Microtubule-acting Drugs Lead to the Nonpolarized Delivery of the Influenza Hemagglutinin to the Cell Surface of Polarized Madin-Darby Canine Kidney Cells

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Abstract. The synchronized directed transfer of the envelope glycoproteins of the influenza and vesicular stomatitis viruses from the Golgi apparatus to the apical and basolateral surfaces, respectively, of polarized Madin-Darby canine kidney (MDCK) cells can be achieved using temperature-sensitive mutant viruses and appropriate temperature shift protocols (Rindler, M. J., I. E. Ivanov, H. Plesken, and D. D. Sabatini, 1985, J. Cell Biol., 100:136–151). The microtubule-depolymerizing agents colchicine and nocodazole, as well as the microtubule assembly-promoting drug taxol, were found to interfere with the normal polarized delivery and exclusive segregation of hemagglutinin (HA) to the apical surface but not with the delivery and initial accumulation of G on the basolateral surface.

Immunofluorescence analysis of permeabilized monolayers of influenza-infected MDCK cells treated with the microtubule-acting drugs demonstrated the presence of substantial amounts of HA protein on both the apical and basolateral surfaces. Moreover, in cells infected with the wild-type influenza virus, particles budded from both surfaces. Viral counts in electron micrographs showed that ~40% of the released viral particles accumulated in the intercellular spaces or were trapped between the cell and monolayer and the collagen support as compared to <1% on the basolateral surface of untreated infected cells.

The effect of the microtubule inhibitors was not a result of a rapid redistribution of glycoprotein molecules initially delivered to the apical surface since a redistribution was not observed when the inhibitors were added to the cells after the HA was permitted to reach the apical surface at the permissive temperature and the synthesis of new HA was inhibited with cycloheximide. The altered segregation of the HA protein that occurs may result from the dispersal of the Golgi apparatus induced by the inhibitors or from the disruption of putative microtubules containing tracks that could direct vesicles from the trans Golgi apparatus to the cell surface. Since the vesicular stomatitis virus G protein is basolaterally segregated even when the Golgi elements are dispersed and hypothetical tracks disrupted, it appears that the two viral envelope glycoproteins are segregated by fundamentally different mechanisms and that the apical surface may be incapable of accepting vesicles carrying the G protein.

The biogenetic processes that determine the exclusive incorporation of distinct sets of plasma membrane proteins into the apical or basolateral surfaces of polarized epithelial cells can be conveniently studied using epithelial cell cultures infected with enveloped viruses that bud from one or the other cell surface (24). It is now well established that the asymmetric budding of influenza and vesicular stomatitis virus (VSV) from the apical or basolateral surfaces of infected Madin-Darby canine kidney (MDCK) cells, respectively, is preceded by the prior accumulation of the envelope glycoprotein of each virus in the corresponding plasma membrane domain (23). This segregation is a consequence of the direct delivery of both proteins to the correct surface and therefore the result of an intracellular sorting process which seems to take place as the proteins emerge from the Golgi apparatus, apparently in separate vesicles (22). It is not known, however, if the post-Golgi vesicles containing the two types of viral proteins differ in their intrinsic capacity to fuse with one or the other cell surface domain or if each type of vesicle follows a distinct intracellular pathway which leads to only one aspect of the cell surface.

Many studies have indicated that microtubules play an important role in directing organellar movement (16, 31), as well as in secretion and the transporting of cytoplasmic vesicles involved in endocytosis and transcytosis (17, 20). In part, these studies have relied on the use of drugs, such as colchi-
cine and nocodazole (8) or taxol (30), that lead to the depolymerization of microtubules or promote the assembly of abnormally large microtubule bundles. These agents also have profound effects on the organization of the location of the Golgi apparatus, which normally is located in a perinuclear region closely apposed to the microtubule-organizing center (12, 26, 33). The microtubule-depolymerizing drugs lead to fragmentation and dispersal of Golgi elements throughout the cell, whereas in taxol-treated cells larger fragments of the Golgi apparatus are produced which are displaced to the cell periphery.

It has long been known that the insertion of plasma membrane proteins at the cell surface does not take place at random but is directed to specific restricted domains (2, 11, 13, 22, 27). This directed transfer is obliterated in cells treated with microtubule assembly inhibitors (27), and this effect has been attributed to the fragmentation of the Golgi apparatus.

