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The Effect of Ribonuclease on Polysomes and Ribosomes of Bacteria and Animal Cells

By M. L. FENWICK
Sir William Dunn School of Pathology, University of Oxford

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The mildest treatment with ribonuclease that causes any disaggregation of the polysomes of *Escherichia coli* or HeLa cells simultaneously attacks the RNA of the constituent ribosomes. It is concluded that the susceptibility to ribonuclease of polysomes does not suggest that they are held together by a strand of messenger RNA. The RNA of the larger sub-unit of bacterial ribosomes has particularly sensitive regions resulting in a non-random degradation. The RNA of the smaller sub-unit of *E. coli* ribosomes is relatively resistant to ribonuclease attack. The same may be true of the respective sub-units of the intact HeLa-cell ribosome, but both sub-units become very sensitive to ribonuclease on dissociation from each other.

Lysates of many different sorts of living cells contain clusters of up to 30 or more ribosomes. It is widely believed that these polysomes are the sites of protein synthesis and that the ribosomes comprising a polysome are linked by a strand of messenger RNA. The evidence for this interpretation, which has been reviewed by Singer & Leder (1966), is based on the following facts: during short periods of incubation with radioactive precursors of either protein or RNA the polysomes are labelled, presumably in their growing protein chains and perhaps in their relatively unstable linking strand of messenger RNA; the presence and size-distribution of polysomes depends upon the metabolic state of the cell from which they were extracted; the clusters are readily broken down to single ribosomes, but not further, by ribonuclease. The last of these and some suggestive electron micrographs are the most direct evidence for the presence of a linking RNA strand.

However, ribonuclease is not alone in its ability to break down polysomes: a similar effect has been achieved with proteolytic enzymes (Rabinowitz, Zak, Beller, Rampersad & Wool, 1964; Nair, Zak & Rabinowitz, 1966; Tsiapalis, Hayashi & Chefurka, 1967). Further, it has been shown (Shakulov, Aitkhozin & Spirin, 1962; Santer, 1963; Santer & Smith, 1966) that, although ribonuclease has no obvious effect on the shape of individual ribosomes, at substantial concentrations (5 μg./ml. or more) it does attack the RNA of the intact ribosomes of *Escherichia coli*. The present work was undertaken with the object of comparing the sensitivity to ribonuclease of polysomes with that of the RNA of the ribosomes themselves. If a high concentration of ribonuclease is needed to produce a measurable effect on the RNA of the ribosomes relative to that required to influence the state of aggregation of polysomes, it would be reasonable to conclude that the two effects were distinct. But if the minimum ribonuclease treatment that disaggregates polysomes was found to degrade the RNA of the constituent ribosomes, the evidence for the existence of a linking strand of messenger RNA would be weakened. The most straightforward conclusion would then be that scission of ribosomal RNA results in the breakdown of polysomes.

MATERIALS AND METHODS

Crystalline pancreatic ribonuclease was obtained from British Drug Houses Ltd. (Poole, Dorset), once-crystallized pancreatic deoxyribonuclease from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.) and crystalline egg-white lysozyme from Koch–Light Laboratories Ltd. (Colnbrook, Bucks.).

Strain Hfr_1 of *Escherichia coli* K12 was grown in the synthetic medium TCG, containing 0·15% casein hydrolysate and 0·5% glucose (see Erikson, Fenwick & Franklin, 1964).

HeLa cells were grown in suspension (Harris & Watts, 1962).

Preparation of polysomes from E. coli. Spheroplasts were made by a modification of the method used by Guthrie & Sinsheimer (1960). The concentration of EDTA was decreased to a minimum, which was found to be about 0·5 mM. A 20 ml. culture of bacteria was grown to a density of 4×10^8–5×10^8 cells/ml. Chloramphenicol was added to give a concentration of 100 μg./ml. and incubation at 37° was continued for 4 min. The cells were chilled in ice, centrifuged and resuspended in 0·8 ml. of 40% (w/v) sucrose in 0·025 M-tris, pH 8·1, with 100 μg. of chloramphenicol/ml.

Lysozyme (0·1 ml. of a 2 mg./ml. soln.) was added, followed at once by 0·1 ml. of 5 mM-EDTA, pH 7. Within 15 sec. the mixture was diluted with 9 vol. of cold TCG medium, containing 10% (w/v) sucrose and chloramphenicol (100 μg./ml.), and then centrifuged for 10 min. at 2000g in the cold. Spheroplast formation continued during the centrifugation, the lysozyme having become adsorbed to the cells before dilution. The pellet of spheroplasts was resuspended in 0·9 ml. of lysis medium (Mangiarotti & Schlessinger, 1966) containing 6 mM-magnesium acetate, 0·04 M-NaCl, 0·01 M-tris, pH 7·2, deoxyribonuclease (5 μg./ml.), chloramphenicol (100 μg./ml.) and sodium deoxycholate (0·5%) (final concentrations). Deoxycholate was added just before use to avoid precipitation of the magnesium salt. After a few minutes in ice the viscosity was decreased to a manageable level and 0·3 ml. of the lysate was pipetted gently on to a 4·8 ml. sucrose gradient of 25–8% (w/v) sucrose in 0·01 M-magnesium acetate–0·04 M-NaCl–0·01 M-tris, pH 7·2, and centrifuged in the SW 39 or SW 50 rotor (which both have the same geometry) of the Spinco model L centrifuge. The same polysome pattern was obtained if chloramphenicol was added to the spheroplasts for 4 min. at 37° in TCG containing 10% sucrose, instead of to the intact bacteria. The distribution of extinction at 254 mμ was automatically recorded by an Isco density-gradient fractionator model 180 (Instrumentation Specialities Co. Inc., Lincoln, Nebr., U.S.A.), which displaced the contents of the tube upwards through a flow cell with an optical path of 2 mm.

