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Possible Physical Mechanisms of tRNA Pre-Selection in the Cytoplasm of *Escherichia coli* Bacteria

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In a previous publication, we used kinetic models to simulate the physical movement of the transfer RNA (tRNA) in the cytoplasm of *Escherichia coli* and concluded that tRNA molecules are not able to approach the ribosome by random Brownian motion at a sufficient rate for protein synthesis. In this paper, we propose three mechanisms to explain tRNA pre-selection (to distinguish it from initial selection, we refer to it in this article as "pre-selection") in prokaryotes. The first hypothesis is that the ribosome stores tRNA molecules inside its structure and aids in the pre-selection process. Because no previous reports in literature support such a pre-selection process, we believe that this hypothesis is unlikely. The second hypothesis suggests distant signaling between the ribosome and the cognate-transfer RNA (ctRNA) that allows the correct ctRNA to approach the ribosome and bind to the "A site." Again, no experimental proof of a signaling mechanism between the ribosome and ctRNA exists when they are distant from each other, which renders this hypothesis invalid. Third, we hypothesize that the messenger RNA (mRNA) could act as a "comb" which is able to filter out the consecutive tRNAs from the cytoplasm, thus allowing the correct ctRNA to reach the "A site" in the ribosome. Although the functions of tRNAs, how they are assembled, and how they get charged by amino acids are widely studied and well understood, few articles report on the mechanism by which tRNAs are pre-selected and how they reach the site of amino-acid assembly, the ribosome. This article suggests several mechanisms to explain this pre-selection process.

1. INTRODUCTION

The functions of transfer RNA (tRNA) molecules are well known. Also well known and documented is how tRNAs are assembled and how they get charged by amino acids. Studies are scarce, however, on the existence of any signaling between tRNAs and ribosomes. Possible physical forms of communication between these two entities could be mechanical vibrations through the liquid cytoplasm of *Escherichia coli (E. coli)* Bacteria, and/or electromagnetic waves. If tRNAs are able to receive any signal emitted by the ribosome, they should have special receptors for this purpose. tRNAs' tertiary structures contain four loops namely, D, TΨC, anticodon, and "variable" loops. Their shape and diameter are specific to each type of tRNA. During activation, the tRNA type is recognized by the aminoacyl-tRNA synthetase enzyme according to the geometry of these loops.[1] In as far as the tRNA pre-selection in *Escherichia coli* bacteria is concerned, the question remains as to where exactly tRNAs are getting charged in the bacterial cytoplasm. Are they being charged in a specific place in the bacteria or does the charging happen throughout the cytoplasm?

The relatively small quantity of tRNAs compared to the number of ribosomes per bacterial cells is another important fact. According to literature, there are approximately ten times more tRNAs present in *E. coli* than ribosomes.[2] If the quantity of each amino acid specific tRNA is about 2%, then there is only one tRNA molecule for every five ribosomes. Thus, tRNAs are quite "busy" and well utilized in providing amino acid molecules to ribosomes.

In a previous publication,[3] we concluded that tRNAs are not able to approach the ribosome by random Brownian motion at a sufficient rate for protein synthesis. To come to this conclusion, we used kinetic models to simulate the physical movement of the tRNA in the cytoplasm. In this paper, we proposed three mechanisms to explain tRNA pre-selection in prokaryotes. First, we summarize the findings of available studies that deal with tRNA movements in the vicinity of the ribosome.

Studies on tRNA selection for protein synthesis emphasize the accuracy and speed with which the ribosome is able to carry out this function. These studies agree on the existence of two stages in this process. Thus, codon–anticodon recognition has two main

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stages, namely, initial selection and proofreading.[4–7] These articles give a detailed description of the "double-trigger" mechanism (in tRNA approaching the ribosome) and the geometric constraints (of the tRNA movement) on a molecular and atomic level. Based on our kinematic simulations, we believe that in addition to the two stages mentioned above, it is possible that the tRNA selection process could have a preliminary stage that we refer to in this paper as "pre-selection."

