs in human RNase P. None of these proteins shares obvious sequence similarity to the bacterial RNase P proteins. The different proteins vary greatly in size, ranging from 15.5 to 100.5 kDa in the yeast holoenzyme and showing similar variation in the human holoenzyme (Figure 2). The protein content of the bacterial RNase P holoenzyme is ~10% of the total mass, whereas that of the eukaryal holoenzyme is at least 70%. Genetic knockout experiments have shown that each of the nine yeast RNase P protein subunits is essential for viability [44].

Although the specific functions of the eukaryal RNase P protein subunits are not currently known, potential roles include those proposed for the bacterial RNase P protein (see earlier), in addition to global RNase P RNA stabilization, localization [46], processing of precursor RNase P RNA, and contributing to the active site [47]. Binding to the RNase P RNA *in vivo* has been demonstrated for human and yeast protein subunits in a yeast three-hybrid genetic screen [48,49]. The binding of the *S. cerevisiae* Pop1 protein subunit has been shown to require the bulge-loop in the P3 stem of the *S. cerevisiae* RNA, and this interaction is required for maturation of the RNase P RNA [50].

Because so much of the eukaryal RNase P is protein, specific interactions among the protein components are expected. Potential binary protein–protein interactions in both *S. cerevisiae* and human RNase P have been investigated by directed yeast two-hybrid genetic screens [49,51]. Each of the protein subunits of the *S. cerevisiae* RNase P has been found to make specific contact with at least one other protein component of the holoenzyme. The *S. cerevisiae* Pop4 protein has been suggested to be a central component of the yeast holoenzyme because it interacts with seven of the other eight protein subunits and with the RNase P RNA. When examined in a similar manner, several human protein subunits have been found to show extensive, albeit weak, protein–protein interactions.

The completion of several archaeal genome sequences has opened the door to a bioinformatic search for genes encoding RNase P protein subunits. Although no genes encoding proteins similar to the bacterial RNase P protein have been found, open reading frames with homology to four eukaryal RNase P proteins, Rpr2, Pop4, Pop5 and Rpp1 (Figure 2), have been identified and subsequently shown to be associated with RNase P activity [52]. Maps of protein interactions for *Methanothermobacter thermoautotrophicus* and *Pyrococcus horikoshii* RNase P enzymes have been generated with the yeast two-hybrid screen [53,54]. The strongest protein–protein interactions observed in both of these archaeal RNase P enzymes (Pop4 with Rpr2 and Pop5 with Rpp1) are generally consistent with results from the eukaryal two-hybrid screens.

RNase P activity: functional in vitro reconstitution

Functional reconstitution of eukaryal and archaeal holoenzymes has proved difficult, but a few successes have been recently reported. For example, the reconstitution of some activity has been demonstrated with human RNase P RNA and the proteins Pop4 and Rpr2 [55]. Data from *S. cerevisiae* are inconsistent with the notion that Rpr2 is necessary for pre-tRNA processing, however, because a *S. cerevisiae* RNase P precursor holoenzyme lacking this protein retains *in vitro* activity [56].

The functional reconstitution of an archaeal RNase P activity from *Pyrococcus horikoshii* has been reported recently, but the results differ in comparison to those obtained with the human enzyme. The pre-tRNA processing activity of *P. horikoshii* RNase P was reconstituted from the corresponding RNase P RNA and the *P. horikoshii* Rpr2, Pop4, Pop5 and Rpp1 proteins [57]. Only three of the four protein subunits (Rpr2, Pop4 and Pop5) were required for minimal detectable activity. The fourth subunit, Rpp1, was reported to improve catalytic efficiency. A comparative approach using variants of RNase P from more diverse organisms is needed

to provide a clearer picture of the eukaryal and archaeal holoenzymes.

Although the eukaryal and archaeal RNase P holoenzymes lack an obvious homolog to the bacterial RNase P protein [58], some archaeal RNase P RNAs are weakly activated by the bacterial RNase P protein [32,59] and, conversely, the human Pop4 protein can activate the *E. coli* RNase P RNA under conditions of low salt [55,60]. These results suggest that a common structure and function – so far undetected – is shared among these proteins.

