Review

Ribosome evolution: Emergence of peptide synthesis machinery

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Proteins, the main players in current biological systems, are produced on ribosomes by sequential amide bond (peptide bond) formations between amino-acid-bearing tRNAs. The ribosome is an exquisite super-complex of RNA-proteins, containing more than 50 proteins and at least 3 kinds of RNAs. The combination of a variety of side chains of amino acids (typically 20 kinds with some exceptions) confers proteins with extraordinary structure and functions. The origin of peptide bond formation and the ribosome is crucial to the understanding of life itself. In this article, a possible evolutionary pathway to peptide bond formation machinery (proto-ribosome) will be discussed, with a special focus on the RNA minihelix (primordial form of modern tRNA) as a starting molecule. Combining the present data with recent experimental data, we can infer that the peptidyl transferase center (PTC) evolved from a primitive system in the RNA world comprising tRNA-like molecules formed by duplication of minihelix-like small RNA.

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1. The ribosome and tRNA

A ribosome consists of large and small subunits, and each ribosomal subunit has separate functions (Moore 1991; Tamura and Alexander 2004). Peptide bond formation occurs at the peptidyl transferase center (PTC) of the large subunit (figure 1), whereas mRNA sequences are decoded on the small subunit (Noller 1993). mRNA decoding contributes to the specificity of protein synthesis on the ribosome. The evolutionary standpoint indicates that the simple function of peptide bond formation came first, and that the specifications based on the codon sequence came later (Schimmel and Alexander 1998).

Corresponding to the functionally separate regions of the ribosome, tRNA is constructed by the assembly of dependent domains. A minihelix is a half-sized tRNA molecule composed of an acceptor stem and TΨC stem-loop (without an anticodon), which corresponds to one arm of the L-shaped structure of tRNA (Schimmel *et al.* 1993; Schimmel and Ribas de Pouplana 1995; Tamura 2008, 2009, 2010, 2011; Tamura and Schimmel 2004, 2006) (figure 1). Aminoacyl minihelices can work as substrates in peptide bond formation on the large ribosomal subunit (Sardesai *et al.* 1999), and also minihelices can work as substrates of many aminoacyl tRNA synthetases (Francklyn and Schimmel 1989; Frugier *et al.* 1994; Martinis and Schimmel 1997; Musier-Forsyth and Schimmel 1999), which are key enzymes catalysing amino acid attachment to tRNAs (Schimmel 1987). The other arm of the L-shape tRNA contains an anticodon, which interacts with a codon on mRNAs (figure 1). Hence, the origin of minihelix-based peptide bond formation and its evolution into the ribosome (large subunit) are key issues for better understanding of the foundation of biological peptide bond formation.

2. Peptide bond formation on the ribosome

The current biological system may have been derived from an RNA-based system. This is clearly evidenced by the atomic-level structure of the modern ribosome (Ban *et al.* 2000; Schluenzen *et al.* 2000; Wimberly *et al.* 2000; Korostelev and Noller 2007; Ramakrishnan 2008; Steitz 2008). The PTC is composed of only RNA molecules, and no proteins was detected closer than approximately 18 Å to

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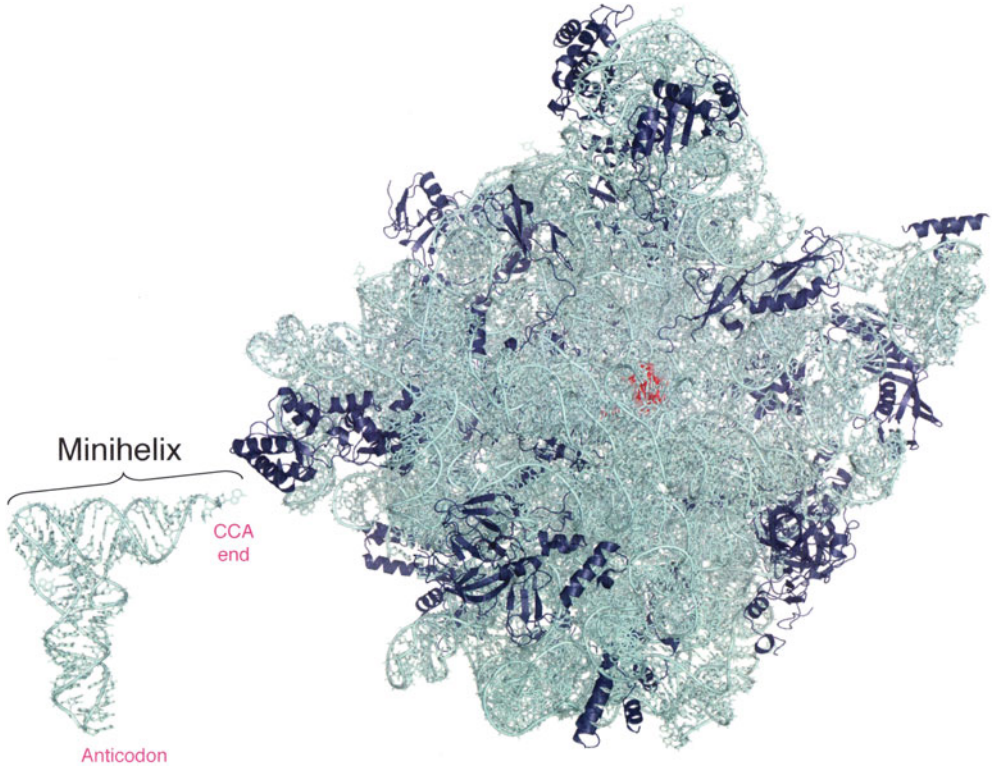

