RNA tetraplex as a primordial peptide synthesis scaffold

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ABSTRACT

Peptide bond formation at the peptidyl transferase center on the ribosome is a crucial phenomenon in life systems. In this study, we conceptually propose possible roles of the RNA tetraplex as a scaffold for two aminoacyl minihelices that enable peptide bond formation. The basic rationale of this model is that “parallel” complementary templates composed of only 10-mer nucleotides can position two amino acids in close proximity, which is conceptually and essentially similar to the situation observed in ribosomes. Using supportive experimental data, we discuss the origin and evolution of peptide bond formation in early biological systems.

Keywords:

tRNA

Minihelix

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Peptide bond formation

1. Introduction

X-ray crystallographic images clearly indicate that the ribosome is a ribozyme (BAN ET AL., 2000; KOROSTELEV AND NOLLER, 2007; NOLLER, 1993; NOLLER et al., 1992; RAMAKRISHNAN, 2008; SCHLUENZEN, 2000; STEITZ, 2008; WIMBERLY ET AL., 2000); no proteins were detected at a distance of less than ~ 18 Å from the peptidyl transferase center (PTC) (BAN ET AL., 2000; NISSEN ET AL., 2000). Complicated folding of 23S rRNA constitutes the PTC, and domain V of 23S rRNA interacts with the CCA ends of two tRNAs. Recent experimental data suggest the importance of the “proton shuttle,” including the ribose 2'-OH group of A2451 (in *Escherichia coli*), in the accelerated catalysis of peptide bond formation (ERLACHER AND POLACEK, 2008; PECH AND NIERHAUS, 2008). The ribosome is now utilized even for nonstandard peptide synthesis (Kang and Suga, 2008). However, such a complicated structure could not have existed from the beginning of biological evolution.

Since the free energy for aminoacyl esters (aminoacyl tRNAs) hydrolysis is greater than that for amide (peptide) bonds (CARPENTER, 1960), peptide bond formation between the two acyl esters attached to tRNAs occurs spontaneously in principle, once the amino group of the aminoacyl-tRNA and the carbonyl carbon of the peptidyl-tRNA are positioned properly. In fact, earlier attempts using aminoacyl mononucleotide esters (analogs of aminoacyl-tRNAs), in the absence of templates, resulted in peptide bond formation at high concentrations (0.4 M) (WEBER AND ORGEL, 1978, 1979). Relatively lower concentrations of the molecules (1-10 mM) can also work in the presence of a template that effectively increases the proximity of two amino acids (CHUNG ET AL., 1978; WEBER AND ORGEL, 1980). Thus, the rationale is already clear that proper

positioning of two activated amino acids is essential for peptide bond formation, and therefore, it is important to elucidate how these situations can be satisfied.

Nature has chosen aminoacyl esters (aminoacyl-tRNAs) from many candidates of activated amino acids for ribosomal peptide synthesis (Ibba and Söll, 2000; Schimmel, 1987), and from an evolutionary point of view, the following two facts should be highly considered. First, ribosomal peptide bond formation is performed between two tRNAs (Moore, 1991; Tamura, 2011a; Tamura and Alexander, 2004). All tRNAs have a conserved single-stranded CCA sequence (Jühling et al., 2009), which is important for the interactions with 23S rRNA during peptide bond formation (Moazed and Noller, 1991; Nissen et al., 2000; Tamura, 1994). The CCA-containing hairpin region is especially considered a primordial part of tRNA and known as a minihelix, which corresponds to one arm of the L-shaped structure of tRNA (Schimmel and Ribas de Pouplana, 1995; Schimmel et al., 1993; Tamura, 2008, 2009, 2010, 2011b; Tamura and Schimmel, 2004, 2006). Therefore, evolutionary models of peptide bond formation should be constructed on the basis of tRNA features. Second, the nucleotides constructing the PTC of the ribosome are distributed around the 23S rRNA (Ban et al., 2000; Nissen et al., 2000), which is composed of thousands of nucleotides (2904 nucleotides in case of *E. coli*). Exquisite folding of the 23S rRNA, along with ribosomal protein folding, enables proper spatial arrangement of the two CCA ends of tRNAs for efficient peptide bond formation (Nissen et al., 2000). However, such intricate RNA folding arrangements causing emergence of the ribosome PTC could not occur during the early period of evolution of biological systems.

