

Immunocytochemical Analysis of the Transfer of Vesicular Stomatitis Virus G Glycoprotein from the Intermediate Compartment to the Golgi Complex

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Abstract. We performed an immunocytochemical analysis to study the transfer of a marker protein (G glycoprotein coded by vesicular stomatitis virus ts 045 strain) from the intermediate compartment to the Golgi stacks in infected Vero cells. The intermediate compartment seemed to consist of about 30–40 separate units of clustered small vesicles and short tubules. The units contained Rab2 protein and were spread throughout the cytoplasm, with a ratio of about 6:4 in the peripheral versus perinuclear site. Time-course ex-

periments revealed a progressive transfer of G glycoprotein from the intermediate compartment to the Golgi stacks, while the tubulo-vesicular units did not appear to change their intracellular distribution. Moreover, the labeling density of peripheral and perinuclear units decreased in parallel during the transfer. These results support the notion that the intermediate compartment is a station in the secretory pathway, and that a vesicular transport connects this station to the Golgi complex.

SEVERAL independent lines of evidence suggest that the traffic between the ER and the Golgi complex involves other membrane-bound structures that may form distinct compartment(s). These structures have been variously named: pre-Golgi vacuoles (33, 36), budding compartment (42), intermediate compartment (37–39), intermediate elements (34), salvage compartment (14, 22, 45), and *cis*-Golgi network (9, 13, 26, 32). However, the morphology of these structures has yet to be defined, and the mechanism of the transport between the ER and Golgi complex is still largely unknown (1, 25, 30, 32).

To address an aspect of this problem we used the G glycoprotein synthesized by the VSV ts-045 mutant strain, which is one of the best tools available for the study of ER export in animal cells. Indeed, at the non-permissive temperature (39°C) G glycoprotein accumulates in the ER as a multimeric aggregate (11), and there is very little protein export. When the temperature is lowered to 31°C, the aggregated protein trimerizes and leaves the ER (8, 16). At 39°C, high amounts of G glycoprotein are synthesized by the infected cell, and good anti-G antibodies are available. Thus, detailed morphological studies on the export of G glycoprotein from the ER have been performed looking at the first minutes after temperature shift-down to 31°C (3, 4).

We have already applied this approach to study, with light microscopy, infected cells previously kept at 39°C and then shifted to 15°C (5). Conventional and confocal immunofluorescence microscopy showed a dotted labeling spread throughout the cytoplasm, a pattern totally different from the reticu-

lar (ER) and the perinuclear (Golgi) staining obtained at 39 and 31°C, respectively. Using a similar protocol, Schweitzer et al. showed that at 15°C G glycoprotein accumulates in tubulo-vesicular structures assumed to be the intermediate compartment (38). Here we describe an immunocytochemical study of the transfer of G glycoprotein from this intermediate compartment to the Golgi stacks.

Materials and Methods

Materials

All culture reagents were supplied by Gibco Laboratories (Grand Island, NY) and all culture plasticware by Falcon (Becton Dickinson, Meylan Cedex, France). Cycloheximide was obtained from BDH Chemicals Ltd. (Poole, U.K.). Rhodamine-conjugated goat anti-mouse IgG were from Jackson Immunoresearch (West Grove, PA). Protein A gold (5 nm) was obtained from Amersham International (Buckinghamshire, U.K.). Moviol was purchased from Thomas Scientific (Philadelphia, PA). Affinity purified polyclonal anti VSV G glycoprotein (27) and anti Rab2 protein (6) antibodies were kindly provided by Drs. K. Simons and M. Zerial (European Molecular Biology Laboratory, Heidelberg, Germany), respectively.

Cell Culture, Virus Infection, and Indirect Immunofluorescence

Propagation of VSV ts045 strain, culture and infection of Vero cells, and indirect immunofluorescence were performed exactly as described (5).

Immunoelectron Microscopy

Cells were fixed with 1.0% glutaraldehyde in PBS for 1 h at room temperature, partially dehydrated in ethanol, and embedded in LR White resin.

Thin sections were collected on nickel grids, immunolabeled with antibodies and then with Protein-A gold (18 nm) prepared by the citrate method (19, 20, 31, 40). In double labeling experiments the sections were first incubated with anti-Rab2 antibody, followed by 18 nm Protein-A gold, and then with anti G protein antibody followed by 5 nm Protein-A gold particles (2). All sections were stained with uranyl acetate and lead citrate before examination with EM.

Conventional Electron Microscopy

Cells were post-fixed in 1% osmium tetroxide in veronal acetate buffer, pH 7.4, for 2 h at 4°C, stained with uranyl acetate (5 mg/ml), dehydrated in acetone, and finally embedded in Epon 812. Thin sections were examined unstained and post-stained with uranyl acetate and lead hydroxide.

Quantitative Evaluation of Immunolabeling

Density of labeling, determined as gold particles divided by the area (in μm^2) of the relevant organelles, and statistical analysis were performed with the Sigma Scan Measurement System (Yandel Scientific, Corte Madera, CA). Vero cells usually show an elongated, fibroblast-like morphology with the nucleus roughly in the center. To study the intracellular distribution of the tubulo-vesicular structure units we used low magnification images and scored as "perinuclear" those found within 2 μm of the nuclear envelope; all others were defined "peripheral."

