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(54) Titre : ARN CATALYTIQUES A ACTIVITE D'AMINOACYLATION
(54) Title: CATALYTIC RNAS WITH AMINOACYLATION ACTIVITY

(57) **Abrégé/Abstract:**

The present invention provides catalytic RNA molecules having cis or trans aminoacylation activity. The catalytic RNA molecules having cis aminoacylation activity comprise a catalytic domain and an aminoacylation domain. The catalytic RNA molecules having trans aminoacylation activity only have the catalytic domain. A method is provided for constructing and screening of these molecules. These molecules are suitable for aminoacylating tRNA-like molecules with specific amino acids.



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(54) Title: CATALYTIC RNAs WITH AMINOACYLATION ACTIVITY

(57) Abstract: The present invention provides catalytic RNA molecules having cis or trans aminoacylation activity. The catalytic RNA molecules having cis aminoacylation activity comprise a catalytic domain and an aminoacylation domain. The catalytic RNA molecules having trans aminoacylation activity only have the catalytic domain. A method is provided for constructing and screening of these molecules. These molecules are suitable for aminoacylating tRNA-like molecules with specific amino acids.



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CATALYTIC RNAs WITH AMINOACYLATION ACTIVITY

This application claims the priority of US provisional application number 60/167,331 filed on November 24, 1999 and US provisional application number
5 60/214,382 filed on June 28, 2000, the disclosure of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to the
10 field of catalytic RNA molecules, and more particularly to catalytic RNA molecules having the ability to aminoacylate tRNA-like molecules in *cis* or in *trans*.

BACKGROUND OF THE INVENTION

15 Proteins containing non-natural amino acids hold great promise for biomedical and therapeutic purposes. Such amino acids may be particularly useful in the structural and functional probing of proteins, construction of peptide libraries for combinatorial
20 chemistry, and in proteomics. However, the synthesis of such proteins has not heretofore been easy. In the translation system that is known to occur currently in nature, genetic coding is carried out by aminoacyl-tRNA synthetases (ARSs). They exist in 20 different forms,
25 each of which specifically catalyzes the esterification of a single amino acid to its cognate tRNA isoacceptor, thereby directly connecting the amino acid with its corresponding anticodon triplet. Because misacylation of noncognate amino acids to tRNAs causes misincorporation
30 of amino acids into cellular proteins which can be fatal to their intracellular activity, the fidelity of the aminoacylation reactions by the ARSs must be extremely high. To achieve this important task, the ARSs use very sophisticated mechanisms to selectively recognize the
35 cognate amino acids and tRNAs. The recognition determinants of tRNAs are diverse ranging from the anticodon loop to the acceptor-Ti~C stem and the

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phosphate-ribose backbone. Because of these complexities, engineering of ARSs with desired specificities toward nonnatural tRNAs and amino acids has not been achieved. As a result attention has turned
5 to the nucleic acids.

For many years, nucleic acids were considered to be only informational molecules. However, the pioneering work of Cech and coworkers (Cech, 1987, *Science*, 236:1532-1539; McCorkle et al., 1987, *Concepts Biochem.*
10 64:221-226) demonstrated the presence of naturally occurring RNAs that can act as catalysts (ribozymes). However, although these natural RNA catalysts have only been shown to act on ribonucleic acid substrates for cleavage and splicing, recent development of artificial
15 evolution of ribozymes has expanded the repertoire of catalysis to various chemical reactions. For example, RNAs have been reported to catalyze phosphodiester cleavage on DNA (Beaudry et al., 1992, *Science*, 257:635), hydrolysis of aminoacyl esters (Piccirilli et
20 al., 1992, *Science*, 256:1420-1424), self-cleavage (Pan et al., 1992, *Biochemistry*, 31:3887), ligation of an oligonucleotide with a 3'OH to the 5'triphosphate end of the catalyst (Bartel et al., 1993, *Science*, 261:1411-1418), biphenyl isomerase activity (Schultz et al.,
25 1994, *Science*, 264:1924-1927), and polynucleotide kinase activity (Lorsch et al., 1994, *Nature*, 371:31-36).

To identify novel catalysts, Brennen et al. (1992, *Proc. Natl. Acad. Sci., USA*, 89:5381-5383) constructed a heterogenous pool of macromolecules and used an in vitro
30 selection process to isolate molecules that catalyze the desired reaction. A variation of this approach has been used by Gold et al. (U.S. patent no. 5,475,096). This method, known as Systematic Evolution of Ligands by Exponential enrichment (SELEX), identifies nucleic acids
35 that have the ability to form specific, non-covalent interactions with a variety of target molecules. A related patent (U.S. patent no. 5,990,142) is based on

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the SELEX method, but can potentially identify modified and non-modified RNA molecules that can catalyze covalent bond formation with a target. Recently, a similar approach was used to identify catalytic RNA molecules having phosphodiesterase, amidase activity (U.S. patent no. 6,063,566 to Joyce).

Additionally, studies have identified RNA molecules that can catalyze aminoacyl-RNA bonds on their own (2')3'-termini. (Illangakekare et al., 1995 Science 267:643-647), or where an RNA molecule can transfer an amino acid from one RNA molecule to another (Lohse et al., 1996, Nature 381:442-444).

However, there has been no demonstration heretofore of catalytic tRNA-like molecules that can cause aminoacylation of RNA molecules which are physiologically significant in modern protein translation processes.

SUMMARY OF THE INVENTION

The present invention provides catalytic RNAs with *cis*-aminoacylation activity. The catalytic RNAs comprise a tRNA-like domain and a ribozyme domain. The ribozyme domain has the catalytic activity and also confers amino acid specificity for aminoacylation. Thus, these catalytic RNAs have the ability to selectively aminoacylate their own 3'-termini with specific amino acids (termed herein as *cis*-aminoacylating RNA molecules). These catalytic RNAs can be used to aminoacylate tRNA-like sequences that are not naturally aminoacylated.

The present invention also provides catalytic RNA molecules that can aminoacylate tRNA-like molecules in *trans* (termed herein as *trans*-aminoacylating RNA molecules). The *trans*-aminoacylating RNAs correspond to the ribozyme domain of the *cis*-aminoacylating RNAs. These catalytic RNA molecules can be used to

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aminoacylate tRNA-like molecules with desired natural or non-natural amino acid in *trans*.

The present invention also provides a method of constructing self-aminoacylating RNA molecules. This method comprises the steps of attaching a sequence to the 5' end of a tRNA-like molecule, said sequence comprising a ribozyme sequence. This method can be used to generate catalytic RNAs that can catalyze the aminoacylation of their own 3' ends. These catalytic molecules can be cleaved by RNase P to produce a different species of catalytic RNA molecules which have the ability to aminoacylate tRNA-like molecules in *trans*.

The present invention also provides a method for the identification of RNA sequences having aminoacylation activity. The method comprises the steps of providing a pool of RNA sequences having a tRNA-like domain and a ribozyme domain, contacting the RNA sequences of the pool with the desired natural or non-natural amino acid, partitioning the aminoacylated RNA molecules from the non aminoacylated molecules, amplifying and sequencing the aminoacylated RNA molecules.

The present invention also provides a method for aminoacylating the self-aminoacylating catalytic molecules provided herein. The method comprises the steps of providing RNA molecules having the catalytic activity of self aminoacylating, contacting the RNA molecules with the desired natural or non-natural amino acid, and isolating the aminoacylated RNA molecules.

The present invention also provides a method for aminoacylating tRNA-like molecules. The method comprises the steps of providing a catalytic RNA molecule having a trans-aminoacylating activity, contacting the RNA molecule with a tRNA-like molecule

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and the desired amino acid, and isolating the aminoacylated RNA molecules.

BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1A is a schematic representation of a catalytic RNA with self-aminoacylation activity (left) and a catalytic RNA with trans-aminoacylation activity (right). The amino acid substrate (the amino acid side chain and leaving group are shown with aa and X, 10 respectively) binds to the 5'-leader ribozyme domain, and the nucleophilic attack of the tRNA 3'-hydroxyl (indicated by a curved arrow) is accelerated. The cleavage site of RNase P RNA is shown by the straight arrow.

15 Figure 1B is a representation of the Secondary structure of otRNA.

 Figure 1C is a representation of the chemical structure of substrates cyanomethyl ester (CME), adenosine monophosphate (AMP) (adenylate), and thioester 20 (TE), respectively.

 Figure 2 is a representation of an autoradiogram showing self-aminoacylation activity as a function of selection cycle. a, Biotin-Phe-RNA complexed with SAV; b, RNA pool.

25 Figure 3 is a representation of the sequence alignment of active clones isolated from round 17 RNA. For the alignment of the tRNA domain, the wild-type otRNA is shown together with the selected rtRNA sequences. Consensus deletions and mutations appearing 30 in the tRNA domain are highlighted by boxes. The abbreviations for tRNA loops are: AC, anticodon; V, variable; T, T C.

 Figure 4A is a representation of the self-aminoacylation activity and amino acid specificity of 35 pre-24: a, Biotin-aminoacyl-pre-24 complexed with SAV; b, pre-24; c, Biotin-Phe-otRNA complexed with SAV; d, otRNA.

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Figure 4B is a representation of comparison of self-aminoacylation activity of pre-24 using three distinct esters. a, Biotin-Phe-pre-24 complexed with SAV; b, pre-24; c, Biotin-Phe-otRNA complexed with SAV; d, otRNA. Reactions were performed in the presence of 0.5 μ M pre24 and 5 mM Phe-CME (lane 1), 5 mM Phe-AMP (lane 2), or 10 mM Phe-TE (lane 3) at 25°C (lanes 1 and 3) or 0°C (lane 2) for 30 min.

Figure 4C is a representation of comparison of self-aminoacylation activity of pre-24 and its mutants containing different degrees of mutation and deletion in the tRNA domain. The wild-type pre-24 (lane 1) and pre-24^{otRNA} (lane 5) contain the rtRNA and otRNA sequences of the tRNA domain, respectively.

Figure 5A is a representation of the trans-aminoacylation activity of the 5'-leader ribozyme. Cleavage of pre-24 otRNA by RNase P RNA. a, pre-24 otRIIA ; b, 5' leader segment; c, otRNA.

Figure 5B is a representation of an autoradiogram showing the time course of 5'-leader ribozyme-catalyzed aminoacylation of otRNA. a, Biotin-Phe-otRNA complexed with SAV; b, otRNA. The RNase P-digested RNA fragments of pre-24^{otRNA} were used for aminoacylation (k.b. = $1.0 \times 10^{-3} \text{ min}^{-1}$).

Figure 5C is a representation of the time course of 5'-leader ribozyme-catalyzed aminoacylation of a minihelix RNA. a, Biotin-Phe-minihelix RNA complexed with SAV; b, minihelix RNA (SEQ ID NO:19) (consisting of the acceptor- T stem-loop region of otRNA).

Figure 6 shows aminoacylation states before and after mild base hydrolysis of aminoacyl-pre-24 with potassium carbonate. Lane 1-3: same as lanes 1-3 in Fig. 4A. Lane 4: Biotin-Phe-pre-24 RNA (same as RNA in lane 1) was treated with 50 mM K₂CO₃ for 15 min at 37°C. Lane 5: RNA recovered from lane 4 was used for aminoacylation under the same conditions as lane 1.

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Figure 7 shows aminoacylation of pre-24 and otRNA in the presence of Phe-AMP and Phe-TE. Lane 1: Aminoacylation of pre-24 in the presence of 5mM Phe-AMP followed by biotinylation. Lane 2: Aminoacylation only. Lane 3: Aminoacylation of otRNA with 5mM Phe-AMP followed by biotinylation. Lane 4: Aminoacylation of pre-24 in the presence of 10mM Phe-TE followed by biotinylation. Lane 5: Aminoacylation only. Lane 6: Biotinylation only. Lane 7: Aminoacylation of otRNA with 10mM Ohe-TE followed by biotinylation.

Figure 8 shows ribozyme-catalyzed aminoacylation on tRNA variants *in trans*. Reactions were carried out for 3 hours. The v1 and v3 (lanes 2 and 3) are the fragment of the tRNA domain described in Figure 4C.

DETAILED DESCRIPTION OF THE INVENTION

The phrase "tRNA-like molecules" or "tRNA-like domains" or "tRNA-like sequences" as used herein for the purposes of specification and claims, means RNA molecules that have sequences consistent with the formation of a cloverleaf structure typically associated with tRNAs. An example of a tRNA-like molecule is otRNA (SEQ ID NO:18). Other t-RNA-like molecules are the tRNA domains of SEQ ID NO:5-15 i.e., nucleotides 86-146 of SEQ ID NO:5, nucleotides 90-151 of SEQ ID NO:6, nucleotides 90-150 of SEQ ID NO:7, nucleotides 89-150 of SEQ ID NO:8, nucleotides 90-150 of SEQ ID NO:9, nucleotides 89-149 of SEQ ID NO:10, nucleotides 89-149 of SEQ ID NO:11, nucleotides 89-149 of SEQ ID NO:12, nucleotides 89-149 of SEQ ID NO:13, nucleotides 90-150 of SEQ ID NO:14, and nucleotides 89-148 of SEQ ID NO:15. Still other tRNA-like molecules are SEQ ID NO:20-22.

The phrase "ribozyme" as used herein for the purposes of specification and claims, means an RNA molecule that is capable of catalyzing a chemical reaction.

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The phrase "natural amino acid" refers to any amino acid among the twenty amino acids that are normally aminoacylated onto tRNAs in living cells. Such amino acids are alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, glycine, serine, threonine, tyrosine, cysteine, glutamine, asparagine, lysine, arginine, histidine, aspartic acid, and glutamic acid. Consequently, the phrase "non-natural amino acids" means any amino acid other than the natural amino acids or modification of a natural amino acid.

The term "cis" means within the same molecule. The term "trans" means on or to a different molecule.

The term "5'-leader domain" or "catalytic domain" or "5'-leader ribozyme" means the 5' region of the catalytic molecules having *cis*-aminoacylating activity. Examples of such 5'-leader domains are nucleotides 1-85 of SEQ ID NO:5, nucleotides 1-89 of SEQ ID NO:6, nucleotides 1-89 of SEQ ID NO:7, nucleotides 1-88 of SEQ ID NO:8, nucleotides 89 of SEQ ID NO:9, nucleotides 1-88 of SEQ ID NO:10, nucleotides 1-88 of SEQ ID NO:11, nucleotides 1-88 of SEQ ID NO:12, nucleotides 1-88 of SEQ ID NO:13, nucleotides 1-89 of SEQ ID NO:14 and nucleotide 1-88 of SEQ ID NO:15.

The present invention is directed to catalytic RNA molecules that can catalyze aminoacylation reactions. The catalytic RNA molecule having self-aminoacylating activity has two domains, a catalytic domain and an aminoacyl acceptor domain. The catalytic domain contains a sequence which has ribozymal activity. This domain also confers amino acid specificity.

