BIOLOGICAL PROPERTIES OF TRANSFER RNA, SYNTHESIZED DURING ARGININE DEPRIVATION IN STRINGENT AND RELAXED AUXOTROPH STRAINS OF *ESCHERICHIA COLI*

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Received 27 August 1971
Revised version received 15 December 1971

1. Introduction

Recent experiments from several laboratories reveal that deprivation of amino acids in amino acid auxotrophs of bacteria with stringent control (RC^{str}) over RNA synthesis allows the transcription of mRNA whereas the synthesis of stable RNA is inhibited [1]. The messenger RNAs which are transcribed for amino acid biosynthetic enzymes however are not necessarily translated indicating that translational control can be superimposed on the transcriptional control [2–6]. Accumulating evidence exists that one type of regulation of protein synthesis, occurring at the translational level, involves conformational or structural modifications of transfer RNAs resulting in changes in their biological activities [7].

Two isogenic arginine-requiring strains of *E. coli* with stringent or relaxed control were used to study: 1) whether the biological properties of tRNAs change upon deprivation of the required amino acid and 2) whether the tRNAs accumulating in relaxed strains during amino acid starvation differ in biological properties from the tRNAs formed in the isogenic stringent strain. The acceptor activities for phenylalanine, lysine and arginine and the activities in the poly U, respectively poly A, directed amino acid incorporating system – "transfer activities" – of the tRNAs synthesized during arginine deprivation in both mutants were measured. The results show that both the acceptor activities and transfer activities of the isolated tRNAs are dependent on the concentration of arginine at which the cells were grown. This dependence is different in the two RC mutants.

2. Materials and methods

2.1. *Chemicals*

Uniformly labeled ^{14}C-L-phenylalanine and ^{14}C-L-lysine: Radiochemical Centre, Amersham; tRNA from *E. coli* MRE 600, ATP (disodium salt), phosphoenolpyruvate (monosodium salt), pyruvatekinase crystal suspension in 3.2 M ammonium sulfate solution and GTP (trisodium salt): Boehringer Mannheim; poly U and poly A: Miles Chemical Company; deoxyribonuclease I: Worthington Biochemical Company; β-mercaptoethanol: Sigma Chemical Company; DEAE-cellulose: Whatman. Ammonium sulfate was removed from the solution of pyruvatekinase by passage through a column of Sephadex G-25 at 0°.

2.2. *Bacterial strains and growth*

E. coli arginine$^-$ thiamine$^-$ of the genotype RC^{str} (PA2) and the isogenic strain RC^{rel} (PA1) were gifts from Dr. Galibert [8]. The bacteria were grown in a minimal medium containing per liter: 5 g NaCl, 2.5 g $(NH_4)_2PO_4$, 1.5 g KH_2PO_4, 3 g sodium glutaminate, 3 g glucose, 0.1 g $MgSO_4 \cdot 7\ H_2O$, 0.1 mg thiaminehydrochloride and varying amounts of arginine (10–60 mg).

2.3. *tRNAs*

These were isolated from cells harvested 1 hr after reaching the stationary phase by cold phenol extraction and purified [9]. Polyacrylamide gel electrophoresis revealed that the tRNA was devoid of rRNA and rRNA degradation products. The contamination with 5 S RNA was negligible.

2.4. *Ribosomes and cell-free extracts*

(S-100) were prepared from log-cells of *E. coli* 9637 grown in maximal medium [10]. The S-100 fractions were dialysed overnight, and the tRNAs were removed [11].

