Glycosylation Does Not Determine Segregation of Viral Envelope Proteins in the Plasma Membrane of Epithelial Cells

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ABSTRACT Enveloped viruses are excellent tools for the study of the biogenesis of epithelial polarity, because they bud asymmetrically from confluent monolayers of epithelial cells and because polarized budding is preceded by the accumulation of envelope proteins exclusively in the plasma membrane regions from which the viruses bud. In this work, three different experimental approaches showed that the carbohydrate moieties do not determine the final surface localization of either influenza (WSN strain) or vesicular stomatitis virus (VSV) envelope proteins in infected Madin-Darby Canine Kidney (MDCK) cells, as determined by immunofluorescence and immunoelectron microscopy, using ferritin as a marker. Infected concanavalin A- and ricin I-resistant mutants of MDCK cells, with alterations in glycosylation, exhibited surface distributions of viral glycoproteins identical to those of the parental cell line, i.e., influenza envelope proteins were exclusively found in the apical surface, whereas VSV G protein was localized only in the basolateral region. MDCK cells treated with tunicamycin, which abolishes the glycosylation of viral glycoproteins, exhibited the same distribution of envelope proteins as control cells, after infection with VSV or influenza. A temperature-sensitive mutant of influenza WSN, ts3, which, when grown at the nonpermissive temperature of 39.5°C, retains the sialic acid residues in the envelope glycoproteins, showed, at both 32°C (permissive temperature) and 39.5°C, budding polarity and viral glycoprotein distribution identical to those of the parental WSN strain, when grown in MDCK cells. These results demonstrate that carbohydrate moieties are not components of the addressing signals that determine the polarized distribution of viral envelope proteins, and, possibly of the intrinsic cellular plasma membrane proteins, in the surface of epithelial cells.

Post-translational addressing of membrane proteins to different membranous compartments appears to be an essential feature of organelle biogenesis in the eucaryotic cell. Madin-Darby Canine Kidney (MDCK) cells infected with enveloped RNA viruses provide a suitable model system for elucidating aspects of this process (38). As is typical of epithelial cells, MDCK cells segregate different sets of membrane proteins into two plasma membrane domains, apical and basolateral, which are morphologically, physiologically, and biochemically distinct (5, 6, 27, 28, 36). The G protein of vesicular stomatitis virus (VSV) is treated by such cells as a basolateral membrane protein, whereas the envelope glycoproteins of the WSN strain of influenza (WSN), Sendai, and simian virus 5 (SV5) are segregated to the apical plasma membrane (37; M. Rindler, D. D. Sabatini, E. J. Rodriguez-Boulan, unpublished results).

Because these viral membrane glycoproteins are produced in large amounts by infected cells, and because they utilize host mechanisms for their synthesis, glycosylation, and intracellular transit (24), they serve as excellent probes to study the mechanisms involved in addressing.

The molecular basis of this process is still unknown. It has been postulated that membrane proteins contain structural information ("sorting-out signals", "addressing signals", "zip codes") that act in concert with specific cellular targeting systems to effect proper segregation (1, 2, 21, 37; G. Palade, personal communication). Given that many cellular and viral integral plasma membrane proteins are glycosylated (24, 45), it was interesting to find out whether carbohydrate moieties participate in addressing. A phosphorylated sugar present on several lysosomal enzymes, mannose-6-phosphate, has been
proposed as a “recognition component” in endocytic processes, specifically routing lysosomal proteins to their destination (13). In epithelial cells, the fact that sialic acid is present on cellular and viral glycoproteins that are basolaterally directed (Na-K-ATPase, VSV G protein, 9, 19), but absent from apical glycoproteins (envelope glycoproteins of WSN, Sendai, and SV5; 14, 15), suggests that sialic acid may act as a signal for addressing in this system (38).

