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Primitive templated catalysis of a peptide ligation by self-folding RNAs

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ABSTRACT

RNA–polypeptide complexes (RNPs), which play various roles in extant biological systems, have been suggested to have been important in the early stages of the molecular evolution of life. At a certain developmental stage of ancient RNPs, their RNA and polypeptide components have been proposed to evolve in a reciprocal manner to establish highly elaborate structures and functions. We have constructed a simple model system, from which a cooperative evolution system of RNA and polypeptide components could be developed. Based on the observation that several RNAs modestly accelerated the chemical ligation of the two basic peptides. We have designed an RNA molecule possessing two peptide binding sites that capture the two peptides. This designed RNA can also accelerate the peptide ligation. The resulting ligated peptide, which has two RNA-binding sites, can in turn function as a *trans*-acting factor that enhances the endonuclease activity catalyzed by the designed RNA.

INTRODUCTION

RNA–polypeptide complexes (also called RNA–protein or ribonucleoprotein complexes and abbreviated as RNP) play crucial roles in various aspects of extant cellular biosystems (1–5). RNP has also been suggested to have played pivotal roles in the evolution of the modern DNA–protein world from the hypothetical RNA world (1,6–8).

In the RNA world hypothesis, the RNP world was proposed to exist as an intermediate stage between the RNA world and the modern DNA–protein world (1,6–8). Several RNPs, which still play crucial roles in extant cellular systems, have been suggested to have evolved from RNA enzymes (ribozymes) by gradual replacement of their structural RNA modules with *trans*-acting polypeptides (9–17). Through gradual replacement of structural modules, polypeptides could be incorporated into the RNA-based primitive life system.

After the isofunctional replacement of RNA modules with polypeptides, the ancient RNPs would have further evolved to more complex and sophisticated forms through developing reciprocal and cooperative evolution systems (18–24). To provide a simple model system for cooperative evolution between RNAs and polypeptides in the RNP world, several RNP systems have been designed in which the processing (ligation or cleavage) of RNA component is facilitated through binding with polypeptide components (25–30). On the other hand, the complementary system, in which processing of the polypeptide component was facilitated by RNA components, has not been reported.

MATERIALS AND METHODS

General

DNase I was purchased from Promega Japan (Tokyo, Japan). Sephadex G-50 spin column was purchased from GE Healthcare Japan (Tokyo, Japan). FAM-labeled substrate RNA and *Human* let-7a RNA were purchased from JBIOS (Tsukuba, Japan). Preparation of peptides is described in the supporting information.

Preparation of RNAs

Plasmids encoding the *Tetrahymena* ribozyme, the TetboxB/RRE RNA and its mutants were described previously (27,28). These plasmids were used for template for PCR reactions with a set of primers that are Sense-L21 (5′-CTAATACGACTCACTATAGGAGGGAAAA GTTATCAGGCA-3′) and Antisense-L21 (5′-ACTCCA AAACTAATCAATAT-3′). The resulting PCR products were used as templates for *in vitro* transcription. A template DNA for the *Homo sapiens* tyrosine-tRNA was PCR amplified using plasmid pHSTY (31) as a template and a set of primers that are T7pro (5′-CTAATACGACTCACT ATAGG-3′) and kyi-55 (5′-TGGTCCTTCGAGCCGGA ATCGAAC-3′). A template DNA for *Escherichia coli*

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RNaseP RNA was PCR amplified using genomic DNA of *E. coli* JM109 as a template and a set of primers kyi-53 (5′-CTAATACGACTCACTATAGGGAGCTGACCAG ACAGTCGCCGCTTCGTC-3′) and kyi-54 (5′-GGGAA CTGACCGATAAGCCGGGTTCTGTCGT-3′). *In vitro* transcription reactions with T7 RNA polymerase were performed as described (27,28). The transcription products were purified on 5% denaturing polyacrylamide gels. RNAs isolated from the gels were passed through Sephadex G-50 spin columns. The concentrations of RNAs were determined from intensities of UV absorption at 260 nm.

Peptide ligation experiments

The chemical ligations of peptides were performed as follows. After denaturation of RNA (7.5 μM) in water for 5 min at 80°C, concentrated reaction buffer [final compositions of the reaction mixture were 40 mM HEPES (pH 8.0), 80 mM KCl and 2 mM $MgCl_2$] was added and the mixture was heated for 5 min at 50°C. The solution was cooled at room temperature. Reaction, which was started by adding peptide (7.5 μM) and 1% (w/v) 2-mercaptoethanesulfonate (MESNA), was performed at 37°C. Samples (20 μl) were quenched with 2 μl of 40% aqueous TFA and the reaction mixtures were analyzed by using analytical HPLC with CosmoSil 5C18-AR-α (4.6 x 25 mm). Gradient conditions for CH_3CN in 0.1% aqueous TFA were as follows: 5–60% in 45 min; flow rate: 1 ml/min. The fractions corresponding to major products were collected and their molecular weights were analyzed by mass spectrometry. All experiments were repeated at least twice. Their mean values are shown in figures wherein error bars indicate their minimal and maximal values.

Assay for endonuclease ribozyme activity

L21-WT, L21-boxB/RRE or L21-boxB/RRE mut 11 nt dissolved in H_2O was heated to 80°C for 5 min and then pre-folded in a solution containing 44 mM HEPES (pH 8.0), 88 mM KCl, appropriate concentration of $MgCl_2$ with or without the ligated peptide at 37°C for 1 min. After 5 min at 37°C, the reaction was started at 37°C by adding the FAM-labeled substrate RNA (5′-FAM-GGCCCUCCAAAAA-3′). Final composition of the reactions mixture was as follows: 40 mM HEPES (pH 8.0), 80 mM KCl, appropriate concentration of $MgCl_2$, 1 μM L21 form RNA, 1 μM the ligated peptide and 0.5 μM FAM-labeled substrate RNA. Aliquots of 10 μl were removed from the reaction mixtures at specified times and quenched by adding an equal volume of 90 mM EDTA in 82% formamide. Substrates and products were separated by electrophoresis on 20% polyacrylamide gels containing 8 M urea. The ratios of products to substrates were quantitated using a Bio-Rad PharosFX Molecular Imager. All experiments were repeated at least twice. Their mean values are shown in figures wherein error bars indicate their minimal and maximal values.

