Adjacent codon-anticodon interactions of both tRNAs present at the ribosomal A and P or P and E sites

Hans-Jörg Rheinberger and Knud H. Nierhaus*

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Ihnestr. 73, D-1000 Berlin 33 (Dahlem), Germany

Received 2 June 1986

A labeled tRNA present at the A, P or E site can be partially chased from the ribosome, a cognate non-labeled tRNA as chasing substrate being 3–12-times more efficient than non-cognate tRNA at a molar ratio tRNA: 70 S = 10:1. These findings indicate that a tRNA bound to a programmed ribosome undergoes codon-anticodon interaction at all three sites (A, P and E site). Furthermore, both labeled tRNAs present on the ribosome can be chased more effectively with cognate than with non-cognate substrate at the same time. This finding provides strong evidence that both tRNAs present on the ribosome exhibit simultaneous codon-anticodon interaction. This is valid for both the pretranslocational state (Ac[^{3}H]Lys-tRNA^{Lys} in the A and [^{14}C]tRNA^{Lys} in the P site) as well as the posttranslocational state (Ac[^{3}H]Lys-tRNA^{Lys} in the P and [^{14}C]tRna^{Lys} in the E site).

ribosomal tRNA binding *Ribosomal A site* *Ribosomal E site* *Ribosomal P site* *Translocation* *Codon-anticodon interaction*

1. INTRODUCTION

The ribosomal A and P sites can bind tRNA via codon-anticodon interaction [1–4]. However, there is a controversy concerning the codon contact at the E site. First evidence for a codon-anticodon interaction at that site [5] was questioned [6,7]. Recently the coded nature of tRNA binding at the E site was positively demonstrated (see [8]).

The problem as to whether both tRNAs on one ribosome can simultaneously undergo codon-anticodon interaction has not yet been settled. In principle, the L-form of the tRNAs enables two adjacent codon-anticodon interactions to take place, in spite of the steric difficulty that both tRNAs, each with a diameter of 20 Å, bind to two adjacent codons, each of length 10Å [9]. Here we provide evidence that the ribosome indeed accommodates two adjacent codon-anticodon interactions at either A and P or P and E sites.

* To whom correspondence should be addressed

2. EXPERIMENTAL

Tightly coupled 70 S ribosomes were isolated from mid-log phase cells of *Escherichia coli* K1, strain D10 (RNase I$^-$, Met$^-$) as described [10]. 1 A_{260} unit of 70 S ribosomes was taken to be equivalent to 24 pmol. Poly(A) and tRNA^{Phe} were from Boehringer Mannheim and tRNA^{Lys} from Sigma (Lys acceptance 1385 pmol/A_{260} unit). The preparation of deacylated [^{14}C]tRNA^{Lys} followed the procedure of [5], and the preparation of Ac[^{3}H]Lys-tRNA^{Lys} that of [11]. The specific activities were 60 and 250 cpm/pmol for [^{14}C]tRNA^{Lys} and Ac[^{3}H]Lys-tRNA^{Lys}, respectively, in the binding reaction and 384 cpm/pmol for Ac[^{3}H]Lys-tRNA^{Lys} in the puromycin reaction. 1 A_{260} unit of tRNA was taken to be equivalent to 1500 pmol. EF-G was isolated as described in [12]. The test system for tRNA binding and translocation has been described in [13], and the procedure of the puromycin reaction with Ac[^{3}H]Lys-tRNA^{Lys} in [8].

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00145793/86/$3.50

3. RESULTS AND DISCUSSION

We make use of the chasing procedure [8], i.e. we occupy A, P or E sites with radiolabeled tRNAs, and then chase the bound material with increasing amounts of non-labeled deacylated tRNA using either cognate or non-cognate material. If the labeled tRNA is bound via codon-anticodon interaction, the cognate chasing substrate should be much more effective than the non-cognate one. On the other hand, if no codon-anticodon interaction exists, then both cognate and non-cognate substrates should be equally effective.

[^{14}C]tRNA^{Lys} is bound to the P site of poly(A)-programmed ribosomes (fig.1, step 1). 72% of the ribosomes carry a [^{14}C]tRNA^{Lys} (see occupation number ν = number of tRNAs bound per 70 S). The cognate chasing substrate (non-labeled tRNA^{Lys}) is 3 times more effective than the non-cognate one, indicating the coded nature of the P-site binding (see curves below step 1). When Ac[^{3}H]Lys-tRNA^{Lys} is bound to the A site (step 2) again a predominant effect of the cognate chasing substrate is seen (dashed curves below step 2). Interestingly, the chasing pattern of the [^{14}C]tRNA^{Lys} present at the P site does not change. We conclude that the coded nature of the P-site bound tRNA is not affected by the occupation state of the A site. It is evident that both tRNAs present at the A and P site simultaneously undergo codon-anticodon interaction.

