Allosteric mechanism for codon-dependent tRNA selection on ribosomes

(tRNA conformations/translation errors/suppressors)

C. G. KURLAND*, R. RIGLER†, M. EHRENBERG‡, AND C. BLOMBERG‡

*Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden; † Department of Medical Physics, Karolinska Institute, Stockholm, Sweden; and ‡ Department of Theoretical Physics, The Royal Institute of Technology, Stockholm, Sweden

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ABSTRACT We suggest that the interaction between a codon and its cognate tRNA induces conformational changes in the tRNA. We further suggest that sites on the ribosome preferentially bind tRNA in those conformations which require proper matching of codon and anticodon. According to this model, the codon functions as an allosteric effector which influences the conformation at various sites in the tRNA. This is made possible by the ribosome, which we suggest traps tRNA molecules in those conformation states that maximize the energy difference between cognate and noncognate codon-anticodon interactions.

Studies of the interactions between tRNA molecules and their cognate codons in the absence of the ribosome have suggested that the triplet-triplet interaction between codon and anticodon is far too weak to account for the specificity of the tRNA selection mechanism during protein synthesis. In contrast, we suggest that such affinity measurements do not adequately describe the interaction between a codon and its cognate tRNA. Thus, such experiments can not detect conformational changes in the tRNA, and, in particular, those stabilized by the ribosome.

One remarkable aspect of protein biosynthesis is its precision. Indeed, estimates of the error frequency of translation suggest that a wrong amino acid is inserted into a protein only once for every three thousand incorporated amino acids (1). Such precision has been inexplicable in terms of what is known about the mechanism of aminoacyl-tRNA selection. Thus, measurements of the affinity of trimucleotides for their complementary triplet sequences in the anti-codon of tRNA molecules indicate that such interactions are quite weak and can be characterized by equilibrium constants of the order of 10^3 M^-1 at 0°C (2–4). Furthermore, a given trimucleotide will interact with its cognate tRNA molecule with an affinity constant that is only 10 times greater than that for a related, noncognate triplet and the same tRNA. Such observations suggest that a simple triplet-triplet interaction at equilibrium can not by itself account for the fidelity of tRNA selection.

Several authors have recently attempted to circumvent this problem with models that describe the tRNA selection mechanism as a nonequilibrium process (5–7). Here, the kinetics of the tRNA interaction with the codon are used to discriminate tRNA molecules through a process that must be driven by an exogenous energy supply. Nevertheless, strong, preferential binding of a specific tRNA by a mRNA-programmed ribosome in the absence of an energy source cannot be explained by such mechanisms (8–10). Here, we describe a model for tRNA selection which will operate at equilibrium, and which has two additional virtues. One is that it is consistent with recent experiments concerning the interaction between tRNA and the ribosome. The other is that this model will account for a previously inexplicable class of tRNA suppressor mutants.

Our model is based on the following observations: In order for a tRNA molecule to be stably bound by the ribosome, sites on the tRNA such as the T-Ψ-C loop are used to supplement the codon-anticodon interaction (11–13). However, these ribosomal binding sites on the tRNA are masked when the tRNA is not bound to the ribosome (14–16). Therefore, it seems likely a priori that the binding of tRNA molecules to the ribosome is accompanied by structural rearrangements of the tRNA which expose ribosomal binding sites. Indeed, Gassen and his coworkers (17) have found that the binding of an aminoacyl-tRNA to the 30S ribosomal subunit is accompanied by the exposure of a site or sites which can bind the tetranucleotide C-G-A-A; this sequence is the complement to the T-Ψ-C sequence. Most important was their demonstration that the appropriate codon enhances the accessibility of the C-G-A-A binding site.

We propose that the structure of tRNA has evolved in such a manner that when the anticodon interacts with its cognate codon on the ribosome, the fold of the anticodon loop is altered. Such a conformational transition corresponds to the first step in the tRNA discrimination process. The rearrangement of the backbone of the anticodon loop is then thought to initiate a series of conformational changes in the tRNA that raise the probability of unmasking binding sites for the ribosome at other positions in the tRNA such as the D loop and the T-Ψ-C loop. Since these conformational changes are dependent on a proper codon-anticodon interaction, they will contribute to the codon specificity of the tRNA binding reaction. Here, enhancement of the cognate tRNA interaction with the programmed ribosome is obtained through nonspecific binding sites that are brought into play by the codon-dependent conformational changes of the tRNA. We first show that such a mechanism will operate at equilibrium. Then the implications and support for such a mechanism are further discussed.