Results

Immunofluorescence Analysis of the Transfer of G Glycoprotein from the Intermediate Compartment to the Cell Surface

Parallel cultures of Vero cells grown on glass coverslips were infected and incubated at 39°C to allow synthesis and ac-

cumulation in the ER of G glycoprotein. 3.5-h post-infection, cycloheximide was added to inhibit further synthesis and the cultures were brought down to 15°C for 1 h. At this stage, the immunofluorescence showed a dotted staining throughout the cytoplasm, and concentration tended to vary in the perinuclear area (Fig. 1 *a*). This pattern reflected the accumulation of G glycoprotein in a location intermediate between the ER and Golgi complex (5), a finding confirmed by the demonstration that p53 and β -COP proteins colocalize with G protein at 15°C (10, 38). We next determined the effect of shifting the temperature for different lengths of time to 31°C, the permissive temperature for G glycoprotein transport. As shown in Fig. 1 (*b–e*) the labeling pattern remained practically unchanged after 2.5 min of incubation, whereas after 5 min the signal was still largely dotted but more concentrated in the perinuclear area. After 10 min most of the signal was confined to the perinuclear area, and after 30 min it was detectable also on the cell surface. Immunoelectron microscopy has shown that G glycoprotein reaches the Golgi complex when the cells are shifted for 10 min to the permissive temperature (3, 4); thus, the pattern shown in Fig. 1 *d* is probably due to Golgi staining. Conversely, we could not draw any conclusion from the images obtained 2.5 and 5 min after the temperature shift because it was not known in which structures G glycoprotein was present. To address this question we conducted an immunoelectron microscopical analysis, starting with the characterization of the structures that accumulate G glycoprotein at 15°C.

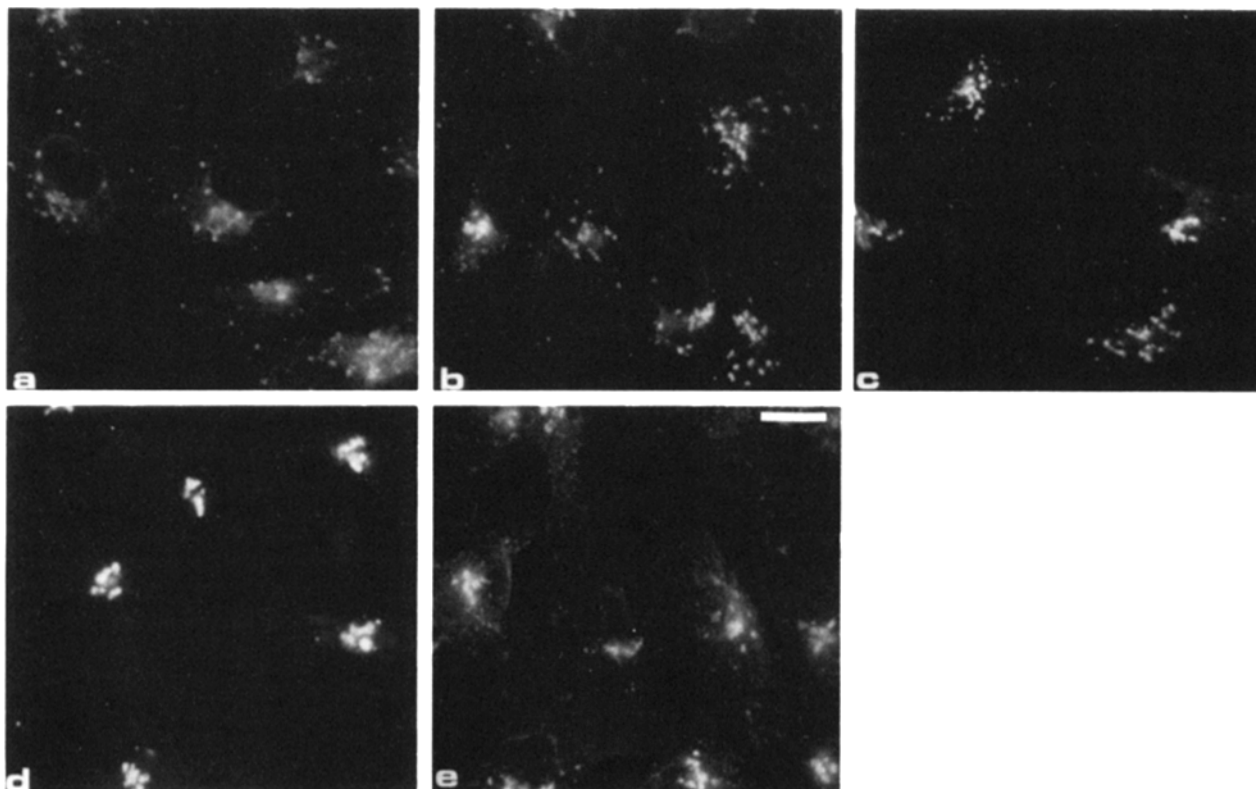


Figure 1. Immunofluorescence analysis of the transfer of G glycoprotein from the intermediate compartment to the cell surface. Vero cells were infected and treated as detailed in the text. After 1 h of incubation at 15°C, parallel cultures were shifted to 31°C for 0 min (*a*), 2.5 min (*b*), 5 min (*c*), 10 min (*d*), 30 min (*e*). Bar, 5 μm .

