Archaeal Homologs of Human RNase P Protein Pairs Pop5 with Rpp30 and Rpp21 with Rpp29 Work on Distinct Functional Domains of the RNA Subunit

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We examined the functional equivalency between Escherichia coli RNase P protein (C5) and Pyrococcus horikoshii RNase P proteins (PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38) for RNase P activity. The C5 protein and P. horikoshii RNase P proteins were unable to activate non-cognate RNase P RNAs, P. horikoshii RNase P RNA (PhopRNA) and E. coli RNase P RNA (M1 RNA) respectively. Two chimeric RNAs, in which functional C- and S-domains of M1 RNA and PhopRNA were exchanged, were prepared and characterized with respect to the cleavage of P. horikoshii pre-tRNA in the presence of C5 or P. horikoshii proteins. The results suggest that PhoPop5 and PhoRpp30 function equivalently to the C5 protein in the E. coli RNase P, being involved in activation of the PhopRNA C-domain. On the other hand, PhoRpp21 and PhoRpp29 are implicated in stabilization of the PhopRNA S-domain.

Key words: chimeric RNA; precursor tRNA (pre-tRNA); Pyrococcus horikoshii; ribonuclease P

Ribonuclease P (RNase P) is a ubiquitous trans-acting ribozyme that processes the 5' leader sequence of precursor tRNA (pre-tRNA).1,2 Although the functionality of RNase P remains similar from bacteria to humans, the chemical composition of this enzyme differs in the three domains of life.3 Eubacterial RNase P is composed of a catalytic RNA and a single protein cofactor, both of which are required for pre-tRNA processing in vivo.4 The RNase P from Escherichia coli contains a catalytic RNA subunit termed M1 RNA and a single protein cofactor known as the C5 protein. Since Altman and co-workers discovered that M1 RNA itself contains a catalytic RNA subunit termed M1 RNA and a single protein cofactor known as the C5 protein. Since Altman and co-workers discovered that M1 RNA itself can hydrolyze pre-tRNA in vitro,5 biochemical and structural studies of RNase P have been focused mainly on eubacterial RNase P proteins.3,6 These studies indicate that eubacterial RNase P RNAs are composed of two domains, the substrate binding domain (S-domain) and the catalytic domain (C-domain).7 These domains can fold independently, and the catalytic domain alone retains RNase P activity at high Mg2+ concentrations.8

The three-dimensional structures of the eubacterial protein subunit9-11 and the RNA subunit12,13 have been established. Several findings indicate that the main functions of the protein component in eubacterial RNase P are to interact with the 5’ leader sequence, which enhances the affinity of pre-tRNA and specific Mg2+ ions bound to the RNase P - pre-tRNA complex, and to promote the transition from the intermediate to the native fold.9 Very recently, it was reported that the E. coli C5 protein functions as a metabolic stabilizer of M1 RNA.10

Archaeal and eukaryotic RNase Ps comprise a single RNA moiety with 4–5 proteins15 and 9–10 proteins16,17 respectively. We showed by reconstitution experiments that RNase P RNA (PhopRNA) alone in the hyperthermophilic archaeon Pyrococcus horikoshii OT3 had no endonuclease activity, but PhopRNA and five archaeal homologs, PhoPop5, PhoRpp38, PhoRpp21, PhoRpp29, and PhoRpp30, of human proteins hPop5, Rpp38, Rpp21, Rpp29, and Rpp30 respectively, reconstituted RNase P activity that exhibits enzymatic properties like those of the authentic enzyme.8 A single protein omission test indicated that the proteins contribute to RNase P activity in the order PhoPop5 > PhoRpp30 > PhoRpp21 >> PhoRpp29 > PhoRpp38.19 Furthermore, Tsai et al. have reported that archaeal Rpp21 with Rpp29 or Pop5 with Rpp30 in Pyrococcus furiosus are sufficient for RNase P RNA-based catalysis.20 As for an eukaryotic RNase P, human proteins Rpp21 and Rpp29 were found to play a key role in the activation of a catalytic conformation of the RNA subunit H1 RNA.21 Although biochemical information has become available on archaeal and eukaryotic RNase P proteins, their functional roles have yet to be elucidated.