FORMAL PROPERTIES

Our model for the conformational changes of tRNA during translation can be represented as follows:

\[ T_1 + R \rightleftharpoons T_2 + R \rightleftharpoons T_3 + R \]

\[ K_1 \]
\[ K_2 \]
\[ K_3 \]

\[ T_1 R \rightleftharpoons T_2 R \rightleftharpoons T_3 R \]

\[ K_{1r} \]
\[ K_{2r} \]
\[ K_{3r} \]

Here, \( T_1 \), \( T_2 \), and \( T_3 \) are different conformations of tRNA which correspond to different functional states: \( T_1 \) is the conformation of tRNA in the unperturbed state; \( T_2 \) is the conformation of tRNA when it is properly bound at the anticodon loop by a cognate codon; and \( T_3 \) is the conformation of tRNA bound both by the codon as well as one or more do-
mains of the ribosome. The free tRNA distributed over these states can interact with the mRNA-programmed ribosome (R) as indicated in scheme 1 by the vertical arrows. Each of the corresponding bimolecular interactions is characterized by an equilibrium constant, \( K_a, K_b, \) or \( K_c. \) The transitions between the different tRNA conformations are represented by the horizontal sequences: The sequence of conformational transitions for free tRNA is characterized by the equilibrium constants \( K_I \) and \( K_{II}, \) while the conformational transitions that take place on the ribosome are characterized by \( K_1 \) and \( K_2. \)

Although it is possible for a tRNA molecule to spontaneously adopt the conformations \( T_2 \) and \( T_3, \) our model explicitly requires that the interactions between a cognate tRNA and a programmed ribosome raise the probability of finding that particular tRNA in the state \( T_9 R. \) This postulate is expressed in the assumption that \( K_1 \) and \( K_2 \) are much larger than \( K_I \) and \( K_{II}, \) respectively, for the cognate tRNA. The physical interpretation of this assumption is that the cognate interaction of codon and anticodon, as well as the codon-dependent interaction between the ribosome and its binding sites on the tRNA, will stabilize the structure \( T_3. \) Accordingly, at equilibrium the principle of detailed balance requires that:

\[
K_c = \frac{K_a K_b K_c}{K_I K_{II}}
\]  

This shows that the interaction with the ribosome of the cognate tRNA in state \( T_3 \) is enhanced over that for state \( T_1 \) by the ratio \( K_1 K_2/K_I K_{II}. \)

Our model, furthermore, introduces the idea that such enhancement cannot be brought fully into play for the noncognate tRNA. This specificity will be reflected in the ratio of \( K_c \) for a cognate tRNA (\( K_c^c \)) over \( K_c \) for a noncognate tRNA (\( K_c^n \)). This ratio can be used as an index of the relative stability of \( T_3 R \) for the cognate and noncognate ternary complexes. Thus,

\[
\frac{K_c^c}{K_c^n} = \frac{K_a K_b K_c^c}{K_a K_b K_c^n}
\]

Here, the superscripts \( c \) and \( n \) refer to the cognate and noncognate parameters. An optimal selection mechanism would require that the codon-independent constants \( K_I \) and \( K_{II} \) are very similar for different tRNA molecules so that only codon-dependent interactions would influence the distribution of the conformational states for tRNA. This as well as the fact that it would simplify our equations prompts us to assume that \( K_I \) is the same for all tRNAs, as is \( K_{II}. \)

Such an assumption reduces Eq. 3 to a form in which the stabilities of the cognate and noncognate ternary complexes are determined by the products of three equilibrium constants: \( K_a, K_1, \) and \( K_2. \) These constants characterize, respectively, the first bimolecular interaction of tRNA and programmed ribosome, the codon-dependent conformational change of the anticodon loop, and, finally, the codon-dependent unmasking of additional binding sites for the ribosome on the tRNA. The extent to which the first bimolecular interaction of \( T_1 \) with \( R \) involves a codon-anticodon interaction will determine the relative advantage of cognate tRNA over noncognate tRNA for the formation of \( T_9 R. \) We suspect that this step plays a small if significant role in the discriminations of tRNAs. However, the subsequent steps characterized by \( K_1 \) and \( K_2 \) are the ones which we postulate are very strongly dependent on a proper matching of codon and anticodon. Consequently, our model states that both \( K_c^c/K_c^n \) and \( K_c^c/K_c^n \) are much greater than one.

During protein synthesis the reversible steps in scheme 1 will be coupled to the enzymatic steps corresponding to peptide bond formation and translocation. We have depicted the latter steps simply as \( k_{irr} \) and we next investigate their effects on the tRNA discrimination steps.

