acid; T, thymine, deoxythymidine, or deoxythymidylate; dATP, dCTP, dGTP, and dTTP, deoxynucleoside triphosphates of adenine, cytosine, guanine, and thymine, respectively. Designations for a polynucleotide chain are those described in *J. Biol. Chem.*

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POLYRIBOSOME-BOUND NUCLEOSIDE TRIPHOSPHATASES IN *ESCHERICHIA COLI**

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It was first shown by Warner *et al.*^{1, 2} and Gierer³ for reticulocytes, and by Wettstein, Staehelin, and Noll⁴ for liver, that when carefully prepared crude extracts were centrifuged on a sucrose gradient, large amounts of RNA, i.e., ultraviolet-absorbing material, appeared in components having a sedimentation constant from one hundred to several hundred svedbergs. It was further shown by these authors that these heavy structures were far more radioactive after *in vivo* labeling with C¹⁴-amino acids than the 70s ribosomes, and it was therefore proposed that these aggregates were the real site of protein synthesis *in vivo*. Evidence was also presented to show that these structures were composed of several 70s ribosomes held together by a strand of RNA with the properties of messenger RNA, and they were therefore termed polyribosomes or polysomes.²

Structures resembling polysomes have been found in several animal cells.^{5, 6} In bacterial extracts, however, where the formation of polysome-like complexes with synthetic polynucleotides can easily be shown,⁷⁻¹⁰ the existence of naturally occurring polyribosomes is more difficult to demonstrate, since in extracts prepared in the normal manner from cells in the logarithmic phase of growth there is very little material heavier than the 70 or 100s ribosomes. Moreover, when crude extracts were labeled with C¹⁴-amino acids and then separated on a sucrose gradient,

most of the nascent protein was found to be associated with a fraction but slightly heavier than the 70s or 100s ribosomes.¹¹ This failure in the earlier experiments to find any significant amount of nascent protein in the polysome region, however, can now be explained by the extreme lability of the bacterial messenger RNA^{12, 13} as compared to animal systems.^{14, 15} Furthermore, according to the "moving tape" hypothesis,¹⁶⁻¹⁸ polysome formation is a dynamic process, and during the process of peptide formation polysomes first increase in size, as the moving end of the messenger picks up new ribosomes, and then again decrease, as ribosomes are sloughed off after the messenger has passed through. Each of the discarded 70s ribosomes carries a molecule of transfer RNA with an attached chain of nascent protein, and hence, as observed, sediments slightly faster than a free 70s particle. Recently, however, these difficulties have been circumvented, partly by carefully controlling the temperature to prevent enzymatic degradation of messenger RNA, and partly by adjusting the assay conditions so that labeled polysomes, rather than their breakdown products, could be observed. Gilbert,¹⁷ for example, by separating the extract first on a sucrose gradient and then assaying tube by tube for amino acid incorporation, showed that the latter occurred exclusively in the heavy fractions. Similar observations were also recently reported by Staehelin and co-workers¹⁹ who, in addition, showed that when extracts are labeled for very short periods of time and then separated on a gradient, labeling of structures other than the monomer could be observed, and that only when labeling was prolonged did the label appear exclusively in the monomer as observed by the earlier workers.

In the present communication evidence is presented that naturally occurring polyribosome fractions in *E. coli* extracts, which can be detected by their amino acid incorporating activity, have a high protein content, i.e., a low RNA to protein ratio. These same structures also have a high content of nucleoside triphosphatase activity. Moreover, both the excess protein, as well as most of the enzymatic activity, are displaced to lighter fractions when the extracts are incubated under conditions known to destroy natural messengers.^{12, 19} Detailed reports on the distribution of RNA relative to protein, as well as of all four ribonucleoside triphosphatase activities in crude extracts and in isolated ribosomes of *E. coli*, are being presented elsewhere.²⁰

Experimental.—Crude extracts were prepared from DNAase-treated *E. coli* B, disrupted by means of a French Press as described elsewhere.²⁰

Two types of buffer were used: 0.005 *M* Tris, pH 7.4 containing 0.01 *M* Mg acetate (buffer A), and 0.005 *M* Tris, pH 7.4, containing 0.0175 *M* Mg(Cl)₂ and 0.086 *M* KCl,¹⁷ designated as Buffer B.