The crystal structures of all five archaeal RNase P proteins have become available.18,22-25 Comparisons of these structures with that of the C5 protein revealed no similarity between eubacterial and archaeal RNase P proteins, although PhoPop5 and PhoRpp38 share an RNA-binding motif (the RNP motif) with the C5 protein.18,25 It was, however, reported that the Bacillus...
subtilis RNase P protein activated Haloferax volcanii \(26\) as well as Methanobacterium RNAse P RNAs. \(27\) Furthermore, human RNAse P protein Rpp29 was reported to activate M1 RNA. \(21\) These findings suggest that some archaeal and eukaryotic RNAse P proteins are functionally equivalent to the eubacterial protein in RNAse P, though they are not structurally related to each other. Hence we attempted to determine the functional equivalence between the \(E. coli\) C5 protein and the \(P. horikoshii\) RNAse P proteins in expectation that it would aid in elucidating the functional role of archaeal as well as eukaryotic proteins in RNAse P.

In this study, we first examined to determine whether the C5 protein and \(P. horikoshii\) RNAse P proteins can activate non-cognate RNAse P RNAs. Next, two chimeric RNAse P RNAs, in which the S- and C-domains in M1 RNA and PhoRNA were exchanged, were prepared and tested to determine whether they were activated by the C5 protein or the \(P. horikoshii\) proteins. We found that the C5 protein and the \(P. horikoshii\) RNAse P proteins did not activate non-cognate RNAse P RNAs. Furthermore, reconstitution experiments using the chimeric RNAs suggested that the \(P. horikoshii\) proteins PhoRpp5 and PhoRpp30 are functionally equivalent to the C5 protein in the roles they play in RNAse P activity, being involved in the activation of the PhoRNA C-domain, while PhoRpp21 and PhoRpp29 are responsible for stabilization of the PhoRNA S-domain.

### Materials and Methods

**Materials.** Five RNAse P proteins (PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38), RNAse P, and pre-RNA \(^{32}\) in \(P. horikoshii\) and M1 RNA were prepared, as described previously. \(16,28\) \(E. coli\) BL21 (DE3) plasmid harboring a pET-15b-derived plasmid encoding the C5 protein was a gift of Professor Y. Kikuchi (Toyohashi University of Technology). The protein was overproduced and purified as described previously. \(29\) The plasmid vector used was pUC19 from Novagen (Darmstadt, Germany), and \(E. coli\) strain JM109 was used as a host for cloning. Restriction enzymes were purchased from MB1 Fermentas (Baltimore, MD). Oligonucleotides were purchased from Sigma-Aldrich (St. Louis, MO). Ex Taq DNA polymerase and the DNA ligation kit were purchased from Takara Bio (Shiga, Japan). All the other chemicals were of analytical grade for biochemical use.

**Preparation of chimeric RNAse P RNAs.** The gene encoding a chimeric RNAse P RNA, ECPS, composed of the M1 RNA C-domain and the PhoRNA S-domain, was constructed as shown in Fig. 1. The gene fragments encoding the M1 RNA C-domain (positions 1–87 and positions 242–377) and the PhoRNA S-domain (positions 62–221) were amplified individually by PCR using specific primers. The M1 RNA C-domain positions 1–87 and positions 242–377 are shown as thin black bars in Fig. 1. The PCR primers were designed so that the gene encoding the PhoRNA S-domain was between the gene fragments encoding the M1 RNA C-domain (positions 1–87 and positions 242–377), that is, the resulting gene contained an artificial site for Eco RI and the T7 phage promoter at the 5’-end and a Bam HI site at the 3’-end. Thus nucleotide sequences encoding the PhoRNA S-domain 5’-(positions 62–79) and 3’-(positions 204–221) terminal regions were fused to the 5’-terminal and 5’-terminal ends of the gene fragments encoding the M1 RNA C-domain positions 1–87 and 242–377 respectively. These terminal regions are shown as thin black bars in Fig. 1. After purification of the PCR products, the gene fragment encoding the M1 RNA C-domain (positions 1–87) and that encoding the PhoRNA S-domain (positions 62–221) were linked by PCR using themselves as primers and templates, then the resulting PCR product and the gene fragment encoding the M1 RNA C-domain (pos. 242–377) were linked by amplification, as described above. The resulting gene encoding ECPS was inserted into the pUC19 plasmid vector at a multi-cloning site between Eco RI and Bam HI. The gene encoding the other chimeric RNAse P RNA, PCES, composed of the PhoRNA C-domain and the M1 RNA S-domain, was prepared in a manner similar to that described above for the construction of ECPS. Thus the gene fragments encoding the PhoRNA C-domain 5’-(positions 1–61) and 3’-(positions 222–329) terminal regions were ligated to the 5’- and 3’-terminal ends respectively of the gene encoding the M1 RNA S-domain (positions 88–241). The resulting plasmid templates encoding the chimeric RNAs, ECPS and PCES, were linearized with Bam HI, and transcription in vitro was conducted using RiboMAX Large scale RNA production system-T7 (Promega, Madison, WI), as described previously. \(20\)