First, we consider scheme 1 when there is only weak coupling between the reversible and irreversible steps, i.e., when \( k_{irr} \) is small compared to all other rate constants in the system. Similarly, we assume that \( K_I \) and \( K_{II} \) are negligibly small. Next, we define an error function, \( E, \) as the ratio of the rate of peptide formation for a noncognate amino acid over that for the cognate amino acid when the ribosome is programmed by a messenger containing a single repetitive codon. Under these conditions, it can be shown that:

\[
E = \frac{T_0^a K_a K_1 K_2}{T_0^c K_c^c K_c^c}
\]

Here, \( T_0^a \) and \( T_0^c \) are the total amounts of tRNA, noncognate and cognate, which are present in large excess over \( R. \) Accordingly, the loosely coupled system discriminates cognate from noncognate tRNA with a fidelity which is proportional to the ratio of the concentrations of cognate to noncognate tRNA and, as expected, proportional to the relative values of the equilibrium constants which control each discriminating step.

So far we have described this system with the assumption that there are only two conformational changes involved in the discrimination of a cognate from a noncognate tRNA. This restriction is an arbitrary one, and for the more general case of \( m \) conformational changes, and with the equilibria only loosely coupled to the irreversible step, we obtain:

\[
E = \frac{T_0^a K_a^m K_1^n}{T_0^c K_c^c K_c^c}
\]

This means that even with a relatively modest advantage at each discriminating step, the end result will be that the cognate tRNA has an overwhelming advantage over the noncognate tRNA for the formation of a stable complex with the programmed ribosome.

The situation described above in which the enzymatic steps are only loosely coupled to the discriminating equilibria is such that the error measured in a tRNA binding experiment will be very nearly the same as that obtained in a polypeptide synthesis experiment. However, this will not be true when the rates of the enzymatic steps are large compared to the rates of conformational equilibration. Here, the discrimination during polypeptide synthesis will be poorer than that obtained in a tRNA binding experiment. This is a consequence of the fact that in the tightly coupled situation there is insufficient time for the noncognate tRNAs to disassociate from the programmed ribosome. As a consequence, the discrimination process becomes dependent on forward rate constants rather than the equilibrium constants. This can be illustrated by examining the error function under conditions of tight coupling, i.e., when \( k_{irr} \) is large compared to all other \( k_i's \) with \( K_i = k_i/k_{irr}. \)

For example, with tight coupling and with the assumption that \( k_{-1} \) is much greater than \( k_2 \) the error function has the form:

\[
E = \frac{T_0^a K_a K_1 k_2}{T_0^c K_c K_c K_c^c}
\]
Alternatively, with the assumption that \( k_2 \) is much greater than \( k_{-1} \) we obtain:

\[
E = \frac{T_0^n K_a^n k_1^n}{T_0^n K_a^n k_2^n}.
\]

[7]

It seems reasonable to assume that the backward rate constants \( k_{-1} \) and \( k_{-2} \) would be responsible for much of the potential discrimination in our model. Therefore, the condition in which \( k_{-n} \) is large compared to the other \( k_i \)’s is unlikely to obtain in a tRNA selection system such as described in scheme I.

DISCUSSION

We have attempted to explain how the apparently weak interactions between tRNA molecules and trinucleotides can give rise to quite specific, strong interactions between the same tRNA molecules and codon-programmed ribosomes. Our approach to this problem begins with the assumption that the affinity constants measured in equilibrium dialysis experiments with trinucleotides and tRNAs in solution do not fully describe these interactions. In particular, we propose that the structure of the tRNA molecule has evolved so that it assumes characteristic conformations when it is associated with a cognate codon. We further postulate that ribosomal sites preferentially bind tRNA molecules in those conformations that are dependent on proper codon–anticodon matching. Here, the structure of the tRNA molecule is such that overall conformational changes, e.g., the unmasking of the D and T-Ψ-C loops, are strongly coupled to the conformational states of the anticodon loop. In effect, the binding energy provided by the ribosome traps tRNA molecules in conformations which maximize the energy difference between cognate and noncognate codon–anticodon interactions. Clearly, more work is required to determine whether or not such a mechanism is used to discriminate tRNA molecules during protein synthesis. Nevertheless, there are a number of observations which indicate that our proposed mechanism is not an unrealistic one.

