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Molecular handedness of life: significance of RNA aminoacylation

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Determination of the rationale behind the origin of homochirality in biological molecules has roused the interest of many for a long time. Natural proteins comprise α-amino acids that are exclusively left-handed (L-amino acids). Efforts have been directed to elucidate the reason underlying this phenomenon from various perspectives. 'Symmetry' is known to play a crucial role in physical laws, which are primarily invariable under translation and rotation (parity conservation). However, parity violation has been observed in the β-decay of nuclei, suggesting that a slight enrichment of the L- over the D-enantiomer ($<10^{-11}$) might be the origin of the exclusive homochirality of the L-enantiomer in the real biological world (Hegstrom 1987). This may be related to the enantiomeric enrichment in an interstellar environment (Oró 1961; Chyba *et al.* 1990; Chyba and Sagan 1992) and also to the polarized synchrotron radiation from neutron stars (Bonner 1996). From the standpoint of chemistry, the concept of enantioselective autocatalysis by chiral materials has been suggested and experimentally confirmed (Soai *et al*. 1995; Blackmond 2004; Kawasaki *et al.* 2009).

In the biological system, tRNAs are the key molecules that carry amino acids for protein synthesis. Amino acid attachment to tRNA is facilitated by protein catalysts known as aminoacyl-tRNA synthetases (Schimmel 1987). Since the discovery of ribozymes (Kruger *et al.* 1982; Guerrier-Takada *et al.* 1983), tRNAs have been investigated with keen interest to understand their evolution from the putative RNA world to the protein theatre. Thus, amino acid homochirality could be closely related to the evolution of tRNA aminoacylation. Peptide bond formation occurs on the ribosome, preceded by the aminoacylation of tRNAs with left-handed amino acids. This step provides an important clue about the composition of amino acids in the natural protein that is exclusively of the L-type and not the D-type. Irrespective of the physical or chemical perspectives described above, chiroselectivity in tRNA aminoacylation would be immensely important in terms of the continuity of evolution of biological systems on the Earth.

Structure of tRNA and origin of aminoacylation

Typically, tRNAs form three-dimensional, L-shaped and secondary cloverleaf structures with 76 nucleotides (Kim *et al.* 1974; Robertus *et al.* 1974). One arm of the L-shape contains the 3′-ends of the single-stranded sequence, NCCA, where the terminal OH of the adenosine residue is the site of aminoacylation by tRNA synthetases, while the other arm contains the trinucleotide anticodon at its end, which interacts with the codon in the mRNA on the ribosome.

The amino acid attachment site and the anticodon are 76 Å apart, and these two helical arms have distinct functions. The former, known as the 'minihelix', is thought to be a primordial part of tRNA. Minihelices can function as substrates for tRNA synthetases and undergo efficient aminoacylation. Possibly, minihelices have evolved to the present-day tRNAs by the addition of another arm containing anticodon along with the development of the corresponding tRNA synthetase domains, such as anticodon-binding domains (Schimmel *et al.* 1993; Schimmel and Ribas de Pouplana 1995) (figure 1).

Presently, tRNA synthetases are classified into two groups according to their sequences and catalytic domain structure (Eriani *et al.* 1990). However, regardless of the group, tRNA aminoacylation primarily occurs in two consecutive steps. Each amino acid is first activated to form aminoacyl adenylate, and then transferred to the terminal adenosine of its corresponding tRNA. Here, the possibility

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of prebiotic formation of aminoacyl adenylate (Paecht-Horowitz and Katchalsky 1973) and oligonucleotides (Lohrmann *et al.* 1980) is noteworthy, suggesting the prebiotic existence of aminoacyl phosphate oligonucleotide. Thus, non-enzymatic aminoacylation of primordial tRNAs (minihelices) could have been performed by aminoacyl phosphate oligonucleotides prior to the establishment of aminoacylation by tRNA synthetases.

Origin of amino acid homochirality through primordial aminoacylation

In a proposed non-enzymatic aminoacylation model, an aminoacyl phosphate oligonucleotide and the universal CCA sequence at the 3′-end of the minihelix were bridged in close proximity to each other by another oligonucleotide (figure 1). This system showed adequate aminoacylation of the minihelix with specificity for 3′-OH as the aminoacylation site (Tamura and Schimmel 2004). The rationale for this was that the greater free energy change of aminoacyl phosphate hydrolysis than that of aminoacyl ester hydrolysis would cause a spontaneous reaction. Thus, aminoacyl transfer from the 5′-phosphate of the oligonucleotide to the minihelix is a 'downhill' reaction. In addition, the formation of an L-aminoacyl-minihelix was significantly preferred over that of a D-aminoacyl-minihelix in a ratio of approximately 4: 1 (Tamura and Schimmel 2004) (figure 1). The validity of chiroselectivity was confirmed from the 'experiment in a mirror world' using L-ribose RNAs (Tamura and Schimmel 2004). The above preferences form a sufficient basis for the manufacture of homochiral proteins in repetitive evolutionary processes.