RESULTS

Effects of RNA on chemical ligation of two arginine-rich peptides

Some ribozymes and other functional RNAs found in extant biosystems have been proposed to be molecular fossils of the hypothetical RNA world (1,32,33). Therefore, we first investigated the effects of RNA molecules on the chemical ligation between two model peptides (Figure S1). We designed and employed two positively charged peptides derived from phage λ N-protein and HIV Rev-protein (Figure 1A). The minimal RNA-binding regions of the two proteins (positions 1–19 of N-protein and positions 34–50 of Rev-protein) have several arginine residues and specifically recognize their target RNA motifs. The 3D structures of λN_{1-19} and Rev_{34-50} fragments have been elucidated by nuclear magnetic resonance (NMR) as complexes with their target RNAs (34,35). To design a peptide ligation system, we added several amino acids to the carboxy terminal of λN_{1-19} fragment and the amino terminal of the Rev_{34-50} fragment to enhance the flexibility of the two termini. For the chemical reaction between the two peptides, we also introduced a reactive thioester onto the carboxy terminal of the N-peptide and a thiol moiety to the amino terminal of the Rev-peptide. The two termini can perform 'native chemical ligation', which produces a native peptide bond (Figure S1) (36).

Before examining the template effects of RNA molecules, we tested the basal reactivity of the chemical peptide ligation without RNA (Figure 1B and C). The reaction was monitored by reverse-phase HPLC and a new chromatography peak (peak 3 in Figure 1B) was assigned to the ligated peptide by Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry. However, the product yield of the ligation reaction was low (5.5%) even after 7 h incubation, presumably because the concentration of each peptide (7.5 μM) was much lower than the typical concentration for ligations in peptide chemistry (millimolar range) (36).

We then investigated the effects of RNA molecules using four noncoding RNAs; *Human* let-7a microRNA (37), the *Human* tyrosine-tRNA (31), *E. coli* RNaseP RNA (38) and a shortened form of the group I intron from *Tetrahymena thermophila* (abbreviated as L21-WT) (39). When the ligation reactions were carried out in the presence of the respective RNA at 7.5 μM, the amount of the ligated product was markedly improved (Figure 1B). Judging from the product yield after 7 h reaction (Figure 1C), L21-WT RNA was most effective, whereas the effects of miRNA and tRNA were modest. These results demonstrated primitive ability of self-folding RNAs to facilitate the chemical ligation of two arginine-rich peptides. As these four RNAs have no specific binding sites for the N- and Rev-peptides, the ligation should be enhanced through nonspecific association between arginine-rich peptides and RNAs, which would be supported by electrostatic interaction between positively charged arginine side chains in peptides and negatively charged phosphates in the RNA backbone (40,41).

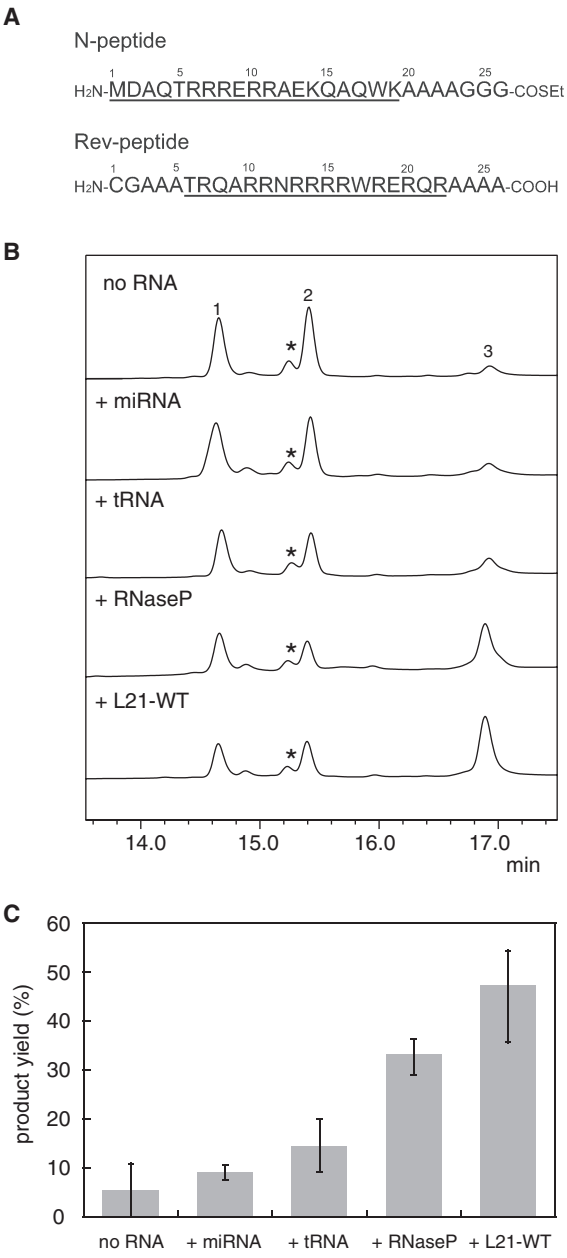

Figure 1. Effects of self-folding RNAs on chemical peptide ligation. (**A**) Amino-acid sequences of N-peptide, and Rev-peptide employed in this study. The sequences of λN_{1-19} and Rev_{34-50} are underlined in the N-peptide and Rev-peptide, respectively. Chemical ligation was designed to proceed between Gly26 of the N-peptide and Cys1 of Rev-peptide. (**B**) Effects of RNAs on chemical ligation between the N- and Rev-peptides. Reactions were carried out for 7 h and the products were analyzed by reverse-phase HPLC. Peaks 1, 2 and 3 correspond to the Rev-peptide, N-peptide and ligated peptide, respectively. The asterisk indicates N-peptide possessing C-terminal carboxylic acid produced by thioester hydrolysis. (**C**) Product yields of the ligated peptide in the absence or presence of RNAs. Reactions were carried out for 7 h.