For the separation of ribosomal sub-units, spheroplasts, without chloramphenicol, were lysed in 0·5 mM-magnesium acetate – 0·01 M - tris – deoxyribonuclease (5 μg./ml.), and centrifuged in gradients containing 0·5 mM-magnesium acetate and 0·01 M-tris.

Preparation of polysomes from HeLa cells. A growing culture of 10^8 cells at a density of $0·7 \times 10^6$–$1·5 \times 10^6$ cells/ml. was chilled in ice and centrifuged, and the cells were resuspended in 2·85 ml. of 1·5 mM-magnesium acetate–0·01 M-KCl–0·01 M-tris, pH 7·2 (Penman, Scherrer, Becker & Darnell, 1963). Deoxycholate was added (0·15 ml. of a 4%, w/v, soln.) and the lysate was immediately centrifuged for 10 min. at 8000g in the cold to remove nuclei, unbroken cells and large particles such as mitochondria. A portion (1 ml.) of the supernatant was pipetted on to a 28 ml. gradient of 30–5% (w/v) sucrose in 1·5 mM-magnesium acetate–0·01 M-KCl–0·01 M-tris, pH 7·2, and centrifuged in the Spinco rotor SW 25.1. E_{254} was monitored as described above, using an optical path of 5 mm.

For the separation of HeLa ribosomal sub-units, 10^8 cells were washed in 0·15 M-NaCl and resuspended in 1·0 ml. of cold 0·01 M-tris, pH 7·2. After five strokes of a motor-driven Teflon pestle, nuclei and surviving cells were removed by centrifuging for 10 min. at 8000g. One-tenth volume of 4 M-NaCl was added to the supernatant, which was left in an ice bath for 5 min. and then divided between three 4·8 ml. gradients of 25–8% sucrose in 0·1 M-NaCl–0·01 M-tris, pH 7·2.

Extraction of RNA. Phenol treatment either at room temperature or in the cold is not very effective in destroying ribonuclease, as can be seen from the slight degradation of the control sample of Fig. 4(*d*). In experiments in which ribonuclease was added to ribosomes it was important to inactivate the enzyme rapidly at the time of liberation of the RNA. The following procedure, based on that of Shakulov *et al.* (1962), was effective with ribonuclease concentrations of up to 0·5 μg./ml. The ribosome suspension was poured on to an equal volume of water-saturated phenol at 60° together with enough sodium dodecyl sulphate to give a final concentration in the aqueous phase of 1·0%, and EDTA to give one of 0·01 M. The mixture was shaken intermittently at 60° for 5 min. and then cooled in ice and centrifuged. The RNA was precipitated from the aqueous layer with 2 vol. of cold ethanol. The sedimented RNA was dissolved in 0·1 M-NaCl–0·01 M-EDTA, pH 7, and precipitated again with ethanol. It was finally dissolved in 0·1 M-NaCl–0·01 M-EDTA, and centrifuged in 4·8 ml. sucrose gradients containing 0·1 M-NaCl and 0·01 M-EDTA.

RESULTS

Experiments with E. coli

Observation of polysomes. The demonstration of polysomes by sedimentation analysis of lysates of *E. coli* has been described recently by a number of workers (Mangiarotti & Schlessinger, 1966; Kiho & Rich, 1964, 1965; Dresden & Hoagland, 1965; Godson & Sinsheimer, 1967; Hotham-Iglewski & Franklin, 1967). The importance of quick chilling of the growing cells, rapid lysis, gentle handling of the lysate and suppression of ribonuclease activity has been emphasized. The presence of any 70s single ribosomes has been taken as evidence of ribonuclease attack (Mangiarotti & Schlessinger, 1966), though most published sedimentation patterns show a prominent 70s peak. In a large

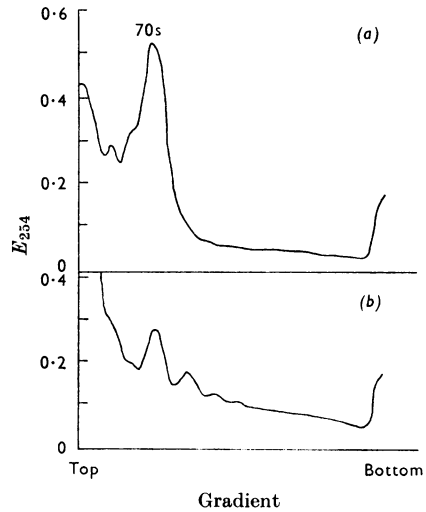

Fig. 1. Effect of chloramphenicol on *E. coli* polysomes: (*a*) spheroplasts lysed in the cold without chloramphenicol; (*b*) spheroplasts incubated for 2 min. at 37° with chloramphenicol (100 μg./ml.) and chilled, centrifuged and lysed in the presence of chloramphenicol at 20°. The lysate was centrifuged for 50 min. at 37 500 rev./min. at 5°.

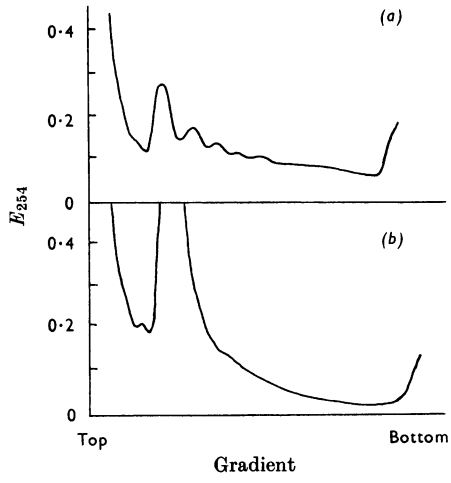

Fig. 2. Effect of actinomycin on *E. coli* polysomes. Spheroplasts were incubated at 37°. (*a*) Actinomycin (0·25 μg./ml.) and chloramphenicol (100 μg./ml.) were added at 20 min., and the preparation was chilled at 24 min. and lysed with chloramphenicol. (*b*) Actinomycin was added at zero time and chloramphenicol at 20 min., and the preparation was chilled at 24 min. and lysed with chloramphenicol. The lysate was centrifuged for 50 min. at 37 500 rev./min. at 5°.

