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Roles of Protein Subunits in RNA–Protein Complexes: Lessons from Ribonuclease P

Abstract: *Ribonucleoproteins (RNP) are involved in many essential processes in life. However, the roles of RNA and protein subunits in an RNP complex are often hard to dissect. In many RNP complexes, including the ribosome and the Group II introns, one main function of the protein subunits is to facilitate RNA folding. However, in other systems, the protein subunits may perform additional functions, and can affect the biological activities of the RNP complexes. In this review, we use ribonuclease P (RNase P) as an example to illustrate how the protein subunit of this RNP affects different aspects of catalysis. RNase P plays an essential role in the processing of the precursor to transfer RNA (pre-tRNA) and is found in all three domains of life. While every cell has an RNase P (ribonuclease P) enzyme, only the bacterial and some of the archaeal RNase P RNAs (RNA component of RNase P) are active in vitro in the absence of the RNase P protein. RNase P is a remarkable enzyme in the fact that it has a conserved catalytic core composed of RNA around which a diverse array of protein(s) interact to create the RNase P holoenzyme. This combination of highly conserved RNA and altered protein components is a puzzle that allows the dissection of the functional roles of protein subunits in these RNP complexes.* © 2003 Wiley Periodicals, Inc. Biopolymers 73: 79–89, 2004

Keywords: *RNA-protein interaction; ribozyme; RNase P; catalysis*

INTRODUCTION

The observation that RNA alone can catalyze site-specific phosphodiester bond cleavage reactions twenty years ago by Cech and co-workers has dramatically changed our perception of RNA.[1] Indeed, many important biological processes are carried out by RNA–protein (ribonucleoprotein, RNP) complexes. These complexes vary greatly in size and function. For example, more than 40 protein and several RNA components are present in the ribosome, the protein synthesis machinery.[2] Furthermore, protein targeting to the membrane of the endoplasmic reticulum is initiated by the cotranslational recognition of targeting signals by the signal recognition particle (SRP), which is a ribonucleoprotein particle.[3,4] The spliceosome, which catalyzes the removal of introns from premessenger RNA (pre-mRNA), consists of 30–100 proteins and several RNA species.[2,5] Additionally, in most eukaryotes, the ends of

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chromosomes are replicated by telomerase, in which an RNA subunit acts as a template, and a protein subunit catalyzes the synthesis of telomeric DNA.[6] Therefore, one important question in the study of RNP complexes has been how to distinguish the different roles of each component, particularly to differentiate the functions of the RNA and protein components. While catalysis carried out by either RNA or protein enzymes has been extensively studied, investigation of catalysis by RNP complexes is still at an early stage and is a new frontier in biochemistry.

CATALYTIC REQUIREMENTS OF RNA AND PROTEIN ENZYMES

Catalysis by RNA enzymes differs from protein enzymes in several ways.[7] First, RNA lacks the diverse functional groups characteristic of protein enzymes. This fact potentially limits the range of mechanisms that can be catalyzed by RNA; however, in vitro selection experiments demonstrate that small RNAs can catalyze a wide range of reactions.[8] In addition, whereas the amino acid side chains are mainly located on the outside of protein secondary structures (α-helices or β-sheets) where they can form tertiary and quaternary contacts, the chemical groups of the RNA bases are largely on the interior of base-paired duplexes.[9] Thirdly, the high density of negative charges and the flexibility of the phosphate backbone of RNA could prevent RNA from forming stable active-site pockets. Whereas the protein folds around a central hydrophobic core, RNA usually folds by the packing of domains around a central conserved region.[7] Because of the high density of the negatively charged phosphate backbone, the folding of RNA requires cations to screen charge repulsion and to compensate for the electrostatic penalty of bringing the backbone phosphate ions into proximity with each other.[10–12] Therefore, RNA folding can be stimulated by the presence of cations (monovalent or divalent) and basic proteins.[13–16]

In many cases, divalent cations such as magnesium are strictly required for folding RNA into a functional conformation[17,18]; in a few cases, monovalent cations, such as potassium, make important contributions to RNA folding.[11] The hairpin and hammerhead ribozymes can both fold and catalyze cleavage in the presence of monovalent ions such as Na^+, Li^+, and NH_4^+, without any divalent cations.[19,20] Narlikar and Herschlag have pointed out that the rigidity of enzymes could be important for maximizing the specificity of interactions and catalysis.[7] Some of the most efficient ribozymes, such as RNase P RNA (RNA component of RNase P) and Group I introns, achieve catalytic enhancement for phosphodiester bond cleavage similar to that of protein enzymes by tightly binding and precisely positioning their substrate for catalysis. However, these ribozymes are less efficient in comparison to protein enzymes in multiple turnover reactions due to slow product dissociation and product inhibition.[7] Narlikar and Herschlag conclude that the limitations of RNA packing may lead to a fundamental weakness in RNA catalysis. This observation may explain why there are so many examples of functional RNP complexes but only a few ribozymes. To date, protein subunits appear to be necessary for naturally occurring ribozymes to carry out multiple turnover reactions under in vivo conditions.

RIBONUCLEOPROTEINS

While protein enzymes greatly outnumber ribozymes, processes catalyzed by RNA–protein complexes remain at the core of the living cell. The question arises as to which of the RNA-requiring reactions in the cell use RNA as a catalyst. This is a challenging question because most functional RNAs do not have any catalytic activity in the absence of protein. Recent atomic resolution structures of both subunits of the ribosome provide convincing evidence that the active site of the ribosome is composed of RNA, which catalyzes peptide bond formation.[21–25] A persuasive, but less definitive, argument has also been made that the small nuclear RNAs (snRNAs) in the spliceosome catalyze the transesterification reactions in pre-mRNA splicing.[26] The splicing of introns are RNA catalyzed, but in some cases the reaction can be enhanced by binding of specific proteins to these introns.[27,28] To date, the best biochemically characterized RNP enzyme where the RNA can bind, cleave, and release substrate is bacterial ribonuclease P (RNase P). In this review, we will briefly discuss the role of proteins in the ribosome, then focus on the function of the protein subunit of bacterial RNase P.