To determine whether microtubules play an important role in the segregation of viral glycoproteins to the distinct surface domains of polarized MDCK cells, we have examined the effect of microtubule inhibitors on the synchronized transfer of the viral glycoproteins from the Golgi apparatus to the plasma membrane that can be achieved in cells infected with viral temperature-sensitive mutants of this virus. We found that the integrity of the microtubule system is required for proper segregation of the influenza hemagglutinin (HA) to the apical surface, but not for accumulation of the G protein of VSV on the basolateral plasma membrane domain.

Materials and Methods

Cell Culture

MDCK cells, originally obtained from Dr. Joseph Leighton of the University of Pennsylvania, were cultured as previously described (5, 21). For immunofluorescence experiments, cells removed by trypsinization were plated at 1-2 x 10^5 cells/cm^2 in a 24-well dish (Costar, Cambridge, MA) containing 13-mm No. 1 glass coverslips (VWR Scientific, South Plainfield, NJ) at 1-2 x 10^4 cells/cm^2 in a 24-well dish (Costar, Cambridge, MA) containing 13-mm No. 1 glass coverslips (VWR Scientific, South Plainfield, NJ) and incubated until 1-3 d past confluency. For electron microscopy, placing 13-mm No. 1 glass coverslips (VWR Scientific, South Plainfield, NJ) at 1-2 x 10^4 cells/cm^2 in a 24-well dish (Costar, Cambridge, MA) contain-

Viral Infections

Monolayers were washed twice in MEM and incubated for 1-1.5 h in the same medium containing virus (5-10 pfu/cell). The viral inoculum was then removed and replaced with MEM. For infection with the wild-type viruses, VSV (strain Indiana, obtained originally from Dr. J. Vilcek, New York University Medical Center) or influenza WSN (originally from Dr. P. Palese, Mt. Sinai School of Medicine, NY), all manipulations were performed at 37°C. When the ts045 mutant of VSV (obtained from Dr. J. Lenard, Rutgers University School of Medicine, New Brunswick, NJ) or the ts61s mutant of influenza WSN (32; provided by Dr. Palese, Mt. Sinai School of Medicine, NY) were used, infection was carried out at 32.5°C and the subsequent incubation was at 39.5°C. After 5 h (ts045) or 5.5 h (ts61s) at 39.5°C, the medium on some samples was replaced with cold MEM (at 18.5°C) lacking bicarbonate. The cultures were then maintained at 18.5°C in an incubator placed in a cold room. It has previously been shown (13, 14, 22) that at this temperature viral glycoproteins are still transported from the endoplasmic reticulum to the Golgi apparatus but their transport toward the cell surface is blocked. In the case of HA this block appears to be complete, but small amounts of G appear to be transported to the surface during incubation at 18.5°C. When the temperature is raised to 32.5°C, normal passage of both proteins to the plasma membrane is restored (14, 22). Other samples were transferred directly from 39.5°C to MEM at 32.5°C, without an intermediate incubation at 18.5°C. All VSV infection media contained 100 µg/ml DEAE-dextran (Pharmacia Fine Chemicals, Piscataway, NJ). Unless stated otherwise, the microtubule inhibitors were added when the cultures were shifted to 39.5°C. Colchicine and nocodazole were purchased from Sigma Chemical Co. (St. Louis, MO) and stored as solutions in ethanol. Taxol was obtained from the National Cancer Institute (Bethesda, MD), dissolved in DMSO, and stored at -20°C.

Immunofluorescence Labeling

This was performed essentially as described (21). Samples were washed in Dulbecco's PBS (Gibco, Grand Island, NY), fixed at 4°C in 4% paraformaldehyde (Fisher Scientific Co., Springfield, NJ) for 30 min and then washed in PBS. When necessary, cells were permeabilized by incubation in 0.2% Triton X-100 for 10 min. The primary antibodies were used as a monoclonal anti-HA (a gift of Dr. R. Webster, St. Jude Children's Hospital, Memphis, TN) applied at 1:1,000 dilution and a monospecific rabbit anti-G protein, used at ~50 µg/ml IgG. TRITC-conjugated goat anti-mouse and anti-rabbit IgG (CooperBiomedical, Inc., Malvern, PA) were used as secondary antibodies at a 1:50 dilution. Coverslips were mounted on glass slides with gelvatol and viewed in a Leitz Orthoplan microscope equipped with a Xenon lamp and a Wild camera.