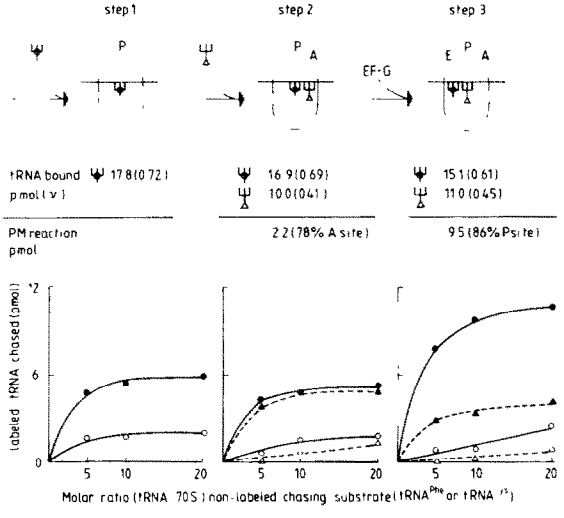

Fig.1. Chasing of labeled tRNA from poly(A)-programmed ribosomes by either non-labeled cognate tRNA^{Lys} (——) or non-cognate tRNA^{Phe} (– – –). For the binding of the labeled tRNAs [^{14}C]tRNA^{Lys} (Ψ) was added in a molar ratio of tRNA:70 S = 1:1 and Ac[^{3}H]Lys-tRNA^{Lys} (Ψ with A) in a molar ratio of 1.7:1. One aliquot contained 24 pmol 70 S per 50 μl. (● and ○) Amount of [^{14}C]tRNA^{Lys} chased with cognate and non-cognate chasing substrate, respectively. (▲ and △) Amount of Ac[^{3}H]Lys-tRNA^{Lys} chased with cognate and non-cognate substrate, respectively. Incubation time for chasing was 15 min at 37°C.

In the third step 7.3 (9.5–2.2) pmol Ac[^{3}H]Lys-tRNA is translocated upon EF-G addition, and the translocation is accompanied by a small release of 1.8 (16.9–15.1) pmol [^{14}C]tRNA^{Lys}. It follows that more than 75% of the translocated Ac[^{3}H]Lys-tRNA must have a [^{14}C]tRNA^{Lys} at the adjacent E site (release and rebinding of the deacylated tRNA could be excluded in previous experiments [5]). The chasing pattern of Ac[^{3}H]Lys-tRNA does not change qualitatively, although the effectiveness of the cognate chasing substrate is enlarged (table 1; cf. chasing factor = effect of cognate/effect of non-cognate chasing substrate of Ac[^{3}H]Lys-tRNA at the A and P site, 5.8 and 11.7, respectively). However, the chasing pattern of [^{14}C]tRNA^{Lys} is drastically altered after translocation to the E site. The effect of the cognate chasing substrate is significantly increased, indicating a lower affinity for [^{14}C]tRNA at the E site as compared to the P site. In contrast, the effect of the non-cognate substrate remains at its low level thus increasing

Table 1

Chasing factors for [^{14}C]tRNA^{Lys} and Ac[^{3}H]Lys-tRNA for the various ribosomal sites

Chased tRNA	Chasing factor (ribosomal site)		
	Step 1	Step 2	Step 3
[^{14}C]tRNA^{Lys}	3.1 (P)	3.3 (P)	9.7 (E)
Ac[^{3}H]Lys-tRNA^{Lys}	–	5.8 (P)	11.7 (P)

The chasing factor is defined as the amount of labeled tRNA chased with cognate non-labeled tRNA^{Lys} divided by the amount chased with non-cognate non-labeled tRNA^{Phe}. The values were calculated from fig.1 using the data obtained with a 10-fold excess of chasing substrate over ribosomes

the chasing factor for [^{14}C]tRNA^{Lys} from 3.3 (P site) to 9.7 (E site, see table 1). Clearly, most if not all [^{14}C]tRNA at the E sites undergoes codon-anticodon interaction. This argues for adjacent codon-anticodon interactions of the corresponding tRNAs at the P and E site.

However, let us assume the alternative possibility that on one ribosome only one codon-anticodon interaction at a time exists, at either the P or E site, and that the adjacent tRNA lacks this interaction. As a consequence we would have a ribosome population (E) with a codon-anticodon interaction at the E and not at the P site, and a population (P) with interaction at the P but not at the E site. We find that both [^{14}C]tRNA^{Lys} at the E site and Ac[^{3}H]Lys-tRNA^{Lys} at the P site can be chased about 10-times better with the cognate substrate than with the non-cognate one. This fact would mean – if the above assumption were correct – that in population (E) only the coded [^{14}C]tRNA at the E site could be chased in contrast to the non-coded Ac[^{3}H]Lys-tRNA at the P site, and correspondingly in population (P) only the coded Ac[^{3}H]Lys-tRNA^{Lys} at the P site in contrast to the non-coded [^{14}C]tRNA^{Lys} at the E site. The extremely unlikely consequence would follow that a tRNA with codon-anticodon interaction has a lower binding affinity and thus can be chased more easily than a tRNA present on the ribosome without codon-anticodon interaction. On the contrary, a wealth of data has demonstrated that codon-anticodon interaction significantly increases the binding affinities (association constants) for tRNAs on the ribosome. Therefore, our results strongly indicate that both tRNAs present at the P and E site simultaneously undergo codon-anticodon interaction. A corresponding experiment has been performed with poly(U)-programmed ribosomes yielding equivalent results.

In conclusion, we confirm here that a tRNA at all three sites (A, P and E site) can undergo codon-anticodon interaction. Furthermore, we provide striking evidence that both tRNAs present at the A and P or P and E site, respectively, simultaneously undergo codon-anticodon interaction.

ACKNOWLEDGEMENTS

We thank Drs H.G. Wittmann and R. Brimacombe for help and advice.

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