BACTERIAL RIBOSOME

The ribosome catalyzes the translation of mRNA for protein synthesis. The bacterial ribosome, a 70S particle, is composed of two subunits: the small (30S) subunit, containing 16S rRNA (ribosomal RNA) and about 20 proteins, and the large (50S) subunit, containing 23S rRNA, 5S rRNA, and over 30 proteins. The 30S subunit plays a crucial role in decoding mRNA by monitoring base pairing between the codon

on mRNA and the anticodon on tRNA (mature transfer RNA), and the 50S subunit catalyzes peptide bond formation (the peptidyl transfer reaction).[29–32]

Mutagenesis and affinity-labeling results have identified regions in 23S rRNA that may be involved in the peptidyl transferase reaction.[33] Noller and colleagues prepared particles that retain peptidyl transferase activity by vigorous proteinase K and SDS treatment of large ribosomal subunits.[34] Additionally, in vitro methods have selected small RNAs that can catalyze the peptidyl transfer reaction.[35,36] Finally, high-resolution x-ray structures of the 50S subunit of the *H. marismortui* ribosome co-crystallized with transition state analogs show that the peptidyl transferase center of the ribosome is largely devoid of protein and is composed of Domain V of the 23S rRNA, the most highly conserved sequence of rRNA.[21,22] Interestingly, a similar RNA sequence has been shown to catalyze peptide bond synthesis in vitro.[37] In the 50S subunit structure, tRNA analogs are found to interact with phylogenetically conserved nucleotides biochemically identified as tRNA binding determinants.[38,39] These data indicate that the RNA component of the ribosome is likely the catalytic component.[40–42] A high-resolution x-ray crystallographic structure of the small (30S) subunit has also been solved.[24,25] Together, these crystal structures illustrate that many of the ribosomal proteins appear to intertwine with the secondary folds in rRNA and thereby help the rRNA to fold into compact structures in both subunits. In addition, most of the proteins bind to multiple domains of rRNA and stabilize the correct three-dimensional structure. Recent mutagenesis studies have begun investigating the role of nucleotides at the active site of the ribosome.[43–48] For more information on the structure and function of the ribosome, please refer to recent reviews on this field.[31,32,41,49,50]

RIBONUCLEASE P

Ribonuclease P (RNase P) catalyzes the 5′ maturation of precursor tRNA (pre-tRNA) in all organisms by the cleavage of a specific phosphodiester bond that generates 5′-phosphate and 3′-hydroxyl end groups (Figure 1). In contrast, many small ribozymes catalyze cleavage reactions that produce 2′,3′-cyclic phosphate and 5′-hydroxyl termini.[51] Nearly all RNase Ps from the three major kingdoms of life (Archaea, Bacteria, and Eukarya) contain both essential RNA and protein subunits. Possible exceptions include RNase Ps from organelles (mitochondria and chloroplasts) and from *Aquifex* that are proposed to not contain an RNA component (for recent reviews, see Refs. 52–54).

The RNase P RNAs from divergent organisms differ remarkably in both sequence and length. However, some conserved elements throughout the RNA structure have been identified to be functionally important.[55] The smallest naturally occurring RNase P RNA sequence is from *Microplasma fermentans*, which is 276 nucleotides in length, about 2/3 of the size of RNase P RNAs from bacteria or the yeast.[56] Many RNase P RNAs can be truncated and still retain catalytic activity. RNase P RNA constructed with either the minimal amount of conserved elements (Micro P) or just the C domain of the *B. subtilis* P RNA (Figure 2) are catalytically active.[56,57]

RNase P holoenzyme from bacteria (e.g., *E. coli* and *Bacillus subtilis*) consists of a single RNA subunit and a single protein subunit. This configuration represents the simplest system of a catalytic heterodimeric RNP enzyme. However, RNase Ps from the other domains of life are more complicated. The yeast nuclear RNase P consists of one RNA subunit and nine protein subunits,[58] and is representative of eukaryotic nuclear RNase P. Similarly, human RNase P from HeLa cells consists of one RNA subunit and at least seven protein subunits that are homologous to the yeast proteins.[59,60] Archaeal RNase P RNAs are similar to those of bacteria in sequence and structure, but the protein subunits are homologous to eukaryotic nuclear RNase P proteins (protein component of RNase P) (Table I).[61,62] Interestingly, RNase Ps from *B. subtilis* and *E. coli* are similar enough that addition of the *B. subtilis* RNase P protein can stimulate the steady state activity of *E. coli* RNase P RNA, and vice versa.[63–65] Additionally, the *B. subtilis* protein modestly enhances the catalytic activity of some archaeal RNase P RNAs.[64] These data suggest that functions of the RNA and protein subunits are conserved among diverse RNase P's. (See Table II.)

No high-resolution crystal structure has yet been determined for the entire RNase P RNA or holoenzyme. However, a recent crystal structure of the specificity domain of RNase P RNA provides a detailed visualization of the structure of this region of P RNA.[66] Phylogenetic comparative analysis, crosslinking, and mutational studies of the bacterial form of the enzyme have led to a detailed understanding of RNase P RNA secondary structure.[67,68] Based on biochemical data, three-dimensional models of RNase P RNA have been proposed for both *E. coli* and *B. subtilis* enzymes.[68,69] The ternary structure of the protein subunits of RNase P from *B. subtilis*,[70] *Thermotoga maritima*,[71] and *Staphylococcus aureus*,[72] have been determined using x-ray crystallography or

