Relative Stoichiometry of Ribosomal Proteins in HeLa Cell Nucleoli

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Total protein was released from isolated HeLa cell nucleoli by guanidine hydrochloride, purified by cesium chloride density gradient centrifugation, and analyzed by two-dimensional polyacrylamide gel electrophoresis. Conditions of electrophoresis restricted attention to proteins that are positively charged at pH 8.6. Most of the major nucleolar protein spots co-electrophoresed with ribosomal proteins; the majority of ribosomal proteins from both the large and small ribosomal subunits were represented. Several proteins found in association with polysomes but not on ribosomal subunits and several proteins unique to the nucleolus were also identified in these nucleolar protein patterns.

In order to determine whether the ribosomal proteins found in the nucleolus represented sizable pools of ribosomal proteins, or merely ribosomal proteins contained in the preribosomal particles, [35S]methionine-labeled nucleoli were mixed with [3H]methionine-labeled polysomes. From analysis of isotopic ratios in individual protein spots it was possible to determine the stoichiometry of individual ribosomal proteins in the nucleolus relative to their complement on cytoplasmic ribosomes. All but a few proteins exhibited relative nucleolar stoichiometry values of approximately one, indicating that there are not significant pools of most ribosomal proteins in isolated nucleoli.

The rate of ribosome synthesis in HeLa cells appears to be closely coordinated with the rate of ribosomal protein synthesis. Inhibition of protein synthesis by a variety of means results in a reduction in the rate of ribosomal RNA synthesis and RNA maturation (1-3). It has been suggested that the supply of one or more ribosomal proteins may limit the overall rate of ribosome synthesis (2, 3). However, it has been established that some ribosome synthesis continues even if protein synthesis is inhibited by more than 99% (4-6). This has been interpreted as indirect evidence for the existence of cellular pools of the essential ribosomal proteins (2, 4, 5). In response to inhibition of RNA synthesis by more than 99% (6-8). This has been interpreted as indirect evidence for the existence of cellular pools of the essential ribosomal proteins. Consequently, if pools of newly synthesized ribosomal proteins exist, they should be found bound to nucleoli isolated in the presence of 0.5 M NaCl (7). Consequently, if pools of newly synthesized ribosomal proteins exist, they should be found bound to nucleoli isolated under these conditions. This paper describes experiments which measure the amounts of individual ribosomal proteins in isolated nucleoli relative to their stoichiometry on cytoplasmic ribosomes. It is found that only one ribosomal protein, L5, has a large nucleolar pool.

EXPERIMENTAL PROCEDURES

Cell Culture and Labeling—HeLa S3 cells were grown in spinner culture in Eagle’s Minimal Essential Medium (Grand Island Biological Co., F14) containing 10% calf serum. Cell concentration was maintained at 2 x 10⁴ to 5 x 10⁴ cells/ml. RNA was labeled by supplementing the culture medium with [3H]uridine (New England Nuclear) and adjusting total uridine concentration to 10⁻³ M. Protein was labeled by resuspending cells in fresh medium containing one-half (5.5 µg/ml) the normal methionine concentration, supplemented with [3H]methionine (New England Nuclear) or [35S]methionine (American/Seakle).

Cell Fractionation—Cells were harvested by pouring cultures over frozen 0.14 M NaCl and centrifuging the chilled cell suspension for 5 min at 150 x g (average). This and all subsequent steps were carried out at 0 to 4°C. Cells were fractionated into cytoplasmic, nucleoplasmic, and nucleolar portions by a modification of the method of Penman et al. (8). Cells were rinsed twice with 0.14 M NaCl and homogenized in 10 mM Tris-HCl, pH 7.4, at 0°C, 10 mM KCl, 1 mM MgCl₂, and 1 mM dithiothreitol (homogenization buffer), using 10 ml of buffer/g wet weight of cell pellet. One-ninth volume of 3 M KCl/20 mM MgCl₂ was added slowly with constant stirring, and nuclei were collected by centrifugation for 5 min at 500 x g. The supernatant was termed crude cytoplasm. Nuclei were resuspended by gentle homogenization in one-half the previous volume of homogenization buffer. One-twentieth volume of 10% (w/v) Brij 58 and 1/10 volume of 10% (w/v) sodium deoxycholate were added sequentially with constant stirring, and the nuclei were immediately collected by centrifugation for 5 min at 500 x g. The resulting detergent-washed nuclei were disrupted by gentle homogenization in 0.5 M NaCl, 50 mM MgCl₂, and 10 mM Tris-HCl, pH 7.4, using 10 ml/g original cell pellet. DNase (Sigma DN-EP) was added to 50 µg/ml, and the viscosity was reduced by incubation at 0°C for 10 to 20 min. At this point aliquots of the nuclear lysate were removed for alkali-labile absorbance measurements (described under "Quantitation of RNA"). Nucleoli were collected from these aliquots and the main sample by centrifugation for 10 min at 7,000 x g.