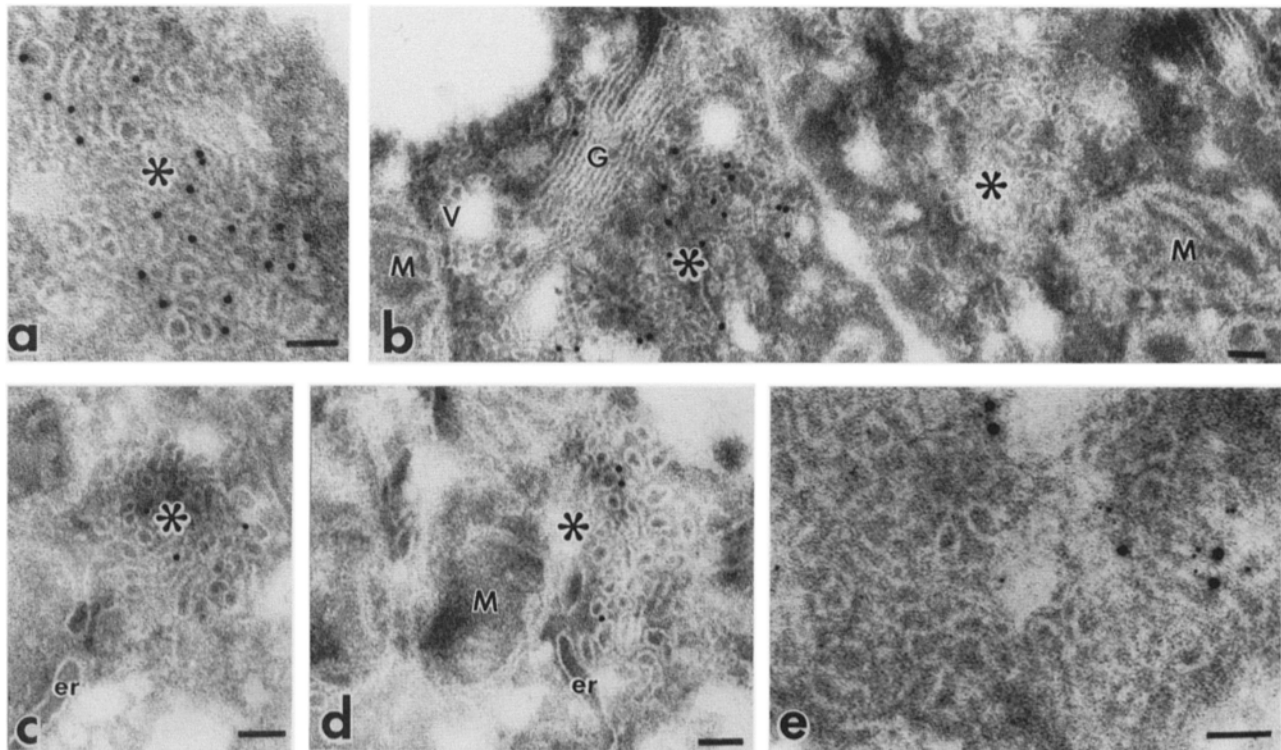


Figure 2. Immunoelectron microscopy of infected cells incubated 1 h at 15°C. (*a* and *b*) Anti G protein antibody. Labeling is rich in short tubules and small vesicles. Tubules and vesicles are clustered together to form roughly circular areas (*asterisks*). Golgi cisternae (*G*) are almost unlabeled. Note in *b* an adjacent cell, probably not infected, showing an unlabeled tubulo-vesicular structure. (*c* and *d*) Anti Rab2 protein antibody. Labeling is specifically localized on the tubulo-vesicular clusters: the average number of colloidal gold particles divided by the area was 28.8 ± 3.1 for the tubulo-vesicular clusters, 1.3 ± 0.9 for the ER, and 0.7 ± 0.3 for the mitochondria. (*e*) Double labeling with anti Rab2 protein antibody (18 nm gold particles) and anti G protein antibody (5 nm gold particles). Labeling colocalizes on the same tubulo-vesicular structure. *M*, mitochondria; *V*, vacuole; *er*, endoplasmic reticulum. Bars, 0.1 μm .

Immunoelectron Microscopy of Infected Cells Incubated at 15°C

Immunoelectron microscopy of cells manipulated as described above showed labeling specifically localized in circular areas characterized by numerous small vesicles (average diameter 80 nm) in close proximity to short tubules (Fig. 2, *a* and *b*). These labeled structures were observed in the perinuclear region of the cells, near the Golgi complex (Fig. 2 *b*), as well as at the cell periphery. In general, they occurred close to the ER cisternae and, in some instances, to vesicular protrusions of the ER (data not shown, but see below, Fig. 3, *a* and *b*), which resembled the transitional elements of the ER described in the exocrine pancreas (15, 21, 35, 41). The tubulo-vesicular structures were morphologically distinguishable from the ER and Golgi stacks thanks to the circular appearance of the area (average diameter <1 μm), and the close clustering of the vesicles and tubules. The ER cisternae were weakly immunolabeled at 15°C, and Golgi cisternae were practically unlabeled (see below, Table I A). Examination of serial sections suggested that the tubulo-vesicular structures labeled at 15°C were formed by small vesicles and short tubules (data not shown); however, we cannot rule out the possibility that these structures were formed by extremely convoluted and narrow tubules that had a constant diameter of ~ 80 nm. Next, we performed single and double immunolabeling with an anti-peptide antibody directed against Rab2 protein, a GTP binding protein that

has been recently localized in membrane structures intermediate between the ER and Golgi complex and in the *cis*-side of the Golgi stacks (6). As shown in Fig. 2, *c* and *d*, immunolabeling with the anti Rab2 antibody was weak but specifically associated with the tubulo-vesicular structures

Table I. Quantitation of the Immunoelectron Microscopical Analysis of the Time-course of G Protein Transfer from the Intermediate Compartment to the Golgi Complex

min at 31°C	A		B	
	Density of labeling \pm SEM		Percent distribution of the tubulo-vesicular structures	
	Golgi complex	Tubulo-vesicular structures	Peripheral location	Perinuclear location
0.0	13 \pm 2.4	88 \pm 5.0	60 (86.0)	40 (91.1)
2.5	49 \pm 8.6	88 \pm 11.0	55 (80.2)	45 (99.8)
5.0	96 \pm 9.1	55 \pm 5.0	58 (59.0)	42 (49.9)
10.0	158 \pm 4.2	35 \pm 5.3	53 (34.3)	47 (35.7)

(A) Average total number of colloidal gold particles (see Materials and Methods) on Golgi complexes and tubulo-vesicular structures after incubation with anti G glycoprotein antibody. In uninfected cells treated in parallel, 2.42 ± 1.36 gold particles were detected on Golgi stacks and 1.95 ± 1.52 on tubulo-vesicular structures. (B) Intracellular distribution of the tubulo-vesicular structure units during the transfer of G protein to the Golgi complex (see Materials and Methods). Values are given as percent of total; the corresponding density of labeling, calculated as in A, is indicated in parentheses. Each time-point value derives from the analysis of at least 15 profiles.

