Evidence for Heterogeneity of Ribosomes within the HeLa Cell*

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SUMMARY
The proteins of several subsets of ribosomal subunits isolated from HeLa cells were separated by high resolution electrophoresis in polyacrylamide slab gels containing sodium dodecyl sulfate. Comparisons were made among native subunits, and subunits derived from single ribosomes, free polysomes, and membrane-bound polysomes. At least 10 heterogeneously distributed proteins were identified among the 40 S subunit proteins, compared with 17 to 20 proteins that occurred in all 40 S subunit classes. All 60 S subunits shared 34 separable proteins. One protein was missing only from membrane-bound 60 S subunits and another protein was present only in membrane-bound 60 S subunits. It is suggested that these two proteins regulate the binding of 60 S subunits to the endoplasmic reticulum.

One of the major unresolved problems about the nature of ribosomes is the question of heterogeneity versus homogeneity. In bacteria, the existence of "fractional" ribosomal proteins (i.e., those not present on every ribosome) has been interpreted as evidence of dynamic heterogeneity, which may reflect distinct steps in the protein synthesis process (1, 2). Other types of evidence suggest bacterial ribosomes may be functionally differentiated, intracellularly (3, 4) or in different growth states (40). In eukaryotes, a wide variety of observations have pointed to the same possibilities (5-14), but there is no direct rigorous proof of heterogeneous ribosomes in a single eukaryotic organism as yet, although the existence of fractional proteins has been suggested (15, 16).

Our interest in the biosynthesis of mammalian ribosomes has involved us in a long-term study of ribosomal proteins in HeLa cells. Although the cell populations are homogeneous, several subsets of intracellular ribosomes are well known. These are native subunits, single ribosomes, free polysomes, and bound (membrane-associated) polysomes. We thought it advisable to make a general comparison of the ribosomal proteins of the subunits derived from each of these four categories, before proceeding to a more quantitative study of the individual proteins. Evidence for intracellular heterogeneity of ribosomes in terms of their protein components is presented in this report.

EXPERIMENTAL PROCEDURE
Cell Cultures and Growth Conditions—HeLa-S3 cells were maintained in spinner culture in Eagle's minimal essential medium supplemented with penicillin-streptomycin and 5% calf serum. Cultures were gassed with a mixture of 5% CO2-95% air and diluted daily with fresh prewarmed medium to a concentration of 1.5 to 2.0 X 10^6 cells per ml. Cell growth was monitored with a Zeiss spectrophotometer at 700 nm.

Buffers—Buffer designations and concentrations are as follows:
Buffer A, 0.01 M Tris-HCl, pH 7.1, at 25°, 0.01 M KCl, 0.00015 M MgCl2; Buffer B, 0.01 M Tris-HCl, pH 7.1, at 25°, 0.0001 M MgCl2; Buffer C, 0.01 M Tris-HCl, pH 7.1, at 25°; Buffer D, 0.05 M triethanolamine-HCl, pH 7.5, at 25°, 0.50 M KCl, 0.003 M MgCl2; saline wash, 0.13 M NaCl, 0.005 M KCl, 0.001 M MgCl2.

Cell Fractionation—Cells were pelleted at 800 rpm for 5 min and resuspended in fresh prewarmed medium with 10% calf serum at 3 to 4 X 10^6 cells per ml for 1 hour. This step served to enrich the polysome fractions. All further steps were carried out at 4° according to a previously published technique for homogenization and preliminary fractionation of HeLa cells (17).

Cells were pelleted and washed three times with saline wash solution and resuspended in approximately 6 volumes of Buffer A. The cells were allowed to swell 2 to 5 min and then were broken in a Dounce homogenizer with 5 strokes of the A pestle (loose fitting) and 20 strokes of the B pestle (tight fitting). Sucrose (1.25 M) in Buffer A was added to a final concentration of 0.25 M sucrose to preserve nuclei. The homogenate was spun at 1100 X g for 3 min to remove whole cells and nuclei. Under these conditions approximately 85 to 95% of the cells were broken with no visibly lysed nuclei.