SDG centrifugation of the extracts, either before or after incubation, was carried out by layering 1 ml of extract on 29 ml of a linear sucrose gradient, 7–18%, and containing the same buffer as that used in the preparation of the extracts. The gradients were centrifuged in a SW 25.1 swinging bucket of a Model L Spinco ultracentrifuge at 25,000 rpm for 2½ hr. After centrifugation, the bottoms of the tubes were pierced, and fractions of 0.9–1 ml each collected by hand into chilled test tubes.

Each fraction of the gradients was analyzed for RNA and for protein, and assayed for ATPase and for UTPase activity, some being assayed for GTPase and CTPase activities as well. Details of the analytical procedures as well as the

enzyme assays are given elsewhere.²⁰ The standard deviation of the assay is ± 0.005 O.D. units. One O.D. unit corresponds to $0.256 \mu\text{mole } P_i$.

Preincubation of the extracts, as well as determination of the amino acid incorporating activity of the separated gradient, were carried out essentially as described by Gilbert,¹⁷ with the exception that incorporated radioactivity was determined according to the method of Mans and Novelli²¹ by placing an aliquot of the chilled reaction mixture on paper disks, and counting the washed disks directly in a liquid scintillation counter with an efficiency of 48%. In order to increase the sensitivity, $50\text{-}\mu\text{l}$ aliquots of the same reaction mixture were placed on two separate disks, which were then counted in the same vial. The counts were additive. The actual compositions of the incubation mixtures are given in the legends to the figures.

ATP, UTP, GTP, CTP, PEP (sodium salt), PEP kinase, and the 1-C^{14} -phenylalanine (10.3 mc/mmole) were obtained from the California Biochemical Research Corp. C^{14} -algal protein hydrolysate, with a specific activity of 1.43 mc/mg was obtained from the New England Nuclear Corp. Poly U was purchased from the Miles Company, and *E. coli* sRNA from General Biochemicals.

Results.—From the distribution of amino acid incorporating activity in crude extracts of *E. coli* (Fig. 1A and B), it can be seen that heavy, amino acid incorporating structures are present in these extracts. It is further seen that while the presence of salt does not appreciably alter the distribution of the active complex itself, it nevertheless enhances the latter by removing nonfunctional aggregates from the same region. These results are entirely similar to those reported by Gilbert.¹⁷ It is also seen that incubation in the presence of KCl is more effective in destroying the active complex than incubation in the absence of this salt.

The next set of experiments was designed to compare the distribution of protein relative to RNA, as well as the distribution of triphosphatase activities before and after incubation, first in the presence of salt, and then in the absence of KCl. In some experiments an aliquot of the incubated extract was subjected to an additional incubation with poly U. It can be seen (Fig. 2) that there is relatively little dif-

FIG. 1.—Amino acid incorporation pattern in crude extracts of *E. coli* after separation by SDG centrifugation. (A) Tris and $10^{-2} M$ $\text{Mg}(\text{Ac})_2$; (B) Tris, $0.0175 M$ MgCl_2 and $0.086 M$ KCl. The dotted line represents RNA concentration; $\bullet\text{---}\bullet\text{---}\bullet$, amino acid incorporation of untreated extract; and $\triangle\text{---}\triangle\text{---}\triangle$, amino acid incorporation of incubated extract. Incubation of the whole extract was carried out for 10 min at 30°C , in a total volume of 0.8 ml containing, in addition to the buffer salts, 1 mM ATP, 5 mM PEP, $32 \mu\text{g}$ PEP kinase, and 0.72 ml of extract. The concentration of protein in the extracts was 23 mg per ml . The amino acid incorporation assay was carried out in a total volume of 0.1 ml , containing $0.1 M$ Tris buffer, pH 7.4, 1 mM ATP, 5 mM PEP, $4 \mu\text{g}$ PEP kinase, 10 mM $\text{Mg}(\text{Ac})_2$ (or 17.5 mM MgCl_2 and 86 mM KCl), $10 \mu\text{g}$ *E. coli* sRNA, $3 \mu\text{l}$ C^{14} -algal hydrolysate with $6.66 \times 10^6 \text{ cpm}$, $10 \mu\text{l}$ of supernatant in addition to 0.05 ml of gradient-separated ribosome fraction. Incubation was carried out at 30°C for 30 min. Duplicate aliquots of $50 \mu\text{l}$ each were placed on filter paper disks and processed for counting as described.