To construct the catalytic RNA molecules having self aminoacylation activity, a selection process is used on a randomly synthesized RNA pool. By attaching the RNA molecules of this pool to the 5' end of a tRNA-like molecule and contacting the complex with a substrate molecule (natural or non-natural amino acid),

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self-aminoacylating RNA molecules are identified. These molecules can then be selectively amplified. The self-aminoacylating nature of these molecules can be confirmed by standard assays such as mobility-gel-shift assays. To facilitate the isolation of the active aminoacylated RNA species, a biotin tag may be attached to the target molecule. Alternatively, a water soluble form of biotin may be used to label the aminoacylated RNAs subsequent to generation of the aminoacylated RNA species. These biotin labeled species can be isolated by using strepavidin coated agarose. Repeated rounds of selective amplification yield pure species of the catalytic RNA with self aminoacylating activity. A round of selection comprises in part reverse - transcribing RNAs collected from the SAV bound portion of the reaction in order to produce cDNAs. The cDNAs are subjected to the polymerase chain reaction (PCR) followed by transcription of the PCR products to generate RNAs used in the next round of selection. The RNAs obtained from the final selection round are subjected to mobility gel-shift assays to demonstrate self-aminoacylation and trans-aminoacylation activity.

The catalytic molecules of the present invention can also aminoacylate RNA species in trans. To produce such catalytic molecules, the aminoacyl acceptor domain (the tRNA-like domain) can be cleaved from the catalytic domain by bacterial RNase P digestion. The catalytic domain is able to aminoacylate the aminoacyl acceptor domain or a tRNA-like molecule in *trans*. The catalytic molecules can also be synthesized by *in vitro* run-off transcription catalyzed by an appropriate RNA polymerase from the corresponding DNA templates by methods known to those skilled in the art.

The aminoacyl acceptor domain is a tRNA-like molecule. A suitable example of a tRNA-like molecule is an artificial orthogonal suppressor tRNA (otRNA see Fig.

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IB). Alternatively, an amber supressor tRNA derived or isolated from different species, such as human, can be used for this purpose. This tRNA sequence is derived from an amber suppressor tRNA^{G1n2} but is not recognized by
5 bacterial ARSs. The otrNA can be attached to unique catalytic domains that confer aminoacylation activity and amino acid specificity.

Once sequences known to possess aminoacylating activity are isolated, they can be used in a scaffold-
10 based approach to isolating additional aminoacylating sequences. The pre-24^{phe} sequence region (except for the 5'-primer sequence) in DNA form is randomly mutagenized such that each base has a certain percentage chance of being non-wild-type. This DNA pool is then annealed with
15 the anistense DNA of the otrNA, and the annealed pool is extended to full-length duplex DNA. New copies of the templates are generated by PCR, these are transcribed and subject to selection as previously described.

Taking advantage of the catalytic scaffold of the
20 pre-24^{phe} allows screening of a focused sequence space in which most sequences can maintain the secondary or even tertiary structures of the pre-24^{phe}. Despite this narrowed focus, the scaffold pool has enough random mutations to give the desired alteration of amino acid
25 specificity dependant upon the amino-acid chosen to be the amino acid component of the substrate. Therefore, the likelihood of encountering active sequences can become higher than the selection using a completely random pool. For the scaffolding pool, it is
30 unnecessary to deal with a high complexity pool because fewer nucleotides are randomized (depending upon the mutation rate, its complexity can be two or three orders of magnitude lower). Therefore, the selection is much less labor-intensive and the outcome can be obtained
35 more rapidly.

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These and other embodiments will become more clear from the examples described below, which are to be construed as illustrative and not limiting.

5

EXAMPLE 1

This embodiment describes the construction of a pool of RNA molecules for screening of aminoacylation activity. In an illustration of this embodiment, randomized sequences were generated, attached to a tRNA-like molecule. Thus, a random pool of 70 nucleotides was attached to the 5' end of a tRNA-like molecule (otrRNA) as follows.

Pool construction: Four synthetic oligonucleotides were used for the pool construction: the random pool DNA template (5'-GGATCGTCAGTGCATTGAGA-N70-GGTGGTATCCCCAAGGGGTA-3') (SEQ ID No. 1), the DNA template complementary to the orthogonal tRNA (otrRNA) (SEQ ID No. 2), the 5'-primer containing T7 promoter sequence (SEQ ID No. 3), and 3'-primer (SEQ ID No. 4). A large-scale Taq DNA polymerase-extension of the DNA templates was performed under thermocycling conditions (95°C for 10 min, 55°C for 10 min, and 72°C for 10 min). The full-length product was then amplified by seven cycles of large-scale PCR in the presence of the 5'- and 3'-primers. Four equivalents of the pool DNA with an approximately 10^{15} complexity were transcribed by T7 RNA polymerase in the presence of α -[^{32} P]-UTP, and purified by 6% polyacrylamide gel electrophoresis (PAGE).

30

EXAMPLE 2

This embodiment describes the selection of active sequences from the RNA molecules constructed in Example 1. As illustrations, the following substrates were used to select aminoacylating species of RNA.

35

Substrates

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N-biotinyl-L-aminoacyl-cyanomethyl esters (Biotin-aa-CME) were chosen as the aminoacyl donor substrates because the CME group has a balance of activation and hydrolytic stability. Furthermore, CME has no hydrogen-bonding functionalities, which helps to ensure that the primary interaction with RNA will occur through the amino acid side chain of the substrate.

Other substrates included Pheylalanyl adenylate (Phe-AMP) and pheylalanyl thioester (Phe-TE). These substrates were also chosen for reasons described above and to demonstrate specificity to the amino acid rather than other components of the substrate. The biotin tag facilitates the isolation of active, i.e. aminoacylated, sequences on immobilized streptavidin (SAv) agarose via an interaction between the biotin moiety of the substrate and the SAv. N-biotinyl-L-phenylalanyl-cyanomethyl esters (Biotin-Phe-CME) and Boc-Phe-CME (Boc is tert-butoxy carbonyl) were synthesized essentially by the procedure as previously described (Suga, et al. *J. Am. Chem. Soc.* 120: 1151-1156 1998). Synthesis of Phe-CME was carried out as follows: 9:1 TFA/anisole solution (500 mL) was added to Boc-Phe-CME (385 mg, 1.26 mmol) under argon atmosphere and the mixture was stirred at room temperature for 30 min. The solvent was removed in vacuo and ca. 4 M hydrogen chloride in dioxane (4 mL) was added to the residue. The solution was concentrated in vacuo, and the addition of anhydrous ether to the residue yielded precipitate. The precipitate was dissolved in a minimal amount of MeOH, and to this solution anhydrous ether was added to reprecipitate the product. Purity of the product was determined using nuclear magnetic resonance (NMR).

Synthesis of phenylalanyl adenylate (Phe-AMP) was carried out essentially as described previously (Berget al. *Bio. Chem.* 253: 608-611, 1958). ³²P NMR analysis of the product indicated that the purity of Phe-AMP was

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approximately 50% and the remaining side-product was unreacted AMP. The product was dissolved in water, and used for aminoacylation without further purification.

Synthesis of phenylalanyl thioester (Phe-TE) was as follows: N,N-bis[2-oxo-3-oxozolidiyl]phosphordiamitic chloride (238 mg, 0.94 mmol) was added to a solution of Boc-Phe (307 mg, 1.16 mmol) and triethylamine (175 mL, 2.26 mmol) in CH₂Cl₂ (10 mL). To this mixture, ethyl 2-mercapto acetate (100 mL, 0.91 mmol) was slowly added, and the reaction mixture was then stirred vigorously for 5 h at room temperature. The reaction was quenched by the addition of 20% NaHCO₃ aqueous solution. After standard aqueous work-up, Boc-Phe-TE was isolated by column chromatography. 9:1 TFA/anisole solution (500mL) was added to Boc-Phe-TE (200 mg, 0.30 mmol) under argon atmosphere, and the mixture was stirred at room temperature for 30 min. The solvent was removed in vacuo and ca. 4M hydrogen chloride in dioxane (4 mL) was added to form the hydrochloride salt. The solution was concentrated in vacuo, and the residue was dissolved in ether. The addition of petroleum ether to this solution resulted in the formation of precipitate, which was rinsed with ether and filtered to yield Phe-TE.