The postnuclear supernatant was spun at 8,100 X g for 10 min to pellet the crude membrane fraction, which was then resuspended in 0.25 M sucrose in Buffer B (one-half the volume of the original homogenate) and recenterfuged at 1,100 X g for 2 min. The supernatant was centrifuged a second time at 8,100 X g for 10 min. This pellet was resuspended in 4 ml of 0.25 M sucrose in Buffer C to which was added 1 ml of 5% sodium deoxycholate in 0.25 M sucrose in buffer C. This solubilized the majority of bound polysomes from the membrane fraction. The solution was centrifuged at 15,800 X g for 20 min to remove the particulate membrane components. The supernatant was then layered onto 33-ml linear 10 to 30% sucrose gradients made in Buffer A. The gradients were centrifuged in an SW 27 rotor (Beckman) for 18 hours at 14,900 rpm at 4°. Absorbance at 260 nm was monitored by pumping the gradients through a flow cell in a Gilford spectrophotometer during fractionation.
Free polysomes, single ribosomes, and native subunits were prepared from the supernatant of the first 8,100 × g centrifugation described above. This supernatant, containing no detergents, was freed of rapidly sedimenting material by centrifugation at 15,800 × g for 20 min, and was then layered onto sucrose gradients and fractionated as described for polysomes derived from the membrane fraction. In both cases, polysomes were pelleted to the bottom of the sucrose gradient tubes. Fractions of the gradients that contained single ribosomes or native subunits were pooled (Fig. 1A), and the particles were sedimented by overnight centrifugation at 4°C (Fig. 1B).

High Salt and Puromycin Treatment of Polysome Fractions—Single ribosomes and native 40 S and 60 S subunits pelleted from Buffer D or centrifuged in an SW 27 rotor for 15 hours at 17,500 rpm, at 4°C (Fig. 1R).

**High Salt Treatment of Native Ribosomes and Subunits**—Single ribosomes and native 40 S and 60 S subunits pelleted from Buffer A sucrose gradients were resuspended in Buffer A and each centrifuged individually through identical 33-ml 10 to 30% linear sucrose gradients made in Buffer A. This second gradient served to purify the individual ribosomal fractions from cross-contamination with the other fractions.

Following gradient fractionation and pelleting of pooled peak fractions, individual pellets were resuspended in Buffer D and layered onto high salt sucrose gradients identical with those used for puromycin-treated polysomes. Fractions containing subunits were collected and pelleted as above.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis**—Electrophoresis was performed in polyacrylamide gels containing sodium dodecyl sulfate according to Laemmli (19). Gels were formed as slabs 0.5 cm thick, 6 inches wide, and 3½ inches high; electrophoresis was carried out in an apparatus designed by Reid and Bieleni (20).

Separation gels containing 14%, 10%, 18%, and 20% acrylamide were prepared from a stock solution containing 30 g of acrylamide and 0.8 g of N,N'-methylenebisacrylamide made up to 100 ml with water (called "30% acrylamide"). The final concentrations of other components in the separation gels were 0.375 M Tris-HCl, pH 8.8, at 25°C, and 0.1% sodium dodecyl sulfate.

The gels were polymerized by the addition of 10% ammonium persulfate (w/v) to a final concentration of 0.5% (w/v) and TEMED to a final concentration of 0.05% (v/v). Following the mixing of all components the final gel solution was degassed and poured into the gel slab apparatus. Water was then gently layered over the gel solution with a syringe to provide a flat gel surface. Polymerization was allowed to proceed overnight. Stacking gels containing 4.5% acrylamide, 0.12% bisacrylamide, 0.125 M Tris-HCl, pH 8.8, at 25°C, and 0.1% dodecyl sulfate, height 1 cm, were prepared. Polymerization was initiated by the addition of ammonium persulfate to 0.337% (w/v) final concentration and TEMED to 0.11% (w/v) final concentration. Polymerization of stacking gel was allowed to proceed for 18 min at room temperature; then the plastic comb used to form the sample wells was removed. The electrode tank buffer (pH 8.3 ± 0.1) contained 0.025 M Tris, 0.192 M glycine, and 0.1% dodecyl sulfate.

