Contemporary models for protein translocation in the mammalian endoplasmic reticulum (ER) identify the termination of protein synthesis as the signal for ribosome release from the ER membrane. We have utilized morphometric and biochemical methods to assess directly the fate of membrane-bound ribosomes following the termination of protein synthesis. In these studies, tissue culture cells were treated with cycloheximide to inhibit elongation, with pactamycin to inhibit initiation, or with puromycin to induce premature chain termination, and ribosome-membrane interactions were subsequently analyzed. It was found that following the termination of protein synthesis, the majority of ribosomal particles remained membrane-associated. Analysis of the subunit structure of the membrane-bound ribosomal particles remaining after termination was conducted by negative stain electron microscopy and sucrose gradient sedimentation. By both methods of analysis, the termination of protein synthesis on membrane-bound ribosomes was accompanied by the release of small ribosomal subunits from the ER membrane; the majority of the large subunits remained membrane-bound. On the basis of these results, we propose that large ribosomal subunit release from the ER membrane is regulated independently of protein translocation.

The endoplasmic reticulum (ER) membrane, the site of nascent secretory and integral membrane protein translocation, contains an abundance of membrane-bound ribosomes. As is now well established, the association of biosynthetically active ribosomes with the ER membrane occurs through the activity of the signal recognition particle/signal recognition particle receptor targeting machinery (1–3). By this process, ribosome-nascent chain complexes bearing secretory or membrane protein precursors are recognized early in synthesis and are trafficked from the cytosol to the ER membrane (4). Following the binding of the ribosome-nascent chain complexes to the resident translocon complex of the ER membrane, protein translocation proceeds and nascent chains are translocated across or integrated into the ER membrane. Subsequently, the termination of protein synthesis is thought to elicit the release of the ribosomal subunits from the ER membrane to the free, cytoplasmic pool (5, 6). In the cytoplasmic pool, the ribosomal subunits are free to participate in the protein synthesis initiation sequence. Should they engage in the synthesis of a secretory or membrane protein precursor, targeting to the ER membrane again occurs, thus describing a cycle of ribosome binding and release.

In the pioneering studies on ribosome-membrane interactions in the ER, it was observed that ribosomes bind asymmetrically, with the binding interaction being mediated entirely through the large subunits (7–10). Thus, if isolated ER microsomes were extracted with increasing concentrations of EDTA, the small ribosomal subunit was preferentially released from the membrane, whereas the large ribosomal subunit remained membrane-bound (7, 8). Confirmation of the nature of ribosome binding to the ER was later obtained by direct ultrastructural analysis of intact cells, where it was observed that the large ribosomal subunit contains the site of membrane attachment (10). On the basis of these observations, it may be predicted that the termination of protein synthesis by membrane-bound ribosomes would be accompanied by the regulated dissociation of large ribosomal subunits from the ER membrane.

Do ribosomes cycle between a membrane-bound and free state? This question was first addressed in biosynthetic labeling and exchange studies and yielded differing and somewhat contradictory conclusions (9, 11). In the study of Baglioni et al. (9), it was proposed that large ribosomal subunits bind to the ER membrane prior to their assembly into polysomes. In contrast, Mechler and Vassalli (11), using a similar isotope incorporation protocol, concluded that small and large ribosomal subunits enter the membrane-bound polysome fraction from the cytosolic pool at similar rates. Although these studies are in disagreement regarding the entry path for large subunits into membrane-bound polysomes, two points are evident. 1) The biosynthetic labeling and exchange kinetics of membrane-bound large subunits differ from those of membrane-bound small subunits. 2) Membrane-bound and cytosolic ribosome pools constitute a common population. However, because ribosome synthesis and assembly are relatively slow and complex processes, the resolution offered by ribosome biosynthetic labeling studies is not sufficient to analyze directly the temporal coupling between protein synthesis on membrane-bound ribosomes and ribosome exchange between free and bound ribosome pools.

The purpose of this study was to investigate experimentally the compartmental fate of membrane-bound ribosomes following the termination of protein synthesis. Do ribosomes dissociate from the ER membrane coincident with the termination of protein synthesis? By using biochemical and morphometric methods in tissue culture cells, we report that in vivo termination yields the preferential release of ribosomal small subunits.
from the membrane, whereas the large ribosomal subunits remain predominantly in the bound state.