In this work, we investigated the role of glycosylation in the segregation of VSV and WSN envelope glycoproteins, localized in infected MDCK cells and, therefore, are not components of tunicamycin (TM), which inhibits the initial events in glycosylation (3, 10), and viral glycoproteins that are basolaterally directed (Na, K-ATPase, VSV G protein, 9, 19), but absent from apical glycoproteins (envelope glycoproteins of WSN, Sendai, and SV5; 14, 15), suggests that sialic acid may act as a signal for addressing in this system (38).

In this work, we investigated the role of glycosylation in the segregation of VSV and WSN envelope glycoproteins, localized in infected MDCK cells and, therefore, are not components of tunicamycin (TM), which inhibits the initial events in glycosylation of asparagine residues and results in the synthesis of totally nonglycosylated proteins (47, 48); and (c) infection by a temperature-sensitive mutant of WSN, ts3, which retains sialic acid on its glycoproteins at nonpermissive temperatures (34). Our results indicate that carbohydrate moieties are not determinants of the polarized distribution of viral glycoproteins in infected MDCK cells and, therefore, are not components of the addressing signals. In addition, they confirm and extend the recent findings of Roth et al. (39), showing that the budding polarity of enveloped viruses from MDCK cells is preserved after treatment with TM.

MATERIALS AND METHODS

Cells, Viruses, and Antibodies

MDCK cells were grown as described (38). Isolation and characterization of mutant lines of MDCK cells resistant to the plant lectins concanavalin A (con A) and Ricinus communis agglutinin (RCA), which show alterations in the addressing signals. In addition, they confirm and extend the recent findings of Roth et al. (39), showing that the budding polarity of enveloped viruses from MDCK cells is preserved after treatment with TM.

Infection and In Vivo Labeling

Cells were routinely infected with either VSV or WSN at a multiplicity of 5-10 CPE 50 U/cell in a small volume of Eagle’s minimal essential medium (MEM) without serum. DEAE-dextran (100 µg/ml) was routinely included in VSV inocula, but only where indicated with WSN. Viruses were allowed to adsorb 45 min–1 h (37°C, CO2 incubator), after which the inoculum was removed and replaced with either MEM-2% fetal calf serum (FCS), MEM-2% FCS containing 50 µCi/ml [35S]methionine (1,145 Ci/mmol, Amersham Corp., Arlington Heights, Ill.), or low-amino acid MEM (a gift of Dr. S. Rohlich, containing one-tenth the usual amount of all amino acids), supplemented with 2% dialyzed FCS and 10-25 µCi/ml [3H]glucosamine (19 Ci/mmol, New England Nuclear, Boston, Mass.), or low-amino acid MEM (a gift of Dr. S. Rohlich, containing one-tenth the usual amount of all amino acids), supplemented with 2% dialyzed FCS and 10-25 µCi/ml [3H]glucosamine (19 Ci/mmol, Amersham Corp., Arlington Heights, Ill.)

Processing of Labeled Samples

Media from 35-mm petri dishes (1 ml/dish) were harvested and centrifuged at low speed (Damon IEC Div., Damon Corp., Needham Heights, Mass., 200 rpm, 10 min) to remove cellular debris. In some cases, the supernates were layered over 10-50% sucrose gradients in TNE buffer (100 mM Tris HCl, pH 7.4, 50 mM NaCl, 10 mM EDTA), and centrifuged in a Beckman SW 41 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 28,000 rpm for 90 min. Gradients were collected manually after bottom puncture; fractions at the peaks of viral distribution were pooled and diluted in TNE buffer. Viruses were collected by centrifugation in an SW 56 rotor at 50,000 rpm for 2 h. In other

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FIGURE 1 Altered electrophoretic mobilities of VSV and WSN glycoproteins synthesized in lectin-resistant MDCK cells. Confluent monolayers of wild-type (Wt), RCA-resistant (RCA⁎), and Con A-resistant (Con A⁎) MDCK cells grown on 35-mm petri dishes were infected with WSN or VSV at a multiplicity of 10 CPE 50 U/cell. Labeling media were added after 1 h of adsorption. Incubation was continued for 12-15 h. Media were harvested and diluted in TNE buffer, viruses were sedimented in a Beckman SW 56 rotor (50,000 rpm, 2 h), and resuspended in gel buffer before processing for electrophoresis on 5-12% gradient polyacrylamide SDS gels. Viruses were labeled with [35S]methionine (10-15 μCi/ml) or with [3H]glucosamine (50 μCi/ml) as indicated. Arrows indicate the position of HA 1, HA 2, and G bands.