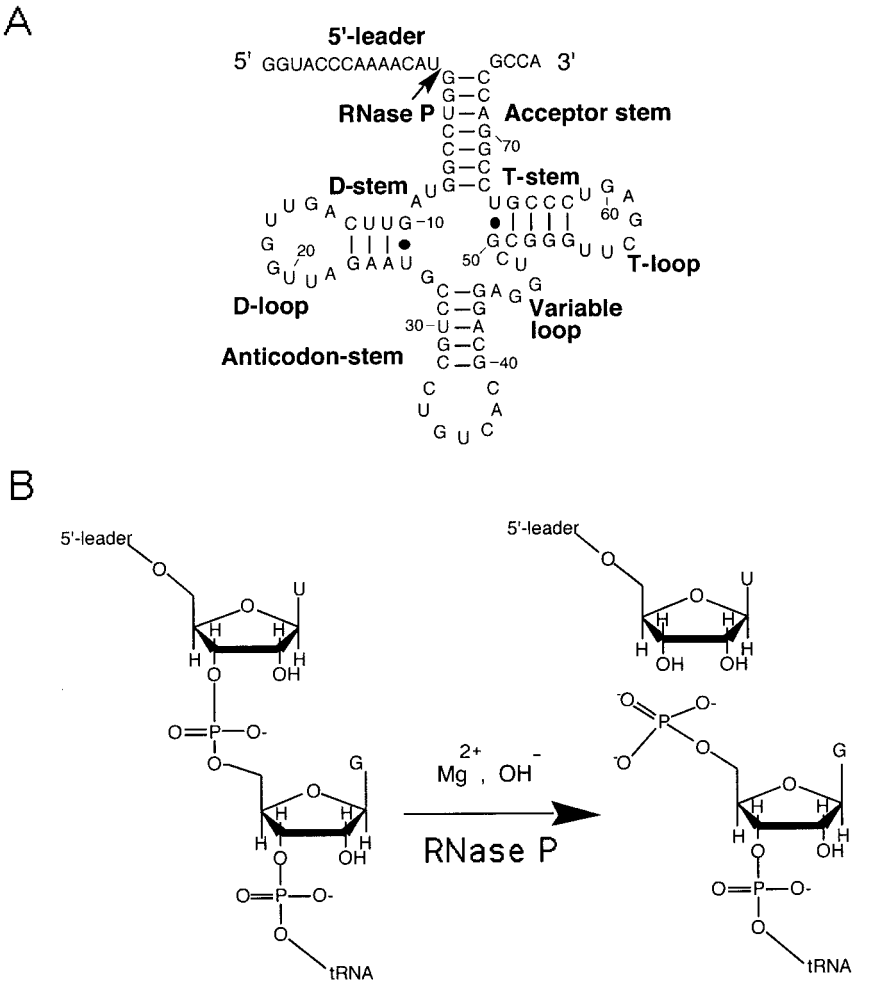


FIGURE 1 Cleavage reaction catalyzed by RNase P. (A) Secondary structural representation of *B. subtilis* pre-tRNA^{ASP}. The arrow indicates the phosphodiester bond that is cleaved by RNase P. (B) The pre-tRNA cleavage reaction catalyzed by RNase P generates a 3′-OH and a 5′-phosphate ends in the 5′ leader and the mature tRNA, respectively.

NMR spectroscopy. Finally, three-dimensional models of the RNase P holoenzymes from *E. coli* and *B. subtilis* have also been derived from hydroxyl radical-mediated footprinting studies and molecular modeling.[73] However, in this review we will focus on the biochemical evidence for the function of the P protein (protein component of RNase P) subunit in the RNase P holoenzyme.

BACTERIAL RNase P RNA

The bacterial RNase P RNA (from *E. coli* and *B. subtilis)* is about 400 nucleotides (Figure 2), and is catalytically active in vitro in the presence of high concentrations of monovalent and divalent cations.[74] The RNase P RNA catalyzed cleavage reaction is pH dependent, and requires several magnesium ions, suggesting that hydroxide or metal-bound hydroxide might function as a nucleophile in the cleavage reaction.[75,76]

The sequence of bacterial RNase P RNA can be divided into 18 double-stranded regions[77] (Figure 2). RNase P RNA contains two distinctive domains that fold independently: a substrate binding domain (S-domain) and a catalytic domain (C-domain).[78,79] The S-domain interacts with the T-stem and loop of the pre-tRNA substrate, while the C-domain has been associated with recognition of the acceptor stem, the cleavage site, and the conserved 3′-CCA sequence.[80] In fact, RNase P RNA and RNase P holoenzyme can efficiently cleave a variety of non-tRNA substrates as

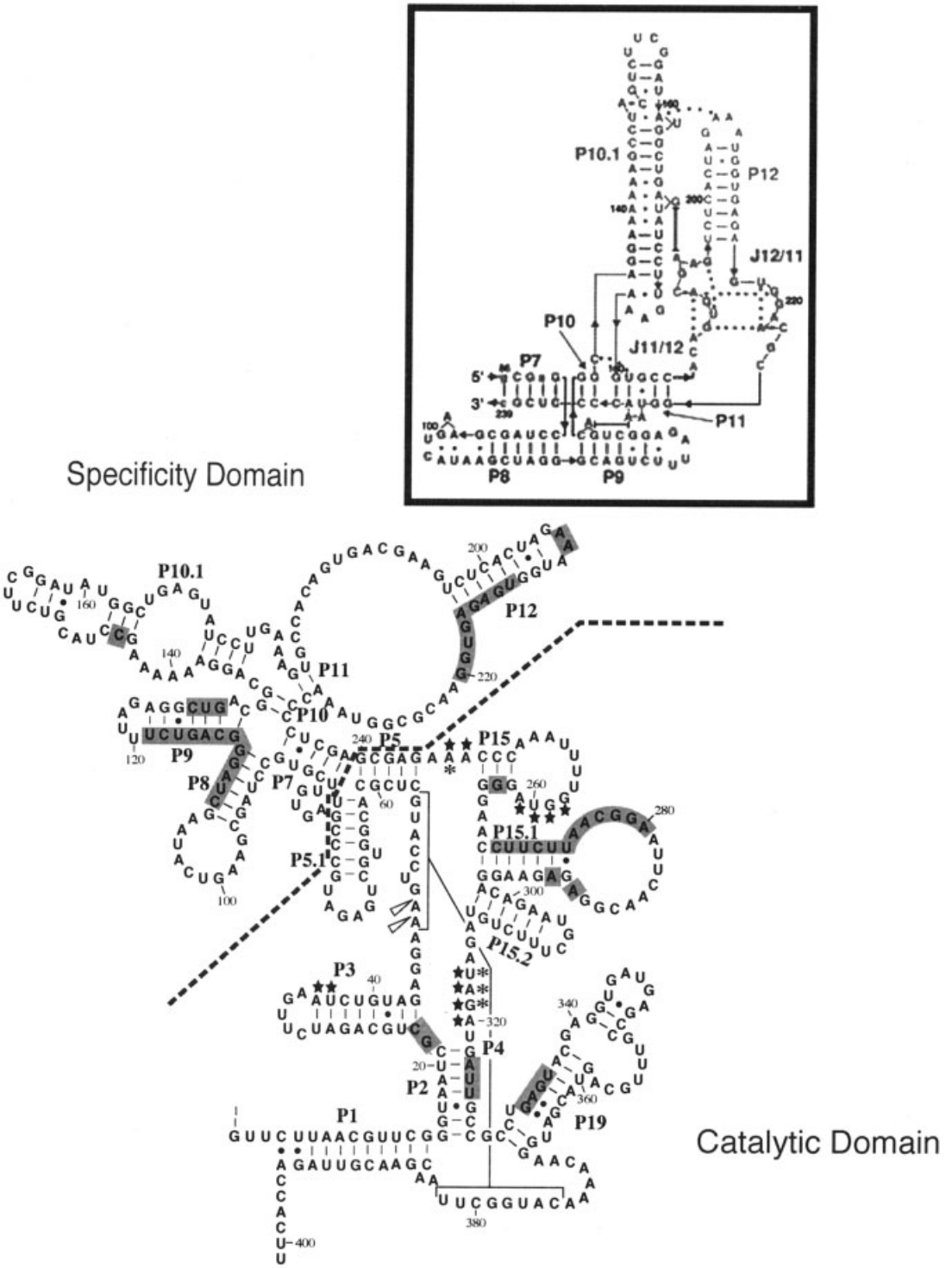
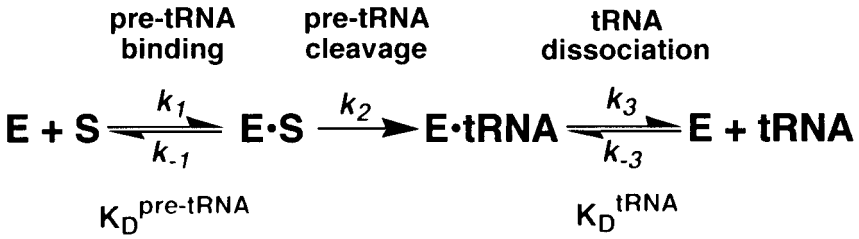


