The G Protein of Vesicular Stomatitis Virus Has Free Access into And Egress from the Smooth Endoplasmic Reticulum of UT-1 Cells

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Abstract. We have investigated the role of the smooth endoplasmic reticulum (SER) of UT-1 cells in the biogenesis of the glycoprotein (G) of vesicular stomatitis virus (VSV). Using immunofluorescence microscopy, we observed the wild type G protein in the SER of infected cells. When these cells were infected with the mutant VSV strain ts045, the G protein was unable to reach the Golgi apparatus at 40°C, but was able to exit the rough endoplasmic reticulum (RER) and accumulate in the SER. Ribophorin II, a RER marker, remained excluded from the SER during the viral infection, ruling out the possibility that the infection had destroyed the separate identities of these two organelles. Thus, the mechanism that results in the retention of this mutant glycoprotein in the ER at 39.9°C does not limit its lateral mobility within the ER system. We have also localized GRP78/BiP to the SER of UT-1 cells indicating that other mutant proteins may also have access to this organelle.

Upon incubation at 32°C, the mutant G protein was able to leave the SER and move to the Golgi apparatus. To measure how rapidly this transfer occurs, we assayed the conversion of the G protein's N-linked oligosaccharides from endoglycosidase H-sensitive to endoglycosidase H-resistant forms. After a 5-min lag, transport of the G protein followed first order kinetics ($t_{1/2}$ = 15 min). In contrast, no lag was seen in the transport of G protein that had accumulated in the RER of control UT-1 cells lacking extensive SER. In these cells, the transport of G protein also exhibited first order kinetics ($t_{1/2}$ = 17 min). Possible implications of this lag are discussed.

THE rough ER (RER)[1] of eukaryotic cells is an extensive ribosome-studded organelle that encompasses the outer membrane of the nuclear envelope and ramifies extensively throughout the cytoplasm. It is the site of synthesis of plasma membrane, secretory, and lysosomal proteins as well as the resident proteins of "intermediary organelles" such as the Golgi apparatus (35, 58, 59).

The ER is also the site of lipid synthesis (11). In cells engaged in the synthesis of large quantities of lipids, a second ribosome-free or smooth form of the ER is prominent. The membranes of the RER and smooth ER (SER) are continuous and, in hepatocytes, serum albumin is found in the lumen of both the rough and smooth domains of the ER system (27, 62). Much less is known about the role of the SER in the intracellular transport of integral proteins of the plasma membrane. In spite of the continuities between these two regions of the ER, the SER lacks some of the integral proteins found in the RER. As might be expected, the SER lacks the signal recognition particle receptor (docking protein) that is required for cotranslational protein import (33). It also lacks two major transmembrane glycoproteins, ribophorins I and II, that may also be involved in ribosome binding (33, 37, 38). It is thus an open question whether integral membrane proteins bound for the plasma membrane have access to the membranes of the SER.

To investigate the role of the SER in plasma membrane protein biogenesis, we have taken advantage of a mutant chinese hamster cell line UT-1. These cells have multiple copies of the gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (18). When grown in the absence of exogenous cholesterol and challenged with mevinolin or other inhibitors of HMG-CoA reductase, these cells are induced to synthesize this enzyme and also to elaborate an extensive crystalloid SER system in which the enzyme accumulates (18, 24). Here we report on the ability of the G protein of vesicular stomatitis virus (VSV) to enter and exit the SER system of these cells.

Materials and Methods

Growth and Infection of UT-1 Cells

Cells were grown in medium containing equal parts DME and Ham's F-12 in an atmosphere of 5% CO_2/95% air. The medium of control UT-1 cells (devoid of SER) was supplemented with 5% FBS. SER was induced and maintained in UT-1 cells by growth in medium supplemented with 40 μM mevinolin (1) and either with 5% lipoprotein deficient serum (LPDS) or 5% fatty acid free FBS (16, 52). These cholesterol-free sera gave identical results. Mevinolin was generously provided by A. Alberts of Merke and prepared as previously described (23). To stimulate the production of SER, UT-

1. *Abbreviations used in this paper*: ACAT, acyl coenzyme A:cholesterol acyltransferase; G protein, glycoprotein; LPDS, lipoprotein deficient serum; RER, rough ER; SER, smooth ER; VSV, vesicular stomatitis virus.

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1 cells were plated at a density of $6 \times 10^3/cm^2$ and incubated at 37°C for at least 3 d. They were maintained with SER by subsequent passage in the same medium.

VSV stocks were maintained as previously described (8). The day before an experiment, cells were plated at 3.5×10^5/35-mm dish. (For immunofluorescence experiments, the cells were plated on No. 1.5 cover slips). The next day, the cells were infected at a multiplicity of 20 pfu/cell. The virus was allowed to adsorb to the cells for 30 min at 32°C (8). At the end of the adsorption period, the inoculum was replaced with 1 ml of growth or labeling medium (see below).

Fixation and Labeling for Immunofluorescence Microscopy

Procedures were essentially as described previously (48). Glycoprotein (G protein) was localized using three successive labeling steps: 10 μg/ml affinity-purified rabbit antibody to the G protein, biotin conjugated goat anti-rabbit (Vector Laboratories, Inc., Burlingame, CA), and streptavidin conjugated to Texas red (Bethesda Research Laboratories, Gaithersburg, MD). Each labeling step was for 20 min and was separated by two 10-min washes in PBS (25 mM sodium phosphate, 25 mM potassium phosphate, 150 mM NaCl, pH 7.5). Unless otherwise indicated, commercially available staining reagents were always used at the concentrations recommended by the supplier. SER was labeled by addition of 4 μg/ml fluorescein-conjugated concanavalin A (Sigma Chemical Co., St. Louis, MO) to the streptavidin Texas red. To localize simultaneously ribophorin II and G protein, the three successive labeling solutions contained: (*a*) monoclonal antibody to ribophorin II (No. 18A4) diluted 1:50 in PBS and affinity-purified rabbit anti-VSV G protein; (*b*) biotin-conjugated horse anti-mouse IgG (Vector Laboratories, Inc.) and fluorescein-conjugated goat anti-rabbit IgG (Boehringer Mannheim Biochemicals); (*c*) Texas red-conjugated streptavidin and fluorescein-conjugated swine anti-goat IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN). Pilot experiments using UT-1 cells that lacked extensive SER revealed that the distribution of both ribophorin II and the ts045 G protein at 39.9°C were the same as that seen previously in normal Chinese hamster ovary (CHO) cells (10) (data not shown). Monoclonal antibody No. 18A4 was a kind gift of David I. Meyer. The same staining protocol was used to localize simultaneously the G protein and HMG-CoA reductase except that monoclonal antibody A9 to HMG-CoA reductase (a generous gift from R. G. W. Anderson) was used instead of antibody 18A4.