2. PROPOSED HYPOTHESES ON tRNA PRE-SELECTION

As we mentioned in the introduction, our kinetic model proved that it is virtually impossible for the tRNA to reach the "A site" (aminoacyl-tRNA binding site) of the ribosome by random motion. The purpose of this paper is to provide possible explanations/mechanisms for tRNAs approaching the ribosome. The following three scenarios are envisioned:

(1) The ribosome stores tRNA molecules and preselects them,
(2) Signaling between the ribosome and the cognate tRNA exists,
(3) tRNA molecules reach the ribosome in a preselected manner.

Before we discuss these three proposed mechanisms, we refer to the importance and definition of the "reading frame" concept. To illustrate this concept we use |EG10906| rpsG: 540 bp–30S ribosomal subunit's S7 protein (a portion of the *E. coli* mRNA gene),[8] shown on Figures 1 and 2. Messenger RNA (mRNA) contains the information (the amino-acid sequence) in the form of triplets of nucleotide bases. The difficulty in decoding the mRNA message arises because the beginning of the code is not obvious. Thus, depending on where deciphering starts, three possible "senses" could be interpreted. This is referred to as a "reading frame."[1] Once the ribosome is attached to the mRNA, the position of the reading frame is fixed. During translation, the ribosome moves along triplet by triplet until it reaches the "stop" codon. Next, we discuss our three hypotheses.

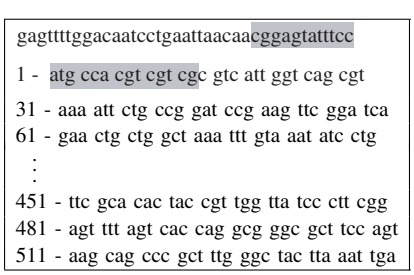

Fig. 1. A portion of the ribosomal 30S subunit protein S7.[8]

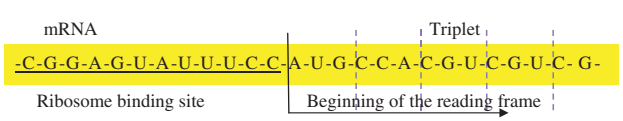

Fig. 2. A portion of an *E. coli* mRNA gene namely |EG10906|rpsG: 540 bp–30S ribosomal subunit's S7 protein[8] used to illustrate the reading frame concept.

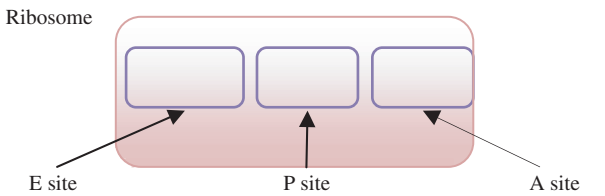

Fig. 3. The figure shows the three ribosomal sites: The "A site" (aminoacyl-tRNA binding site), the "P site" (peptidyl-tRNA binding site), and the "E site" (Exit site).

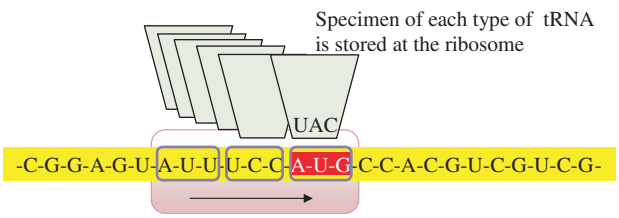

Fig. 4. The figure illustrates the first hypothesis proposed in our article, whereby the ribosome is capable of capturing any tRNA molecule present in the vicinity and selecting the cognate.

2.1. Transfer RNAs are Stored in or at the Ribosome

The results of our previously published simulations show that statistically, the number of consecutive amino acids delivered to the ribosome by random motion in a given time frame is slower than the rate at which proteins are synthesized.[3] Suppose the ribosome collects available tRNA molecules in its neighborhood and stores them for future amino acid assembly. This could happen only when all types of tRNAs are readily available to provide the proper amino-acid molecule for protein synthesis. The problem is that there are no studies in literature that report the ability of ribosomes to store tRNA molecules. Another fact that renders this hypothesis impractical is the lack of time for a trial and error process for all types of tRNAs. The duration of one trail is about 3 ms, so the ribosome is capable of attempting to fit an average of 6 or 7 "probes" within the allowable 20 ms time interval.[5] The number of possible tRNA molecules is 42 (the actual number of different tRNAs in *E. coli* bacteria is more than 80, but some are redundant).[9] In order for this hypothesis to be viable, tRNAs should have, on average, enough time for 21 trials, or 63 ms, but nature allows 20 ms only. Therefore, this hypothesis seems to be less likely, as illustrated in Figure 4.