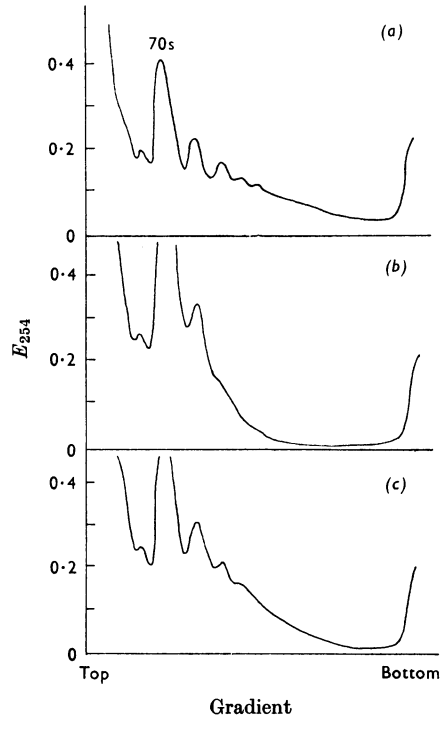

Fig. 3. Effect of ribonuclease on *E. coli* polysomes. Spheroplasts were incubated for 4 min. at 37° with chloramphenicol before lysis. The lysate was untreated (*a*) or treated for 10 min. at 20° with (*b*) 0·1 μg. of ribonuclease/ml. or (*c*) 0·01 μg. of ribonuclease/ml.; it was then chilled, applied to gradients and centrifuged for 25 min. at 48 000 rev./min. at 10°.

number of experiments in the present work, with various strains of *E. coli*, the one essential condition for the reproducible observation of polysomes was the presence of chloramphenicol for a period of 2–4 min. at 37° before lysis. The speed of chilling and lysis was then immaterial. Chloramphenicol has been used to preserve polysomes during isolation (Mangiarotti & Schlessinger, 1966) or during glucose starvation (Dresden & Hoagland, 1967), supposedly by fixing the protein-synthesizing structure in mid-synthesis. Typical patterns obtained with and without chloramphenicol are shown in Fig. 1. After treatment with chloramphenicol the optically dense material extended to about the 10-ribosome region. the major peak of 70s ribosomes suggested endogenous ribonuclease, and therefore a direct test for ribonuclease activity was made by adding HeLa-cell RNA, pulse-labelled for 20 min. with tritiated uridine, to a lysate of *E. coli*. After 5 min. at 37° the mixture was treated with phenol and sodium dodecyl sulphate, and the total RNA was precipitated and analysed on sucrose gradients. The labelled RNA was undamaged, although other samples were degraded by as little as 0·01 μg. of added ribonuclease/ml., but not by 0·001 μg. of added ribonuclease/ml. Thus the effective nuclease activity of the *E. coli* lysate was less than the equivalent of 0·01 μg. of ribonuclease/ml. Of course, the existence of a specific nuclease attacking the postulated messenger RNA linking the ribosomes cannot be excluded.

The polysomes behaved as expected (Singer & Leder, 1966) in that they did not appear if spheroplasts were preincubated with actinomycin (Fig. 2). This is generally interpreted as a result of the depletion of messenger RNA, although the possible effects of degradation of ribosomal RNA, observed in animal cells treated with actinomycin (Weisner, Acs, Reich & Shafiq, 1965), must also be considered. Polysomes were broken down (except for the 100s peak of dimers) with the liberation of 70s ribosomes by ribonuclease, but not by Pronase (33 μg./ml. for 10 min. at 20°).

Effect of ribonuclease on polysomes. By varying the concentration of ribonuclease it was found that 0·1 μg./ml. (for 10 min. at 20° plus a further 5–8 min. for application of samples to sucrose gradients and acceleration) caused a marked effect (Fig. 3*b*). Therefore polysomes isolated from a sucrose gradient were treated with ribonuclease at a

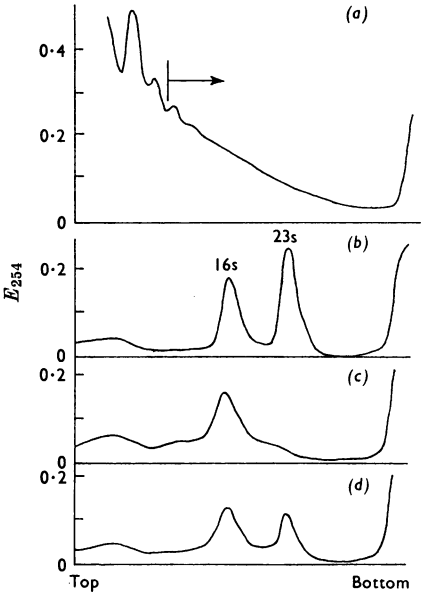

Fig. 4. RNA from *E. coli* polysomes. (*a*) Polysomes from spheroplasts treated with chloramphenicol for 4min. at 37° were centrifuged for 30min. at 40000rev./min. at 10°. The contents of three gradients, from the point shown by the arrow to the bottom, were pooled and divided into portions, which were treated as follows: (*b*) one portion was shaken with phenol; (*c*) ribonuclease (0·1 μg./ml.) was added to another portion for 10min. at 20°, followed by phenol treatment at 20°; (*d*) ribonuclease was added to another portion immediately after phenol. RNA extracted from (*b*), (*c*) and (*d*) was centrifuged for 3hr. at 48000rev./min. at 5°.