Polysomal pellets were prepared from crude cytoplasm by centrifugation through 2.5-ml cushions of 1.75 M sucrose, 100 mM KCl, 10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, and 1 mM dithiothreitol for 16 hours at
40,000 rpm in a Spinco 65 rotor. The supernatant was carefully removed, and the polysomal pellets were frozen in polycarbonate tubes in -70°C hexane and stored at that temperature. For mixing experiments, polysomal pellets were used within 4 days of preparation.

**Extraction of Protein**—Nucleolar or polysomal pellets representing about 500 μg of protein were suspended in 2.0 ml of 6 M guanidine hydrochloride, 10 mM EDTA, pH 7.4, and 2.5% β-mercaptoethanol and homogenized gently for 10 min in a glass-Teflon homogenizer. If required, nucleolar and ribosomal samples were mixed at this stage in the procedure. The suspension was clarified by centrifugation for 15 min at 15,000 × g, layered over 3.0-ml cushions of 4.75 M CsCl, 10 mM EDTA, pH 7.4, and 2.5% β-mercaptoethanol, and centrifuged to equilibrium density for 40 to 80 hours in a Spinco SW 50L rotor at 40,000 rpm. The tube was punctured from the bottom and the gradient was fractionated by gravity. Fractions from the top fourth of the gradient, which contained >90% of the total load, were pooled, dialyzed against 0.1 M acetic acid, and lyophilized.

For polysomal protein reference gels, protein was extracted from pelleted polysomes by modification (9) of the acetic acid extraction procedure of Hardy et al. (10).

**Two-dimensional Polyacrylamide Gel Electrophoresis of Protein**—Protein was analyzed by two-dimensional polyacrylamide gel electrophoresis as described (9) of the method of Hirs and Wittmann (11). For analysis of nucleolar protein, 200 to 300 μg of protein was used per gel. In experiments involving labeled protein, two duplicate gels were run, the spots were located by staining, and the radioactivity of individual spots was determined by hydrogen peroxide digestion and liquid scintillation counting as described elsewhere (9).

**Quantitation of RNA**—The total RNA content of the polysomal and nucleolar pellets used for protein extraction was determined by spectrophotometry. Aliquots of the suspension of polysomal pellets in 6 M guanidine hydrochloride (see above) were diluted with water and their absorbance at 260 nm was measured on a Zeiss spectrophotometer. Nucleolar pellets from 0.2-ml aliquots of the nuclear lysate were homogenized in 1.0 ml of 1 M perchloric acid, after 10 min the precipitates were collected at 15,000 × g for 10 min and rinsed with cold 1 M perchloric acid. RNA in the pellets was determined from their alkali-labile absorbance at 260 nm as previously described (12).

To determine the proportion of total nucleolar RNA present as various high molecular weight species, a culture of cells was labeled to about 1010 of the total load. The relative contents of the smaller RNA species (20 S and 18 S) were also present in the 45 S + 41 S, 32 S, 28 S, 20 S, and 18 S peaks were determined by spectrophotometry. Peaks of various RNA species were located by positioning the trough at selected locations relative to the ultraviolet beam, switching to a visible light source, and marking the gels at those positions. Gel slices up to 1 cm long were digested for 18 h at 37°C in 1 ml of a mixture of 30% hydroxylamine and concentrated ammonium hydroxide (50:1), and the samples were counted in 20 ml of Aquasol in a liquid scintillation counter.