Whole ribosome subunits were dissolved directly in sample buffer to a final concentration of 1 mg per ml. Sample buffer contained the following concentrations: 0.0625 M Tris-HCl, pH 6.8, at 25°C, 2.5% dodecyl sulfate, 10% glycerol, and 5% 2-mercaptoethanol. The ribosomal proteins then underwent electrophoresis individually to determine the location of small contaminating bands, then a stock solution of each marker was run on all gels in parallel to the ribosomal protein samples. Standard curves were plotted for each gel as a control against irregularities between gels (Fig. 2).

Values of relative mobility (Rf) were plotted against molecular weight on semilog paper. We assigned myoglobin an arbitrary
results are similar to those reported by Neville (22), who used another type of dodecyl sulfate gel system including a stacking gel. The relationship between $R_F$ and molecular weight is clearly different for proteins above molecular weight 40,000 and those below 30,000. In the transition area of 30,000 to 40,000 the curve is somewhat uncertain, but reproducibility of results has been very good.

RESULTS

Purification of Subunits—HeLa cells growing in fresh medium have very few single ribosomes (Fig. 1A). Even so, a second low salt sucrose gradient was usually necessary to separate native subunits completely from each other and from single ribosomes. The final stage, sucrose gradients containing 0.5 M KCl, produces very homogeneous peaks (Fig. 1B). Cross-contamination of the 40 S subunits by co-sedimenting forms of the 60 S particle, and vice versa, has been found to be undetectable by CsCl gradient analysis (not shown) and by the absence of characteristic proteins on the electropherograms (see below).

Proteins Common to All 40 S Subunits—All subunits were subjected to dodecyl sulfate gel electrophoresis as described under "Experimental Procedures," with the use of 14%, 16%, 18%, and 20% acrylamide in the gels. This permitted us to detect several components that separate poorly or not at all in gels of one concentration, but are clearly resolved in gels of a different concentration. We find 17 electrophoretically identifiable components in all 40 S subunits, regardless of the cell fraction from which the subunits were obtained. An example of a complete gel pattern of 40 S subunit protein is given in Fig. 3. We shall refer to these 17 components as "primary ribosomal proteins" because of their ubiquity.

Molecular weights of the 40 S subunit proteins are summarized in Table I. The range of molecular weights represented by the 17 primary proteins is 17,000 to 33,700, which agrees well with the measurements of Bickle and Traut (23) on mouse cell ribosomal proteins. Two other features of the data in Table I are worth noting. First, the molecular weight estimates were remarkably reproducible, as indicated by the narrow range of values found for all components. Even in the range of 30,000 to 40,000, where the slope of the calibration curve changes radically (Fig. 2), reproducibility was excellent. Absolute errors of measurement appear to be more likely in this molecular weight range than relative errors. Secondly, the resolving power of this gel electrophoresis technique is very high. Components 1 and 2, for example, differ by at least five amino acids, but they are almost completely separated. Components 13 and 14 or components 15 and 16 differ by only three to four amino acids out of a total of more than 250 amino acids, but they are distinguishable. We conclude that very few proteins can have escaped detection in this survey, although the number is probably not zero (see "Discussion").

Proteins That Have Heterogeneous 40 S Subunit Distributions—The simplest gel pattern is shown in Fig. 3. It is characteristic of 40 S subunits from single ribosomes. The two small bumps just above the 40,000 mark occur frequently, but are extremely variable. Otherwise, there are no protein components that distinguish this class of 40 S subunits. Instead, the single ribosome 40 S subunits are characterized by the simplicity of the gel pattern in the high molecular weight range; the densitometer tracing is literally at the baseline level almost everywhere above 33,000.

All four types of 40 S subunits give exactly the same gel pattern for components 1 to 11. However, the gel pattern of the native 40 S subunit proteins contrasts strikingly (Fig. 4A) with the
single ribosome pattern in the high molecular weight region. Numerous proteins larger than component 17 are present. These vary from one preparation to other, and therefore have not been assigned numbers. None is quantitatively significant individually, but the total constitute a substantial fraction of the protein in the sample. We suspect, although we cannot prove, that this heterogeneous group of proteins consists mostly of co-sedimenting nonribosomal materials. Native subunits are collected from a low salt sucrose gradient, concentrated by centrifugation, and sedimented into another sucrose gradient containing 0.5 M KCl. It is also unlikely that they represent contaminants and then changing to a 40 S sedimentation rate upon exposure to 0.5 M KCl. Some or all of components 16a to 16d might be that extra protein.

