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Function of Aggregated Reticulocyte Ribosomes in Protein Synthesis

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The ribosome fraction of rabbit reticulocytes contains ribosomal units with a sedimentation coefficient of 80 s and some smaller components. In addition there is a spectrum of at least five more rapidly sedimenting fractions, comprising of the order of 30% or more of the material. The fast fractions are considered to be aggregates of ribosomes consisting of up to six ribosomal units. They disintegrate into the 80 s ribosomal units upon incubation with RNase. The fast fractions, separated from the slow fraction by sucrose gradient centrifugation, are shown to be more active both in whole cell and in cell-free incorporation of radioactive amino acids. Incorporation of [^{14}C]phenylalanine is stimulated by the addition of polyuridylic acid as messenger RNA. The stimulation of activity is maximum for unaggregated ribosomes. Part of the active products of stimulation appear to be aggregates. The results indicate that many of the aggregated ribosomes contain messenger RNA, but are not receptive to added messenger RNA; whereas many of the unaggregated ribosomes do not contain messenger RNA, but are receptive to added messenger RNA molecules. The simplest interpretation is that the linkage of the ribosomes into aggregates is due to the messenger RNA itself. The findings support a theory of protein synthesis whereby several ribosomes can operate simultaneously on one molecule of messenger RNA.

1. Introduction

Ribosomes are known to be sites of protein synthesis. At any given time only some of the ribosomes are active in protein synthesis. Attachment of messenger RNA is necessary for a ribosome to be active.

Most of the evidence on messenger RNA concerns bacterial ribosomes. The active fraction of ribosomes from bacteria may be distinguished from the inactive fraction by its relatively high stability at low Mg^{2+} concentrations (Tissières, Schlessinger & Gros, 1960). Recently, a distinction in terms of physical properties was discovered by Risebrough, Tissières & Watson (1962). It was shown that the active fraction has a higher sedimentation coefficient than most of the ribosomes. This was attributed to the increased molecular weight caused by messenger RNA and to possible aggregation of ribosomes.

It is of interest to establish that ribosomes from mammalian cells also contain a rapidly sedimenting active fraction, and to study its properties. For this purpose rabbit reticulocytes are particularly well suited. In reticulocytes, protein synthesis does not seem to be accompanied by a large turnover or synthesis of RNA. Messenger RNA, if existing in the same sense as in bacteria, seems to be rather stable (Marks,

Willson, Kruh & Gros, 1962). Most of the protein synthesized is of one type: haemoglobin. Protein synthesis can be investigated *in vitro* by incorporation of radioactive amino acids into whole cells (Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1952; Kruh & Borsook, 1956) and into a cell-free system (Schweet, Lamfrom & Allen, 1958). As in bacteria, only some of the ribosomes, which are relatively stable at low Mg^{2+} concentrations, are active in protein synthesis (Lamfrom & Glowacki, 1962).

Most of the ribosomes are of one type, with a sedimentation coefficient of 80 s. In addition, two slower and three or four faster minor components have been reported (Dintzis, Borsook & Vinograd, 1958; T'so & Vinograd, 1961; Arnstein, 1961; Lamfrom & Glowacki, 1962).

In our studies attention has been focused on the rapidly sedimenting fractions, their structure and their function in protein synthesis.

2. Materials and Methods

[^{14}C]Valine (about 5 mc/mM) and [^{14}C]phenylalanine (9 mc/mM) were purchased from the Radiochemical Centre, Amersham. Polyuridylic acid (poly-U) was a product of the California Corporation, Los Angeles.

Medium A (with sucrose) contains 0·35 M-sucrose, 0·035 M-$KHCO_3$, 0·004 M-$MgCl_2$, 0·025 M-KCl (Keller & Zamecnik, 1956); solution A (without sucrose) contains 0·14 M-KCl, 0·001 M-$MgCl_2$, 0·01 M-tris (pH 6·9).

Preparation of reticulocytes, incorporation of ^{14}C-labelled amino acids into whole cells, lysis, cell fractionation and incorporation into the cell-free system were performed essentially as described by Borsook *et al.* (1952) and by Schweet *et al.* (1958) with the specifications given below. All preparations were made at 5°C unless otherwise stated.