RNase P proteins: 3D structures

The archaeal RNase P protein subunits have proved to be useful targets for structural study because of their solubility and stability. The structures of four archaeal RNase P proteins (Pop4, Rpp1, Rpr2 and Pop5) are shown with a bacterial protein for comparison in Figure 5. The Pop4 protein from three archaea (*M. thermoautotrophicus*, *P. horikoshii* and *Archaeoglobus fulgidus*) has been solved by NMR and/or crystallography [61–64] (Figure 5). This protein adopts an oligonucleotide- or oligosaccharide-binding (OB) fold or an Sm-like fold – a structure that is present in many RNA-binding proteins and is consistent with its role in binding RNase P RNA in the eukaryal holoenzyme, as shown by three-hybrid analysis. The *P. horikoshii* Rpp1 homolog, also determined by X-ray crystallography, adopts an αβ barrel that is similar to a structure found in the metallo-dependent hydrolase superfamily [65] (Figure 5). As mentioned earlier, this protein is not required for minimal *in vitro* RNase P activity, but stimulates the activity of the reconstituted enzyme [57]. The crystal structure of the *P. horikoshii* Rpr2 homolog consists of two long α helices that interact with each other through hydrophobic amino acids at the N terminus, a central domain comprising an unstructured loop, and a C-terminal zinc ribbon [66] (Figure 5).

Structural studies of the *P. furiosus* and *P. horikoshii* Pop5 homologs show that it adopts an α/β sandwich fold similar to the single-stranded RNA-binding RNA recognition motif (RRM), or ribonucleoprotein domain, seen in the bacterial RNase P protein [67,68] (Figure 5). Although the *P. furiosus* Pop5 protein resembles the bacterial RNase P protein in overall shape to some extent, these two proteins share no obvious sequence similarity, suggesting that they have different evolutionary origins [67]. Because of the homology in both protein and RNA elements of the archaeal and eukaryal holoenzymes, the archaeal system is expected to reflect the essence of the eukaryal version.

Evolutionary journey: from ribozyme to ribonucleoprotein

Primordial RNase P possessed a core RNA structure that persists in contemporary versions of the enzyme. The bacterial RNase P RNAs arrived at an intramolecular solution to the problem of global structural stability by using RNA struts, whereas archaeal and eukaryal RNase P RNAs followed a different evolutionary course during