Figure 1. Structures of tRNA and the large ribosomal subunit. The tertiary structure of yeast $tRNA^{Phe}$ (PDB ID 1EHZ) and the large ribosomal subunit from *Haloarcula marismortui* (PDB ID 1JJ2) are shown. RNA is shown in light blue in the stick-ribbon model, and the proteins are shown in deep purple. The PTC located on the large ribosomal subunit is indicated in red. One arm of the L-shaped tRNA contains the CCA end, which is called a 'minihelix,' and the other arm of the L-shaped tRNA contains an anticodon. Figures of the same scale were rendered using PyMOL.

the PTC (figure 1) (Ban *et al.* 2000; Nissen *et al.* 2000), which strengthens the hypothesis that the ribosome is a ribozyme (Noller *et al.* 1992). Mainly, the PTC is constituted around the domain V of 23S rRNA, with which the CCA ends of two tRNAs interact, and peptide bond formation occurs (Nissen *et al.* 2000).

However, despite the completion of the high-resolution structure of the ribosome, the real mechanism underlying peptide bond formation remains unclear. The role of A2451 (in *Escherichia coli*) of 23S rRNA was suggested from the ribosome structure as A2451 was closest to the PTC (Nissen *et al.* 2000). From the standpoint of the acid–base catalysis mechanism, it seemed that the N1 or N3 of the adenine ring of A2451 was a good candidate for abstracting the proton from the α-amino group of aminoacyl-tRNA and that the nitrogen atom with increased nucleophilicity facilitates the attack on the carbonyl carbon of the ester of peptidyl-tRNA (figure 2a) (Nissen *et al.* 2000). However, base substitutions in A2451 had no critical effects on the activity (Polacek *et al.* 2001; Thompson *et al.* 2001; Bieling *et al.* 2006). On the other hand, recent experimental data strongly suggest that the hydrogen bond network, including the ribose 2′-OH group of A2451, facilitates proper orientation of the α-amino group for nucleophilic attack (figure 2b), because the lack of a hydroxyl group at the 2′-position of A2451 inhibits peptide bond formation (Erlacher and Polacek 2008; Pech and Nierhaus 2008).

The mechanistic models described previously are based on the crystallographic structural feature of the PTC, and no proteins were shown to be closer than approximately 18 Å from the PTC. However, because a ribosome is a dynamic entity, the conformation of ribosomal constituents that was not evident in the X-ray crystallographic image must also be taken consideration. In fact, neutron scattering analysis suggested the dynamic movement of ribosomal proteins. The L2 protein (a protein of the large subunit) moved approximately 30 Å into the PTC when comparing the large (50S) ribosomal subunits and the whole (70S) ribosome (Willumeit *et al.* 2001). In terms of the chemical reactions, abstracting the proton from the α-amino group of aminoacyl-tRNA increases the chemical reactivity that stimulates peptide bond formation, as mentioned previously in the discussion on the N1 or the N3 of the adenine ring of A2451. It has long been suggested that histidine residue in the C-terminal region of L2 plays a significant role in the translational activity of ribosomes (Cooperman *et al.* 1995). The pK_a value of the conjugate acid of imidazole is approximately 7, and if the dynamic movement of L2