The free polysome 40 S profiles (Fig. 4B) also show a large number of high molecular weight components, none of which can definitely be identified with the components of the 40 S native subunit profile. Four small peaks, designated 17a to 17d, occur regularly in the free polysome 40 S profiles; all higher molecular weight peaks occur erratically.

The origin of the variable high molecular weight proteins cannot easily be ascribed to co-sedimenting contaminants in this case, which would have to combine the unusual properties of sedimenting with polysomes through a low-salt sucrose gradient and then changing to a 40 S sedimentation rate upon exposure to 0.5 M KCl. It is also unlikely that they represent contaminants adsorbed during homogenization, because they do not occur with single ribosome 40 S particles. We suggest that small amounts of elongation factors, aminoaacyl-tRNA synthetases, and other proteins associated with active ribosomes are attached to the free polysome 40 S in such a way that they resist our usual purification procedure. This group of high molecular weight contaminants may be partly responsible for the apparent difference in protein content between eukaryotic and prokaryotic small ribosomal subunits (31).

Two additional components should be noted in the free polysome 40 S subunit profile; these are designated 11a and 12a. They do not occur in the 40 S native subunit or single ribosome

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### Table I

Molecular weights of proteins from 40 S subunits

The numbers given for average molecular weights and range of molecular weights refer to all classes of 40 S subunits and to data obtained from 14%, 16%, 18%, and 20% sodium dodecyl sulfate-polyacrylamide gels as described under “Experimental Procedures.”

| Protein | Molecular Weights | Occurrence in Subunit Classes |
|---------|------------------|------------------------------|
| Protein | Average | Range | NS 40S | SR 40S | FP 40S | BP 40S |
| 1       | 17,000 | 16,500-17,500 | + | + | + | + |
| 2       | 17,500 | 17,000-17,800 | + | + | + | + |
| 3       | 17,900 | 17,800-18,000 | + | + | + | + |
| 4       | 18,200 | 18,000-18,400 | + | + | + | + |
| 5       | 18,600 | 18,400-18,800 | + | + | + | + |
| 6       | 19,100 | 18,700-20,000 | + | + | + | + |
| 7       | 19,600 | 19,000-20,000 | + | + | + | + |
| 8       | 20,400 | 19,700-20,000 | + | + | + | + |
| 9       | 23,700 | 23,100-24,300 | + | + | + | + |
| 10      | 24,400 | 24,200-24,700 | + | + | + | + |
| 11      | 25,200 | 25,000-25,500 | + | + | + | + |
| 11a     | 26,100 | 25,400-26,600 | - | + | + | + |
| 12      | 26,800 | 26,000-27,200 | + | + | + | + |
| 12a     | 27,500 | 26,800-27,800 | - | + | + | + |
| 13      | 27,800 | 27,000-28,500 | + | + | + | + |
| 14      | 28,400 | 28,200-28,900 | + | + | + | + |
| 15      | 29,300 | 29,000-29,700 | + | + | + | + |
| 16      | 29,600 | 28,600-30,200 | + | + | + | + |
| 16a     | 30,400 | 30,000-30,900 | + | - | - | - |
| 16b     | 31,000 | 30,700-31,500 | + | - | - | - |
| 16c     | 31,800 | 31,500-32,200 | + | - | - | - |
| 16d     | 32,600 | 32,000-33,100 | + | - | - | - |
| 17      | 33,700 | 33,000-34,700 | + | + | + | + |
| 17a     | 35,500 | 34,900-36,500 | + | - | - | - |
| 17b     | 37,600 | 36,800-38,800 | - | - | - | - |
| 17c     | 38,900 | 37,100-42,000 | + | - | - | - |
| 17d     | 40,700 | 37,800-44,000 | - | - | - | - |
40 S subunit patterns, but they are present in subunits from bound polysomes (Fig. 4C). Thus, they may be proteins found only on ribosomes that were active in protein synthesis at the time the cells were harvested. It should be noted that components 11a and 12a cannot be dismissed on the grounds of quantitative insignificance, although they appear as minor shoulders on peaks 12 and 13, respectively. When proteins overlap like this, it is impossible to estimate how much of a compound peak is due to any individual component. A different separation technique would be required to settle this question.