Radioactive Labeling

Virus was adsorbed to the cells as described above. Immediately after the adsorption period, the inoculum was replaced with 1 ml of labeling medium and the cells were incubated at 39.9°C for 2 h. The labeling medium for cells with extensive SER was MEM lacking methionine and glutamine (Flow Laboratories, Inc., McLean, VA) supplemented with 2 mM glutamine, 20 mM Hepes (pH 7.2), 1% DME, 40 μM mevinolin, 5% fatty acid-free FBS, and 50 μCi/ml [^{35}S]methionine. For pulse chase studies, these cells were infected and incubated in their normal growth medium for 2 h at 37°C. The cells were then rinsed once in medium without methionine and incubated with prewarmed pulse medium (MEM without methionine, 40 μM mevinolin, 20 mM Hepes, pH 7.2, 5% LPDS, 50 μCi/ml [^{35}S]methionine) at 40°C. The chase was initiated by replacement of the pulse medium with normal growth medium supplemented with 10 μg/ml cycloheximide. The control cells (without SER) were labeled with the same media except that mevinolin was omitted and dialyzed FBS was substituted for fatty acid-free serum.

Cell Lysis and Immunoprecipitation of the G Protein

After incubation for the times indicated in the text, the cells were lysed in 1 ml PBS (150 mM NaCl, 10 mM sodium phosphate, and 10 mM potassium phosphate, pH 7.5) with 1% Triton X-100 (Sigma Chemical Co.), 0.5% sodium deoxycholate (Sigma Chemical Co.), and 0.2 mM PMSF (Sigma Chemical Co.). The nuclei were removed by centrifugation for 1 min in a microfuge (model No. 5414; Eppendorf Instruments made by Brinkmann Instruments Co., Westbury, NY). The supernatant was adjusted to 0.3% SDS and 10 μg/ml affinity-purified rabbit anti-VSV G protein. Nonspecific aggregates were removed by centrifugation at 19,000 rpm for 20 min in a rotor (SS-34; Sorvall). The supernatants were transferred to new tubes and 50 μl immunoprecipitin (Bethesda Research Laboratories) was added to each tube. After a 15-min incubation at 0°C, the immunoprecipitin was collected by centrifugation for 1-min in a microfuge. The immunoprecipitates were washed, disaggregated, treated with endoglycosidase H (endo H), and analyzed by PAGE as previously described (48). The fraction of G proteins that contained endo H sensitive oligosaccharides was determined by densitometric scanning of the autoradiogram.

Results

The VSV G Protein Has Free Access to the SER

The crystalloid ER of UT-1 cells is a highly condensed structure that completely fills large domains within the cytoplasm and excludes all other organelles (3). It has the two major attributes of smooth ER. It is connected to the surrounding RER and it lacks ribosomes. Although both the RER and SER contain HMG CoA reductase, HMG CoA reductase is more concentrated in the SER. Additionally, the SER is more highly condensed than the RER (3, 18, 43, 44). Thus, by immunofluorescence microscopy using antibodies to HMG CoA reductase, labeling of the SER dominates that of the RER and gives a distinctive pattern (18, 44).

During infection with VSV, the G protein is synthesized on the RER (19, 29, 30, 41). It is subsequently transported to the Golgi apparatus and then to the plasma membrane (7, 9, 10, 60). To see whether such a membrane glycoprotein is able to enter the SER, we compared the distribution of HMG-CoA reductase with that of the ts045 G protein (a form of the G protein that is retained in the ER system at 40°C). Fig. 1 demonstrates that at 40°C these proteins have a common intracellular distribution. The arrows point to examples of discrete "patches" of SER that are labeled with both HMG-CoA reductase and G protein.

The SER of UT-1 Cells Does not Contain Ribophorin II

To discover whether infection with VSV had altered the ability of resident RER components to enter the SER, we compared the distributions of ribophorin II and the G protein of ts045 at 40°C (Fig. 2). As above, the bulk of the G protein is localized in very discrete and compact regions of the cell. In contrast, ribophorin II labeling is specifically reduced in these G protein containing structures (*arrows*). Rather, it is labeled primarily in regions immediately surrounding the SER. This is consistent with the electron microscopic observations of Anderson and co-workers that elements of the RER surround the SER of UT-1 cells, but are excluded from the densely packed crystalloid domains of the SER (3, 43). As in hepatocytes, the integral membrane protein ribophorin II is excluded from the SER (33, 37, 38). We conclude that the mechanism(s) that exclude ribophorin II from the SER do not restrict the movement of the G protein. In addition, it is clear that this mutant form of the G protein moves from the RER and accumulates in the SER of these cells.

The ts045 G Protein freely Exits the SER

The ability of this mutant G protein to resume its intracellular transport at 32°C has made it especially useful in the study of intracellular transport (5, 8, 47). We used this feature of the ts045 G protein to examine the kinetics of its export from the SER. Again we infected UT-1 cells that had extensive SER with ts045 and incubated them at 40°C. After 2 h, we added cycloheximide and shifted the cells to the permissive temperature for 0, 15, or 30 min before fixation (Fig.

