SPECIFICITIES IN MESSENGER RNA AND RIBOSOMES FROM FREE AND BOUND POLYRIBOSOMES

K. Uenoyama and T. Ono

Department of Chemistry, Cancer Institute,
Japanese Foundation for Cancer Research,
Toshima-ku, Tokyo 170, Japan

Received September 7,1972

SUMMARY: The specificity of RNA from free and bound polyribosomes for free and bound ribosomes was examined using the system for cell-free protein synthesis dependent on RNA. RNA from free polyribosomes can stimulate amino acid incorporation in the system with free ribosomes in the presence of KCl extract from free polyribosomes, which was prepared by treatment of polyribosomes with 0.8M KCl. Whereas RNA from bound polyribosomes can stimulate amino acid incorporation in system with bound ribosomes. This suggests that in course of protein synthesis a distinct specificity is present between messenger RNA and ribosomes.

INTRODUCTION

In all animal cells, polyribosomes are divided into two classes by their situation; either attached to membrane of endoplasmic reticulum or free of membrane. It has been demonstrated that bound polyribosomes can synthesize exportable proteins (1-10), and in secretory cells major part of ribosomes is in bound polyribosomes (11). Free polyribosomes can synthesize non-exportable proteins (12), and in rapidly multiplying cells most is in free polyribosomes. While there are numerous investigations on characteristic proteins synthesized by these two classes of polyribosomes, it has not become apparent which factor(s) is involved in the segregation of ribosomes into two classes of polyribosomes. An approach which we adopted was to examine the specificity of messenger RNA for ribosomes from free and bound polyribosomes in the course of protein synthesis dependent on RNA.

MATERIALS AND METHODS

Preparation of bound and free polyribosomes Membrane-bound and free polyribosomes were prepared by the method of Webb et al. (13). The actual procedure was described in our previous report (14). The pelleted polyribosomes were suspended in medium containing 0.25M sucrose, 50mM Tris-HCl, pH 7,4, 5mM $MgCl_2$, 25mM KCl (TKMS buffer).

Preparation of RNA from free and bound polyribosomes RNAs were extracted by SDS-phenol method according to Scherrer and Darnell (15) and designated as bound- or free- polyribosomal RNA.

Preparation of KCl extract from bound and free polyribosomes Suspension of bound or free polyribosomes was adjusted to 0.8M KCl and, after standing for 3 hr at 0°C, it was centrifuged at 150,000 g for 1.5 hr. The supernatant was dialyzed against TKMS buffer and designated as bound- or free-KCl extract.

System for cell-free protein synthesis dependent on RNA The supernatant used for this experiment was prepared by centrifugation of post-mitochondrial fraction at 150,000 g for 1.5 hr. The procedure and the reaction mixture for cell-free protein synthesis dependent on RNA were described in a previous paper (16). The polyribosomes were incubated at 37°C for 60 min to convert them into monosomes and to the mixture were added RNA, KCl extract and 5 μCi of ^{3}H-phenylalanine (spec. act. 660 mCi/m-mole, Daiichi Pure Chemical Co., Tokyo). At the indicated time, an aliquot of the reaction mixture was added to 5%-trichloroacetic acid (TCA) and the insoluble precipitate was collected, washed by centrifugation and dissolved in 0.04N NaOH after heating twice at 90°C for 20 min. Its radioactivity was determined by a liquid scintillation counter with toluene-Triton scintillator.

RESULTS AND DISCUSSION

The present experiment was carried out to examine whether RNA extracted from free or bound polyribosomes had the specificity for ribosomes and KCl extract prepared from free or bound polyribosomes in the course of protein synthesis dependent on RNA. As described in our previous paper (16), messenger RNA added to the system for cell-free protein synthesis, in which polyribosomes had been converted to monosomes as completing the translation, could stimulate amino acid incorporation in the presence of KCl extract, which was prepared by treatment of polyribosomes with 0.8M KCl.

Fig. 1 shows the incorporation of radioactive amino acid into TCA precipitable materials dependent on free-polyribosomal RNA after free polyribosomes (a) or bound polyribosomes (b) were preincubated in the system for cell-free protein synthesis. As seen in Fig. 1 (a), free-KCl extract can stimulate the amino acid incorporation dependent on free-polyribosomal RNA added to the system with free ribosomes, but bound-KCl extract cannot stimulate amino acid incorporation in this system. Fig. 1 (b) shows that free-polyribosomal RNA added to the system with bound ribosomes cannot direct the amino acid incorporation in the presence of either free- or bound-KCl extract.