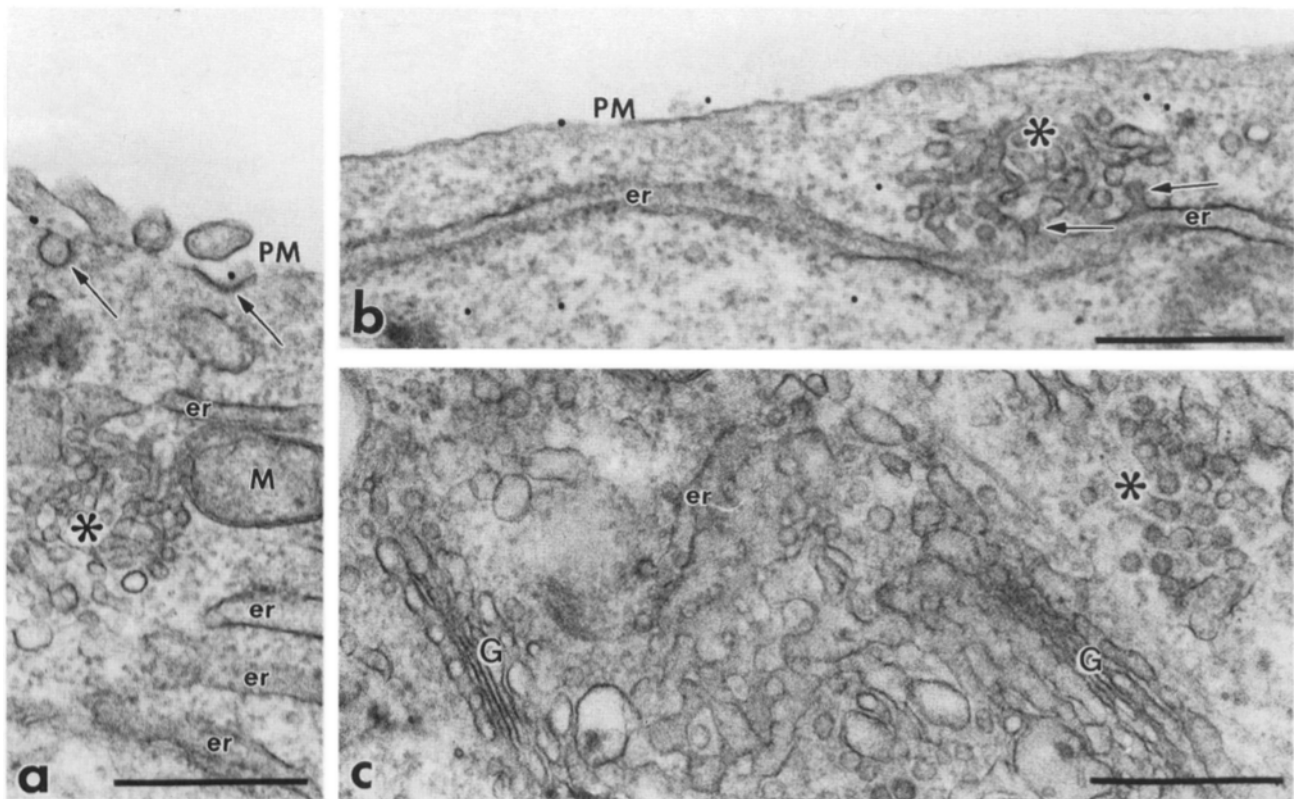


Figure 3. Conventional thin sections of Vero cells. (*a* and *b*) Infected cells incubated for 1 h at 15°C. (*a*) Note the tubulo-vesicular structure that appears uncoated in comparison with the coated pits at the cell surface (*arrows*). (*b*) An ER cisterna with protrusions (*arrows*) facing a tubulo-vesicular structure. (*c*) A tubulo-vesicular structure in uninfected cells kept at 37°C; the morphology and size are similar to those seen in infected cells at 15 or 31°C. PM, plasma membrane; all other symbols as in Fig. 2. The examples in (*a* and *b*) derive from a pre-embedding labeling. Bars, 0.5 μm .

described above. Some immunolabeling was also seen over the *cis*-most Golgi cisternae (not shown), whereas the ER was always unlabeled (Fig. 2 *d*). Double immunolabeling with the anti-VSV G and the anti-Rab2 antibodies, and then with protein-A gold particles of different sizes (5 and 18 nm, respectively), showed colocalization of the two proteins over the tubulo-vesicular structures (Fig. 2 *e*).

Constant Presence and Intracellular Distribution of the Tubulo-vesicular Structures

To exclude the possibility that the immunoelectron microscopic method used altered the morphology of the tubulo-vesicular structures, we analyzed parallel samples using conventional EM. Here, again, these structures were present and clearly detectable even without immunolabeling (Fig. 3). In addition, the conventional thin sections confirmed the apparent uncoated nature of the vesicles (Fig. 3 *a*), and the presence of the tubulo-vesicular structures at the cell periphery (Fig. 3 *b*), as well as in the perinuclear area (Fig. 3 *c*). Immunolabeling with anti Rab2 protein antibody of uninfected Vero cells cultured at 37°C confirmed the presence of these tubulo-vesicular clusters (data not shown). Moreover, no labeling was obtained with anti-G glycoprotein antibody on uninfected cells (Fig. 2 *b*). We next performed a quantitative analysis by comparing the immunolabeling observations made in the various conditions. We detected an average of one structure per ultrathin cell section, and thus we esti-

mated the total number of these structures in a single cell to be 30–40. Approximately 40% of the structures were detected in a perinuclear area (see Materials and Methods), where most of the Golgi complex is located, whereas the other 60% was found in a peripheral area, close to ER membranes, and only rarely close to recognizable Golgi elements. Under our experimental conditions, the morphology of both the vesicles and the tubules, the roughly circular area of the clusters, and the total number and the distribution of the clusters in the cytoplasm, did not differ in infected and uninfected cells immunolabeled with anti Rab2 antibody, or in infected cells kept at 37, 31, or 15°C and immunolabeled with anti-G protein antibody.