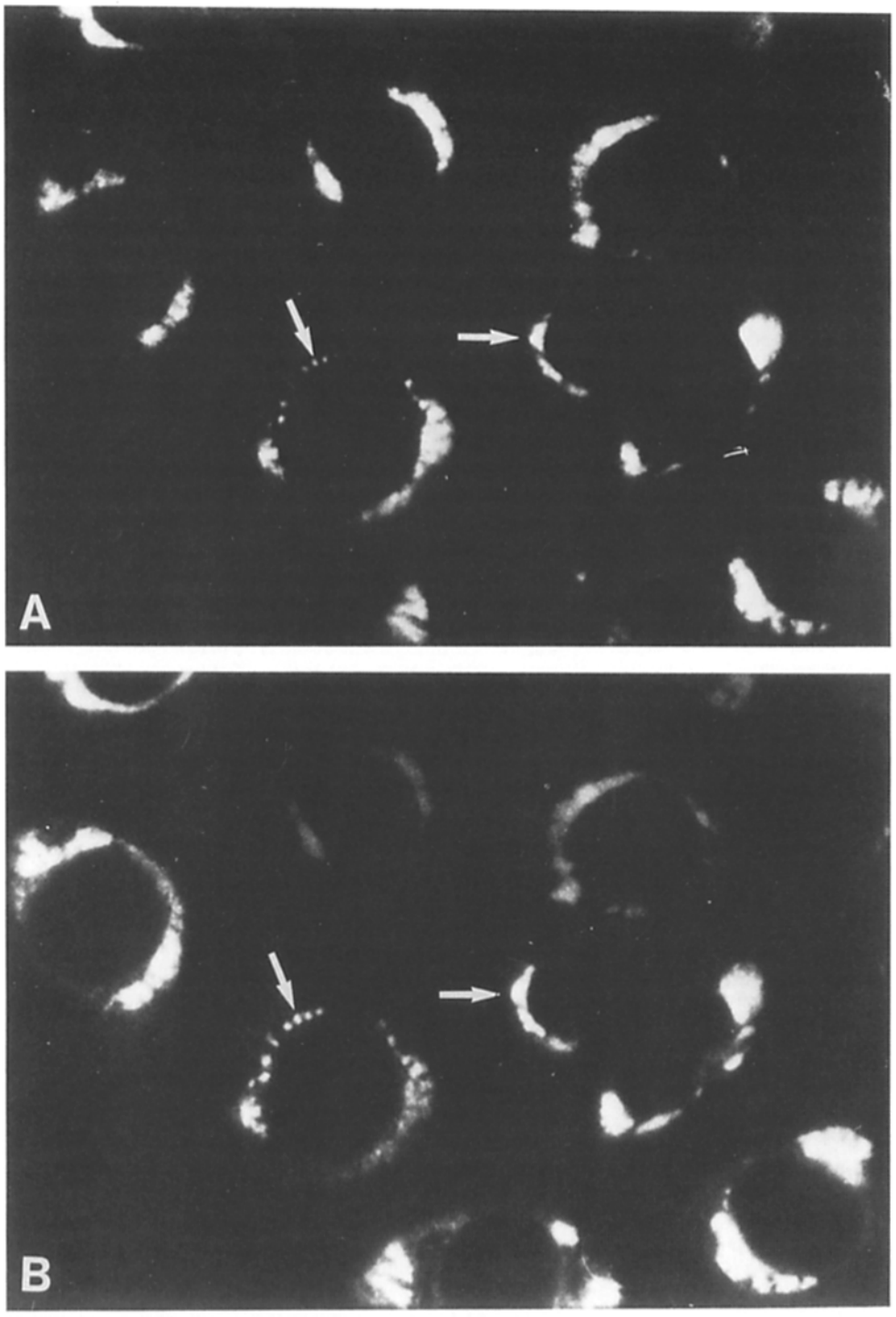

Figure 1. Colocalization of HMG CoA reductase and G protein. UT-1 cells cultured and infected as in Fig. 3 were fixed 2 h post-infection. They were double labeled for HMG-CoA reductase (*A*) and G protein (*B*) as described in the methods section. Arrows indicate examples of SER structures containing both proteins.