In this study, in order to identify how simple the earliest protein-building machinery could have been, we have proposed a simple scaffold that enables the positioning of two apposed CCA ends of aminoacyl-tRNAs. Here, we focus on a plausible scaffold based on the RNA tetraplex structure that tethers the two CCA ends in a parallel fashion, because, as mentioned above, the rationale for peptide bond formation in terms of energy has already been solved.

2. Materials and methods

2.1. Preparation of oligonucleotides

Non-labeled oligodeoxyribo- and oligoribo-nucleotides were synthesized by Operon Biotechnologies Inc. (Tokyo, Japan). HPLC-purified dye-labeled oligonucleotides with fluorescein and DABCYL (where fluorescein is a fluorophore, and DABCYL, a quencher) were prepared by Japan Bio Services Co. LTD. (Saitama, Japan). The concentrations of the oligonucleotide solutions were determined from the absorbance at a wavelength of 260 nm.

2.2. CD spectroscopy

CD spectra (Balagurumoorthy et al., 1992; Hardin et al., 1991) of the oligodeoxyribo- or oligoribo-nucleotide solutions in Buffer A (100 mM Tris-HEPES (pH 7.4), 200 mM NaCl, 10 mM MgCl₂, and 0.1 mM EDTA) at room temperature were recorded on a J-805 Circular Dichroism Spectrometer (JASCO Corporation, Tokyo, Japan). The cell path length was 1 mm. The scanning speed was 50 nm·min⁻¹. The concentration of the oligodeoxyribo- or oligoribo-nucleotides was 10 mM.

2.3. Electrophoretic mobility shift assay (EMSA)

Oligodeoxyribo- and oligoribo-nucleotides (5'-d(TGGGGTTGGT)-3' and 5'-r(UGGGGUUGGU)-3') were prepared to achieve a final concentration of 200 mM in Buffer A, and incubated at room temperature for 1 h. The oligonucleotides were loaded on a 15% nondenaturing polyacrylamide gel with Buffer B (45 mM Tris-borate, 100 mM NaCl, and 5 mM MgCl₂). Electrophoresis was carried out in Buffer B at a constant

current of 15 mA and 4°C for 16 h, and stained with toluidine blue. The oligodeoxy-homonucleotides, dT₁₀, dT₂₀, and dT₃₀ were used as markers.

2.4. Fluorescence resonance energy transfer (FRET) analyses

The fluorophore, fluorescein, and the quencher DABCYL were attached to the 3'-termini of 5'-d(ACCA)-3', 5'-d(GTGT)-3', 5'-r(ACCA)-3', and 5'-r(GUGU)-3'. ACCA is a part of the 3'-terminal sequence of typical tRNAs, and GTGT or GUGU is selected as a negative control that does not bind to TGGT or UGGU of the 10-mer construct that can form a tetraplex structure. FRET measurements (Mergny, 1999; Mergny and Maurizot, 2001) in Buffer A were carried out on an FP-6200 spectrofluorometer (JASCO Corporation, Tokyo, Japan) using a 3-×-3-mm quartz cuvette. For emission spectra, the excitation wavelength was set at 490 nm. We mixed 100 mM non-labeled oligodeoxyribo- or oligoribo-nucleotides (5'-d(TGGGGTTGGT)-3' or 5'-r(UGGGGUUGGU)-3') with 0.1 or 0.15 mM fluorescein- and 50 mM DABCYL-labeled 5'-d(ACCA)-3' or 5'-r(ACCA)-3'. As negative controls, 100 mM non-labeled oligodeoxyribo- or oligoribo-nucleotides (5'-d(TGGGGTTGGT)-3' or 5'-r(UGGGGUUGGU)-3') were also mixed with 0.2 or 0.07 mM fluorescein- and 50 mM DABCYL-labeled 5'-d(GTGT)-3' or 5'-r(GUGU)-3'. The solution was preincubated at 4°C for 24 h before measurements.