FIGURE 2 Secondary structure of the *B. subtilis* RNase P RNA.[53] The proposed folding domains are separated by dashed line.[78,98] Stars indicate nucleotides in RNase P RNA that crosslink with the 5′ leader of pre-tRNA.[119] White arrows identify positions of importance for catalysis, as determined by phosphorothioate interference in *B. subtilis* RNase P holoenzyme[86] and *E. coli* RNase P RNA.[85,119] Shading indicates regions of potential interactions with the protein component, as determined by chemical protection.[100] The insert is the secondary structure for the specificity domain derived from the x-ray structure.[66]

Table I P Protein Dependence of Kinetic and Thermodynamic Parameters for Pre-tRNA Processing Catalyzed by RNase P[a]



	P-RNA	Holoenzyme	Protein Effect	Divalent Ions
k_{cat}	$(0.03 \pm 0.01)\ s^{-1}$	$(0.27 \pm 0.02)\ s^{-1}$	↑ 9-fold	Mg^{2+}
K_M	$(21 \pm 9)\ \mu M$	$(0.08 \pm 0.02)\ \mu M$	↓ 260-fold	Mg^{2+}
k_{cat}/K_M	$(1.4 \pm 0.1)\ mM^{-1}\ s^{-1}$	$(3400 \pm 600)\ mM^{-1}\ s^{-1}$	↑ 2400-fold	Mg^{2+}
k_2	$(0.09 \pm 0.04)\ s^{-1}$	$(0.30 \pm 0.01)\ s^{-1}$	↑ 3-fold	Mg^{2+}
k_2	$(3.1 \pm 0.3) \times 10^{-6}\ s^{-1}$	$(1.8 \pm 0.3) \times 10^{-5}\ s^{-1}$	↑ 6-fold	Ca^{2+}
$K_D^{pre\text{-}tRNA}$	$4 \pm 1\ \mu M$	$(4 \pm 2) \times 10^{-4}\ \mu M$	↓ 10,000-fold	Ca^{2+}
$K_D^{(tRNA)}$	$(12 \pm 3)\ \mu M$	$(1.2 \pm 0.1)\ \mu M$	↓ 10-fold	Mg^{2+}
$K_D^{(tRNA)}$	$(0.3 \pm 0.1)\ \mu M$	$(0.20 \pm 0.06)\ \mu M$	↓ 1.5-fold	Ca^{2+}

[a] Measured at 100 mM NH_4Cl, 50 mM Tris, and 50 mM MES, pH 6.1, at 37°C with either 10 mM $MgCl_2$ or $CaCl_2$. Data are taken from Ref. 102.

long as some or all of these structural elements are present.[81,82] Specific interaction between nucleotides in RNase P RNA and the nucleotide preceding the cleavage site on the 5′leader[83] and the 3′-RCCA sequence[80,84] of the pre-tRNA substrate have also been identified through mutagenesis and biochemical studies. In addition, studies of the activity of RNase P with specific phosphorothioate modifications in the P RNA have identified nonbridging phosphate oxygens that may coordinate catalytically important magnesium ions.[85,86] These data indicate that RNase P RNA contains most of the essential elements required for catalysis of pre-tRNA hydrolysis.

Table II Comparison Between RNase Ps from Different Organisms[a]

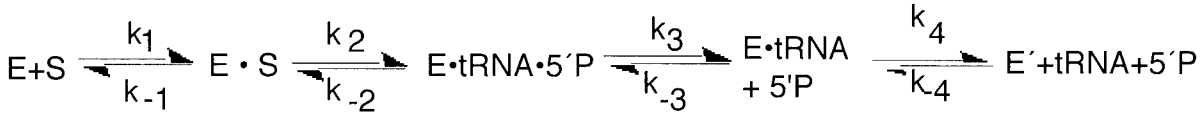
	Core RNase P RNA Helices				P RNA Activity	Number of Protein Subunits
	P4	P5	P15	P3		
Bacterial	+	+	+	+	Yes	1
Eukaryotic	+	−	−	+	No	9
Archaeal	+	+	D	+	Some	≥4

[a] A "+" means presence, "−" means absence, and "D" means that the helix is found in some but not all known RNase P RNA examined (Data from Refs. 52, 61, 120, and 121, and references therein).

Under steady state turnover conditions, the rate-limiting step for pre-tRNA cleavage catalyzed by RNase P RNA is tRNA dissociation, not phosphodiester bond cleavage.[87–89] Therefore, mechanistic information about the catalytic mechanism is not easily derived from steady-state kinetic experiments. Transient kinetic techniques were used in order to derive the minimal kinetic mechanism for pre-tRNA hydrolysis catalyzed by RNase P RNA.[89] The minimal kinetic scheme for RNase P RNA in high salt includes (1) rapid and essentially irreversible binding of pre-tRNA, (2) irreversible cleavage of pre-tRNA, (3) rapid dissociation of the 5′-RNA fragment, and (4) slow dissociation of tRNA (Scheme 1).[89] This mechanism therefore provides a platform for discussing the effects of the protein component on the function of RNase P.