**EXPERIMENTAL PROCEDURES**

Reagents—35S-Labeled Pro-Mix (125S)methionine and -cysteine and 5,6-3H]uridine were obtained from Amersham Pharmacia Biotech. 

Pactamycin was obtained from the Upjohn Co. Cell culture media and reagents were from Life Technologies, Inc. Digitonin was from Wako Chemicals (Richmond, VA). Antibodies to human albumin were from Roche Molecular Biochemicals. Reagents for electron microscopy were obtained from Electron Microscopy Sciences (Fort Washington, PA). All other reagents were from Sigma.

**Cell Culture**—Human hepatocarcinoma cells (HepG2) and Buffalo Rat liver cells (BRL 3A) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum at 37 °C and 5% CO2. Cells were subcultured at 3-day intervals.

Analysis of Protein Synthesis Inhibitor Activity—Cells were plated at 1 x 10^6 cells/well on 6-well plates and used at 70% confluency. For pulse labeling studies, cells were first starved of methionine by incubation in serum- and methionine-free DMEM for 20 min at 37 °C. Pulse labeling was subsequently initiated by replacement of the serum-free and methionine-free DMEM supplemented with 100 μCi/ml 35S-labeled Methyl-[35S]methionine with 35S-labeled Methyl-[35S]methionine added directly to the pulse medium to final concentrations of 200 μCi/ml.

Other reagents were from Sigma.

**Characterization of Experimental System**—To study the temporal coupling of ribosomal exchange and protein translation on the ER membrane, tissue culture cells were treated with protein synthesis inhibitors selective for either the elongation or initiation cycle of protein translation, and ribosome-membrane interactions were measured by morphometric and biochemical methods. Three protein synthesis inhibitors were investigated as follows: cycloheximide, puromycin, and pactamycin. The mode of action, site of action, and the predicted effects of each inhibitor on polysome structure are listed in Table I.
Because tissue culture cell lines often display differences in their sensitivity to protein synthesis inhibitors, each inhibitor was assayed to identify effective concentrations and to screen for secondary effects on protein secretion. In these experiments, methionine-starved HepG2 cells were pulsed with [35S]methionine in the presence or absence of inhibitor, and the rate of isotope incorporation into albumin was determined by immunoprecipitation, SDS-PAGE, and PhosphorImager analysis. As shown in Fig. 1A, in the absence of inhibitor, [35S]methionine incorporation increased linearly over the time course of the assay (7.5 min). At the concentrations used, cycloheximide and puromycin rapidly and effectively inhibited incorporation of [35S]methionine into albumin. In the presence of pactamycin, [35S]methionine incorporation into albumin proceeded linearly for a short time and then ceased. For pactamycin, the pattern of isotope incorporation was as predicted for an inhibitor of initiation; only those nascent chains undergoing elongation at the time of pactamycin addition were completed, and subsequent de novo protein synthesis was blocked. An additional series of controls was performed to identify any secondary effects of the protein synthesis inhibitors on protein secretion. In these experiments, pulse-chase studies of albumin synthesis and secretion were performed in the presence of each of the inhibitors. The kinetics of albumin secretion in the presence of either cycloheximide, puromycin, or pactamycin were essentially identical, with a half-time for secretion of approximately 20–30 min (Fig. 1B). In summary, the three inhibitors used in this study disrupt the protein synthesis activity of HepG2 cells in the manner predicted from their established mode of action. Furthermore, these data demonstrate that the experimental manipulations necessary to evaluate the fate of ribosomal subunits arising from premature (puromycin) or natural (pactamycin) termination do not adversely alter ER function or the activity of the protein secretion machinery.

