

Testing the classical two-tRNA-site model for the ribosomal elongation cycle

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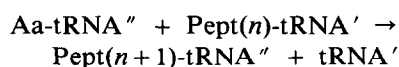
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An experimental system where the elongation of a polypeptide (polyphenylalanine) is performed stepwise and synchronously by purified *Escherichia coli* ribosome in a matrix-coupled poly (U) column is proposed for testing the number of non-overlapping tRNA binding sites on the elongating ribosome. If phenylalanyl[^3H]tRNA is introduced into the column and bound with the ribosomes at the beginning of a given elongation cycle, deacylated [^3H]tRNA is shown to be released from the ribosomes and comes out from the column at the translocation step of the next elongation cycle. The result obtained is fully predicted by the classical two-tRNA-site model and contradicts any model involving more than two non-overlapping high-affinity tRNA binding sites in the ribosomal elongation cycle.

<i>Ribosome</i>	<i>Matrix-coupled messenger</i>	<i>tRNA binding</i>	<i>Elongation cycle</i>
	<i>Two-site model</i>	<i>Translocation</i>	

1. INTRODUCTION

According to the classical model, the elongation of a polypeptide on the ribosome proceeds by means of repeating the cycles each consisting of 3 successive steps: aminoacyl-tRNA binding (I), transpeptidation (II) and translocation (III) [1,2]. Two tRNA binding sites on the ribosomes, the so-called A and P (or D) sites, are postulated. In step I an aminoacyl-tRNA binds to the A site containing a vacant codon; the binding is catalysed by the elongation factor T_u (EF- T_u) with GTP. A peptidyl-tRNA, i.e. another tRNA coupled to the nascent peptide, is situated at this moment in the P site. In step II the aminoacyl-tRNA of the A site reacts with the peptidyl-tRNA of the P site, so that transpeptidation takes place:



The reaction is catalyzed by the peptidyl transferase center of the ribosome itself. Now the elongated peptidyl-tRNA (its tRNA residue) occupies the A site, and the deacylated tRNA is in the

P site. This is the so-called pre-translocation state. In step III the peptidyl-tRNA (its tRNA residue) together with the template codon is displaced from the A site to the P site, and the deacylated tRNA is released from the P site into solution; the displacements are catalysed by the elongation factor G (EF-G) with GTP. As a result, the so-called post-translocation state is again achieved when a peptidyl-tRNA is in the P site, and the A site with a new vacant codon is ready to accept the next aminoacyl-tRNA.

The above model is well consistent with the majority of experimental data obtained in the last 15 years. Nevertheless, contradictory reports appeared where the existence of more than two tRNA binding sites on the ribosome was claimed [3-9]. Recently, a new three-site model for the ribosomal elongation cycle has been proposed [10,11]. According to the model two tRNA molecules are present on the ribosome at both the pre- and post-translocation state, and deacylated tRNA is not released from the P site during translocation but moved to a non-overlapping E ('exit') site. (The proposed E site is assumed to have an affinity to tRNA comparable with that of the P site [6,7].)