2.2. Signaling Between Ribosome and tRNA

In this hypothesis, we assume that cognate tRNAs (ctRNA) are capable of following a beacon signal emitted by the ribosome

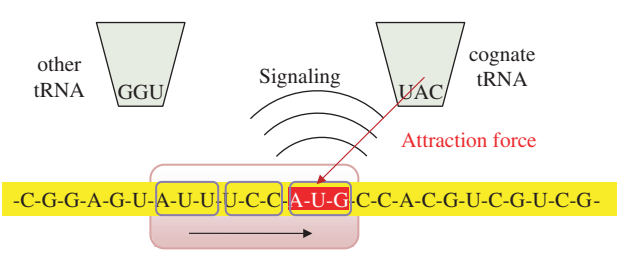

Fig. 5. The figure illustrates the second hypothesis whereby the ribosome emits a signal in the cytoplasm, to guide the cognate tRNA towards the ribosome.

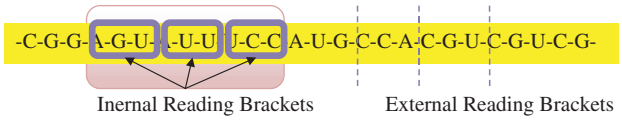

Fig. 6. The figure illustrates the concept of "Reading Brackets." The ribosome's A, P, and E sites are mechanically constraining tRNAs to follow the reading frame. Therefore, these sites act as brackets.

which propels them towards its "A site" (Fig. 5). Simulation results reported earlier[3] showed that when the velocities of ctRNA molecules are increased, more cognate transfer RNA molecules can reach the ribosome. In order to justify this hypothesis, we need to prove that ctRNAs are moving faster than other non-cognate tRNAs in the cytoplasm. There are no reports in literature that show evidence of differences in speed between ctRNA and other tRNA molecules. Additionally, no data is available on the existence of specific forces or signals acting between the ribosome and tRNAs when both are relatively far from each other. Cells are known to emit electromagnetic radiation, but there is no data that support the existence of such signaling between these two entities. Thus, based on the lack of evidence of any signaling between tRNA molecules and ribosomes, we conclude that this hypothesis is also unlikely.

2.3. Transfer RNAs are Preselected Using a Different Mechanism

RNA molecules and nucleotides tend to form pairs with their antisense counterpart. For example, the 16S ribosomal RNA (rRNA) component is able to bind to the Shine-Dalgarno sequence of the ribosomal binding site, which is its antisense. Once the ribosome subunits are fixed on the mRNA, the beginning of the actual reading frame is determined by its position. The ribosome has three sites to accommodate a tRNA molecule, namely the "A site" (aminoacyl-tRNA binding site), "P site" (peptidyl-tRNA binding site) and "E site" (Exit site). These sites act as mechanical constraints. In this paper, we will refer to them as "Reading Brackets" (see Fig. 6). The ribosome translocates along the mRNA, triplet by triplet, maintaining the correct translation of the entire reading frame from the "start" codon (AUG) to the terminating "stop" codon (UAA, UAG or UGA). We posit that the ribosome is capable of determining "an external reading bracket" in addition to its normal "internal reading brackets" (Fig. 7). The ribosome's body may be capable of mechanically constraining tRNA molecules by its internal sites and external shape (Fig. 8). The free floating tRNAs have an equal chance of binding to any part of the mRNA where the three consecutive bases are complementing its anticodon, regardless of the

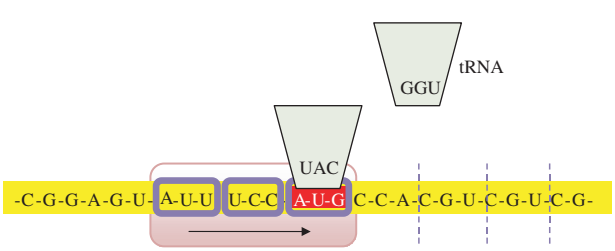

Fig. 7. The figure shows the "initiation" step proposed in our third hypothesis. The first tRNA to start protein synthesis reaches the ribosomal "A site" by diffusion.