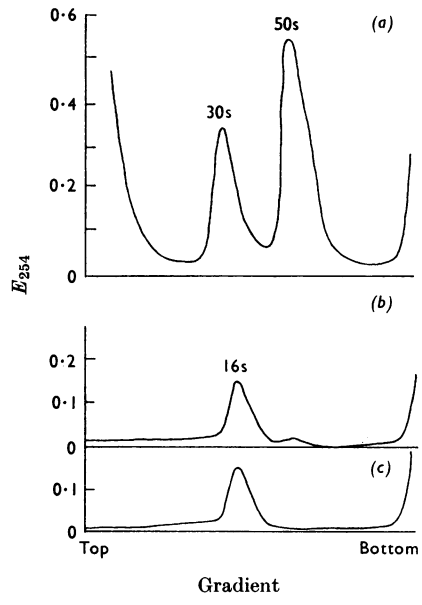

Fig. 5. RNA from *E. coli* 30s sub-units. The lysate of spheroplasts from 40ml. of cells ($3·5 \times 10^8$ cells/ml.) was divided between three 4·8ml. gradients containing 0·5mM-magnesium acetate and 0·01M-tris. Sub-units were collected after centrifugation for 90min. at 48000rev./min. at 10° (*a*). After addition of magnesium acetate to give a final concn. of 0·01M, 30s sub-units were incubated for 10min. at 20° without (*b*) or with (*c*) 0·1 μg. of ribonuclease/ml. RNA extracted with hot phenol (*b*, *c*) was centrifuged for 3hr. at 48000rev./min. at 10°.

concentration of 0·1 μg./ml. for 10min. at 20° and then shaken with phenol, and their RNA was extracted and analysed on further gradients as shown in Fig. 4. The absence of 23s RNA was striking (Fig. 4*c*) although a 16s peak of approximately normal size remained. This could have been unharmed 16s ribosomal RNA or a breakdown product of the 23s RNA, the original 16s having been completely degraded. To distinguish between these possibilities, ribosomal sub-units were isolated and treated separately with ribonuclease.

Effect of ribonuclease on sub-units. Ribosomes broke down to yield their constituent 30s and 50s sub-units in 0·5mM-Mg^{2+} (Fig. 5*a*). These were collected separately from a sucrose gradient and treated with ribonuclease after addition of Mg^{2+} to a concentration of 10mM. The 30s sub-units yielded intact 16s RNA without ribonuclease (Fig. 5*b*) or after 10min. with ribonuclease (0·1 μg./ml. at 20°) (Fig. 5*c*), and there was only a small degradation after 2hr. incubation with ribonuclease (not shown). In contrast, the 23s RNA of the 50s sub-units was very susceptible to ribonuclease attack, as expected from the earlier experiment with polysomes (Fig. 4). By gradually intensifying the ribonuclease treatment the progressive breakdown of the 23s RNA to more slowly sedimenting pieces was observed (Figs. 6*a*–6*f*). The degradation was not random: several species of breakdown product could be distinguished, and the three most prominent are labelled *A*, *B* and *C* in Fig. 6. Initially product *A* predominated, but it was gradually itself broken down, contributing to peaks *B* and *C*.

Experiments with HeLa cells

Observation of polysomes. Polysomes have been isolated from homogenates of HeLa cells (Penman *et al.* 1963) as well as many other animal cells and tissues. To avoid the shearing stresses involved in homogenization, attempts were made to lyse HeLa cells, like *E. coli* spheroplasts, with deoxycholate. It was found that 0·2% deoxycholate quickly

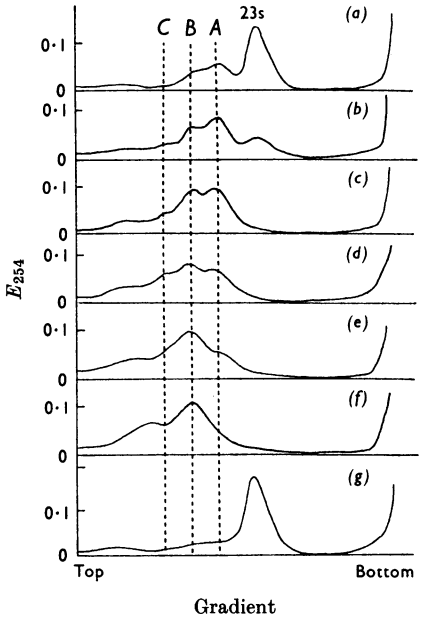

Fig. 6. RNA from *E. coli* 50s sub-units. 50s Material from Fig. 5(*a*) was treated with ribonuclease and then RNA was extracted with hot phenol and centrifuged for 3hr. at 48000rev./min. at 10°. Ribonuclease treatment was at 20° for various times: (*a*) 0·033μg./ml., 10min.; (*b*) 0·1μg./ml., 10min.; (*c*) 0·1μg./ml., 20min.; (*d*) 0·1μg./ml., 34min.; (*e*) 0·1μg./ml., 1hr.; (*f*) 0·1μg./ml., 2hr.; (*g*) control, incubated for 2hr. before the addition of ribonuclease (0·1μg./ml.) for 5sec. Peaks *A*, *B* and *C* are the most prominent peaks of breakdown products (see the text).

disrupted most of the cells in hypo-osmotic medium (1·5mM - magnesium acetate – 0·01M - potassium chloride–0·01M-tris), leaving nuclei with a certain amount of adhering cytoplasmic material. Concentrations of deoxycholate above 0·3% had the undesirable effect of rupturing the nuclei and releasing their viscous nucleoprotein. Polysomes were observed by sucrose-gradient analysis of 0·2% deoxycholate lysates after removal of nuclei and unbroken cells. However, the anti-shear precautions may have been unnecessary, since the polysome pattern was unchanged after vigorous pipetting with a fine-tipped pipette. The rate of cooling of the cell culture before lysis was also unimportant: the same polysome pattern resulted whether the cells were poured on to crushed frozen medium or allowed to cool slowly to room temperature.

