FACTOR DEPENDENT BREAKDOWN OF POLYSOMES[1]

Akikazu Hirashima and Akira Kaji

Department of Microbiology, School of Medicine, University of Pennsylvania
Philadelphia, Pennsylvania 19104

Received October 6, 1970

SUMMARY: When isolated polysomes from E. coli were incubated with soluble protein, most of the polysomes were converted to monosomes. GTP and its generator stimulated the breakdown of polysomes. The requirement for the soluble factor could not be met by the addition of purified T and G factor.

It is well known that the half-life of messenger RNA in a bacterial system is relatively short and constant turnover of messenger RNA takes place (1,2,3,4). In spite of this established concept, the mechanism through which the breakdown of polysome takes place has remained obscure. During the studies on the release of tRNA from polysomes, we observed that the breakdown of polysomes isolated from E. coli extract was dependent on the presence of soluble factor(s). This factor could not be polynucleotide phosphorylase (5,6) because the breakdown was not dependent on the presence of inorganic phosphate. The factor could not be replaced by two polymerization factors for protein synthesis, namely T or G factor (7). These observations indicated that this factor(s) was distinctly different from already known factors for RNA hydrolysis such as ribonuclease - I ~ V (8,9,10,11,12).

MATERIALS AND METHODS

Preparation of polysomes from E. coli: E. coli B was grown to Klett Unit 70 (with filter 42) in 500 ml of minimum tris-glycerol medium as previously described (13).

---

[1] Supported by U.S.P.H.S. grant GM-12,053, National Science Foundation Grant GB-18355 and Damon Runyon Memorial Fund for Cancer Research, grant DRG-799.

At this point, tetracycline-HCl (obtained from Calbiochem Co.) was added to the final concentration of 3×10^{-4} M. After the addition of this drug, the culture was shaken for 10 seconds, chilled quickly by immersing container into the mixture of acetone and dry ice, and centrifuged at 7000 g for 5 minutes. Cells thus collected were lysed according to the method of Flessel et al. (14) and cell debris was removed by centrifugation. The supernatant fluid of the lysate was passed through Sepharose 4B column (1.3 x 40 cm) (obtained from Pharmacia Co.), which had been equilibrated with buffer A containing 10 mM Tris-HCl (pH 7.4), 10 mM $MgSO_4$, 50 mM NH_4Cl, and 6 mM β-mercaptoethanol. Polysomes appeared as the first peak of 260 mμ absorbing materials and this fraction was pooled and stored in the liquid nitrogen for further use. For routine studies, polysomes were prepared from the culture of E. coli strain Q13 in a similar fashion except that the cells were grown to 80 Klett units (filter 66) in 1.5 liter of nutrient broth (8 gram per one liter of distilled water).

Preparation of soluble protein and polymerization factor (G and T factors) from E. coli strain Q13. A frozen cake of E. coli Q13 cells (obtained from General Biochem. Co.) was ground with alumina and the fraction containing soluble protein (S-150) was prepared as described (15).

Soluble protein fraction A, free of tRNA but containing aminoacyl-tRNA synthetases and amino acid polymerization factors (T and G factors), was prepared from S-150 as described with a partial modification, that is, the proteins were precipitated between 35 and 60 per cent ammonium sulfate saturation (16). Starting from this fraction A, the polymerization factors were prepared according to Lucas-Lenard and Lipmann (17).

RESULTS

In the experiment shown in Fig. 1 the isolated polysome preparation was incubated with various soluble components. It can be seen from Fig. 1A that incubation