(a) *Preparation of ribosomes and pH 5 fraction*

After lysis of the reticulocytes the solution was centrifuged for 10 min at 20,000 rev./min to remove the debris. The supernatant solution was centrifuged for 120 min at 30,000 rev./min in the Spinco no. 30 rotor, using stainless steel tubes containing 25 ml./tube. The sediment of ribosomes was suspended either in solution A (for sucrose gradient or analytical centrifugation) or in medium A (for direct cell-free incubation).

The supernatant solution was titrated to pH 5·1 with acetic acid. The precipitate was centrifuged and dissolved in 0·1 M-tris, pH 7·5, to a concentration of 12 mg protein/ml. (pH 5 fraction). The remaining supernatant solution was titrated back to pH 7 (pH 5 supernatant).

(b) *Incorporation of [^{14}C]valine into whole cells and fractionation of radioactive ribosomes*

Reticulocytes were incubated with 0·2 μc/ml. [^{14}C]valine for 4 min at 37°C and ribosomes were prepared as described above. Ribosomes (about 2 mg in 0·2 ml. solution A) were layered on a 5 to 20% sucrose gradient and centrifuged in the refrigerated Spinco SW39 rotor for 60 min at 35,000 rev./min. Fractions were collected from a hole in the bottom of the tube and diluted to 2·5 ml. with solution A to measure the optical density. Two mg of carrier protein (serum albumin) were added to each sample, followed by TCA† precipitation. The precipitates were washed once with TCA, dried, dissolved in formic acid and plated. The radioactivity was measured in a windowless gas-flow counter.

(c) *Fractionation of ribosomes for cell-free incorporation*

A preparation of ribosomes in solution A was layered on a sucrose gradient and centrifuged in a refrigerated Spinco SW25 rotor for 110 min at 23,000 rev./min. Three ml. samples were collected from the tube and centrifuged for 60 min at 40,000 rev./min in stainless steel tubes of the Spinco rotor no. 40. The sediments of ribosomal particles were dissolved in medium A for cell-free incorporation.

† Abbreviation used: TCA = trichloracetic acid.

(d) *Incorporation of [^{14}C]amino acids into a cell-free system*

Cell-free incubation mixtures contained ribosomes in amounts specified, dissolved in about 0·5 ml. medium A, plus 0·1 ml. pH 5 fraction, 0·3 ml. pH 5 supernatant solution, 0·25 μM-GTP, 1 μM-ATP, 20 μM-creatine phosphate, 80 μg creatine phosphate kinase, 0·06 ml. of a complete amino acid mixture minus valine or phenylalanine (Borsook, Fischer & Keighley, 1957) and 0·2 μC [^{14}C]valine or [^{14}C]phenylalanine. Forty μg poly-U was added if specified. After incubation for 15 min at 37°C, protein was precipitated by TCA and prepared for measurement of radioactivity.

In some cases, ribosomes were re-isolated after incubation by centrifugation (60 min at 40,000 rev./min with 3 ml. per stainless steel tube of the Spinco no. 40 rotor).

Sedimentation coefficients of ribosomes were measured in solution A using schlieren or u.v. optics of the Spinco model E analytical ultracentrifuge. Most measurements were performed at about 5°C; some were made at 20°C. All values were corrected to 20°C.

3. Results

(a) *Spectrum of components in the analytical ultracentrifuge*

The sedimentation pattern of freshly prepared reticulocyte ribosomes shows at least eight components (Plate I(a) and (c)). A similar pattern is obtained if packed cells are lysed as usual, the debris removed by short centrifugation and the supernatant solution analysed immediately in the ultracentrifuge (Plate I(b)). The pattern is therefore not an artefact of the further steps in the preparation of the ribosomes. For unknown reasons the absolute value of sedimentation coefficients deviates by about 2% in different preparations. There is a main component with a sedimentation coefficient of 78 to 80 s (extrapolated to zero concentration) which is the ribosomal unit corresponding to the 70 to 80 s ribosomes of bacteria, plants and animal tissues. Two slower components (43 and 61 s) are probably subunits analogous to the 30 and 50 s fraction of bacteria. In addition, there are at least five fast components with sedimentation coefficients of 120, 153, 180, 205 and 220 s. (In some preparations only traces of the 220 s component are visible.) Altogether, the fast components of freshly prepared ribosomes constitute a considerable proportion (30% or more) of the total amount.