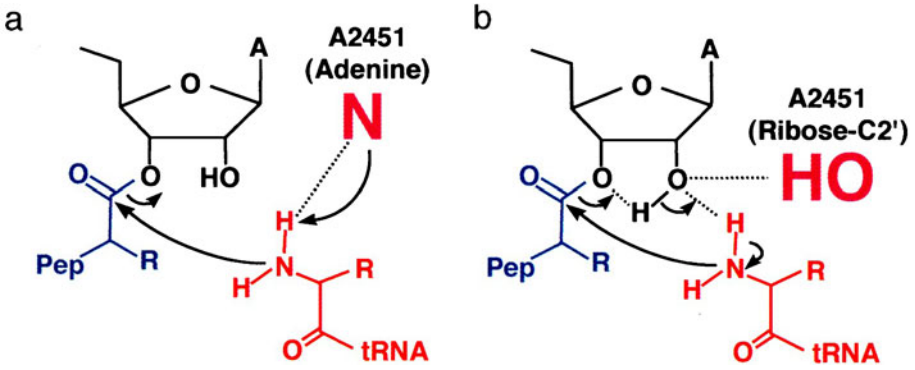

Figure 2. Proposed mechanisms of peptide bond formation on the ribosome. (**a**) N1 or N3 of A2451 (adenine ring) abstracts a proton from the α-amino group of the amino acid and increases the nucleophilicity of the nitrogen atom. (**b**) The 2′-OH of the A2451 hydrogen-bonds the 2′-O of the peptidyl-tRNA, and a proton moves from the α-amino group of the aminoacyl-tRNA to the 2′-O of the peptidyl-tRNA, giving its own proton to the 3′-O of the peptidyl-tRNA. The mechanism is called 'proton shuttle.'

allows the histidine to position close to the PTC during the reaction, it may contribute to improving peptide bond formation. Thus, it is important to analyse biomolecular interactions in terms of the dynamic nature of the structure.

3. Origin of peptide bond formation and the RNA world

Minihelix-based peptide bond formation may have evolved in a simple manner. However, similar to how difficult it is to explain the emergence of minihelix, it is difficult to explain the origin of the RNA world. Hence, before discussing the peptide bond formation in the RNA world, I will present a plausible scenario that illustrates the peptide bond formation at a simpler stage (pre-RNA world).

According to the second law of thermodynamics, peptide bond formation does not occur spontaneously. For it to occur, amino acids must be 'activated'. For activation, geographical energy sources on the Earth, i.e. light from the sun, geothermal energy and pressure in thermal vents, could have been used. Reactions on clay (Paecht-Horowitz *et al.* 1970) and/or dry mixtures of amino acids (Fox and Harada 1958) were effective in stimulating peptide formation. Iron sulphate may have been used to originate autotrophic metabolism in an 'iron-sulphur world' (Wächtershäuser 1992). Sulphur may have played an important role as thioesters. Thioesters are high-energy compounds and used in modern biological systems (e.g. acetyl-CoA); they can serve as a driving force in peptide bond formation. According to this rationale, non-ribosomal peptide synthesis occurs along with the ribosome-based system in some organisms. In addition to the simple activated form of amino acids as thioesters, the congruence and the compatibility of thioesters with the modern biological system should be emphasized. The 'thioester world' hypothesis (de Duve 1995) is a good bridge to the ribosome-based peptide synthesis, and elucidation of the process from the thioester world to the RNA world will cast new light on the origin and evolution of life.

In modern biological systems, DNA is used to generate proteins and is also generated by proteins (Crick 1970). Discoveries by Cech's and Altman's groups (Kruger *et al.* 1982; Guerrier-Takada *et al.* 1983) suggested that RNA not only carries genetic information but also functions as a catalyst, leading to the RNA world hypothesis (Gilbert 1986). The RNA world hypothesis proposes that a self-contained biological world that evolved from RNA molecules acting as genetic materials and biocatalysts predates the emergence of the current biological system based on DNA, RNA and proteins. This hypothesis also provided an answer to the so-called contemporary version of the classic chicken-or-egg conundrum; that is, which evolved first, nucleic acids or proteins? Therefore, the origin of modern biological systems should be discussed taking this standpoint into consideration. Template-dependent peptide bond formation and *in vitro* selection methods lead to the possibility of the RNA world. Activated amino acids attached to mononucleotide analogs can form peptide bonds (Weber and Orgel 1978, 1979). Furthermore, a ribozyme capable of catalysing peptide bond formation with 196 nucleotides was isolated (Zhang and Cech 1997). However, spontaneous formation of such long ribozymes is quite unthinkable.