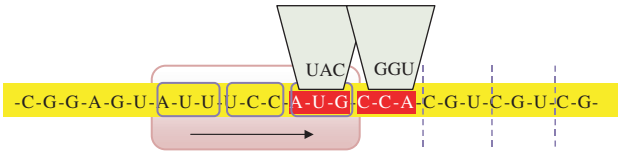

Fig. 8. The figure illustrates the "beginning of the elongation" step proposed in the third hypothesis. The figure shows that other tRNAs are randomly attached to the naked portion of the mRNA, highly increasing the probability that the suitable tRNA for the next step is the nearest to the ribosome.

position of the reading frame. The ribosome is able to determine, or increase the possibility, that the three bases next to its location will be occupied by a suitable charged tRNA molecule. Our above hypothesis (illustrated in Figs. 7, 8 and 9) is proposed to account for the simulation results reported earlier.[3]

Unlike eukaryotes, *E. coli* cells have no compartments. Therefore, the only mean of material transport viable is through diffusion.[10, 11] Despite this fact, in all the articles explaining the translation process, tRNA molecules are assumed to be floating directly to the "A site" of the ribosome.[12] Moreover, the tRNA cognate always reaches the required site. In reality, at any given moment, only one type of tRNA's anticodon (out of more than 40 possible tRNA molecules) matches the actual codon. The ration of the number of mismatches to one tRNA successful proofreading[13] is estimated of 1 in 10^3–10^4.

The essence of our theoretical proposal is that the mRNA itself is capable of attracting tRNA molecules and storing them. These two processes, attracting and attaching the tRNA molecules to the mRNA template itself, are proceeding in a parallel manner contrarily to what was hypothesized earlier (in the first and second hypotheses the imagined process is taking place in a serial manner at the ribosome). Thus, the timing is not critical in this case. Moreover, tRNAs are preselected automatically if they are able to align themselves with each other without gaps before reaching the ribosome. In other words, the chance that three types of tRNAs are present next to the ribosome is increased dramatically before the next tRNA molecule is preselected. tRNA molecules are moving randomly (by diffusion) in the cytoplasm and are interacting with the mRNA. Once the ribosome is assembled and attaches itself to the mRNA, the "reading brackets" are established. As the ribosome moves along, these external reading brackets move forward as well.

2.4. Proposed Explanation of Protein Assembly in More Detail

After synthesis, mRNA is ready for translation (Fig. 10). The first step in translation starts when the ribosome is assembled from existing rRNAs and proteins. Specifically, 16S rRNA attaches itself to the Shine-Dalgarno sequence, and the ribosome is ready to start protein polymerization (Fig. 11). The first amino-acid

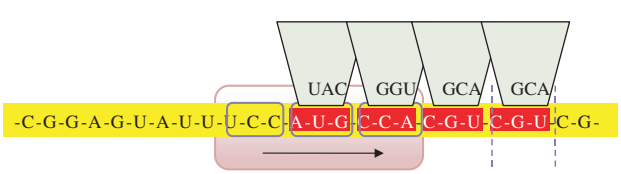

Fig. 9. The figure illustrates the "elongation in progress" step proposed in the third hypothesis. The figure also shows that more and more tRNAs are deposited on the mRNA.

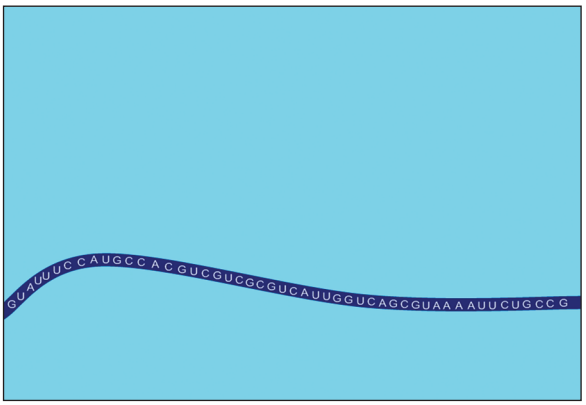

Fig. 10. The figure shows a growing mRNA chain.

