Selection and Analysis of Genomic Sequence-Derived RNA Motifs Binding to C5 Protein

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Escherichia coli RNase P is a ribonucleoprotein composed of M1 RNA and C5 protein. Previously, analysis of RNA aptamers selected for C5 protein from a synthetic RNA library showed that C5 protein could bind various RNA molecules as an RNA binding protein. In this study, we searched cellular RNA motifs that could be recognized by C5 protein by a genomic SELEX approach. We found various C5 protein-binding RNA motifs derived from *E. coli* genomic sequences. Our results suggest that C5 protein interacts with various cellular RNA species in addition to M1 RNA.

Key Words : C5 protein, Genomic SELEX, Aptamer, RNA-protein interaction, M1 RNA

Introduction

RNase P has been initially characterized as an RNA processing enzyme that removes the 5' leader sequence of precursor tRNAs.[1] In addition to precursor tRNAs, non-tRNA substrates of RNase P, such as the 4.5 S RNA, tmRNA precursors, messenger RNAs are also found in *Escherichia coli*.[2-5] RNase P enzymes from diverse organisms have been shown to contain both essential RNA and protein components.[6,7] The *E. coli* holoenzyme consists of two subunits,[8] a large RNA subunit (M1 RNA, 377 nucleotides), and a small basic protein (C5 protein, 119 amino acids). *In vitro*, M1 RNA itself correctly cleaves precursor tRNAs.[9,10] Therefore, M1 RNA is a ribozyme or an RNA enzyme. However, both M1 RNA and C5 protein are essential for the RNase activity *in vivo*.[11,12] Furthermore, C5 protein stabilizes the catalytically active conformation of M1 RNA and modulates substrate specificity in RNase P reaction.[13-15]

Our previous SELEX (systematic evolution of ligands by exponential enrichment) experiment using a synthetic RNA pool showed that C5 protein can bind RNA aptamers displaying diverse RNA sequences with dissociation constants comparable to that of M1 RNA.[16] Furthermore, it was reported that various RNA molecules in addition to M1 RNA were co-isolated with C5 protein during purification,[17] although these molecules are yet to be identified. Therefore, C5 protein may participate in other metabolic reactions in the cell as an RNA-binding protein besides the protein component of RNase P.

To identify cellular RNA motifs that bind to C5 protein, we performed genomic SELEX,[18] in which the library contained sequences derived from the *E. coli* genome. We selected and characterized several RNA motifs that bound to C5 protein with dissociation constants comparable to that of M1 RNA. They displayed diverse sequences, but some contained sequences homologous to the M1 RNA sequence. This finding suggests that C5 protein interact with various

RNA molecules as an RNA binding protein in the cell.

Experimental Section

Library construction. The template DNA for the *E. coli* genomic RNA library was constructed by random priming with chromosomal DNA from *E. coli* K-12 strain W3110, as described previously.[19,20] The RNA library was generated by *in vitro* transcription using T7 RNA polymerase. Two libraries RNA G120 and G150 with genomic inserts of about 70 and 100 nucleotides, respectively, were prepared (Fig. 1). The analysis of the end distribution[19] using an *rnpB* specific primer suggested that the libraries contained overlapping sets of inserts for every segment of the *E. coli* genome (data not shown).

Preparation of MBP-C5 protein. C5 protein was expressed in *E. coli* as a fusion to maltose-binding protein (MBP-C5 protein), as described previously.[16] The total proteins of cells expressing MBP–C5 protein were applied to an amylose column and extensively washed with washing buffer (20 mM Tris–HCl, pH 7.4, 0.2 M NaCl, 1 mM EDTA, pH 8.0, 0.1 M 2-mercaptoethanol and 1 mM NaN₃). MBP–C5 protein immobilized on amylose resin was used for selection or analysis of C5 protein-binding RNA motifs.

120 nt (G120) and 150 ng (G150) RNA pools
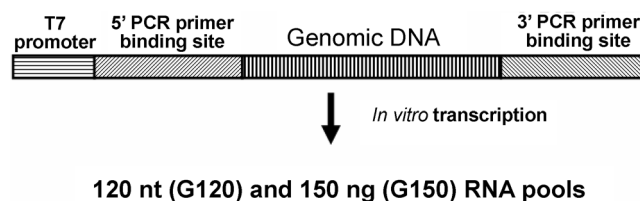
Figure 1. Schematic representation of template DNA for a library comprising genomic sequence-derived RNA motifs. Genomic DNA fragments were flanked by 5' and 3' primer binding sequences. A T7 promoter is linked to the 5' primer binding sequence at the 5' end. The 120-nt and 150-nt RNA pools were prepared by *in vitro* transcription.