In contrast to this ribozyme, tRNA is typically composed of about 75 nucleotides (Jühling *et al.* 2009). Even in this case, direct formation of full-length tRNA is not likely to occur because then the mass of the required materials would be one-hundredth of that of the whole Earth when considering all combinations of nucleotide sequences

required for forming full-length tRNA (4^{75}). However, a minihelix is a half-sized molecule of tRNA (typically 35 nucleotides); it corresponds to one arm of the L-shaped three-dimensional structure of tRNA (Schimmel *et al.* 1993). The total mass of the nucleotides required for all combinations during minihelix formation (4^{35}) is only 22 g. Therefore, nature could actually have utilized all combinations of nucleotide sequences in the elongation process of the minihelix.

4. Model of peptide bond formation based on minihelix

A model system has been proposed by focusing on the single-stranded CCA sequence that equally exists in a minihelix and full-length tRNA (Tamura and Schimmel 2001). The single-stranded CCA sequence is conserved in all tRNAs (Jühling *et al.* 2009), and the peptidyl transferase activity of the PTC of the large ribosomal subunit can be produced by base pairing between 23S rRNA nucleotides and the universal CCA sequence of tRNA (Moazed and Noller 1991; Tamura 1994; Nissen *et al.* 2000). The crystal structure of the complex of the large subunit with an analog of the tetrahedral intermediate (CCdA-p-Puromycin) clearly showed the Watson–Crick base pairings of CC from CCA and GG from 23S rRNA (Nissen *et al.* 2000). In the model system, the interaction between CCA of tRNA and 23S rRNA is retained, and other components (RNAs and proteins) are eliminated (Tamura and Schimmel 2001).

Puromycin (Pm) has structure similar to 3′-L-tyrosyl-adenosine, which can be an analog of aminoacyl-tRNA. Pm attached to UGGU through a 5′-5′ phosphodiester linkage produces Pm-UGGU, which represents the minimum essence of both aminoacyl-tRNA and rRNA (Tamura and Schimmel 2001). On the other hand, L-alanyl-minihelix was acetylated at the amino group, and the resulting *N*-acetyl-L-alanyl-minihelix was used as an analog of peptidyl-tRNA, because its structure has an amide bond (peptide bond) arising from the *N*-acetylation. On the mixing of *N*-acetyl-L-alanyl-minihelix with Pm-UGGU, a lone pair of the amino group of *O*-methyl-L-tyrosine residue of Pm should attack carbonyl carbon of *N*-acetyl-L-alanyl-minihelix. The trial resulted in peptide bond formation (figure 3). It was true that the formation was dependent on base complementarity between Pm-UGGU and the minihelix 3′-CCA (Tamura and Schimmel 2001). In addition, the imidazole in the reaction mixture acted as a stimulant that improved the efficiency of peptide bind formation. If this is intrinsic, the nitrogen atom of imidazole could act as a general base catalyst to abstract the proton from the amino group of the *O*-methyl-L-tyrosine residue of Pm. Thus, the origin of the ribosome should be investigated taking into consideration both proximity and catalyst effects.

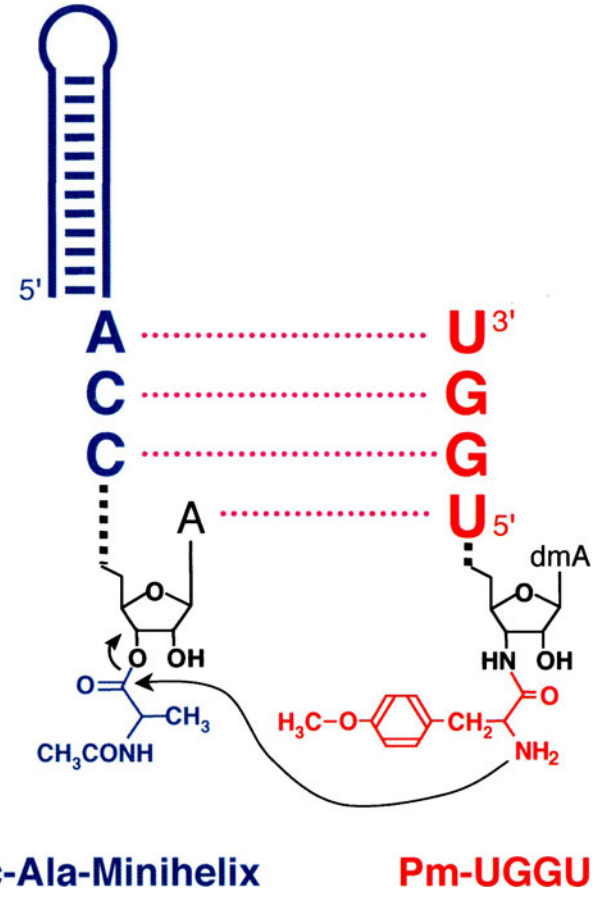

Figure 3. Minihelix-based model reactions on peptide bond formation. Puromycin-containing oligonucleotide substrates (Pm-UGGU) and *N*-acetyl-L-alanyl-minihelix can be hybridized for peptide bond formation. dmA denotes *N,N*-dimethyladenosine.