Effect of ribonuclease on polysomes. HeLa-cell lysates (8000*g* supernatants; see the Materials and Methods section) were incubated with various concentrations of ribonuclease for 10min. at 20° and then chilled and applied to sucrose gradients. The polysomes appeared somewhat less susceptible than those of *E. coli*, 1μg. of ribonuclease/ml. being required to cause a substantial breakdown (Figs. 7*a*–7*d*). RNA was extracted from the pooled contents of the sucrose gradient from the 70s region (single ribosomes) to the bottom (aggregates of perhaps 20 ribosomes). After addition of bentonite (1mg./ml.) (Fraenkel-Conrat, Singer & Tsugita, 1961) the polysomes were poured on to hot phenol as described in the Materials and Methods section, and their extracted RNA was analysed in further gradients as shown in Figs. 7(*e*)–7(*h*). The polysomes showing only a very small degree of breakdown (Fig. 7*b*) yielded slightly degraded RNA (Fig. 7*f*), whereas in Figs. 7(*g*) and 7(*h*) the degradation of RNA was extensive and related to the state of aggregation of the polysomes. There was a suggestion that, as with *E. coli* ribosomes, the faster-sedimenting (30s) RNA was more susceptible than the slower (18s), and that the breakdown resulted in a specific series of RNA fragments which formed distinct peaks in the gradient pattern. In this experiment the volume of the pooled polysome region was more than ten times that of the sample applied to the gradient. Thus the concentration of ribonuclease present during phenol treatment must have been less than one-tenth of that in the sample, even if all the enzyme had sedimented with the polysomes.

Another test was carried out in which a similar lysate was treated with ribonuclease (0·2μg./ml.) for 10min. at 20°. Then bentonite was added and the mixture transferred to hot phenol with sodium dodecyl sulphate and EDTA as before. Ribonuclease was added to the control sample, followed within 5sec. by bentonite, and the mixture was immediately transferred to hot phenol. Comparison of the RNA from these samples (Fig. 8) confirmed that some breakdown of 30s RNA had occurred during the incubation of the polysomes with this low concentration of ribonuclease.

Separation of sub-units. The ribosomes of HeLa cells were more difficult to dissociate into their component sub-units than were those of *E. coli*. Even when the cells were homogenized in 0·01M-tris, pH7·2, without added Mg^{2+} the sub-units did not separate cleanly (Fig. 9*a*). Separation in a medium of high ionic strength has been described by Vaughan, Warner & Darnell (1967), but this technique was not successful with our HeLa cells. Separation may be achieved by treating the ribosomes with EDTA, but lest this should cause damage to the particles a test was carried out to determine a concentration of sodium chloride that would cause the dissociation, presumably by displacing bound Mg^{2+} ions. As shown in Fig. 9, the addition of 0·2M-sodium chloride (or greater concentrations)

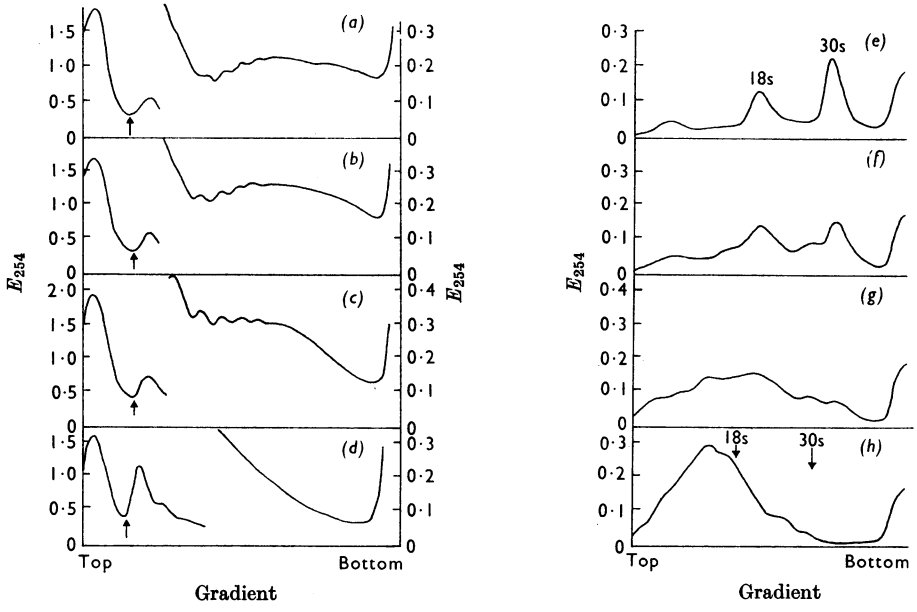

Fig. 7. Effect of ribonuclease on HeLa-cell polysomes. Lysates were untreated (*a*) or treated for 10 min. at 20° with ribonuclease [(*b*) 0·2 μg./ml., (*c*) 0·5 μg./ml., (*d*) 1·0 μg./ml.], and chilled and centrifuged for 90 min. (*a–c*) or 70 min. (*d*) at 25000 rev./min. at 10° in the SW 25.1 rotor. In (*a*)–(*d*) the contents of the gradients were pooled from the point shown by the arrow to the bottom, and RNA was extracted with hot phenol after addition of bentonite (1 mg./ml.); the left-hand scale applies as far as the break in the curves, and then the right-hand scale applies. Extracted RNA (*e–h*) was centrifuged for 3 hr. at 48000 rev./min. at 10° (*e–g*) or for 15 hr. at 22000 rev./min. at 5° (*h*) in the SW 50 rotor. The arrows in (*h*) show the positions of 18 s and 30 s RNA corresponding to the peaks in (*e*).

