EFFECTS OF CHLORAMPHENICOL AND FUSIDIC ACID ON POLYRIBOSOME METABOLISM IN ESCHERICHIA COLI

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1. Introduction

A functional cycle for ribosomes was first suggested by Mangiarotti and Schlessinger [1], and has been elaborated and supported by various studies [2–4]. In this cycle, a 30S and a 50S ribosomal subunit periodically are coupled to mRNA, move along it directing the synthesis of a protein chain, and dissociate on the release of the completed chain.

While the addition of ribosomal subunits at initiation of protein synthesis and their release at termination have been studied in some detail (see reviews, refs. [5,6]), information has remained fragmentary about other major features of polyribosome metabolism, such as mRNA formation and destruction, and movement of ribosomes along mRNA. Here we report relevant results on the effects of chloramphenicol and fusidic acid on polyribosome metabolism.

When chloramphenicol is added to cells, peptide bond formation stops, but newly-formed mRNA continues to enter polyribosomes at rates comparable to control cells for several hours; i.e., polyribosome formation is uncoupled from protein synthesis. In contrast, when fusidic acid is added to cultures, most of the polyribosomes are "frozen", and the rate of entry of new messenger RNA into polyribosomes is decreased 5 to 10-fold. Since fusidic acid specifically blocks the GTPase action of the G factor required for amino acid polymerization we conclude that GTPase action, but not peptide bond formation, is specifically required *in vivo* for movement of ribosomes on mRNA.

2. Methods and results

Strain *sud* 24 was grown exponentially in fragile form and stable RNA was uniformly labeled with ^{14}C uracil. (For details concerning this strain, see ref. [2].) At zero-time, portions of the culture were treated with chloramphenicol (200 to 1000 μg/ml) or fusidic acid (50 to 500 μg/ml), concentrations that cause nearly total arrest of protein synthesis. (Throughout the dose range tested, similar results were observed.) At intervals up to 120 min after addition of drug, pulse labeling of newly-formed RNA with ^{3}H-uracil was carried out for 90 sec in 10 ml portions of culture to observe the flow of the newly-formed RNA into polyribosomes and free RNA. Cells were then harvested and lysed [1,2,7], and extracts analyzed by zonal sedimentation in sucrose gradients.

Fig. 1 shows a typical result observed 30 min after addition of 500 μg/ml chloramphenicol; fig. 2 shows the corresponding pattern after addition of fusidic acid.

Lysates of chloramphenicol-treated cells show a pattern much like that from untreated cultures (see, for example, ref. [7]), except that in presence of the drug, small polyribosomes tend to increase at the expenses of larger ones. The increase of small polyribosomes is correlated with the increase in cellular mRNA; for further details and discussion, see [8]. Here we emphasize that new mRNA continues to join to ribosomes in presence of chloramphenicol, and to a roughly comparable extent ($^{3}H/^{14}C$ ratio in

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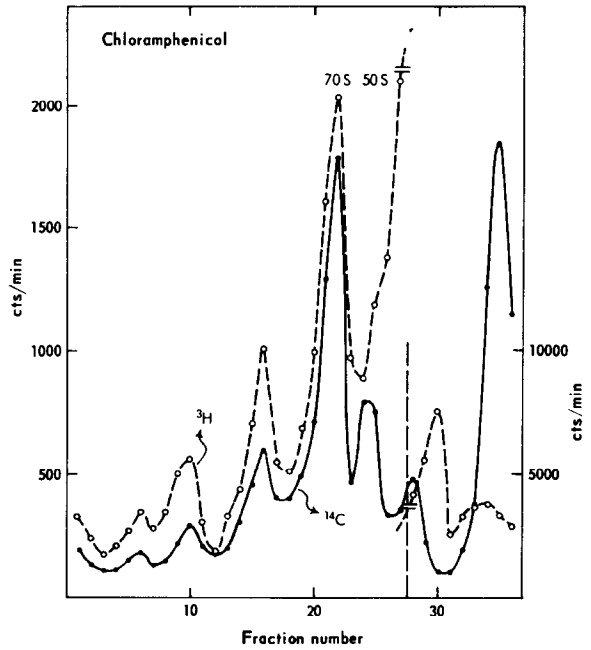