Although the RNase P RNA alone is catalytically active at high salt, the protein cofactor is essential for growth of *E. coli*.[90] The RNase P protein has been proposed to play a structural role by stabilizing the active conformation of the P RNA,[69,91] or to alleviate charge repulsion, perhaps by enhancing the affinity of cation-binding sites on the P RNA.[87] Recently, the protein has been proposed to play a direct role in substrate recognition.[92]

Is the main role of the protein subunit of RNase P to promote P-RNA folding into a catalytically active



SCHEME 1

form? Fe(II)-induced hydroxyl-radical cleavage has been used to probe RNA secondary structures to investigate this question for several RNA–protein complexes. Although some Group I and Group II introns self-splice in vitro, many require protein factors for efficient splicing in vivo to help the intron RNA fold into the catalytically active structure.[27] For example, *Saccharomyces cerevisiae* Group I bI5 intron and the *Neurospora crassa* mitochondial Group I intron require specific proteins (CBP2 and CYT-18 proteins, respectively) for splicing under physiological conditions.[93–95] In these cases, the functional role of these proteins is to stabilize the tertiary structure of the Group I intron active site, which enhances splicing.[15,96,97] In contrast, the *E. coli* and *B. subtilis* RNase P RNAs are fully folded at equilibrium in 6 m*M* Mg^{2+}.[15,96,97] Furthermore, in 2–10 m*M* $MgCl_2$ the folded RNase P RNA is active immediately upon mixing with the pre-tRNA substrate, indicating that no slow folding step is required to gain catalytic activity.[99] The presence of P protein has modest effects on the global folding of P RNA.[100,101] Therefore, the primary function of the RNase P protein is not to promote RNase P RNA folding. In contrast, the protein component of RNase P has multiple effects on the kinetics of pre-tRNA hydrolysis. Under steady state turnover at moderate divalent and monovalent concentrations, addition of the *B. subtilis* protein subunit modestly enhances steady state turnover (k_{cat}) by about 10-fold, but decreases the steady state Michaelis–Menten constant (K_M) for the cleavage reaction by 120-fold (Table I).[102] This leads to a 2000-fold increase in the bimolecular rate constant (k_{cat}/K_M), which sets a lower limit for the second-order rate constant for substrate association.[103] A preliminary interpretation of these steady state data suggested that the protein component enhances the association rate constant for pre-tRNA. However, interpretation of these steady-state kinetic data is complicated by the fact that addition of the protein component alters the rate-limiting step in steady-state turnover. Therefore, transient kinetic measurements were required to definitively determine the effect of the protein component of RNase P on individual steps in the pre-tRNA kinetic pathway. These data indicate that the protein component affects pre-tRNA recognition without having a drastic effect on the phosphodiester cleavage rate constant as detailed below.[92,100,102,104,105]

Tallsjö and Kirsebom first demonstrated that the protein component has little effect on the cleavage rate constant for pre-$tRNA^{TyrSu3}$ catalyzed by *E. coli* RNase P under single turnover conditions.[88] Further, detailed investigation of the effect of the protein subunit on individual rate constants definitively demonstrated that the *B. subtilis* P protein has a modest effect (<10-fold increase) on the rate constant for phosphodiester cleavage of pre-$tRNA^{Asp}$ catalyzed by RNase P at saturating magnesium and substrate.[102] These data clearly show that the protein component of RNase P does not directly stabilize the transition state for pre-tRNA hydrolysis. Therefore, the RNA component of RNase P is the main catalytic subunit of this enzyme.

In order to directly investigate whether the protein subunit affects substrate recognition, thermodynamic analysis of pre-tRNA and mature tRNA affinity of RNase P RNA and RNase P holoenzyme were performed in buffers using Ca^{2+} rather than Mg^{2+} as a divalent cation. RNase P RNA folds effectively in Ca^{2+},[106] and retains the ability to bind both protein and tRNA under these conditions.[91,107,108] However, the rate constant for phosphodiester bond cleavage is reduced by four orders of magnitude.[75] Ligand binding was measured by separating the bound and unbound ligand using a gel filtration centrifuge column.[89] The protein component of RNase P increased the affinity of pre-tRNA substrates (>5 nucleotides leader length) by a factor of 10^4 while having a more modest increase (≤10-fold) on the affinity of tRNA (Table I).[102] Kurz et al.[102] demonstrated that a similar increase in substrate affinity occurred in Mg^{2+}-containing buffers and the main effect of the *B. subtilis* protein component was to decrease the pre-$tRNA^{Asp}$ dissociation rate constant with little effect on the association rate constant. In contrast, the protein component has little effect on either the association or dissociation rate constant of the mature tRNA. These data clearly demonstrate that the protein component does not have a generalized effect on pre-tRNA affinity but that it specifically enhances recognition of the pre-tRNA leader either indirectly by altering the P RNA conformation or directly by interacting with the

leader. Therefore, addition of the protein subunit increases the specificity of RNase P for binding pre-tRNA substrates compared to the tRNA product.

However, the effect of the RNase P protein on the pre-tRNA affinity does not completely explain all of the functional effects of the protein component. The protein component also decreases the concentration of magnesium required for optimal activity.[76] Magnesium ions fulfill several functional roles in RNase P: stabilizing the folded RNA tertiary structure, enhancing the affinity of RNase P RNA for pre-tRNA and tRNA, and stabilizing the transition state for pre-tRNA cleavage.[75,98,109] There are greater than 100 magnesium ions that associate nonspecifically with P RNA,[109] which is a common feature for all RNAs.[12,110,111] The effects of the P protein on the metal requirement of *B. subtilis* RNase P enzyme have been addressed in detail using transient kinetics and equilibrium binding measurements.[76] The protein component does not change the apparent number of magnesium ions that associate with the RNase P RNA, or decrease the number of classes of magnesium ions that are required for pre-tRNA cleavage.[76] However, the protein subunit does increase the affinities of metal ions in at least four magnesium binding sites that stabilize pre-tRNA binding. This stabilizing effect is coupled to the direct contact between P protein and the 5′ leader in the P holoenzyme–pre-tRNA complex.[76] It is currently proposed that this effect is mediated indirectly by altering the structure of P RNA or pre-tRNA to "preorganize" the metal sites, thereby enhancing their affinity. However, it is still possible that a protein side chain could directly coordinate one or more magnesium ions. An increase in Mg^{2+} ion affinity by the protein component has also been observed in the mitochondrial intron COB from *Aspergillus nidulans*,[112] suggesting a common theme among metal-dependent RNP enzymes.