Selection of Active Sequences

Selection reactions were carried out under the following conditions: A mixture of 10 μ M (first round only) or 1 μ M RNA pool, 1 mM Biotin-Phe-CME, in an EK buffer (50 mM EPPS, 500 mM KCl, pH 7.5), 100 mM MgCl₂, and ethanol (25% of the total volume) The pool RNA was preincubated in the EK buffer, heated at 95°C for 5 min, and cooled to 25°C over 5 min. MgCl₂ was then added followed by a 5 min equilibration. The reaction was initiated by the addition of the substrate solution in ethanol, and incubated for 3 h at 25 °C (30 min in the 15-17th rounds). The reaction was stopped by adding 2 volumes of cold ethanol, and the RNA was ethanol-precipitated twice. The RNA pellet was dissolved into

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EKE buffer (50 mM EPPS, 500 mM KCl, 5 mM EDTA, pH 7.5), then incubated with 200 μ L (1 mL for the first round) of streptavidin agarose for 30 min at room temperature. Unbound RNAs were eluted with 20-resin volumes of the
5 EKE buffer, 40-resin volumes of 4 M urea, then 10-resin volumes of water. The resin-bound RNAs were eluted by heating at 95°C for 10 min in the presence of 10 mM biotin at pH 7. The collected RNAs were reverse-transcribed using 100 units M-MLV reverse transcriptase
10 (PromegaTM) in the presence of 1 μ M 3'primer (SEQ ID No. 4), 125 μ M dNTPs, 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, pH 8.3 at 42°C for 1 h (for 1-14 rounds) or 10 min (for 15-17 rounds). The cDNAs were subjected to PCR followed by transcription under standard conditions.

15 Fifteen rounds of selective amplification of self-aminoacylating RNA molecules in the pool yielded an enrichment of active sequences, which was confirmed by a SAV-dependent mobility-gel-shift assays (Figure 2, lanes 1-3). Approximately 10% of the total input RNA
20 molecules from round 15 showed aminoacylation after 3 h (lane 3). Two rounds of selection with shorter incubation times were employed in order to further enhance the activity in the pool (lanes 4-6). The absence of SAV or substrate resulted in loss of the
25 retarded band (lanes 7,8), indicating that self-aminoacylation of active RNAs with the Biotin-Phe group is occurring. Periodate oxidation of the 3' terminal diol or deletion of the 3' adenosine resulted in near complete inhibition of aminoacylation (lanes 9,10),
30 strongly suggesting that the 3'terminal hydroxyl group is the aminoacylation site.

Thirty-six individual clones from the round 17 pool were screened for self-aminoacylation (self aminoacylation assay described in Example B), and 11
35 clones exhibited appreciable activity (SEQ ID NO:5-15). Alignment of their sequences revealed an approximately 95% identity in the 5' leader domains (Fig. 3, top). A

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representative clone, termed as the pre-24 or rtRNA (SEQ ID NO:9), was selected for confirmation of the self-aminoacylation activity.

5

EXAMPLE 3

The self-aminoacylation activity of pre-24 in the presence of Biotin-Phe-CME was assayed under conditions similar to those of the selection except that 12.5 mM KCl and 5% ethanol were used. At each time point, an aliquot of the reaction was ethanol-precipitated twice, and the pellet was dissolved into 7 μ L a MEUS buffer (25 mM MOPS, 5 mM EDTA, 8 M Urea, 10 μ M streptavidin, pH 6.5). The reactions were carried out in the presence of 1 μ M RNA and 1 mM Biotin-Phe-CME (Fig. 4, lanes 1, 2, 4, and 5) or 1 mM Biotin-aa-CME (lanes 6-10) or in the absence of substrate (lane 3). For periodate oxidation (lane 4), pre-24 was treated with 10 mM NaOH at 0 °C for 1 h and ethanol-precipitated prior to the aminoacylation reaction. The reactions were incubated for 30 min (lanes 1-4) or 2 h (lanes 5-10). The resulting solution was analyzed by 10% PAGE in a cold room in order to keep the gel temperature below 20 °C (Fig. 4). A plot of initial rates vs. substrate concentration revealed Michaelis-Menten behavior with kinetic parameters of $k_{cat} = 0.10 + 0.01 \text{ min}^{-1}$ and $K_m = 6.3 \pm 1.2 \text{ mM}$, while the solubility limit of substrate constrains its actual working concentration to below 5 mM. The background rate was determined by incubating otRNA with 1 mM Biotin-Phe-CME for 3 h in the same reaction buffer as above, giving a yield of 0.01% of the aminoacyl-otRNA (lane 11). The background rate is thus estimated to be an approximately $5.5 \times 10^{-7} \text{ Min}^{-1}$. The observed rate acceleration by ribozyme is approximately 10^5 -fold greater than background.

35

The self-aminoacylation activity was confirmed by the SAV-dependent mobility-gel-shift assay (Fig. 4A, lanes 1-3). Periodate oxidation completely eliminated

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activity, strongly suggesting that the aminoacylation site is the 3'-end (lane 4). Mild base hydrolysis of aminoacyl-pre-24 with potassium carbonate resulted in loss of the retarded band, i.e. Biotin-Phe was

5 hydrolyzed from pre-24. When re-exposed to the aminoacyl substrate this deacylated pre-24 still showed full self-aminoacylation activity (see Fig. 6). This suggests that a 3' or 2'-ester bond is the only plausible linkage of the aminoacyl-pre-24.

10 The amino acid specificity of catalytic RNAs was investigated by using five distinct Biotin-aminoacyl-CMEs (Fig. 4, lanes 6-10). For substrates of Phe-CME, Phe-AMP and Phe-TE, self-aminoacylation reactions were carried out with the same procedures as those described
15 as above, except that the aminoacyl-RNA pellet was resuspended in an acidic EPPS buffer (0.3 M, pH 5.5). 0.3 M EPPS-KOH was then added to this solution, which brought the pH to 8.0.

Reaction rates for these amino acids were drastically
20 reduced compared with phenylalanine, indicating that the ribozyme domaine has a remarkable specificity toward Biotin-Phe-CME.

To further define the primary recognition element in the substrate, three synthetic phenylalanyl esters
25 (Fig. 1C) were tested for activity (Fig. 4B) (16). Reactions were performed in the presence of 0.5 μ M pre24 catalytic RNA and 5 mM Phe-CME (lane 1), 5 mM Phe-AMP (lane 2), or 10 mM Phe-TE (lane 3) at 25 °C (lanes 1 & 3) or 0 °C (lane 2) for 30 min. The absence of biotinylation
30 (lane 4) or substrate (lane 5) for Phe-CME resulted in loss of the retarded band, indicating that the aminoacylation is necessary for the retarded band. The background aminoacylation was monitored using otRNA in the presence of 5 mM Phe-CME (lane 6). The same control
35 experiments for Phe-AMP and Phe-TE were also performed (see figure 7). Omission of biotin from the α -amino group, i.e. Phe-CME, gave almost the same catalytic rate

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as that observed for Biotin-Phe-CME (Fig. 4B, lane 1) when the concentration of Phe-CME increased by 5-fold. This illustrates that the biotinyl group, presumably the amide functionality on the α -amino group, interacts with ribozyme, but it is not an essential element for substrate recognition. The ribozyme also accommodated the adenylate (Phe-AMP) and a thioester (Phe-TE) in place of the CME leaving group (lanes 2&3). This demonstrated that the critical recognition element of the substrate is the phenylalanyl side chain, not the leaving group.