Upon incubation with RNase (1 μg/ml. for 10 minutes at 20°C) the fast fractions disappear whereas the 80 s fraction increases in amount; traces of the 120 s fraction are resistant to the concentration of RNase used (Plate I(c) and (d)).

(b) *Incorporation of radioactive amino acids into whole reticulocyte cells*

Ribosomes were prepared from reticulocyte cells which had been incubated *in vitro* with [^{14}C]valine. About 2 mg of ribosomes were layered on a sucrose gradient and centrifuged for 60 minutes at 35,000 rev./min. Fractions were collected and their u.v. absorption and radioactivity measured. The results are given in Fig. 1(a). In accordance with the results on analytical ultracentrifugation, the pattern contains slow and fast fractions. The slow main fraction consists mainly of 80 s ribosomes as measured with the u.v. optics of the analytical ultracentrifuge. The distribution of radioactivity does not follow the distribution of the optical density; the radioactivity resides mainly in the fractions much faster than 80 s.

Treatment of ribosomes with RNase (1 μg/ml. for 10 minutes at 20°C) before sucrose gradient centrifugation leads to disappearance of the fast fractions and a concentration of nearly all u.v.-absorbing material and nearly all the radioactivity in one band corresponding to about 80 s (Fig. 1(b)). By comparing Fig. 1(a) and 1(b),

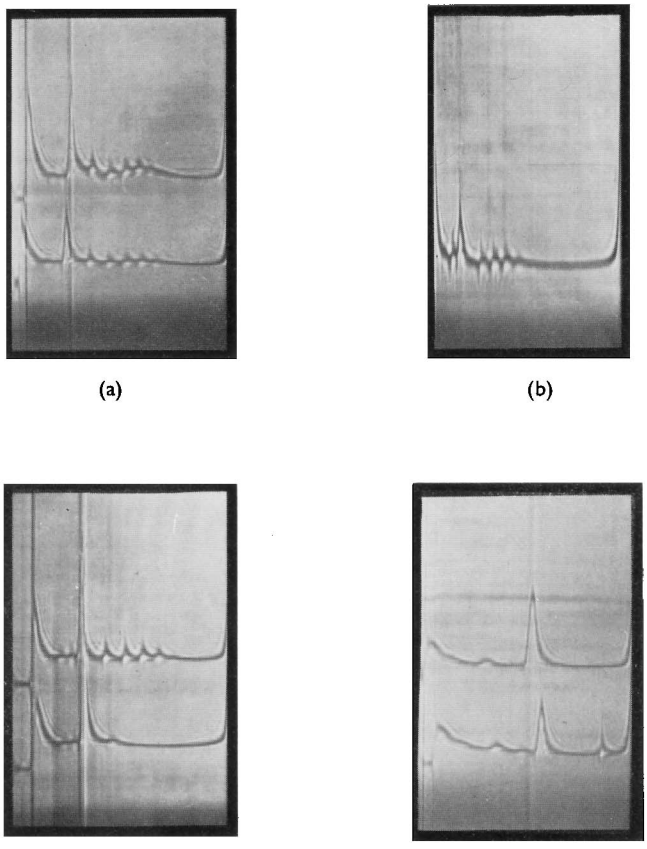

PLATE I. Schlieren sedimentation patterns of reticulocyte ribosomes in solution A in the analytical centrifuge. Sedimentation is from left to right. In (a), (c) and (d) two samples were examined simultaneously, using normal and wedge cells.

(a) 8 mg/ml. (top) and 4 mg/ml. (bottom), 4°C. The main peak corresponds to the 80 s component. At least five more rapidly sedimenting fractions occur.

(b) Lysed cells after removal of the debris by short centrifugation (10 min at 20,000 rev./min).

(c) Short centrifugation, 20°C, 4 mg/ml. top: without RNase; bottom: after incubation with 1 μg/ml. RNase for 10 min at 20°C. The faster fractions disappear upon incubation with RNase. Traces of the 120 s fraction remain visible.