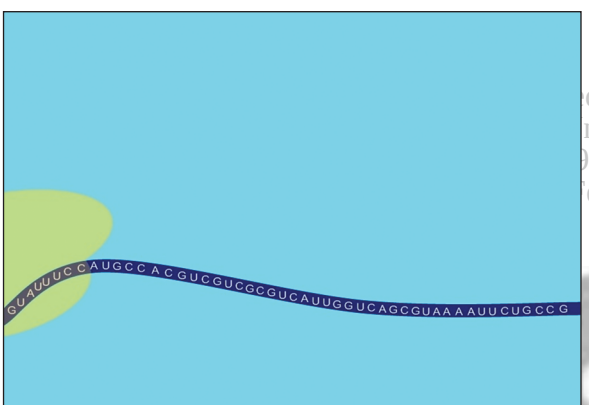

Fig. 11. The figure shows a ribosome assembled on the mRNA chain.

to start a protein chain is always methionine.[1] The first tRNA, Met-tRNA, enters into the ribosomal "A site" (Figs. 12 and 13), waiting for the arrival of the next amino-acid. The next codon in our example is CCA. Three types of tRNA have the highest chance of being present in the vicinity of the ribosome; two of them are out of the reading frame. Threonine-tRNA (Thr-tRNA) matches with ACG (Fig. 14) and Histidine-tRNA (His-tRNA) matches with CAC base sequences (Fig. 15), but not with the CCA. It is important to note here that other tRNAs are capable of randomly attaching themselves to the mRNA. Proline-tRNA

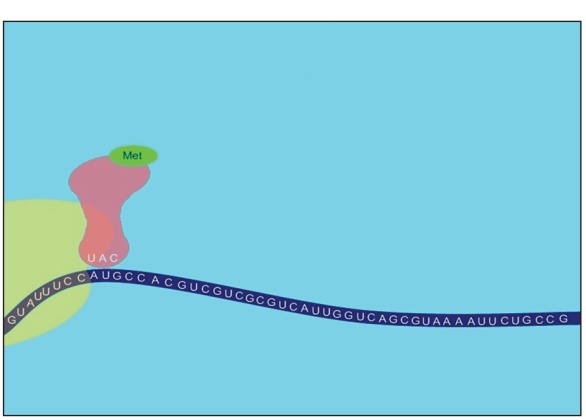

Fig. 12. The figure shows the ribosome waiting for the first tRNA.

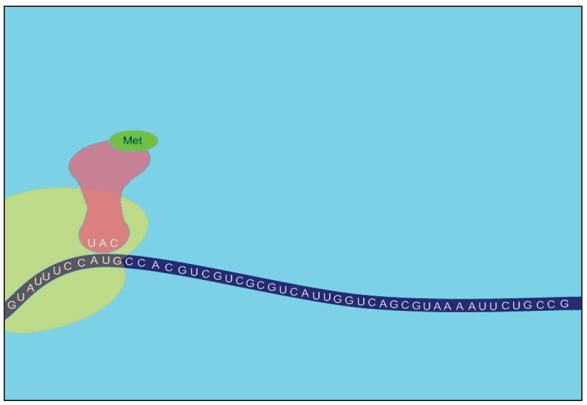

Fig. 13. The figure shows the first tRNA entering the "A site" of the ribosome.

(Pro-tRNA) (GGU) is the only anticodon that matches the CCA codon as show in Figure 16.

The pre-selection process ends when the ctRNA is found at the ribosomal "A site." The following detailed explanation, shown on Figures 17–20, is purely speculative. tRNA molecules, according to their anticodon, line up outside the ribosome. The nearest tRNA is leaning against the ribosome and bonds with the first two bases of the codon's triplet using hydrogen bonding. Therefore, the interaction between the codon in the third position and its anticodon diminishes, which could account for the wobbling effect observed. The ribosome steps forward to capture the next ctRNA and the polypeptide chain grows. Therefore, as the ribosome progresses along the mRNA, more and more tRNA molecules are deposited, and then selected, following the reading frame. Any other tRNA molecule previously attached to the mRNA, off the reading frame, could be washed away by water molecules, the latter continuously bombarding the system. But tRNAs that are touching the ribosome or leaning towards each other could cling tightly to it and remain attached to the mRNA. Additionally, the progression of the ribosome along the mRNA during protein assembly can easily scrape these tRNA molecules off the mRNA. The secondary shape of the RNA molecule is helical; one full turn is made of 11 bases. When the ribosome steps forward relative to the mRNA, it should turn 33° to the