INTERACTIONS BETWEEN RNase P PROTEIN AND THE PRE-tRNA SUBSTRATE

The x-ray crystal structure of the *B. subtilis* P protein alone shows a globular protein that shares structure homology with two RNA binding proteins, the C-terminus domain of ribosomal protein 5S and domain VI of elongation factor G.[70] The *B. subtilis* RNase P protein contains three possible RNA binding regions: (1) a central cleft formed by one α-helix and four β-sheets, (2) a negatively charged metal-binding loop, and (3) a base-rich conserved RNA binding motif (RNR motif) in an unusual left-handed $\beta/\alpha/\beta$ cross-over connection (Figure 3). Despite the low sequence similarities among the RNase P proteins, the structure of the *B. subtilis* RNase P protein is also very similar to the structures of the homologous proteins from *S. aureus* [72] and *T. maritima*.[71] This observation indicates that bacterial RNase P proteins share a homologous structure.

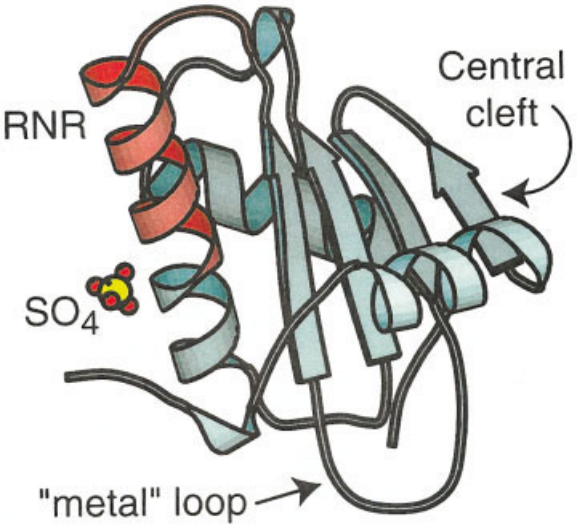


FIGURE 3 Structure of the *B. subtilis* RNase P protein.[70]

To determine whether the protein subunit of RNase P directly interacts with the bound pre-tRNA substrate, cross-linking experiments were performed with pre-tRNA bound to RNase P holoenzyme reconstituted with single-cysteine *B. subtilis* RNase P protein variants labeled with photocross-linkers.[92] These data demonstrate that the central cleft of the protein directly interacts with the single-stranded 5′ leader sequence of pre-tRNA (4–8 nucleotides away from the cleaved phosphodiester bond). Pre-tRNA binding studies examining the dependence of affinity on the length of the 5′ leader indicate that a 4- or 5-nucleotide leader is required to achieve the enhanced affinity conferred by the protein component.[104] Furthermore, aromatic residues on the central cleft of the *E. coli* P protein have been identified in mutational studies as important for substrate specificity.[82] The cross-linking and biochemical data demonstrate that the protein component is located in close proximity (less than 4 nucleotides) to the cleavage site, consistent with the proposal that RNase P protein plays a role in substrate recognition. These data also place the protein component near the active site of RNase P.[73,92,113] Therefore, the main functional role of the protein component in bacterial RNase P is to enhance substrate and metal affinity, not RNA folding.

YEAST NUCLEAR RNase P

In comparison with the bacterial RNase P holoenzyme, the nuclear RNase P of yeast has 9 tightly associated essential protein subunits, with molecular masses ranging from 15.5 to 100.5 kDa.[58] The RNase P RNA from yeast has not yet been shown to catalyze the cleavage of pre-tRNA in vitro, but it is essential for yeast survival.[58] There is little known about the functions of individual protein subunits of the yeast nuclear RNase P, and none of the proteins have recognizable homology to the bacterial P protein. In addition, it is not clear whether the proteins bind to the RNA as a preformed complex or individually.[52] Yeast two- or three-hybrid studies have led to a model of subunit interactions in the yeast nuclear RNase P complex.[52] Additionally, yeast nuclear RNase P requires a 3′-trailer sequence rather than a 5′-leader sequence for optimal steady-state activity,[114] and is inhibited by single stranded RNAs.[114] These data suggest that substrate recognition in yeast RNase P has altered along with the protein subunit composition.[114]

Yeast nuclear RNase P is closely related to RNase MRP, which is only found in eukaryotes. RNase MRP plays an important role in pre-rRNA (precursor to ribosomal RNA) processing.[115,116] Interestingly, these two RNP complexes share 8 of the protein subunits.[58] The importance of this observation is not clear. However, it suggests that these two RNP complexes may share a common ancestor, and that some of these proteins may have the same function in the two systems, such as subcellular localization of these enzymes.[58]

CONCLUSIONS

Using various genetic and biochemical approaches, many insights into the mechanism of bacterial RNase P have been gained. Current evidence places the protein in close proximity to the active site of the RNase P holoenzyme, and indicates that the protein directly plays a role in enhancing substrate recognition. Thermodynamic and kinetic analysis have shown that addition of the protein component dramatically enhances the discrimination of RNase P binding for pre-tRNA compared to tRNA, with only a modest increase in the rate constant for catalysis.

In other RNP complexes, the protein subunits may have significantly different functions, including the following: (1) Binding of the protein subunit may enhance the correct folding of the RNA subunit.[15,24] (2) The protein component is the catalytic subunit while the RNA subunit serves as a template for replication (e.g., telomerase). (3) The protein subunit induces and/or stabilizes dramatic conformational changes in the RNA.[15,16] Furthermore, proteins such as RNA helicases and RNA chaperones assist RNA refolding and/or conformational changes by disrupting small regions of RNA duplexes.[5,117,118] Differentiating among these possible roles requires a systematic and multidisciplinary approach. Structural data obtained from NMR or x-ray crystallography is instructive to complement biochemical and genetic studies. Atomic resolution structures of the ribosome, one of the most complex RNP particles, has verified results from decades of biochemical studies, but also provides the most direct support for RNA as the catalytic component. The RNase P studies illustrate how thermodynamic and kinetic techniques in combination with cross-linking and mutation analysis can yield detailed information on the functional roles of a protein subunit in a simple RNP complex. These approaches can also be applied to differentiate functional properties of RNA and protein subunits in other complex RNA systems.

