Hypothesis

A stereospecific mechanism for the aminoacyl-tRNA selection at the ribosome

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Translation fidelity *Aminoacyl-tRNA selection* *Codon–activation complex*
Ribosome–aa-tRNA binding *Ternary complex* *Stereospecific selection*

1. INTRODUCTION

The apparent error frequency for protein synthesis in vivo is close to 10^{-4} mistakes/amino acid residue [1,2]. Such a high fidelity of translation is substantially greater than the specificity of codon–anticodon recognition which would be expected from the known energetics of base-pairing in solution [3,4]. Moreover, the codon-dependent binding constant of cognate aa-tRNA to the ribosome is $> 10^{11}$ M^{-1} [5]. The interactions between tRNAs and their cognate codons in the absence of the ribosome can be characterized by an equilibrium constant of the order 10^3 M^{-1} only [6–8]. It is not clear how such a weak triplet–triplet interaction between codon and anticodon can regulate the process of strong binding of aa-tRNA to the ribosome in a highly specific way. Several attempts have been made to solve this problem [4,9,10].

Here, a new hypothetical mechanism is proposed for the aa-tRNA selection at the ribosome. It postulates the direct interaction between the ribosome and the codon–anticodon complex. The ribosome is suggested to select the correct codon–anticodon complex according to the degree of its geometrical perfection.

Abbreviation: aa-tRNA, aminoacyl-tRNA

2. GENERAL STEREOCHEMICAL PRINCIPLE FOR SELECTION OF aa-tRNA AT THE mRNA-PROGRAMMED RIBOSOME

One of the possible ways to solve the problem of high fidelity of aa-tRNA selection is to suggest a suitable mode of specific enhancement of the initially low stability of the correct aa-tRNA–mRNA complex at the ribosome. The main problem here is that such enhancement should be highly specific and at the same time universal for a great number of different aa-tRNAs.

aa-tRNA selection at the ribosome is based on the complementarity between the anticodon of aa-tRNA and the codon of mRNA. One of the most remarkable features of the Watson–Crick complementarity is a weak dependence of the corresponding double helix geometry on its base-pair composition. X-ray diffraction studies of the natural and synthetic complementary RNA double helices show that they can exist in the same conformation [11].

The following suggestions may be taken into consideration:

(i) Different correct complexes of the mRNA codons and the cognate tRNA anticodons have a common structure whereas incorrect complexes consisting of non-cognate tRNA anticodons lack this structure;

(ii) The ribosome can recognize this structure and participate in the direct interaction with the

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correct codon–anticodon double helix forming the ternary complex X • (codon–anticodon), where X is the corresponding region of the ribosome;

(iii) Such ternary complex formation is an obligatory stage of the aa-tRNA selection. It occurs at the very first steps of this process and it is the trigger for a number consecutive interactions between the selected aa-tRNA and the ribosomal A site;

(iv) The ternary complex formation is based mainly on interactions between some proteins of the ribosome X region and codon–anticodon sugar–phosphate backbones as the RNA elements which are common for different codon–anticodon complementary complexes.

So, the aa-tRNA selection process is suggested to be grounded on both RNA–RNA and RNA–protein recognitions in the system X • (codon–anticodon). Hence, a general stereochemical principle for the aa-tRNA selection at the ribosome may be postulated. Accuracy of the aa-tRNA selection depends not only on the codon–anticodon interaction energy but on the codon–anticodon sugar–phosphate backbone geometry and the stereospecificity of the ribosome X region towards selected codon–anticodon structure as well.

The proposed mechanism fully satisfies the requirement for the selective enhancement of the correct codon–anticodon complex stability. At the same time it is universal for different codon–anticodon pairs. Such aa-tRNA selection is based on the ability of tRNA to form the universal standard structure with the mRNA codon. Only this structure is recognized by the monospecific ribosome X region. So, the ribosome which is known to function as a multisubstrate enzyme with a programmed substrate specificity can work actually as a monospecific enzyme.

3. DISCUSSION

The proposed stereospecific selection mechanism, in contrast to the kinetic proofreading mechanism [4,9], is intrinsic to the ribosome itself and does not depend strictly on GTP hydrolysis. It can explain not only the high accuracy of translation in vivo but the low level of miscoding in the GTP-factor-free system of translation in vitro as well [12].

The tRNA–mRNA interaction specificity is known to depend strongly on the ribosome structure [13]. Mutational alterations of some ribosomal proteins proved to be responsible for either decreased or increased misreading in translation. In agreement with the discussed suggestions these data demonstrate the key importance of some ribosomal proteins at the very first steps of the aa-tRNA selection process.