Electron Microscopy

Infected monolayers on 25-mm collagen-coated coverslips were scraped off into PBS and sedimented in Eppendorf centrifuge tubes before fixation with 2% glutaraldehyde (Polysciences, Inc., Warrington, PA) in PBS for 3 h at 4°C. Samples were then processed for electron microscopy by standard procedures and viewed in a Phillips 301 microscope at 80 kV. Virion counts were made on low magnification photographs taken at random of 50-100 cells. Viral particles were scored for apical or basolateral budding.

Electrical Resistance Measurements

MDCK cells were plated on 13-mm nitrocellulose filters of 0.45 µm pore size (Millipore Corp., Bedford, MA) at a density of 2-3 x 10^5 cells/cm^2 and used 2 d later. Infections and drug treatments were carried out as described above. Measurements were made in a modified Ussing chamber (5).

Results

Effect of Drugs That Induce Microtubule Disassembly on the Polarized Delivery of Influenza HA to the Cell Surface

MDCK cells infected with the ts61s mutant of influenza WSN were used to examine the effect of colchicine and nocodazole on the transport of the influenza HA from the endoplasmic reticulum to the cell surface. It has previously been shown (22, 25) that at the nonpermissive temperature (39.5°C), the ts61s mutant produces a defective HA, which cannot be transported out of the endoplasmic reticulum and therefore accumulates in this organelle. The accumulation of HA in the endoplasmic reticulum yields a diffuse lattice-like cytoplasmic pattern of staining when the infected cells are examined by immunofluorescence with anti-HA antibodies (22). A synchronized transport of the defective protein out of the endoplasmic reticulum takes place after transfer of the infected cultures to 32.5°C, a temperature which permits passage of the glycoprotein through the Golgi apparatus and its subsequent appearance at the cell surface (22). If, on the other hand, the infected cultures are transferred from 39.5°C to 18.5°C, passage of the protein toward the cell surface is halted intracellularly in a crescent-shaped juxtanuclear region of the cytoplasm (Fig. 1 a) which, by electron microscopy, has been shown to correspond to the Golgi region (22). The distribution of HA in cultures of ts61s-infected cells that received colchicine (20 µg/ml) or nocodazole (10 µg/ml) during the incubation period at 39.5°C (4 h) and were then transferred to 18.5°C was strikingly different from that in cultures...
Figure 1. Effect of microtubule-acting drugs on the intracellular distribution of the influenza HA in cells incubated at 18.5°C. MDCK monolayers were infected with the ts61s mutant of influenza WSN for 1.5 h at 32.5°C and subsequently incubated in the presence or absence of inhibitors for 5.5 h at 39.5°C. After this incubation, samples were then shifted to 18.5°C for 90 min and then fixed, permeabilized with detergent, and immunolabeled with anti-influenza HA antibodies. (a) In cells not treated with the inhibitors, the HA accumulated near the nucleus (N) in regions of the cytoplasm that have been shown to correspond to the Golgi apparatus (arrows). (b and c) In cells treated with colchicine (20 μg/ml) (b) or nocodazole (10 μg/ml) (c) the HA is contained in brightly fluorescent small elements dispersed throughout the cytoplasm. (d) In taxol (10 μM)-treated cells, the fluorescent regions (arrows) containing HA appear more compact and near the cell periphery. Bar, 5 μm.

not treated with the drugs. In drug-treated cultures, after the temperature shift, the HA was found to concentrate in small and irregularly shaped elements dispersed throughout the cytoplasm (Fig. 1, a–c). In light of previous studies (14, 22) showing that upon a temperature shift to 18.5°C HA accumulates in Golgi components, and the known effect of colchicine in dispersing the Golgi apparatus (12, 26), it can be presumed that the small fluorescent elements (Fig. 1, b and c) seen after drug treatment are components of this organelle.