**Results**

**Extraction of Nucleolar Protein**—Because the experiments described in this report involved quantitative measurements made on electrophoretically purified nucleolar proteins, the procedure used for the preparation of nucleolar protein had to fulfill two requirements. First, it had to extract total protein directly from isolated nucleoli with high yield. Second, the resulting protein extract had to be sufficiently free of aggregates and nucleic acids to enter polyacrylamide gels. Since existing techniques failed to meet these requirements, a new method was developed for the extraction of total protein from isolated nucleoli. This method (see "Experimental Procedures") involves the suspension of isolated nucleoli in 8 M guanidine hydrochloride in the presence of EDTA and mercaptoethanol. The resulting suspension is clarified by centrifugation, layered over cesium chloride, and subjected to equilibrium density gradient centrifugation. The protein which is recovered from the top of the density gradient contains less than 1.5% contaminating nucleic acid. Recovery of nucleolar protein throughout the procedure is also good. Virtually all protein remains in suspension following clarification (>95%), although little ribosomal precursor RNA remains (<20%). Recovery of total protein from equilibrium density gradient centrifugation is better than 80%. If recovery of nucleolar ribosomal protein is estimated by following small amounts of added labeled ribosomal protein, similar figures are obtained.
Identification of Nucleolar Ribosomal Proteins—By the criteria of two-dimensional co-electrophoresis the majority of nucleolar proteins seen in Fig. 1B may be identified as ribosomal proteins. Proteins from both the small and large ribosomal subunit are seen. Ribosomal protein spots are identified by a nomenclature intended to correspond to the nomenclature of Sherton and Wool (9, 15). Proteins from the large and small ribosomal subunit are designated by an L or S prefix, respectively. In addition, a number of proteins which appear on gels of pelleted polysomes but are absent from gels of both ribosomal subunits are designated by a P prefix. Several of these are present in significant amounts in isolated nucleoli. Finally, a number of nucleolar proteins are found on these gels which do not correspond to proteins found on pelleted 80 S particles. These nonribosomal nucleolar proteins are designated by the prefix N.

Although the resolution of this two-dimensional gel system is good, the possibility of co-electrophoresis of nonidentical proteins cannot be eliminated. Such co-electrophoresis would have to be precise, since direct comparison of autoradiograms with stained parent gels allows accurate matching of spot positions. It should be noted that there are several regions of the gel where resolution is poor; quantitative data regarding the spots in these regions must be regarded with caution (proteins L6, L7, L21, L23, L24, S1, S3, S13, S16, S18, S21). Finally, there are several instances where proteins from the large and small subunits are not resolved (proteins L13 and S8, L27 and S24, and L32 and S25). It is not clear whether the nucleolar proteins found in such positions represent one or both ribosomal subunit proteins, if in fact two different proteins are involved. Answers to these questions must await further characterization of the proteins in these gel positions.

Relative Stoichiometry of Nucleolar Ribosomal Proteins—Direct analysis of nucleolar protein patterns such as the one shown in Fig. 1B does not provide quantitative information about the nucleolar content of ribosomal proteins. Even if the amount of protein per gel spot were measured and the molecular weight of each protein were known, uncertainty about preferential loss of individual proteins would make interpretation difficult. Consequently, we sought an experimental approach that would provide unambiguous quantitative information relevant to the question of nucleolar pools of ribosomal proteins.

Diagrammed in Fig. 2 is an experiment designed to measure the stoichiometry of ribosomal proteins in isolated nucleoli relative to their stoichiometry on cytoplasmic ribosomes. Two cultures of HeLa cells were grown for three generations, one in the presence of [3H]methionine and the other in the presence of [35S]methionine. Cells were harvested and polysomes and nucleoli were prepared as described under "Experimental Procedures." A portion of the polysomes from the [3H]methionine-labeled culture was mixed with the nucleoli from the [35S]methionine-labeled culture. Protein was extracted by the GuHCl-CsCl procedure and subjected to two-dimensional polyacrylamide gel electrophoresis (see "Experimental Procedures"). Migration in the first dimension is toward the right (cathode) at pH 8.6. Migration in the second dimension is toward the bottom (cathode) at pH 4.0. A, stained gel pattern. Due to proportion of polysomes and nucleoli mixed, all but the faintest spots are polysomal proteins. B, autoradiogram obtained by exposing x-ray film for 1 week to the gel shown in A. C, reference gel pattern of 50 μg of polysomal protein obtained by acetic acid extraction (see "Experimental Procedures"). Refer to text for discussion of nomenclature. Spots visible in the original gel patterns but too faint to be seen in the published diagrams are enclosed by dashed lines.
dimensional electrophoresis. The resulting gel was stained, individual spots were digested with hydrogen peroxide, and the isotopic ratios of these spots were determined by liquid scintillation counting. In addition, several other measurements were made. Cytoplasmic ribosomes derived from each culture were used to determine the specific activity of total ribosomal protein (specific activity $^3$H-r-protein and specific activity $^{35}$S-r-protein, respectively). Aliquots of both the pelleted ribosomes and the isolated nucleoli used for mixing were removed and their ribonucleic acid content was measured. Finally, in an independent set of experiments, the proportions of total nucleolar RNA found in various high molecular weight ribosomes and the isolated nucleoli used for mixing were measured.