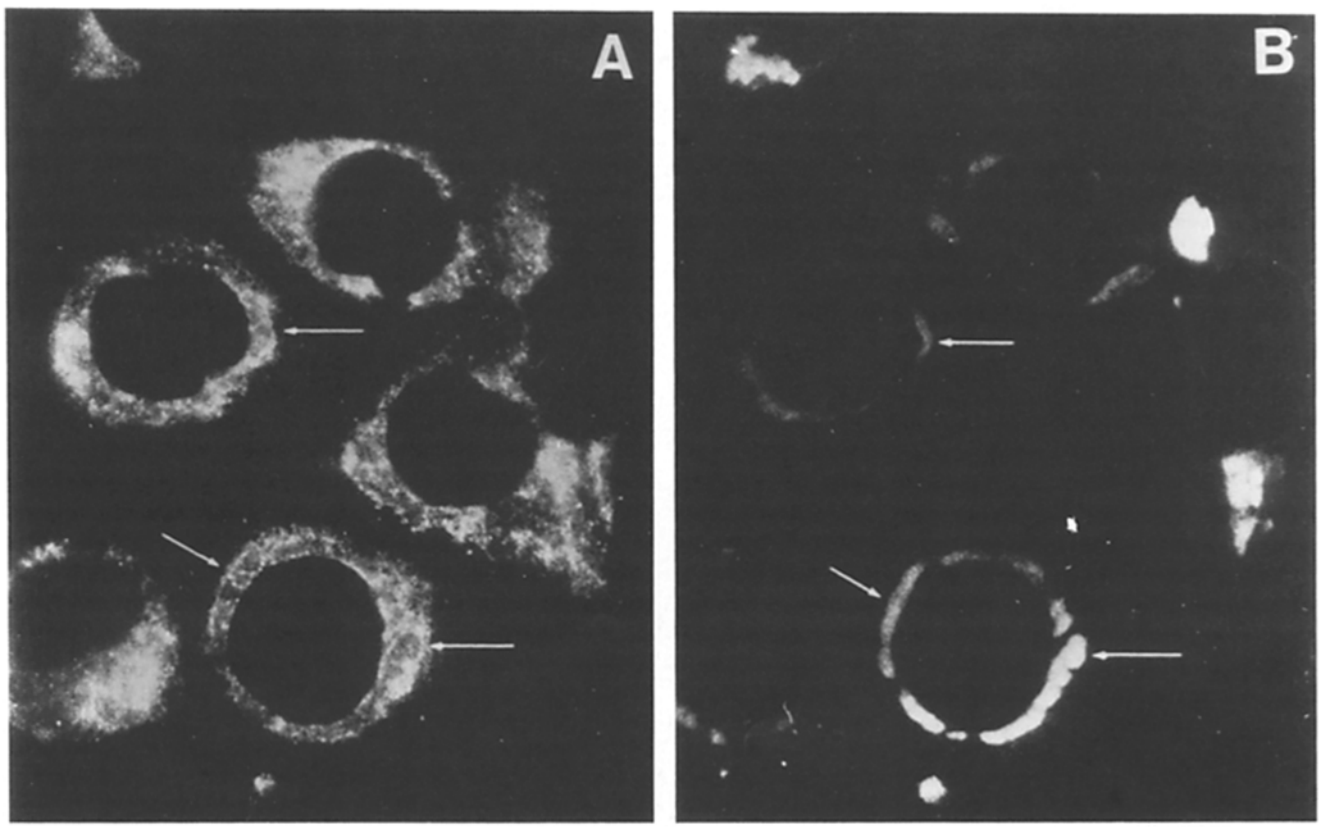

Figure 2. Simultaneous localization of the G protein and ribophorin II. UT-1 cells cultured and infected as in Fig. 3 were fixed 2 h postinfection. Double immunofluorescence labeling was carried out as described in Materials and Methods. G protein was localized in the SER (*B*, *arrows*). In contrast, ribophorin II staining was reduced in these regions (*A*, *arrows*).

3). As concanavalin A (Con A) specifically labels the ER and exhibits the same pattern of labeling as HMG-CoA reductase (39, 50), we double labeled the SER with fluorescein-Con A rather than with HMG-CoA reductase.

Before shifting to the permissive temperature, the ts045 G protein is again seen to be localized primarily in the SER (Fig. 3). However, 15 min after a shift to the permissive temperature, the G protein was in perinuclear structures that did not label with concanavalin A (Fig. 3, *C* and *D*, *large arrows*). The timing of the movement of G protein to these structures and their intracellular distribution indicated that these structures were probably the Golgi apparatus (9, 10). After 30 min, labeling of these structures was even more pronounced and very little G protein remained in the SER. Most of the structures labeled intensely with fluorescein–Con A were no longer labeled with G protein (compare small arrows in Fig. 3, *E* and *F*).

The Wild-type G Protein also Enters the SER

The data presented above suggest that an integral membrane protein does not require special sorting signals to gain entry into the SER. However, these data do not rule out the possibility that at 40°C the ts045 G protein has the conformation of a SER resident protein. The ability of this mutant G protein to resume its intracellular transport at 32°C might then be attributed to its loss of this special conformation. If this latter hypothesis were correct, we would not expect the wild-type G protein to have access to the SER. To test this possibility, we infected UT-1 cells with wild-type VSV and simultaneously localized the G protein and concanavalin A ligands by indirect immunofluorescence microscopy (Fig. 4). At 1 h postinfection, the most intense G protein labeling was again found primarily in the SER (Fig. 4, *arrows*).

GRP78/BiP Has Free Access to the SER of UT-1 Cells

GRP78/BiP is an interesting resident protein of the ER. It is not an integral membrane protein and is retained in the ER by a tetrapeptide sequence (KDEL) present at its extreme carboxy-terminus (42). In addition, it appears that such KDEL-containing proteins may be retained by an active recycling mechanism that returns them to the ER from a later compartment (45). GRP78/BiP has in turn been implicated in the ER retention of a variety of incompletely assembled and improperly folded proteins (21, 22, 25, 28, 32, 36, 45, 55). Although there is no direct evidence that GRP78/BiP retains the ts045 G protein in the ER at 40°C, we wished to