Ribosomes Remain Membrane-bound after Termination, Morphometric Analysis—To characterize any changes in ribosome-membrane interaction that might accompany the termination of protein synthesis, a series of ultrastructural and biochemical studies were conducted with cultured cells. In experiments on rough ER ultrastructure, HepG2 and BRL cell monolayers were treated with the described protein synthesis inhibitors for 10 min. This time period is sufficient to allow complete inhibition of protein synthesis and, in the case of puromycin and pactamycin, the discharge of nascent chains from the ribosome. Samples were then placed on ice and fixed with glutaraldehyde. Ultrathin sections were prepared, stained, and viewed by electron microscopy. A representative section from untreated HepG2 cells is illustrated in Fig. 2A. In this micrograph an abundant and extensive ER network can be readily distinguished from cytosol by the relatively dark staining of the membrane-bound ribosomes and the granular, lumenal reticuloplasm. The ER cisternae are seen in both transverse transversely sectioned and obliquely sectioned membrane sheets. Two high magnification views of polypyribosomes on the rough ER (Fig. 2, B and C) illustrate the resolution observed in oblique and transverse sections. In the oblique view, a polysome is visible in the commonly observed rosette pattern (14). The spatial orientation of ribosomal subunits can also be discerned in these panels, where large subunits are oriented toward the outside of the polysome. Small subunits appear atop the large subunits and face the polysome interior. The morphological analysis of ribosomal-membrane interactions following translation inhibition was restricted to transverse views of the ER membrane. Extensive sets of micrographs were prepared for cells that had been treated with cycloheximide, pactamycin, and puromycin, as well as untreated cells. Representative micrographs of the kind used in the analysis are depicted in Fig. 3A. A total magnification of

![FIG. 1. Analysis of protein synthesis inhibitor activity. A, protein synthesis. HepG2 cells were incubated in methionine-free DMEM at 37 °C and subsequently pulse-labeled with 100 \( \mu \)Ci of [35S]methionine for 1 min prior to addition of each inhibitor. At the indicated time points, which represent the time after inhibitor addition, cells were harvested, and radiolabeled albumin was collected from the cell lysate by immunoprecipitation. Albumin content in each sample was quantitated from SDS-PAGE gels by PhosphorImager analysis. B, protein secretion. Cells were starved of methionine and subsequently pulsed with 100 \( \mu \)Ci of [35S]methionine; the chase period was initiated by replacing the labeling medium with serum-free DMEM supplemented with 2 mM methionine and the indicated inhibitors. At each time point, the medium was removed, and cells were chilled, rinsed, and solubilized. Albumin was immunoprecipitated from the cell lysate (C) and chase medium (M) as described under “Experimental Procedures.” Final inhibitor concentrations were 200 \( \mu \)M cycloheximide, 200 \( \mu \)M puromycin, or 0.2 \( \mu \)M pactamycin.

| Inhibitor     | Mode of action                      | Effect on polysome structure       | Site of action | References |
|--------------|-------------------------------------|------------------------------------|---------------|------------|
| Cycloheximide| Inhibits elongation factor-dependent translation | Stabilizes polysome structure       | Large subunit | 25, 26     |
| Puromycin    | Ejects nascent peptide chain from ribosome | Induces premature polypeptide termination and polysome breakdown | Peptidyltransferase center, large subunit | 27, 28     |
| Pactamycin   | Blocks initiation complex assembly with large subunit | Elicits polysome breakdown           | Small subunit | 29, 30     |

Table I: The effects of protein synthesis inhibitors on protein translation and polysome structure.
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approximately 100,000 afforded clear views of individual ribosomes along the length of transversely sectioned ER in untreated cells (Fig. 3A). The ribosomes are clustered in groups of 3–5 along some regions, which indicates the association of these ribosomes in polyribosomes at the time of fixation. Although it was not possible to consistently resolve individual subunits at this magnification, the asymmetric positive staining of the membrane-bound particles suggests that both large and small subunits are bound in untreated cells. A similar distribution of ribosomes along the ER membrane was observed in cells after treatment with cycloheximide (not shown).

Visual inspection of transverse ER sections from pactamycin- and puromycin-treated BRL cells revealed an extensive number of membrane-bound ribosomes, as shown in Fig. 3, B and C. This finding was surprising in light of the activity of the inhibitors and the predicted behavior of ribosomes upon termination. Following run-off translation or premature termination, the ribosomes would be expected to dissociate into subunits and discharge from the membrane. However, we observed a distribution of membrane-bound ribosomes which approximated that seen in untreated and cycloheximide-treated cells, where the majority of membrane-bound ribosomes contained translocating nascent chains. Along the plane of the membrane, the ribosomes of pactamycin- and puromycin-treated cells did not have the same degree of lateral organization found in control cells (Fig. 3A). A more random distribution of ribosomes in pactamycin or puromycin-treated cells may be attributed to the loss of polyosome structure. Thus, following pactamycin or puromycin treatment, no rosette or linear polysome patterns in the cytoplasm or on the rough ER membrane were observed (data not shown).