Alternatively, MBP–C5 protein was eluted with the washing buffer containing 10 mM maltose, according to the manufacturer's instructions (New England Biolabs), and used for RNA analysis.

SELEX experiment. The selection and amplification was performed as previously described[16] with minor modifications. Prior to the main selection, the G120 or G150 RNA pool (1 nmol) in binding buffer containing 20 mM K-HEPES, pH 8.0, 400 mM ammonium acetate, 10 mM magnesium acetate, and 0.01% (v/v) Nonidet P-40 was passed through an MBP-immobilized amylose resin for negative selection. The pass-through fraction was incubated with MBP-C5 protein-immobilized amylose resin (10 nmol protein) in 100 μL binding buffer. From the 1st to 8th rounds of selection, a 1:1 molar ratio of RNA to MBP-C5 protein was used, and from the 9th to final rounds of selection, a 2:1 molar ratio of RNA to MBP-C5 protein was used. The RNA samples were allowed to equilibrate with the protein for 30 min during the 1st to 8th rounds of selection, and for 10 min in the subsequent cycles. Each binding reaction was filtered through a nitrocellulose filter (2.5 cm Millipore, 0.22 μm) pre-wetted with binding buffer in a Millipore filter binding apparatus. MBP-C5 protein-linked amylose resin collected on a nitrocellulose filter was washed three times with 3 mL binding buffer. RNA ligands bound to MBP-C5 protein were eluted with 400 μL binding buffer supplemented with 10 mM maltose. The eluted RNA fraction was phenol-extracted and ethanol-precipitated. The RNA was reverse-transcribed with AMV reverse transcriptase (Promega), amplified by PCR with *Taq* DNA polymerase (Promega) and used for additional rounds of selection. After the 13th and 11th rounds of selection from the G120 and G150 RNA pools, respectively, cDNA was amplified and cloned into pGEM®-T vector (Promega). Individual C5 protein-binding aptamer sequences were determined by DNA sequencing.

Preparation of aptamer RNA. Aptamer RNA was regenerated by *in vitro* transcription with T7 RNA polymerase using PCR-amplified cDNA, as described previously.[20] The PCR-amplified cDNA was prepared with primers a and b from plasmid DNA clones. Alternatively, only genomic sequences in selected aptamers were amplified by PCR from *E. coli* chromosomal DNA with oligonucleotide primers listed in Table 1. The PCR-amplified DNA was used as template for *in vitro* transcription with SP6 RNA polymerase. *In vitro* transcribed RNAs were purified by gel-elution.

RNA-protein binding assays. Gel-mobility shift assay was performed as described previously.[21] RNA (1 nM) internally labeled with [α-^{32}P]CTP was incubated in standard binding buffer containing 20 mM K–HEPES, pH 8.0, 400 mM NH₄OAc, 10 mM Mg(OAc)₂, 0.01% (v/v) Nonidet P-40 and 5% glycerol at 37 °C for 10 min in the presence of a 100-fold excess of unlabeled tRNA. Excess amounts of tRNA (5 μg) were used to prevent non-specific interactions of target RNA with protein. The binding assay was initiated by adding protein to the RNA-containing solution at a final volume of 20 μL. After 20 min incubation at 37 °C, 10 μL of

each reaction mixture was loaded onto a 5% non-denaturing polyacrylamide gel (acrylamide : bisacrylamide = 29 : 1). Electrophoresis was performed at a constant current of 13 mA at room temperature in 0.25x TBE (1x TBE comprises 90 mM Tris–borate containing 2 mM EDTA). Following electrophoresis, the gel was dried and analyzed with Bas-1500 phosphoimage analyzer (Fuji). For filter-binding assay, the protein-RNA mixtures were filtrated through a membrane (Protran, BA85 0.45 μm pore size, Schleicher & Schuell Bioscience). The RNA quantitation was performed using Bas-1500 (Fuji).