5. Route to the ribosome

What is the ultimate route to the modern ribosome? The answer remains unclear and many studies need to be performed. However, the crystal structure of the large ribosomal subunit of *Deinococcus radiodurans* may provide further insight into ribosome origin (Agmon 2009; Agmon *et al.* 2005).

The PTC is formed by a pocket-like symmetrical RNA dimer that is composed of two L-shaped RNA units (Agmon 2009; Agmon *et al.* 2005). The L-shaped RNA is similar in size to tRNA; this suggests the roles of tRNA as an amino acid carrier and in amino acid elongation. Proto-tRNA can be produced by duplicating the minihelix-like structures (Tanaka and Kikuchi 2001). The L-shape is quite important in modern tRNA, but it may have come from the original requirement of the foundation of the peptide bond forming system itself. The two tRNAs can be positioned properly on the ribosome because of the L-shape of tRNA, which allows for interaction between protein factors at each step of the

ribosomal cycle (Moore 1991). In a similar fashion, two L-shaped RNAs can be placed symmetrically to build a scaffold that facilitates proper positioning of the two reactants (figure 4). In that sense, the first functional RNA in the proto-ribosomal system comprised tRNA-like molecules formed by duplication of small RNA like minihelices. Then, some of the tRNA molecules would have evolved to contribute to the formation of PTC, and other molecules would have evolved to function as tRNA. The tRNA L-shape is formed by a combination of tertiary interactions between D-arm and T-arm. The interaction of GG in the D-loop and ΨC in the T-loop is essential (Kim *et al.* 1974; Robertus *et al.* 1974). Such kinds of interactions are important in elucidating the evolution of RNA structure formation. The symmetrical association of the two units produces a stereochemistry favourable for peptide bond formation to occur in a manner similar to that in the modern ribosome.

6. Conclusion

Peptide bond formation on the ribosome is a type of a unique phenomenon in biological systems. Both RNA molecules and proteins work co-operatively to perform the task of maintaining life. The modern crystallographic images elucidated the complex and phenomenal structure of the ribosome. However, wherever life originated, we are certain that the modern biological system is a product of a