Interpretation of the measurements of the relative nucleolar stoichiometries was calculated from the following relationship:

$$Q_i = \frac{R_i - R_t}{(specific\ activity\ ^{35}S\ r\ protein)(moles\ ribosomes\ mixed)}$$

where $R_t$ is the $^{35}$S/$^3$H isotopic ratio for protein $i$ at the time of mixing. Since nucleoli and ribosomes were mixed under strongly denaturing and reducing conditions, it was assumed that this isotopic ratio was not altered during subsequent protein extraction and electrophoresis, and the isotopic ratio determined following chromatography was used. In calculating the moles of pre-rRNA mixed, the measurement of total nucleolar RNA was used in conjunction with the percentage and molecular weights of the various RNA species listed in Table I. For large subunit proteins the $45\ S + 41\ S$, $32\ S$, and $28\ S$ RNA species were considered; for the small subunit the $45\ S + 41\ S$, $20\ S$, and $18\ S$ species were considered (16). The specific activity figures appearing in this equation are the average values measured for total ribosomal protein; since they appear as a ratio, no assumption need be made about the amino acid composition of the protein in question.

Table II shows the results of such an experiment. This table lists the relative nucleolar stoichiometries of proteins from the large and small ribosomal subunits (L and S proteins). Only those proteins are listed that were adequately resolved by electrophoresis and that yielded relative stoichiometries consistent with a second identical experiment (not shown). The majority of ribosomal proteins listed in Table II exhibit relative nucleolar stoichiometries approximately equal to one. Our interpretation of these results is that most nucleolar particles have a content of ribosomal protein much like that of cytoplasmic ribosomes, and that this particle-associated protein makes up the bulk of the ribosomal protein found in isolated nucleoli. Only one large subunit protein, L5, has a markedly high relative nucleolar stoichiometry, suggestive of a sizable nucleolar pool. A number of other ribosomal proteins have relative nucleolar stoichiometries somewhat higher or lower than one. This may reflect small differences in the relative amounts of these proteins in the nucleolus; other possible interpretations will be discussed in the following section.

**DISCUSSION**

Interpretation of the measurements of the relative nucleolar stoichiometry of ribosomal proteins involves three basic assumptions. The first of these is the assumption that nucleolar and ribosomal proteins are sufficiently dissociated in the presence of guanidine hydrochloride, $\beta$-mercaptoethanol, and EDTA to allow complete mixing of polypeptides with respect to isotopic label. If such dissociation were incomplete, undisassociated protein bearing predominantly one isotope might be
nuclear stoichiometry would result. We suspect the latter possibility might explain the higher values measured for some ribosomal proteins. However, Howard et al. (20) have suggested that most ribosomal proteins may be present in one copy per ribosome based on the distribution of their molecular weights. These uncertainties should be resolved when direct information regarding the stoichiometry of ribosomal proteins on cytoplasmic ribosomes becomes available.

Our third assumption is that the protein content of isolated nucleoli reflects in a meaningful way the actual protein content of that organelle in vivo. The Penman method for nuclear isolation employed in these experiments involves disruption of nuclei in 0.5 M sodium chloride. Such high salt conditions are likely to remove proteins loosely associated with the organelle in vivo. Thus, the set of ribosomal proteins which we identify in isolated nucleoli is a minimum set. Clearly, it would not include pools of ribosomal proteins present in the nucleolus or nucleoplasm in a soluble form. Conversely, it is possible that some proteins which we have found in isolated nucleoli are not associated with the nucleolus in vivo but are rather adventitiously bound to nucleoli or contained in other cellular contaminants of the nucleolar pellet. Uncertainties of these sorts would be encountered in any measurements made on isolated nucleoli, regardless of the isolation procedure employed. The Penman procedure was chosen because it provides a compromise between protein contamination and protein loss, and because it has been employed in a number of other investigations relevant to this study (7, 8, 21, 24).