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REFERENCES

1. Kruger, K.; et al. Cell 1982, 31(1), 147–157.
2. Wilson, K. S.; Noller, H. F. Cell 1998, 92(3), 337–349.
3. Rapoport, T. A.; Jungnickel, B.; Kutay, U. Annu Rev Biochem 1996, 65, 271–303.
4. Keenan, R. J.; et al. Annu Rev Biochem 2001, 70, 755–775.
5. Staley, J. P.; Guthrie, C. Cell 1998, 92(3), 315–326.
6. Greider, C. W.; Blackburn, E. H. Cell 1987, 51(6), 887–898.
7. Narlikar, G. J.; Herschlag, D. Annu Rev Biochem 1997, 66, 19–59.
8. DeRose, V. J. Chem Biol 2002, 9(9), 961–969.
9. Saenger, W. Principles of Nucleic Acid Structure. Springer Advanced Texts in Chemistry; Springer-Verlag: New York, 1984; p 556.
10. Misra, V. K.; Draper, D. E. J Mol Biol 2002, 317(4), 507–521.
11. Shiman, R.; Draper, D. E. J Mol Biol 2000, 302(1), 79–91.
12. Misra, V. K.; Shiman, R.; Draper, D. E. Biopolymers 2003, 69(1), 118–136.
13. Pyle, A. M.; Green, J. B. Curr Opin Struct Biol 1995, 5(3),303–310.

14. Pyle, A. M. J Biol Inorg Chem 2002, 7(7-8), 679–690.
15. Weeks, K. M. Curr Opin Struct Biol 1997, 7(3), 336–342.
16. Williamson, J. R. Nat Struct Biol 2000, 7(10), 834–837.
17. Gonzalez, R. L., Jr.; Tinoco, I., Jr. J Mol Biol 1999, 289(5), 1267–1282.
18. Tinoco, I., Jr.; Bustamante, C. J Mol Biol 1999, 293(2), 271–281.
19. Hanna, R.; Doudna, J. A. Curr Opin Chem Biol 2000, 4(2), 166–170.
20. Murray, J. B.; et al. Chem Biol 1998, 5(10), 587–595.
21. Ban, N.; et al. Science 2000, 289(5481), 905–920.
22. Nissen, P.; et al. Science 2000, 289(5481), 920–930.
23. Schluenzen, F.; et al. Cell 2000, 102(5), 615–623.
24. Wimberly, B. T.; et al. Nature 2000, 407(6802), 327–339.
25. Carter, A. P.; et al. Nature 2000, 407(6802), 340–348.
26. Collins, C. A.; Guthrie, C. Nat Struct Biol 2000, 7(10), 850–854.
27. Lambowitz, A. M.; Perlman, P. S. Trends Biochem Sci 1990, 15(11), 440–444.
28. Saldanha, R.; et al. FASEB J 1993 7(1) 15–24.
29. Green, R.; Noller, H. F. Annu Rev Biochem 1997, 66, 679–716.
30. Ramakrishnan, V.; White, S. W. Trends Biochem Sci 1998, 23(6), 208–212.
31. Ramakrishnan, V. Cell 2002, 108(4), 557–572.
32. Moore, P. B.; Steitz, T. A. Ann Rev Biochem 2003, 72(1), 813–850.
33. Noller, H. F. Annu Rev Biochem 1991, 60, 191–227.
34. Noller, H. F.; Hoffarth, V.; Zimniak, L. Science 1992, 256(5062), 1416–1419.
35. Illangasekare, M.; Yarus, M. RNA 1999, 5(11), 1482–1489.
36. Yarus, M.; Welch, M. Chem Biol 2000, 7(10), R187–R190.
37. Zhang, B.; Cech, T. R. Chem Biol 1998 5(10), 539–553.
38. Moazed, D.; Noller, H. F. Nature 1989, 342(6246), 142–148.
39. Green, R.; Switzer, C.; Noller, H. F. Science 1998, 280(5361), 286–289.
40. Lilley, D. M. Chembiochem 2001, 2(1), 31–35.
41. Moore, P. B.; Steitz, T. A. RNA 2003, 9(2), 155–159.
42. Cech, T. R. Science 2000 289(5481), 878–879.
43. Polacek, N.; et al. Nature 2001, 411(6836), 498–501.
44. Katunin, V. I.; et al. Mol Cell 2002, 10(2), 339–346.
45. Muth, G. W.; et al. RNA 2001, 7(10), 1403–1415.
46. Xiong, L.; et al. RNA 2001, 7(10), 1365–1369.
47. Thompson, J.; et al. Proc Natl Acad Sci USA 2001, 98(16) 9002–9007.
48. Polacek, N.; et al. Mol Cell 2003 11(1), 103–112.
49. Ramakrishnan, V.; Moore, P. B. Curr Opin Struct Biol 2001, 11(2), 144–154.
50. Ogle, J. M.; Carter, A. P.; Ramakrishnan, V. Trends Biochem Sci 2003, 28(5), 259–666.
51. Doudna, J. A.; Cech, T. R. Nature 2002, 418(6894), 222–228.
52. Xiao, S.; et al. Annu Rev Biochem 2002, 71, 165–189.
53. Brown, J. W. Nucleic Acids Res 1999, 27(1), 314.
54. Kurz, J. C.; Fierke, C. A. Curr Opin Chem Biol 2000, 4(5), 553–558.
55. Pace, N. R.; Brown, J. W. J Bacteriol 1995, 177(8), 1919–1928.
56. Siegel, R. W.; et al. RNA 1996, 2(5), 452–462.
57. Loria, A.; Pan, T. Nucleic Acids Res 2001, 29(9), 1892–1897.
58. Chamberlain, J. R.; et al. Genes Dev 1998, 12(11), 1678–1690.
59. Bartkiewicz, M.; Gold, H. Altman, S. Genes Dev 1989, 3(4), 488–499.
60. Eder, P.S.; et al. Proc Natl Acad Sci USA 1997, 94(4), 1101–1106.
61. Frank, D. N.; et al. RNA 2000, 6(12), 1895–1904.
62. Hall, T. A.; Brown, J. W. RNA 2002, 8(3), 296–306.
63. Guerrier-Takada, C.; et al. Cell 1983, 35(3, Pt 2), 849–857.
64. Pannucci, J. A.; et al. Proc Natl Acad Sci USA 1999, 96(14), 7803–7808.
65. Hansen, A.; et al. Mol Microbiol 2001, 41(1), 131–143.
66. Krasilnikov, A. S.; et al. Nature 2003, 421(6924), 760–764.
67. Brown, J. W.; et al. Bacteriology 1991, 173(12), 3855–3863.
68. Harris, M. E.; et al. EMBO J 1994, 13(17), 3953–3963.
69. Westhof, E.; Altman, S. Proc Natl Acad Sci USA 1994, 91(11), 5133–5137.
70. Stams, T.; et al. Science 1998, 280(5364), 752–755.
71. Kazantsev, A. V.; et al. Proc Natl Acad Sci USA 2003 100(13):7497–7502.
72. Spitzfaden, C.; et al. J Mol Biol 2000, 295(1), 105–115.
73. Tsai, H.Y.; et al. J Mol Biol 2003 325(4), 661–675.
74. Guerrier-Takada, C.; Altman, S. Science 1984, 223(4633), 285–286.
75. Smith, D.; Pace, N. R. Biochemistry 1993, 32(20) 5273–5281.
76. Kurz, J. C.; Fierke, C. A. Biochemistry 2002, 41(30), 9545–9558.
77. Haas, E. S.; Brown, J. W. Nucleic Acids Res 1998, 26(18), 4093–4099.
78. Loria, A.; Pan, T. RNA 1996, 2(6), 551–563.
79. Pan, T.; Loria, A.; Zhong, K. Proc Natl Acad Sci USA 1995, 92(26), 12510–12514.
80. Kirsebom, L. A.; Svard, EMBO J 1994, 13(20), 4870–4876.
81. Hartmann, R. K.; et al. Proc Natl Acad Sci USA 1995, 92(13), 5822–5826.
82. Gopalan, V.; et al. J Mol Biol 1997, 267(4), 818–829.
83. Zahler, N. H.; Christian, E. L.; Harris, M. E. RNA 2003, 9(6),734–745.