Boundary determination analysis. RNA was labeled with ^{32}P, at either the 5' end with T4 polynucleotide kinase and [γ-^{32}P]ATP or the 3' end with T4 RNA ligase and [^{32}P] pCp, as described.[22] Labeled RNA (1 nM) and 5 μg unlabeled yeast tRNA were suspended in alkaline hydrolysis buffer (50 mM NaHCO₃/Na₂CO₃, pH 9.5), and subjected to partial hydrolysis by incubation samples at 95 °C for 5 min. Hydrolyzed RNA fragments were ethanol precipitated and resuspended in 100 μL of binding buffer containing MBP–C5 protein-amylose beads (1 μg protein). RNA fragments binding to C5 protein were isolated, as described in the SELEX procedure described above, and recovered by phenol–chloroform extraction and ethanol precipitation. The RNA fragments were fractionated on an 8% sequencing gel containing 8 M urea.

Results

Isolation of RNA aptamers from *E. coli* genomic libraries. RNA aptamers were selected from RNA libraries carrying overlapping sets of inserts for every segment of the *E. coli* genome. We prepared two RNA libraries, G120 (the 120-bp-library) and G150 (the 150-bp-library) that contained central stretch of about 70 and 100 nucleotide-genome sequences, respectively. After 13 and 11 rounds of affinity selection from G120 and G150, respectively, the RNA species binding to MBP-C5 protein significantly increased (Fig. 2). The selected pools were cloned and 38 clones were sequenced (Table 2). We identified 14 clones with unique sequences. Some clones occurred with multiple frequencies. Clone 120-2 occurring with the highest frequency of 16 times displayed sequences that are derived from within the coding region of *dinD* gene. The clones carrying the intergenic region of *ygfA-rygC* were selected from both G120 and G150. Eleven of 14 clones were derived from within coding region of known genes or open reading frame of unknown function. Surprisingly, 5 clones had matches within a gene but in antisense orientation. Sequence analysis revealed that sequences of 11 clones were related to the M1 RNA sequences between positions 81 to 101 and 174 to 194 in M1 RNA. A footprinting analysis showed that these M1 RNA regions are protected by C5 protein.[23] Among M1 RNA-related sequences, the pentanucleotide sequence of GGUAA was highly conserved. In addition, some homologies, not related to M1 RNA, existed in the primary sequence among the clones and they were categorized into

Table 1. Sequences of oligonucleotide primers used in this study

| Primer	Sequences [a]	Description
Aran	AGGGAGGACGATGCGGNNNNNNNNN	random priming
Bran	TCCCGCTCGTCGTCTGNNNNNNNNN	
A	**GAAATTAATACGACTCACTATAGGG**AGGACGATGCGG	Amplification of library
B	TCCCGCTCGTCGTCTG	
120-2_A	**GGGTATTTAGGTGACACTATAGAA**GCAAGACAACTTGGGAAACTA	Amplification of 120-2g
120-2_B	GCGCGCGTTAATACAGGTATA	
120-4_A	**GGGTATTTAGGTGACACTATAGAA**TGGTTACACCGTCGAAAGTCT	Amplification of 120-4g
120-4_B	GTCAAAGAATCAACGATGTCA	
120-6_A	**GGGTATTTAGGTGACACTATAGAA**CTGTAAACCGTCGTCGGTTAC	Amplification of 120-6g
120-6_B	GCGGTAAAAGCCATTCTGGCG	
120-17_A	**GGGTATTTAGGTGACACTATAGAA**CGCTGGGGTAAATTTCGTCC	Amplification of 120-17g
120-17_B	**GGGTATTTAGGTGACACTATAGAA**AGGCCAGTACACAGACGATCC	
120-23_A	**GGGTATTTAGGTGACACTATAGAA**CGCGTGAATGTTACCCCATTT	Amplification of 120-23g
120-23_B	CCAAAGCGAACGACAAGGTT	
120-25_A	**GGGTATTTAGGTGACACTATAGAA**CTGGTAATACAGCTCGCCACC	Amplification of 120-25g
120-25_B	GCGGTAAATCGACCCTCGGTC	
150-1_A	**GGGTATTTAGGTGACACTATAGAA**TGGCACAATTGGGAGGCGCAC	Amplification of 150-1g
150-1_B	CGGTTGCGAGATAGATGTGGT	
150-2_A	**GGGTATTTAGGTGACACTATAGAA**GAAGAGTGGGATATCCCTCTT	Amplification of 150-2g
150-2_B	ATCAACGATGTCAATCAGGGC	
150-3_A	**GGGTATTTAGGTGACACTATAGAA**CTGCGAAATAAAAAACGCCGCCGT	Amplification of 150-3g
150-3_B	CAGCACCACCGCGTCGGGCA	
150-9_A	**GGGTATTTAGGTGACACTATAGAA**GGATATCTGGTCGATGGCACA	Amplification of 150-9g
150-9_B	GCGCAACGCGGCGGAAATT	
150-10_A	**GGGTATTTAGGTGACACTATAGAA**TGGCCCAGATTTCCATTGGGT	Amplification of 150-10g
150-10_B	CACGATGCCAATATCATTAGC	
150-14_A	**GGGTATTTAGGTGACACTATAGAA**GAAGAGTGGGATATCCCTCTT	Amplification of 150-14g
150-14_B	ATCAACGATGTCAATCAGGGC	
150-19_A	**GGGTATTTAGGTGACACTATAGAA**GTCTTTAATGCCTGTTTTGCT	Amplification of 150-19g
150-19_B	CTGGAAGACGCTAAACGTTT	

