# STUDY OF TRANSLOCATION IN POLYPEPTIDE SYNTHESIS

S.Bresler, R.Grajevskaja, E.Saminsky

Institute of High Molecular Weight Compounds, Leningrad

Received June 18, 1969

Summary

Preincubation of the polylysyltRNA-ribosomal complex with lysyltRNA effected a strong inhibition of the puromycin reaction. This reaction can be resumed by addition of supernatant enzymes and GTP.

Tetracycline at $10^{-3}$M inhibited the formation of the polylysyltRNA-ribosomal complex.

The modern conception of protein synthesis distinguishes a donor and acceptor site in the ribosome. PeptidyltRNA is alternating between both. It occupies the donor site immediately before the act of peptide bond synthesis. After the synthesis took place peptidyltRNA is in the acceptor site and must be translocated back into the donor site to free the acceptor or decoding site for a new aminoacyltRNA. We developed an experimental test for translocation.For this purpose we used a cell-free system from E.coli, containing ribosomes and polylysyltRNA Rychlik[1,2] showed that this particular peptidyltRNA yields a specific complex with ribosomes in the presence of its template,i.e. polyA.In this state it reacts readily with puromycin to give polylysylpuromycins. Gottesman[3] showed additionally that with polylysyltRNA and lysyltRNA one single act of chain elongation takes place,i.e. one single peptide bond is synthesized in the absence of supernatant factors and energy source(GTP).We found that

after this reaction polylysyltRNA is unable to react with puromycin, obviously because it occupies the acceptor site[4]. It must be translocated into the donor site to resume reactivity towards puromycin. For this purpose it needs GTP and some specific enzyme(s) from the supernatant solution. This course of events gives us a simple test for translocation.

Tetracycline at $10^{-3}$M inhibits the formation of the peptidyltRNA-ribosome complex. According to Sarkar and Thach[5] this proves that polylysyltRNA is bound at first in the acceptor site and translocated thereafter to the donor site.

## MATERIALS AND METHODS

A cell-free system from E.coli Q I3 was incubated with $^{14}$C lysine(9.$10^{7}$cpm/$\mu$mole). PolylysyltRNA was purified according to Gottesman[3] with one substantial modification: 200$\mu$g/ml of chloramphenicol were added to medium prior to incubation to decrease the length of polylysine chains[6]. Our purpose was to make the influence of the peptide chain length insignificant[7].

Ribosomes prepared from the same strain of E.coli were washed by a $NH_4Cl$ solution according Nishizuka and Lipmann[8] and stored at -70°. Their amount was estimated by optical density at 260 m$\mu$. The formation of the active complex and the reaction with puromycin were studied in samples of 0.I ml, incubated at 37°. All other details are given in the legend to Table I. After incubation we measured the residual amount of polylysyltRNA by precipitation with trichloroacetic acid(TCA) in the cold.[9] To measure the binding of polylysyl--tRNA to ribosomes the samples were subjected to ultracentrifugation at 5° C through a gradient of $^{2}H_2O$(90-33%) in tris-buffer pH 7.5, containing $10^{-2}$M $Mg(OAc)_2$ and 6.$10^{-2}$M KCl.

## RESULTS AND DISCUSSION

Experiments in the ultracentrifuge showed that in conditions adopted by us 3 $A_{260}$ units of ribosomes are more than enough to bind at least 80% of the peptidyltRNA added. A threefold increase of the amount of ribosomes did not influence the binding of peptidyltRNA and the completeness of the puromycin reaction. One can see from the data presented in Table I, that the release of polylysine from polylysyltRNA is effected by puromycin with an efficiency 60-70%. GTP and supernatant enzymes are of no consequence. But a preincubation of the polylysyltRNA-ribosome complex with the aminoacyltRNA effects a strong inhibition of the puromycin reaction. As shown by special controls in the ultracentrifuge, the peptidyltRNA-ribosome complex is preserved after preincubation but is inert. After addition of GTP and supernatant factors the reactivity of the complex towards puromycin is restored entirely.The reaction of peptidyltRNA with puromycin is possible only in case when peptidyltRNA occupies the donor site [4]. Therefore the data presented show that the reaction of peptidyltRNA with the monomer, aminoacyltRNA, transfers polylysyltRNA from the donor site to the acceptor site. The subsequent translocation requires GTP and some protein factors. The fact that preincubation of peptidyltRNA with aminoacyltRNA inhibits the subsequent liberation of peptides by puromycin was noticed recently by Scogerson and Moldave [10] and Schneider et al.[11] working with endogenous templates. But in these systems the effect is much less pronounced. It is easy to study different antimetabolites for their action on translocation.As was shown by Sarkar and Thach [5] the

Table I

Influence of lysyltRNA on the reaction of polylysyltRNA with puromycin.