3. Results

3.1. Concept of the design

Peptide bond formation on the ribosome occurs between the peptidyl residues of the peptidyl-tRNA and the aminoacyl residues of the aminoacyl-tRNA. Here, the two single-stranded CCA ends (terminal adenosine residues) of the tRNAs are positioned in close proximity through the interaction performed especially by ribosomal RNA. To substitute the role of the ribosomal RNA with a simple naturally formed scaffold, we designed an RNA tetraplex scaffold (G-rich sequence) to tether two aminoacyl tRNAs, by hybridizing the CCA ends to the appendix sequence complementary to the ACCA of tRNA. In contrast to the Watson-Crick (antiparallel) duplex, our tetraplex scaffold enables “parallel” positioning of two CCA ends that could be favorable for peptide bond formation between the two amino acids attached at the 3'-terminal adenosine (Fig. 1).

3.2. CD spectroscopy of a series of oligonucleotides that forms tetraplex structure

To reveal the conformational properties, we measured CD spectra of the G-rich oligodeoxyribonucleotides, which were used because of technical convenience for the initial survey. The CD spectra of 5'-d(TGGGGT)-3', 5'-d(TGGGGTGG)-3', 5'-d(TGGGGTGGT)-3' and 5'-d(TGGGGTTGGT)-3' exhibited a positive peak around 260 nm, and a negative peak around 240 nm (Fig. 2). These types of spectra are typical of a parallel tetraplex DNA consisting of a parallel four-stranded tetrameric structure (Balagurumoorthy et al., 1992; Hardin et al., 1991). On the other hand, the CD spectra of 5'-d(TTTTTTAA)-3' exhibited a positive peak around 276 nm, and a negative peak around 250 nm (Fig. 2), typical of unstructured single-stranded DNA (Balagurumoorthy

et al., 1992; Hardin et al., 1991). The 5'-d(TGGGGTTTGGT)-3' nucleotide exhibited a positive peak around 260 nm, and a negative peak around 240 nm, but also displayed a positive peak around 295 nm (Fig. 2), which is typical of tetraplex DNA consisting of an antiparallel four-stranded tetrameric structure (Balagurumoorthy et al., 1992; Hardin et al., 1991).

Considering the results of oligo DNAs, we also measured the CD spectra of oligoribonucleotides. The CD spectra of 5'-r(UGGGGU)-3', 5'-r(UGGGGUUGGU)-3', and 5'-r(UGGGGUUGGU)-3' exhibited a positive peak around 260 nm, and a negative peak around 240 nm (Fig. 3), indicating the formation of parallel tetraplex RNA. A mixture containing equal amount (5 mM) of 5'-r(UGGGGU)-3' and 5'-r(UGGGGUUGGU)-3', and that of 5'-r(UGGGGU)-3' and 5'-r(UGGGGUUGGU)-3', also showed a positive peak around 260 nm, and a negative peak around 240 nm (Fig. 3). These results suggest that these RNAs can form tetraplex RNA consisting of a parallel four-stranded tetrameric structure (Balagurumoorthy et al., 1992; Hardin et al., 1991).

3.3. Electrophoretic mobility analyses of d(TGGGGTTGGT) or r(UGGGGUUGGU)

In the following two sections, we focused on the 10-mer construct (5'-d(TGGGGTTGGT)-3' or 5'-r(UGGGGUUGGU)-3'). Here, dT or rU is inserted between the G-rich sequence and the appendix sequence (TGGT or UGGU), because the sixth dT or rU could provide proper structural flexibility.