(d) Longer centrifugation, 20°C, 4 mg/ml., bottom: without RNase; top: after incubation with 1 μg RNase for 10 min at 20°C. The sedimentation rates of the main components are equal, the shift of the position of the main peaks being due to a different position of the meniscus. Components faster than 120 s are already in the sediment. RNase treatment has increased the amount of 80 s ribosomes.

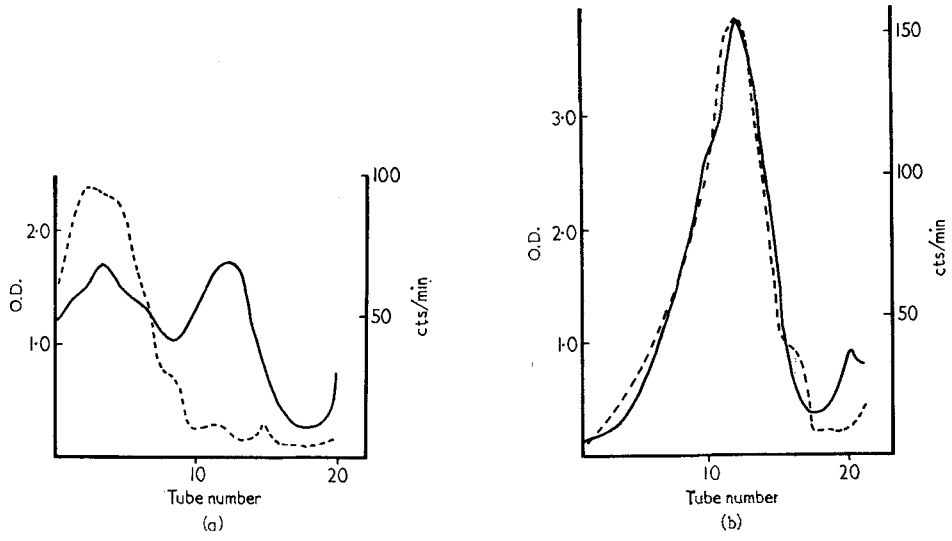

FIG. 1. Incorporation of radioactive amino acids into whole reticulocyte cells. After incorporation with [^{14}C]valine into whole cells, ribosomes were isolated and fractionated by sucrose gradient centrifugation (60 min at 35,000 rev./min, 5°C). Fractions were collected, and the optical density and radioactivity were measured.

————: Optical density (O.D.) (given as total O.D. of each fraction in 1 ml. at 260 mμ); – – – – –: Radioactivity (measured as cts/min).

(a) Ribosomes not treated with RNase. (b) Ribosomes treated with RNase for 10 min at 20°C (0·2 μg RNase in 0·2 ml. ribosome solution).

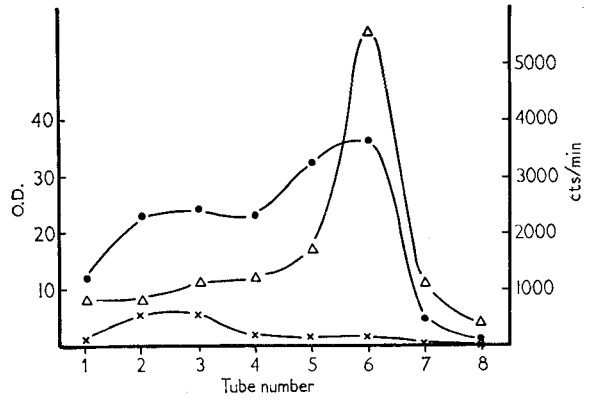

FIG. 2. Cell-free incorporation of [^{14}C]phenylalanine into fractionated ribosomes and stimulation of incorporation by poly-U. Fractionation of ribosomes was performed by sucrose gradient centrifugation (110 min at 23,000 rev./min). Ribosomes were prepared from 8 equal fractions and used for cell-free incorporation. Fraction 6 contains mainly 80 s ribosomes, faster fractions 1 to 5 are rich in aggregated ribosomes.

●—●—●—optical density (O.D.), given as total O.D. at 260 mμ of the fraction in 1 ml.; ×—×—×—incorporation (cts/min) without poly-U; △—△—△—incorporation with poly-U (40 μg/sample).

it is seen that of the order of 30% or more of the optical density residing in the fast fraction is shifted to the 80 s band after treatment with RNase, whereas only a small amount (5% or less) is released as degraded RNA into the slowest fractions.