First, Robertus et al. (16) have acknowledged that two alternative conformations for the anticodon loop of tRNA\(^{\text{Phe}}\) from yeast are possible. Accordingly, the preferential binding to the ribosome of tRNA in one of these conformational states associated with proper codon matching would correspond to the sort of conformational selection that we have postulated. If sufficient strain in the backbone fold of the tRNA develops, other structural transitions would be possible. The important point here is that conformations which the tRNA cannot easily adopt by itself could very well be stabilized by interactions with the ribosome and messenger RNA. Furthermore, there are indications that aminoaclation of tRNA at the 3' terminus can effect the conformation of the anticodon loop (18, 19), which supports the idea that conformational perturbations at one site can influence the tRNA conformation at other sites.

One important aspect of our model has been evident for quite some time. This is the notion that sites on tRNA other than the anticodon loop contribute to the codon specificity of tRNA selection. Thus, a mutation effecting the D stem of tRNA\(^{\text{Phe}}\), which normally decodes the triplet UGC, converts this tRNA into a suppressor of the UGA nonsense codon (20, 21). Such a result is difficult to reconcile with the earlier view of tRNA discrimination as a consequence solely of simple codon–anticodon interactions. Furthermore, recent measurements of the affinities of tRNA\(^{\text{Phe}}\) as well as the suppressor form of this tRNA for the UGA codon show that they are indistinguishable according to the stability of this interaction (22). We suggest that this apparent paradox is readily explained by our model.

We have postulated that the tRNA discrimination mechanism consists of a series of steps at which tRNA molecules are tested by virtue of the relative ease with which they undergo a series of conformational transitions that are coupled to the codon–anticodon interaction. Were a mutation to alter a tRNA molecule in such a fashion that the tertiary interactions which normally stabilize the unperturbed structure of the molecule become destabilized, the tight coupling between the conformation of the anticodon loop and the fold of the tRNA of other sites might be weakened. Consequently, such a mutant tRNA might bind stably to the ribosome even when only two out of three of the nucleotides in a noncognate codon match the triplet in its anticodon, just as in the case of the Hirsh UGA suppressor tRNA.

It is still not possible to decide with any certainty which of the several potential binding sites on the tRNA are involved in the amplification mechanism that we have described here. The data discussed above suggest that the T-Ψ-C loop may be one such site. However, recent data indicate that the tRNA interaction with the 30S ribosomal subunit is quite stable (23). Since the proposed binding site for the T-Ψ-C loop is on the 50S ribosomal subunit, it seems likely that other sites on the tRNA can also interact with the 30S subunit. The only relevant aspect of the data concerning the T-Ψ-C loop in the present context is the idea that it must become unmasked during the tRNA binding reaction. Hence, this unmasking phenomenon serves as a paradigm for what we suggest is a more general conformational change in the tRNA resulting from the interaction with a cognate codon and stabilized by interactions with the ribosome.

The codon according to our model serves its principal function by initiating a series of conformational changes that alter the functional state of the tRNA. From this point of view the codon is functioning as an allosteric effector which selectively enhances the stability of interaction between the cognate tRNA and the ribosome. A similar model based on the selection of conformational states has also been developed for the recognition of tRNA molecules by their cognate aminocyl ligases(5). Indeed, we suspect that such a discriminating function may be a general one for allosteric effectors. Thus, similar allosteric influences of small molecules on the functional states of proteins may also have the effect of enhancing or changing the substrate specificity of the affected enzymes (24).

Finally, our model sets in perspective two aspects of the evolution of the present protein synthetic mechanism. One of these concerns the rate of peptide bond formation, which we suggest may not be a maximum. Thus, the full potential of the tRNA selection system according to our model can not be brought into play unless the rates of the tRNA conformational changes are fast compared to the rates of the enzymatic steps which follow. Therefore, part of the structure of the ribosome may have evolved in such a fashion as to adjust the rates of peptide bond formation and translocation so that the coupling between these steps and the tRNA binding reactions remains weak.

In addition, our model may explain in part why the aminoacl adapters in protein synthesis are so much larger than trinucleotides, which are the minimum size adapters required in the original statement of the adapter hypothesis

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4 R. Rigler and M. Ehrenberg, submitted for publication.
(25). Thus, we have based the development of the present theory on the assumption that triplet–triplet interactions cannot by themselves provide the known fidelity of translation. Instead we suggest that the triplet anticodon is incorporated into a very special molecular setting that can, together with the ribosome, enhance the effectiveness of the codon–anticodon interaction. Here, conformational rearrangements of the tRNA resulting from the proper codon–anticodon interaction bring into play universal sites on the tRNA which can interact with conjugate structures on the ribosome. In this way, the stability of interaction of a cognate tRNA is preferentially enhanced. Clearly, such a mechanism would not be possible for a trinucleotide adapter.