Rational installation of peptide binding sites into a self-folding RNA

Based on the observation that L21-WT RNA can be used for primitive templated catalysis of the ligation of arginine-rich peptides, we then attempted to improve the ability of self-folding RNA. To engineer a variant of L21-WT RNA capable of capturing the substrate peptides specifically, we rationally introduced two peptide binding sites to L21-WT RNA (Figure 2A). Previously, the *Tetrahymena* group I intron ribozyme (which is the parental form of L21-WT) was rationally converted to a peptide-dependent allosteric ribozyme by inclusion of two peptide binding sites in two particular regions in the RNA (P5b and P6b, see Figure 2A) (28). On a model 3D structure of the resulting derivative (abbreviated as Tet-boxB/RRE), N- and Rev-peptides can bind Tet-boxB/RRE simultaneously to form a ternary RNA–peptide complex, in which the two reactive peptide termini could be placed in close proximity (Figure 2B). Based on this molecular modeling, we decided to use the L21 form of Tet-boxB/RRE ribozyme (designated L21-boxB/RRE) as an RNA component for RNA-dependent peptide ligation.

Before the peptide ligation experiment, we investigated the structural stability of L21-boxB/RRE in the buffer employed in the peptide ligation assay. We analyzed the stability of L21-boxB/RRE and L21-WT by assaying their intrinsic ribozyme activities. L21-WT acts as an RNA catalyst for site-specific cleavage of a short RNA oligomer and this activity sensitively reflects the stability of the correctly folded L21-WT RNA (39). As L21-WT has been shown to fold into a catalytically proficient structure in the presence of 2 mM Mg^{2+} (42,43), it can be used as a positive control to evaluate the stability of the L21-boxB/RRE. In the presence of 2 mM Mg^{2+}, endonuclease activities of the two RNAs were virtually identical (Figure 2C). On the other hand, L21-UUCG, a variant known to require a higher concentration of Mg^{2+} ions for folding, was markedly less active (Figure 2C) (44). These results indicated that in the presence of 2 mM Mg^{2+}, the L21-boxB/RRE can fold into the correct 3D structure and its stability was comparable to that of L21-WT.

Ligation reactions between N- and Rev- peptides (7.5 μM each) without RNA, with 7.5 μM L21-WT and 7.5 μM L21-boxB/RRE were examined (Figure 3). Ligation proceeded better in the presence of L21-boxB/RRE than the reactions without RNA and with L21-WT. For 7 h reaction, the yield of ligated peptide with L21-boxB/RRE was 64%, which was 12-fold or 1.4-fold higher than the yields without RNA (5.5%) or with L21-WT (47%), respectively (Figure 3C). More importantly, the template effect of L21-boxB/RRE was more remarkable in 1 h reaction, in which the product yield (35%) was 70- or 6.6-fold higher than those without RNA (0.5%) or with L21-WT (5.3%), respectively (Figure 3C).

The peptide ligation proceeded more efficiently in the presence of L21-boxB/RRE than in the presence of other RNAs although the effects of installation of the two peptide binding sites were modest.

Peptide ligation with mutant L21-boxB/RRE

To examine the importance of the ternary complex composed of L21-boxB/RRE RNA and the two substrate