FIG. 2.—Comparison of RNA profiles of untreated and incubated extracts. Both extracts and gradients contained Tris, 0.0175 *M* MgCl₂, and 0.086 *M* KCl, and had a protein concentration of 23 mg per ml. ●—●—, untreated extract; X—X—X—, incubated extract (see legend to Fig. 1); o...o..., incubated extract plus 50 µg of poly U in 1 ml.

ference in the optical density profiles, although, after incubation, the loss of a small amount of RNA-containing material from the heavy side of the main peak can be discerned.

The differences between untreated and incubated extracts are somewhat larger when protein profiles are compared, but because of the small amount of material in the heavy region of the gradient, even a large relative difference appears as a small absolute difference on the graphs, and is therefore hard to appreciate. By determining ratios of RNA to protein, on the other hand, the relative differences become independent of the absolute values (although, naturally, the error would be larger for small numbers than for large ones), and any differences can be more readily visualized. In untreated extracts the RNA to protein ratio is very low in the polysome region, whereas after incubation it becomes high. This shows that incubation results in the displacement of the excess protein from the polysome region.

After addition of poly U, the ratio is again somewhat lowered, showing that the formation of artificial polysomes causes some protein to be readsorbed. The curves pertaining to this experiment are shown in Figure 3.

The displacement of protein from the heavy region of the gradient under conditions leading to polysome destruction is still more dramatically demonstrated by the results of enzyme assays. Figure 4 shows the effect of incubation on the UTPase and ATPase activities of crude extracts containing KCl. It is seen that in both cases there is a drastic decrease in the total activity of these enzymes in the region of the heavy ribosomes, and an increase in activity in the lighter fractions, demonstrating a redistribution of the enzyme. As shown for UTPase, the addition of poly U did not cause a readsorption of the enzyme, the same being true in the case of ATPase (not shown). Figure 5 shows the same type of results for

FIG. 3.—Comparison of RNA to protein ratio profiles of untreated and incubated extracts. o—o—o—, untreated; X—X—X—, incubated; ■—■—, incubated, plus poly U. Conditions as in Fig. 2.

FIG. 4.—Comparison of ATPase (A) and UTPase (B) activities in untreated and incubated extracts. Conditions as in Fig. 2. ●...●... untreated; X—X— incubated; o—o—o— treated with 2 µg of pancreatic ribonuclease in the cold; ▲—▲— incubated, plus poly U.

GTPase and CTPase. Again, the CTPase activity disappears from the heavy components. The effect is small for GTPase, although a decrease is also observed.

Another very effective way to cause the destruction of polysomes is treatment with small amounts of ribonuclease in the cold.^{1, 4} In Figure 4B it can be seen that such a treatment does indeed cause a decrease in the ATPase activity to the same level as that observed after incubation.

That the displacement of enzyme is proportional to the displacement of total protein is shown clearly in Figure 6, which shows a plot of specific activity (μ moles P_i liberated per mg protein) of ATPase before and after incubation. It is seen that while there was a considerable loss in total activity upon incubation, the enzyme which is left has essentially the same specific activity as that in the untreated extract. Furthermore, when a series of the above experiments was carried out in the absence of salt, under conditions where less destruction of the active complex occurs during incubation (Fig. 1A), a smaller difference in the RNA to protein ratios is observed (Fig. 7A and B), as well as a less severe loss in total enzyme activities (Fig. 8). Again, the addition of poly U, while causing some protein to be readsorbed, does not result in the readsorption of enzyme to the heavy fractions.

Discussion.—Results presented elsewhere²⁰ showed that the low RNA to protein ratios in the heavy regions of the gradient observed with crude extracts of *E. coli* are also observed when crude and partially purified ribosomes from the same organism, as well as ribosomes from pea seedlings, are separated by SDG centrifugation. It was also generally observed that the protein in the heavy region was extraordinarily high in ribonucleoside triphosphatase activity. As can be seen from Figure 6, specific activity of ATPase in *E. coli* extracts, for example, is about two orders of magnitude higher in the heavy region than in the supernatant. It is also low in the region of the 70s ribosomes.

