Sequence Motifs of rRNAs Interacting with Yeast tRNA^{Phe}

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The eukaryotic ribosome consists of two subunits, 60S large and 40S small. The 60S large subunit contains 5S rRNA, 5.8S rRNA and 28S rRNA, and the 40S small subunit has 18S rRNA. Presently, the rRNAs are thought to be directly involved in the ribosome function. There is extensive biochemical, genetic and phylogenetic evidence for the functional role of ribosomal RNAs. This includes the participation of rRNA in mRNA selection, tRNA binding (in A, P and E sites), ribosomal subunit association, proofreading, factor binding, antibiotic interaction, termination and the peptidyltransferase function.[1-9]

An *E. coli* 50S, large ribosomal subunit without 5S rRNA loses the binding ability of tRNA to the A-site.[10] So the interaction between tRNA and rRNA is thought to be important in the translation. Although it was suggested that a consensus sequence C42CGAAC47 in loop C of 5S rRNA which is conserved in all prokaryotes can interact with a sequence G53TΨCR57 conserved in tRNA,[11] there is no experimental evidence for that. However, an important piece of the information for the interaction between rRNA and tRNA in ribosome was obtained from the cleavage sites of 5S rRNA, 16S rRNA and 23S rRNA by hydroxyl radical (·OH) generated from Fe(II) which was attached to 5'-terminus of anticodon stem-loop (ASL) analog of tRNA.[12] According to their results, bases of the 1230, 1300 and 1330 of 16S rRNA, the 40 of 5S rRNA and the 2600 of 23S rRNA were cleaved by P site-bound ASLs. This result definitely shows that tRNA can interact with ribosomal RNAs inside the ribosome. But there is still no direct evidence for the interaction between tRNA and rRNAs. So it is very important to get the information on tRNA-rRNA interactions for the understanding of the function of ribosomal RNAs in translation.

We performed a SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method to screen the RNA motifs interacting with yeast tRNA^{Phe}. A DNA library which contains a region of randomized 48-mer oligonucleotides flanked by conserved sequence primers was transcribed into RNA pool using T7 RNA polymerase. Selection was performed with tRNA^{Phe}-immobilized affinity column which was prepared by coupling the 3'-terminal sugar of tRNA^{Phe} to the commercial Sepharose-adipic acid hydrazide resin. The affinity of selected RNA pools for tRNA^{Phe} increased as the number of the selection cycle increased. After 12 rounds of SELEX, the selected RNA aptamers were reverse-transcribed into DNA and cloned into the pGEM-T^{TM} vector for sequence analysis (Fig. 1). The aptamers were categorized into several groups according to the features of the consensus sequences. Since loop regions of RNA are generally accepted as the sites capable of interacting with other RNAs or proteins, we searched consensus sequences of the aptamers that were homologous to the sequence of loop regions in a secondary structure model of three rRNAs.[13] We found a consensus sequence homologous to C40GAUC44 sequence of 5S rRNA, G1114UAUGG119 sequence of 18S rRNA, and G2609UGUA2613 and A2613GAAU2617 sequnces of 28S rRNA (Fig. 2). Our result that there are RNA aptamers having CGAUC sequences which are complementary to GTΨCR sequences conserved in tRNA is identical to the Erdmann group's result that CCGAAC sequences of 5S rRNA can interact with GTΨCR sequences of tRNA.[11]