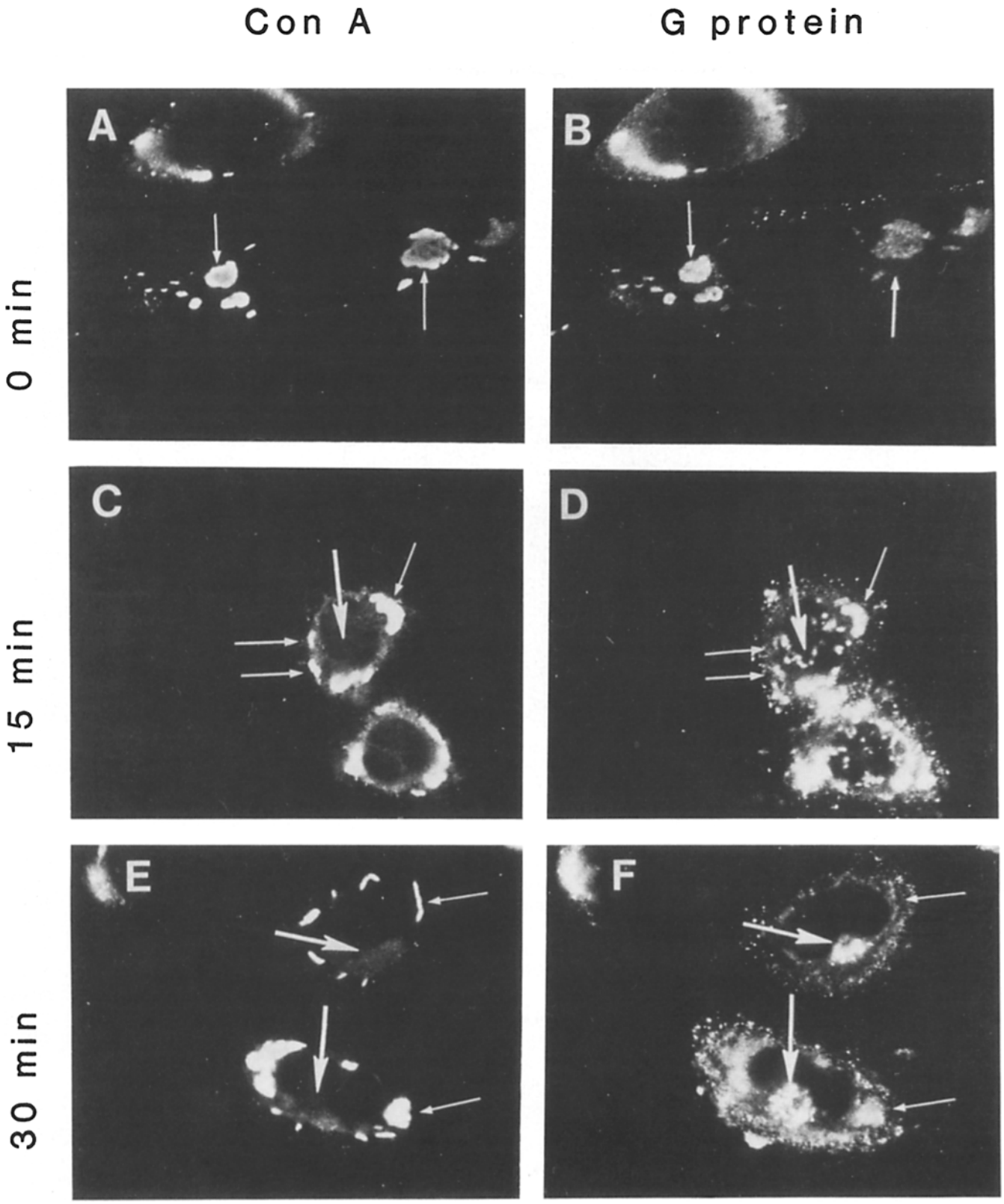

Figure 3. Synchronized transport of the ts045 G protein into and out of the SER of UT-1 cells. UT-1 cells cultured in DME/F12 with 5% fatty acid–free serum plus 40 μM mevinolin were infected with ts045 and incubated at 39.9°C. 2 h postinfection, the cells were either fixed (*A* and *B*) or transferred to the same medium containing 20 μg/ml cycloheximide at 32°C and incubated for an additional 15 min (*C* and *D*) or 30 min (*E* and *F*) before fixation. The concanavalin A ligands (*A*, *C*, and *E*) and G protein (*B*, *D*, and *F*) were simultaneously localized as in Fig. 1. Small arrows indicate condensed SER. Large arrows indicate presumptive Golgi apparatus (*D* and *F*).

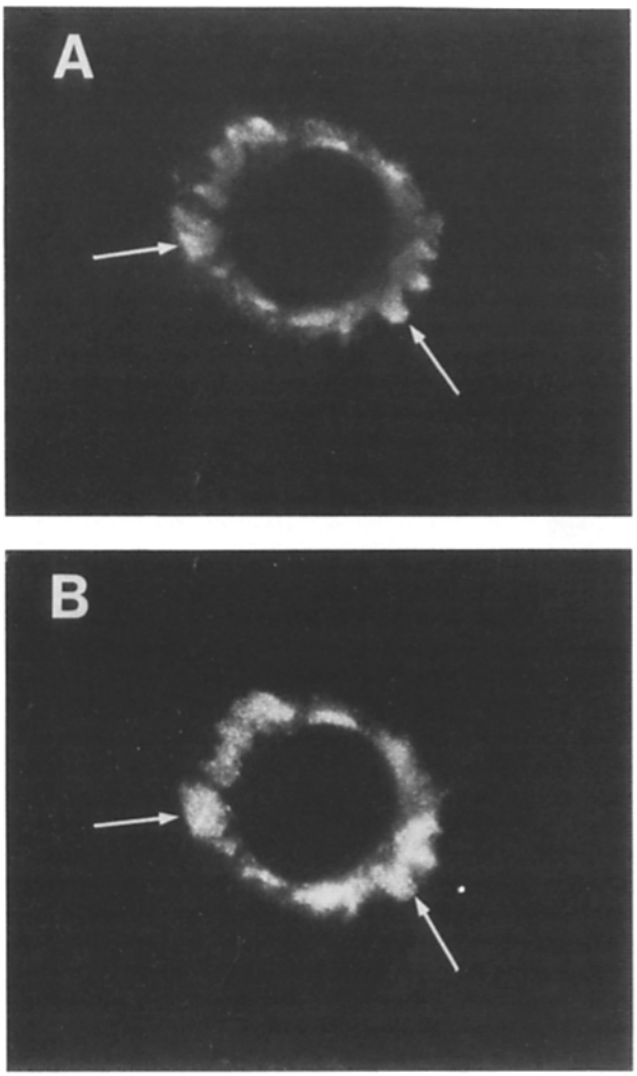

Figure 4. Localization of G protein in UT-1 infected cells. UT-1 cells cultured in FAFS plus 40 μM mevinolin were infected with wild-type VSV and incubated at 37°C for 2 h. After fixation, G protein and concanavalin A ligands were simultaneously localized as described in Materials and Methods. *A*, Distribution of G protein; arrows indicate G protein in SER. *B*, Distribution of concanavalin A ligands; arrows indicate SER structures.

learn whether GRP78/BiP also has access to the SER. We therefore fixed UT-1 cells with extensive SER and simultaneously labeled them for GRP78/BiP and concanavalin A ligands. As seen in Fig. 5, the concanavalin A ligands and GRP78/BiP codistribute. We conclude that GRP78/BiP also has free access to the SER.

The G Protein Is Transported Rapidly from the SER to the Golgi Apparatus

The immunofluorescence experiments described above indicate that the bulk of the ts045 G protein that is synthesized at 40°C accumulates in the SER of infected UT-1 cells. These experiments further indicate that upon reversal of the temperature block, the G protein leaves the SER. To quantitate directly the transfer of G protein to the Golgi apparatus, we examined the rate and extent of remodeling of the asparagine-linked oligosaccharides of the ts045 encoded G protein. This was most conveniently accomplished by examining the fraction of the G protein which changed electrophoretic mobility on SDS polyacrylamide gels after treatment with endoglycosidase H (endo H). This enzyme can remove the high mannose-type oligosaccharides present on G proteins that remained in the RER, but fails to remove the oligosaccharides from G proteins that have passed through the medial compartment of the Golgi apparatus (26, 34, 40, 56).