Fig. 2 shows amino acid incorporation dependent on bound-polyribosomal RNA in the system with free ribosomes (a) or bound ribosomes (b). As seen in this figure, bound-polyribosomal RNA can direct amino acid incorporation only in system with bound ribosomes in the presence of bound-KCl extract, but not in the presence of free-KCl extract, and also cannot direct amino acid incorporation in the system with free ribosomes in the presence of either free or bound-KCl extract. These results seem to in-

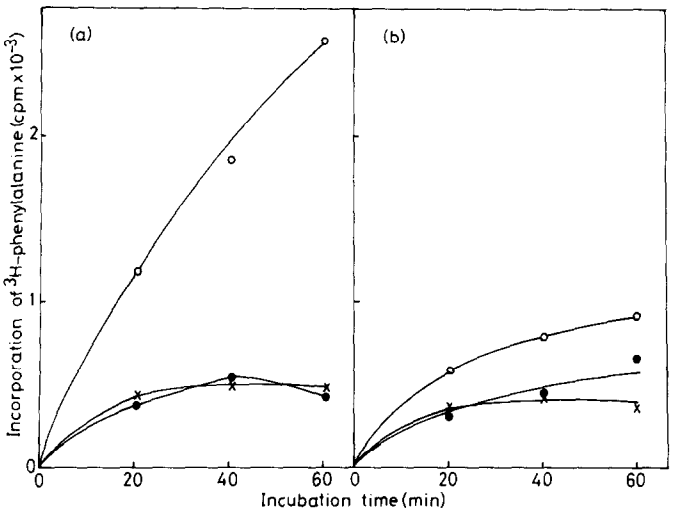
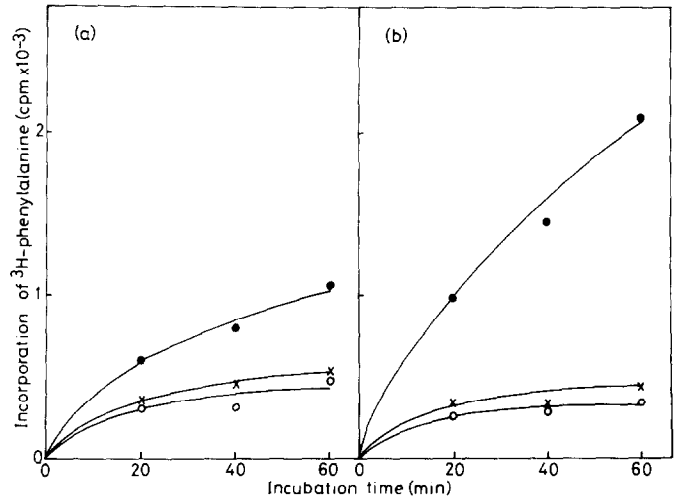


Fig. 1. Amino acid incorporation dependent on free-polyribosomal RNA in system with free (a) and bound (b) ribosomes.

Free and bound polyribosomes were prepared as described in previous report (14). Free (a) and bound (b) polyribosomes equivalent to 1.2 mg RNA were incubated with supernatant in the reaction mixture as described in a previous report (16) at 37°C for 60 min, and 5 μCi of ^{3}H-phenylalanine, 25 μg protein of free- or bound-KCl extract, and 50 μg of free-polyribosomal RNA were added and again incubated at 37°C. Final volume was 0.85 ml. At the indicated time, an aliquot (0.2 ml) was added to 5% TCA and the radioactivity in hot TCA precipitate was determined as described in MATERIALS AND METHODS. o——o , amino acid incorporation in the system with free-KCl extract. ●——● , amino acid incorporation in the system with bound-KCl extract. x——x , amino acid incorporation in the system without KCl extract.



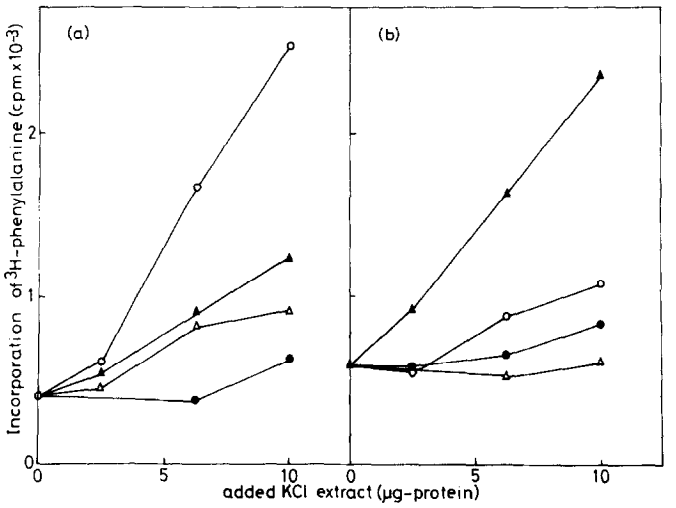


Fig. 3. Stimulation of amino acid incorporation in response to the dose of KCl extract added to the system with free (a) and bound (b) ribosomes.
Reaction was carried out as described in legend to Fig. 1, except that KCl extract of the indicated amount was added. Final volume was 0.25 ml. Reaction was carried out at 37°C for 60 min. o——o , amino acid incorporation in the system with free-KCl extract and free-polyribosomal RNA. Δ——Δ , amino acid incorporation in the system with free-KCl extract and bound-polyribosomal RNA. ●——● , amino acid incorporation in the system with bound-KCl extract and free-polyribosomal RNA. ▲——▲ , amino acid incorporation in the system with bound-KCl extract and bound-polyribosomal RNA.

dicate that free and bound-polyribosomal RNA undergo the translation by a distinct species of ribosomes.