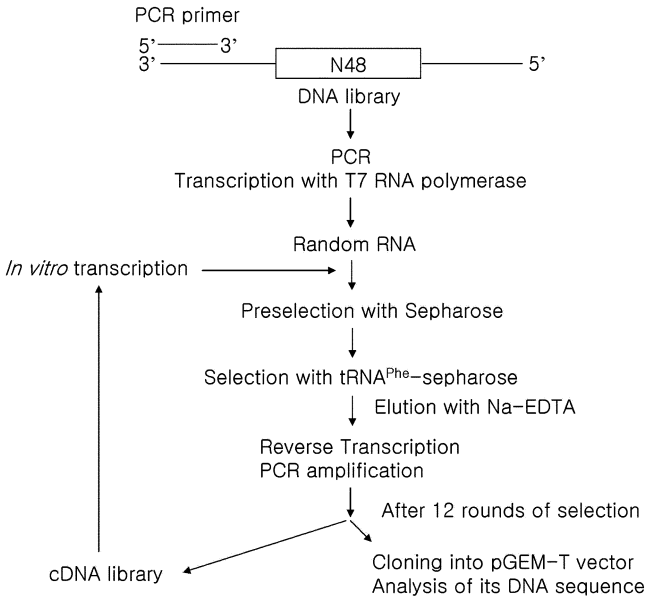

Figure 1. Outline of the experimental strategy for SELEX. The RNA pool was prepared by *in vitro* transcription of the amplified DNA library carrying a randomized 48-mer DNA region. RNA aptamers binding to tRNA^{Phe} were selected with tRNA^{Phe}-attached affinity column chromatography. After the 12th round of selection, cDNA of each RNA aptamer was cloned into the pGEM-T^{TM} vector and its sequence was analyzed.

In conclusion, we have here selected RNA aptamers

a) Aptamers identical to C40GAUC44 sequence of 5S rRNA
UCACGUCAAAGCUUACCCUGGAUAUUUAGUAGAGC**CGAUC**UCGCUAU
AUGUGAUGAAUCGUGACGCUCUGCUA**CGAUC**GUACGCAGUGUUGUAGU
GCUGUU**CGAUC**CGUAAU**CGAUC**AGUGUUUGCCUCUCUCGAUACUGAGU
UUGUCAGGUAUGGAUAAUCGCAGGGCCUUA**CGAUC**AUCAGUAUAGGGU
CGAUCUAUACGGAUAAGUCGCGUUUCGACAUCACCAUGAACUCUUGA
AUGACUAUGUCCCGUGGAGUGGGGACUGGAGAUUGAACC**CGAUC**GAU

b) Aptamers identical to G1114UAUGG1119 sequence of 18S rRNA
CGACAACGGACUUCUACUUAUCCGUUCGAGA**GUAUGG**AUUCGAGGGG
CCACUACGAAUUAAUUAGUACAUCGCGAAACUGCUAU**GUAUGG**AAGU
AACAGUAUACACCAAAG**GUAUGG**UUGACAUUCGUUCACGAACGAUGU
AAAAUUCCAGGUGUAUUGUGACCUCCCGAUUAAGGAA**GUAUGG**CUAG
UUGUCAG**GUAUGG**AUAAUCGCAGGGCCUUACGAUCAUCAGUAUAGGGU
AAGUCUAGAGAUAUGUUAAACUCUAAAGAAUAACGC**GUAUGG**UCGCUAC
UGUAGA**GUAUGG**AUUUAUCCAGAGUAGAGUCUAAGUGGUUAUGUAAGUA

c) Aptamers identical to A2613GAAU2617 sequence of 28S rRNA
AUGAUCGUGCUUGAUCACUGGGCGCUAAGCUUG**AGAAU**ACCACUCUU
UUCCAUGAGCACACGAU**AGAAU**UGACCUAAGUCUGGAAGAUGAAUGUA
GUAAGCGUCCCAAUCGACUUCCUGAAUUGCUCUG**AGAAU**CGCGUGUU
GCGAGAUGUUAUCG**AGAAU**ACGUUAUUGAUAAAUUCCUACUGUUCC
GAUAAAGUAUGUCUUCACGGAUGAUAACUCUC**AGAAU**UGACGUUGC
AAGUCUAGAGAUAUGUUAAACUCUAA**AGAAU**AACGCGUAUGGUCGCUAC
UAAAAGAUAA**AGAAU**AAACUGAAACUGUCCUGAAACUUGGGCUCUCUU