EXAMPLE 4

This embodiment demonstrates the preparation and trans-aminoacylating activity of the catalytic RNA molecules. The pre-24 catalytic RNA from Example 1 and 2 was used in this illustration. First, pre-24 otRNA was subjected to RNase P scission (Fig. 5A) to liberate the 5'-catalytic domain from the 3'-tRNA domain. A [^{32} P]-body-labeled pre-24^{otRNA} was treated with RNase P RNA for 2 h, resulting in the cleavage of approximately 23% of pre-24^{otRNA} (lane 1). The absence of RNase P yielded no cleaved product (lane 2). The marker RNAs (5'-leader segment in lane 3 and otRNA in lane 4) were prepared by in vitro transcription using the corresponding DNA segments. E. coli RNase P RNA was in vitro transcribed using a PCR-amplified DNA template from the M1 gene of the pDW27 plasmid as previously described (Ziehler et al. (1996) *Biotechniques* 20, 622-624), then purified on 6% PAGE. The cleavage of pre-24^{otRNA} (1 μ M) by the RNase P RNA (1 μ M) was carried out in 1 M NH₄OAc, 50 mM MgCl₂ and 0.1 % SDS at 37 °C (14). After the reaction, the solution was ethanol-precipitated twice and the resulting solution was analyzed by 10% PAGE.

Treatment of pre-24^{otRNA} with E.coli RNase P RNA

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produced two fragments of lengths corresponding to the 5'-leader segment and the otRNA (Fig. 5A, lanes 1 and 2, compare with in vitro transcripts of each fragment in lanes 3 and 4). This demonstrates that pre-24^{otRNA} is susceptible to RNase P RNA hydrolysis. Thus, the catalytically active pre-tRNA can be segmented tRNA-like molecule and a 5' leader segment.

Next, it was examined whether the 5'-leader fragment could aminoacylate the otRNA fragment in trans (Fig. 1A, right). For the analysis of the trans-aminoacylation activity (Fig. 5B), an unlabeled pre-24^{otRNA} was cleaved, and the individual segments of otRNA and 5'leader domain were purified by 10% PAGE. The tRNA segment was treated with calf intestinal alkaline phosphatase and then phosphorylated using T4 polynucleotide kinase in the presence of [³²P]-a-ATP. The 5'-leader and otRNA fragments generated by RNase P RNA action on pre-24^{otRNA} were treated with Biotin-Phe-CME (Fig. 5B). The 5'-leader fragment transaminoacylated otRNA at a rate similar to the cis reaction of pre-24^{otRNA}. The in vitro transcribed 5'-leader fragment also exhibited trans-activity similar to that observed above. Thus, the RNase P RNA-digested 5'leader fragment can independently fold into its functional structure, and act as a trans-acting aminoacylation enzyme.

The substrate properties of a minihelix RNA consisting of the acceptor- T stem-loop region of otRNA (Fig. 5C) were also tested. The 5'-leader ribozyme and minihelix RNA were in vitro transcribed, then purified on PAGE. Prior to the reaction, each of the RNAs (4 μM of 5' leader ribozyme and 3 μM of minihelix RNA) were folded independently. The remaining procedures were the same as described in the method section. The minihelix RNA was still aminoacylated by 5'-leader ribozyme, indicating that the anticodon loop is not essential for activity. An approximately 4-fold reduction of the observed rate as compared to otRNA, however, suggests

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that 5'-leader ribozyme interacts with additional elements present in the full-length of otRNA.

Additionally, the 5'-leader fragment *trans*-aminoacylated otRNA at a rate similar to the *cis* reaction of pre-24^{otRNA}. The *in vitro* transcribed 5'-leader fragment also exhibited *trans*-activity similar to that observed above. In addition, this fragment showed activity toward rtRNA and its variants at similar rates to the respective *cis* reactions (Fig. 8).

In conclusion, the present invention provides modified tRNAs with the ability to catalyze the aminoacylation of their own 3' ends. The 5'-leader sequences may exist independently of the 3'-tRNA domains and still retain their catalytic property.

EXAMPLE 5

A scaffolding strategy was used for the selection of Leu-specific ribozymes using a 15%-mutagenized scaffold pool. The 15%-mutagenized scaffold RNA pool was synthesized as follows: Each nucleotide position of ribozyme, except for the 5'-primer region and the 5'-overlapped region of tRNA, is mutagenized on the corresponding DNA template by using an automated DNA synthesizer. Prior to the synthesis, each phosphoramidite base was mixed with the other three bases with the reactivity ratio of 85:5:5:5. The DNA template was synthesized according to the ribozyme sequence. The deprotection and purification of oligonucleotide was employed as standard methods. The DNA was amplified by the same method as described in Example 1, except that the PCR was carried out in a 200 μ L scale. *In vitro* transcription of this DNA template followed by purification on PAGE gave the 15%-mutagenized scaffold RNA pool. After only five rounds of selection, active sequences appeared in the pool (whereas selection of pre-24^{Phe} from a completely random

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pool required 15 rounds). It should be noted that the observed activity toward orthogonal tRNA (otRNA) is even higher than that of the original pre-24^{Phe}, indicating that this strategy is effective not only for switching
5 the specificity but also for optimizing activity toward otRNA.

By using this approach, leucine specific cis aminoacylating RNAs were obtained. Two examples are presented as SEQ ID NO:17 and SEQ ID NO;18.

10

Although preferred embodiments of the present invention have been described and illustrated herein, the present invention is not limited to such preferred embodiments. Those skilled in the art will appreciate
15 that various changes may be made without departing from the spirit of the invention.

SEQUENCE LISTING

GENERAL INFORMATION

APPLICANT: The Research Foundation of State University of New York

TITLE OF INVENTION: CATALYTIC RNAs WITH AMINOACYLATION ACTIVITY

NUMBER OF SEQUENCES: 22

CORRESPONDENCE ADDRESS: c. Brian Barlow & Associates
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COMPUTER-READABLE FORM

COMPUTER: Dell Dimension 4700
OPERATING SYSTEM: Windows xp
SOFTWARE: PatentIn 2.1

CURRENT APPLICATION DATA

PCT APPLICATION NUMBER: PCT/US00/32184
CANADIAN APPLICATION NUMBER: 2,391,433
FILING DATE: 2000-11-22
CLASSIFICATION:

PRIOR APPLICATION DATA

APPLICATION NUMBER: 60/167,331
FILING DATE: 1999-11-24

APPLICATION NUMBER: 60/214,382
FILING DATE: 2000-06-28

PATENT AGENT INFORMATION

NAME: Dr. C. B. Barlow

REFERENCE NUMBER: 1355

INFORMATION FOR SEQ ID NO.: 1

SEQUENCE CHARACTERISTICS

LENGTH: 110
TYPE: nucleic acid
STRANDEDNESS:
TOPOLOGY:
MOLECULE TYPE:DNA
HYPOTHETICAL:
ANTI-SENSE:
FRAGMENT TYPE:
ORIGINAL SOURCE: Artificial Sequence
IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

NAME/KEY: N represents A,T,G or C.