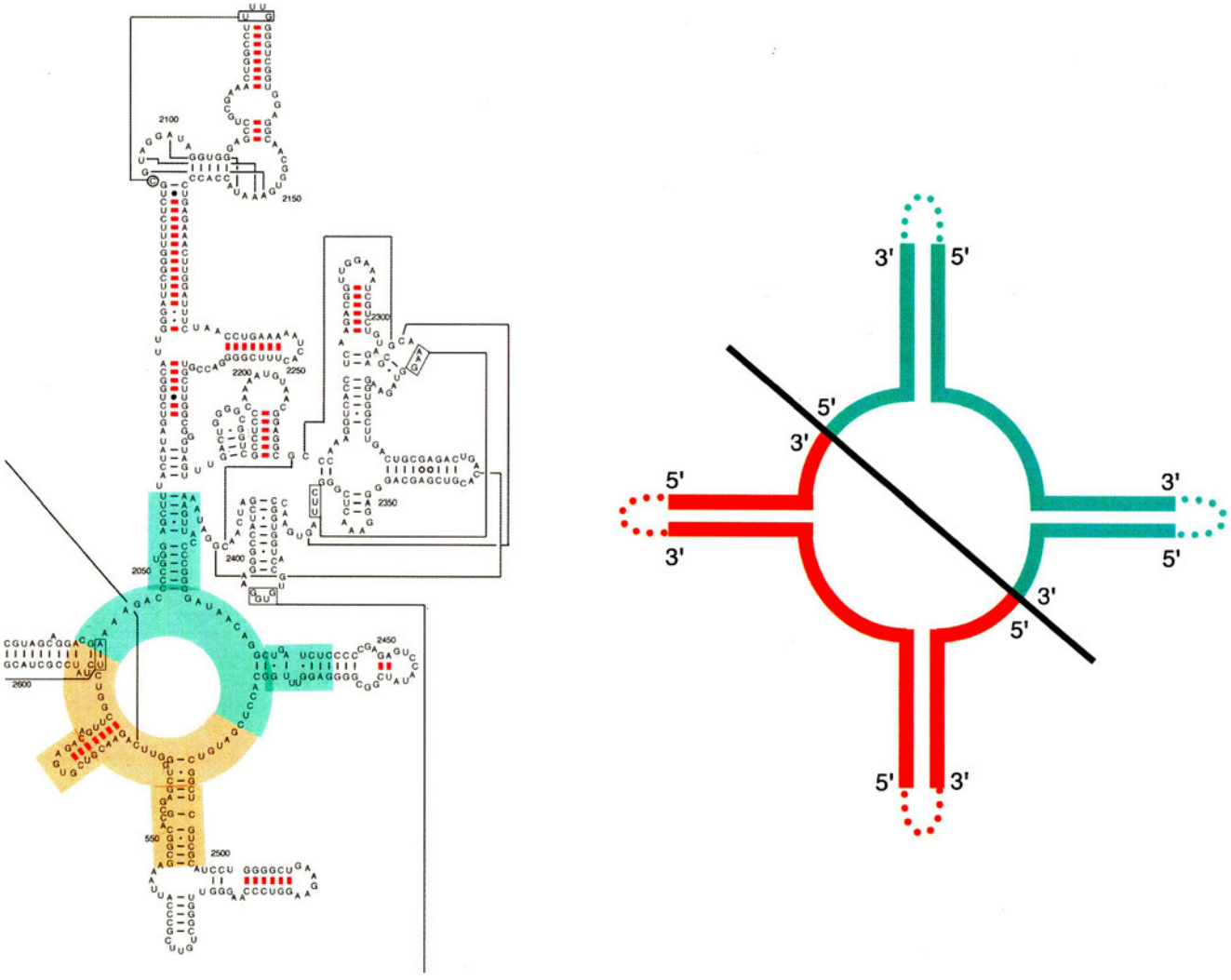

Figure 4. Domain V of 23S rRNA from *Deinococcus radiodurans*. The secondary structure is derived from the Comparative RNA Web (CRW) site of the University of Texas, Austin (*http://www.rna.ccbb.utexas.edu/SIM/4C/mfold_Eval/structures/23S_efn2/d.233.b.D.radiodurans.rrnC.pdf/*) (Cannone *et al.* 2002; Doshi *et al.* 2004). The following modifications were made: symmetrical region of the central loop of domain V is marked in light blue and orange. The conceptual drawing is shown on the right.

long evolutionary history. Therefore, this study aimed to illustrate a possible evolutionary route to the modern ribosome by especially focusing on peptide bond formation.

To attain a goal, we had to determine the appropriate starting point of this study. As we currently know, a high-resolution image of the crystal structure of the ribosome is available and shows that the symmetrical RNA composed of two L-shaped RNA units forms the PTC. The interaction between the PTC and tRNA led to peptide bond formation. If life started or went through the initial stages of evolution in the manner proposed by the RNA world hypothesis, tRNA and rRNA (the PTC) should have the same origin. In this perspective, the proto-tRNA and proto-PTC originated from the same source. Modern tRNA has an L-shaped structure. Similarly, the modern PTC constitutes two L-shaped RNA units. Therefore, it is possible that both tRNA and the RNA composed of the PTC have the same origin.

On the other hand, many experimental data suggest that modern tRNA evolved from a simple hairpin RNA, like a minihelix. The minihelix can be a vestige of modern tRNA, and it can be substrates of modern aminoacyl tRNA synthetase and of peptidyl transferase on the ribosome. If the modern biological system evolved with a concrete continuity from the primitive one, considering the RNA minihelix as the molecule of origin can definitely lead us to attaining our goal of elucidating the emergence of the modern peptide synthesis machinery, the ribosome.

Thus, combining these facts, it is plausible that the tRNA-like molecules are primordial ones composed of both proto-PTC and proto-tRNAs. The symmetrical RNA dimer composed of two L-shaped RNA units should be verified experimentally in future studies.

In the evolutionary process from proto-ribosomes to modern-ribosomes, proteins played the same roles as those of RNAs. Because release factors mimic the tRNA structure and recognize stop codons in mRNA, the concept of structural mimicry between translational protein factors and tRNA is a key factor in the transition from the RNA world to the protein world (Ito *et al.* 1996, 2000).

Elucidation of the origin of peptide bond forming machinery is directly related to the understanding 'life' itself. There are many obstacles before us, but future research will overcome these. Going back to the ribosome, it is a complex structure made of many proteins and RNAs. Although the crystal structure of the PTC strongly suggests that the ribosome is a ribosome, peptide bond formation catalysed by rRNA alone has not been demonstrated yet. In this sense, we may conclude that the ribosome evolved from a long period of RNA-protein interactions. However, to reach the intrinsic key point that the emergence of peptide synthesis machinery is based on the RNA, it is inevitable to conduct an experiment that demonstrates peptide bond formation using rRNA alone. In addition, a trial that extracts the essential part of the ribosome, i.e., the PTC, would be quite important. Therefore, it is crucial to demonstrate that L-shaped tRNA-like molecules can function as proto-ribosomes without the help of proteins.