Immunoelectron Microscopical Analysis of the Transfer of G Glycoprotein from the Intermediate Compartment to the Golgi Complex

Having established the morphology and the intracellular distribution of the tubulo-vesicular structures accumulating G glycoprotein at 15°C, we repeated, at the ultrastructural level, part of the analysis reported in Fig. 1. As shown in Fig. 4, *a* and *b*, and in Table I *A*, after 2.5 min the amount of G protein in the tubulo-vesicular structures was unchanged, but there was some labeling over the Golgi complex. At 5 min, the amount of labeling on the Golgi complex doubled and was mostly present on the first cisternae, whereas labeling of the tubulo-vesicular structures decreased. Finally, at 10

min, all Golgi cisternae were heavily labeled, while the tubulo-vesicular structures showed a dramatic decrease of the initial labeling. The timing, and the asymmetrical entry of G glycoprotein into the Golgi stacks fully confirmed previous results (3, 4). Analysis of low-magnification images at all time points failed to reveal immunolabeled morphologically distinct structures, other than from the tubulo-vesicular structures, the Golgi complex and the ER. Quantitative analysis of the intracellular distribution of the tubulo-vesicular structures during the transfer of G protein into the Golgi complex is reported in Table I B. The ratio of about 6:4 in the peripheral versus perinuclear location of tubulo-vesicular structure units was constant throughout the time course, whereas the labeling density of perinuclear and peripheral units decreased in parallel. Therefore, as yet there is no evidence that the peripheral and perinuclear units play different roles in the transfer of G glycoprotein to the Golgi complex.

Discussion

We performed an immunocytochemical analysis to follow the export of G glycoprotein synthesized by the VSV ts-045 strain from the intermediate compartment to the Golgi complex. With this approach we were able to characterize further the intermediate compartment (9, 13, 14, 22, 26, 32, 33, 34, 36–39, 42, 45), and to analyze the qualitative and quantitative features of the transfer of G glycoprotein to the Golgi stacks.

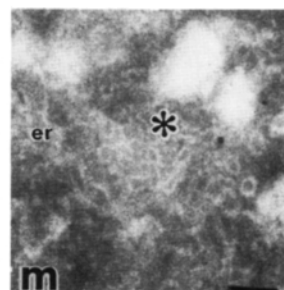
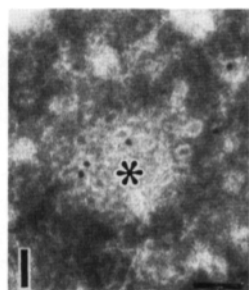
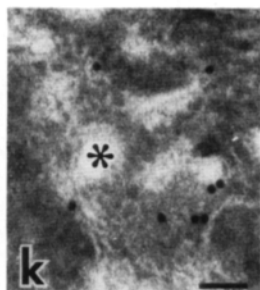
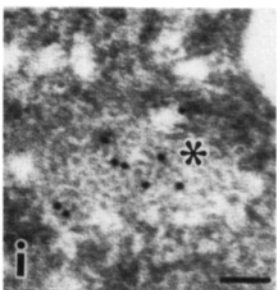
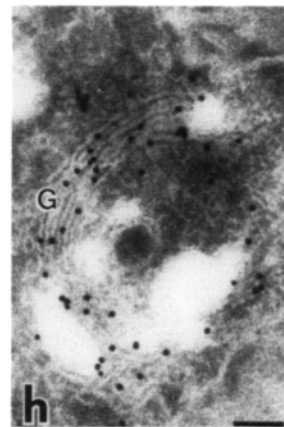
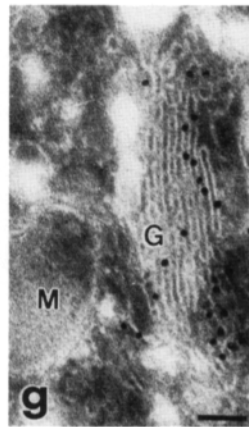
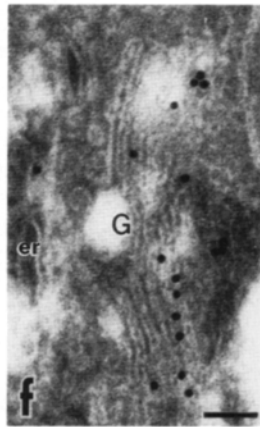
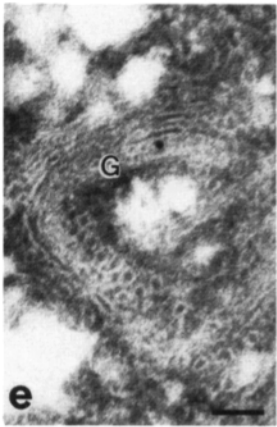
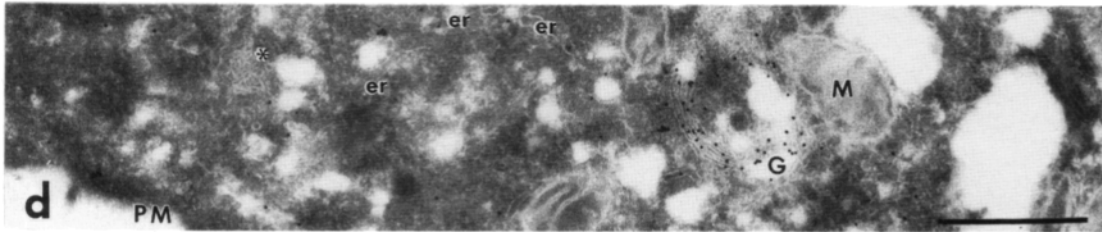
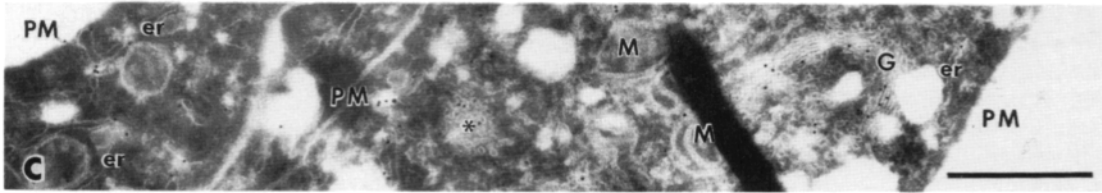
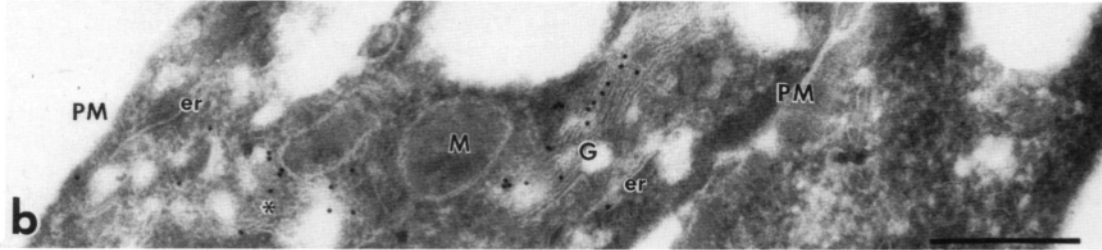
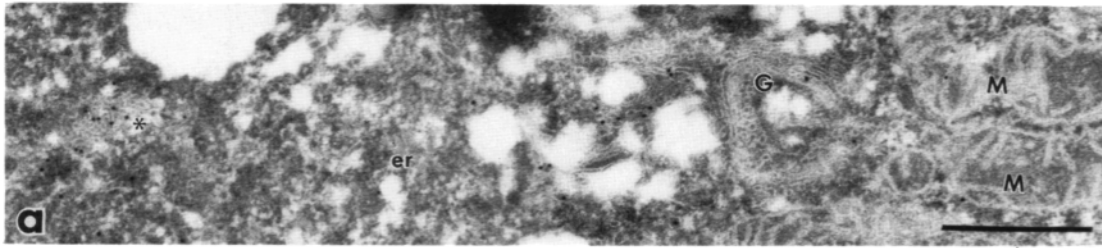
We confirm the presence, along the pathway leading from the ER to the Golgi complex, of tubulo-vesicular structures apparently distinct from both organelles (6, 18, 33, 34, 36–38, 42, 43). Very few marker proteins for these structures have been identified (see below), and all are quantitatively minor proteins. Thus, the structures are studied with immunocytochemical methods at 15°C, because, at this temperature, the transfer to the Golgi complex of all newly made, non ER-resident proteins is blocked in these structures (5, 33–36, 38, 41). We provide evidence that the tubulo-vesicular structures are not a result of, or significantly modified by, the low temperature alone or in combination with viral infection. In addition, we quantified these structures and determined their intracellular distribution. We also demonstrate that Rab2 protein is localized mainly in the structures. The intermediate compartment is formed by about 30–40 apparently independent tubulo-vesicular structure units spread throughout the cytoplasm of Vero cells. By the rough criterion of being more distant than 2 μm from the nuclear envelope, $\sim 60\%$ of the units may be considered peripheral. Therefore, there appears to be no preferential localization in the perinuclear area, and the more frequent topological association was with the ER. In a few instances, the tubulo-vesicular units were near a specialized region of the ER that shows several features of the transitional elements of the ER as described in the exocrine pancreas (15, 21, 35, 41). The ER in tissue cultured cells has yet to be morphologically characterized, thus it remains to be seen whether the tubulo-vesicular units we described are related to the “transitional elements” of the ER present in Vero cells. Our characterization differs slightly from the observations reported by Schweitzer et al. (38), who, with the same model, demonstrated that G and p53 proteins co-localize at 15°C. They reported a preferential perinuclear location and found that