LOCATION: 21-99

OTHER INFORMATION:

synthetic oligonucleotide containing random pool of 70 nucleotides

SEQUENCE DESCRIPTION: SEQ ID NO: 1

GGATCGTCAG TGCATTGAGA NNNNNNNNNN NNNNNNNNNN	40
NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN	80
NNNNNNNNNN GGTGGTATCC CCAAGGGGTA	110

INFORMATION FOR SEQ ID NO.: 2

SEQUENCE CHARACTERISTICS

LENGTH: 76

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: DNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: completely synthesized primer complementary to the otrNAGln

SEQUENCE DESCRIPTION: SEQ ID NO: 2

TGGCTGCGGT ACGAGGATTC GAACCTCGGA ATGCCGGATT	40
TAGAAATCCG GTCCCTTACC CCTTGGGGAT ACCACC	76

INFORMATION FOR SEQ ID NO.: 3

SEQUENCE CHARACTERISTICS

LENGTH: 52

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: DNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: 5' primer containing T7 promoter sequence

SEQUENCE DESCRIPTION: SEQ ID NO: 3

GGTAACACGC ATATGTAATA CGACTCACTA TAGGATCGTC 40
 AGTGCATTGA GA 52

INFORMATION FOR SEQ ID NO.: 4

SEQUENCE CHARACTERISTICS

LENGTH: 20

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: DNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: 3'completely synthesized primer

SEQUENCE DESCRIPTION: SEQ ID NO: 4

TGGCTGCGGT ACGAGGATTC 20

INFORMATION FOR SEQ ID NO.: 5

SEQUENCE CHARACTERISTICS

LENGTH: 146

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: RNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: pre-12 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 5

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU CAUGGGUAUU GGCGUUAGGU GCGGGAUGCU	80
ACGCUGGUGG UAUCCCCAAG GGUACGGGAC CGGACAUUCG	120
AGAUUCGAU CCUCGUACCG CAGCCA	146

INFORMATION FOR SEQ ID NO.: 6

SEQUENCE CHARACTERISTICS

LENGTH: 151
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 NAME/KEY: N represents A,T,G or C.
 LOCATION: 21-99

OTHER INFORMATION: pre-38 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 6

GGAUCGUCAG UNCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU UCAUGGGUAU UGGCGUUAGG UGCGGGAUGC	80
UACUACGCUG GUGGUAUCCC CAAGGGUACG GGACCGGAUC	120
AUUCGAGAUU CGAAUCCUCG UACCGCAGCC A	151

INFORMATION FOR SEQ ID NO.: 7

SEQUENCE CHARACTERISTICS

LENGTH: 150
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-29 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 7

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGG CCCUUCUCAC	40
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UAACGGUGGG UCAUGGGUAU UGGCGUUAGG UGCGGGAUGC	80
UACUACGCUG GUGGUAUCCC CAAGGGUACG GGACCGGACA	120
UUCGAGAUUC GAAUCCUCGU ACCGCAGCCA	150

INFORMATION FOR SEQ ID NO.: 8

SEQUENCE CHARACTERISTICS

LENGTH: 150
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:

MOLECULE TYPE: RNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: pre-36 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 8

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU CAUGGGUAU UGGCGUUAGG GCGGGAUGCU	80
ACUACGCUGG UGGUAUCCCC AAGGGUACGG GACCGGAUCA	120
UUCGAGAUUC GAAUCCUCGU ACCGCAGCCA	150

INFORMATION FOR SEQ ID NO.: 9

SEQUENCE CHARACTERISTICS

LENGTH: 150
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:

MOLECULE TYPE: RNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: pre-24 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 9

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGG CCCUUCUCAC	40
UAACGGUGGG UCAUGGGUAU UGGCGUUAGG UGCGGGAUGC	80
UACUACGCUG GUGGUAUCCC CAAGGGUACG GGACCGGACA	120
UUCGAGAUUC GAAUCCUCGU ACCGCAGCCA	150

INFORMATION FOR SEQ ID NO.: 10

SEQUENCE CHARACTERISTICS

LENGTH: 149
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-25 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 10

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU CAUGGGUAUU GGCGUUAGGU GCGGGAUACU	80
ACUACGCUGG UGGUAUCCCC AAGGGUACGG GACCGGACAU	120
UCGAGAUUCG AAUCCUCGUA CCGCAGCCA	149

INFORMATION FOR SEQ ID NO.: 11

SEQUENCE CHARACTERISTICS

LENGTH: 149
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-22 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 11

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU CAUGGGUGUU GGCGUUAGGU GCGGGAUGCU	80
ACUACGCUGG UGGUAUCCCC AAGGGUACGG GAUCGGACAU	120
UCGAGAUUCG AAUCCUCGUA CCGCAGCCA	149

INFORMATION FOR SEQ ID NO.: 12

SEQUENCE CHARACTERISTICS

LENGTH: 149
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE:RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 NAME/KEY: N represents A,T,G or C.
 LOCATION: 21-99

OTHER INFORMATION: pre-5 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 12

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCCUCUCACU	40
AACGGUGGGU CAUGGGUAUU GGCGUUAGGU GCGGGAUGCU	80
ACUACGCUGG UGGUAUCCCC AAGGGUACGG GNCCGGACAU	120
UCGAGAUUCG AAUCCUCGUA CCGCAGCCA	149

INFORMATION FOR SEQ ID NO.: 13

SEQUENCE CHARACTERISTICS

LENGTH: 149
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-19 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 13

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUAGGU CAUGGGUAUU GGCGUUAGGU GCGGGAUGCU	80
ACUACGCUGG UGGUAUCCCC AAGGGUACGG GACCGGACAU	120
UCGAGAUUCG AAUCCUCGUA CCGCAGCCA	149

INFORMATION FOR SEQ ID NO.: 14

SEQUENCE CHARACTERISTICS

LENGTH: 150
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-8 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 14

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU UCAUGGGUAU UGGCGUUAGG UGCGGGAUGC	80
UACUACGCUG GUGGUAUCCU CAAGGGUACG GGACCGGACA	120
UUCUAGAUUC GAAUCCUCGU ACCGCAGCCA	150

INFORMATION FOR SEQ ID NO.: 15

SEQUENCE CHARACTERISTICS

LENGTH: 148
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: pre-23 catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 15

GGAUCGUCAG UGCAUUGAGA UUUCCGCAGC CCUUCUCACU	40
AACGGUGGGU CAUGGGUAU UGGCGUUAGG GCGGGAUGCU	80
ACUACGCUGG UGGUAUCCCA AGGGUACGGG ACCGGACA UU	120
CGAGAUUCGA AUCCUCGUAC CGCAGCCA	148

INFORMATION FOR SEQ ID NO.: 16

SEQUENCE CHARACTERISTICS

LENGTH: 75
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Escherichia coli
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: otrRNA

SEQUENCE DESCRIPTION: SEQ ID NO: 16

GGUGGUAUCC CCAAGGGGUA AGGGACCGGA UUCUAAAUCC	40
GGCAUUC CGA GGUUCGAAUC CUCGUACCGC AGCCA	75

INFORMATION FOR SEQ ID NO.: 17

SEQUENCE CHARACTERISTICS
 LENGTH: 160
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: H2 Leu catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 17

GGAUCGUCAG UGCAUUGAGA UGCCCCAAAGC CCUUCUCACU	40
UCCGGUGGGU CAUGCGUUAU UGCGUUAGGU GAGGAAUGCU	80
AGUAUGCGGG UGGUAUCCAA GGGGUAAGGG ACCGGAUUCU	120
AAAUCCGCAU UCCGAGGUUC GAAUCCUCGU ACCGCAGCCA	160

INFORMATION FOR SEQ ID NO.: 18

SEQUENCE CHARACTERISTICS
 LENGTH: 156
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:

ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: D1-Leu catalytic RNA

SEQUENCE DESCRIPTION: SEQ ID NO: 18

GGAUCGUCAG UGCAUUGAGA UAGUGUCACU AGGCGGGGGG	40
UGAUAGCGCA UUUUGAGGUU UGGUUUGGGG GGUUAUGCGU	80
GAGUUCUUGG GUGGUAUCCA AGGGGUAAGG GAUCUAAAUC	120
CGACAUUCCG AGGUUCGAAU CCUCGUACCG CAGCCA	156

INFORMATION FOR SEQ ID NO.: 19

SEQUENCE CHARACTERISTICS

LENGTH: 35
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: RNA forming a minihelix