**Assay for RNAse P activity.** RNAse P activity for the reconstituted particles was analyzed using in vitro transcription \(^{32}\)-labeled \(P. horikoshii\) pre-RNA \(^{37}\), as described previously, \(18,28\) with some modifications. \(P. horikoshii\) pre-RNA \(^{37}\) was used as a substrate throughout the study, since the reconstituted \(P. horikoshii\) RNAse P as well as the \(E. coli\) M1 RNA cleaved a single phosphodiester bond between positions US9 and C66 in the pre-RNA \(^{37}\). \(20\) The reaction mixtures were incubated in 50 mm Tris–HCl (pH 7.6) containing 0–100 mm MgCl\(_2\), 600 mm NH\(_2\)OAc, 60 mm NHCl, RNAse P RNA (10 pmol), proteins (30 pmol each), and \(32\)-labeled pre-RNA \(^{37}\) (2.6–4.16 pmol/\(\mu\)L) at 50°C for the specified periods. To examine the dependency on Mg\(^{2+}\) of the RNAse P activity of the reconstituted particles, RNAse P activity was analyzed as described above in the presence of 0–100 mm MgCl\(_2\), for 30 min. The reactions were stopped by adding phenol, and the reaction products were separated on 15% polyacrylamide denaturing gels in TBE buffer (90 mm Tris-borate containing 1 mm EDTA) at 47 W for 1 h. The gels were exposed to an Imaging Plate and the reaction products were visualized by a PhosphorImager FLA-5000 (Fuji Film, Tokyo). For time-course analysis, the resulting phosphorimager was used to obtain values for the pre-RNA \(^{37}\) processing activity at various incubation times. The cleavage activity was calculated as follows: the quantity of (matured tRNA \(^{37}\) + leader fragment) \(\times 100\)/the quantity of (pre-RNA \(^{37}\) + mature tRNA \(^{37}\) + leader fragment), and the percentage was plotted against the incubation times, as described previously. \(30\)

**Nomenclature.** The RNAse P proteins from \(P. horikoshii\) OT3 were designated according to their homology to the corresponding eukaryotic proteins, with the prefix \(Pho\) to differentiate them from homologous proteins of other organisms, as described previously. \(39\) This new nomenclature replaces the old one: Ph1481p, Ph1496p, Ph1601p, Ph1771p, and Ph1877p, the names of which were based on gene ID numbers, became PhoPop5, PhoRpp38, PhoRpp21, PhoRpp29, and PhoRpp30 respectively. The reconstituted particles of chimeric RNAs are denoted with the RNA given first, followed by the protein number (e.g., the reconstituted particle ECPS/C5-PhoPop5-Rpp21 is composed of ECPS with the C5 protein, PhoPop5, and PhoRpp21).

### Results

**Exchange of archaeal and eubacterial RNAse P proteins**

First we examined to determine whether \(P. horikoshii\) RNAse P proteins can activate \(E. coli\) M1 RNA in the presence of 10 mm MgCl\(_2\). In this experiment, we had to choose a reaction temperature for measuring RNAse P activity with a heterologous complex, M1 RNA with \(P. horikoshii\) proteins. We have found that reconstituted particles composed of PhoPop5, PhoRpp21, PhoRpp29, and PhoRpp30 with PhoRNA have an optimal temperature of about 50°C. \(28\) First we tested to determine whether M1 RNA could be activated by the C5 protein with 10 mm MgCl\(_2\) at 50°C for 3 h. As shown in Supplemental Fig. 1 (see Biosci. Biotechnol. Biochem. Web site, lane 2), pre-RNA \(^{37}\) was completely processed, giving rise to mature pre-RNA \(^{37}\), indicating that...
the *E. coli* RNase P subunits, M1 RNA and the C5 protein, are capable of interacting with each other and retain considerable processing activity toward *P. horikoshii* pre-tRNA at 50 °C. Hence M1 RNA was first mixed with individual *P. horikoshii* proteins, and then the mixtures were subjected to enzymatic assay at 50 °C. No mixture exhibited cleavage activity under the conditions tested (Supplemental Fig. 1, lanes 3–7). Then pairwise mixing of proteins with M1 RNA was done, and the resulting mixtures were analyzed for cleavage activity. None of mixtures containing *P. horikoshii* proteins activated M1 RNA under the conditions used (Supplemental Fig. 1).