To reproduce accurately the conditions seen in Figs. 1–4, cells were continuously labeled with [^{35}S]methionine beginning at the time of infection. 2 h postinfection, the cells were transferred to standard growth medium and shifted to 32°C. Fig. 6 shows representative autoradiograms from such an experiment. It is readily evident that in cells containing extensive SER the G protein matures more slowly than in control cells. In cells with extensive SER, 50% of the G protein oligosaccharides are remodeled during the first 20 min at 32°C. In the control cells, this process requires only 15 min.

Using densitometric analysis of such autoradiograms, we were able to quantify the percent of the G protein that had reached the medial compartment of the Golgi apparatus at each time point (Fig. 7). In cells containing extensive SER there is a lag of ~5 min during which very few molecules of G protein exit the ER system. No such lag is seen in the movement of G protein out of the RER system of control cells. Interestingly, after this initial lag, the movement of G protein from the ER to the Golgi apparatus follows first order kinetics in both cell types, indicating that exit from the ER is a stochastic process. The time constant for this transfer was essentially the same in both cell types (15 min in cells with SER, 17 min in control cells). In these experiments, all the G proteins synthesized during the infection were labeled. In the UT-1 cells cultured in fatty acid free serum and 40 μM mevinolin, the bulk of these G proteins had accumulated in the SER. Thus, if one ignores the lag, the G protein is able to leave the SER and move to the Golgi apparatus as quickly as it leaves the RER of control cells.

The difference in the initial kinetics of G protein movement in these two cell types was quite reproducible (see the error bars in Fig. 7 *A*). If the lag were because of a requirement for the bulk transfer of protein from the SER back to the RER, we would have expected the distribution of G protein seen after a 15-min chase (Fig. 3, *C* and *D*) to approximate that of ribophorin II (Fig. 2). Instead, the G protein was seen either in the SER or in a concentrated perinuclear structure (Fig. 3 *D*, *large arrow*). We therefore hypothesized that at 40°C, cells with extensive SER might not sustain some maturational steps supported by the control cells. If this were the case, cells without extensive SER should also exhibit a lag in the transport of G proteins that had not had time to mature at 40°C. To test this hypothesis, we pulse-labeled both cell types for 2 min at the nonpermissive temperature and then immediately examined their transport rates. We reasoned that 2 min is shorter than the lag seen in Fig. 7 *A* and should not allow maturation in either cell type. As predicted, both cell types transported pulse-labeled G protein to the Golgi apparatus after a 5-min lag (Fig. 7 *B*). After the initial lag, newly synthesized G protein left the ER with kinetics similar to those seen with long term labeled G protein. The $t_{½}$ for transfer from the ER to the Golgi apparatus was 11 min for control cells and 17 min for cells with extensive SER.

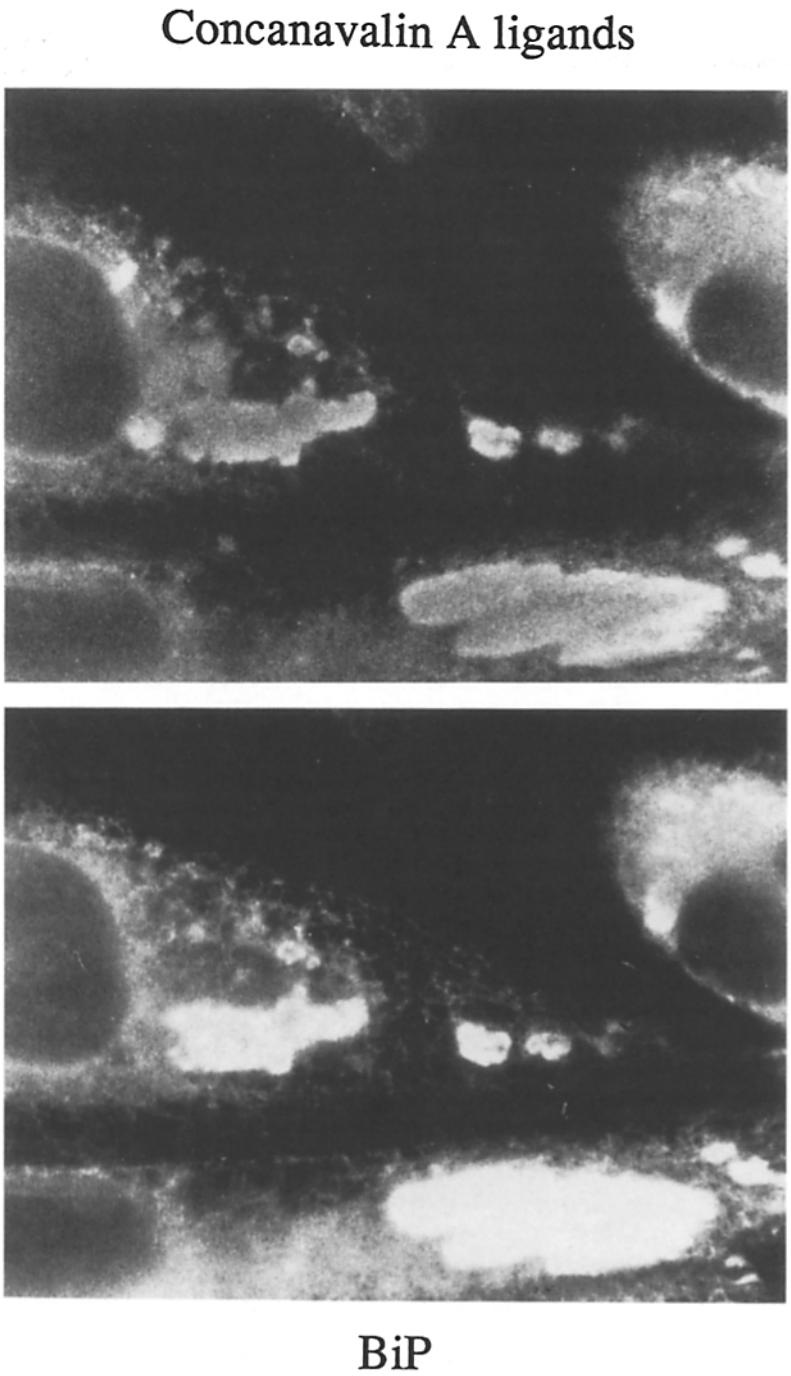

Figure 5. Subcellular localization of GRP78/BiP. Uninfected UT-1 cells were fixed, permeabilized, and labeled with fluorescein-concanavalin A (*upper panel*) or GRP78/BiP (*lower panel*).