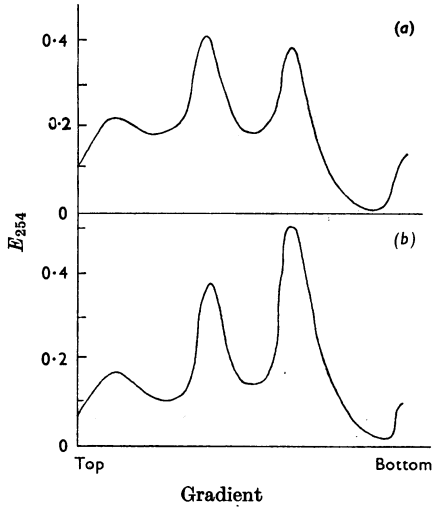

Fig. 8. RNA extracted from HeLa-cell lysate: (*a*) lysate treated with ribonuclease (0·2 μg./ml.) for 10 min. at 20°; (*b*) control, 0·2 μg. of ribonuclease/ml. for 5 sec. at 20°. RNA was extracted with hot phenol after the addition of bentonite, and centrifuged for 15 hr. at 22000 rev./min. at 5°.

to a homogenate in 0·01 M-tris, pH 7·2, caused a clear separation of the ribosomal sub-units. Increasing amounts of sodium chloride also caused a decrease in sedimentation rate of the sub-units, probably indicating a loosening of structure. In the absence of precise measurements they are designated '30 s' and '50 s' respectively.

Effect of ribonuclease on sub-units. In an experiment similar to that described above (Figs. 5 and 6) for *E. coli*, HeLa-cell ribosomal sub-units were isolated from a sucrose gradient containing 0·1 M-sodium chloride (Fig. 10*a*) and treated with ribonuclease after addition of Mg^{2+} to a concentration of 0·01 M. Subsequent analysis of the RNA (Figs. 10*b*–10*g*) showed that 0·01 μg. of ribonuclease/ml. (which did not appreciably affect either the polysome pattern or the RNA within polysomes) caused much degradation of both the 18 s RNA of the 30 s sub-unit and the 30 s RNA of the 50 s sub-unit. Thus both sub-units of HeLa ribosomes, and particularly the smaller, were more susceptible to ribonuclease than were those of *E. coli*. This difference may be related to a loosening of their structure during preparation, referred to in the preceding section.

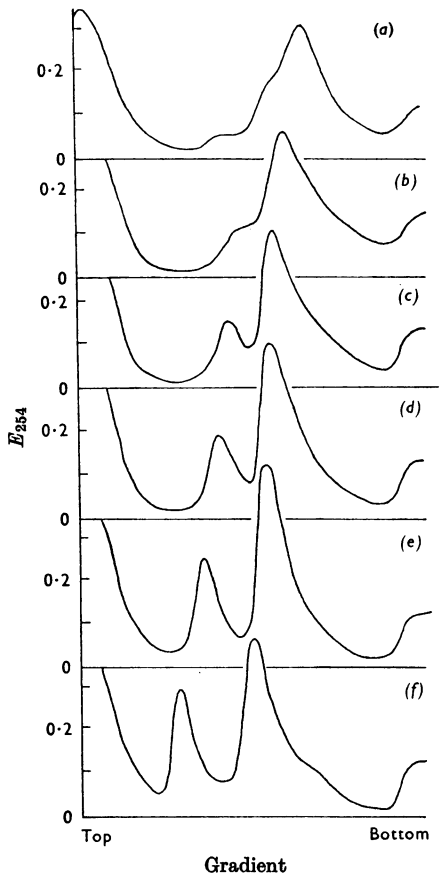

Fig. 9. Separation of HeLa-cell sub-units: effect of increasing NaCl concentration. A homogenate in 0·01 M-tris was centrifuged for 10 min. at 2000*g*, and NaCl was added to the supernatant: (*a*) none; (*b*) 0·02 M; (*c*) 0·05 M; (*d*) 0·1 M; (*e*) 0·2 M; (*f*) 0·4 M. The mixture was centrifuged in gradients containing the same NaCl concentration for 75 min. at 48000 rev./min. at 10°.