2.5. *Acceptance activity*

This was measured as described [12]. The reaction mixture, final vol 250 μl, contained: 25 μmoles Tris-HCl pH 7.2; 4 μmoles magnesium acetate; 2.5 μmoles KCl; 1.5 μmoles β-mercaptoethanol; 0.25 μmoles ATP; 0.0075 μmoles GTP; 1.25 μmoles PEP; 5.0 μg pyruvate kinase; 19 C-12 amino acids (lysine, phenylalanine or arginine omitted), 5 nmoles each; 4 $A_{260\ units}$ tRNA; *E. coli* S-100 fraction chromatographed over a DEAE-cellulose column = 30 μg protein, (30 μg of S-100 protein were found to be the optimal concentration in the acceptor system); ^{14}C-phenylalanine, specific activity 47.7 mCi/mmole or ^{14}C-lysine, specific activity 31.2 mCi/mmole, each nmoles, or ^{14}C-arginine, specific activity 32.4 mCi/mmole, 7.9 nmoles. Incubation: 8 min at 25°.

2.6. *Amino acid incorporation*

Tested in an equal system except that the concentration of KCl was raised to 12.5 μmoles per 250 μl and the pH to 7.8. Ribosomes, 9–10 nmoles, and 300–400 μg S-100 protein were added. After 10 min preincubation at 37°, the incorporation was started by the addition of 20 μg poly U or poly A. Incubation time: 20 min at 37°. At a concentration of tRNA corresponding to 4 $A_{260\ units}$, the system was strictly dependent on added tRNA. The radioactivity in both assays was measured by the filter paper disc technique [13].

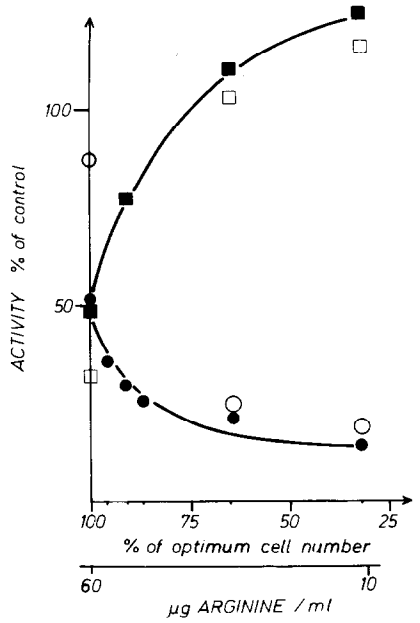

Fig. 1. Acceptor and transfer activities for phe or lys of total tRNAs isolated from cultures of *E. coli* thiamine$^-$ arginine$^-$ RC^{str} grown at different levels of arginine. Acceptor activity was measured in a final vol of 250 μl, which contained: 25 μmoles Tris HCl pH 7.2; 4 μmoles magnesium acetate; 2.5 μmoles KCl; 1.5 μmoles β-mercaptoethanol; 0.25 μmoles ATP; 0.0075 μmoles GTP; 1.25 μmoles PEP; 5.0 μg pyruvatekinase; 19 C-12 amino acids (lys or phe omitted), 5 nmoles each; 4 A_{260} units tRNA; 30 μg protein *E. coli* S-100 fraction (chromatographed over a DEAE-cellulose column); ^{14}C-phe (47.7 mCi/mmole) or ^{14}C-lys (31.2 mCi/mmole), each 5 nmoles. Incubation: 8 min at 25°. Transfer activity was measured in 250 μl of the same buffer except that the concentration of KCl was raised to 12.5 μmoles and the pH to 7.8. Ribosomes of *E. coli* B 9–10 nmoles per 250 μl, and S-100 fraction 300–400 μg protein per 250 μl were added. After 10 min preincubation at 37° the incorporation was started by the addition of 20 μg poly U or poly A. Incubation: 20 min at 37°. The radioactivity was measured in both test systems following the method of Mans and Novelli [13]. Both the acceptor activities and the transfer activities are referred to the activity of a control tRNA (Boehringer) set at 100%. The acceptor and transfer activities were referred to the relative decrease in the cell number, caused by arginine starvation. Optimal cell number obtained at 60 μg arginine/ml medium was set at 100%. (■—■—■): Acceptor activity for phe; (●—●—●): transfer activity for phe; (□—□—□): acceptor activity for lys; (○—○—○): transfer activity for lys.