A mixture for the first incubation contained in 0.Iml: 3 $A_{260}$ units of washed ribosomes, I5 μg of polyA, tris-HCl buffer (pH 7.4) -50 mM, $NH_4Cl$ -I60mM, $Mg(OAc)_2$ - I5 mM, EDTA -I.0 mM, β-mercaptoethanol -0.6 mM, polylysyltRNA -2300-2800 cpm(about I0μμmoles).

When the second and third incubations were performed the amounts of components added were : $^{12}C$-lysyltRNA -20 μl(I2mg/ml), GTP -5 μl(2 mM), puromycin -5 μl(4mg/ml), supernatant enzymes, obtained after 4 hours of centrifugation at I05 000g -5 μl(I0 mg of protein per ml). All solutions contained buffer and $Mg(OAc)_2$.

| The conditions of incubation | | The amount of polylysyltRNA insoluble in TCA | The extent of release by the puromycin reaction |
|---|---|---|---|
| 2d incubation (20 min) | 3d incubation (I0 min) | | |
| | | cpm | % |
| No additions | No additions | I225 | - |
| No additions | + puromycin | 364 | 70 |
| + enzymes, GTP | No additions | I020 | - |
| + enzymes, GTP | + puromycin | 244 | 80 |
| + lysyltRNA | No additions | II25 | - |
| + lysyltRNA | + puromycin | II80 | 4 |
| + lysyltRNA | + enzymes, GTP, puromycin | I92 | 84 |

action of tetracycline enables to find in what site of the ribosome aminoacyltRNA is bound. Only the binding in the

acceptor site is subject to inhibition by this poison.The same is valid for formylmethionyltRNA, only the concentration of the antimetabolite must be increased in this case to $10^{-3}$M instead of $10^{-4}$M. Table II shows that the reaction of polylysyltRNA with puromycin is strongly inhibited by

Table 2

Influence of tetracycline on the release of polylysine from peptidyltRNA by puromycin.

All the conditions and concentrations of the components were the same as in Table I. The amount of tetracycline was 0.02 ml(2.4 mg/ml). The amount of polylysyltRNA in the sample was 420 cpm in experiment I and 625 cpm in experiment 2.

| N | The conditions of incubation | | The amount of polylysyltRNA insoluble in TCA | The extent of release by puromycin |
|---|---|---|---|---|
| | 2d incubation (20 min) | 3d incubation (10 min) | cpm | % |
| I | No additions | No additions | 220 | - |
| | No additions | + puromycin | 79 | 64 |
| | tetracycline ($10^{-3}$M) | + puromycin | 183 | 16 |
| | No additions | + tetracycline ($10^{-3}$M), and puromycin | 79 | 64 |
| 2 | No additions | No additions | 327 | - |
| | No additions | + puromycin | 127 | 61 |
| | tetracycline ($10^{-3}$M) | + puromycin | 342 | 0 |
| | tetracycline ($10^{-3}$M) | + enzymes, GTP, puromycin | 292 | 10 |
| | No additions | tetracycline ($10^{-3}$M), puromycin | 139 | 57 |

tetracycline(at $10^{-3}$M),if the poison is added before the formation of the peptidyltRNA-ribosome complex.If tetracycline is added to the preformed complex simultaneously with puromycin, its action is much weaker. Hence we conclude that polylysyltRNA is bound initially in the acceptor site. Its subsequent translocation to the donor site is effected either spontaneously or under the action of some remainders of GTP and the corresponding enzyme(translocase), which escaped the washing procedure during the preparation of ribosomes. If an alternative method of preparation of the polylysyltRNA-ribosome complex is used [7],i.e. polylysyltRNA is bound at first to 30 s subunits and afterwards mixed with 50 s subunits, then the reaction of the complex with puromycin requires GTP from the very beginning. Probably peptidyltRNA has to be translocated to the donor site and no energy source is avalable in this case to effect the transfer.

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