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References

Agmon I 2009 The dimeric proto-ribosome: Structural details and possible implications on the origin of life. *Int. J. Mol. Sci.* **10** 2921–2934

Agmon I, Bashan A, Zarivach R and Yonath A 2005 Symmetry at the active site of the ribosome: structural and functional implications. *Biol. Chem.* **386** 833–844

Ban N, Nissen P, Hansen J, Moore PB and Steitz TA 2000 The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289** 905–920

Bieling P, Beringer M, Adio S and Rodnina MV 2006 Peptide bond formation does not involve acid-base catalysis by ribosomal residues. *Nat. Struct. Mol. Biol.* **13** 423–428

Cannone JJ, Subramanian S, Schnare MN, Collett JR, D'Souza LM, Du Y, Feng B, Lin N, *et al.* 2002 The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BioMed Central Bioinformatics* **3** 2

Cooperman BS, Wooten T, Romero DP and Traut RR 1995 Histidine 229 in protein L2 is apparently essential for 50S peptidyl transferase activity. *Biochem. Cell Biol.* **73** 1087–1094

Crick F 1970 Central dogma of molecular biology. *Nature (London)* **227** 561–563

de Duve C 1995 *Vital dust: Life as a cosmic imperative* (New York, NY: Basic Books)

Doshi KJ, Cannone JJ, Cobaugh CW and Gutell RR 2004 Evaluation of the suitability of free-energy minimization using nearest-neighbor energy parameters for RNA secondary structure prediction. *BioMed Central Bioinformatics* **5** 105

Erlacher MD and Polacek N 2008 Ribosomal catalysis: the evolution of mechanistic concepts for peptide bond formation and peptidyl-tRNA hydrolysis. *RNA Biol.* **5** 5–12

Fox SW and Harada K 1958 Thermal copolymerization of amino acids to a product resembling protein. *Science* **128** 1214

Francklyn C and Schimmel P 1989 Aminoacylation of RNA minihelices with alanine. *Nature (London)* **337** 478–481

Frugier M, Florentz C and Giegé R 1994 Efficient aminoacylation of resected RNA helices by class II aspartyl-tRNA

synthetase dependent on a single nucleotide. *EMBO J.* **13** 2219–2226

Gilbert W 1986 The RNA world. *Nature (London)* **319** 618

Guerrier-Takada C, Gardiner K, Marsh T, Pace N and Altman S 1983 The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35** 849–857

Ito K, Ebihara K, Uno M and Nakamura Y 1996 Conserved motifs in prokaryotic and eukaryotic polypeptide release factors: tRNA-protein mimicry hypothesis. *Proc. Natl. Acad. Sci. USA* **93** 5443–5448

Ito K, Uno M and Nakamura Y 2000 A tripeptide 'anticodon' deciphers stop codons in messenger RNA. *Nature (London)* **403** 680–684

Jühling F, Mörl M, Hartmann RK, Sprinzl M, Stadler PF and Pütz J 2009 tRNAdb 2009: compilation of tRNA sequences and tRNA genes. *Nucleic Acids Res.* **37**(Database-Issue) 159–162

Kim SH, Suddath FL, Quigley GJ, McPherson A, Sussman JL, Wang AH, Seeman NC and Rich A 1974 Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. *Science* **185** 435–440

Korostelev A and Noller HF 2007 The ribosome in focus: new structures bring new insights. *Trends Biochem. Sci.* **32** 434–441

Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE and Cech TR 1982 Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. *Cell* **31** 147–157

Martinis SA and Schimmel P 1997 Small RNA oligonucleotide substrates for specific aminoacylations; tRNA: Structure, biosynthesis, and function (Washington, DC: ASM Press) pp 349–370

Moazed D and Noller HF 1991 Sites of interaction of the CCA end of peptidyl-tRNA with 23S rRNA. *Proc. Natl. Acad. Sci. USA* **88** 3725–3728

Moore PB 1991 Ribosomes. *Curr. Opin. Struct. Biol.* **1** 258–263

Musier-Forsyth K and Schimmel P 1999 Atomic determinants for aminoacylation of RNA minihelices and relationship to genetic code. *Acc. Chem. Res.* **32** 368–375