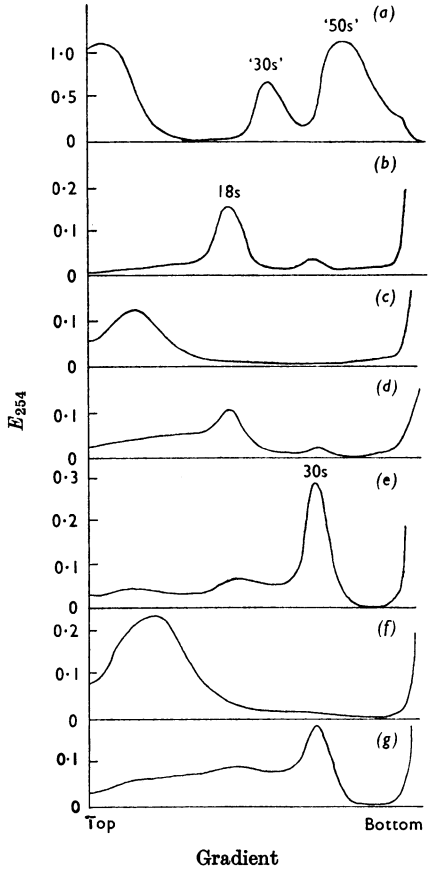

Fig. 10. RNA from HeLa-cell sub-units. Sub-units were collected after centrifuging for 90 min. at 48000 rev./min. at 5° in 0·1 M-NaCl–0·01 M-tris (*a*). After addition of magnesium acetate to a final concn. of 0·01 M, sub-units were incubated for 10 min. at 20°: (*b*) 30 S sub-units, no ribonuclease; (*c*) 30 S sub-units with 0·1 μg. of ribonuclease/ml.; (*d*) 30 S control, treated with ribonuclease for 5 sec. at 20° before phenol treatment; (*e*) 50 S sub-units, no ribonuclease; (*f*) 50 S with 0·1 μg. of ribonuclease/ml.; (*g*) 50 S control, treated with ribonuclease for 5 sec. at 20°. RNA was extracted with hot phenol, and centrifuged for 15 hr. at 22000 rev./min. at 5° (*b–g*).

DISCUSSION

From the results of these experiments it appears that even the mildest ribonuclease treatment that has any disaggregating effect on polysomes simultaneously splits the RNA of the larger ribosomal sub-unit of both *E. coli* and HeLa cells, although the smaller sub-units of *E. coli*, and possibly of HeLa cells also, is more resistant. This suggests that the ribosomal RNA is involved in binding the ribosomes together in polysomes. It provides no evidence for or against the presence of a strand of RNA linking the ribosomes, but it does show that if there is such a strand it is not more susceptible to ribonuclease than is the ribosomal RNA. This is contrary to the experience of Marbaix & Burny (1964), who reported that the polysomes of rabbit reticulocytes were disaggregated by incubation for 5 min. at 37° with 0·01 μg. of ribonuclease/ml. without the enzyme affecting the RNA of the ribosomes. Assuming that incubation alone, without ribonuclease, was not responsible for the disaggregation in their experiments, attack by ribonuclease near the end of the ribosomal RNA would probably not cause a noticeable change in the sedimentation pattern of the extracted RNA.

The evidence is inconclusive, but taking into consideration other reports that polysomes can be broken down by trypsin and chymotrypsin (Rabinowitz *et al.* 1964; Nair *et al.* 1966; Tsiapalis *et al.* 1967), perhaps the simplest hypothesis would be that polysomes are aggregates of ribosomes held together by the Mg^{2+}-dependent interaction between an exposed section of their RNA and a protein component. The protein concerned might be ribosomal protein or perhaps partly synthesized polypeptides, making active ribosomes more prone to aggregation than inactive ones, or perhaps part of a cytoplasmic fibrillar network (Schlessinger, Marchesi & Kwan, 1965; van Iterson, 1966).

The question remains: if the polysomes are supporting protein synthesis, where is the RNA message? Labelled 10s RNA has been found in HeLa polysomes (Penman *et al.* 1963) and in reticulocyte polysomes (Huez, Burny, Marbaix & Lebleu, 1967), and viral (35s) RNA in the larger polysomes from HeLa cells infected with poliovirus (Penman, Becker & Darnell, 1964). In contrast it has been reported that the messenger activity of reticulocyte polysomes cannot be dissociated from ribosomal RNA with EDTA (Gould, Arnstein & Cox, 1966); nor could the pulse-labelled RNA associated with ribosomes in *E. coli* be detached from the particles by lowering the concentration of Mg^{2+} (Artman, Silman & Engelberg, 1967). It is still possible that each ribosome carries its own template, either attached or built in (Harris, 1968; Gould *et al.* 1966; Hadjiolov, 1967).

The case for an extraribosomal messenger RNA has been strengthened by studies with cell-free systems, which have of course revealed the nature of the nucleic acid code that determines protein structure. In the few instances in which the synthesis of a recognizable protein has been directed *in vitro* the added template has been a viral (Nathans, Notani, Schwartz & Zinder, 1962; Nathans, 1965; Capecchi & Gussin, 1965; Capecchi, 1966) or a virus-specific (Salser, Gesteland & Bolle, 1967) RNA, and the possibility must be considered that viral protein synthesis is a special case and not identical in every detail with the process of the host cell (Harris, 1965, 1968). The host mechanism may be operating in those cases where the addition of RNA to ribosomes *in vitro* stimulates synthesis of protein characteristic of the cell which provided the ribosomes (Drach & Lingrel, 1966; Hunt & Wilkinson, 1967). A distinction between host- and virus-controlled synthesis is suggested by the work of Dr. L. Thiry (personal communication), who found it is easier to induce mistakes in amino acid incorporation into the protein of cells infected with Newcastle-disease virus than into that of uninfected cells. A difference in mechanism, such as a different relationship of message to ribosome, might also explain the remarkable specificity of interferon in preventing the synthesis of viral protein [by stopping the association (Joklik & Merigan, 1966; Carter & Levy, 1967) or the functioning (Marcus & Salb, 1966) of viral RNA with host ribosomes] while allowing host protein synthesis to continue on ribosomes already bearing a message. In cells infected with the transforming virus SV 40, a change from sensitivity to resistance to interferon occurs as the viral DNA becomes integrated in the cell (Oxman *et al.* 1967). Perhaps transformation involves a change from the viral mechanism to the host-cell mechanism of virus-directed protein synthesis.