(c) *Cell-free incubation*

The relatively high activity of the rapidly sedimenting fractions of ribosomes can also be shown by cell-free incubation of fractions of ribosomes. A ribosome solution (about 20 mg in 1·5 ml.) was layered on a sucrose gradient and centrifuged in the

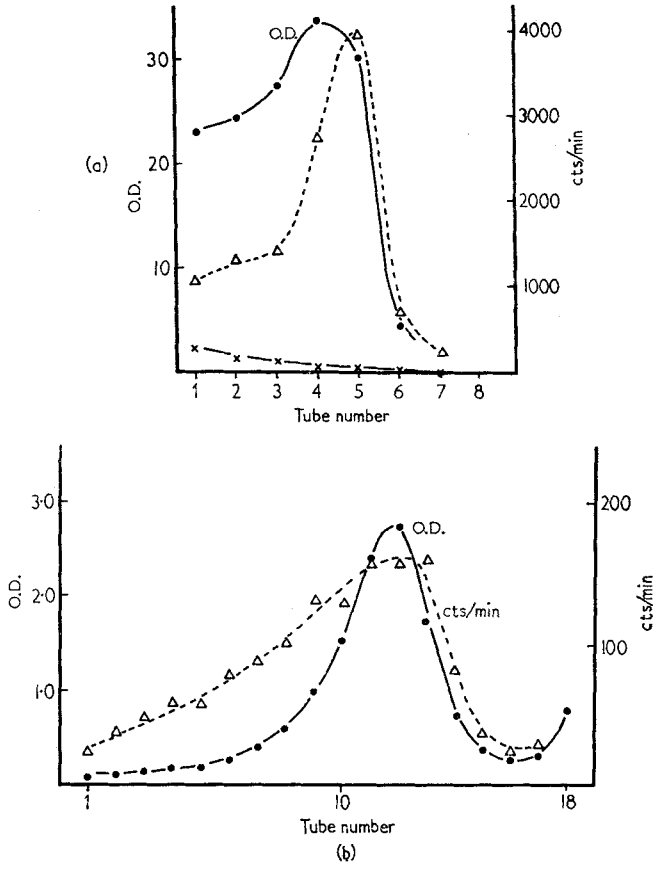

FIG. 3. Cell-free incorporation of [^{14}C]phenylalanine into fractionated ribosomes, followed by sucrose gradient centrifugation. Optical density (O.D.) is given as total O.D. at 260 mμ of the fraction in 1 ml., radioactivity as cts/min.

(a). Fractionation of ribosomes for cell-free incorporation was performed by sucrose gradient centrifugation (220 min at 23,000 rev./min). Ribosomes were prepared from 8 equal fractions and used for cell-free incorporation. The increased time of centrifugation implies that Fig. 3(a) corresponds with only the right half of Fig. 2; thus there is a corresponding increase in resolution. Fraction 6 contains, in addition to some 80 s ribosomes, slower components. Fractions 3 to 5 contain mainly 80 s ribosomes.

●—●—● O.D.; ×—×—× cts/min without poly-U; △—△—△ cts/min with poly-U.

(b) After the incubation with poly-U described in Fig. 3(a), fractions 3 to 5 were pooled, and the ribosomes were reisolated and refractionated by sucrose gradient centrifugation (60 min at 35,000 rev./min). Fractions were collected, measured for O.D. and counted. Scale and position of the diagram (b) were chosen to correspond approximately to the diagram (a) with respect to sedimentation coefficients.

●—●—● O.D.; △—△—△ cts/min.

Spinco SW25 rotor for 110 minutes at 23,000 rev./min. Eight equal fractions were collected in stainless steel tubes and were then centrifuged for 60 minutes at 40,000 rev./min; the ribosomal sediments were dissolved and used for cell-free incubation experiments. Figure 3 gives the result of a cell-free incorporation experiment with [^{14}C]phenylalanine. Similar results were obtained using [^{14}C]valine. Control experiments in the analytical ultracentrifuge showed that the fast fractions (Fig. 2, tube nos. 1 to 3) contained the components of higher sedimentation coefficients, although some decay into 80 s particles had occurred. The slower fractions (tube no. 6) contained almost exclusively 80 s particles. The fast fractions were much more active in causing incorporation of valine or phenylalanine into the cell-free system than the slower 80 s fraction (Figs. 2, 3(a); Table 1).