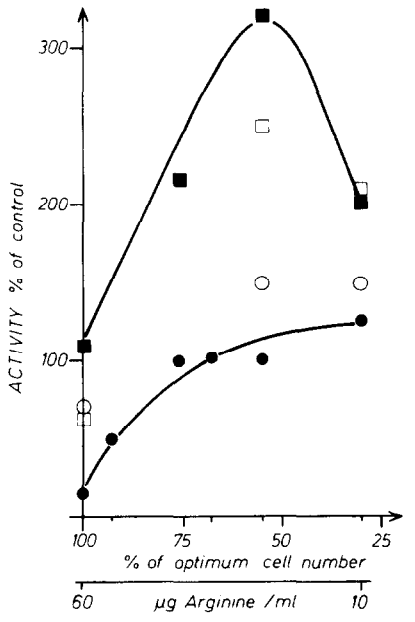

Fig. 2. Acceptor and transfer activities for phe or lys of total tRNAs isolated from cultures of *E. coli* thiamine⁻ arginine⁻ RC^{rel} grown at different levels of arginine. For further details see legend of fig. 1. (▪—▪—▪): Acceptor activity for phe; (●—●—●) transfer activity for phe; (□—□—□): acceptor activity for lys; (○—○—○): transfer activity for lys.

3. Results

The arginine and thiamine auxotrophs of *E. coli* RC^{str} and RC^{rel} chosen for the experiments have optimal growth at concentrations of 100 µg thiamine and 60 µg arginine/ml growth medium. In the relaxed strain RNA accumulates under starvation conditions.

3.1. *tRNAs from E. coli* RC^{str}

The functional properties of tRNAs formed in cells grown at different levels of arginine (60–10 µg/ml) are shown in fig. 1. For comparison the acceptor activities or transfer activities were referred to the biological activity of a control tRNA, which was tested in each series of experiments. Acceptor activity for phe of the control tRNA (Boehringer) was in the range of 6.5–8.0 pmoles phe/1 A_{260} tRNA, respectively 11–12 pmoles lys/1 A_{260} tRNA. Transfer activity was in the range of 300–500 pmoles phe or lys/nmole ribosome. The activity of the control tRNA in each series of experiments was set at 100% and all other values referred to the control.

At sufficient doses of arginine the *"acceptor activities"* of total tRNA for phe or lys are about 1/2 or 1/3 lower than the acceptor activities for both amino acids of the control tRNA. Upon successive deprivation of arginine the acceptor activities for both amino acids increase, reaching equal or little higher values than those observed for the control tRNA.

The tRNA function at the ribosome was tested in the poly U, respectively poly A, directed amino acid incorporating system. It is impossible to measure selectively the degree of binding of differently aminoacylated specific tRNAs to ribosomes with a mixture of total tRNA. The *"transfer activity"* for phe at sufficient doses of arginine is 50% less, and for lys slightly less, than the activity of the control tRNA. If only the acceptor activities of the tRNAs for phe or lys were changed, dependent on the arginine concentration the increase in acceptor activities at starvation could mimic a corresponding increase in the poly U directed incorporation of phe or respectively poly A directed incorporation of lys in the "transfer assay". The transfer activities for both amino acids decrease, however, on stepwise deprivation of arginine down to arginine concentrations of 10 µg/ml of the growth medium.

3.2. *tRNAs from E. coli* RC^{rel}

The functional properties of the tRNAs formed in the relaxed strain are also dependent on the arginine concentration in the growth medium (fig. 2). At sufficient doses of arginine the acceptor activities for phe are equal and for lys only slightly lower than those of the control tRNA. As in the stringent strain the acceptor activities increase when cells were grown at reduced levels of arginine. Upon further deprivation of arginine the ability to accept amino acids decreases, but remains still higher than the amino acid acceptance activity for lys or phe of the control tRNA.

As in the stringent strain the tRNAs formed at sufficient levels of arginine are much less active in the poly U directed phe and slightly less active in the poly A directed lys incorporation than the control tRNA, though the degree of aminoacylation is the same as in the control system. In contrary to the tRNAs from stringent strains, the tRNAs from relaxed

Table 1
Arginine and phenylalanine acceptance of total tRNA of *E. coli* thiamine⁻ arginine⁻ RC^{str} and RC^{rel} grown at different concentrations of arginine.

