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A three-dimensional model of RNase P in the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3

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ABSTRACT

Ribonuclease P (RNase P) is an endoribonuclease involved in maturation of the 5′-end of tRNA. We found previously that RNase P in the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 consists of a catalytic RNase P RNA (*Pho*pRNA) and five protein cofactors designated *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30, and *Pho*Rpp38. The crystal structures of the five proteins have been determined, a three-dimensional (3-D) model of *Pho*pRNA has been constructed, and biochemical data, including protein-RNA interaction sites, have become available. Here, this information was combined to orient the crystallographic structures of the proteins relative to their RNA binding sites in the *Pho*pRNA model. Some alterations were made to the *Pho*pRNA model to improve the fit. In the resulting structure, a hetero-tetramer composed of *Pho*Pop5 and *Pho*Rpp30 bridges helices P3 and P16 in the *Pho*pRNA C-domain, thereby probably stabilizing a double-stranded RNA structure (helix P4) containing catalytic Mg^{2+} ions, while a heterodimer of *Pho*Rpp21 and *Pho*Rpp29 locates on a single-stranded loop connecting helices P11 and P12 in the specificity domain (S-domain) in *Pho*pRNA, probably forming an appropriate conformation of the precursor tRNA (pre-tRNA) binding site. The fifth protein *Pho*Rpp38 binds each kink-turn (K-turn) motif in helices P12.1, P12.2, and P16 in *Pho*pRNA. Comparison of the structure of the resulting 3-D model with that of bacterial RNase P suggests transition from RNA-RNA interactions in bacterial RNase P to protein-RNA interactions in archaeal RNase P. The proposed 3-D model of *P. horikoshii* RNase P will serve as a framework for further structural and functional studies on archaeal, as well as eukaryotic, RNase Ps.

1. Introduction

Ribonuclease P (RNase P) is an endoribonucleolytic *trans*-acting ribozyme required for the correct processing of tRNA precursors (pre-tRNA) into their 5′-phosphorylated mature forms [1]. In all domains of life, RNase P is composed of a catalytic RNA component and a variable number of RNase P protein cofactors [2]. A characteristic feature of archaeal and eukaryotic RNase P RNAs is that they alone have little catalytic activity, but their interaction with protein cofactors leads to an active ribozyme [3]. Hence, archaeal and eukaryotic RNase Ps may serve a good model ribozyme to show how a catalytic RNA is activated by protein cofactors.

We earlier found in a reconstitution experiment that RNase P RNA (*Pho*pRNA) of the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 is enzymatically active only when bound to five proteins: *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30, and *Pho*Rpp38 [4,5]. Crystal structures of the five proteins were determined at a high resolution [6], and a 3-D model of *Pho*pRNA was constructed by combining sequence and structure comparisons [7]. Moreover, biochemical and structural studies revealed that a heterotetramer composed of *Pho*Pop5 and *Pho*Rpp30 is involved in structural formation of the catalytic domain (C-domain), while a heterodimer of

Abbreviations: 3-D, three-dimensional; C-domain, catalytic domain; CR, conserved region; K-turn, kink-turn; PDB, protein data bank; *Pho*pRNA, *Pyrococcus horikoshii* ribonuclease P RNA; pre-tRNA, precursor tRNA; RNase P, ribonuclease P; S-domain, specificity domain.

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*Pho*Rpp21 and *Pho*Rpp29 is implicated in an appropriate conformation of the specificity domain (S-domain) [8–10]. Recently, we showed that *Pho*Rpp30 functions as a molecular chaperone for dimerization of *Pho*Pop5, and that the C-terminal helix in *Pho*Pop5 binds helices P3 and P16 in the C-domain [11,12]. Moreover, we found that *Pho*Rpp21 primarily functions as a scaffold for *Pho*Rpp29 by binding to a single-stranded loop linking helices P11 and P12 in *Pho*pRNA, and that Lys residues on the N-terminal helix and Arg residues on the C-terminal strand in *Pho*Rpp21 and the C-terminal basic residues in *Pho*Rpp29 are responsible for activation of *Pho*pRNA [13]. As for the fifth protein, *Pho*Rpp38, chemical footprinting localized its binding sites on stem-loops containing helices P12.2 and P16 [5]. Furthermore, crystal structures of *Pho*Rpp38 in complex with kink-turn (K-turn) motifs in helix P12.1 and P12.2 have been determined [[14] and Oshima et al. unpublished results]. In this study, we constructed a 3-D model of *P. horikoshii* RNase P on the basis of this information. In the resulting 3-D structure, interactions of α-helices in proteins with double-stranded RNA structures appear to play an important role in stabilization of an appropriate *Pho*pRNA conformation. Furthermore, comparison of the resulting 3-D model with the crystal structure of the bacterial RNase P suggests that RNA-RNA interactions in bacterial RNase P are replaced by protein-RNA interactions in archaeal RNase P.