To determine if the HA accumulated in the dispersed compartment could be transferred with the proper polarity to the apical plasma membrane, infected cultures maintained at 18.5°C in the presence of colchicine were shifted for 80 min to 32.5°C, a temperature which allows rapid passage of material accumulated in the Golgi apparatus to the cell surface (22). Under these conditions, HA reached the cell surface (Fig. 2) but it was no longer confined to its normal apical location and appeared in both plasma membrane domains. The accumulation of HA on the lateral surfaces of the colchicine-treated monolayers gave rise to highly fluorescent polygonal patterns when the antibodies were applied after permeabilization with detergent (Fig. 2 b). This appearance of the HA on the basolateral surfaces results from the treatment with microtubule inhibitors, since it was not observed in control cultures that were infected with influenza and submitted to the same synchronization procedure, but were not incubated with the drugs (Fig. 2 a). In this case, the labeling pattern corresponded almost entirely to the punctate fluorescent spots representing the microvilli on the apical pole of the cells and very little label could be found on the lateral domains. In the inhibitor-treated samples, the simultaneous labeling of both apical and lateral surfaces was easily demonstrated by changing the plane of focus of the microscope (see Fig. 3). Essentially the same patterns of cell surface staining were obtained when infected monolayers treated with colchicine were transferred directly from 39.5° to 32.5°C, without an intermediate incubation at 18.5°C (not shown).

The effect of nocodazole on the sorting of HA was essentially indistinguishable from that of colchicine (Fig. 3). Similar minimal concentrations (10 μg/ml) of these drugs were required to produce the apparent dispersion of Golgi elements, observed at 18.5°C, and the nonpolarized appearance of HA at the cell surface found after transfer to 32.5°C. When added <30 min before transfer of the cultures to either 18.5° or 32.5°C, both inhibitors failed to markedly affect the polarized distribution of HA. However, the accumulation of HA in the dispersed cytoplasmic elements observed at 18.5°C did not require the continued presence of the inhibitors during incubation at this temperature. When nocodazole was present for 4 h and then removed 2 h before shiftdown to 18.5°C or 32.5°C for 80 min, its effect on the Golgi apparatus and the redistribution of HA were completely reversed (not shown) as would be expected from studies in other cell types (26). In addition, the nonpolarized surface distribution of HA, obtained in colchicine-treated cultures shifted to 32.5°C for 90 min after incubation at 39.5°C, was not altered during subsequent incubation at 39.5°C for 2 h in the absence of inhibitors (not shown).

Effect of Taxol, an Inhibitor of Microtubule Disassembly, on the Delivery of Influenza HA to the Cell Surface

In experiments similar to those just described, ts61s-infected cultures maintained at 39.5°C were incubated with taxol, a drug which inhibits microtubule disassembly and leads to the buildup of large bundles of microtubules (7, 29, 30, 33). When these cultures were transferred to 18.5°C, the distribu-
Figure 3. Visualization of HA on both the apical and basolateral surfaces of cells treated with nocodazole. MDCK cells were treated as in Fig. 2 but nocodazole (10 μg/ml) was used as an inhibitor. (a) The plane of focus was placed on the apical pole of the cells. The punctate fluorescence corresponding to the microvillar localization of HA as well as the somewhat diffuse fluorescent basolateral outlines of the same cells are apparent. (b) The plane of focus is placed in the middle of the cells (as in Fig. 2) and the high concentrations of HA present on the basolateral domains yield a clear polygonal pattern. Bar, 5 μm.

Figure 2. Distribution of the influenza HA on the plasma membrane of infected MDCK cells treated with microtubule inhibitors. Monolayers infected as described in the legend to Fig. 1 were shifted to 18.5°C for 90 min and then incubated at 32.5°C for 80 min before fixation, permeabilization, and immunolabeling with anti-influenza HA antibodies. (a) In cells not treated with inhibitors, very little HA is found on the lateral surface (arrows), whereas in colchicine- (b) or taxol- (c) treated cells, HA fluorescence in the basolateral surfaces is intense (arrows), yielding the polygonal pattern characteristic of a basolateral localization. The plane of focus in each case is on the lateral plasma membrane. Bar, 5 μm.

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When the taxol-treated ts61s-infected cultures were shifted from 18.5°C to 32.5°C, to allow progression of HA to the cell surface, the protein appeared not only in the apical but also on the basolateral domain (Fig. 2c). After short times of incubation at 32.5°C, the HA was concentrated in limited regions of both plasma membrane domains (Fig. 5a and b) from where it later diffused over the remainder of each. The clustered distribution of HA suggests that taxol treatment causes a restriction in the diffusion of the glycoprotein in the plasma membrane. As was the case with the other microtubule inhibitors, taxol was not effective when added only 30 min before the temperature shift, and the same effect on the delivery of HA to the cell surface was observed when cells treated with taxol were shifted directly from 39.5°C to 32.5°C (not shown).