Next we examined to determine whether the C5 protein would replace *P. horikoshii* RNase P proteins for activation of PhopRNA. Since the addition of a fifth archaeal protein to particles containing any four archaeal proteins with PhopRNA activated RNase P activity at 50 °C, the C5 protein was added to mixtures containing PhopRNA and any four archaeal proteins, and the
cleavage activity of the reconstituted mixtures was measured in 50 mM Tris–HCl (pH 7.5) containing 50 mM MgCl\(_2\) at 50°C for 3 h. The reaction products were resolved on 15% acrylamide/8 M urea and visualized by autoradiography, as described in “Materials and Methods.” Lanes 1 and 2 indicate the reaction products of pre-tRNA\(^{32P}\) digested with six proteins in the absence of PCES and with PCES alone respectively. Slanting numbers C5, 5, 21, 29, 30, and 38 indicate the C5 protein, PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38 respectively; e.g., the lane 3 indicates the reaction product digested with the reconstituted particle composed of PCES with C5, PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38.

**Preparation and characterization of chimeric RNase P RNAs**

The results described above indicate that the C5 protein and *P. horikoshii* RNase P proteins did not activate non-cognate RNase P RNAs, indicating that they have no influence on the non-cognate RNase P RNAs under the conditions tested. On the other hand, it is known that the C5 protein binds the M1 RNA C-domain,\(^{30}\) and that both S- and C-domains of the eubacterial RNase P RNA can fold independently at high Mg\(^{2+}\) concentrations.\(^{8}\) Taking this information into account, we anticipated that if the *P. horikoshii* RNase P proteins could activate a chimeric RNA composed of parts of M1 RNA and PhopRNA, this should provide new insight into their functional role in RNase P activity. Based on this assumption, we attempted to prepare two chimeric RNase P RNA gene, in which the gene fragments encoding the C- and S-domains in M1 RNA and PhopRNA were exchanged, and conducted transcription of them *in vitro*, as described in “Materials and Methods.” To evaluate their ribozymatic potential, the chimeric RNAs were mixed with all six proteins (C5 and the five *P. horikoshii* proteins), and the cleavage of pre-tRNA\(^{32P}\) at 50°C was analyzed. Both mixtures exhibited cleavage activity, as shown in Fig. 2, lane 3 and Fig. 3, lane 3. It is known that *E. coli* M1 RNA and *P. horikoshii* RNase P cleave pre-tRNA\(^{32P}\) at a single phosphodiester bond between positions U59 and C60.\(^{28}\) To determine whether that is also the case for these two mixtures, we conducted a primer extension analysis on the cleavage products, as described previously.\(^{28}\) We found that both mixtures cleaved efficiently at the same site between positions U59 and C60 of pre-tRNA\(^{32P}\) (data not shown). This finding indicates that both chimeric RNAs could be correctly folded and activated by interacting with the proteins.

The mixtures were further characterized with respect to cleavage activity at 50°C under various Mg\(^{2+}\) concentrations. ECPS, composed of the M1 RNA C-domain and the PhopRNA S-domain, exhibited optimal activity at about 10–30 mM Mg\(^{2+}\), while the other chimeric RNA, PCES, composed of the PhopRNA C-domain and M1 RNA S-domain, had optimal activity at about 40–60 mM Mg\(^{2+}\) (Supplemental Fig. 3). Hence we further tested to determine which proteins can activate ECPS and PCES in the presence of 10 mM and 50 mM Mg\(^{2+}\) respectively at 50°C for 3 h.