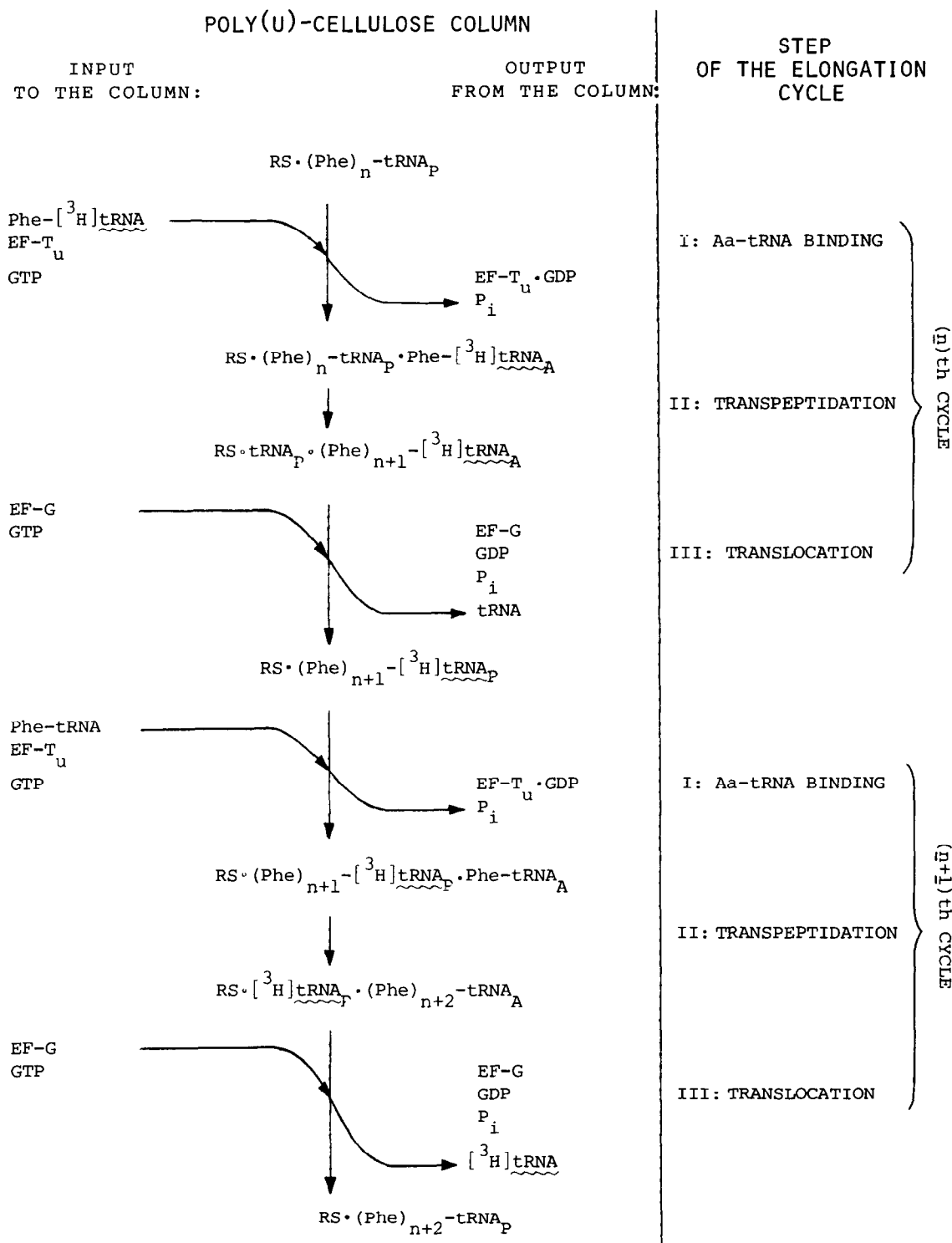


Fig. 1. Scheme of the experiment with the prediction of the moment of the deacylated tRNA ($[^3H]tRNA$) release from the elongating ribosomes according to the classical two-tRNA-site model for the elongation cycle.

To test the classical two-site model, as well as any other model using n non-overlapping tRNA binding sites for the elongation cycle, an experimental system has been used where the elongation is performed stepwise and synchronously by ribosomes in the column with poly(U) covalently coupled to cellulose through its 3'-end [12,13]. The results obtained are consistent with the two-site model and contradict the three-site one.

2. METHODS AND RESULTS

If the classical two-tRNA-site model is correct, a labelled tRNA introduced in the form of the aminoacyl-tRNA into the column at the beginning of the elongation cycle (fig.1, step I of the n -th cycle) should come out from the column at the end of the next cycle during the translocation step (fig.1, step III of the $(n+1)$ -th cycle). This prediction is fully realized in the experiment done here.