when the viruses were grown in wild-type (wt), RCA⁎, and Con A⁎ cells in the presence of tunicamycin (data not shown). Preliminary experiments showed incorporation of [3H]galactose into VSV glycoprotein produced by infected wild type and lectin-resistant cells. In a similar fashion, various lectin-resistant Chinese hamster cells ovary have been characterized with respect to glycosylation of VSV G protein (36, 48).

VSV G protein synthesized in Con A⁎ MDCK cells was an exception, because it showed no obvious alteration in mobility in gels (Fig. 1), whereas the hemagglutinin of WSN synthesized in the same cells exhibited a clear reduction in molecular weight. This discrepancy is possibly due to differences between G and HA in oligosaccharide complement. The former contains only complex-type oligosaccharide side chains, with galactose and sialic acid as well as mannose and N-acetylglucosamine (9); whereas the latter has simple (mannose-rich) oligosaccharides, in addition to complex chains (30). Because Con A appears to have a greater specificity for simple oligosaccharides (18), the mutation conferring Con A resistance might involve some biosynthetic step exclusive for the mannose-rich chains. In fact, a similar phenomenon has been observed in the case of class E Thy 1 lymphoma cell mutants, in which an inability to synthesize mannosylolichophosphate results in abnormal lipid-linked oligosaccharide and resistance to Con A (7, 8). When Sindbis virus (which contains both simple and complex oligosaccharides) was grown in these cells, only the high mannose residues were affected (51). These data suggest that a similar defect may be responsible for Con A resistance in our Con A⁎ mutant, and would account for the differential effect of the lesion on glycosylation of HA and G proteins. If this is the case, the G protein produced in this mutant would be identical to wild-type G.

Quantitation of production of VSV and WSN in lectin-resistant MDCK cells with respect to infectious units and [35S]methionine incorporation allowed an estimation of specific infectivities (infectivity per particle, in this case, CPE-50 U/acid-precipitable cpm in virions; Table I). By both parameters, the lectin-resistant cells manufactured both viruses with similar efficiencies as did the parental cells, and the resultant viruses retained normal infectivities (0.67-2.6 times the control levels) in spite of having altered envelope glycoproteins.

However, it should be noted that in one case lectin-resistance did decrease the cell’s susceptibility to infection. WSN production by RCA⁎ cells was negligible, even with a multiplicity of infection 10-fold higher than what was used on wild-type cells (data not shown). When 100 μg/ml DEAE-dextran was added to the inoculum, viral production reached wild-type levels (Table I). Because DEAE-dextran is thought to increase viral adsorption (49), these data suggest that the lesion which resulted in RCA-resistance in this cell line has affected a carbohydrate-containing cell surface receptor for WSN, thereby decreasing binding, uptake, or uncoating of infecting virions.
Polarity of Viral Glycoproteins in Lectin-resistant MDCK Cells

Localization of viral envelope glycoproteins on apical and basolateral plasma membranes was accomplished by indirect immunofluorescence and immunoelectron microscopy. In both procedures, special measures were taken to ensure access of the reagents to basal and lateral surfaces (37). In the case of immunofluorescence, antibody staining of infected, fixed monolayers was performed with or without preincubation of slides in Triton X-100 (see Materials and Methods). WSN glycoproteins, being apical, can be detected without the use of detergent (Fig. 2a). In contrast, staining by anti-VSV IgG requires detergent pretreatment (Fig. 2d), which exposes basolateral as well as intracellular antigenic sites. WSN infection results in a spotty fluorescence pattern, given by the staining of the apical microvilli (Fig. 2a); on the other hand, VSV-infected cells frequently show characteristic ringlike images (Fig. 2d) caused by the accumulation of viral antigens and virions at intercellular spaces and basolateral plasma membranes (37), or may exhibit diffuse intracellular staining. As can be seen (Fig. 2b, c, e, and f), lectin-resistant MDCK infected with WSN or VSV showed staining patterns similar to those of wild-type MDCK cells.