The results presented in this paper, which show a displacement from the heavy regions of both total protein and enzymatic activity under certain conditions, make it unlikely that this extra protein is due to the presence of ill-defined structures, such as membrane fragments, or to denatured, aggregated ribosomes which have lost part of their RNA. On the other hand, this displacement of protein and enzymatic activity from the region of the amino acid incorporating complex under conditions leading to the destruction of the latter, lends support to the conclusion that both the excess protein and the triphosphatase activities are bound to structures operationally identical to polysomes. The notion that the intact polysomes,

FIG. 5.—Comparison of CTPase (A) and GTPase (B) activities in untreated and incubated extracts. Conditions as in Fig. 2, but with extracts prepared on a different day. 24 mg protein in 0.8 ml of extract were used for each run. ●—●— untreated; ⊗ ⊗ incubated.

FIG. 6.—Specific activities of ATPase before (solid line) and after incubation (broken line) of crude extracts. Same extract as in Fig. 4A.

rather than their constituent monomers, are responsible for the adsorption of the enzyme is further strengthened by the fact that after destruction of the polysome structure the enzyme is transferred to the light fractions, rather than remaining bound to the 70s ribosomes.

FIG. 7.—Comparison of RNA (A) and RNA to protein ratio (B) profiles of extracts prepared in the absence of KCl (0.01 M $Mg(Ac)_2$) before and after incubation. 0.8 ml extract, containing 27 mg protein, were used for each run. The incubation was carried out as in Fig. 2, but there was an additional incubation of 5 min at $30^\circ C$ after the addition of poly U (50 μg). $\circ \dots \circ \dots$, untreated; $\times \dots \times \dots$, incubated; $\blacksquare \dots \blacksquare \dots$, incubated, plus poly U.

FIG. 8.—Comparison of (A) CTPase, (B) ATPase, and (C) GTPase activities before and after incubation in the absence of salt. Conditions as in Fig. 7. $\circ \dots \circ \dots$, untreated; $\times \dots \times \dots$, incubated; $\odot \dots \odot \dots$, incubated, plus poly U.

An important corollary to the above conclusion is that naturally occurring polyribosomes contain not only 70s ribosomes, transfer and messenger RNA's, but also hold adsorbed to them a large complement of protein high in certain kinds of enzymatic activities. As has been pointed out elsewhere,²⁰ the large relative amount of these proteins and the very high specific activity of the enzymes (Fig. 6) preclude the possibility that these are nascent enzymes. Since polyribosomes have attached to them two relatively long strands of RNA, they are chemically very different from "naked" monomeric units, and it is therefore not overly surprising that the former can adsorb proteins not held by the latter.

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The following abbreviations are used: SDG, sucrose density gradient; ATP, GTP, UTP, and CTP, the triphosphates of adenosine, guanosine, uridine, and cytidine, and ATPase, GTPase, UTPase, and CTPase, the respective triphosphatases; PEP, phosphoenolpyruvate; P_i , inorganic phosphate; and poly U, polyuridylic acid.

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PHYSICAL AND IMMUNOLOGICAL PROPERTIES OF A SOLUBLE PRECURSOR OF THE POLIOVIRUS CAPSID*

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Two types of particles are produced during the replication of poliovirus in HeLa cells: infectious virions in which the capsid encloses a single RNA molecule of 2×10^6 molecular weight units, and empty capsids.¹⁻³ Although both particles presumably contain the same protein subunits, they are immunologically unrelated, the empty capsids in this respect resembling virus denatured by heat, radiation, and certain chemicals.⁴ The antigenic property of infectious virus has been designated D antigen; that of empty capsids, C antigen.¹ The present experiments show: (a) that when virions are degraded with guanidine, yet a third antigenic specificity, which will be called S antigen, appears; (b) that treatment with guanidine leads to a relatively homogeneous product with a sedimentation constant of about 2S in guanidine; and (c) that antibody against the guanidine-degraded virus can be used to identify a soluble protein in the infected cell which is a precursor of the virus particles.

Materials and Methods.—*Cells and virus:* Suspension cultures of strain S3 HeLa cells were grown in Eagle's medium with 5% horse serum, and infected in serum-free medium with Type 1