The electrophoretic mobilities of 5'-d(TGGGGTTGGT)-3' and 5'-r(UGGGGUUGGU)-3' in Buffer A were analyzed on a 15% nondenaturing polyacrylamide gel (Fig. 4). The 5'-d(TGGGGTTGGT)-3' and 5'-r(UGGGGUUGGU)-3'

migrated with different electrophoretic mobilities from the same 10-mer control (dT₁₀). Based on homonucleotide markers (dT₂₀ and dT₃₀), the migrated 10-mer nucleotides correspond to a high molecular weight. These results, combined with those of the CD spectra, suggest that 5'-d(TGGGGTTGGT)-3' and 5'-r(UGGGGUUGGU)-3' can form parallel tetraplex structures.

3.4. FRET analysis of the interaction between the tetraplex construct and tetranucleotides

To show that the plausible tetraplex structure works as a scaffold, FRET analysis was used (Mergny, 1999; Mergny and Maurizot, 2001). The experimental system was composed of three molecules (Fig. 5): (A) 5'-d(TGGGGTTGGT)-3', 5'-d(ACCA)-fluorescein-3', and 5'-d(ACCA)-DABCYL-3', (B) 5'-d(TGGGGTTGGT)-3', 5'-d(GTGT)-fluorescein-3', and 5'-d(GTGT)-DABCYL-3', (C) 5'-r(UGGGGUUGGU)-3', 5'-r(ACCA)-fluorescein-3', and 5'-r(ACCA)-DABCYL-3', (D) 5'-r(UGGGGUUGGU)-3', 5'-r(GUGU)-fluorescein-3', and 5'-r(GUGU)-DABCYL-3'. In each case, the effect of causing plausible tetraplex scaffolds was monitored by measuring the resultant fluorescence. In cases of (A) and (C), FRET-mediated quenching between the fluorophore and the quencher was observed. However, in cases of (B) and (D), no significant change was observed in the fluorescence emission spectra (Fig. 5). These results are consistent with the inability of 5'-d(GTGT)-3' and 5'-r(GUGU)-3' to bind with 5'-d(TGGGGTTGGT)-3' and 5'-r(UGGGGUUGGU)-3', respectively. Combining the results, we have concluded that the 10-mer construct can work as a scaffold that tethers two CCA ends of the tRNAs.

4. Discussion

The constructs based on the RNA tetraplex, consisting of parallel four-stranded oligonucleotides, can be scaffolds that position the two aminoacyl-CCA ends of the tRNAs to facilitate peptide bond formation. The CCA and the discriminator (the fourth nucleotide from the 3'-terminal) are single-stranded structures, and this region (ACCA) can form base pairs with appending nucleotides (UGGU). The present results demonstrate that the tetraplex structure is a possible scaffold for efficient peptide bond formation between the two aminoacyl-tRNAs. Previously, a grossly simplified system with similar interactions between the ACCA of aminoacyl minihelix and the modified puromycin containing UGGU was proved to form peptide bond. However, this system used “artificial” 5'-5' phosphodiester linkage to connect puromycin to a short RNA chain (TAMURA AND SCHIMMEL, 2001). In contrast, in the present study, we have used a simplified nucleotide that can “naturally” form tetraplex scaffolds.

The DNA or RNA oligonucleotides containing a GGGG sequence can form tetraplex structures (ABOUL-ELA ET AL., 1992; KIM ET AL., 1991; LAUGHLAN ET AL., 1994; SEN AND GILBERT, 1988). Our idea is that the appendix sequence (UGGU) attached to the GGGG region can work as “parallel” templates of the ACCA (discriminator and the CCA end). The success of the present experiments was based in part on the presence of such “parallel” appendix templates attached to the G-rich tetraplex structure (Fig. 1). FRET analysis clearly demonstrated that ACCA specifically binds to the UGGU (TGGT) sequence, but GUGU (GTGT) does not. Similar principle is used in the modern ribosome, where the CCA end interacts with specific nucleotides in 23S rRNA (MOAZED AND NOLLER, 1991; NISSEN ET AL., 2000; TAMURA, 1994).