The measurements of the relative nuclear stoichiometries of individual ribosomal proteins reported in this paper indicate that, for most ribosomal proteins, isolated nucleoli contain about as much protein per ribosomal RNA sequence as do cytoplasmic ribosomes. In other words, we do not find significant pools of most ribosomal proteins associated with isolated nucleoli. We would have expected to have detected such pools if they constituted as much as 2% of any individual ribosomal protein in the cell. Nevertheless, a number of individual ribosomal proteins were found to exhibit higher than average nuclear stoichiometries (L5, L11, L31, S8, S14, S18, and S21). Protein L5 stands out in this regard with an exceptionally high nucleolar stoichiometry suggestive of a large pool of this individual protein. There is independent evidence that a sizable pool of protein L5 does exist in HeLa cells (9). The other proteins in this group may have small nucleolar pools; alternatively, they may be present in fractional amounts on cytoplasmic ribosomes. A number of proteins were identified with lower than average nuclear stoichiometries (L8, L10, S2, S8, S15, and S17). One of the two large subunit proteins in this group (L10) has been identified as a protein that can add directly to ribosomes in the cytoplasm of HeLa cells (9); such a protein might be expected to be absent from the nucleolus. The rest of the proteins in this group have been identified by kinetic measurements as proteins that appear to add late in the assembly process and might be expected to show reduced nuclear stoichiometries.

The experiments described in this report by themselves do not exclude the possibility that pools of most ribosomal proteins exist in the cell but are not found in isolated nucleoli. However, we feel that several lines of evidence argue against the existence of soluble pools for most ribosomal proteins. It has been observed in several laboratories that when ribosomal proteins are preferentially lost or recovered during extraction and electrophoresis. As a consequence, the isoionic ratio measured would differ from the true ratio in the original mixture. However, it should be noted that 6 M guanidine hydrochloride is a very strong denaturing agent, capable of converting most polypeptides to random coil configuration (17). It has been used elsewhere for extraction of protein from nucleoli (18). In addition, mixing and subsequent cesium chloride centrifugation was carried out in the presence of 2.5% β-mercaptoethanol, strongly reducing conditions in excess of those used to dissociate ribosomal precursor particles from nucleoli (19). These considerations, in combination with the high overall yield of protein during extraction, lead us to believe that serious error due to incomplete mixing is very unlikely.

The second assumption made is that the ribosomal protein content of the crude polysomal pellet used in mixing accurately reflects the protein content of mature ribosomes. If an excess of a particular ribosomal protein were bound adventitiously to polysomes, a reduced nuclear stoichiometry would be measured, even if that protein were present in average amounts in the nucleus. The fact that polysomes are exposed to moderately salt (0.3 M KCl) before sedimentation through sucrose cushion argues against such adventitious binding. Conversely, if a given ribosomal protein were present in unit stoichiometry on newly completed ribosomes, but were lost or cleaved during ribosome aging or polysome purification, an increased relative

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**Table II**

**Relative stoichiometry of nucleolar proteins**

The relative nucleolar stoichiometries of individual ribosomal proteins are defined as the ratio of the stoichiometry of these proteins in isolated nucleoli to their stoichiometry in cytoplasmic polysomes (see text). Individual spots were cut from each of two two-dimensional polyacrylamide gels from the experiment described in Fig. 1. 1H and 3H radioactivities were determined and corrected for counting efficiency and radioactive decay. The average 3H/1H ratios were used to calculate relative nucleolar stoichiometries (see formula in text) using the following additional measurements (see "Experimental Procedures" and "Results"): specific activity 1H-r-protein = 1.37 x 10^12 dpm/μg; specific activity 3H-r-protein = 2.43 x 10^16 dpm/μg; mol of pre-rRNA mixed (large subunit proteins) = 2.29 x 10^-14 mol; mol of pre-rRNA mixed (small subunit proteins) = 1.15 x 10^-14 mol; mol of ribosomes mixed = 2.28 x 10^-15 mol. Protein S8, which co-migrates with L13, contains no methionine and therefore does not affect the stoichiometry calculated for L13 (6).