the intermediate structures present in infected cells were larger than those in uninfected cells, although they did not quantitate their data. The different incubation time at 15°C, 1 h in our work and 3 h for Schweitzer et al., may explain these minor differences.

The intracellular transport mechanism underlying exit from the intermediate compartment and entry into the Golgi stacks of membrane and luminal proteins is unknown. Although the static images of electron microscopic immunocytochemistry may be misleading in the context of intracellular movement, they are supported by the time-course experiments shown in Table I. In principle, transfer to the Golgi stacks may occur in one of three ways: (a) Via vesicular transport originating from each of the intermediate compartment units; (b) direct movement of the intermediate compartment units; or (c) lateral diffusion of G glycoprotein through membrane bridges that join the intermediate compartment units in a network, and this network to the Golgi complex. Our findings seem to discount the “network” hypothesis, because with our methodology, we did not detect any membrane continuity between the organelles involved (12, 18). Similarly, we found no evidence that the tubulo-vesicular structures may be formed by single, narrow, and very convoluted membrane tubules. Conversely, our data favor the first hypothesis. We observed a progressive transfer of G glycoprotein from the intermediate compartment to the Golgi stacks, with no detectable changes in the intracellular distribution of the tubulo-vesicular structure units that form the compartment; at the same time the density of labeling of peripheral and perinuclear units decreased in parallel, and no other structures were labeled by G protein during the transfer to the Golgi stacks. Based on our results, it is difficult to envisage the en bloc transfer of a cohort of vesicles and short tubules to the Golgi complex. The majority of Golgi stacks were concentrated perinuclearly in Vero cells, whereas the single tubulo-vesicular units that form the intermediate compartment were spread throughout the cytosol, similarly to ER cisternae, with a slight preference for more peripheral locations. During an en bloc transfer, one would expect to see a higher density of labeling in the perinuclear located units, which was not the case. To reconcile our quantitative data with the en bloc transfer hypothesis one must invoke an extremely fast generation and diffusion throughout the cell of new tubulo-vesicular units, a possible but unlikely event.