For immunoelectron microscopy, fixed monolayers were scraped off their substrata before incubation with antibody and ferritin conjugates in order to expose basolateral membranes. Fig. 3a shows wild-type MDCK cells sectioned perpendicular to the plane of their substratum, with apical plasma membranes containing microvilli, junctional areas, lateral interdigitations, and desmosomes. The apical and basolateral plasma membranes of one cell are shown at higher magnification in Fig. 3b. Virus budding and ferritin labeling were localized only on the apical plasma membrane; infection of RCA and Con A cells with WSN gave identical results (Fig. 3c and d). On the other hand, lectin-resistant cells infected with VSV were labeled primarily on their basal and/or lateral surfaces, where budding of VSV was also localized (Fig. 3e-g). Coated vesicles and numerous coated pits (Fig. 3p), which have been implicated in the delivery of VSV G protein to the plasma membrane of infected Chinese hamster ovary cells (40), were observed in lectin-resistant as well as wild-type MDCK cells. Areas showing increased ferritin labeling were often seen in plasma membrane regions adjacent to coated pits, and occasionally on the coated pits themselves. The results of these experiments demonstrate that constitutive modifications of the carbohydrate moieties of MDCK cells, introduced by mutations that confer lectin-resistance, do not affect the polarized distribution of viral glycoproteins.

Viral Replication in the Absence of Glycosylation

To examine the effect of total lack of carbohydrate on addressing, we treated MDCK cells with TM before and during infection. TM inhibits the synthesis of dolichol-linked oligo-
FIGURE 3 Polarized distribution of viral glycoproteins in lectin-resistant MDCK cells. Confluent monolayers of wild-type, RCA, and Con A MDCK cells grown on 35-mm plastic dishes were infected with VSV or WSN as described in Materials and Methods. 3.5-5 h after infection by VSV, or 6-8 h after infection by WSN, monolayers were fixed in paraformaldehyde (4% in PBS, 20 min, rt), scraped off the substratum, and incubated with biotin-conjugated antiviral IgG (50 μg/ml in PBS, 30 min, rt), followed, after several washes in PBS, by avidin-conjugated ferritin (1 mg/ml in PBS, 1.5 h, rt). Samples were washed in PBS three times; 0.1 M Na cacodylate, pH 7.4, once; fixed in glutaraldehyde (2% in cacodylate); and processed for thin-section electron microscopy. The boxed areas in a are shown at higher magnification in b. Each pair of figures represents the apical (top) and basolateral (bottom) region of a single cell. Arrows indicate budding or released virions; arrowheads point at patches of ferritin on the plasma membrane. Ap, apical plasma membrane; Bas, basal plasma membrane; Lat, lateral plasma membrane; TJ, tight junctional areas; mv, microvilli; cp, coated pits. (a) wt and WSN (x 14,500); (b) wt and WSN; (c) RCA and WSN; (d) Con A and WSN; (e) wt and VSV; (f) RCA and VSV; (g) Con A and VSV (b-g, x 43,700).

saccharide precursors and thus effectively blocks glycosylation of asparagine residues (47, 50). The action of TM was monitored by SDS-PAGE of purified viruses labeled in vivo with [3H]leucine (for VSV) or cell lysates labeled with [35S]methionine (for WSN). Because correct interpretation of ferritin-labeling experiments (next section) required us to account for all viral glycoproteins synthesized from the onset of infection, cells were incubated with labeling medium from the end of the adsorption period. Initial experiments using this labeling protocol, in which TM was added at infection and not earlier, demonstrated synthesis of fully glycosylated, as well as totally nonglycosylated, viral glycoproteins (data not shown). The fact that Roth et al. (39) did not detect fully glycosylated envelope proteins under similar experimental conditions (addition of
TM at the time of infection) may be attributed to the late isotope addition in their experiments (6 h after infection), which presumably allowed for a depletion of the dolichol-linked precursor pool before a significant amount of HA was labeled. Pretreatment of cells with TM for 2 h before infection eliminated this residual glycosylation. This protocol actually represents a 5-h preexposure to TM because, in this system, glycoproteins of both VSV and WSN are not synthesized in detectable amounts until 3 h postinfection (data not shown).