[a]Primer sequences for T7 or SP6 RNA polymerase are shown in bold.

four groups (Fig. 3).

We determined dissociation constants of complexes between RNA aptamers and MBP-C5 protein using a gel mobility shift assay (Table 3). The dissociation constants (K_{DS}) varied from 5 to 10 nM. Someone may argue that the primer-annealing sequences of the library could form C5 protein-binding motifs along with genomic sequences.[24] Therefore, we tested whether RNA motifs comprising only genomic sequences bound to C5 protein. For this purpose, we generated RNA molecules by initiating and terminating transcription at the corresponding genomic sequences with SP6 RNA polymerase, and rename them by adding 'g' after each original clone's name. The binding affinity of these RNA molecules to MBP-C5 protein was tested using filter binding assays (Fig. 4). All the tested RNA molecules showed the binding affinity for MBP-C5 protein, whereas

precursor tRNA[Phe] did not. The binding ability of RNA aptamers carrying no primer binding sequences was further verified by gel retardation analysis. The dissociation constants of aptamers 150-1g and 150-2g, which carried only genomic sequences of aptamers 150-1 and 150-2, respectively, were 3.0 nM and 6.0 nM (Fig. 5 and Table 3). These values are comparable to that of W2, a representative RNA aptamer to C5 protein, which was selected from a chemically synthesized RNA pool.[16] These results suggest that the C5 protein-binding ability of the RNA aptamers selected from the genomic libraries is originated from their genomic sequences rather than combined sequences of genomic and primer binding sequences.

Determination of minimal binding domains. Aptamers 150-1g and 150-2g were chosen as representative aptamers for further analysis of binding domains for C5 protein. The
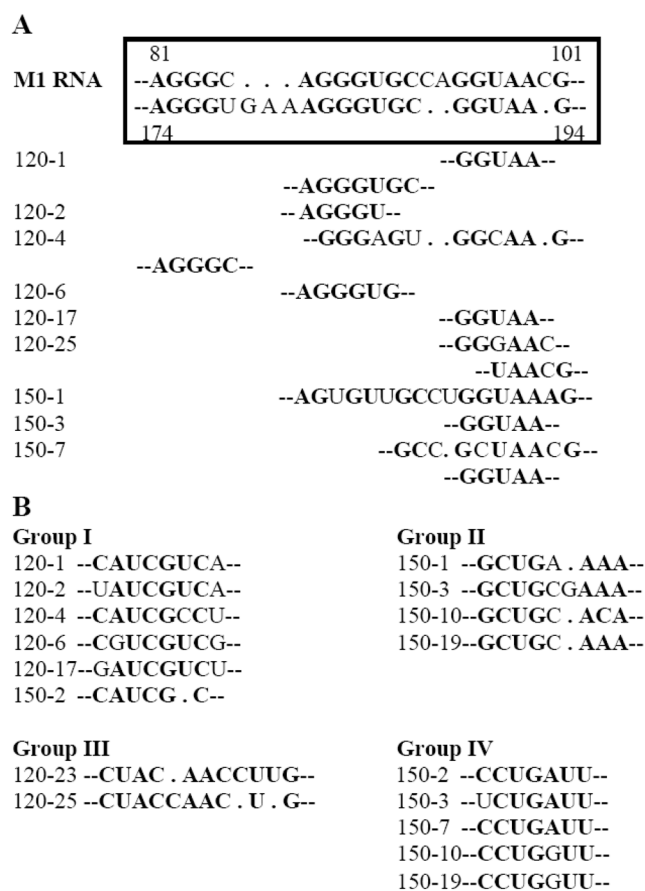
A