What appears to be a contradiction between the images obtained by light and electron microscopic immunocytochemistry (compare Fig. 1 c with Table I) can be explained as follows: the concentration of the signal in the most perinuclear area shown in Fig. 1 c is probably due to G glycoproteins that have already reached the Golgi complex, and to the simultaneous decrease of G glycoproteins in all the tubulo-vesicular units. In conclusion, we favor the view that the tubulo-vesicular units represent a station in the secretory pathway between the ER and Golgi complex, and that a vesicular transport system connects this station with the Golgi stacks. This leads to the question: how does the intermediate station interact with both the ER and the Golgi complex?

There are only three proteins that mark the intermediate compartment: p58, p53, and Rab2 proteins (6, 36, 37; this study). The function of the first two proteins is unknown,



whereas it has been suggested that Rab2 protein is part of the transport machinery connecting the ER with the Golgi complex (6, 29). All three proteins are localized both in the intermediate compartment and in the *cis*-most cisternae of the Golgi stacks (6, 34, 36, 37). This dual localization may indicate that the intermediate compartment and the *cis*-most part of the Golgi form a single functional unit or, alternatively, that these proteins perform their function in more than one compartment. Indeed, p53 may be transiently moved into the ER (18), and β -COP protein, which is normally associated with non-clathrin coated vesicles and the Golgi complex, may be moved into the intermediate compartment when the temperature is lowered to 15°C (10). These observations strongly suggest that the intermediate compartment is functionally connected to the ER and to the Golgi complex in both directions of intracellular transport.

It has been proposed that the intermediate compartment is the site where the salvage function takes place (the retrieval of escaped ER resident proteins back to the ER) (14, 22, 25, 26, 43, 45). However, subsequent data showed that this function may involve also the Golgi complex (7, 17, 24). None of the several maturation events thought to occur late in the ER or early in the Golgi complex may be attributed to the intermediate compartment. It seems that palmitylation, mannose trimming (a result of mannosidase I activity), and initiation of O-glycosylation (addition of N-acetyl-galactosamine to serine/threonine residues), all take place after exit from the intermediate compartment (5, 23, 28; M. C. Pascale et al., 1992). Neither are the enzymes responsible for the phosphorylation of lysosomal proteins present in membrane fractions enriched in the intermediate compartment (39), whereas it remains to be investigated whether or not the intermediate compartment has any relation with the calciosomes, the organelles thought to be involved in the intracellular storage of calcium (44).

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References

- Balch, W. E. 1990. Molecular dissection of the early stages of the eukaryotic secretory pathway. *Curr. Opin. Cell Biol.* 2:634-641.
- Bendayan, M. 1982. Double immunocytochemical labeling applying the protein A-gold technique. *J. Histochem. Cytochem.* 30:691-696.
- Bergmann, J. E., and S. J. Singer. 1983. Immunoelectron microscopic studies of the intracellular transport of the membrane glycoprotein (G) of vesicular stomatitis virus in infected Chinese Hamster ovary cells. *J. Cell Biol.* 97:1777-1787.
- Bergmann, J. E., K. T. Tokuyasu, and S. J. Singer. 1981. Passage of an integral membrane protein, the vesicular stomatitis virus glycoprotein, through the Golgi apparatus en route to the plasma membrane. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
- Bonatti, S., G. Migliaccio, and K. Simons. 1989. Palmitylation of viral membrane glycoproteins takes place after exit from the endoplasmic reticulum. *J. Biol. Chem.* 264:12590-12595.
- Chavrier, P., R. G. Parton, H. P. Hauri, K. Simons, and M. Zerial. 1990. Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. *Cell.* 62:317-329.
- Dean, N., and H. R. B. Pelham. 1990. Recycling of proteins from the Golgi compartment to the ER in yeast. *J. Cell Biol.* 111:369-377.
- Doms, R. W., D. S. Keller, A. Helenius, and W. E. Balch. 1987. Role of adenosine triphosphate in regulating the assembly and transport of vesicular stomatitis virus G protein trimers. *J. Cell Biol.* 105:1957-1969.
- Duden, R., V. Allan, and T. Kreis. 1991. Involvement of β -COP in membrane traffic through the Golgi complex. *Trends Cell Biol.* 1:14-19.
- Duden, R., G. Griffiths, R. Frank, P. Argos, and T. E. Kreis. 1991. β -COP, a 110 kD protein associated with non-clathrin-coated vesicles, and the Golgi complex, shows homology with β -adaptin. *Cell.* 64:649-665.
- Gallione, C. J., and J. K. Rose. 1985. A single amino acid substitution in a hydrophobic domain causes temperature-sensitive cell-surface transport of a mutant viral glycoprotein. *J. Virol.* 54:374-382.
- Heuser, J. H. 1989. Mechanism behind the organization of membranous organelles in cells. *Curr. Opin. Cell Biol.* 1:98-102.
- Hsu, V. W., L. C. Yuan, J. G. Nuchtern, J. Lippincott-Schwartz, G. J. Hammerling, and R. D. Klausner. 1991. A recycling pathway between the endoplasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. *Nature.* 352:441-444.
- Jackson, M. R., T. Nilsson, and P. A. Peterson. 1990. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3153-3162.
- Jamieson, J. D., and G. E. Palade. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. *J. Cell Biol.* 34:577-596.
- Kreis, T. E., and H. F. Lodish. 1986. Oligomerization is essential for transport of vesicular stomatitis viral glycoprotein to the cell surface. *Cell.* 46:929-937.
- Lewis, M. J., and H. R. B. Pelham. 1990. A human homolog of the yeast HDEL receptor. *Nature (Lond.)* 348:162-163.
- Lippincott-Schwartz, J., J. G. Donaldson, S. Schweizer, E. G. Berger, H. P. Hauri, L. C. Yuan, and R. D. Klausner. 1990. Microtubule-dependent retrograde transport of proteins into the ER in the presence of Brefeldin A suggests an ER recycling pathway. *Cell.* 60:821-836.
- Lotti, L. V., and A. R. Hand. 1988. Endocytosis of parotid salivary proteins by striated duct cells in streptozotocin-diabetic rats. *Anat. Rec.* 221:802-811.
- Lotti, L. V., and H. R. Hand. 1989. Endocytosis of native and glycosylated bovine serum albumin by duct cells of the rat parotid gland. *Cell Tissue Res.* 255:333-342.
- Merisko, E. M., M. Fletcher, and G. Palade. 1986. The reorganization of the Golgi complex in anoxic pancreatic cells. *Pancreas.* 1:95-109.
- Munro, S., and H. R. B. Pelham. 1987. C-terminal signal prevents the secretion of luminal ER proteins. *Cell.* 48:899-907.
- Pascale, M. C., N. Malagolini, F. Serafini-Cessi, G. Migliaccio, A. Leone, and S. Bonatti. 1992. Biosynthesis and oligosaccharide structure of human CD8 glycoprotein expressed in a rat epithelial cell line. *J. Biol. Chem.* In press.
- Pelham, H. R. B. 1988. Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:913-918.
- Pelham, H. R. B. 1988. Control of protein exit from the endoplasmic reticulum. *Ann. Rev. Cell Biol.* 5:1-23.
- Pelham, H. R. B. 1991. Recycling of proteins between the endoplasmic reticulum and Golgi complex. *Curr. Opin. Cell Biol.* 3:585-591.
- Pfeiffer, S., S. D. Fuller, and K. Simons. 1985. Intracellular sorting and basolateral appearance of the G protein of vesicular stomatitis virus in Madin-Darby canine kidney cells. *J. Cell Biol.* 101:470-476.
- Piller, V., F. Piller, and M. Fukuda. 1990. Biosynthesis of truncated O-glycans in the T cell line Jurkat. *J. Biol. Chem.* 265:9264-9271.