In all the experiments using the microtubule inhibitors, temperature shifts were used to synchronize the transfer of HA between compartments and, in particular, its delivery to the plasma membrane. Under these conditions, the possibility could not be excluded that the basolateral accumulation of HA observed in the presence of inhibitors resulted from a very rapid redistribution of the glycoprotein that took place after it had reached the surface. To examine this possibility, control ts61s infected cultures, in which the HA had accumulated in the apical surface (90 min after a shift to 32.5°C), were incubated for 3 h with colchicine or nocodazole in the presence of cycloheximide (10 μg/ml), to eliminate the con-

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Figure 4. Relationship of the Golgi apparatus to microtubule bundles in a taxol-treated cell. Uninfected MDCK monolayers grown on collagen-coated coverslips were treated with taxol (10 μm) for 4 h at 37°C and processed for electron microscopy. A flattened Golgi apparatus (GA) is found closely apposed to a large microtubule bundle (arrowheads) and to the lateral intercellular space. Bar, 0.5 μm.

Figure 5. Focal distribution of HA on the surface of taxol-treated cells soon after the temperature shift to 32.5°C. Cells infected with influenza ts61 and incubated with (a and b) or without (c and d) taxol were shifted to 18.5°C for 90 min (see legend to Fig. 1), and then transferred to 32.5°C for 30 min. Anti-HA antibodies were added directly to fixed monolayers to visualize the apical surfaces only (a and c), or to monolayers which were first permeabilized with detergent (b and d) to allow access of the antibodies to the intercellular spaces and intracellular structures. (a and b) In taxol-treated cells, HA appears in discrete regions (arrows) of the apical (a) and lateral (b) plasma membranes where it is still concentrated 30 min after the shift to the permissive temperature. (c and d) In monolayers not treated with the inhibitor, there is very little HA on the lateral cell surfaces (d) and after 30 min at 32.5°C, the HA is distributed over the whole apical surface (c). Bar, 5 μm.
absence of the inhibitors, and subsequently shifted to 18.5°C for 90 min and 32.5°C. All samples were then shifted to 39.5°C for 4.5 h in the presence or absence of the inhibitors, and subsequently shifted to 18.5°C for 90 min and 32.5°C for an additional 90 min. The filters were then mounted in a modified Ussing chamber and their electrical resistances determined. Each value represents the mean and standard deviation of five filter measurements.

**Polarity of Viral Budding in Cells Treated with Microtubule Inhibitors**

Since the accumulation of HA in the apical surface precedes budding of the influenza virus from this domain and is, in fact, thought to be a determinant of the polarized budding (23), it was of interest to assess if the polarity of viral budding was affected in cells treated with microtubule inhibitors. Very few virions were produced, however, from ts61s-infected cells that were transferred to 32.5°C after a prolonged incubation at the nonpermissive temperature. The effect of the inhibitors on the polarity of viral budding was therefore examined in cultures infected with the wild-type influenza virus. In each infected culture, >1,500 viral particles were counted and their apical or basolateral location was recorded. Electron microscopic examination of colchicine-treated infected cultures, showed that nearly 40% of the virus particles were present in the intercellular spaces or trapped between the cell monolayer and the collagen support. As previously reported (24) in control cultures, virions were present at extremely low levels (≤0.5%) at these sites. In many cases, actual budding from the basolateral plasma membrane was observed (Fig. 6) and, in fact, 40% of virions clearly in the process of budding and still attached to the membrane (625 counted) were found here as well. Moreover, in the cell cytoplasm, near the basolateral surface, endocytic vesicles and/or lysosomes containing influenza virions were also found. Similar observations were made in taxol-treated monolayers, where ≤40% of the virions were also found associated with the basolateral surfaces (Fig. 7). The effect was substrate independent since identical results were obtained on MDCK grown on Millipore filters (not shown).

**Effect of Microtubule Inhibitors on the Distribution of the VSV G Protein**

To determine if the microtubule inhibitors also affect the polarized delivery of VSV G to the basolateral domain, the ts045 mutant of VSV was used in experiments similar to those just described for the influenza virus. In analyzing the results of these experiments it is important to note that, although G protein is normally delivered in a polarized fashion to the basolateral surface, subsequently some redistribution of the protein takes place that brings G molecules to the apical surface (22). Consequently, in these experiments the location of the G protein was assessed very soon after the shift to 32.5°C, before a significant redistribution could affect the results.