Materials, preparations and experimental conditions were the same as in [12,13]. The standard buffer, 10 mM Tris-HCl (pH 7.2, at 25°C), with 10 mM MgCl₂, 100 mM NH₄Cl and 1 mM dithiothreitol was used in all procedures. The sequence of the procedures was as follows:

(1) All ribosomes (not less than 10 pmol) synthesizing polyphenylalanine in a 0.12 ml po-

ly(U)-cellulose column were brought to the post-translocation state by passing EF-G with GTP at 25°C;

- (2) the column was washed with the buffer in the cold;
- (3) the mixture containing [¹⁴C]phenylalanyl-^{[3}H]tRNA, EF-T_u and GTP was passed through the column in the cold (step I, aminoacyl-tRNA binding, and the automatically following step II, transpeptidation);
- (4) the column was washed with the buffer in the cold;
- (5) the mixture of EF-G and GTP at 25°C was passed through the column (step III, translocation);
- (6) the column was washed with the buffer in the cold;
- (7) the mixture containing unlabelled phenylalanyl-tRNA, EF-T_u and GTP was passed through the column in the cold (step I, aminoacyl-tRNA binding, and step II, transpeptidation, of the next cycle);
- (8) the column was washed with the buffer in the cold;
- (9) the mixture of EF-G and GTP at 25°C was passed through the column (step III, translocation); in the case of the control column EF-G was omitted.

Table 1

Release of deacylated tRNA from ribosomes as a result of translocation

Column No.	Conditions during translocation step in the $(n+1)$ th cycle	[¹⁴ C]Phe incorporated (pmol)	[³ H]tRNA		
			Total bound (pmol)	Released during the translocation step in the $(n+1)$ th cycle (pmol)	(%)
1	+ EF-G, + GTP (experiment)	11.6	11.3	9.2	81
2	- EF-G (control)	11.8	12.1	0.5	4

[¹⁴C]Phe-^{[3}H]tRNA is introduced into poly(U)-programmed post-translocation ribosomes carrying polyphenylalanyl-tRNA (Phe_{*n*}-tRNA) (step I of the n -th cycle in fig. 1). The amount of elongating ribosomes is indicated by the amount of [¹⁴C]phenylalanine incorporated. The total amount of [³H]tRNA retained by the ribosomes coincides with the above (within experimental error). The EF-G-induced translocation in the next cycle (step III of the $(n+1)$ th cycle in fig.

1) releases the majority of this [³H]tRNA in the deacylated form

All the fractions eluted from the column, as well as the material retained, were analysed as in [12,13].

The results are presented in table 1.

3. DISCUSSION

In the above experiment the elongating ribosome behaves in accordance with the classical two-tRNA-site model: the tRNA bound with the A site at the beginning of a given elongation cycle seems to be displaced first to the P site as a result of translocation in the same cycle, and then it is released from the ribosome as a result of translocation in the next cycle (see fig.1). This experiment contradicts any model where the existence of more than two non-overlapping tRNA binding sites is postulated provided the additional sites are thought to be functionally involved in the elongation cycle and possess an affinity to tRNA of the same order of magnitude that is ascribed to the A and/or P site.

The experiments presented do not exclude the possible existence of other tRNA binding sites overlapping with the A or P site, such as the common intermediate complex site [14] or the recognition (R) site [15]. Moreover, in view of the complexity of the ribosomal machinery and the evident multi-center character of the binding and retention of the substrates and the products, each step of the elongation cycle should consist of sub-steps, so that intermediate states and positions of tRNA seem to be likely.

Of course, the existence of codon-independent low-affinity sites on the elongating ribosome cannot be ruled out by the experiment. Besides, it cannot be excluded that some tRNA binding sites not directly involved in the elongation cycle may exist on the ribosome; the site suggested by authors in [16] could be such an example.

In conclusion, I would like to point out that the release of deacylated tRNA from the ribosome during translocation has been well established [17-23]. However, in the earlier reports either non-translating ribosomes precharged with deacylated tRNA or initiation ribosomal complexes with Ac-Phe-tRNA or F-Met-tRNA were used as a starting material for demonstration of the subsequent tRNA release. The release of a non-initiator tRNA during continuing elongation was not

demonstrated. That is why the experiment with elongating ribosomes presented here seems to be a necessary complement to confirm both the deacylated tRNA release during translocation and the classical two-tRNA-site model of the elongating ribosome.

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