Figure 4. Immunoelectron microscopic analysis of the time-course of G glycoprotein transfer from the intermediate compartment to the Golgi complex. (A) Low magnification images of parallel cultures of infected cells incubated for 1 h at 15°C and then shifted to 31°C for 0 min (a), 2.5 min (b), 5 min (c), 10 min (d). (B) Higher magnification images of tubulo-vesicular structures and Golgi stacks at various time-points. (e and i) 0 min, (f and k) 2.5 min, (g and l) 5 min, and (h and m) 10 min. (a, e, and i) Labeling appears in the tubulo-vesicular structures, but is almost absent from the Golgi complex. (b, f, and k) No change in the labeling on the tubulo-vesicular structures, but labeling on the first Golgi cisternae. (c, g, and l) Labeling decreases on the tubulo-vesicular structures and increases on the first Golgi cisternae. (d, h, and m) Labeling is very weak on the tubulo-vesicular structures but present on all Golgi cisternae. Symbols as in Fig. 2. Bars: (a-d) 1 μ m; (e-l) 0.1 μ m.

29. Plutner, H., R. Schwaninger, S. Pind, and W. E. Balch. 1990. Synthetic peptides of the Rab effector domain inhibit vesicular transport through the secretory pathway. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2375-2383.
30. Rose, J. K., and R. W. Doms. 1988. Regulation of protein export from the endoplasmic reticulum. *Ann. Rev. Cell Biol.* 4:257-288.
31. Roth, J., M. Bendayan, and L. Orci. 1978. Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. *J. Histochem. Cytochem.* 26:1074-1081.
32. Rothman, J. E., and L. Orci. 1992. Molecular dissection of the secretory pathway. *Nature (Lond.)*. 355:409-415.
33. Saraste, J., and E. Kuismanen. 1984. Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. *Cell*. 38:535-549.
34. Saraste, J., and K. Svensson. 1991. Distribution of intermediate elements operating in ER to Golgi transport. *J. Cell Sci.* 100:415-430.
35. Saraste, J., G. E. Palade, and M. G. Farquhar. 1986. Temperature-sensitive steps in the transport of secretory proteins through the Golgi complex in exocrine pancreatic cells. *Proc. Natl. Acad. Sci. USA*. 83:6425-6429.
36. Saraste, J., G. E. Palade, and M. G. Farquhar. 1987. Antibodies to rat pancreas Golgi subfractions: identification of a 58-kD cis-Golgi protein. *J. Cell Biol.* 105:2021-2029.
37. Schweizer, A., J. A. M. Fransen, T. Bachi, L. Ginsel, and H. P. Hauri. 1988. Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. *J. Cell Biol.* 107:1643-1653.
38. Schweizer, A., J. A. M. Fransen, K. Matter, T. E. Kreis, L. Ginsel, H. P. Hauri. 1990. Identification of an intermediate compartment involved in protein transport from endoplasmic reticulum to Golgi apparatus. *Eur. J. Cell Biol.* 53:185-196.
39. Schweizer, A., K. Matter, C. M. Ketcham, and H. P. Hauri. 1991. The isolated ER-Golgi intermediate compartment exhibits properties that are different from ER and cis-Golgi. *J. Cell Biol.* 113:45-54.
40. Slot, J. W., H. J. Geuze. 1981. Sizing of Protein A-colloidal gold probes for immunoelectron microscopy. *J. Cell Biol.* 90:533-536.
41. Tartakoff, A. M. 1986. Temperature and energy dependence of secretory protein transport in the exocrine pancreas. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:1477-1482.
42. Tooze, S. A., J. Tooze, and G. Warren. 1988. Site of addition of N-acetylgalactosamine to the E1 glycoprotein of mouse hepatitis virus-A59. *J. Cell Biol.* 106:1475-1487.
43. Vaux, D., J. Tooze, and S. D. Fuller. 1990. Identification by anti-idiotypic antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. *Nature (Lond.)*. 345:495-501.
44. Villa, A., P. Podini, D. O. Clegg, T. Pozzan, and J. Meldolesi. 1991. Intracellular Ca⁺⁺ stores in chicken Purkinje neurons: differential distribution of the low affinity-high capacity Ca⁺⁺ binding protein, calsequestrin, of Ca⁺⁺ ATPase and of the ER luminal protein, Bip. *J. Cell Biol.* 113:779-791.
45. Warren, G. 1987. Signal and salvage sequences. *Nature (Lond.)*. 327:17-18.