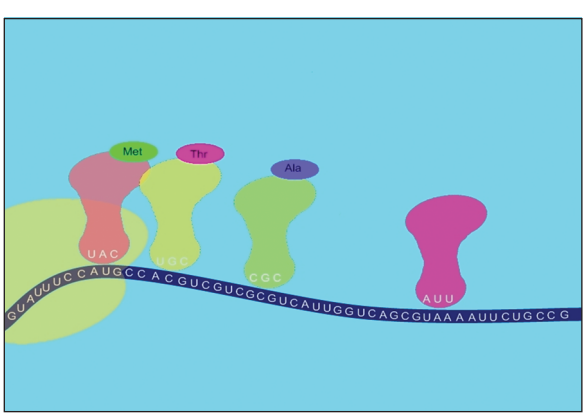

Fig. 14. The figure shows the first step in "pre-selection." RNAs are randomly stuck to the mRNA including the Thr- and Ala-tRNA molecules.

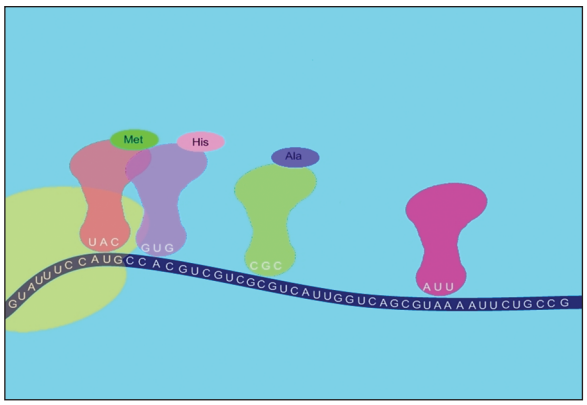

Fig. 15. The figure illustrates the second step in our pre-selection example. His-tRNA anticodon (GUG) is not attached to the correct (CAC) mRNA codon.

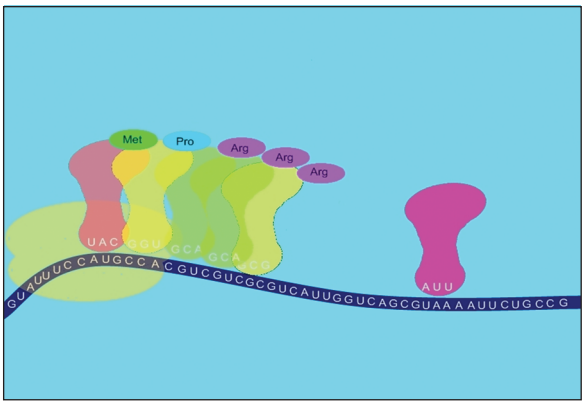

Fig. 18. The figure illustrates the "elongation" step proposed in the third hypothesis. More and more tRNA molecules could line up along the mRNA, increasing the chance that the correct cognate is one of the nearest tRNAs to the ribosome for the next initial selection.

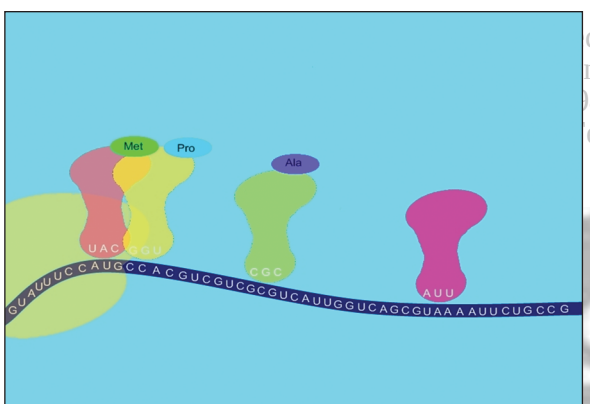

Fig. 16. The figure illustrates the next step in pre-selection. Pro-tRNA has the highest chance of being the nearest to the ribosome for the next tRNA selection process.