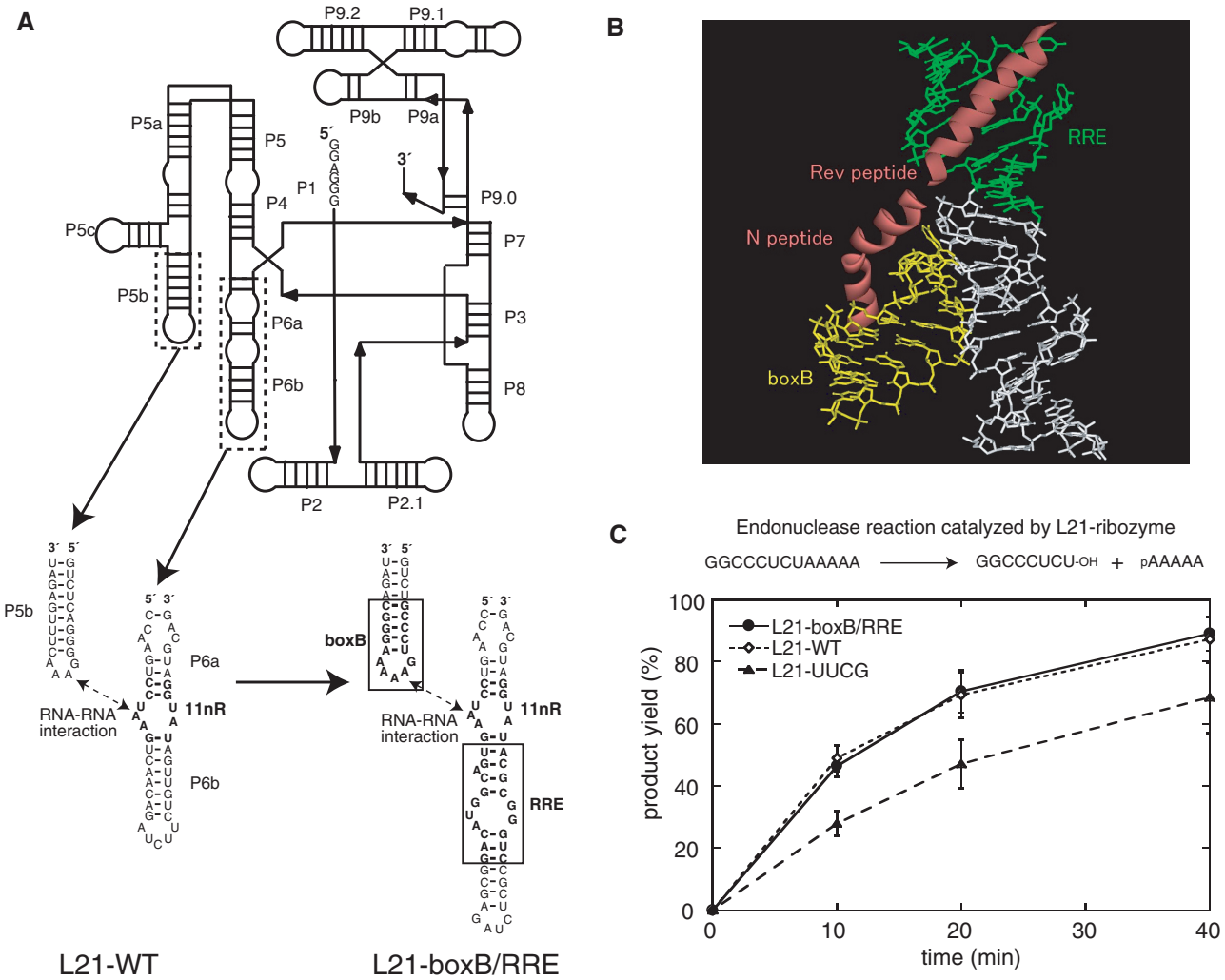

Figure 2. A variant of the *Tetrahymena* ribozyme RNA designed for chemical ligation of RNA-binding peptides. (**A**) The secondary structures of L21-WT and a designed RNA (L21-boxB/RRE). Sequences of the structural regions engineered to construct L21-boxB/RRE are indicated. The boxB and RRE motifs were included in P5b and P6b of L21-WT RNA, respectively. (**B**) 3D model for the ternary complex of L21-boxB/RRE, N-peptide, and Rev-peptide. (**C**) RNA cleavage reaction catalyzed by L21-WT or its derivative RNA.

peptides in promoting their ligation, we prepared variants of L21-boxB/RRE by disrupting one of the two peptide binding motifs (Figure 4A). L21-UUCG/RRE and L21-boxB/mutRRE were incapable of recognizing the N- and Rev-peptide, respectively, because of mutations in the corresponding peptide binding motifs (28). The product yields after 1 h reaction showed that the ligation reactions with L21-UUCG/RRE (6.0%) and L21-boxB/mutRRE (5.6%) were less efficient than that with the L21-boxB/RRE (35%) but comparable to that with L21-WT (5.3%) (Figure 4B).

Effects of RNAs on ligation of peptides missing arginine

To determine the importance of the RNA-binding abilities of the peptide component, substitutions were introduced into the peptide side chains to eliminate their RNA-binding abilities. In the mutant N-peptide and Rev-peptide, several important arginine residues were replaced with alanines (Figure 5A) (27).

In the presence of L21-boxB/RRE, ligation between mutant N-peptide and Rev-peptide (4.6%) or between N-peptide and mutant Rev-peptide (15%, Figure 5B) was much less efficient than the parental combination (N- and Rev-peptides: 64%) (Figure 5C). Consistent with these results, reaction between the mutant N- and Rev-peptides was also inefficient (7.4%) even in the presence of L21-boxB/RRE. The product yields of these reactions (4.6–15%) were similar to the ligation reaction without RNA component (5.5%), and lower than that in the presence of L21-WT (47%) (Figure 5C). This observation indicated that substitution of positively charged arginines with uncharged alanines in one peptide canceled the template effects of RNA, presumably because the mutant peptides abolished electrostatic affinity to RNA components.

L21-boxB/RRE suppressed ligation of peptides bearing reactive groups in opposite termini

In the ternary complex between L21-boxB/RRE and two peptides, relative orientation and distance of the reactive