KCl extract contains the initiation factor for protein synthesis as described in our previous paper (16). Free- and bound-KCl extracts were found to have the specificity for both messenger RNA and ribosomes. To ascertain this further, amino acid incorporation in response to the increasing dose of each KCl extract

Fig. 2. Amino acid incorporation dependent on bound-polyribosomal RNA in system with free (a) and bound (b) ribosomes
Reaction was carried out as described in legend to Fig. 1. o——o , amino acid incorporation in the system with free-KCl extract. ●——● , amino acid incorporation in the system with bound-KCl extract. ×——× , amino acid incorporation in the system without KCl extract.

was examined, and Fig. 3 shows this result. As seen in Fig. 3 (a) free-KCl extract stimulate amino acid incorporation in the system with free ribosomes and free-polyribosomal RNA in response to the added dosage, but not in the system with bound-polyribosomal RNA. Bound-KCl extract can stimulate amino acid incorporation dependent on neither free- nor bound-polyribosomal RNA added to the system with free ribosomes. Bound-KCl extract can stimulate amino acid incorporation only in the system with bound ribosomes and -RNA as shown in Fig. 3 (b). Thus, a distinct specificity was observed between messenger RNA, ribosomes and KCl extract. However, it is not demonstrated whether initiation factor(s) itself contains the specificity for the binding of ribosomes to messenger RNA, since KCl extract was a crude fraction which contained proteins released by treatment of polyribosomes with 0.8M KCl.

We were interested on which factor(s) was involved in the determination of bound and free polyribosomes. The results presented here indicate that messenger RNA from free and bound polyribosomes contains the specificity for a distinct species of ribosomes. Although this does not demonstrate the factor(s) which determines the segregation of free and bound polyribosomes, this appears to give a key. Shires et al. (17) reported that membrane-bound polyribosomes had greater affinity than free polyribosomes to smooth membrane. Recently, Rosbash (18) reported that messenger RNA was present as free messenger RNP-ribosome complex with about 120 s in size before formation of bound polyribosomes, and subsequently this complex could bind with the membrane. We have not yet found which of the ribosomes, messenger RNA or proteins in KCl extract has affinity to the membrane, and the mechanism by which polyribosomes attach to the membrane, but

it appears to be certain that there exist messenger RNA and ribosomes which are destined to form bound polyribosomes.

REFERENCES

1. Siekevitz, P., and Palade, G.E., J. Biophys. Biochem. Cytol., 7, 619 (1960).
2. Campbell, P.N., Greengard, O., and Kernot, A.B., Biochem. J., 74, 107 (1960).
3. Peters, T., J. Biol. Chem., 237, 1181 (1962).
4. Peters, T., J. Biol. Chem., 237, 1186 (1962).
5. Ganoza, M.C., Williams, C.A., and Lipmann, F., Proc. Natl. Acad. Sci. U. S., 53, 619 (1965).
6. Williams, C.A., Ganoza, M.C., and Lipmann, F., Proc. Natl. Acad. Sci. U. S., 53, 622 (1965).
7. Redman, C.M., Biochem. Biophys. Res. Commun., 31, 845 (1968).
8. Takagi, M., and Ogata, K., Biochem. Biophys. Res. Commun., 33, 55 (1968).
9. Ganoza, M.C., and Williams, C.A., Proc. Natl. Acad. Sci. U. S., 63, 1370 (1969).
10. Takagi, M., Tanaka, T., and Ogata, K., Biochim. Biophys. Acta, 217, 148 (1970).
11. Palade, G.E., J. Biophys. Biochem. Cytol., 2, 85 (1956).
12. Hicks, S.J., Drysdale, J.W., and Munro, H.M., Science, 164, 584 (1969).
13. Webb, T.E., Blobel, G., and Potter, V.R., Cancer Res., 24, 1229 (1964).
14. Uenoyama, K., and Ono, T., Biochim. Biophys. Acta, in press.
15. Scherrer, K., and Darnell, J.E., Biochem. Biophys. Res. Commun., 7, 486 (1962).
16. Uenoyama, K., and Ono, T., J. Mol. Biol., 65, 75 (1972).
17. Shires, T.K., Narurkar, M.L., and Pitot, H.C., Biochem. Biophys. Res. Commun., 45, 1212 (1971).
18. Rosbash, M., J. Mol. Biol., 65, 413 (1972).