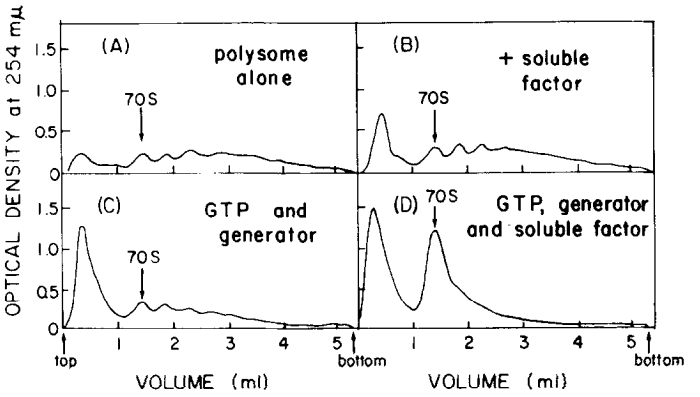

Figure 1. Effect of various factors on the sedimentation behavior of polysome. Reaction mixture (A) (0.27 ml) for the breakdown of polysomes contained 10 mM Tris-HCl (pH 7.4), 8.3 mM $MgSO_4$, 50 mM NH_4Cl, 6 mM β-mercaptoethanol, and polysome preparation from E. coli B (1.85 OD_{260} units). The following were added in addition to the above components: (B), 146 μg of S-150; (C), 0.16 mM GTP, 3.2 mM phosphoenolpyruvate, 20 μg pyruvatekinase; (D), 0.16 mM GTP, 3.2 mM phosphoenolpyruvate, 20 μg of pyruvatekinase and 146 μg of S-150. The reaction mixtures were incubated at 30° C for 25 minutes, chilled with ice, and layered on 5 ml of 15-30% sucrose gradient in Buffer A. The tubes were centrifuged in Beckman SW 50.1 rotor with the speed of 38,000 rpm for 50 minutes at 4° C. After the centrifugation, absorbancy at 254 mμ of the gradient was monitored with ISCO ultraviolet analyzer. Relative absorbancy at 254 mμ was plotted against the volume from the top of the tube.

---

of polysomes alone did not result in the breakdown of polysomes and major portions of the polysomes remained intact after incubation at 30° C for 25 minutes. On the other hand, when the same preparations of polysome were incubated with S-150, GTP and its generator, a considerable breakdown of polysomes was observed and most of the ribosomes were sedimented at the position where 70 S ribosome was expected. In the absence of GTP and its generator or S-150 from E. coli, much less breakdown of polysome was observed. This indicates that for the complete breakdown of polysomes, soluble protein, energy and its generator were required. Table I summarizes the result of this experiment. It is shown in this table that when polysomes alone were incubated, only 9.6% of total ribosomes were found as monosome (70 S ribosome). When polysomes were incubated with S-150, GTP and its generator, more than 50% of ribosomes were found

as 70 S ribosome. From the calculated increase of the monosome due to the incubation, it is clear that the maximum increase of monosome was observed when soluble protein, GTP and its generator were present. A separate experiment indicated that the effect of soluble protein or GTP and its generator on the breakdown of polysome was not due to the formation of inorganic phosphate which might have stimulated polynucleotide phosphorylase (5,6) in the soluble protein fraction. Addition of inorganic phosphate (8.0 mM) in the absence of GTP and its generator did not cause any appreciable breakdown of polysome. This indicates also that the energy generating system was not stimulating the breakdown through its production of inorganic phosphate which may activate RNase by chelating Mg^{++}.

It is well established that for peptide chain elongation two soluble factors, namely T and G factor (7), are involved. In order to determine whether our soluble factor contained in S-150 can be replaced by purified T and G factor or not, the experiment shown in Fig. 2 was carried out. In this experiment the polysomes were isolated from E. coli Q13 in a similar fashion, and incubated with purified T and G factor. In some cases, aminoacyl-tRNA was added. It is noted in this figure that even in the presence of

TABLE 1. Effects of Soluble Factors on the Breakdown of Polysomes

Reaction System	Monosomes (% of total ribosome)	Increase of Monosome
Polysome alone	9.2	--
Polysome, S-150	13.0	3.8
Polysome, GTP and its generator	24.0	14.8
Polysome, S-150, GTP and its generator	55.2	46.0

Values were calculated from Fig. 1

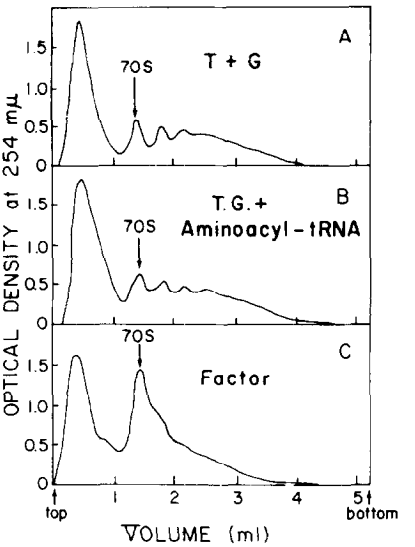

Figure 2. Effect of two polymerization factors (T and G) and aminoacyl-tRNA on the breakdown of polysome. The complete reaction mixture (0.25 ml) for the breakdown of polysome was identical to that of Fig. 1D except that polysomes (3.68 OD_{260}units) prepared from E. coli Q13 and 150 μg of soluble protein fraction A were used. Sedimentation patterns of polysomes were studied as in Fig. 1. (A) The reaction mixture was as in the complete system except that instead of E. coli soluble fraction A, purified G factor (15 μg) and T factor (20 μg) were added; (B), the reaction mixture was as in the complete system except that mixture of aminoacyl-tRNA (50 μg), G factor (15 μg), and T factor (20 μg) were added instead of protein fraction A; (C) complete reaction mixture for the breakdown of polysomes.