B
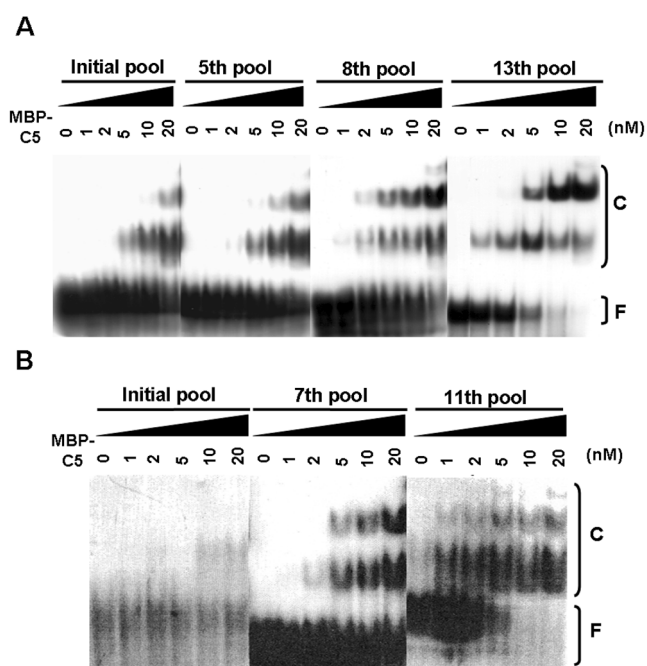
Figure 2. Selection of RNA aptamers binding to MBP-C5 protein by genomic SELEX. Selected RNA pools during SELEX were internally labeled with ^{32}P and tested for binding ability to MBP-C5 protein. The RNA pools of 5 nM for libraries G120 (A) and G150 (B) were incubated with MBP-C5 protein in binding buffer at 37 °C and electrophoresed on 5% non-denaturing polyacrylamide gel. The amounts of protein were indicated above each lane. C, complex. F, free RNA.

sequence of aptamer 150-1g resides within the DNA topoisomerase I coding region of *topA* mRNA, while that of aptamer 150-2g spans *ygfA* and *rygC*, carrying *ygfA-rygC* intergenic region. Therefore, both aptamers could be used as model RNA molecules for future works to determine various cellular functions of C5 protein as a RNA-binding protein. To determine the minimal binding domain of 150-1g and 150-2g, we performed a boundary determination analysis. Partially hydrolyzed RNAs were incubated with MBP–C5 protein immobilized on amylose resin, and RNA fragments binding to the protein were analyzed. The RNA regions spanning 74 nt from 51G to 124G of aptamer 150-1g and 43 nt from A52 to C94 of 150-2g were required for binding to C5 protein (Fig. 6). The consensus sequences to M1 RNA as well as some homologies not related to M1 RNA are located within these minimal binding domains.

Discussion

In this study, we demonstrate that C5 protein interacts with various RNA motifs derived from *E. coli* genomic sequences. Our results support the hypothesis that C5 protein is an interacting partner protein for some cellular RNA molecules, which was previously proposed through a SELEX experiment with an RNA library composed of random sequences.[16] Therefore, C5 protein can function not only as a protein component of RNase P but also as a regulator of cellular