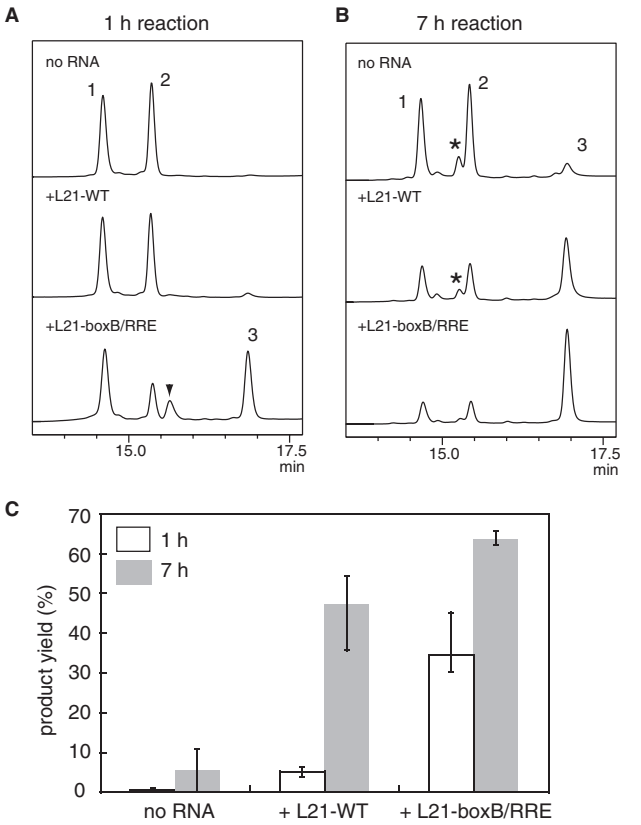

Figure 3. Effects of the designed RNA on chemical peptide ligation. Chemical ligation between N-peptide and Rev-peptide was carried out for 1 h (**A**) or 7 h (**B**). The products were analyzed by reverse-phase HPLC. Peaks 1, 2 and 3 correspond to the Rev-peptide, N-peptide and ligated peptide, respectively. The asterisk indicates N-peptide possessing C-terminal carboxylic acid produced by thioester hydrolysis. Filled arrowheads indicate unidentified product that was not observed in 7 h reaction. (**C**) Product yields of the chemical ligations.

groups in two peptides must be a factor contributing for modest acceleration of the reaction. To see contribution of this factor, we designed variant N- and Rev-peptides that can be specifically recognized by the L21-boxB/RRE RNA (Figure 6A). However, the two reactive groups were attached at the opposite termini of the original peptides (Figure 6B). The variant N- and Rev-peptides (termed cysN- and thioRev-peptides, respectively) have cysteine and thioester at their amino- and carboxy-termini, respectively (Figure 6C). Therefore, cysN- and thioRev-peptides should interact with RNAs as strongly as the original N- and Rev- peptides because the amino-acid sequences contributing directly to RNA recognition were completely preserved.

We first evaluated the nonspecific association between the variant peptides and the self-folding RNA using L21-WT. In the presence of 7.5 μM L21-WT, ligation reaction between cysN- and thioRev-peptides proceeded as efficiently as that between the original N- and Rev-peptides (Figure 6D).

We then examined the effects of L21-boxB/RRE, which was expected to modestly impair the ligation because the variant peptides would be fixed in unfavorable orientations. After 1 h ligation reaction in the presence of L21-boxB/RRE, the yield of ligation between cysN- and thioRev-peptides (3.2%) was 11-fold less than that between N- and Rev-peptides (35%) (Figure 6E). After 17 h ligation reaction, the yield of ligated product in the reaction in the presence of 7.5 μM L21-boxB/RRE (24%) was 3.3-fold less than the yield of the reaction of the original N- and Rev-peptides with L21-boxB/RRE (79%) (Figure 6E).

The ligated peptide can enhance ribozyme activity of L21-boxB/RRE

Tet-boxB/RRE RNA derived from the full-length *Tetrahymena* group I intron ribozyme with 5′ and 3′

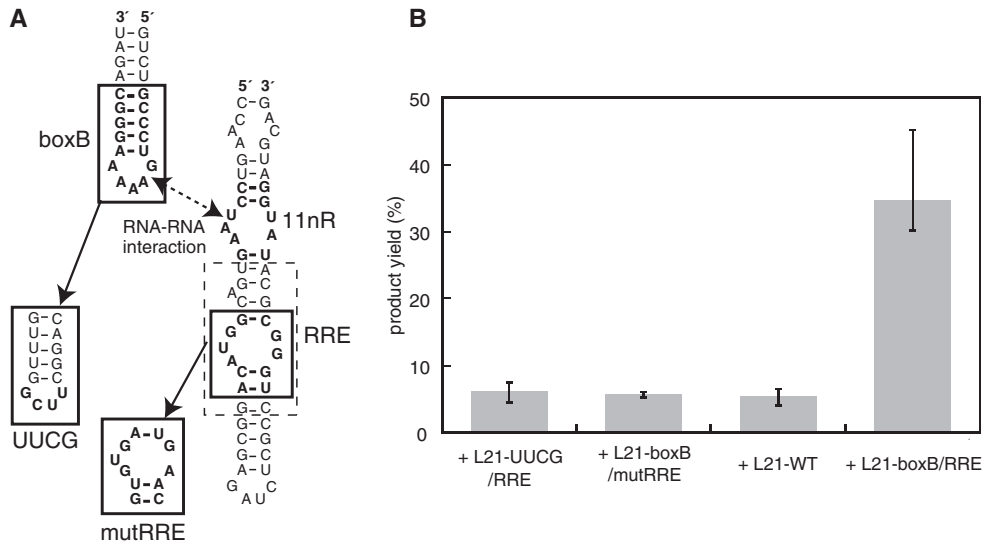

Figure 4. Peptide ligation with mutants of the designed RNA. (**A**) Mutations introduced to abolish specific RNA-binding abilities of L21-boxB/RRE. L21-UUCG/RRE and L21-boxB/mutRRE possess a UUCG tetraloop and mutated RRE motif in place of the GAAAA pentaloop and RRE, respectively, which are crucial for specific recognition of their cognate peptides. (**B**) Product yields of the peptide ligations in the presence of mutant RNAs. Reactions were carried out for 1 h.