VSV G protein synthesized under these stringent conditions of TM treatment migrated faster than control G (Fig. 4), and lacked any detectable [3H]glucosamine labeling, even after considerable overexposure of the fluorograms. Scans of fluorograms from [3H]leucine-labeled samples revealed that VS virions grown in cells treated with TM contained 40% of the control level of G, when normalized to radioactivity in the N + NS band. The production of VSV in the presence of TM (Table II) was 4.5–14.5% of control when measured as infectivity, and 10–38% of control [3H]leucine incorporation. The specific infectivity (CPE 50/cpm) of VSV was thereby reduced by TM to 1/3 of control, perhaps due to the decrease in the absolute amount of G per viral particle. The infectivity data essentially agree with those of Gibson et al. (11), who found that TM inhibited assembly of VSV in baby hamster kidney cells to 0.1–15% of control at 37°C and to 30–95% of control at 30°C, depending on the virus strain used. The amount of reduction in specific infectivities also depended on the viral strain and temperature (35–55% at 38°C, 35–93% at 30°C; 10, 11).

In previous reports, the detection of nonglycosylated HA protein in total cell lysates of TM-treated, influenza-infected cells was hampered by its reportedly low intracellular stability (44), and by the fact that its electrophoretic mobility is close to that of the viral nucleoprotein (NP) in SDS-PAGE (30). We demonstrated the presence of nonglycosylated HA in [35S]methionine-labeled lysates of MDCK cells infected with WSN in the presence of TM by immune precipitation with anti-WSN IgG. The immune precipitates (Fig. 4) showed the disappearance of two larger molecular weight bands (HA 1 and HA 2) and the appearance of at least one (and on occasion two) band migrating slightly slower than the NP band. Two lower molecular weight bands (HA 1 and HA 2) also shifted to a faster mobility or disappeared in the presence of TM. Production of infectious WSN in the presence of TM varied from 0.15 to 4.4% of control. Incorporation of radioactivity into virions was reduced to 5.8–20% of control by TM, which caused a concomitant decrease in specific infectivities.

In summary, VSV G and WSN HA were synthesized as nonglycosylated proteins in TM-treated MDCK cells. The experimental protocol allowed us to rule out the possibility that fully glycosylated viral glycoproteins synthesized early in
infection contaminated the population of unglycosylated molecules which were localized by immunocytological means later during the infection (next section).

Polarized Distribution of Nonglycosylated Viral Glycoproteins in MDCK Cells

Viral glycoproteins synthesized in the presence of TM were detected by immunofluorescence on the surface of infected cells. Staining by anti-VSV IgG required detergent pretreatment in both TM-treated and control samples (Fig. 5a and b), indicating that G was not present on the apical plasma membrane in significant amounts. In the case of WSN, TM treatment reduced the overall level of specific fluorescence, with or without detergent pretreatment. However, isolated patches of TM-treated cells (10–20% of the cells) showed positive staining of the apical surface similar to control (Fig. 5c and d).

Immunoelectron microscopy confirmed these findings. In TM-treated, VSV-infected cells, budding virions were observed only on basal and lateral surfaces. Ferritin binding was seen primarily on budding or released virions (Fig. 6a and c), and on occasional patches in the plasma membrane.

Monolayers infected with WSN in the presence of TM exhibited a decreased number of virus-producing cells, which, in turn, showed lower virion budding and ferritin binding as compared with untreated cells. However, the observed ferritin labeling was exclusively localized on apical plasma membranes, as isolated patches, and on budding or released virions (Fig. 6d and e). No staining was seen on basal or lateral surfaces. In addition, WSN virions released from cells treated with TM were frequently seen in aggregates, in contrast to the control situation.