A

M1 RNA	81 101	
	--AGGGC . . . AGGGUGCCAGGUAACG--	
	--AGGGU G A A AGGGUGC . . GGUAA . G--	
	174 194	

120-1		--GGUAA--
		--AGGGUGC--
120-2		-- AGGGU--
120-4		--GGGAGU . . GGCAA . G--
	--AGGGC--	
120-6		--AGGGUG--
120-17		--GGUAA--
120-25		--GGGAAC--
		--UAACG--
150-1		--AGUGUUGCCUGGUAAAG--
150-3		--GGUAA--
150-7		--GCC . GCUAACG--
		--GGUAA--

B

Group I	Group II
120-1 --CAUCGUCA--	150-1 --GCUGA . AAA--
120-2 --UAUCGUCA--	150-3 --GCUGCGAAA--
120-4 --CAUCGCCU--	150-10--GCUGC . ACA--
120-6 --CGUCGUCG--	150-19--GCUGC . AAA--
120-17--GAUCGUCU--	
150-2 --CAUCG . C--	

Group III	Group IV
120-23 --CUAC . AACCUUG--	150-2 --CCUGAUU--
120-25 --CUACCAAC . U . G--	150-3 --UCUGAUU--
	150-7 --CCUGAUU--
	150-10--CCUGGUU--
	150-19--CCUGGUU--

Figure 3. Groups of genomic aptamers showing similarity with M1 RNA motifs binding to C5 protein (A) or among themselves (B). The predicted homologous sequences are shown in bold.
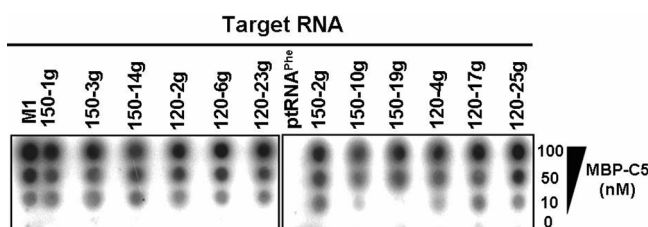
Figure 4. Filter binding assay for genomic sequence-derived RNA motifs. RNA molecules (1 nM) comprising only genomic sequences were internally labeled with ^{32}P and were incubated with MBP-C5 protein in binding buffer at 37 °C. The protein-RNA mixtures were filtrated through a nitrocellulose membrane. M1 RNA (M1) and precursor tRNA[Phe] (ptRNA[Phe]) were used as positive and negative controls, respectively. The amounts of RNA bound to the membrane were analyzed with Bas-1500 (Fuji).

metabolism by binding to various non-M1 RNA targets *in vivo*.

C5 protein interacts with selected RNA motifs with about 20-fold lower affinity than M1 RNA, This lower affinity is reasonable considering that enough RNase P holoenzyme could not be formed in the cell if non-M1 RNA motifs with similar or higher affinity are highly expressed. Since RNase P holoenzyme is essential *in vivo*, this should not happen.