The effect of ribonuclease on the RNA of the larger sub-unit of *E. coli* provides some information about the structure of this particle, which is thought to consist of a coiled RNA–protein thread (Spirin, Kisselev, Shakulov & Bogdanov, 1963; Hart, 1965; Nanninga, 1967). The pattern of breakdown products suggests that there is a particularly sensitive region of the RNA chain in the intact particle, perhaps about one-third of the distance from one end, and attack in this region results in peaks *A* and *B* of Fig. 6. There is a somewhat smaller or less susceptible naked region in the larger section *A* and ribonuclease attack here breaks it down further, producing peak *C*. In contrast, the RNA of the smaller sub-unit does not seem to be exposed in the same way, except possibly near the ends of the chain, removal of which would not be detected by the techniques used here. The 5s RNA that has been found associated with the larger ribosomal sub-unit in some species (Galibert, Lelong, Larsen & Boiron, 1967) must also be considered as a possible specific intraribosomal degradation product.

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REFERENCES

Artman, M., Silman, N. & Engelberg, H. (1967). *Nature, Lond.*, **213**, 39.

Capecchi, M. R. (1966). *J. molec. Biol.* **21**, 173.

Capecchi, M. R. & Gussin, G. N. (1965). *Science*, **149**, 417.

Carter, W. A. & Levy, H. B. (1967). *Science*, **155**, 1254.

Drach, J. C. & Lingrel, J. B. (1966). *Biochim. biophys. Acta*, **129**, 178.

Dresden, M. H. & Hoagland, M. B. (1965). *Science*, **149**, 647.

Dresden, M. H. & Hoagland, M. B. (1967). *J. biol. Chem.* **242**, 1065.

Erikson, R. L., Fenwick, M. L. & Franklin, R. M. (1964). *J. molec. Biol.* **10**, 519.

Fraenkel-Conrat, H., Singer, B. & Tsugita, A. (1961). *Virology*, **14**, 54.

Galibert, F., Lelong, J. C., Larsen, Ch. J. & Boiron, M. (1967). *Biochim. biophys. Acta*, **142**, 89.
Godson, G. N. & Sinsheimer, R. L. (1967). *J. molec. Biol.* **23**, 495.
Gould, H. J., Arnstein, H. R. V. & Cox, R. A. (1966). *J. molec. Biol.* **15**, 600.
Guthrie, G. D. & Sinsheimer, R. L. (1960). *J. molec. Biol.* **2**, 297.
Hadjiolov, A. A. (1967). *J. theoret. Biol.* **16**, 229.
Harris, H. (1965). In *Evolving Genes and Proteins*, p. 469. Ed. by Bryson, V. & Vogel, H. J. New York: Academic Press Inc.
Harris, H. (1968). *Nucleus and Cytoplasm*. Oxford: Clarendon Press (in the Press).
Harris, H. & Watts, J. W. (1962). *Proc. Roy. Soc.* B, **156**, 109.
Hart, R. G. (1965). *Proc. nat. Acad. Sci., Wash.*, **53**, 1415.
Hotham-Iglewski, B. & Franklin, R. M. (1967). *Proc. nat. Acad. Sci., Wash.*, **58**, 743.
Huez, G., Burny, A., Marbaix, G. & Lebleu, B. (1967). *Biochim. biophys. Acta*, **145**, 629.
Hunt, J. A. & Wilkinson, B. R. (1967). *Biochemistry*, **6**, 1688.
Joklik, W. K. & Merigan, T. C. (1966). *Proc. nat. Acad. Sci., Wash.*, **56**, 558.
Kiho, Y. & Rich, A. (1964). *Proc. nat. Acad. Sci., Wash.*, **51**, 111.
Kiho, Y. & Rich, A. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 1751.
Mangiarotti, G. & Schlessinger, D. (1966). *J. molec. Biol.* **20**, 123.
Marbaix, G. & Burny, A. (1964). *Biochem. biophys. Res. Commun.* **16**, 522.
Marcus, P. I. & Salb, J. M. (1966). *Virology*, **30**, 502.
Nair, K. G., Zak, R. & Rabinowitz, M. (1966). *Biochemistry*, **5**, 2674.
Nanninga, N. (1967). *J. Cell Biol.* **33**, C1.
Nathans, D. (1965). *J. molec. Biol.* **13**, 521.
Nathans, D., Notani, G., Schwartz, J. H. & Zinder, N. D. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1424.
Oxman, M. N., Baron, S., Black, P. H., Takemoto, K. K., Habel, K. & Rowe, W. P. (1967). *Virology*, **32**, 122.
Penman, S., Becker, Y. & Darnell, J. E. (1964). *J. molec. Biol.* **8**, 541.
Penman, S., Scherrer, K., Becker, Y. & Darnell, J. E. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 654.
Rabinowitz, M., Zak, R., Beller, B., Rampersad, O. & Wool, I. G. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 1353.
Salser, W., Gesteland, R. F. & Bolle, A. (1967). *Nature, Lond.*, **215**, 588.
Santer, M. (1963). *Science*, **141**, 1049.
Santer, M. & Smith, J. R. (1966). *J. Bact.* **92**, 1099.
Schlessinger, D., Marchesi, V. T. & Kwan, B. C. K. (1965). *J. Bact.* **90**, 456.
Shakulov, R. S., Aitkhozin, M. A. & Spirin, A. S. (1962). *Biokhimiya*, **27**, 744 (Engl. trans. p. 631).
Singer, M. F. & Leder, P. (1966). *Annu. Rev. Biochem.* **35**, 195.
Spirin, A. S., Kisselev, N. A., Shakulov, R. S. & Bogdanov, A. A. (1963). *Biokhimiya*, **28**, 920.
Tsiapalis, C. M., Hayashi, Y. & Chefurka, W. (1967). *Nature, Lond.*, **214**, 358.
van Iterson, W. (1966). *J. Cell Biol.* **28**, 563.
Vaughan, M. H., Warner, J. R. & Darnell, J. E. (1967). *J. molec. Biol.* **25**, 235.
Weisner, R., Acs, G., Reich, E. & Shafiq, A. (1965). *J. Cell Biol.* **27**, 47.