(d) *Poly-U as messenger RNA*

Specific stimulation of the incorporation of [^{14}C]phenylalanine by poly-U as messenger RNA has been discovered by Nirenberg & Matthaei (1961), using bacterial ribosomes.

TABLE 1

Incorporation of [^{14}C]valine and [^{14}C]phenylalanine into fractionated ribosomes with and without polyuridylic acid.

Fraction no.	1	2	3	4	Unfractionated ribosomes	Mixture without ribosomes
Mg ribosomes per sample	1·64	2·44	2·64	0·46	2·00	0·00
Incubation mixture with:						
[^{14}C]valine without poly-U	665	811	293	68	2,928	23
[^{14}C]valine with poly-U	431	738	258	37	2,542	11
[^{14}C]phenylalanine without poly-U	900	1,114	269	128	3,426	20
[^{14}C]phenylalanine with poly-U	2,105	4,422	7,385	2,835	10,004	238

Fractionation was performed by sucrose gradient centrifugation in the refrigerated Spinco no. SW25 rotor for 110 min at 23,000 rev./min. Four equal fractions were collected from each tube; the ribosomes were isolated by centrifugation, dissolved in medium A and used for cell-free incorporation. Fractions 1 and 2 are rich in fast components (aggregates), fraction 3 contains mainly 80 s ribosomes, fraction 4 contains some 80 s particles and smaller components. The results are given as cts/min.

In the cell-free incorporation system from rabbit reticulocytes, addition of poly-U (40 μg) to an incubation mixture also resulted in stimulation of [^{14}C]phenylalanine incorporation. For unfractionated freshly prepared ribosomes, poly-U causes about a threefold increase in incorporation. No attempts were made to increase this factor by attaining optimum conditions (e.g. by addition of soluble RNA). The purpose of these studies was to compare the stimulating effect of poly-U for various ribosomal fractions.

To establish the specificity of the stimulating effect with respect to phenylalanine, four fractions of ribosomes were collected from the sucrose gradient (110 minutes, at 23,000 rev./min). The ribosomes of each fraction were prepared by centrifugation. Each fraction was split into four equal samples and incubated (with either [^{14}C]valine or [^{14}C]phenylalanine) both with and without poly-U. As shown in Table 1,

there is a strong stimulation of incorporation of [^{14}C]phenylalanine by poly-U whereas no stimulation of the incorporation of [^{14}C]valine occurs.

Figure 2 shows a similar experiment on [^{14}C]phenylalanine incorporation of eight separate sucrose gradient fractions with and without poly-U. As mentioned above, the fast fractions are the most active ones without poly-U. However, with poly-U there is a stimulation of activity, which is relatively low in the fast fractions but shows a very pronounced maximum in the slow fraction corresponding to about 80 s.

It remains undecided whether only the 80 s fraction, or also the smaller components (61 or 43 s, or both together) are stimulated by poly-U. Even a higher resolution of fractions in the sucrose gradient, attained by doubling the time of sucrose gradient centrifugation (220 minutes), did not reveal a difference in specific activity in the slow fractions up to the main 80 s band (Fig. 3(a)).

(e) *Aggregation of ribosomes by poly-U*

If the most active components are the fast fractions of ribosomes and the 80 s ribosomes the most receptive ones with respect to poly-U as messenger RNA, one may ask whether the activity of 80 s ribosomes, after incubation with poly-U, resides in rapidly sedimenting particles. For this purpose, the slow fraction of ribosomes (80 s) was prepared in a sucrose gradient (Fig. 3(a)) and then used for cell-free incorporation with poly-U and [^{14}C]phenylalanine. Thereafter the ribosomes were re-isolated by centrifugation, again layered on a sucrose gradient and centrifuged in the SW39 rotor for 60 minutes at 35,000 rev./min. Fractions were collected and measured for u.v. absorption and radioactivity. The result is shown in Fig. 3(b). Radioactivity does not follow the distribution of optical density. The specific activity of the faster sedimenting fractions is higher than that of the main band of about 80 s. This indicates that some of the 80 s ribosomes, after incubation with poly-U, have formed fast-sedimenting particles which have actively incorporated [^{14}C]phenylalanine.