As with HA in influenza-infected monolayers, G in colchicine-treated VSV-infected cultures transferred to 18.5°C accumulated in small irregular elements dispersed through the cytoplasm (Fig. 8 b), rather than in the juxtanuclear crescent-shaped areas observed in control cultures (Fig. 8 a). As was also the case with the HA, the G protein in the taxol-treated cultures maintained at 18.5°C accumulated in the more compact Golgi apparatus found near the lateral surfaces (Fig. 8 c). On the other hand, in contrast to the situation with HA, when the temperature was shifted from 18.5° or 39.5° to 32.5°C, the G protein was delivered exclusively to the basolateral domains, where it could only be visualized by applying antibodies after permeabilization with detergent (compare Fig. 9, a and b; Fig. 9, c and d; and Fig. 9, e and f). It should be noted that the cytopathic effect of VSV infection, which eventually leads to loss of polarity, was manifested much sooner in colchicine-treated cultures, where many cells became rounded and frequently detached from the support. Therefore, observations on the polarized distribution of G were only made on regions of the monolayers where the cells had remained well attached to the substrate. Although the G protein was delivered to the basolateral domains, as expected from previous studies (22), after 90 min at 32.5°C significant amounts of G protein were detectable in the apical surfaces of many cells (not shown).

Taxol, a drug which, in contrast to colchicine, did not appear to abet the viral cytopathic effect, also failed to abolish the polarized delivery of G protein to the basolateral surface. After the shift to 32.5°C, the appearance of G protein on the lateral domain was somewhat retarded by taxol; but by 60 min after the shift to 32.5°C the G protein had accumulated sufficiently to determine that it remained exclusively confined to the basolateral surface (Fig. 9, e and f). As in all cells infected with VSV, at later times (90 min) G molecules became detectable on the apical surface although the amount of G protein on the apical surface of taxol-treated cells was less than in untreated cells (not shown).

As expected from these observations, the polarized budding of VSV virions from cells infected with the wild-type virus was also not affected by treatment with the various mi-
Figure 6. Nonpolarized budding of influenza virions from infected MDCK cells treated with colchicine. Monolayers grown on collagen were infected with wild-type influenza WSN for 1.5 h at 37°C, and incubated for 5.5 h with colchicine (20 μg/ml) at 37°C before processing for electron microscopy. (a and b) Viral particles assemble at both the apical (arrowheads) and basolateral surfaces (arrows) and are released into the medium or accumulate in the intercellular spaces. Bars: (a) 0.25 μm; (b) 0.5 μm.
Figure 7. Budding of influenza virions from both surfaces of taxol-treated MDCK cells. MDCK monolayers were infected and treated as described in the legend to Fig. 6, but taxol (10 μM) was used as inhibitor. In the cell depicted, viral particles bud from a limited region of the apical surface (arrowhead) near the intercellular junction or from a region of the basolateral membrane (arrow) near the base of the cells, which is shown at higher magnification in b. Bars: (a) 0.5 μm; (b) 0.1 μm.

cytotubule inhibitors. As is the case with untreated cultures, in cells treated with all these drugs, >90% of the viral particles were released from the basolateral surfaces (not shown).

Discussion

The experiments just described demonstrate that in epithelial cells infected with influenza virus, the microtubule inhibitors colchicine, nocodazole, and taxol interfere with the process that leads to the exclusive segregation of HA to the apical plasma membrane. After treatment with these inhibitors, HA was not only present in the apical surfaces but large amounts of the glycoprotein also accumulated in the basolateral domains. Furthermore, from such cells, budding of influenza virions was found to proceed from both aspects of the plasma membrane, an observation that underscores the role of the segregation of the envelope proteins in determining the site of influenza virion assembly (23).