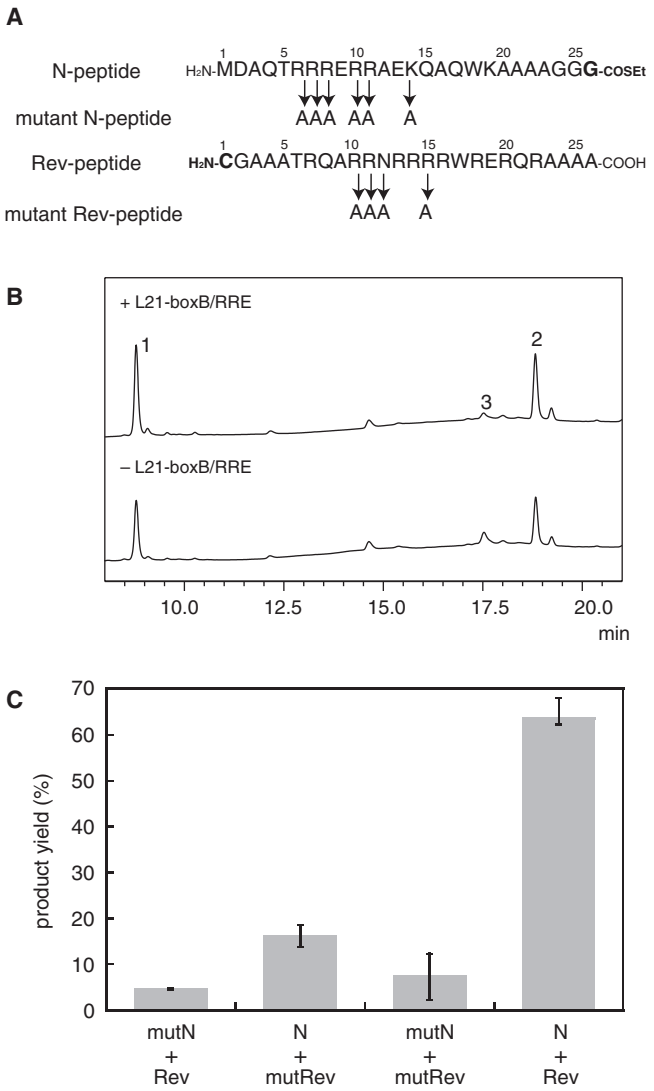

Figure 5. Effects of the designed RNA on the ligation of mutant peptides lacking RNA-binding ability. (**A**) Amino acid sequences of parental and mutant peptides. In mutant N- and mutant Rev-peptides, six and four positively charged amino acids were substituted with uncharged alanines, respectively. (**B**) Chemical ligations between mutant N-peptide and original Rev-peptide with (top) or without (bottom) L21-boxB/RRE. Ligation reactions were carried out for 7 h. The products were analyzed by reverse-phase HPLC. Peaks 1, 2 and 3 correspond to the Rev-peptide, mutant N-peptide and ligated peptide, respectively. (**C**) Product yields of the chemical ligation with L21-boxB/RRE. Reactions were carried out for 7 h.

exons performs *in vitro* splicing reaction triggered by guanosine cofactor (28). Under conditions where the structure of the Tet-boxB/RRE was marginally stable, the population of RNA molecules folded into a splicing-proficient structure was increased by addition of artificial peptide activators (28). The peptide activators have two RNA-binding motifs connected by linker sequences. The two RNA-binding motifs simultaneously bind to the target sites in P5b and P6 regions in Tet-boxB/RRE RNA, enabling the peptide activator to clamp P5b and P6 regions and stabilize the hairpin structure of P4-P6 domain (28). The amino-acid sequence of one peptide