4. Discussion

(a) *Spectrum of ribosomal aggregates*

The main fraction with a sedimentation coefficient of 80 s is considered as the ribosomal unit analogous to the 70 to 80 s ribosomes of other mammalian cells and bacteria. According to Dintzis *et al.* (1958) this particle has a molecular weight of 4×10^6, of which 50% is RNA and 50% is protein. The two slower fractions (43 and 61 s) are probably subunits of these monomers. All three slow fractions are resistant to the doses of RNase applied.

The spectrum of fast fractions shows components sedimenting with 120, 153, 180, 205 and 220 s. The spectrum disappears upon incubation with RNase, the faster components being more sensitive than the 120 s fraction. At the same time there is a corresponding increase in the amount of 80 s particles. No new fraction much slower than 80 s can be observed and only a few percent of the total RNA is released as low molecular weight material. These findings show that the fast components are aggregates of ribosomal monomers (80 s) linked by RNA.

An interpretation of the fast components in terms of structural changes of the monomer is excluded. The shape of the monomer is approximately spherical, the hydration of the order of 80% of the total volume (Dintzis *et al.*, 1958). The maximum

sedimentation coefficient for the most compact shape of equal molecular weight, an unhydrated sphere, would be about 135 s; this is below the sedimentation coefficients observed. Therefore, the sedimentation coefficients of the fast fractions must be attributed to an increased molecular weight.

One can also exclude the possibility that the fast fractions are 80 s ribosomal monomers with attached messenger RNA. To account for the sedimentation coefficients observed, at least 50% of the total RNA would have to be messenger RNA for the 120 s particles and at least 75% for the 205 s particles. To produce the 80 s particles all messenger RNA would have to be released as low molecular weight material upon treatment with RNase. The experiments show, however, that nearly all the optical density appears in the 80 s fraction after treatment with RNase. Therefore, the fast fractions are considered as aggregates of ribosomal monomers, the linkage being due to RNA. The fastest fractions would correspond to aggregates of at least six ribosomal monomers linked by RNA. Perhaps there occur in the cell even higher aggregates which disintegrate upon preparation of the ribosomes.

The spectrum of sedimentation coefficients observed is consistent with this interpretation. A simplified model would be a linear chain of spherical 80 s particles, approximated by the equivalent ellipsoid. The sedimentation coefficient for the n-fold aggregate would be

$$s_n = 80.\frac{n^{\frac{2}{3}}}{f(n)}$$

where $f(n)$ is the frictional coefficient for an ellipsoid of axial ratio n. These values are (for comparison, experimentally determined sedimentation coefficients are given in parentheses):

$s_2 = 122$ (120); $s_3 = 149$ (153); $s_4 = 171$ (180); $s_5 = 187$ (205); and $s_6 = 200$ (220). The actual values may be somewhat higher because RNA is attached to the 80 s ribosomes and because the higher aggregates may not be straight chains. In agreement with these expectations, the experimental values agree closely with the linear chain model for the smaller aggregates ($n = 2$ to 3) and are somewhat higher for $n = 4$ to 6.

(b) *Function of ribosomal aggregates in protein synthesis*

The following conclusions appear to be relevant to the mechanism of protein synthesis.

1. Ribosomal monomers are linked into aggregates up to six or more units. The linkage is dependent on RNA.

2. The aggregates are more active in protein synthesis than the monomers, both in intact cells and in the cell-free system, with respect both to absolute and to specific activity.

3. Complementary to this finding, the monomers (or their subunits) are more active than the aggregates in accepting poly-U as messenger RNA to incorporate [^{14}C]phenylalanine.

4. After cell-free incorporation of the monomers with [^{14}C]phenylalanine and poly-U, part of the radioactivity seems to reside in aggregated ribosomes. Thus, poly-U as messenger RNA seems to be able to link ribosomal units into aggregates.