Some of the selected aptamer sequences are similar to C5

Table 2. Primary sequences of selected RNA

RNA	Frequency	Sequences	Comments[a]
120-1	2	CUGGUAAUACAGCUCGCCACCGGUGGGCGUUUCAAUCAUCGUCAGCAACCG ACCGAGGGUGCAUUUACCGCUGC	(−) Middle of *dppF*
120-2	16	GCAAGGCAACUUGGGAAACUACAGGGUUAUUCAGAGUAUCGUCACUUUAU ACCGUAUUAUCGCGCGC	(+) Middle of *dinD*
120-4	1	UGGUUGCACGUCGAAAGUCUGGGAGUGGCAAGGGCCGAUACUCCCGCAUCG CCUGAUUGACAUCGUUGAUUCUUUGAC	(+) Downstream of *ygfA*
120-6	1	UAAUGCUGUAAACCGUCGUCGGUUACGAUGAUCUGCACAUCAGGGUGUUG CGCGCGAAUGGCUUUUACCGCGUA	(−) Middle of *lpxK*
120-17	3	CGCUGGGGUAAAUUUCGUCCGUGGGUACUGUUUGGCGCACUGCCAUUCGGG AUCGUCUGUGUACUGGCCU	(+) Middle of *yicJ*
120-23	1	CGCGUGAAUGUUACCCCAUUUUAUGUAUGGACAUGUAGUGAAGACUACAA CCUUGCUCGUUCGCUUUGGG	(+) Upstream of *narP*
120-25	1	GUUGGGAACCUGCGACCAAUUGGUUAAAAGUCAACUGCUCUACCAACUGAG CUAACGACCCGGUGGUGUG	(−) Middle of *lysW*
150-1	2	UGGGAGGCGCACUAUGAAGUGUUGCCUGGUAAAGAGAGGGUCGUAUCUGA ACUGAAACAACUGGCUGAAAAGCGACCACAUCUAUCUCGCAACCG	(+) Middle of *topA*
150-2	3	GAAGAGUGGGAUAUCCCUCUUCUGCGGUGUUACACCGUCGAAAGUCUGGG AGUGGUAAGGCGAUACACCCGCAUCGCCCUGAUUGACAUCGUUGAU	(+) Downstream of *ygfA*
150-3	1	CUGCGAAAUAAAAAAAACGCCGCCGUGAACUGGGUAAUCUGAUUAAAGCUU UGCAGCGGCGCGGAGUCGCUGCCCGACGCGGUGGUGCUG	(+) Middle of *phoR*
150-6	2	CUGGAGGGAUAUCUGGUCGAUGGCACGCUCAAAACCGGUACUGCAAUCUGG CGACUAUCCGAUUGCCUGUGCCGUCGAUAUCUCAAUUUCCGCCGCGUUGC	(+) Middle of *yibF*
150-7	1	CGGGAGAAAACUACCGCUCUACUAGCAAUAUUCUGAGCGCCGCUAACGCCC UGAUUGAAAACAAUAACGGGCGUCUGGGUAAAAAACUGUGGACCC	(+) Middle of *uvrD*
150-10	1	UGGCCCAGAUUUCCAUUGGGUGUGUUCUACCCAACCUGGUUUUGGGUAGA UUUGCUCAAAUUCGCGCUGCGACGCUAAUGAUAUUGGCAUCGUG	(−) Middle of *glpK*
150-19	3	CAGGUCUUUAACGCCUGUUUUGCUAACAAAAUGCGGGCCUCAGCUGCUGCA CUUGGCUCUAUCUGCUGCAAACGUUUAGCGUCUUCCAGCGUG	(−) Middle of *ymfJ*

[a]The sense (+) or antisense (−) orientation of RNA sequences in a given gene is indicated.

Table 3. Dissociation constants of selected RNA aptamer

RNA	K_D (nM)
M1	0.4
W2	4.2
120-Random	250
120-1	7.4
120-2	10.2
120-17	9.8
150-Random	290
150-1	5.8
150-2	6.5
150-19	8.1
150-1g	3.0
150-2g	6.0