**Figure 5** Immunofluorescence localization of viral envelope glycoproteins synthesized in the presence of TM. Confluent monolayers of MDCK cells were grown on glass cover slips. 2 h after the addition of TM (2 µg/ml), they were infected with VSV or WSN. Cover slips were fixed in paraformaldehyde, washed, and stained as described in Materials and Methods. (a) VSV-infected cells stained with sheep anti-VSV IgG and fluorescein-conjugated rabbit anti-sheep IgG. (b) VSV + TM, stained as a. (c) WSN-infected cells stained with rabbit anti-WSN IgG and rhodamine-conjugated goat anti-rabbit IgG. (d) WSN + TM, stained as c. The cells in a and b were incubated with Triton X-100 before antibody staining, whereas the cells in c and d were not × 720.

It is clear from these experiments that both WSN HA and VSV G protein, though devoid of carbohydrate, are segregated by MDCK cells to apical and basolateral plasma membranes, respectively, as are their glycosylated forms.

Correct Segregation of WSN Envelope Glycoproteins Containing Sialic Acid

In previous sections, we described the effect of lack or partial deficiency of carbohydrate on the addressing of viral glycoproteins. In the experiments described below, we investigated the effect that retention of sialic acid, a sugar that is usually cleaved from glycoproteins of apically budding viruses (14), has on the polarized distribution of influenza envelope proteins. We used a temperature-sensitive mutant of WSN, ts3, which has been shown to possess a defective viral neuraminidase that does not cleave terminal sialic acid residues of viral glycoproteins at nonpermissive temperatures (34). SDS-PAGE of lysates from MDCK cells infected with ts3 at 32°C and 39.5°C in the presence of [3H]glucosamine confirmed the reported lesion: HA synthesized at 39.5°C migrated slower than at 32°C (data not shown), presumably due to retention of sialic acid residues (34).

Immunofluorescence (not shown) revealed that glycoproteins of ts3 synthesized at 39.5°C could be detected on apical plasma membranes without the use of detergent (see Materials and Methods and Results), suggesting an apical localization. By immunoelectron microscopy (Fig. 7), we observed that specific labeling of cells infected with ts3 at nonpermissive temperature was confined to the apical surface. Ferritin distribution on the cell surface appeared patchy, as distinct from a more uniform labeling of the apical surface typical of WSN infection. However, no labeling was observed on the basal or lateral plasma membranes. In addition, we observed the aggregation of virions on the cell surface reported by Palese et al. (34; Fig. 7b), which they ascribed to interaction of HA molecules with sialic acid residues on the virion surface. In conclusion, WSN glycoproteins that retain sialic acid as their ultimate saccharide residue are directed to the apical surface of MDCK cells and achieve the same distribution as proteins which are normally desialylated.

**DISCUSSION**

The experiments reported here demonstrate that glycosylation is not a critical factor in determining the targeting of viral membrane glycoproteins to specific surface domains in infected epithelial cells. The envelope proteins of two viruses of opposite budding polarity, influenza (WSN) and VSV, retained the capacity to be segregated into their respective plasma membrane domains, apical and basolateral, after glycosylation by the host MDCK cells was modified by three different approaches:

(a) By isolating and infecting lectin-resistant mutant lines of MDCK cells, we were able to study the effect of constitutive changes in glycosylation on the polarized behavior of viral glycoproteins in the same cells. Though the primary lesions in these mutants have not yet been determined, the resulting glycoproteins probably possess truncated and otherwise modified versions of the wild-type carbohydrate structures. The fact that the viral glycoproteins remain properly segregated in lectin-resistant MDCK cells indicates that ultimate or penultimate sugars (presumably affected in RCAⅡ mutants) or glycoprotein cores (presumably affected in Con AⅡ cells; 45) do not constitute addressing signals. This is in agreement with
results indicating that these mutants retain structural and physiological properties of polarized epithelia, such as the ability to form functional tight junctions and domes.  