G-quadruplex structures are typically seen in telomeric DNA that consists of tandemly repeated sequences. The tetraplex structures of G-cluster sequences are stabilized by cations such as Na^+ or K^+ (Williamson et al., 1989). RNA quadruplex structures have been recently reported. G-quadruplex motifs towards the 5'-ends of 5'-UTRs, and immediately after the 3'-end of genes are supposed to control translation (Huppert et al., 2008). The RNA quadruplex is also suggested to link with HIV-1 RNA dimerization, which is crucial for the genomic incorporation of two RNA molecules into infectious virion particles (Linial and Miller, 1990). In addition, the RNA quadruplex is known to be a target structure of the Fragile X Mental Retardation Protein (FMRP), where the RGG box interacts with the RNA quadruplex with a high affinity (Darnell et al., 2001).

From an evolutionary standpoint, the existence of RNA quadruplex structures in mRNA could be significant (Halder and Hartig, 2011; Huppert et al., 2008). The original function of mRNA was probably to tether the two aminoacyl-RNAs to facilitate peptide bond formation (Crick et al., 1976). Codon-anticodon interactions are thought to have appeared later with the development of the second arm of the L-shaped tRNA structure (containing an anticodon region). A minihelix, which contains an amino acid-attachment site, the universal CCA sequence, is thought to be a primitive part of modern tRNA; primordial aminoacylation and peptide bond formation was established first without rigorous specificity. The RNA quadruplex seen in modern mRNA may be a remnant of its original function as a scaffold to tether small RNAs and cause some interactions. Another remnant may be also seen in a 19-mer oligonucleotide derived from the 5S rRNA of *E. coli* (strand III), which forms a tetrameric structure (Kim et al., 1991).

RNA tetraplex might have played an important role from the perspective of studying prebiotic evolution, even at the stage prior to the onset of peptide synthesis. Although, generally, it is quite difficult for the small-sized RNA to fold into a unique structure in water, RNA tetraplex has a specific tertiary structure despite the small size (comprises only ≤ 10 -mer-sized nucleotides). This unique structure could have been useful for interactions with other small molecules, including metal ions. The mass of the materials required during the elongation of all combinations of the nucleotide sequences in order to match even the size of tRNA (only ~ 75 -mer) would be one-hundredth of the mass of the Earth. This incredible fact suggests that long RNA molecules may have been produced by repeating the duplication of small-sized RNA molecules. Thus, the peculiar property of RNA tetraplex is quite noteworthy.

We have just presented the concept of a scaffold, and we did not attempt to form a peptide bond in this study. This is partly because peptide bond formation, in principle, occurs spontaneously between two activated amino acids such as aminoacyl esters in close proximity, and partly because our model did not provide the best positioning between the two CCAs. Although the RNA tetraplex structure shown here enables the formation of parallel scaffolds, the G-quadruplex has a square shape, with ~ 10 Å on each side. To attain close proximity under these constraints, structural flexibility must be added to our simple model. In fact, chemical reactions using DNA templates between two reactive groups at the DNA terminals proceeded efficiently even when one of the reactive regions was far from the other reactive end of the template (CALDERONE AND LIU, 2004; GARTNER ET AL., 2003; LI ET AL., 2004). The next stage is to fine-tune the

model for actual peptide bond formation based on this concept, which could have been used in the initial evolution stages of protein biosynthesis.