d) Aptamers identical to G2609UGUA2613 sequence of 28S rRNA
UAUAUUGGAAUUCUGCCGGAUGUAGAUUCG**GUGUA**GUAGUAUAUUUU
AUGAU**GUGUA**UAUAAAUUGUUCAUGAAACGCCGAAUGUCACAAUUCC
AUC**GUGUA**GAUGGCUUCCUCUAGGUAAUACGUUGAUGAAUUCGGUAA
AUAGAUGACUUAGUACAUUAAGGCAUCCAUUUAUGAUUAGA**GUGUA**U
GAUGAGUUGGGAAAUGCUUAGUUUUCAAAGUCUGACAUUGACUC**GUGUA**
CGUAUAGUCACGUUGCUGUUGUGAGCUCUA**GUGUA**AUCACCCUGAAU
UUGAUGGUGGCAUGGUUGUGUUGAACAUCCGGGAUUUAAUC**GUGUA**

Figure 2. Sequences of the randomized region in RNA aptamers. RNA molecules containing the consensus sequence homologous to those that appeared in the loop regions of three rRNAs in yeast are shown. The consensus sequences are indicated in bold letters and underlined.

which bind to $tRNA^{Phe}$ using $tRNA^{Phe}$-immobilized affinity column. According to our results, yeast $tRNA^{Phe}$ can interact with 5S rRNA, 18S rRNA and 28S rRNA in ribosome. Footprinting studies are in progress to evaluate which sequence of tRNA is important for the interaction between tRNA and rRNAs, and will be discussed later.

Experimental Section

RNA library. The library, 5'-AAGCTTGCATGCGGATCC-(N)48-GAGCTCGAATTCACCTATAGTGAGTCGTATTA-3', contained 48randomized nucleotides flanked by cDNA primer (5'-AAGCTTGCATGCGGATCC-3') and T7 primer (5'-TAATACGACTCACTATAGGTGAATTCGAGCTC-3') at the 5' and 3' regions, respectively. This library was amplified through five cycles of polymerase chain reaction (PCR). One microgram of this population was transcribed with T7 RNA polymerase (RiboMAX™, Promega) in a 20 uL reaction volume according to the manufacturer's directions. Transcripts were purified by 6% denaturing polyacrylamide gel electrophoresis. The purified RNA composed of 81 nucleotides was dissolved in the binding buffer (30 mM Tris-acetate, pH 7.5, 50 mM Mg-acetate, 100 mM K-acetate, 100 mM NH_4-acetate) and brought to 55 °C for 15 min and slowly cooled to room temperature.

SELEX protocol. The affinity column for *in vitro* selection was prepared as described.[14] The gel-purified yeast $tRNA^{Phe}$ was oxidized at the 3'-terminal sugar with $NaIO_4$ and then coupled to Sepharose-adipic acid hydrazide resin (Amersham Pharmacia Biotech). In order to minimize the enrichment of undesirable RNA species binding to the Sepharose resin itself, we pre-selected the RNA pool on the uncoupled Sepharose-adipic acid hydrazide resin. After being passed through the pre-column of Sepharose-adipic acid hydrazide resin, we then loaded the RNA pool onto the $tRNA^{Phe}$-attached affinity column. We washed the column with the binding buffer and then eluted the bound RNA with three column volumes of the elution buffer (25 mM Na-EDTA, pH 8.0). The selected RNAs were recovered by ethanol precipitation and reverse-transcribed with an AMV reverse transcriptase using a cDNA primer. Then, the cDNA was amplified by PCR with the cDNA primer and a T7 primer, and used for the next round of selection. After the 12th round of selection, the amplified cDNAs were cloned into the pGEM-T vector (Promega). The insertions of 98-mer DNAs in plasmid were identified with restriction endonuclease Pvu II (5'-CAG↓CTG-3') and their sequences were determined.

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