![Fig. 2. In Vitro Reconstitution of RNase P Activity with Chimeric RNA, PCES, with the C5 Proteins and *P. horikoshii* RNase P Proteins. Combinations of six proteins, as indicated by the slanting numbers, with PCES were incubated with \(^{32P}\)-labeled pre-tRNA\(^{32P}\) in the presence of 50 mM MgCl\(_2\) at 50°C for 3 h. The reaction products were resolved on 15% acrylamide/8 M urea and visualized by autoradiography, as described in “Materials and Methods.” Lanes 1 and 2 indicate the reaction products of pre-tRNA\(^{32P}\) digested with six proteins in the absence of PCES and with PCES alone respectively. Slanting numbers C5, 5, 21, 29, 30, and 38 indicate the C5 protein, PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38 respectively; e.g., the lane 3 indicates the reaction product digested with the reconstituted particle composed of PCES with C5, PhoPop5, PhoRpp21, PhoRpp29, PhoRpp30, and PhoRpp38.](image-url)
Reconstitution of RNase P activity by chimeric RNase P RNAs and RNase P proteins

PCES was first mixed with individual proteins, and then subjected to enzymatic assay. No mixture exhibited cleavage activity (Fig. 2, lanes 60–65). Then pairwise mixing with PCES was done, and the resulting mixtures were analyzed for cleavage activity in the presence of 50 mM MgCl$_2$ at 50°C. The reconstituted mixture, PCES/Pop5-Rpp30, had cleavage activity, as shown in Fig. 2, lane 56. In contrast, the other mixtures lacking PhoPop5 or PhoRpp30 had no cleavage activity (Fig. 2). Thus PhoPop5 and PhoRpp30 are responsible for the activation of PCES (Fig. 4A).

Next, the other chimeric RNA, ECPS, was mixed with the proteins, and the resulting mixtures were analyzed for pre-tRNA cleavage activity in the presence of 10 mM MgCl$_2$ at 50°C. The particles composed of ECPS with C5 and PhoRpp29 exhibited some cleavage activity, as shown in Fig. 3, lane 46. In contrast, the other mixtures lacking PhoPop5 or PhoRpp30 had no cleavage activity (Fig. 2). Thus PhoPop5 and PhoRpp30 are responsible for the activation of ECPS (Fig. 4A).

In contrast, the other mixtures composed of ECPS/C5-Rpp21-Rpp29 and ECPS/Pop5-Rpp21-Rpp29-Rpp30 exhibited cleavage activity (Fig. 3, lane 40) and the addition of PhoRpp30 strongly enhanced this activity (Fig. 3, lane 18). These findings indicate that the reconstituted particles, ECPS/C5-Rpp21-Rpp29 and ECPS/Pop5-Rpp21-Rpp29-Rpp30, exhibited cleavage activity (Fig. 4B). It should be noted however that the mixtures composed of C5, PhoRpp21, and PhoRpp29 at least PhoRpp38, gave rise to labile matured tRNAs, though the 5'-leader fragment was stably detected (Fig. 3, lanes 5, 10, 17, and 27). This suggests that because of non-cognate interaction between the M1 RNA C-domain with C5 and of the PhopRNA S-domain with PhoRpp21 and PhoRpp29, substrate binding site or catalytic site are improperly formed, cleaving at a phosphodiester bond distinct from the other mixtures (between positions U59 and C60). To address this assumption, we attempted to conduct a primer extension analysis on the cleavage product, but our attempt failed. No further attempt was made in this study.

We found in this reconstitution that the addition of PhoRpp38 to the mixtures that produced the labile tRNAs described above improved cleavage activity, giving rise to stable tRNA (Fig. 3, lane 3 versus lane 5, lane 4 versus lane 10, lane 8 versus lane 17, and lane 13 versus lane 27). In contrast, the addition of PhoRpp38 to mixture ECPS/Pop5-Rpp21-Rpp29 abolished the cleavage activity (Fig. 3, lane 24 versus lane 40). Similarly, PhoRpp38 abrogated the RNase P activity due to ECPS/Pop5-Rpp21-Rpp29-Rpp30 (Fig. 3, lane 9 versus lane 18). The influence of PhoRpp38 is discussed below.