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References

- Aboul-ela, F., Murchie, A.I.H., Lilley, D.M.J. 1992. NMR study of parallel-stranded tetraplex formation by the hexadeoxynucleotide d(TG₄T). *Nature* 360, 280-282.
- Balagurumoorthy, P., Brahmachari, S.K., Mohanty, D. et al. 1992. Hairpin and parallel quartet structures for telomeric sequences. *Nucleic Acids Res.* 20, 4061-4067.
- Ban, N., Nissen, P., Hansen, J., Moore, P.B., Steitz, T.A. 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* 289, 905-920.
- Calderone, C.T., Liu, D.R. 2004. Nucleic acid-templated synthesis as a model system for ancient translation. *Curr. Opin. Chem. Biol.* 8, 645-653.
- Carpenter, F.H. 1960. The free energy change in hydrolytic reactions: the non-ionized compound convention. *J. Am. Chem. Soc.* 82, 1111-1122.
- Chung, S.K., Copsey, D.B., Scott, A.I. 1978. Template-directed organic synthesis. A model for the peptidyl transfer reaction of protein biosynthesis. *Bioorg. Chem.* 7, 303-312.
- Crick, F.H.C., Brenner, S., Klug, A., Piezenik, G. 1976. A speculation on the origin of protein synthesis. *Origins of Life* 7, 389-397.
- Darnell, J.C., Jensen, K.B., Jin, P., Brown, V., Warren, S.T., Darnell, R.B. 2001. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107, 489-499.
- Erlacher, M.D., Polacek, N. 2008. Ribosomal catalysis: the evolution of mechanistic concepts for peptide bond formation and peptidyl-tRNA hydrolysis. *RNA Biol.* 5, 5-12.

- Gartner, Z.J., Grubina, R., Calderone, C.T., Liu, D.R. 2003. Two enabling architectures for DNA-templated organic synthesis. *Angew. Chem. Int. Ed.* 42, 1370-1375.
- Halder, K., Hartig, J. S. 2011. RNA quadruplexes. *Met. Ions Life Sci.* 9, 125-139.
- Hardin, C.C., Henderson, E., Watson, T. et al. 1991. Monovalent cation induced structural transitions in telomeric DNAs: G-DNA folding intermediates. *Biochemistry* 30, 4460-4472.
- Huppert, J.L, Bugaut, A., Kumari, S., Balasubramanian, S. 2008. G-quadruplexes: the beginning and end of UTRs. *Nucleic Acids Res.* 36, 6260-6268.
- Ibba, M., Söll, D. 2000. Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem.* 69, 617-650.
- Jühling, F., Mörl, M., Hartmann, R.K., Sprinzl, M., Stadler, P.F., Pütz, J. 2009. tRNAdb 2009: compilation of tRNA sequences and tRNA genes. *Nucleic Acids Res.* 37(Database-Issue), 159-162.
- Kang, T.-J., Suga, H. 2008. Ribosomal synthesis of nonstandard peptides. *Biochemistry and Cell Biology* 86, 92-99.
- Kim, J., Cheong, C., Moore, P.B. 1991. Tetramerization of an RNA oligonucleotide containing a GGGG sequence. *Nature* 351, 331-332.
- Korostelev, A., Noller, H.F. 2007. The ribosome in focus: new structures bring new insights. *Trends Biochem. Sci.* 32, 434-441.
- Laughlan, G., Murchie, A.I., Norman, D.G., Moore, M.H., Moody, P.C., Lilley, D.M., Luisi, B. 1994. The high-resolution crystal structure of a parallel-stranded guanine tetraplex. *Science* 265, 520-524.
- Li, X.Y., Gartner, Z.J., Tse, B.N., Liu, D.R. 2004. Translation of DNA into synthetic N-acyloxazolidines. *J. Am. Chem. Soc.* 126, 5090-5092.