These results are consistent with our hypothesis that the G protein can readily exit the SER of UT-1 cells, but that the SER is less efficient than the RER in promoting a maturation step required for transport.

Discussion

In this paper, we have investigated the nature of the connections between the rough and smooth domains of the ER. The SER of UT-1 cells displays the major characteristics of the SER in other cells. It is the site of reactions required for lipid synthesis and its membrane is smooth although continuous with that of the RER (3, 43, 44). Like the SER of the hepatocyte, ribophorin II is excluded from its membrane (Fig. 2; references 33, 37, 38). Finally, the highly condensed tubular and cisternal SER structures seen in UT-1 cells have been seen in guinea pig adrenal glands as well as the antebrachial organ of the lemur (12, 13, 53).

Under conditions in which ribophorin II was restricted to the rough domain of the ER, the G protein of VSV readily passed into the SER. As the G protein moves ultimately to the plasma membrane, these data suggest that the ability to enter the SER is not restricted to specific resident proteins of that organelle. It therefore seems likely that the identity of the RER is created by the specific retention of a class of proteins within a restricted domain of the ER system (see be-

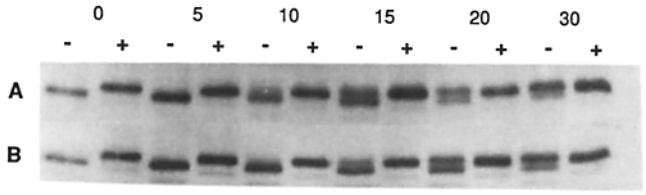

Figure 6. Endoglycosidase H analysis of total G protein transport to the Golgi apparatus. UT-1 cells were cultured in either medium containing 5% FBS (*A*) or 5% LPDS and 40 μM mevinolin (*B*). Immediately after the virus adsorption period, the cells were continuously labeled with [^{35}S]methionine at 39.9°C for 2 h. They were then shifted to 32°C for 0, 5, 10, 15, 20, or 30 min before lysis. G protein was immunoprecipitated from the cell lysates, either treated (+) or mock treated (−) with endoglycosidase H, and subjected to analysis by SDS-PAGE.

low). This proposal is consistent with previous work showing that only a small number of the resident ER proteins are restricted to the RER (37, 38).

Retention of ts045 in the ER Is not Mediated by Immobilization

Ceriotti and Colman (17) found that the rotavirus protein VP10 and a mutant form of the influenza virus hemagglutinin HA_{env} did not readily diffuse within the ER of the *Xenopus* oocyte. In contrast, we found that the ts045 G protein is able to move from the RER to the SER at the nonpermissive temperature even though it appears to form aggregates immediately after synthesis (20). Evidently, these aggregates and whatever mechanisms restrict the ts045 G protein to the ER system (56) fail to inhibit the lateral mobility of this G protein in the ER membrane. The observation that GRP78/BiP is also present in the SER of UT-1 cells suggests that the diverse proteins that associate with GRP78/BiP during their retention in the ER system have access to the SER. In fact, work of Pelham indicates that these proteins may not be strictly retained in the ER but cycle back from a post-ER compartment (45). Thus, ER retention of such proteins cannot be attributed to their immobilization in the ER membrane.

The G Protein is Rapidly Transported from the SER to the Golgi Apparatus

Despite the highly specialized structure of the SER of UT-1 cells, the G protein that accumulated in the SER is readily transported to the Golgi apparatus. Indeed, G proteins that accumulated in the SER reached the Golgi apparatus in approximately the same time as those that accumulated in the RER with a $t_{1/2}$ of ~16 min. Unexpectedly, a 5-min lag was seen in the transport of the SER resident G proteins, but not those resident in the RER. Using immunofluorescence microscopy, we were able to show that the G protein did not quantitatively return from the SER to the RER during these 5 min. Even 15 min after the temperature shift, a sizable fraction of the G protein remained in the SER (Fig. 3, *C* and *D*). However, we could not rule out the possibility that there was a net flow of some of the G protein from the SER to the RER during this time. A quantitative kinetic model of intracellular transport will therefore be required to properly judge the significance of these observations. We have proposed an alternate and equally simple explanation for the observed lag. We hypothesized that at 39.9°C the RER but not the SER supports a required "maturational step" in the biogenesis of the G protein. At 32°C, this maturation might either occur spontaneously or be supported by both the RER and SER. This hypothesis was supported by our observation that newly synthesized G proteins exhibited the same 5-min lag in UT-1 cells with or without extensive SER systems. Balch and coworkers have also seen such a lag in the processing of pulse labeled G protein (5). A priori, it is clear that this lag in the transport of newly synthesized G protein is not because of a requirement for it to move to the RER. It must therefore