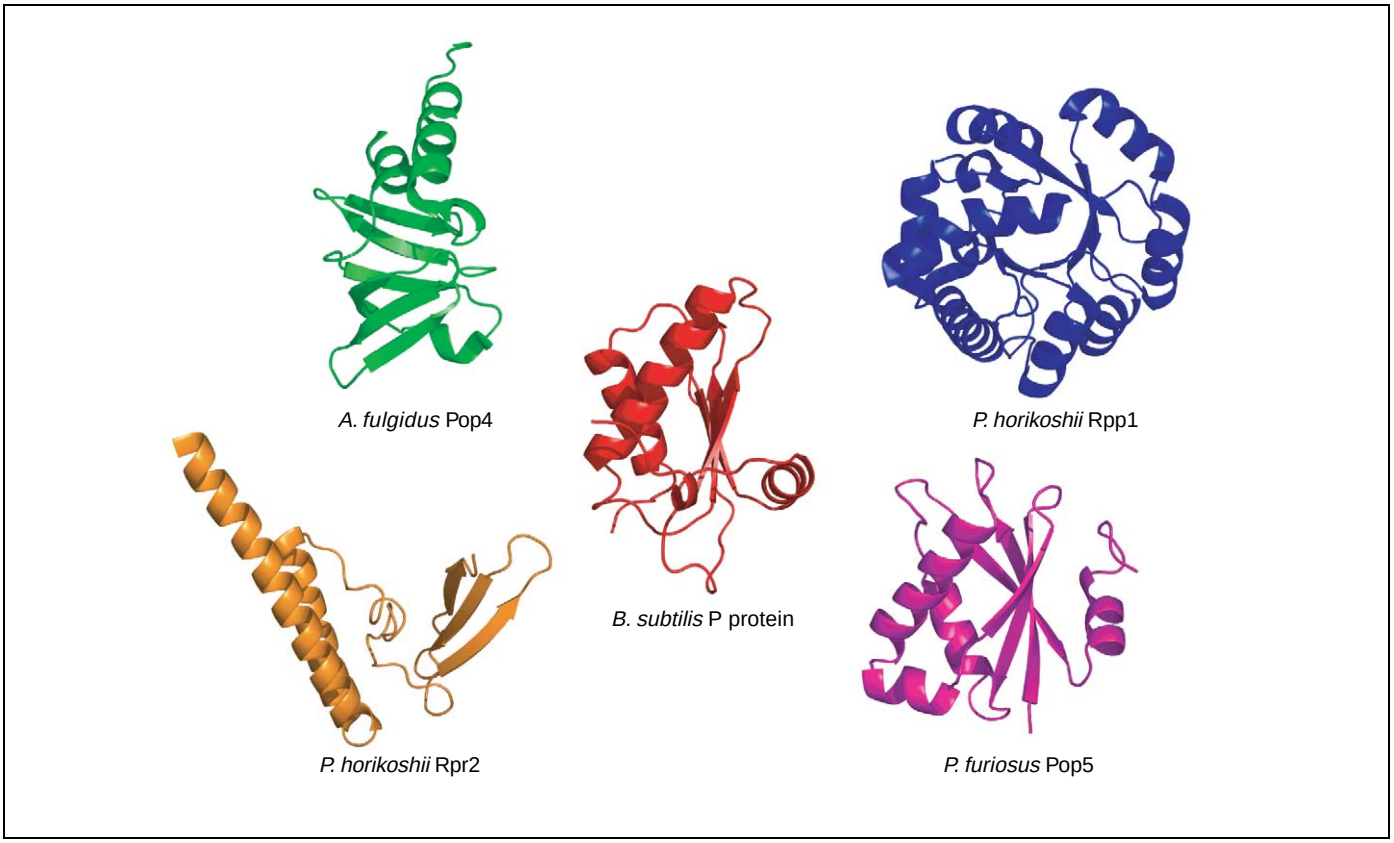

Figure 5. Comparison of bacterial and archaeal RNase P protein structures. Shown are the ribbon structures of *A. fulgidus* Pop4 (PDB code: 1TS9) [64]; *P. horikoshii* Rpp1 (PDB code: 1V77) [65], *P. horikoshii* Rpr2 (PDB code: 1X0T) [66] and *P. furiosus* Pop5 (PDB code: 2AV5) [67], together with the *B. subtilis* bacterial RNase P protein (PDB code: 1A6F) [34]. Although the archaeal Pop5 and the bacterial RNase P protein are not related in sequence, both contain a fold similar to the ribonucleoprotein domain. Figure created with MacPyMol (DeLano Scientific; http://www.pymol.org).

Box 1. Outstanding questions

- What is the mechanism of RNase P catalysis? Higher resolution structures of RNA alone or bound to protein and/or tRNA are needed to shed light on the mechanism.
- Is the RNA only a scaffold on which to bind substrate and to position hydrated Mg^{2+} ions correctly? Although Mg^{2+} is required for catalysis, there are no RNA functional groups in the proposed mechanism. Does the RNA participate chemically?
- What are the roles of the archaeal and eukaryal RNase P protein subunits?

Studies of the archaeal and eukaryal proteins are in their early stages and reconstitution systems are rudimentary. Continued investigation of protein subunits in diverse eukaryal and archaeal systems is needed. Comparative analysis provides a filter to sift out general properties from idiosyncrasies.

- Can RNase P be used to improve human health? RNase P is being pursued both as a tool to control gene expression, especially that of infective agents to prevent disease progression [70], and as a drug target [71].
- Why is RNase P composed of RNA?

which they acquired more RNA–protein and protein–protein interactions. In contrast to the universal RNA subunit, a primordial RNase P protein did not apparently exist before the divergence of the three main lines of descent. This conclusion stems from the lack of a homologous protein in all variants of RNase P, which indicates that the earliest RNase P was catalytic without proteins, thereby supporting the RNA world hypothesis [58].

If the earliest RNase P was solely RNA, then the proteins were obtained in at least three separate stages: first, the acquisition of a single bacterial RNase P protein after the divergence of the bacterial evolutionary line from the one that led to Eukarya and Archaea; second, the acquisition of at least four RNase P proteins before the split of Archaea and Eukarya; and third, the accumulation of additional proteins by the eukaryal version of the holoenzyme.

Concluding remarks

Recent advances have improved our understanding of, and evolutionary perspective on, RNase P. This information has opened the door for future discovery and will assist in providing answers to several outstanding questions (Box 1). In contrast to other catalytic RNAs, RNase P RNA and the ribosome are true enzymes in the sense that they turnover. They might be remnants of some protobiological RNA world, but RNase P RNA and the ribosome must have been retained because of the unique qualities of RNA – qualities that remain indispensable to life.

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