84. Oh, B. K.; Pace, N. R. Nucleic Acids Res 1994, 22(20), 4087–4094.
85. Christian, E. L.; Kaye, N. M.; Harris, RNA 2000, 6(4), 511–519.
86. Crary, S. M.; Kurz, J. C.; Fierke, C. A. RNA 2002, 8(7), 933–947.
87. Reich, C.; et al. Science 1988, 239(4836), 178–181.
88. Tallsjo, A.; Kirsebom, L. A. Nucleic Acids Res 1993, 21(1), 51–57.
89. Beebe, J. A.; Fierke, C. A. Biochemistry 1994, 33(34), 10294–10304.
90. Kole, R.; et al. Cell 1980, 19(4), 881–887.
91. Talbot, S. J.; Altman, S. Biochemistry 1994, 33(6), 1406–1411.
92. Niranjanakumari, S.; et al. Proc Natl Acad Sci USA 1998, 95(26),15212–15217.
93. Weeks, K. M.; Cech, T. R. Cell 1995, 82(2), 221–230.
94. Weeks, K. M.; Cech, T. R. Science 1996, 271(5247), 345–348.
95. Saldanha, R.; Ellington, A.; Lambowitz, A. M. J Mol Biol 1996, 261(1) 23–42.
96. Caprara, M. G.; Myers, C. A.; Lambowitz, A. M. J Mol Biol 2001, 308(2),165–190.
97. Webb, A. E.; et al. J Mol Biol 2001, 309(5), 1087–1100.
98. Pan, T. Biochemistry 1995, 34(3), 902–909.
99. Pan, T.; Sosnick, T. R. Nat Struct Biol 1997, 4(11), 931–938.
100. Loria, A.; et al. Biochemistry 1998, 37(44), 15466–15473.
101. Westhof, E.; Wesolowski, D.; Altman, S. J Mol Biol 1996, 258(4), 600–613.
102. Kurz, J. C.; Niranjanakumari, S.; Fierke, C. A. Biochemistry 1998, 37(8), 2393–2400.
103. Fersht, A. Enzyme Structure and Mechanism, 2nd ed.; W. H. Freeman: New York, 1985; p 475.
104. Crary, S. M.; Niranjanakumari, S.; Fierke, C. A. Biochemistry 1998, 37(26), 9409–9416.
105. Niranjanakumari, S.; Kurz, J. C.; Fierke, C. A. Nucleic Acids Res 1998, 26(13), 3090–3096.
106. Harris, M. E.; et al. RNA 1997, 3(6), 561–576.
107. Smith, D.; et al. J Biol Chem 1992, 267(4), 2429–2436.
108. Hardt, W. D.; et al. Nucleic Acids Res 1993, 21(15), 3521–3527.
109. Beebe, J. A.; Kurz, J. C.; Fierke, C. A. Biochemistry 1996, 35(32), 10493–10505.
110. Conn, G. L.; Draper, D. E. Curr Opin Struct Biol 1998, 8(3), 278–285.
111. Misra, V. K.; Draper, D. E. Biopolymers 1998, 48(2-3), 113–135.
112. Solem, A.; Chatterjee, P.; Caprara, M. G. RNA 2002, 8(4), 412–425.
113. Sharkady, S. M.; Nolan, J. M. Nucleic Acids Res 2001, 29(18), 3848–3856.
114. Ziehler, W. A.; et al. Biochemistry 2000, 39(32), 9909–9916.
115. Lindahl, L.; Zengel, J. M. Mol Biol Rep 1995, 22(2-3), 69–73.
116. Tollervey, D. Mol Biol Rep 1995, 22(2-3), 75–79.
117. Tanner, N. K.; Linder, P. Mol Cell 2001, 8(2), 251–262.
118. Schwer, B. Nat Struct Biol 2001, 8(2), 113–116.
119. Christian, E. L.; Harris, M. E. Biochemistry 1999, 38(39),12629–12638.
120. Harris, J. K.; et al. RNA 2001, 7(2), 220–232.
121. Hall, T. A.; Brown, J. W. Methods Enzymol 2001, 341, 56–77.

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