Support for the idea that the ribosome interacts directly with the correct codon–anticodon complexes may be found in the study of the trinucleotide-dependent binding of aa-tRNA to the ribosome. In agreement with the present suggestion, the ribosome • trinucleotide • aa-tRNA complex assembly shows a clear cooperative behaviour. In the absence of the ribosome the trinucleotide has a comparatively low affinity for its cognate tRNA anticodon [6–8]. In the absence of aa-tRNA such a short template does not interact markedly with the ribosome but becomes bound to it after addition of an appropriate aa-tRNA [14,15]. The increase in the binding of trinucleotide correlates with the increase in the aa-tRNA binding to the ribosome [15].

It is interesting that the position of the terminal phosphate residue in the trinucleotide (pNpNpN or NpNpNp) clearly influences the template efficiency of the trinucleotide [14] and the stability of the ribosome • trinucleotide • aa-tRNA complex [16]. On the other hand, the 2′-hydroxyl groups of mRNA codons are necessary for their reading by aa-tRNAs. Oligodeoxynucleotides, polydeoxynucleotides and single-stranded DNA apparently are inactive as templates in translation [14,17–19]. Polyribothymidylic acid, but not polydeoxyribothymidylic acid, efficiently substitutes for polyribouridylic acid as a messenger for polyphenylalanine synthesis [17,20]. These observations are consistent with the important role of the codon–anticodon sugar–phosphate backbones in the aa-tRNA selection suggested here.

It is remarkable that single-stranded DNA [19] and synthetic single-stranded polydeoxynucleotides [17,18] can act as a direct template for protein synthesis in a cell-free system from *E. coli* when an aminoglycoside antibiotic neomycin B is present. Presumably, the antibiotic interacts with the ribosome [13,21]. So, these data tend to support the hypothesis that the ribosome in some way recog-

nizes the codon–anticodon complex. It seems likely that neomycin modifies the ribosome in such a way that recognition of the codon–anticodon complex is less stringent and no longer restricted to ribonucleotides.

Further, some thermophilic bacteria are capable of growing at 75–80°C [22] where weak triplet–triplet RNA–RNA associations must be unstable. This fact indicates that the selected codon–anticodon complexes may be significantly stabilized by the ribosome. A comparison of the melting temperature of the oligomer polymer complex with that of oligomer aa-tRNA and ribosome oligomer aa-tRNA complexes [16] confirms this view.

The genetic code is known to be a three-letter code in which only the first 2 positions of the codon are read by the anticodon strictly according to the classic Watson–Crick base-pairing. The role of the third position in the codon is not so important as each of the first two positions [23,24]. However, omission of the complementarity in a single position of the 3 positions of the codon seems to weaken considerably the codon–anticodon associations. This functional feature of the genetic code may be interpreted as representing the minor role of the energetics of the codon–anticodon interactions themselves and the predominant role of some other codon–anticodon-dependent energetics have been suggested here as the energetics of the X · (codon–anticodon) interactions.

According to the nature of the genetic code, the type of the correct codon–anticodon complexes which can be recognized by the ribosome may be suggested to have the standard double helix geometry of the sugar–phosphate chains in the first two positions of the codon and a similar geometry of the sugar–phosphate units in the third position. This geometry may be induced partly by the ribosome X region.

High resolution X-ray diffraction data provides strong evidence for the formation of the universal Watson–Crick double helices by sugar–phosphate backbones of short RNA self-complementary dinucleotide fragments: ApU [25], UpA [26,27] and GpC [28]. Hence, the complementarity in the first two positions of the codons seems to be able to provide a certain common structural property of the different correct codon–anticodon complexes.

Thus, it appears that the suggested stereospecific selection mechanism may be regarded as a working hypothesis. To put the discussion on a wider basis new experimental data are necessary. Several predictions follow from this hypothesis:

(1) In the presence of a trinucleotide template the ribosome would have high affinity for the isolated anticodon arm of the template-specific tRNA molecule.
(2) The ribosome would be able to select and bind short RNA double-helical fragments formed by complementary oligoribonucleotides;
(3) aa-tRNA binding to the mRNA-programmed ribosome would be inhibited by both the isolated anticodon arm of the codon-specific tRNA and the codon complementary trinucleotide, which would act as high specific competitive inhibitors.

In this connection the existence of natural eukaryotic oligonucleotides affecting mRNA translation is very interesting. One of them has been shown to block chain elongation by interfering with the ribosomal binding of aa-tRNA and to act primarily on the ribosome [29]. According to these suggestions, the molecular mechanism of its action may be explained as the competition with the aa-tRNA anticodon for the X region of the ribosome.

The proposed stereospecific selection mechanism and its general stereochemical principle seem to be applicable not only to the ribosome but also to all messenger-programmed enzymes including various DNA- and RNA-polymerases whose functioning requires high fidelity.

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