aminoacyl-tRNA, two factors for peptide chain elongation, GTP and its generator, the major portion of ribosomes was found as polysome (Fig. 2B). On the other hand, in the presence of soluble protein fraction from E. coli, GTP and its generator, the large portion of ribosomes was converted to monosomes (Fig. 2C). These results show that for the breakdown of polysomes under the experimental conditions used, two amino acid polymerization factors were not sufficient and some additional factors were required.

DISCUSSION

Results presented in this paper suggested that possible new factor(s) may be involved in the breakdown of polysomes. This factor(s) is apparently different from ribonucleases reported so far. For example, ribonuclease V which is presumably

present on ribosome has been shown to hydrolyse synthetic polynucleotides as well as phage messenger RNA in the presence of GTP, T factor and G factor (12). In contrast, when the naturally occurring polysome was used as substrate, the soluble factor requirement for the breakdown of polysomes could not be met by the presence of T and G factors. Similarity between our factor and ribonuclease V exists, however, in that they are both apparently stimulated by nucleotide and its generator. We have not established the nucleotide specificity in this reaction, but preliminary experiments showed that GTP appeared to be most efficient in the polysome breakdown. The soluble factor can apparently not be a simple ribonuclease, because the action of the soluble factor was apparently stimulated by nucleotide triphosphate and its generator. Ribonucleases I - IV have not been reported to be stimulated by nucleotide triphosphate. The additional evidence that the factor responsible for the breakdown of polysomes present in S-150 cannot be replaced by T and G factor was obtained from the observation that heated S-150 was still mostly active in the breakdown of polysomes. In this experiment S-150 was heated to 50° C for 5 minutes and its activity was tested. Under these conditions, T factor has been reported to lose most of its activity (17). Only about 20% or less of the activity for the breakdown of polysomes was lost by this heat treatment.

With the use of naturally occurring polysomes, it is hoped that the elucidation of the present system for the breakdown of polysomes may lead to an understanding of the metabolism of polysome in a cell. Purification of the factor and establishment of nucleotide specificity for this system is in progress, and will be reported elsewhere.

REFERENCES

1. Gros, F., Dubert, J-M, Tissieres, A., Bourgeois, S., Michelson, M., Soffer, R. and Legault, L., Cold Spring Harbor Sym. Quant. Biol., 28, 299 (1963).
2. Kepes, A., Cold Spring Harbor Symp. Quant. Biol., 28, 325 (1963).
3. Levinthal, C., Fan, D. P., Higa, A., Zimmerman, R. A., Cold Spring Harbor Sym. Quant. Biol., 28, 183 (1963).
4. Nakada, D., and Magasanik, B., J. Mol. Biol., 8, 105 (1964).

5. Grunberg-Manago, M., Ortiz, P. J., and Ochoa, S., Biochim. Biophys. Acta, 20, 269 (1956).
6. Littauer, U. Z., and Kornberg, A., J. Biol. Chem., 226, 1077 (1957).
7. Nishizuka, Y., and Lipmann, F., Proc. Natl. Acad. Sci. 55, 212 (1966).
8. Spahr, P. F., and Hollingworth, B. R., J. Biol. Chem., 236, 823 (1961).
9. Spahr, P. F., J. Biol. Chem., 239, 3716 (1964).
10. Robertson, H. D., Webster, R. E., and Zinder, N., J. Biol. Chem., 243, 82 (1968).
11. Spahr, P. F., and Gesteland, R. F., Proc. Natl. Acad. Sci., 59, 876 (1968).
12. Kuwano, M., Kwan, C. N., Apirion, D., and Schlessinger, D., Proc. Natl. Acad. Sci., 64, 693 (1969).
13. Kaempfer, R. O. R., and Magasanik, B., J. Mol. Biol., 27, 453 (1967).
14. Flessel, C. P., Ralph, P., and Rich, A., Science, 158, 658 (1967).
15. Igarashi, K., and Kaji, A., Proc. Natl. Acad. Sci., 58, 1971 (1967).
16. Momose, K., and Kaji, A., Arch. Biochem. Biophys., 111, 245 (1965).
17. Lucas-Lenard, J., and Lipmann, F., Proc. Natl. Acad. Sci., 55, 1562 (1966).