Chiroselectivity has been shown to be caused by the steric clash of the side chain of amino acids. In the model reaction, the CH_3 of D-Ala was found to be located close to the 3′-OH of the terminal adenosine of the minihelix, while that of L-Ala was located distal to the 3′-OH (Tamura and Schimmel 2006; Tamura 2008). Aminoacylation of a minihelix by an aminoacyl phosphate oligonucleotide is a typical nucleophilic reaction, where the oxygen in the 3′-OH of the terminal adenosine in the minihelix attacks the carbonyl carbon of the aminoacyl phosphate linkage. The approach

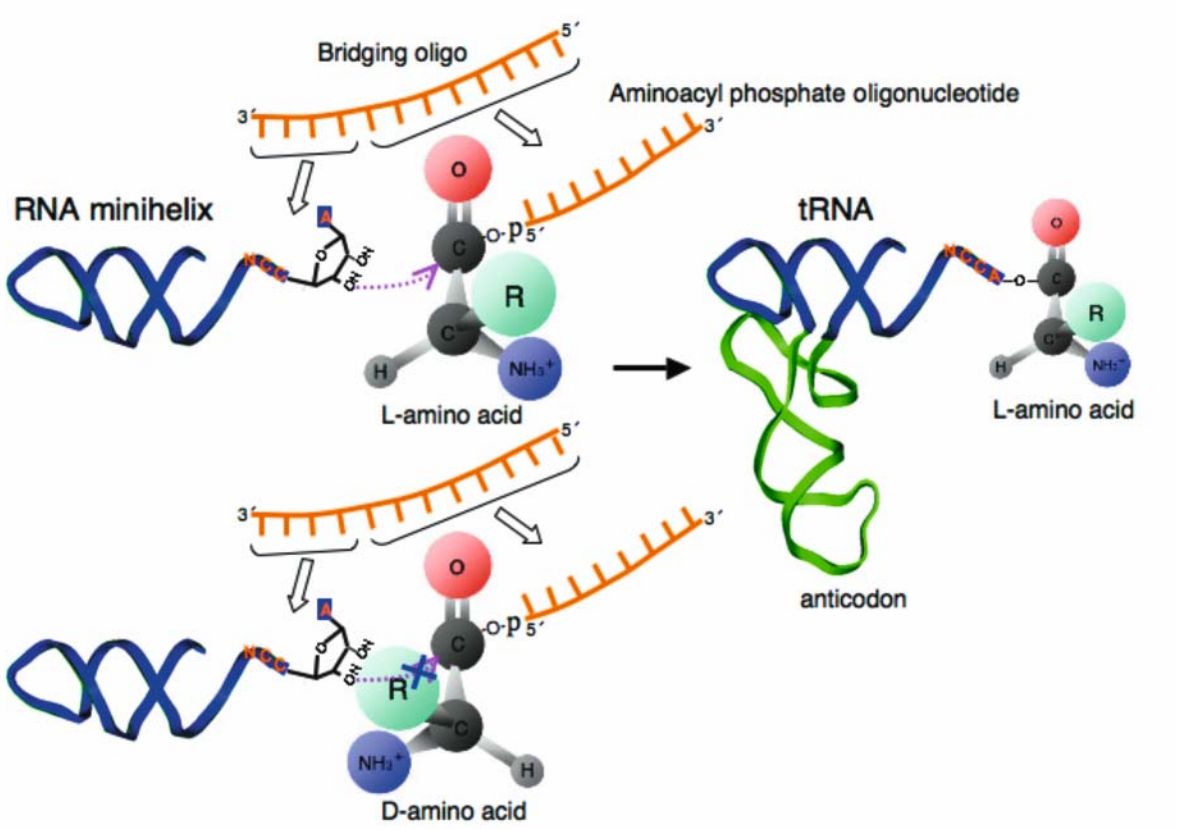

Figure 1. Chiroselective aminoacylation of an RNA minihelix and the evolution of tRNA. Aminoacylation with an aminoacyl phosphate oligonucleotide occurs with a clear preference for L- over D-amino acids. The side chain of the D-amino acid is close to the 3′-OH of the minihelix, while that of the L-amino acid is distal to the 3′-OH. Steric clash of the side chains of the D-amino acid is considered to be the reason for chiroselectivity. Minihelices could have evolved to tRNAs by the addition of the anticodon-containing domain.