Thus we find that active aggregates of ribosomes linked by RNA seem to contain messenger RNA and less active monomers (or their subunits) seem to accept messenger RNA (of course, it is not excluded that a small fraction of the monomers also

contains messenger RNA). Many complicated assumptions can be made to account for these findings, e.g. that there is a secondary effect of messenger RNA on the aggregation properties of ribosomes. The most simple and likely hypothesis would be, however, that the messenger RNA itself is linking the ribosomal units.

(c) *Models of protein synthesis*

These results have some bearing on the elementary process of protein synthesis; the extension of a growing peptide chain by one amino acid, the nature of which is determined by a small group of nucleotides of the messenger RNA. This process is known to require a ribosomal particle as the site of protein synthesis. Two different models of the mechanism of ribosomal function may be discussed (Fig. 4).

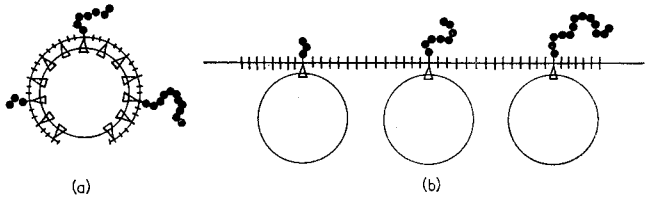

FIG. 4. Two extreme models of protein synthesis. The schematic diagrams are only to illustrate the text, not to postulate details of the mechanism (soluble RNA is omitted from the diagram). Large circles represent ribosomes, △ their active sites, ++++++++++ messenger RNA, ●●●●● the growing peptide chain.

(a) Static attachment of messenger RNA to the ribosome. Growth of the peptide chain is accompanied by shifting the point of growth from one active site on the ribosome to the next. Several peptide chains could grow simultaneously on each ribosome.

(b) Dynamic relation between ribosome and messenger RNA. Growth of the peptide chain is accompanied by shifting the active site of the ribosome from one coding group of nucleotides of the messenger RNA to the next. Several ribosomes could operate simultaneously on each molecule of messenger RNA.

1. Messenger RNA is statically linked to the ribosomal surface. Only one ribosome would operate on one molecule of messenger RNA. The ribosome would contain as many sites where a peptide bond could be formed as there are amino acids in the peptide chain. The growth of the peptide chain by one amino acid is accompanied by a shift of the point of growth from one site on this surface, and therefore from one group of nucleotides of the messenger RNA, to the next. Since the growing peptide needs to be attached to the ribosome only at the point of growth, there would be no *a priori* reason why several peptides could not grow simultaneously on the same ribosome.

2. Messenger RNA is not statically linked to the ribosome but is attached by a small group of nucleotides to the ribosomal surface. There is only one site of synthesis (or possibly very few sites) on the ribosome to which the peptide chain can be attached with its point of growth. The messenger RNA is shifted across this site from one coding group of nucleotides to the next whenever the growing peptide chain is extended by one amino acid. In this model, only one peptide can grow on each ribosome but several ribosomes can operate simultaneously on one molecule of messenger RNA.

Model 1 is more economic with respect to ribosomes, model 2 with respect to messenger RNA. Model 2 allows for a complicated structure of the active site of the

ribosome because the site occurs only once within the large particle. One should realize that many hypotheses can be made by combining properties of the extreme models 1 and 2.

The results on reticulocyte ribosomes described above demonstrate the role of aggregates of ribosomal units linked by RNA in protein synthesis and suggest that the linking RNA is messenger RNA. Thus they support model 2, with several ribosomes operating on one messenger RNA molecule.

Possibly one messenger RNA molecule can consist of several sections each of which codes for a different peptide chain. It remains undecided whether several ribosomes can simultaneously operate on the same section although there is no *a priori* reason against this. One may attempt to correlate the activity of higher aggregates in protein synthesis of reticulocytes with the fact that the main product of protein synthesis, the haemoglobin molecule, contains four peptide chains per molecule. Thus it may be assumed that four ribosomes have to be assembled to produce and to release a haemoglobin molecule. It is unlikely, however, that the function of the aggregates can be explained solely on this basis because aggregates up to at least six ribosomal monomers seem to occur in appreciable amounts.

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