2. Materials and methods

2.1. Materials

Restriction enzymes were purchased from MBI Fermentas (Ontario, Canada). 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 other chemicals were of analytical grade for biochemical use.

2.2. Preparation of proteins and RNAs

Five RNase P proteins (*Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30, and *Pho*Rpp38) were prepared as described previously [4,5]. As for preparation of RNAs, *Pho*pRNA, its mutants, and pre-tRNA were prepared by *in vitro* transcription with T7 RNA polymerase using corresponding double stranded DNA as a template and purified on a DEAE-Sepharose column, as described previously [15]. The purified RNAs were annealed by heating up to 90 °C for 5 min and cooling down to 4 °C over 5 min, and then used for further experiments.

2.3. Assay for pre-tRNA cleavage activity

Pre-tRNA cleavage activity of the *in vitro* reconstituted RNase P containing either the wild-type *Pho*pRNA or its mutants was done in a reconstitution buffer, 50 mM Tris-HCl (pH 7.6) containing 50 mM $MgCl_2$, 600 mM NH_4OAc, and 60 mM NH_4Cl, principally as described previously [16]. The reactions were stopped by adding phenol, and the reaction products were separated on 10% polyacrylamide denaturing gels in TBE buffer (900 mM Tris-borate containing 10 mM EDTA) at 150 V for 1 h. After electrophoresis, the reaction products were visualized by staining in a 0.1% toluidine blue solution. The resulting image was used to obtain values for the pre-$tRNA^{Tyr}$ processing activity with various incubation times. The cleavage efficiency was calculated as follows: the quantity of (matured $tRNA^{Tyr}$ + leader fragment)/the quantity of (pre-$tRNA^{Tyr}$ + matured $tRNA^{Tyr}$ + leader fragment), and the percentage was plotted against the incubation times.

2.4. Modeling of PhopRNA and RNase P

The *Pho*pRNA model structure was reconstructed with the

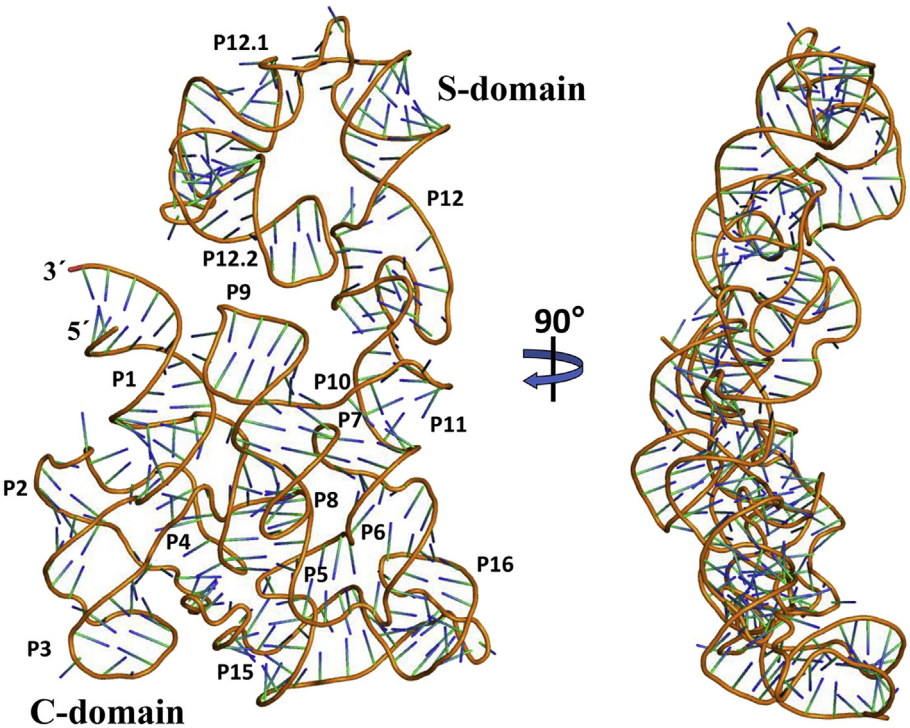

Fig. 1. A 3-D model of *Pho*pRNA. A 3-D structure of *Pho*pRNA was remodeled in order to have two K-turn motifs in helices P12.1 and P12.2. In addition, nucleotides at CRII and CRIII connecting helices P11 and P12 were remodeled in order to interact with invariant nucleotide residues in D loop and TψC loop in tRNA, as shown in the bacterial RNase P structure [18]. Helices are numbered according to the existing RNase P RNA nomenclature [25]. The figure was drawn with PyMol (http://pymol.sourceforge.net).

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