<table>
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<tr>
<th>PROTEIN</th>
<th>RELATIVE NUCLEOLAR STOICHIOMETRIES</th>
<th>( \frac{3H}{1H} )</th>
<th>RELATIVE NUCLEOLAR STOICHIOMETRIES</th>
<th>( \frac{3H}{1H} )</th>
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<tr>
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<td>0.19</td>
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<tr>
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<tr>
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<td>0.19</td>
<td>3542000.19</td>
<td>0.19</td>
</tr>
<tr>
<td>L10</td>
<td>7717700.23</td>
<td>0.23</td>
<td>7717700.23</td>
<td>0.23</td>
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<tr>
<td>L12</td>
<td>3542000.19</td>
<td>0.19</td>
<td>3542000.19</td>
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<tr>
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<tr>
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RNA synthesis is inhibited by low levels of actinomycin D, synthesis of ribosomal proteins continues on cytoplasmic ribosomes at approximately the same rate (22, 23). However, most ribosomal proteins synthesized in the absence of rRNA synthesis do not accumulate in isolated nucleoli (24), nor do they chase into cytoplasmic ribosomes when rRNA synthesis is allowed to resume (25). Similarly, when RNA synthesis is inhibited by the antibiotic camptothecin (21), incorporation of newly synthesized ribosomal protein into isolated nucleoli is almost completely inhibited. Upon removal of the drug only those ribosomal proteins recoverable in isolated nucleoli eventually appear in cytoplasmic ribosomes. The half-life of extranucleolar protein in these experiments has been estimated to be less than 5 min (1). In all of the above experiments nucleoli were isolated in the presence of 0.5 M sodium chloride. These observations argue against the existence of significant pools of ribosomal protein in any cellular compartment; unless their size was extremely well regulated, such pools should accumulate in the presence of either antibiotic, and this label should chase into cytoplasmic ribosomes. The kinetic experiments of Wu and Warner are even more convincing in this regard (7). They measured accumulation of newly synthesized proteins in various cellular compartments after addition of a labeled amino acid precursor. Within 2 min the accumulation of labeled protein (primarily ribosomal protein) within the nucleolus became linear; when a 2-min label was followed by a cold amino acid chase, incorporation in the nucleoli stopped within 1 min. These experiments imply that ribosomal proteins must migrate very rapidly to the nucleolus and there become bound in such a way as to be conserved during nucleolar isolation. Thus, if most of a ribosomal protein passes through a pool before being utilized in ribosome assembly, the protein in this pool could not be freely soluble, but must be stably associated with the nucleolus. These considerations, in combination with the data presented in this paper, lead us to believe that HeLa cells do not contain sizable pools of the majority of ribosomal proteins, although they might contain pools of a few individual ribosomal proteins.

If this conclusion is correct, an alternate explanation must be found for the observations of Warner and others that some RNA synthesis and ribosome maturation continues in the absence of protein synthesis (2, 4, 5). Although the real explanation for this phenomenon is not presently clear, Warner now considers it unlikely that there are pools of most ribosomal proteins (1). One possibility is that when no new ribosomal proteins are available, previously synthesized protein is reutilized from inactivated ribosomes. Any hypothetical scheme for the control of ribosomal RNA synthesis must take into account two general observations (1–9). Partial or complete inhibition of protein synthesis results in inhibition of ribosomal RNA synthesis. However, inhibition of ribosomal RNA synthesis does not noticeably inhibit the synthesis of ribosomal protein on cytoplasmic ribosomes; this protein is not utilized, even if RNA synthesis is allowed to resume. These observations and the results described in this paper are compatible with a simple model of ribosome assembly: newly synthesized ribosomal proteins diffuse rapidly to the nucleolus where they bind to newly synthesized ribosomal precursor RNA to form ribosomal precursor particles. Unbound ribosomal proteins are rapidly degraded. The availability of one or more proteins, possibly ribosomal proteins, limits the rate of ribosomal RNA synthesis.

REFERENCES

*E. H. McConkey, unpublished observations.
Relative stoichiometry in ribosomal proteins in HeLa cell nucleoli.
W F Phillips and E H McConkey


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