The pattern for 40 S subunits from bound polysomes (Fig. 4C) differs conspicuously from the free polysome 40 S pattern in the absence of nearly all the proteins of molecular weight greater than 35,000. This is actually a consequence of technique. If free polysomes are treated with deoxycholate in the same manner as the bound polysomes, the protein patterns subsequently obtained from the 40 S subunits are essentially the same as those for the bound polysome 40 S particles (not illustrated). Apparently the detergent treatment makes the purification procedure more rigorous. It is worth noting, however, that the occurrence and proportions of the primary 17 proteins, plus 11a and 12a, are indistinguishable in the bound and free polysome 40 S subunits, whether the latter are treated with detergent or not (not shown). Thus this group of proteins is resistant to deoxycholate, which strengthens the evidence that they are genuine ribosomal proteins.

The occurrence of component 17c in the 40 S pattern from bound polysomes is reproducible, but its significance is unknown.

60 S Particle—All four classes of 60 S particles gave identical protein patterns on dodecyl sulfate gels, with one exception described below. Therefore, we have illustrated only one complete pattern, that of free polysome 60 S particles (Fig. 5). We find 35 regularly occurring components, in good agreement with the results obtained by two-dimensional electrophoresis of rat liver 60 S ribosomal proteins by Sherton and Wool (32) and others (8, 9, 33, 34). The uniformity of this pattern implies that none of the numbered proteins is a co-sedimenting contaminant, but it does not rule out the possibility that some of these proteins are ubiquitous, adsorbed contaminants that are resistant to 0.5 M KCl. If this occurs, it cannot involve many proteins. For the present, we shall consider all 35 components as "primary" proteins of the large subunit.

The sole evidence of heterogeneity among 60 S subunits that we have found is illustrated in Fig. 6. The particles from bound polysomes lack component 35, and contain a component, 31a, not found elsewhere. The illustration shows tracings obtained from 14% acrylamide gel patterns, which present the difference most clearly. Components 32 and 34 are not distinguished on such gels; we are not certain whether they occur in bound polysome 60 S subunits. We have demonstrated, however, that treatment of free polysome 60 S subunits with deoxycholate does not alter the gel pattern of the proteins in any detectable way (not illustrated). In particular, components 32 to 35 remain in exactly the proportions illustrated in Fig. 5, and component 31a does not appear.

Molecular weights of the 60 S subunit proteins are given in Table II. The sensitivity and reproducibility of the electrophoretic separations are again evident. Components 1 to 31

Fig. 4. Differences in the proteins of 40 S particles from native subunits (NS, Fig. 4A), free polysomes (FP, Fig. 4B), and bound polysomes (BP, Fig. 4C). All three panels show densitometer tracings from the same 16% polyacrylamide gel slab. Only the higher molecular weight proteins are shown; no differences in components 1 to 11 were observed.

Fig. 5. Proteins of 60 S subunits from free polysomes (FP). A densitometer tracing of a 20% polyacrylamide gel is shown.
FIG. 6. Differences in the proteins of 60 S subunits from free polysomes (FP, Fig. 6A) and bound polysomes (BP, Fig. 6B). Densitometer tracings from a 14% polyacrylamide gel slab are shown.

require no special comment. Components 31a to 35 behaved in an unusual manner; their apparent molecular weights increased as the concentration of acrylamide in the separation gel increased. Component 31a, for example, migrated in 14% gels as though its molecular weight were 36,400, in contrast to the estimate of 43,500 obtained from 20% gels, given in Table II. The comparable numbers for component 35 were 41,000 in 14% gels and 58,800 in 20% gels. This type of electrophoretic behavior suggests some unusual feature of the composition of these proteins (22), and this possibility is considered under “Discussion.”