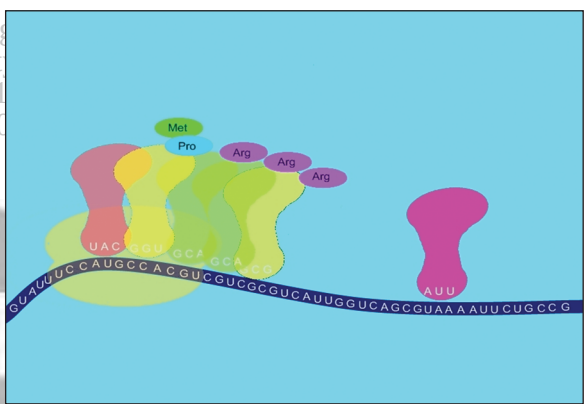

Fig. 19. The figure shows the "peptide bonding" step in protein synthesis. The primary role of the ribosome is to insure that the correct ctRNAs are selected and bind the amino acids together to transform them into a polypeptide chain.

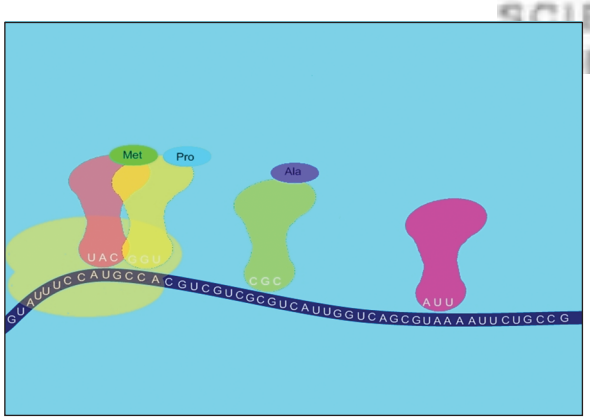

Fig. 17. The figure illustrates the "proofreading" step proposed in the third hypothesis. The ribosome steps forward after the correct tRNA enters the ribosomal "A site" and the "proofreading" verifies the match.

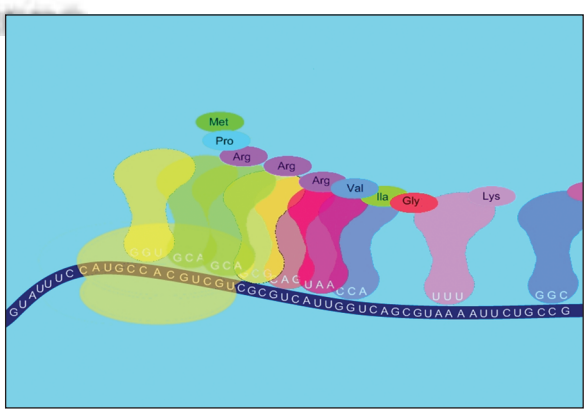

Fig. 20. The figure illustrates how the translation step progresses along the mRNA.

right, giving the tRNA molecule free space to enter the "A site." As can be seen, water molecules are very important in the biological scenario discussed above, primarily because seventy percent of the cytoplasm is water.

3. CONCLUSION

Three hypotheses that attempt to explain tRNAs pre-selection leading to their binding at the ribosomal "A site" have been discussed in this article. The first hypothesis is that the ribosome

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stores tRNA molecules inside its structure and aids in the pre-selection process. Because no previous articles report on such a pre-selection process, this hypothesis is most likely invalid. The second hypothesis suggests the existence of signaling between the ctRNA and the ribosome that permits the correct ctRNA to approach and bind to the ribosomal "A site." As with the first hypothesis, there is no experimental proof of a signaling mechanism between the ribosome and ctRNA, which suggests that the second hypothesis is also unlikely. Our third hypothesis proposes that tRNA molecules reach the ribosome in a preselected manner. Although the functions of tRNAs, how they are assembled, and how they get charged by amino acids are widely studied and well known, few if any studies report on the mechanism by which tRNAs are pre-selected to reach the ribosome This article suggests several mechanisms to explain this pre-selection process.

ABBREVIATION

Abbreviation	Term
Aa	Aminoacyl
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
tRNA	transfer RNA
ctRNA	cognate tRNA
mRNA	messenger RNA
rRNA	ribosomal RNA
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
ms	Millisecond
E. coli	*Escherichia coli*
A site	aminoacyl-tRNA binding site
Met	Methionine
Thr	Threonine
His	Histidine
Pro	Proline

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