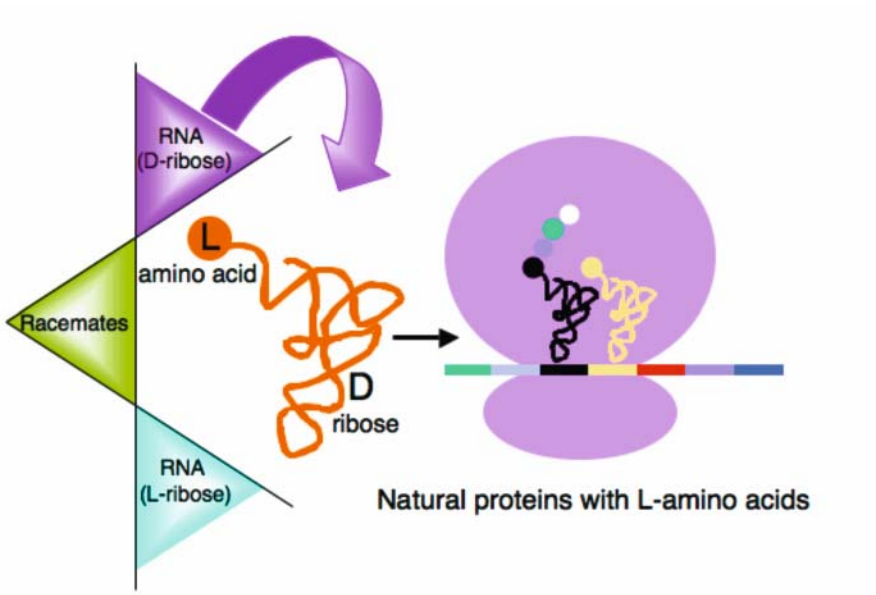

Figure 2. The evolutionary process of the origin of biological homochirality. 'Symmetry violation' due to chirospecific ligation of the oligomer RNAs produced a 'winner' sequence contained only in the D-libraries. Establishment of a D-ribose-based 'RNA world' would lead to homochiral (L) aminoacylation of RNA, thereby producing homochiral (L) natural proteins.

of the oxygen (Nu) to the carbonyl carbon is described by the Bürgi–Dunitz angle that is defined as the Nu–C=O angle measuring approximately 105° (Bürgi *et al.* 1974); in this restriction, the position of the amino acid side chain is crucial in determining chiroselectivity (figure 1). The differences in the positions of the amino acids could be extremely sensitive to the different sugar puckers of the nearest nucleotide (Tamura and Schimmel 2006; Tamura 2008). Systems having a wobble base pair at the position closest to the aminoacyl phosphate bond and/or different puckers strengthen the plausibility of the mechanism based on the steric clash of the side chains of right-handed amino acids (D-amino acids). Very subtle structural parameters appear to affect the chiroselectivity of amino acids in the transfer of aminoacyl from the oligonucleotide to the minihelix (figure 1).

Origin of homochirality in the RNA world

Because D-ribose RNA determines the homochirality of L-amino acids, it is important to determine the origin of D-ribose in RNA. The discovery that RNA can function as a catalyst and store genetic information led to the 'RNA world' hypothesis (Gilbert 1986), which has provided an answer to the classic 'chicken-or-egg' conundrum in biological molecules (what came first, nucleic acids or proteins?). Many believe that the RNA world was followed by the protein theatre, presumed to have been initiated by the encounters between amino acids and RNA. The key step of this encounter is the aminoacylation of RNA.

Prebiotic functional RNA molecules could have been synthesized by repeated oligomerization of short nucleotides, the initial building blocks. In fact, template-directed auto-oligomerization using all possible combinations of homo- and heterochiral diastereomers of short pyranosyl-RNA oligonucleotide 2′,3′-cyclophosphates has been shown to proceed in a chiroselective manner, yielding all D- or L-products (Bolli *et al.* 1997). In a possibly similar process of chirospecific ligation of oligomer ribose-RNAs to all D- or L-libraries, the sequences contained in the D- or L-libraries would not be the same because the number of possible sequences would exceed the number of sequences actually formed during the process of RNA lengthening. Thus, 'symmetry violation' would occur as a matter of course. In such a situation, one 'winner' sequence contained only in the D-libraries would have continued the establishment of the real RNA world composed of homochiral (D) components. In other words, a specific sequence that accidentally exhibited an important chemical ability for the establishment of the RNA world would have been contained only in the D-libraries and this might be the origin of the 'homochiral' RNA world. Establishment of a D-ribose-based RNA world would form the basis for homochiral (L) aminoacylation of RNA, leading to homochiral (L) natural protein synthesis (Tamura 2008) (figure 2).

At present, we cannot specifically determine the factor that could provide the selective advantage to D-ribose-based RNA over L-ribose-based RNA. It might even be 'chance

and necessity'. However, non-enzymatic aminoacylation of RNA minihelices by aminoacyl adenylate analogues (aminoacyl phosphate oligonucleotides) has shown a clear chiral preference for L-amino acids over D-amino acids (Tamura and Schimmel 2004). The rationale for this is in the relationship between time, space and the existence of real chemical substances. The amino acid side chain could be a significant 'discriminator' of the chiral selection of amino acids even if it contains only one additional methyl group (as in Ala) (Tamura and Schimmel 2006; Tamura 2008). The real significance of this discovery is that a part of the RNA structure has the ability to distinguish between the chiral differences in amino acids. Although the plausibility that RNA could serve as the first simple form of life is questionable, the type of molecular discrimination exhibited in chiroselective aminoacylation of an RNA minihelix would have played a crucial role in the establishment of the homochirality of biological systems.

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