(b) TM experiments, though subject to potential drug artefacts not present in studies with lectin-resistant mutants, have a more straightforward interpretation: glycosylation of both apical and basolateral viral glycoproteins was totally inhibited, but the polarized distribution of these proteins was preserved. It should be pointed out that the preservation of virus budding polarity after tunicamycin treatment, demonstrated by Roth et al. (39) and by our laboratory (12), did suggest but not necessarily prove this point. It has recently been demonstrated that virus budding may take place in the absence of envelope glycoproteins, although at highly reduced efficiencies (42). It is possible, then, to imagine a situation in which envelope proteins whose conformation has been changed by the absence of glycosylation do not promote budding as efficiently as their glycosylated counterparts; therefore, budding sites do not represent sites of accumulation of viral glycoproteins. Thus, only the direct immunocytochemical localization of the envelope proteins, as performed in this paper, justifies the conclusion that they remain polarized after drug treatment.

(c) Cleavage of sialic acid residues from HA by viral neuraminidases probably occurs intracellularly, shortly after their addition (16, 30). By infecting MDCK cells (with a temperature-sensitive mutant of WSN, ts3) with a defective neuraminidase, we found that the retention of sialic acid in influenza A during viral biogenesis did not alter its final surface distribution.

The role of glycosylation in the biogenesis of membrane and secretory glycoproteins is controversial at present. Most of the relevant studies to date have focused on the efficiency with which a particular protein reaches its destination after inhibition of glycosylation by TM or other drugs (10, 43). The phenomenon most consistently reported has been an increased degradation of the molecule of interest, with a corresponding reduction in its appearance of the plasma membrane, in the extracellular medium, or in viral envelopes (for discussion and reference, see 31). Lack of carbohydrate could render a protein intrinsically more sensitive to proteolysis, which has been demonstrated directly only in the case of fibronectin (32), or, alternatively, could cause its rerouting to other organelles (i.e., lysosomes), thus exposing it to proteases or other unfavorable conditions. In the case of the G protein of VSV, experimental evidence indicates a change in the physical properties of the nonglycosylated protein, which tends to aggregate in intracellular membranes, thus possibly hindering its migration to the plasma membrane (11, 23). The data in this report and previous work (16, 30) indicate that glycosylation is not essential for intracellular migration of HA. However, the efficiency of its migration varies considerably, according to the particular cell-virus system studied.

The experimental system of virus-infected MDCK cells allowed us to study an unexplored aspect of addressing—directionality—because we could easily monitor one alternative final localization that might be reached by a protein secondary to lack or modification of carbohydrate moieties. Therefore, even though we observed a considerable decrease in the effi-
cytoplasmic domain of membrane proteins appears a logical interaction with the hypothetical cellular targeting system. The nature of the addressing signals. It is of the utmost importance to determine which of the integral membrane protein domains, luminal, intramembranous, or cytoplasmic, is involved in the interaction with the hypothetical cellular targeting system. The cytoplasmic domain of membrane proteins appears a logical candidate for interaction with cytoskeletal elements, actin or clathrin-like proteins for example, that may be involved in distribution, or with organelle-specific peripheral proteins (spectrin-like molecules), that may trap a protein into a specific cell membrane. Organelle-specific integral proteins, such as the ribophorins found in membranes of the rough endoplasmic reticulum (20), may also play a central role in determining the membrane individuality. For example, these proteins may provide a scaffolding specifically recognized by other proteins destined to carry out their function in that specific membrane system.

Future research should attempt to identify the cellular distribution systems involved in addressing. In this regard, it is interesting to consider the role of clathrin-like proteins. It has been recently proposed that coated vesicles participate in the transport of acetylcholine receptors in muscle, whereas a different kind of vesicle transports acetylcholinesterase, a secretory protein (4, 44). Coated vesicles have been recently proposed as carriers of the VSVG protein at two separate intracellular stages (41). The system of virus-infected epithelial monolayers should be of great help to the study of the validity of these models because, if coated vesicles are critically involved in distribution of plasma membrane proteins, two separate vesicle populations could be identified for apical and basolateral viral proteins.

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