Nissen P, Hansen J, Ban N, Moore PB and Steitz TA 2000 The structural basis of ribosome activity in peptide bond synthesis. *Science* **289** 920–930

Noller HF 1993 On the origin of the ribosome: coevolution of subdomains of tRNA and rRNA. The RNA World (Plainview, NY: Cold Spring Harbor Laboratory Press) pp 137–156

Noller HF, Hoffarth V and Zimniak L 1992 Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* **256** 1416–1419

Paecht-Horowitz M, Berger J and Katchalsky A 1970 Prebiotic synthesis of polypeptides by heterogeneous polycondensation of amino-acid adenylates. *Nature (London)* **228** 636–639

Pech M and Nierhaus KH 2008 Ribosomal peptide-bond formation. *Chem. Biol.* **15** 417–419

Polacek N, Gaynor M, Yassin A and Mankin AS 2001 Ribosomal peptidyl transferase can withstand mutations at the putative catalytic nucleotide. *Nature (London)* **411** 498–501

Ramakrishnan V 2008 What we have learned from ribosome structures. *Biochem. Soc. Trans.* **36** 567–574

Robertus JD, Ladner JE, Finch JT, Rhodes D, Brown RS, Clark BF and Klug A 1974 Structure of yeast phenylalanine tRNA at 3 Å resolution. *Nature (London)* **250** 546–551

Sardesai NY, Green R and Schimmel P 1999 Efficient 50S ribosome-catalyzed peptide bond synthesis with an aminoacyl minihelix. *Biochemistry* **38** 12080–12088

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 and 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 and Ribas de Pouplana L 1995 Transfer RNA: from minihelix to genetic code. *Cell* **81** 983–986

Schimmel P and Alexander RW 1998 All you need is RNA. *Science* **281** 658–659

Schluenzen F, Tocilj A, Zarivach R, Harms J, Gluehmann M, Janell D, Bashan A, Bartels H, Agmon I, Franceschi F and Yonath A 2000 Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell* **102** 615–623

Steitz TA 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 2011 Molecular basis for chiral selection in RNA aminoacylation. *Int. J. Mol. Sci.* **12** 4745–4757

Tamura K and Alexander RW 2004 Peptide synthesis through evolution. *Cell. Mol. Life Sci.* **61** 1317–1330

Tamura K and Schimmel P 2001 Oligonucleotide-directed peptide synthesis in a ribosome-and ribozyme-free system. *Proc. Natl. Acad. Sci. USA* **98** 1393–1397

Tamura K and Schimmel P 2004 Chiral-selective aminoacylation of an RNA minihelix. *Science* **305** 1253

Tamura K and Schimmel PR 2006 Chiral-selective aminoacylation of an RNA minihelix: mechanistic features and chiral suppression. *Proc. Natl. Acad. Sci. USA* **103** 13750–13752

Tanaka T and Kikuchi Y 2001 Origin of the cloverleaf shape of transfer RNA-the double-hairpin model: implication for the role of tRNA intro and the long extra loop. *Viva Origino* **29** 134–142

Thompson J, Kim DF, O'Connor M, Lieberman KR, Bayfield MA, Gregory ST, Green R, Noller HF and Dahlberg AE 2001 Analysis of mutations at residues A2451 and G2447 of 23S rRNA in the peptidyltransferase active site of the 50S ribosomal subunit. *Proc. Natl. Acad. Sci. USA* **98** 9002–9007

Wächtershäuser G 1992 Groundworks for an evolutionary biochemistry: the iron-sulphur world. *Prog. Biophys. Mol. Biol.* **58** 85–201

Weber AL and Orgel LE 1978 The formation of peptides from the 2′(3′)-glycyl ester of a nucleotide. *J. Mol. Evol,* **11** 189–198

Weber AL and Orgel LE 1979 The formation of dipeptides from amino acids and the 2′(3′)-glycyl ester of an adenylate. *J. Mol. Evol.* **13** 185–192

Willumeit R, Forthmann S, Beckmann J, Diedrich G, Ratering R, Stuhrmann HB and Nierhaus KH 2001 Localization of the protein L2 in the 50 S subunit and the 70 S E. coli ribosome. *J. Mol. Biol.* **305** 167–177

Wimberly BT, Brodersen DE, Clemons WM Jr, Morgan-Warren RJ, Carter AP, Vonrhein C, Hartsch T and Ramakrishnan V 2000 Structure of the 30S ribosomal subunit. *Nature (London)* **407** 327–339

Zhang B and Cech TR 1997 Peptide bond formation by in vitro selected ribozymes. *Nature (London)* **390** 96–100

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