protein-binding region of M1 RNA. Especially, the pentanucleotide sequence of GGUAA appeared with high frequency of 5 out of 9. This sequence appears in the loop regions of both helices P8 and P13 in the M1 RNA secondary structure,[25] suggesting that GGUAA might be one of C5 protein binding domains. We also found other consensus sequences, which are not related to M1 RNA. If these sequences would play a role in binding to C5 protein, they could be involved in C5 protein binding with different binding modes. The results that C5 protein interacts with various motifs displaying diverse sequence is consistent with
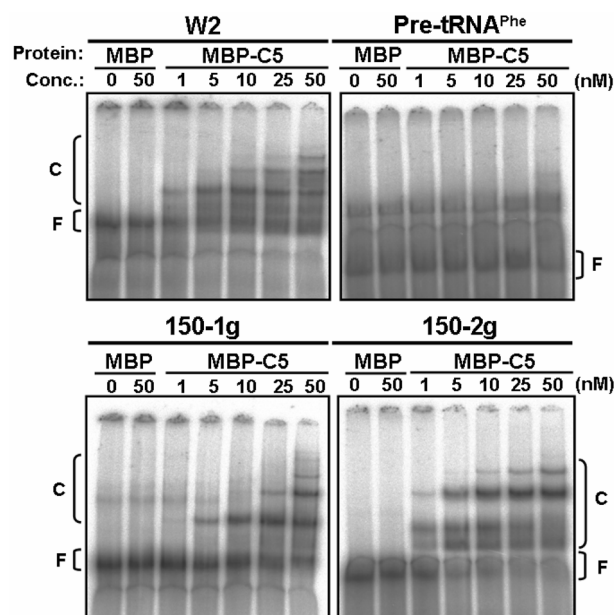
Figure 5. Complex formation of aptamers 150-1g and 150-2g with MBP-C5 protein. ^{32}P-labeled 150-1g and 150-2g (1 nM each) were incubated with increasing amounts of MBP-C5 protein at 37 °C for 10 min. The mixtures were electrophoresed on 5% non-denaturing polyacrylamide gels. Protein concentrations were indicted above each lane. F and C signify free RNA and complex. W2 RNA and precursor tRNA^{Phe} (pre-tRNA^{Phe}) of 1 nM each were used as positive and negative controls, respectively.
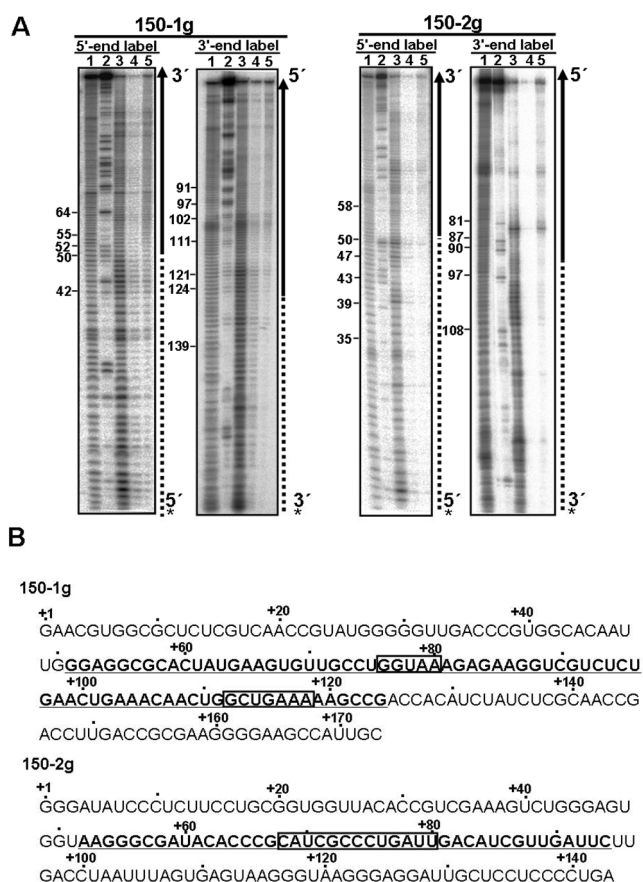
Figure 6. Minimal binding domain of RNA aptamers 150-1g and 150-2g. (A) Boundary determination analysis. Aptamers 150-1g and 150-2g were labeled with ^{32}P, either at the 5' or 3' end. Labeled RNAs were partially digested by alkaline hydrolysis and incubated with MBP–C5 protein immobilized on amylose resin. RNA fragments binding to MBP–C5 protein were analyzed on 8% polyacrylamide gels containing 8 M urea. The dotted line to the right of the boundary assay result of either 5' or 3' labeled RNA represents the essential domains for binding to C5 protein. Lane 1, partial alkaline hydrolytic products; lane 2, RNase T1 digests; lane 3, unbound fraction to amylose resin; lane 4, washed fraction after binding to amylose resin; lane 5, eluted fraction from amylose resin with 10 mM maltose. (B) RNA motifs required for C5 protein. The minimal binding domains are underlined and in bold on each aptamer sequence. The consensus sequences are boxed.

the previous results of RNA aptamers selected from a synthetic RNA pool containing random sequences.[16]

Although we showed that C5 protein can bind to *in vivo* RNA target sequences, biological relevance of its binding is not clear. The binding may facilitate recruiting of RNA substrates for RNase P. Recently, Li *et al.* revealed that a mutation in *rnpA* encoding C5 protein caused the increase of expression of some genes.[26] Therefore, it is possible that the increased expression may be due to failure of recruitment of target mRNAs by the mutated C5 protein. Alternately, binding of C5 protein to target RNAs may affect their *in vivo* stability. In either case, it should be noted that only free C5 protein could bind to non-M1 RNA targets because C5 protein interacts to non-M1 RNA targets less efficiently than M1 RNA. Therefore, C5 protein binding to RNA targets other than M1 RNA may depend on the cellular level of M1 RNA.

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