Discussion

The present study indicates that the _P. horikoshii_ RNase P proteins and the _E. coli_ C5 protein are unable to activate non-cognate RNase P RNAs under the conditions used. It has, however, been reported that human RNase P protein Rpp21 activated M1 RNA.21) A subsequent study indicated that M1 RNA-Rpp29 is catalytically less effective than the M1 RNA-C5 com-
plex, and that the C-terminal domain of Rpp29 is responsible for the activation of M1 RNA, though the N-terminal domain is moderately involved as well.31) PhoRpp29, an archaeal homolog of human Rpp29, can be aligned with the C-terminal two-thirds of Rpp29 when the residues are aligned so as to optimize the similarity.24) Hence PhoRpp29 was expected to have the potential to activate M1 RNA. However, we did not observe any cleavage of pre-tRNA Tyr by the particles composed of M1 RNA with PhoRpp29 (Supplemental Fig. 1, lane 6). The reason for this apparent discrepancy is not known at present. Either PhoRpp29 plays a function non-equivalent to that of Rpp29 in human RNase P or the in vitro conditions used were insufficient to provide PhoRpp29 activity.

The chimeric RNase P RNA, PCES, composed of the PhopRNA C-domain (black) and the M1 RNA S-domain (gray), was activated by PhoPop5 and PhoRpp30 (Fig. 2). Since the M1 RNA S-domain can be folded independently at 50 mM MgCl$_2$,8) it is likely that PhoPop5 and PhoRpp30 play essential roles in the activation of the PhopRNA C-domain (Fig. 4A). On the other hand, the other chimeric RNA, ECPS, composed of the M1 RNA C-domain and the PhopRNA S-domain, was activated by the C5 protein, PhoRpp21, and PhoRpp29 (Fig. 3), though the resulting tRNA appeared to be labile as described above. It is known that the C5 protein binds to the helix P3 stem-loop and helix P4 regions in the M1 RNA C-domain,30) activating the RNase P activity of M1 RNA. It is thus reasonable to predict that PhoRpp21 and PhoRpp29 are involved in the stabilization of the PhopRNA S-domain (Fig. 4B). Taking these findings into consideration, the present results suggest that P. horikoshii proteins PhoPop5 and PhoRpp30 are functionally equivalent to the C5 protein in the roles they play in RNase P activity, being involved in activation of the PhopRNA C-domain, while PhoRpp21 and PhoRpp29 are responsible for stabilization of the PhopRNA S-domain (Fig. 4). Two different observations have some relevance to these assumptions.

First, Tsai et al. reported that one of two specific pairs of P. furiosus proteins (Rpp21 with Rpp29 or Pop5 with Rpp30), together with the RNA subunit, is sufficient to obtain weak cleavage of the 5'-leader sequence of pre-tRNA in vitro, suggesting that the two protein pairs have distinct roles in RNase P.20) They further mapped the binding site for Pop5 and Rpp30 to the C-domain in the P. furiosus RNase P RNA based on footprinting analysis.20) Second, structural and mutational analyses demonstrated that PhoPop5 and PhoRpp30 form a heterotetramer,25) while PhoRpp21 and PhoRpp29 fold into a heterodimer in solution,32) the quaternary structures playing a crucial role in the P. horikoshii RNase P.25,32) It is thus likely that protein complexes PhoPop5-PhoRpp30 and PhoRpp21-PhoRpp29 are involved in the activation and stabilization of the C- and S-domains in PhopRNA respectively (Fig. 4B).

It is known that the C- and S-domains of eubacterial RNase P RNA can fold independently and that the C-domain alone retains RNase P activity.5) Nevertheless, the chimeric RNA, ECPS, composed of the M1 RNA C-domain and the PhopRNA S-domain, was not activated by the C5 protein alone. Recently, Li et al., described based on mutational analysis of the archaean Methanothermobacter thermoautotrophicus that the catalytic capacity of archaenal RNase P RNAs is masked by the poor function of the archaenal S-domain in the absence of archaenal protein cofactors.35) Hence the...
presence of the PhoRNA S-domain in ECPS interfered with activation of the M1 RNA C-domain by the C5 protein.