SEQUENCE DESCRIPTION: SEQ ID NO: 19

GGUGGUACGA GGUUCGAAUC CUCGUACCGC AGCCA	35
--	----

INFORMATION FOR SEQ ID NO.: 20

SEQUENCE CHARACTERISTICS

LENGTH: 73
 TYPE: nucleic acid
 STRANDEDNESS:
 TOPOLOGY:
 MOLECULE TYPE: RNA
 HYPOTHETICAL:
 ANTI-SENSE:
 FRAGMENT TYPE:
 ORIGINAL SOURCE: Artificial Sequence
 IMMEDIATE SOURCE:
 MAP POSITION:
 UNITS: FEATURE:
 OTHER INFORMATION: V1 variant of otrNA

SEQUENCE DESCRIPTION: SEQ ID NO: 20

GGUGGUAUCC CCAAGGGUAC GGGACCGGAU UCUAAAUCCG	40
GCAUUCGAGA UUCGAAUCCU CGUACCGCAG CCA	73

INFORMATION FOR SEQ ID NO.: 21

SEQUENCE CHARACTERISTICS

LENGTH: 75

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: RNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: V2 variant of otrRNA

SEQUENCE DESCRIPTION: SEQ ID NO: 21

GGUGGUAUCC CCAAGGGGUA CGGGACCGGA UUCUAAAUCC	40
GGCAUCCGA GAUUCGAAUC CUCGUACCGC AGCCA	75

INFORMATION FOR SEQ ID NO.: 22

SEQUENCE CHARACTERISTICS

LENGTH: 73

TYPE: nucleic acid

STRANDEDNESS:

TOPOLOGY:

MOLECULE TYPE: RNA

HYPOTHETICAL:

ANTI-SENSE:

FRAGMENT TYPE:

ORIGINAL SOURCE: Artificial Sequence

IMMEDIATE SOURCE:

MAP POSITION:

UNITS: FEATURE:

OTHER INFORMATION: V3 variant of otrRNA

SEQUENCE DESCRIPTION: SEQ ID NO: 22

GGUGGUAUCC CCAAGGGUAA GGGACCGGAU UCUAAAUCCG	40
GCAUUCGAGG UUCGAAUCCU CGUACCGCAG CCA	73

What is claimed is:

1. A nucleic acid molecule comprising a polynucleotide selected from the group consisting of :

a) RNA having a sequence of SEQ ID NO : 5, SEQ ID NO : 6, SEQ ID NO : 7, SEQ ID NO : 8, SEQ ID NO : 9, SEQ ID NO : 10, SEQ ID NO : 11, SEQ ID NO : 12, SEQ ID NO : 13, SEQ ID NO : 14 and SEQ ID NO : 15, and complementary sequences thereof ;

b) DNA having the sequence of a) wherein t is substituted for u.

2. The nucleic acid molecule of claim 1, wherein the RNA sequence has a sequence of SEQ ID NO : 9.

3. A nucleic acid molecule comprising a polynucleotide selected from the group consisting of :

a) RNA having a sequence of nucleotides 1-85 of SEQ ID NO : 5, nucleotides 1-89 of SEQ ID NO : 6, nucleotides 1-89 of SEQ ID NO : 7, nucleotides 1-88 of SEQ ID NO : 8, nucleotides 1-89 of SEQ ID NO : 9, nucleotides 1-88 of SEQ ID NO : 10, nucleotides 1-88 of SEQ ID NO : 11, nucleotides 1-88 of SEQ ID NO : 12, nucleotides 1-88 of SEQ ID NO : 13, nucleotides 1-89 of SEQ ID NO : 14, nucleotide 1-88 of SEQ ID NO : 15, and complementary sequences thereof ; and

b) DNA having the sequence of a) wherein t is substituted for u.

4. A nucleic acid molecule comprising the polynucleotide selected from the group consisting of :

a) RNA having a sequence of nucleotides 86-146 of SEQ ID NO : 5, nucleotides 90-151 of SEQ ID NO : 6, nucleotides 90-150 of SEQ ID NO : 7, nucleotides 89-150 of SEQ ID NO : 8, nucleotides 90-150 of SEQ ID NO : 9, nucleotides 89-149 of SEQ ID NO : 10, nucleotides 89-149 of SEQ ID NO : 11, nucleotides 89-149 of SEQ ID NO : 12, nucleotides 89-149 of SEQ ID NO : 13, nucleotides 90-150 of SEQ ID NO : 14, nucleotides 89-148 of SEQ ID NO : 15, SEQ ID NO : 20, SEQ ID NO : 21, SEQ ID NO : 22, complementary sequences thereof ; and

b) DNA having a sequence of a) wherein t is substituted for u.

5. The nucleic acid molecule of claim 3, wherein the RNA has the sequence of nucleotides 90-150 of SEQ ID NO : 9.

6. A method for constructing cis-aminoacylating catalytic RNA molecules comprising the steps of providing tRNA-like molecules and attaching a ribozyme sequence to the 5' end of the tRNA like molecule, said ribozyme sequence being selected from the group consisting of nucleotides 1-85 of SEQ ID NO:5, nucleotides 1-89 of SEQ ID NO:6, nucleotides 1-89 of SEQ ID NO:7, nucleotides 1-88 of SEQ ID NO:8, nucleotides 89 of SEQ ID NO:9, nucleotides 1-88 of SEQ ID NO:10, nucleotides 1-88 of SEQ ID NO:11, nucleotides 1-88 of SEQ ID NO:12, nucleotides 1-88 of SEQ ID NO:13, nucleotides 1-89 of SEQ ID NO:14 and nucleotide 1-88 of SEQ ID NO:15.

7. A method of identifying cis-aminoacylating catalytic RNA molecules comprising the steps of:

- a) providing a tRNA-like molecule;
- b) providing a ribozyme domain molecule;
- c) attaching the ribozyme domain molecule to the 5' end of the tRNA-like molecule to obtain a pool of ribozyme-tRNA molecules;
- d) contacting the ribozyme-tRNA molecules with an amino acid substrate;
- e) partitioning the aminoacylated ribozyme-tRNA molecules from the remainder of the ribozyme-tRNA molecules to obtain cis-aminoacylating catalytic RNA molecules.

8. The method of claim 6, wherein the tRNA-like molecule has a sequence selected from the group consisting of nucleotides 86-146 of SEQ ID NO:5, nucleotides 90-151 of SEQ ID NO:6, nucleotides 90-150 of SEQ ID NO:7, nucleotides 89-150 of SEQ ID NO:8, nucleotides 90-150 of SEQ ID NO:9, nucleotides 89-149 of SEQ ID NO:10, nucleotides 89-149 of SEQ ID NO:11, nucleotides 89-149 of SEQ ID NO:12, nucleotides 89-149 of SEQ ID NO:13, nucleotides 90-150 of SEQ ID NO:14, nucleotides 89-148 of SEQ ID NO:15, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and complementary sequences thereof.

9. The method of claim 6, wherein the ribozyme domain molecule has a sequence selected from the group consisting of nucleotides 1-85 of SEQ ID NO:5, nucleotides 1-89 of SEQ ID NO:6, nucleotides 1-89 of SEQ ID NO:7, nucleotides 1-88 of SEQ ID NO:8, nucleotides 1-89 of SEQ ID NO:9, nucleotides 1-88 of SEQ ID NO:10, nucleotides 1-88

of SEQ ID NO:11, nucleotides 1-88 of SEQ ID NO:12, nucleotides 1-88 of SEQ ID NO:13, nucleotides 1-89 of SEQ ID NO:14 and nucleotide 1-88 of SEQ ID NO:15.

10. A method of obtaining a trans-aminoacylating catalytic RNA molecule comprising the steps of:

- a) providing a RNA molecule having a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15,
- b) cleaving the RNA molecule with RNase P to obtain the trans-aminoacylating catalytic RNA molecule and an aminoacyl acceptor domain; and,
- c) isolating aminoacylated RNA molecules.