To evaluate quantitatively the distribution of bound ribosomes, micrographs of cells from all experimental conditions were digitized and adjusted to uniform contrast and brightness levels (see “Experimental Procedures”). Because of the small diameter of ribosomes relative to resin sections and the propensity of polyosome-associated ribosomes to extend above and below the plane of section, it is difficult to quantify the number of ribosomes present per unit area of membrane in an oblique section (15, 16). However, transverse sections in which the membrane-bound ribosomes are of similar dimension can be quantitated and the relative ribosome density expressed as ribosomes per linear micron. At least 430 individual data points were collected for each treatment from 7 or more unique micrographs. Differences between results from untreated cells and inhibited cells were assessed using the Student’s t test. The results of the analysis are depicted in Table II. Following elongation arrest with cycloheximide, ribosomes were present at a density of 12.17 ribosomes/μm, a decrease of 35% compared with control. A similar decrease was observed for cells treated with pactamycin, where 8.03 ribosomes/μm were present. Statistical analysis of the data indicate that these differences from control cells are significant, with p values of 0.0001. It is apparent from these results that following release of the nascent chain from the ribosome upon either premature termination or run-off translation, approximately one-third of the ribosomes completely detach from the ER, whereas the majority remain in stable association.

Biochemical Fractionation of Free and Membrane-Bound Ribosomes—Morphometric analysis of the ribosome-membrane association in intact cells indicated that ribosomes and/or ribosomal subunits are found in association with the ER membrane following inhibitor-elicited termination. However, in traditional thin sections, it is difficult to distinguish unequivocally large and small ribosomal subunits. To gain insight into the structural composition of the bound ribosomal particles re-
remaining upon termination, inhibitor-treated cells were fractionated and ribosomal subunit identity determined by velocity sedimentation on sucrose gradients. A method was developed to allow rapid separation of membrane-bound and free ribosomes in BRL cells, based on earlier fractionation procedures with this cell line (17, 18). Cell monolayers were cultured for 18 h with \(^3\)!H|uridine to radiolabel RNA. Semi-intact cells devoid of cytoplasm were subsequently generated by digitonin-based permeabilization in isotonic buffer. The digitonin-extracted cells were then solubilized with nonionic detergent, to release membrane-bound ribosomes, and centrifuged to remove insoluble components. Membrane-derived ribosomes were resolved by centrifugation through continuous sucrose gradients or discontinuous sucrose cushions. The digitonin concentration was optimized to allow efficient removal of cytosolic ribosomes without affecting ER membrane integrity. As shown in Fig. 4, 40 \(\mu\)g/ml digitonin was the minimum detergent concentration necessary to elicit selective plasma membrane permeabilization. Under these conditions, cytoplasmic radiolabeled RNA species were removed with two washes in digitonin-supplemented isotonic buffer. The radiolabeled RNA remaining in the cell was not susceptible to further extraction at 4 \(^\circ\)C and thus was operationally defined as membrane-associated. Integral ER membrane proteins were observed to remain in quantitative association with the cell pellet (data not shown).

Large Ribosomal Subunits Remain Bound after Termination—The digitonin-based cell fractionation procedure was used to determine biochemically the state of ribosome assembly following the termination of protein synthesis. In these experiments, \(^3\)!H|uridine-labeled cells were incubated with inhibitor-supplemented media for 10 min at 37 \(^\circ\)C. For each analysis, 2 \(\times\) \(\times\) \(\times\) \(\times\) \(\times\) \(\times\) \(\times\) cells/ml in isotonic buffer and subsequently treated with digitonin at the indicated concentration for 5 min at 4 \(^\circ\)C. Soluble cellular material was then removed following brief centrifugation, and the cell pellet was subjected to a series of washes in detergent-supplemented isotonic buffer. To recover the membrane-bound ribosomes, cell pellets remaining after the final wash were solubilized in a buffer containing 1\% Nikkol and 0.5\% deoxycholic acid, and remaining nuclei and/or nuclear fragments were removed by centrifugation. Cytosolic ribosomes released upon digitonin extraction, and membrane-bound ribosomes released upon detergent solubilization were recovered by sedimentation through 1.5 \(\times\) sucrose cushions, and ribosomal RNA was quantitated by scintillation counting.