- Linial, M.L., Miller, A.D. 1990. Retroviral RNA packaging: sequence requirements and implications. *Curr. Top. Microbiol. Immunol.* 157, 125-152.
- Mergny, J.L. 1999. Fluorescence energy transfer as a probe for tetraplex formation: the i-motif. *Biochemistry* 38, 1573-1581.
- Mergny, J.L., Maurizot, J.C. 2001. Fluorescence resonance energy transfer as a probe for G-quartet formation by a telomeric repeat. *Chembiochem* 2, 124-132.
- Moazed, D., Noller, H.F. 1991. Sites of interaction of the CCA end of peptidyl-tRNA with 23S rRNA. *Proc. Natl. Acad. Sci. USA* 88, 3725-3728.
- Moore, P.B. 1991. Ribosomes. *Curr. Opin. Struct. Biol.* 1, 258-263.
- Nissen, P., Hansen, J., Ban, N., Moore, P.B., Steitz, T.A. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science* 289, 920-930.
- Noller, H.F. 1993. On the origin of the ribosome: coevolution of subdomains of tRNA and rRNA. *The RNA World*, Cold Spring Harbor Laboratory Press, Plainview, NY, 137-156.
- Noller, H.F., Hoffarth, V., Zimniak, L. 1992. Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* 256, 1416-1419.
- Pech, M., Nierhaus, K.H. 2008. Ribosomal peptide-bond formation. *Chem. Biol.* 15, 417-419.
- Ramakrishnan, V. 2008. What we have learned from ribosome structures. *Biochem. Soc. Trans.* 36, 567-574.
- Schimmel, P. 1987. Aminoacyl tRNA synthetases: general scheme of structure-function relationships in the polypeptides and recognition of transfer RNAs. *Annu. Rev. Biochem.* 56, 125-158.

- Schimmel, P., Giegé, R., Moras, D., Yokoyama, S. 1993. An operational RNA code for amino acids and possible relationship to genetic code. *Proc. Natl. Acad. Sci. USA* 90, 8763-8768.
- Schimmel, P., Ribas de Pouplana, L. 1995. Transfer RNA: from minihelix to genetic code. *Cell* 81, 983-986.
- Schlueder, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., Yonath, A. 2000. Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell* 102, 615-623.
- Sen, D., Gilbert, W. 1988. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. *Nature* 334, 364-366.
- Steitz, T.A. 2008. A structural understanding of the dynamic ribosome machine. *Nat. Rev. Mol. Cell. Biol.* 9, 242-253.
- Tamura, K. 1994. The role of the CCA sequence of tRNA in the peptidyl transfer reaction. *FEBS Lett.* 353, 173-176.
- Tamura, K. 2008. Origin of amino acid homochirality: relationship with the RNA world and origin of tRNA aminoacylation. *Biosystems* 92, 91-98.
- Tamura, K. 2009. Molecular handedness of life: significance of RNA aminoacylation. *J. Biosci.* 34, 991-994.
- Tamura, K. 2010. Amino acid homochirality and the RNA world: necessities for life on Earth. *J. Cosmol.* 5, 883-889.
- Tamura, K. 2011a. Ribosome evolution: Emergence of peptide synthesis machinery. *J. Biosci.* 36, 921-928.

- Tamura, K. 2011b. Molecular basis for chiral selection in RNA aminoacylation. *Int. J. Mol. Sci.* 12, 4745-4757.
- Tamura, K., Alexander, R.W. 2004. Peptide synthesis through evolution. *Cell. Mol. Life Sci.* 61, 1317-1330.
- Tamura, K., Schimmel, P. 2001. Oligonucleotide-directed peptide synthesis in a ribosome- and ribozyme-free system. *Proc. Natl. Acad. Sci. USA* 98, 1393-1397.
- Tamura, K., Schimmel, P. 2004. Chiral-selective aminoacylation of an RNA minihelix. *Science* 305, 1253.
- Tamura, K., Schimmel, P.R. 2006. Chiral-selective aminoacylation of an RNA minihelix: mechanistic features and chiral suppression. *Proc. Natl. Acad. Sci. USA* 103, 13750-13752.
- Weber, A.L., Orgel, L.E. 1978. The formation of peptides from the 2'(3')-glycyl ester of a nucleotide. *J. Mol. Evol.* 11, 189-198.
- Weber, A.L., Orgel, L.E. 1979. The formation of dipeptides from amino acids and the 2'(3')-glycyl ester of an adenylate. *J. Mol. Evol.* 13, 185-192.
- Weber, A.L., Orgel, L.E. 1980. Poly(U)-directed peptidebond formation from the 2'-(3')-glycyl esters of adenosine derivatives. *J. Mol. Evol.* 16, 1-10.
- Williamson, J.R., Raghuraman, M.K., Cech, T.R. 1989. Monovalent cation-induced structure of telomeric DNA: the G-quartet model. *Cell* 59, 871-880.
- Wimberly, B.T., Brodersen, D.E., Clemons, W.M. Jr., Morgan-Warren, R.J., Carter, A.P., Vornrhein, C., Hartsch, T., Ramakrishnan, V. 2000. Structure of the 30S ribosomal subunit. *Nature* 407, 327-339.