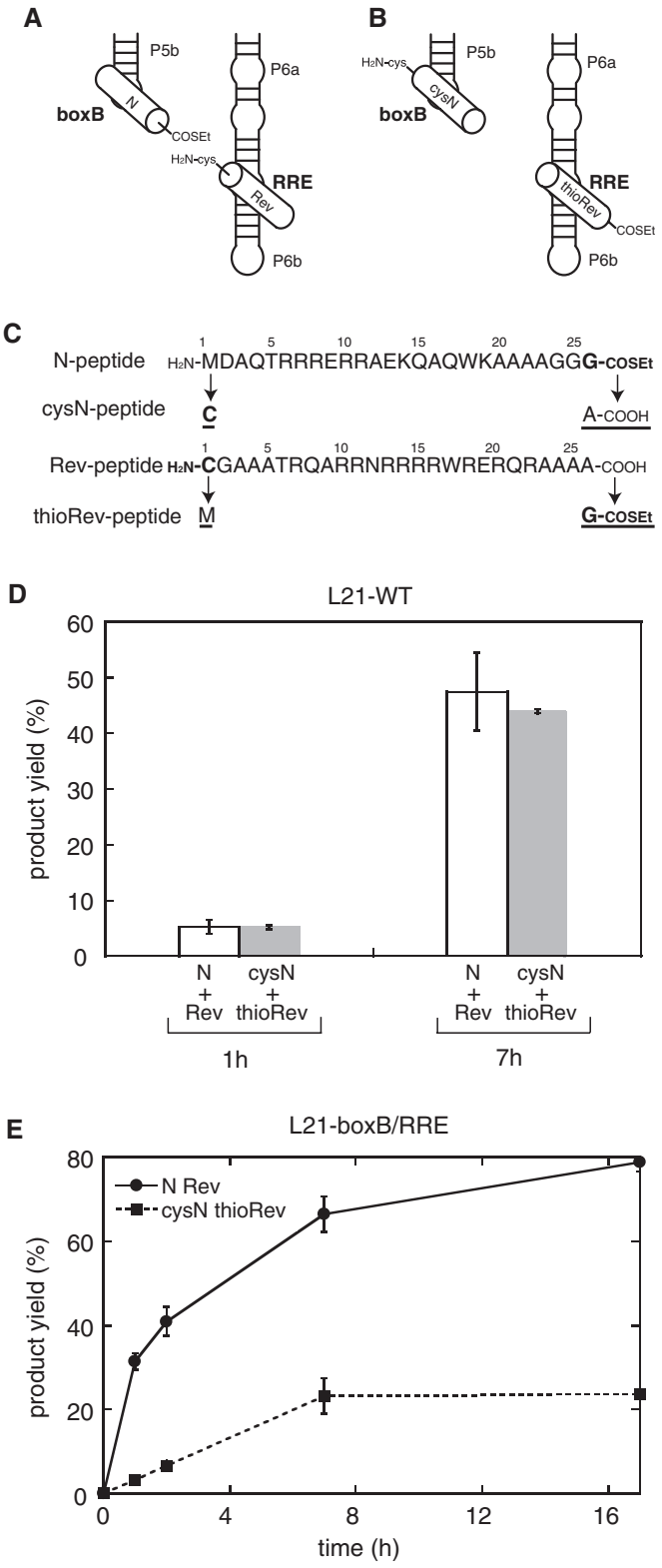

Figure 6. Effects of orientation of substrate peptides on the chemical ligation in the presence of the designed RNA. Schematic representation of ternary complex of L21-boxB/RRE with two parental peptides (**A**) or two variant peptides (**B**). (**C**) Amino-acid sequences of cysN-peptide and thioester Rev-peptide. (**D**) Product yields of the chemical ligation in the presence of L21-WT. (**E**) Time courses of the ligation reaction between N-peptide and Rev-peptide (solid line) and cysN-peptide and thioRev-peptide (broken line) in the presence of L21-boxB/RRE.

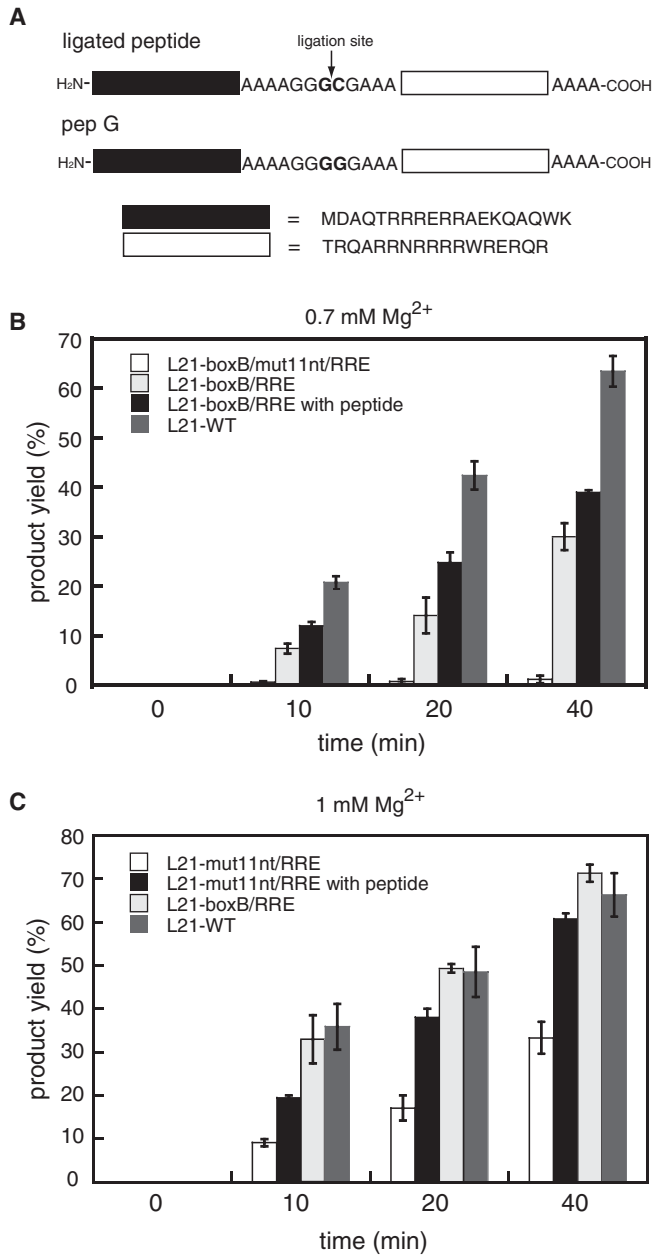

Figure 7. Effects of the ligated peptide on the endonuclease reaction by L21-boxB/RRE RNA. (**A**) Amino-acid sequences of the pepG peptide and ligated peptide. (**B**) The endonuclease reactions of L21-WT and its derivatives in the presence of 0.7 mM $MgCl_2$. (**C**) Endonuclease reactions of L21-WT and its derivatives in the presence of 1 mM $MgCl_2$.

activator (pep-G) was almost identical to the peptide product of the ligation reaction in this study (Figure 7A). This prompted us to examine whether the ligated peptide can increase the population of correctly folded L21-boxB/RRE although the folding process and structural stability may be different between the self-splicing form RNA and L21 form RNA (45,46).

In the presence of 2 mM Mg^{2+}, L21-boxB/RRE was as active as L21-WT in the endonuclease activity assay, indicating that the correct structure of L21-boxB/RRE was stably established. Therefore, we reduced Mg^{2+} ion concentration. At Mg^{2+} concentrations of 1 mM or more, the endonuclease activity of L21-boxB/RRE was indistinguishable from that of L21-WT (Figure 7C). At 0.7 mM Mg^{2+}, however, L-21-boxB/RRE was less active than L21-WT, presumably because the GAAAA pentaloop in the P5b region of L-21-boxB/RRE interacts with 11ntR in the P6 region less strongly than the GAAA tetraloop in L21-WT (34,47). Consistent with this observation, two other variants in which P5b-P6 interaction is weaker (L21-boxB/mut11nt/RRE) or disrupted (L21-UUCG) showed no detectable activity in the presence of 0.7 mM Mg^{2+}.