We found that four P. horikoshii proteins (PhoPop5, PhoRpp21, PhoRpp29, and PhoRpp30) virtually activated ECPS composed of the M1 RNA C-domain and PhoRNA S-domain (Fig. 3, lane 18 and Fig. 4B), although they did not activate M1 RNA (Supplemental Fig. 1, lane 10). Several explanations can be put forward to explain how ECPS is activated by four P. horikoshii proteins. First, the crystal structures of eubacterial pRNAs from Bacillus stearothermophilus and Thermostoga maritima showed that the C- and S-domains interact closely with each other, ensuring that they are positioned correctly and oriented in a manner appropriate for tRNA binding. When the PhoRNA S-domain interacts with PhoRpp21-PhoRpp29, it undergoes a conformational change that alters its interaction with the M1 RNA C-domain in ECPS, an event that might allow PhoPop5-PhoRpp30 to activate the M1 RNA C-domain. Another possibility to account for the activation of ECPS by the four archaeal proteins is that an interaction between the two protein complexes PhoPop5-PhoRpp30 and PhoRpp21-PhoRpp29 would result in activation of the M1 RNA C-domain by PhoPop5-PhoRpp30. Yeast two-hybrid analysis indicated that PhoRpp21 interacts strongly with PhoRpp29 and moderately with PhoPop5, suggesting that PhoPop5-PhoRpp21 functions as a bimodule between the two protein complexes (PhoPop5-PhoRpp30 and PhoRpp21-PhoRpp29). Indeed, the addition of PhoPop5 to complex PhoRpp21-PhoRpp29 produced a stable protein complex (PhoPop5-PhoRpp21-PhoRpp29) with gel filtration on Biogel P100 (Honda et al., unpublished results). Furthermore, the presence of the four proteins significantly enhanced the RNase P activity of the reconstituted particles. Hence it can be postulated that PhoRpp21-PhoRpp29 bound to the PhoRNA S-domain interacts with PhoPop5-PhoRpp30, allowing PhoPop5-PhoRpp30 to activate the M1 RNA C-domain in ECPS.

We found in the reconstitution experiments that the addition of PhoRpp38 to ECPS/C5-Rpp21-Rpp29 (Fig. 3, lane 13 versus lane 27 and Fig. 4B) or mixtures composed of at least the C5 protein, PhoRpp21, and PhoRpp29 with ECPS improved cleavage activity (Fig. 3, lane 3 versus lane 5, lane 4 versus lane 10, and lane 8 versus lane 17). In contrast, the addition of PhoRpp38 to the mixture ECPS/Pop5-Rpp21-Rpp29 abolished the cleavage activity in reverse (Fig. 3, lane 24 versus lane 40 and Fig. 4B). In addition, PhoRpp38 abrogated RNase P activity due to ECPS/Pop5-Rpp21-Rpp29-Rpp30 (Fig. 3, lane 9 versus lane 18). PhoRpp38 is a multifunctional protein, operating in ribosomes as well as RNP complexes (box C/D and box H/ACA). It is involved in elevating the optimum temperature for the P. horikoshii RNase P activity, interacting with two stem-loop structures: nucleotides A116-G201 (S-domain) and G229-C276 (C-domain) in PhoRNA. Loop structure L15/16 at the second binding site (G229-C276) in the PhoRNA C-domain is conserved in the M1 RNA C-domain, but an extra stem structure (helix P17) occurs in the M1 RNA C-domain. Since yeast two-hybrid analysis detected no interaction between PhoRpp38 and the other four proteins, it is unlikely that PhoRpp38 influences the cleavage activity of the mixtures by binding directly to C5 or PhoPop5-PhoRpp30. Rather, it is probable that incorrect binding of PhoRpp38 to the M1 RNA C-domain might have a reverse effect on the interaction of C5 or PhoPop5-PhoRpp30 with the M1 RNA C-domain in ECPS. Although we have no explanation for the reverse effect due to PhoRpp38 at the moment, this suggests that PhoPop5-PhoRpp30 activates the M1 RNA C-domain in a manner distinct from the C5 protein.

In conclusion, the present study indicates that chimeric RNA ECPS was activated by C5, PhoRpp21 and PhoRpp29 and four P. horikoshii proteins. A further characterization of the two particles, ECPS/C5-Rpp21-Rpp29 and ECPS/Pop5-Rpp21-Rpp29-Rpp30, might provide more insight into the functional equivalence of the C5 protein and PhoPop5-PhoRpp30. In addition, further biochemical studies of the interaction between PhoPop5-PhoRpp30 and the PhoRNA C-domain, and between PhoRpp21-PhoRpp29 and the PhoRNA S-domain, should provide a better understanding of the molecular mechanism by which archaeal and eukaryotic RNase P RNAs are activated by protein cofactors.

Note added in proof

Xu et al. recently localized the Rpp21-Rpp29 complex to the specificity domain (S-domain) of the RNA subunit in Methanocaldococcus jannaschii during the review process of this manuscript.

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