Figure Legends

Fig. 1. Concept of RNA tetraplex as a primordial peptide synthesis scaffold. The RNA tetraplex scaffold (G-rich sequence) tethers two aminoacyl tRNAs, by hybridizing the CCA ends of minihelices to the appendix complementary sequence in a “parallel” fashion, where the positioning of the two amino acids attached at the 3'-terminal adenosine could favor peptide bond formation.

Fig. 2. CD spectra of oligodeoxyribonucleotides. (A) 10 mM 5'-d(TGGGGT)-3', (B) 10 mM 5'-d(TGGGGTGG)-3', (C) 10 mM 5'-d(TTTTTTAA)-3', (D) 10 mM 5'-d(TGGGGTGGT)-3', (E) 10 mM 5'-d(TGGGGTTGGT)-3', and (F) 10 mM 5'-d(TGGGGTTTGGT)-3'. The measurements were performed in Buffer A (100 mM Tris-HEPES (pH 7.4), 200 mM NaCl, 10 mM MgCl₂, and 0.1 mM EDTA) at room temperature.

Fig. 3. CD spectra of oligoribonucleotides. (A) 10 mM 5'-r(UGGGGU)-3', (B) 10 mM 5'-r(UGGGGUGGU)-3', (C) 10 mM 5'-r(UGGGGUUGGU)-3', (D) Mixture of 5 mM 5'-r(UGGGGU)-3' and 5 mM 5'-r(UGGGGUGGU)-3', and (E) Mixture of 5 mM 5'-r(UGGGGU)-3' and 5 mM 5'-r(UGGGGUUGGU)-3'. The measurements were performed in Buffer A (100 mM Tris-HEPES (pH 7.4), 200 mM NaCl, 10 mM MgCl₂, and 0.1 mM EDTA) at room temperature.

Fig. 4. Electrophoretic mobility of r(UGGGGUUGGU) (lane 1) and d(TGGGGTTGGT) (lane 2). The oligonucleotides were separated by 15% native PAGE. The gel was stained with 0.04% toluidine blue. dT₁₀, dT₂₀ and dT₃₀ were used as size markers.

Fig. 5. FRET analysis for the formation of complex between quadruplex and d(ACCA) or r(ACCA). d(TGGGGTTGGT) was mixed with (A) d(ACCA) and (B) d(GTGT), which has fluorescein and DABCYL at the 3'-end. r(UGGGGUUGGU) was mixed with (C) r(ACCA) and (D) r(GUGU), which has fluorescein and DABCYL at the 3'-end. Complex formation-induced quenching effects were observed. The excitation wavelength was 490 nm.

Fig 1

G-quadruplex scaffold

Aminoacyl mini-helices

A : d(TGGGGT)

B : d(TGGGGTGG)

C : d(TTTTTTAA)

Fig 2

D : d(TGGGGTGGT)

E : d(TGGGGTTGGT)

F : d(TGGGGTTTGGT)

A : 6mer (r(UGGGGU))

B : 9mer (r(UGGGGUGGU))

C : 10mer (r(UGGGGUUGGU))

Fig 3

D : 6mer + 9mer

E : 6mer + 10mer

Fig4

dT₃₀

dT₂₀

dT₁₀

1

2

A Fig5 **B** **C** **D**