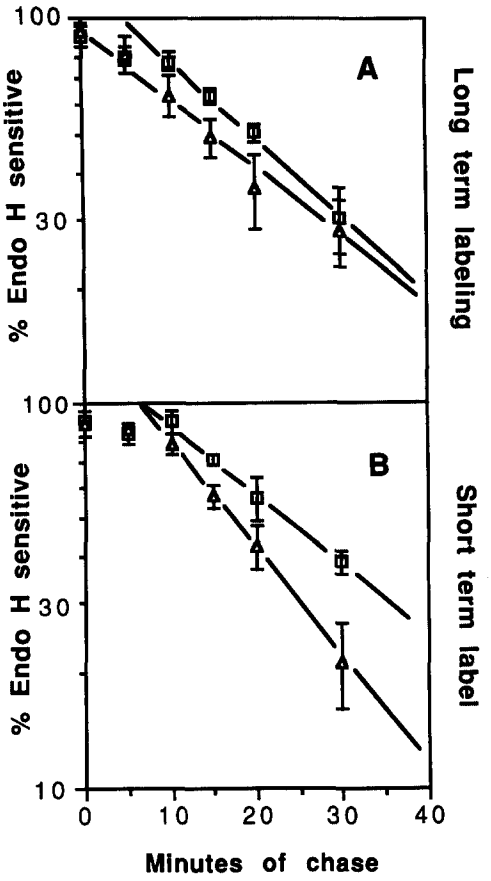

Figure 7. The transport kinetics of newly synthesized G protein. UT-1 cells were cultured in either medium containing 5% LPDS and 40 μM mevinolin (*squares*) or 5% FBS (*triangles*). The fraction of the G protein that was sensitive to endoglycosidase H digestion was quantitated by densitometric scanning of autoradiograms such as those in Fig. 6. *A* shows the mean and standard deviation of data gathered from four experiments carried out as in Fig. 6. *B* shows the results of experiments in which cells were infected, incubated for 2 h at 37°C, pulse-labeled for 2 min with [^{35}S]methionine at 39.9°C, and shifted to 32°C for 0, 5, 10, 15, 20, or 30 min, and then lysed. G protein was immunoprecipitated from the cell lysates and either treated or mock treated with endoglycosidase H as indicated. The fraction of the G protein that was sensitive to endoglycosidase H digestion was quantitated by densitometric scanning of the autoradiograms. The mean and standard deviation of the data from three experiments are plotted. In both *A* and *B*, the data from 10, 15, 20, and 30 min of chase were subjected to linear regression analysis. The lines show the best fit to the data (for both curves in *A*, $R > 0.97$; in *B*, $R > 0.99$).

reflect a requirement either for maturation of the G protein or for G protein's movement to yet another compartment distinct from both the RER and SER.

In conclusion, these data are most consistent with our hypothesis that the observed lag in transport was caused by a requirement for G protein maturation rather than for its return to the RER. However, further work will be required to definitively distinguish between these possibilities. In either case, exit from the SER was rapid and demonstrated that the SER is an active component of the secretory pathway.

Why Should the Cell Maintain a SER System?

The data presented here and in previous studies (27, 62) show that both a soluble protein (serum albumin) and an integral membrane protein (the G protein) have facile access to the SER. In contrast, the proteins involved in ribosome binding are absent from the membranes of the SER (33, 37, 38). If these proteins were not restricted to a "rough" domain of the ER system, the ribosomes would bind to all the ER membranes and no distinct SER would remain. By restricting RER functions to a limited region of the total ER membrane, the cell is able to maintain the local concentration of such complexes while independently regulating the total surface area of the ER system (14, 18, 24, 54). This maintains the efficiency of the ER's protein import system against dilution effects as the membrane system becomes more extensive. Polysomes initiating the insertion of nascent polypeptides are already associated with the rough domain of the ER via more mature growing polypeptides.

Posttranslational functions are not so easily restricted. Unless both the target protein and the modifying enzyme are restricted to the rough domain of the ER, either the enzyme or the substrate will be diluted as the ER enlarges. For example, the ER system of UT-1 cells grown in mevinolin in the absence of exogenous cholesterol is at least four times the size of the ER of control UT-1 cells (43). Many resident ER proteins would therefore be diluted by this factor. In our experiments, we observed a lag in the transport of G protein that had accumulated in the SER and proposed that this lag might be because of the inability of the SER to support a maturational step at 39.9°C (see above). It is quite possible that a "defect" such as we proposed might simply result from the dilution of the G protein and an ER resident maturation factor.

There are other potential advantages of segregating domains of the ER. As with other organelles, compartmentalization of the ER also provides the cell with the potential to order a set of reactions. For example, using indirect immunocytochemistry Alexander et al. found apolipoprotein B (apo B) only in the rough and transitional elements of the ER. They failed to detect apo B in the SER even in association with nascent VLDL particles (2). Also, using cell fractionation techniques, several laboratories have found that acyl coenzyme A:cholesterol acyltransferase (ACAT) also remains segregated in the RER (4, 31, 46, 57). As ACAT is required for the synthesis of the cholesterol esters of VLDL, it is tempting to speculate that the segregation of apo B and ACAT in the RER is important in ordering the stepwise assembly of VLDL particles (6, 15). Reinhart et al. proposed the alternative possibility that ACAT is needed to maintain the low concentration of nonesterified cholesterol in the RER (46).

The Rate of ER to Golgi Transport Is Tightly Regulated

Except for a lag of ~5 min, the rate of transport of the G protein from the ER to the Golgi apparatus was similar in UT-1 cells with and without an extensive SER system. This observation is quite surprising in light of the findings of Orci et al. that the absolute volume of the ER system differs at least fourfold between these cell types (43). Rothman and co-workers have proposed that the transport of proteins between the ER and the Golgi apparatus is mediated by the bulk flow of ER-derived vesicles between these organelles (51, 61). They further proposed that the limiting rate constant supported by such a bulk flow is 10 min implying that 50% of the ER lipid moves through the Golgi apparatus every 10 min (61). As the rate constant for G protein transport is 11–17 min in cells with and without extensive SER, the logical extension of this proposal would be that the absolute flux of lipid between the ER and the Golgi apparatus varies 4-fold between these cell types. As the size of the Golgi apparatus is the same in both cell types (43), the mechanisms that control the transport of lipid back from the Golgi apparatus to the ER would have to regulate directly the size of that organelle.

An alternate and simpler view is that the rate of bulk flow is not the rate limiting event in protein transport between the ER and the Golgi apparatus. Rapid packaging could be mediated by the concentration of proteins in specialized transitional regions of the ER prior to transport (9). Indeed, Rose and Doms have estimated that bulk flow proceeds more slowly than the transport of G protein (49). It is therefore possible that other processes, such as folding, limit the rate of G protein transport from the ER to the Golgi apparatus (21, 22, 25, 28, 32, 36, 45, 55). At least some steps in protein folding should be intrinsic to a single polypeptide and would be independent of the volume of the ER. Other steps such as incorporation of the polypeptide into a multimeric protein would require the participation of other polypeptide chains. Such steps should be sensitive to the concentration of these other polypeptides in the ER. As discussed above, the activity of maturation promoting factors would also be sensitive to the volume of the ER. UT-1 cells should provide a good system in which to examine the dependence of folding, multimerization, and transport of a variety of proteins on the volume of the ER system.

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