SECTION 2
Molecular Weights of Proteins from 60 S Subunits

The numbers given for average molecular weights and range of molecular weights refer to all classes of 60 S subunits and to data obtained from 14%, 16%, 18%, and 20% sodium dodecyl sulfate-polyacrylamide gels as described under “Experimental Procedures.” The exception is that only data from 20% gels are given for the proteins marked with an asterisk (see text).

| Protein | Average | Range |
|---------|---------|-------|
| 1       | 15,100  | 15,000-15,200 |
| 2       | 15,900  | 15,800-16,000 |
| 3       | 16,700  | 16,600-16,800 |
| 4       | 17,400  | 17,300-17,500 |
| 5       | 17,900  | 17,700-18,100 |
| 6       | 18,400  | 18,300-18,600 |
| 7       | 19,100  | 19,000-19,200 |
| 8       | 19,400  | 19,300-19,600 |
| 9       | 19,800  | 19,700-19,900 |
| 10      | 20,200  | 20,100-20,300 |
| 11      | 21,100  | 21,000-21,300 |
| 12      | 21,500  | 21,400-21,700 |
| 13      | 21,900  | 21,700-22,000 |
| 14      | 22,600  | 22,500-22,700 |
| 15      | 23,100  | 23,000-23,200 |
| 16      | 23,500  | 23,400-23,600 |
| 17      | 24,300  | 24,200-24,400 |
| 18      | 24,600  | 24,500-24,800 |
| 19      | 25,300  | 25,200-25,400 |
| 20      | 25,700  | 25,600-25,800 |
| 21      | 26,200  | 26,100-26,500 |
| 22      | 26,800  | 26,700-26,900 |
| 23      | 27,300  | 27,200-27,500 |
| 24      | 28,100  | 27,900-28,200 |
| 25      | 29,000  | 28,900-29,300 |
| 26      | 29,100  | 29,000-29,300 |
| 27      | 29,600  | 29,500-29,900 |
| 28      | 30,100  | 29,900-30,400 |
| 29      | 31,700  | 31,500-31,900 |
| 30      | 33,100  | 33,000-33,500 |
| 31      | 33,400  | 33,300-33,500 |
| 31a     | 43,500* | 43,400-43,900 |
| 32      | 45,200* | 45,100-46,000 |
| 33      | 48,500* | 48,400-51,000 |
| 34      | 52,700* | 51,300-54,000 |
| 35      | 58,800* | 57,000-60,500 |

DISCUSSION

We have compared the proteins found in four major intracellular subsets of ribosomes: native subunits, single ribosomes, free polysomes, and membrane-associated ("bound") polysomes. Seventeen electrophoretically separable components are found in all four classes of 40 S subunits, and these are designated "primary" ribosomal proteins. It is probable that several of the electrophoretic components include more than one protein (e.g., 5, 7, and 15). Thus, there are approximately 20 primary proteins in the HeLa cell small subunit, essentially the same as the number of Escherichia coli 30 S subunit proteins (1, 35).

All classes of 40 S subunits are not identical, however. Co-sedimenting or loosely bound "contaminants" complicate the patterns for the native and free polysome subunits. These may be, in part, remnants of salt-sensitive populations of proteins functionally associated with ribosomes, such as initiation and elongation factors. A more distinctive type of heterogeneity is represented by a variety of minor components, such as 16a to 16d in native 40 S subunits, 17a to 17d in free polysome 40 S particles, and 11a and 12a in both free polysome and bound polysome 40 S particles. The functional significance of these components cannot be assessed at present, but operationally they show that the four classes of ribosomal subunits studied here are distinguishable in terms of their minor proteins. It should also be noted that the total number of 40 S subunit proteins revealed by this analysis is approximately 30 (17 to 20 primary proteins and 10 heterogeneously distributed proteins), which agrees well with two-dimensional electrophoretic studies (e.g., Ref. 32).
In this connection, we would like to point out that the choice between one- or two-dimensional electrophoretic separation of ribosomal proteins inevitably leads to a compromise. Two-dimensional analyses can separate proteins of identical or nearly identical molecular weight very well, but accurate quantitation of the amount of protein in spots on two-dimensional electropherograms is not simple, and molecular weight measurements are not precise. The one-dimensional technique that we have employed is sensitive to molecular weight differences of less than 2% (Tables I and II), and roughly quantitative estimates of the amount of each protein can be easily obtained by densitometric tracing of stained gels. The occurrence of some minor components is more easily detected with the one-dimensional technique because of the sharpness of the bands.