11. A method of cis-aminoacylating a catalytic RNA molecule comprising the steps of:

- a) providing catalytic RNA molecules having an cis-aminoacylation activity;
- b) contacting the RNA molecules with the desired natural or non-natural amino acid; and
- c) isolating aminoacylated RNA molecules.

12. A method of trans-aminoacylating a tRNA-like molecule comprising the steps of:

- a) providing catalytic RNA molecules having a trans-aminoacylation activity;
- b) providing a tRNA-like molecule;

- c) contacting the catalytic RNA molecules and the tRNA-like molecules with the desired natural or non-natural amino acids to effect aminoacylation of the 3' end of the tRNA-like molecules in a one step process; and
- d) isolating aminoacylated tRNA-like molecules.

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Figures: 1A, 1B, 1C, 4A, 4B, 4C, 5A, 5B, 5C, 6, 7, 8

Pages: 1, 3, 4, 5

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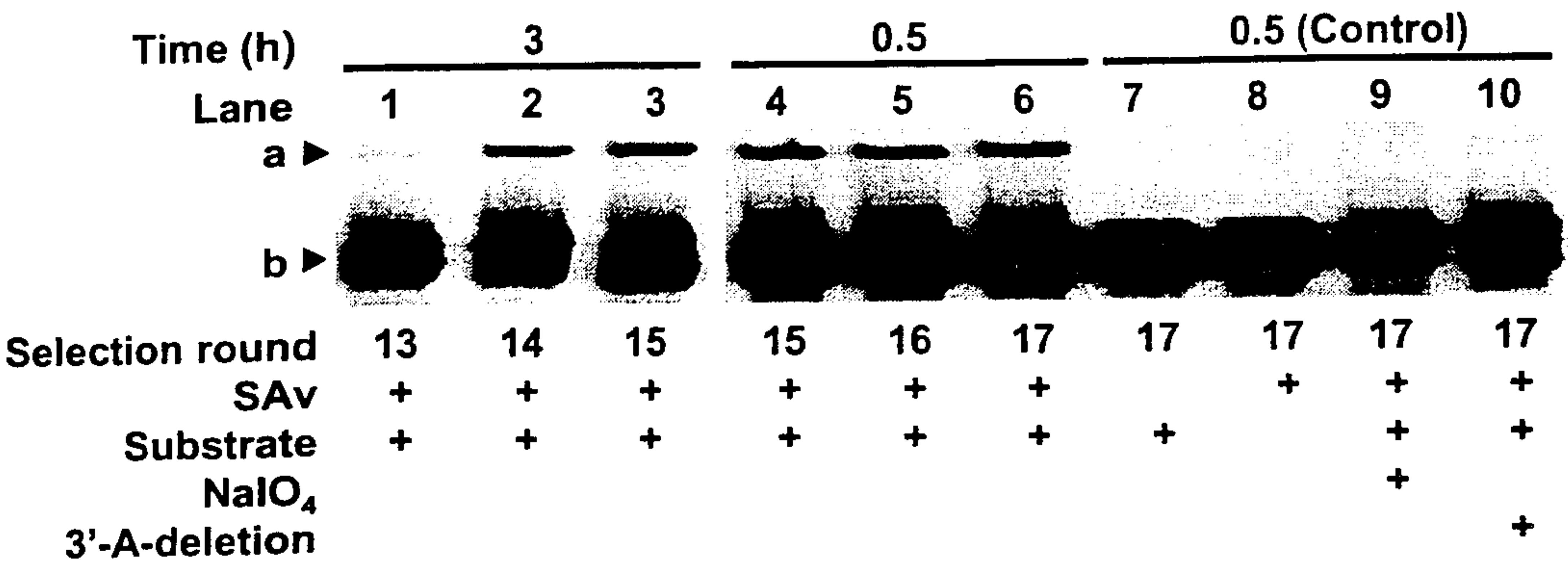


Fig. 2

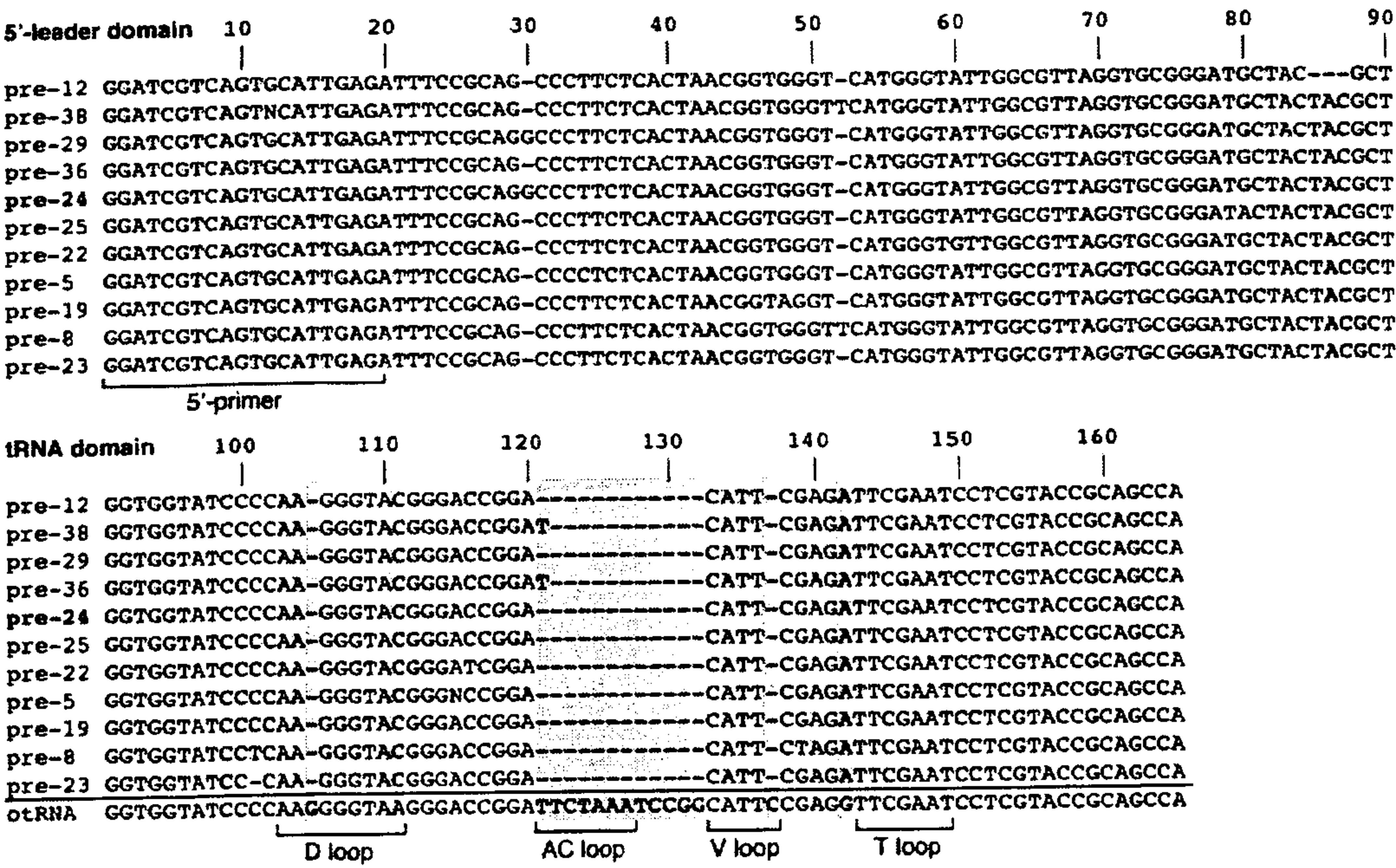


Fig. 3