### Table II

**Morphometric analysis of ribosome-membrane interactions following premature or natural termination**

Micrographs of inhibitor-treated or control BRL cells were digitized and measured as described under “Experimental Procedures.” Membrane-bound ribosomes along each segment of transversely sectioned rough ER were counted individually. Data for number of bound ribosomes per linear micron of membrane were compiled by treatment. \(n\), number of micrographs in set; c.i., 95\% confidence interval for variation from mean. \(p \leq 0.05\) indicates a statistically significant difference between data sets from untreated and experimental populations.

| Treatment     | Length Ribosomes | Ribosomes/\(\mu\)m | c.i. (95\%) | \(p\) value |
|---------------|------------------|-------------------|-------------|------------|
| Control       | 57.56            | 690               | 11          | 12.68      | 1.486      | 0.331 |
| Cycloheximide | 46.29            | 539               | 10          | 12.17      | 2.116      | 0.0001 |
| Pactamycin    | 62.06            | 506               | 9           | 8.03       | 1.727      | 0.0001 |
| Puromycin     | 52.66            | 435               | 7           | 8.24       | 1.329      | 0.0001 |

**Fig. 4. Isolation of membrane-bound ribosomes from tissue culture cells.** BRL cells were labeled with \(^3\)!H|uridine and harvested at 75\% confluence. Cells were washed and resuspended to 2 \(\times\) \(\times\) \(\times\) \(\times\) \(\times\) cells/ml in isotonic buffer and subsequently treated with digitonin at the indicated concentration for 5 min at 4 \(^\circ\)C. Soluble cellular material was then removed following brief centrifugation, and the cell pellet was subjected to a series of washes in detergent-supplemented isotonic buffer. To recover the membrane-bound ribosomes, cell pellets remaining after the final wash were solubilized in a buffer containing 1\% Nikkol and 0.5\% deoxycholic acid, and remaining nuclei and/or nuclear fragments were removed by centrifugation. Cytosolic ribosomes released upon digitonin extraction, and membrane-bound ribosomes released upon detergent solubilization were recovered by sedimentation through 1.5 \(\times\) sucrose cushions, and ribosomal RNA was quantitated by scintillation counting.

**Ultrastructural Analysis of Membrane-bound Ribosomal Subunits**—Biochemical studies with permeabilized cells showed that large subunits remain bound to the ER membrane after termination, whereas small subunits were preferentially released. To evaluate further these findings, a series of experiments was conducted to image the membrane-associated particles after inhibitor treatment. BRL cells were treated with cycloheximide, pactamycin, or puromycin, and a microsomal fraction was prepared. After solubilization of the microsome fraction and centrifugation, the soluble microsomal contents were adsorbed onto copper grids, negatively stained with ura-
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Fig. 5. Ribosomal subunit structure following premature or natural termination. HepG2 cells were labeled with [3H]uridine for 18 h and at the time of the experiment incubated in DMEM supplemented with 200 μM cycloheximide, 200 μM puromycin, or 0.2 μM pactamycin for 15 min at 37 °C. Cells were then harvested, and the cytosolic and membrane-bound ribosome fractions were collected following treatment with an extraction buffer containing 40 μg/ml digitonin, as described in the legend to Fig. 4. Cytosol and membrane-derived ribosome fractions obtained by digitonin treatment were subsequently fractionated by velocity sedimentation on sucrose gradients. The amount of [3H]uridine in each gradient fraction was quantitated by scintillation counting. A, polysomal profile of digitonin-releasable cytosolic contents. B, polysomal profile of solubilized ER membrane fractions of digitonin-treated cells. A and B, ribosomes were separated on a 0.5–1.5 M sucrose gradient. C, ribosomal subunit profile of solubilized ER membrane fractions of digitonin-treated cells. Subunits were separated on 0.3–0.9 M sucrose gradients.

Fig. 6. Negative stain imaging of small and large ribosomal subunits and monomeric ribosomes. Rat liver rough microsomes were prepared by homogenization and centrifugation according to standard protocols. Microsomes were solubilized in 1% Nikkol, and the soluble ribosomes were isolated by centrifugation through sucrose cushions. Ribosomal subunits were separated by treatment with a puromycin-high salt treatment followed by sucrose gradient centrifugation. Preparations containing 40 S subunits, 60 S subunits, and 80 S ribosomes were adsorbed onto carbon-coated copper grids without fixation. The grids were stained with uranyl acetate before viewing in a transmission electron microscope at 100,000–150,000-fold magnification. Representative particles were selected from micrographs of each fraction A, purified 40 S subunits; B, purified 60 S subunits; C, microsome-derived 80 S monomers and polysomes (lower left).