Another reason that led us to prefer one-dimensional sodium dodecyl sulfate gel electrophoresis was its insensitivity to certain modifications of proteins, such as acetylation (36) and phosphorylation (37, 38), which might lead to separate spots on two-dimensional analyses. Our immediate goal was to search for major protein differences among the various classes of subunits. The study of protein modifications is important, but we did not wish to complicate our initial survey by including it.

In the case of the 60S subunits, we have found one obvious example of heterogeneity: component 31a, which is found only in subunits from membrane-bound polysomes, and which is always associated with the absence of component 35. It is possible that these proteins function in the attachment of 60S subunits to the endoplasmic reticulum. This possibility is increased by some evidence that components 31a, 33, and 35 are glycoproteins (components 32 and 34 are too minor to analyze). First, these proteins stain with the periodic acid–Schiff reagent. The reaction is weak, and we have not yet quantitated it. Second, the apparent molecular weight of components 31a, 33, and 35 varies greatly with the concentration of acrylamide used for the separation gel. The presence of sugars attached to the proteins might cause variable electrophoretic behavior. None of the other proteins from either subunit exhibits such behavior.

Differences in at least one protein component of membrane-bound versus free ribosomes have been reported for bacteria (3), chickens (5), and mammals (6, 14). Trivial explanations can be advanced to account for all these observations, including our own, of course. However, we would like to suggest the possibility that, in HeLa cells, component 35 prevents attachment of 60S subunits to the endoplasmic reticulum, while component 31a allows attachment and that both proteins cannot occupy the same subunit at the same time. This hypothesis should be testable.

In this connection, it is interesting to note that Borgese et al. (14) have observed proteins from rat liver free polysomes clearly homologous to our components 33 and 35, and that component 35 is missing from bound polysomes. They did not observe component 31a in 60S subunits from bound polysomes, but this was probably a consequence of technique. Borgese et al. removed ribosomes from the endoplasmic reticulum with a high concentration of KCl, whereas we used sodium deoxycholate. If component 31a actually links 60S subunits to the endoplasmic reticulum, it may remain with the membranes when the subunits are removed by KCl, but remain with the subunits when the membranes are destroyed with detergent.

Two classes of membrane-associated ribosomes have been described from HeLa cells; one is removed from membranes by EDTA or RNase, the other is not (39). The fact that component 35 is completely absent from bound 60S subunits implies that the preparation of bound polysomes is not contaminated significantly with free polysomes, which might have been trapped in the low speed pellet.

All of the above discussion has dealt with qualitative heterogeneity, i.e. the presence or absence of specific bands in specific classes of subunits. Qualitative heterogeneity involving E. coli ribosomal proteins S21, L2, and L20 has been reported by Bickle et al. (4), who carried out a comparison similar to ours. It is also possible that quantitative heterogeneity exists. Deussel (40) has shown that the relative amounts of the "fractional" proteins S6, S21, and L12 are 2- to 3-fold higher in E. coli grown in rich medium than in E. coli grown in minimal medium. We should have been able to detect differences of this magnitude if they occurred in one or more of the ribosomal subunit populations that we examined. It would not have been surprising, for example, to find that inactive single ribosomes have some conspicuous differences of a quantitative nature, when compared to free polysomes, but none was observed. Single ribosomes are known to be more highly phosphorylated than polysomes, but this does not appear to be correlated with detectable difference in protein content (41). Perhaps mammalian ribosomes accomplish, by phosphorylation on other subtle modifications, the functional flexibility suggested by the existence of fractional proteins in bacterial ribosomes.

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