To determine whether the ligated peptide improved the endonuclease activity of L21-boxB/RRE, we examined the effects of the ligated peptide on the reaction at 0.7 mM Mg^{2+} (Figure 7B). In the presence of the ligated peptide, the product yields with 10 and 20 min reaction were 1.6- and 1.8-fold better than those without peptide, respectively. Enhancement by the ligated peptide was similar to that observed in the self-splicing reaction of the Tet-boxB/RRE RNA with pep-G peptide (from 1.4- to 1.7-fold). L21-boxB/mut11nt/RRE, which was less active than L21-boxB/RRE due to weaker P5b–P6 interaction, was more clearly activated by the ligated peptide in the presence of 1 mM Mg^{2+}. These results indicated that the ligated peptide is capable of assisting correct folding of L21-boxB/RRE or its less stable variant under conditions where their structures are marginally stable.

DISCUSSION

In the present study, we investigated the abilities of RNAs as a component for templated catalysis of chemical peptide ligation. We have also developed a designed RNA on which two RNA-binding peptides can be captured and ligated (Figure 2). The resulting ligated peptide can in turn function as a peptide activator to enhance the endonuclease activity of the designed RNA (Figure 7).

Peptide thioester and early evolution of life

In native chemical ligation, the carboxy terminus of one peptide needs to be activated as a thioester. Native chemical ligation was originally developed as a method of peptide chemistry (40). However, similar chemistry is utilized in biological reactions. Peptide carboxy terminal thioesters are used as the activated intermediate in nonribosomal peptide synthesis and peptide ligation step in protein splicing (48,49).

In addition to modern biological reactions, peptide and amino-acid thioesters have been proposed to participate in the prebiotic world because they can be utilized for nonenzymatic synthesis of primitive polypeptides. To investigate the possibility that the thioesters were synthesized under prebiotic conditions, attempts to generate amino-acid thioester and peptide thioester under prebiotic conditions have been reported (50–54). Moreover, the possible roles of thioesters in the hypothetical RNA world were also examined by generating RNA enzymes for CoA-thioester synthesis and aminoacylation with CoA-thioesters through *in vitro* evolution (55,56). Therefore,

the RNA-templated peptide ligation system developed in this study may be useful as a new model system to exploit the possible link between the RNA world and thioester-based prebiotic peptide synthesis.

Nonspecific effects of RNA on peptide ligation

In the present study, self-folding RNAs were shown to modestly but distinctly accelerate chemical ligation between the arginine-rich peptides (Figure 1). This result indicated that RNA molecules are able to serve as primitive templates for RNA ligation through nonspecific association mediated by electrostatic interaction between positively charged guanidyl groups and the negatively charged phosphate moiety. Binding specificities between RNA and polypeptide in RNP complexes are often determined by a limited number of interactions between particular arginines and base-moieties of nucleotides (34,35,57). In such cases, other arginines form electrostatic interactions with phosphate backbone of RNA to improve the stability of RNP complexes (40). In several cases, however, nonspecific interactions are strong enough to exhibit biologically relevant effects *in vitro* and *in vivo* (58,59).

Considering the properties of the arginine-phosphate interaction, it is conceivable that self-folding RNAs possess primitive ability to accelerate the chemical ligation of arginine-rich peptides, in which RNAs would act as templates that increase the concentrations of arginine-rich peptides through nonspecific association.

Templated catalysis of peptide ligation by a designed RNA

Specific RNA–protein interactions play a variety of biological roles in living cells (1). In some RNPs, many proteins bind to one (or a few) RNA molecule(s) to form multimolecular complexes, where RNA component can be regarded as a scaffold that specifically arranges protein components in defined distances and orientations (1,4,5). The designed RNA (L21-boxB/RRE) employed in this study can specifically capture the two arginine-rich peptides to form a defined ternary complex (Figure 2). Through comparison of this specific RNP with those of nonspecific RNA–peptide association, the specific complex was shown to promote the peptide ligation more efficiently than the nonspecific association although effects of the peptide binding sites were relatively modest. While the designed RNA seems to afford entropic advantage modestly to the substrate peptides, the RNA had no catalytic machinery capable of accelerating the chemical step (thioester exchange or acyl transfer) in the ligation. Recent analyses of ribosomal protein synthesis and non-ribosomal peptide synthesis revealed that their catalytic sites play crucial roles in proper alignment of substrates (60,61). On the other hand, there is still debate regarding whether their catalytic sites directly accelerate the chemical step by providing general acid/base moieties or coordinating catalytic metal ions (60–62). Therefore, improved RNA molecules for templated catalysis of peptide ligation may be developed based on our simple RNP if the 3D structures of RNA components can be elaborated to enable two reactive termini of peptides to be fixed highly precisely.

Toward interdependent system of RNA and polypeptide

In addition to peptide ligation in the presence of the designed RNA, this study also demonstrated that the ligated peptide has the ability to enhance the ribozyme activity of the designed RNA (Figure 7). In this RNP model system, RNA and peptide components play dual (active and passive) roles in regulating or transforming each other's functions or structures. The RNA component actively facilitates the ligation of two peptides, whereas its endonuclease activity was passively regulated by the ligated peptide. The peptide component serves passively as a substrate captured by the RNA, whereas the ligated peptide functions actively as a positive regulator of the endonuclease activity by the RNA component.

In the RNP system of this study, the RNA component (L21-boxB/RRE) may not necessarily be suitable for an integrated RNP system because of its nuclease activity. It can be readily imagined that RNA ligation reactions would be more important in the RNA and RNP world (25,63). However, L21-boxB/RRE would have the potential to evolve to a ligase ribozyme because its parental *Tetrahymena* group I ribozyme has been converted to a ligase ribozyme and also used as a parental ribozyme in attempts to create polymerase and replicase ribozymes (64–66).

This study provided a primitive but potentially important RNP complex, from which more complex RNP systems may be developed, that may mimic the ancient system that emerged during the molecular evolution of life.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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