Fig. 1. Distribution of ribosomes and newly-formed RNA in cells treated with chloramphenicol. A fragile growing culture was labeled uniformly in its stable RNA with 0.05 μC and 0.2 μg/ml ^{14}C uracil (obtained from Schwarz Bioresearch). A 10 ml portion was then treated with 200 μg/ml chloramphenicol (Parke-Davis) for 30 min. Then new RNA was pulse-labeled 90 sec with ^{3}H uracil (4 μC and 0.05 μg/ml; also obtained from Schwarz Bioresearch). The drug-treated culture and an untreated control culture were then harvested, lysed and centrifuged through 15 to 30% sucrose gradients in 0.01 M Mg^{2+} [2] for 4 hr at 25000 rpm in the SB110 rotor of an International B60 ultracentrifuge. The polyribosomes and subunits from the treated culture are displayed; the control sample resembled those in previous publications (cf. fig. 10, ref. [7]). Polyribosomes that had sedimented to the bottom of the gradient during the centrifugation were resuspended and contained 3000 cts/min of ^{14}C and 5600 cts/min of ^{3}H. To the right of the vertical dashed line, the ^{3}H cts/min are plotted according to the right-hand scale; other cts/min are plotted according to the left-hand scale. (–●–), ^{14}C stable RNA; (–○–), ^{3}H pulse-labeled RNA.

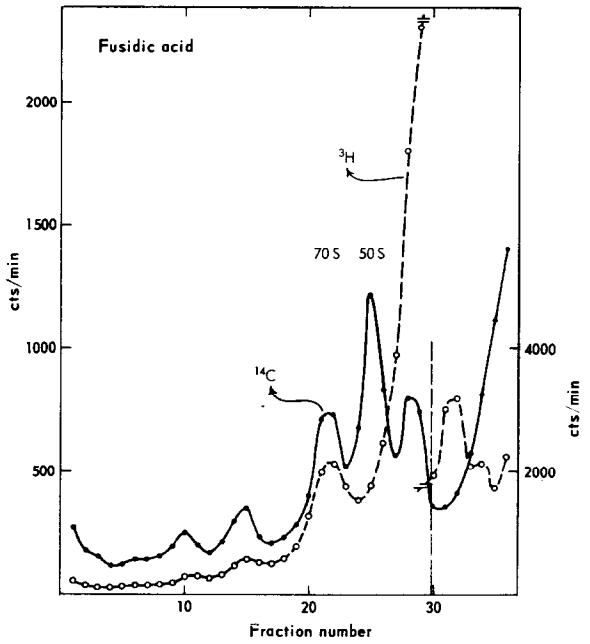

Fig. 2. Polyribosomes and newly-formed RNA in cells treated with fusidic acid. Protocol as in fig. 1, but with the addition of 300 μg/ml fusidic acid (a gift of Barbara Stearns, The Squibb Institute for Medical Research, New Brunswick, N.J.), instead of chloramphenicol. The pelleted polyribosomes contained 4300 cts/min of ^{14}C and 1200 cts/min of ^{3}H. (–●–), ^{14}C stable RNA; (–○–), ^{3}H pulse-labeled RNA.

fig. 1) for various size classes of ribosomes. We infer from the continued entrance of mRNA into polyribosomes that movement of ribosomes on mRNA continues in cells treated with chloramphenicol.

An alternative explanation of the results with chloramphenicol would be that ribosomes periodically are somehow released from one mRNA and join to another. A strong argument against the existence of such a random process is provided by the experiment with fusidic acid (fig. 2), which indicates that blockage of *G* factor function [9] blocks ribosome movement. Fig. 2 shows that most of the pre-existing polyribosomes are preserved and that the percentage of pulse-labeled mRNA entering polyribosomes falls from 30% in the control or chloramphenicol-treated cells (fig. 1 and refs. [2,8]) to 5 to 10%. Since overall RNA synthesis also drops by a factor of two to three, the entry of mRNA into polyribosomes is reduced 5 to 10-fold compared to control cells.