They are identifiable by their distinct bilobal appearance, wherein a deposit of stain is visible at the large subunit-small subunit interface. The interface is visible along the width of the structure or as a dense spot on one side (see Ref. 19, plates IX and XI).

The composition of membrane-derived subunits from inhibitor-treated cells was identified on the basis of these standards. Composites of these subunits are shown in Fig. 7. As expected, treatment with cycloheximide yields polysomes and 80 S monosomes (Fig. 7A). Negatively stained intact polysomes were not as numerous as we predicted from sucrose gradient centrifugation; this is likely due to degradation during the homogenization and isolation procedure. Ribosomes from cycloheximide-treated BRL membranes appear very similar to those from untreated rat liver microsomes. The monomers are asymmetric and contain a stain-filled groove between large and small subunits in the frontal orientation.

In contrast, negatively stained particles from pactamycin- and puromycin-treated BRL cell membranes (Fig. 7, B and C) strongly resemble purified rat liver large subunits. They share common features with large subunits from both our preparation and that described in Ref. 19, including a round appearance and small protuberances on one side marked by heavier stain deposits in the skiff orientation. The particles are also similar in diameter to purified 60 S subunits and noticeably larger than 40 S subunits (Fig. 6A), while lacking the large spot of stain that demarcates intact monomers. We conclude that the distribution and structure of these membrane-bound ribosomal particles after premature or natural termination in vivo is consistent with our biochemical observations of large subunit retention and small subunit dissociation.

DISCUSSION

We report that the termination of protein synthesis on membrane-bound ribosomes in vivo results in the enhanced release
of small ribosomal subunits from the membrane-bound pool; large ribosomal subunits remain in stable association with the ER membrane. Although these data do not support the proposal that the termination of protein synthesis on membrane-bound ribosomes yields the release of large and small ribosomal subunits into the free, cytoplasmic pool, they are consistent with prior data demonstrating that, in vitro, the release of nascent chains from membrane ribosomes results in the enhanced exchange of small but not large ribosomal subunits (21).

From a historical perspective, the question of whether membrane-bound ribosomes participate in a translation-dependent exchange with free ribosomes closely followed the observation that free, cytosolic ribosomal subunits associate and dissociate coincident with the initiation and termination stages of translation (22, 23). Since it had been established that ribosomes were segregated between free and membrane-bound pools, studies were thus performed to determine whether such ribosome pools were kinetically exchangeable (6, 9, 11). Protein synthesis, however, occurs in the time frame of seconds to minutes, and thus the relevant exchange kinetics differ by 1–2 orders of magnitude. With this experimental limitation in mind, we re-examined the fate of membrane-bound ribosomes following the termination of protein synthesis through a combined morphometric and biochemical analysis of ribosome-membrane interactions in intact cells. The results of our studies best support the hypothesis that the termination of protein synthesis on membrane-bound ribosomes results in the free exchange of small ribosomal subunits, with the large ribosomal subunits remaining in stable association with the ER membrane. These data thus confirm and extend the conclusions obtained by Borgese et al. (21) and demonstrate that in the intact cell, large ribosomal subunit release from the ER membrane does not occur coincident with the termination of protein synthesis.

What is the fate of membrane-bound large ribosomal subunits after termination? Should such subunits be competent for protein translation, it is likely that protein synthesis could be initiated on the endoplasmic reticulum membrane. Assuming this to be true, it then becomes necessary to determine whether membrane-bound ribosomes can select mRNA substrates and thus whether membrane-bound ribosomes can catalyze the synthesis of free, cytosolic proteins. Furthermore, as membrane-bound ribosomes are thought to reside in intimate association with the protein conducting channel component of the ER translocon, it is equally important that the compartmental fate of such translation products be determined. As it is known that membrane-bound large ribosomal subunits exchange with the cytoplasmic pool, it is essential that the mechanism of large subunit release be determined and the factors governing this release process be identified. Insights into these questions are presented in the accompanying manuscript (31).

Acknowledgments—We gratefully acknowledge Drs. M. Reedy and J. Corless for access to electron microscopy equipment and supplies; and E. Wonniello, C. Lucaveche, and T. Zheng for excellent technical assistance. We thank M. Potter, R. Lerner, C. Rioja and other members of the laboratory for stimulating discussions. Portions of this study were performed with the electron microscopy facility of the Duke University Comprehensive Cancer Center.

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