Other investigators have previously examined the role of microtubules in the biogenesis and maintenance of epithelial cell polarity. Using cell fractionation (6) and electron microscopic autoradiography (1, 3, 9, 19), it was observed that microtubule-depolymerizing drugs markedly inhibit the incorporation of [3H]fucose-labeled glycoproteins in the microvillar membrane of absorptive intestinal cells, while in most cases leading to their enhanced accumulation in the lateral plasma membrane as well as in Golgi-associated vesicles. This apparent alteration in the routing of newly synthesized plasma membrane proteins is presumably responsible for the striking finding that in enterocytes of colchicine-treated rats (18), nearly regular microvillar borders, as well as partially assembled ones, appear in the basolateral plasma membrane domain near the base of the cells. In this case, microvillar membrane glycoproteins misrouted to the lateral membrane appear to trigger the ectopic assembly of brush border microfilaments. Together with these previous studies on the intestinal epithelium, our results with MDCK cells suggest that functional microtubules may be generally required for the correct segregation of apical membrane proteins in all epithelial cells.

Using cultured cells infected with a temperature-sensitive influenza viral mutant, we have been able to synchronize the transport of the newly synthesized glycoprotein to the cell surface. This allowed us to demonstrate that the microtubule inhibitors directly altered the targeting of the newly synthesized proteins rather than induce a redistribution of pre-existing apical plasma membrane proteins. Such a redistribution could, in principle, have involved the passage of apically delivered proteins through altered tight junctions, however this is unlikely since, when the microtubule inhibitors were applied after HA appeared on the apical surface, the polarized distribution of the protein was not altered. Moreover, judging from the maintenance of the electrical resistance of the monolayer, the inhibitors did not disrupt the
Figure 8. Effect of microtubule-acting drugs on the intracellular distribution of the VSV G protein in cells incubated at 18.5°C. MDCK monolayers were infected with the ts045 mutant of VSV for 1 h at 32.5°C and subsequently incubated in the presence or absence of inhibitors for 5 h at 39.5°C. Samples were then shifted to 18.5°C for 90 min before fixation, permeabilized with detergent, and immunolabeled with anti-VSV G antibodies. (a) Cells not treated with inhibitors. The G protein accumulates near the nuclei (N) in brightly fluorescent regions that correspond to the Golgi apparatus (arrows). (b) Cells treated with colchicine (20 μg/ml). The G protein is contained in brightly fluorescent elements dispersed throughout the cytoplasm. (c) Taxol (10 μM)-treated cells. The fluorescent regions containing G protein appear more compact and near the lateral surface. Bar, 5 μm.
Figure 9. Polarized incorporation of the VSV G protein in the basolateral domain of MDCK cells incubated with colchicine or taxol. ts045-infected monolayers (see legend to Fig. 8) were incubated for 5 h at 39.5°C in the absence of inhibitors (a and b) or in the presence of colchicine (c and d) or taxol (e and f). The cultures were shifted directly to 32.5°C and fixed at the times indicated below. Intact monolayers were incubated with anti-VSV G antibodies with no previous detergent treatment to visualize the apical surface (a, c, and e), or the antibodies were applied after permeabilization with detergent (b, d, and f) to allow access to the basolateral membranes and intracellular structures. (a and b) Cells in monolayers not treated with inhibitors, 30 min after the shift. There is no detectable labeling of the apical surface (a) but G protein is beginning to accumulate in the lateral domain (b; arrows). (c and d) Cells that remain attached to the substrate in colchicine-treated monolayers, 45 min after the temperature shift. There is no detectable labeling of the apical surface (c), but the lateral membranes are labeled (d; arrows). (e and f) Cells treated with taxol, 60 min after the shift. The apical surface is free of label (e) but high concentrations of G protein are found on the lateral domains (f; arrows). Bar, 5 μm.

In contrast with the striking effects on HA, in parallel experiments with VSV-infected cells we found, in agreement with the results of Salas et al. (28), that the microtubule inhibitors did not interfere with the polarized delivery of the G protein to the basolateral domain. This implies that the sorting or delivery mechanisms for the G protein differ fundamentally from those ensuring the segregation of HA to the apical surface. The fact that the microtubule inhibitors did not affect the segregation of the G protein is somewhat surprising since in migrating, VSV-infected normal rat kidney cells the G protein is normally delivered to the leading edge of the motile cell (2) and disassembly of microtubules leads to a uniform initial distribution of G protein over the entire cell surface (27). We must therefore conclude that the apical domain of MDCK cells is incapable of accepting G protein that contains post-Golgi vesicles, even if as a result of the dispersion of the Golgi elements and the elimination of any putative microtubule tracks these vesicles have access to all regions of the cell surface.

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