4. Discussion

We suggest that translocation of ribosomes on mRNA, and therefore polyribosome formation, always requires GTPase function but can proceed without peptide bond formation. In support of this suggestion, two types of interruption of the ribosome cycle are reported here. The first is effected by chloramphenicol;

ribosomes continue to cycle on old and new mRNA, even though protein synthesis has been halted. As analyzed elsewhere in detail [8], chloramphenicol, like streptomycin [7], inhibits mRNA breakdown. mRNA continues to accumulate in the cells, and since there are more mRNA chains and no new ribosomes are made, the average size of polyribosomes decreases (compare fig. 1 to untreated control cultures in refs. [7,8]).

In the presence of chloramphenicol, a new kind of monosome seems to be formed; it contains mRNA, two aminoacyl tRNA's and a 30S and a 50S ribosome, but no peptidyl tRNA in the peptidyl site [8]. We discuss elsewhere [8] the way in which these results can be brought into accord with earlier observations that peptidyl tRNA is retained on ribosomes in cells treated with chloramphenicol [10].

Fusidic acid causes a second and very different interruption of polyribosome metabolism. The ribosome cycle is apparently blocked; pre-existing polyribosomes are preserved, but little new mRNA enters polyribosomes (fig. 2).

Two other modes of interruption of the ribosome cycle have been recognized. One occurs in cultures starved for glucose [7] or treated with levels of tetracycline greater than about 100 μg/ml [8,11]; almost all the ribosomes accumulate as free 30S and 50S subunits. The other mode of interruption is observed when cells are inhibited with streptomycin [7] or neomycin [8]. Protein synthesis is arrested but ribosomes tend to finish polypeptide chains that were started before drug addition [7,12,13]. The ribosomes then accumulate irreversibly, blocked at an early stage in protein synthesis as "str-monosomes" [7,12]. These seem to consist of a 30S and 50S ribosome, joined together and bearing tRNA, each bound to a full-sized chain of stabilized mRNA [7,12]. Studies of polyribosome metabolism in growing and non-growing cells therefore provide diagnostic information about the *in vivo* action of antibiotics that block ribosome function, and give some information about ribosome movement.

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References

[1] G.Mangiarotti and D.Schlessinger, J. Mol. Biol. 20 (1966) 123.
[2] G.Mangiarotti and D.Schlessinger, J. Mol. Biol. 29 (1967) 395.
[3] R.O.R.Kaempfer, M.Meselson and H.Raskas, J. Mol. Biol. 31 (1968) 277.
[4] C.Guthrie and M.Nomura, Nature 219 (1968) 232.
[5] P.Lengyel and D.Soll, Ann. Rev. Biochem., in press.
[6] D.Schlessinger and D.Apirion, Ann. Rev. Microbiol., in press.
[7] L.Luzzatto, D.Apirion and D.Schlessinger, J. Mol. Biol. (1969), in press.
[8] C.Gurgo, D.Apirion and D.Schlessinger, submitted to J. Mol. Biol.
[9] T.Kinoshita, G.Kawano and N.Tanaka, Biochem. Biophys. Res. Commun. 33 (1968) 769.
[10] M.Weber and J.A.DeMoss, Proc. Natl. Acad. Sci. U.S. 55 (1966) 1224.
[11] E.Cundliffe, Biochem. Biophys. Res. Commun. 33 (1968) 245.
[12] L.Luzzatto, D.Apirion and D.Schlessinger, Proc. Natl. Acad. Sci. U.S. 60 (1968) 873.
[13] L.Luzzatto, D.Apirion and D.Schlessinger, submitted to J. Bacteriol.