PUROMYCIN REACTION AT A SITE OF 70S RIBOSOMES

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Antibiotic puromycin (PM), the simplest analog of aminoacyl-tRNA, interacts with the A-site of peptidyltransferase. When the P site is occupied with peptidyl (aminoacyl)-tRNA, a transpeptidation reaction occurs yielding peptidyl (aminoacyl)-PM. The absence of these products means that acylated forms of tRNA are bound in the A sites [1]. The puromycin reaction, at present, is the main test for both correct organization of ribosomal complexes corresponding to different stages of the elongation cycle and the study of events which occur in a ribosome during this cycle.

The situation described appears to be valid for the simplest model systems only, when peptidyl--tRNA comes to ribosomes from a medium. Detailed analysis of the piromycin reaction of peptidyl--tRNA synthesized in ribosomes revealed its new and unexpected property, namely, its ability to react with PM being located on the A site [2].

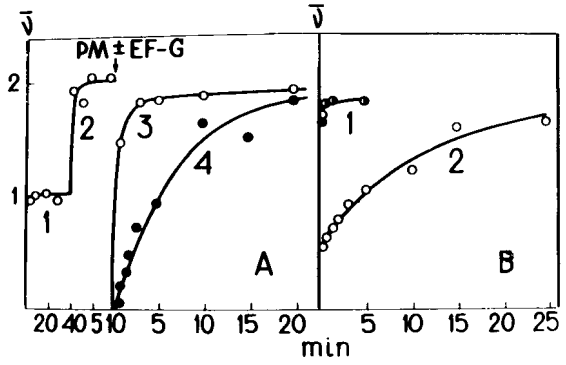

Fig. A. Kinetics of binding AcPhe-tRNA^{Phe} in P sites (*1*) and Phe-tRNA^{Phe}·GTP·EF—T_u in A sites (*2*) followed by measurements of kinetics of $AcPh_2$-PM synthesis (*3*, +EF—G; *4*, —EF—G). B. Kinetics of $AcPhe_2$-PM synthesis after 3 min (+EF—G) or 20 min (—EF—G) preincubation of pretranslocative complex

Fig. A(1, 2) shows the consecutive filling of the P and A sites with AcPhe-tRNA^{Phe} and ternary complex EF-T_u·GTP·Phe-tRNA^{Phe}. According to the HPLC analysis, $AcPhe_2$-tRNA^{Phe} is formed in 80 — 90% of the ribosomes. In the presence of EF-G and PM this newly formed peptidyl-tRNA is readily translocated to the P site (fig. A, 3). Without EF-G the $AcPhe_2$-tRNA^{Phe} appeared to be reactive with PM too, although much slower (fig. A, 4). This result could be ascribed to spontaneous translocation [3]; if so a 3 min (+EF-G) or 20 min (-EF-G) preincubation of the pretranslocative complexes followed by the addition of PM must yield both fast and quantitative formation of $AcPhe_2$--PM. This is the case in the presence of EF-G (fig. B, 1); however, without the EF-G a highly pronounced heterogeneity in the kinetics of the puromycin reaction has been observed (fig. B, 2). As the fast component of this kinetics is characteristic for the P-site location of $AcPhe_2$-tRNA^{Phe}, one can draw the conclusion that a spontaneous translocation has taken place in ~30% of the ribosomes (fig. B, 2, fast phase) whereas in the rest of ribosomes $AcPhe_2$-tRNA^{Phe} has been able to form $AcPhe_2$-PM not being located in the P site. Experiments with the inhibitor of translocation (antibiotic kanamycin) showed that in its presence the spontaneous translocation was stopped completely; nevertheless, $AcPhe_2$-tRNA^{Phe} was able to react with PM with the kinetics similar to that shown in fig. 1 B, 2, slow phase. This means that $AcRhe_2$-tRNA^{Phe} is able to react with PM when bound to the ribosomal A site.

Table

Crosslinks of tRNA located at A site with ribosomal proteins before and after transpeptidation

Transpeptidation	Proteins
—	S5, 7, 9, 11, 14, 18, 19, 21 L9, 21, 29
+	S2, 5, 7, 21 L11, 13

If peptidyl-tRNA is bound to the A site from a medium, peptidyl-PM synthesis cannot be detected. Therefore, the transpeptidation reaction triggers conformational changes in the A-site complex bringing the 3′-end of peptidyl-tRNA molecule in close vicinity to the P-site of the peptidyltransferase center. This conclusion based on functional experiments is consistent with the results on structural studies [4]. When Phe-azido-tRNA^{Phe} is used instead of Phe-tRNA^{Phe} (i. e. the derivative of the latter is able to form crosslinks with ribosomal proteins during UV-irradiation), it is possible to control the changes in the protein arrangement of tRNA after single steps of the elongation cycle. The data table γ show clearly that, indeed, the transpeptidation reaction results in considerable conformational changes in the pretranslocative complex.

REFERENCES

1. *Pestka S.* Molecular Mechanisms of Protein Biosynthesis, Acad. Press. New York. 1977. P. 467 — 504.

2. *Semenkov Yu. P., Shapkina T. G., Kirillov S. V.* LNPI preprint — 1495. Leningrad. 1989. 10 P.

3. *Bergemann K., Nierhaus K.* J. Biol. Chem. 1983. V. 258. P. 15105 — 15113.

4. *Vladimirov S. N., Graifer D. M., Karpova G. G., Semenkov Yu. P., Makhno V. L., Kirillov S. V.* FEBS Lett. 1985. V. 181. P. 367 — 372.