	$tRNA_{control}$	tRNA RC^{str}		tRNA RC^{rel}	
arginine concentration during growth (μg/ml)	–	60	20	60	20
arg	56	68	96	59	94
phe	40	33	41	31	51

Acceptor activities are expressed as pmoles arg or phe/A_{260} tRNA. S-100 fraction was prepared from *E. coli* MRE 600. ^{14}C-arginine (32.4 mCi/mmole) was added at a concentration of 7.8 nmoles/assay. The reaction mixture was incubated for 30 min. at 25°. For further details see legend of fig. 1.

strains increase in transfer activity for phe and lys upon stepwise arginine deprivation.

In another series of experiments the acceptor activities for arg of *tRNAs from stringent or relaxed strains* grown at 60 or 20 μg of arginine per ml culture were tested and compared with that for phe (table 1). The results show that the acceptor activities for arg in both strains increase by arginine deprivation and that the increase of activity in the relaxed mutant is 10% higher than in the stringent strain.

The activities of the S-100 fractions of both strains were tested with control tRNA and found to be not influenced by arginine starvation.

4. Discussion

From the results presented, the following conclusions can be drawn: 1) The biological activities of total tRNAs for phe, lys or arg formed in the 2 arginine auxotroph isogenic strains of *E. coli* with stringent or relaxed control over RNA synthesis are dependent on the arginine concentration at which the cells were grown. 2) In both strains the acceptor activities for phe, lys and arg increase upon arginine starvation. Contrary, the transfer activities for phe and lys decrease in the stringent and increase in the relaxed strain during starvation.

Differences in the biological activities of total tRNA for certain amino acids may be caused by quantitative or qualitative changes of the specific tRNAs. The observed increase in acceptor activity and the concomitant decrease in transfer activities for lys and phe in the stringent strain can not be explained with merely a quantitative change. Furthermore the analogous behaviour of the 3 amino acids in the acceptor system and of the 2 amino acids in the transfer system in both strains indicate qualitative rather than quantitative alterations. (The transfer activity with arginine was not yet tested because the corresponding synthetic messenger was not commercially available.)

Alterations in the activities of specific tRNAs may be caused by: 1) inhibitors or activators binding to tRNA; 2) differences in the total amount of the CCA-end; 3) differences in the red-ox state of sulfur-containing bases; 4) differences in the modification of the adenosine-residue located in the anticodon-region; 5) differences in the degree of methylation [7].

Modifications by activators or inhibitors or differences in the degree of methylation could be plausible explanations for the observed variations in the biological activities of the tRNAs tested.

Arginine could reduce the polyamine content in the *E. coli* strains by repressing the enzymes of the pathway glutamic acid to ornithine [14]. Thus arginine in both strains may control the level of polyamines which have been shown to affect tRNA structure and function [15, 16]. Cohen et al. [17] have found in 2 *E. coli* strains RC^{str} and RC^{rel} (auxotroph for thymine, uracil and arginine) upon arginine starvation differences in the polyamine content. Therefore the observed differences of the transfer function in the stringent and relaxed strains may reflect alterations in the polyamine content of the tRNAs.

Polyamines not only bind to tRNA, but also could influence the methylation of tRNA [18, 19]. We have found that total tRNA from the relaxed strain accepts less methyl groups than the total tRNA from the stringent strain. The overall methylase activity is significantly higher in extracts from the relaxed than in that from the stringent strain [20]. Whether the degree of methylation of the tRNAs is dependent

on arginine and can be correlated to the biological function is under current investigation.

In the stringent mutant, acceptor activities and transfer activities for phe or lys respond opposite to arginine. A balance between these two functions of the tRNAs seems to be a plausible mechanism to regulate the rate of protein synthesis at the translational level, which could be disturbed under relaxed control.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. The expert technical assistance of Ursula Frohn is gratefully acknowledged.

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