Polarized Budding of Measles Virus Is Not Determined by Viral Surface Glycoproteins

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For viruses that mature by a budding process, the envelope glycoproteins are considered the major determinants for the site of virus release from polarized epithelial cells. Viruses are usually released from that membrane domain where the viral surface glycoproteins are transported to. We here report that measles virus has developed a different maturation strategy. Measles virus was found to be released from the apical membrane domain of polarized epithelial cells, though the surface glycoproteins H and F were transported in a nonpolarized fashion and to the basolateral membrane domain, respectively.

Transport of viral envelope glycoproteins to and virus release from the apical plasma membrane are well documented for influenza viruses and for Sendai virus (7–9; for a review, see reference 14). Both viruses cause a localized infection of the respiratory tract. Though measles virus belongs to the same virus family (Paramyxoviridae), it spreads from the respiratory tract to the blood and from there to various organs and tissues. Because of this difference in the course of infection, it was of interest to analyze the infection of polarized cells by measles virus. Studies with monkey kidney cells (Vero C1008) and colon carcinoma cells (Caco-2) indicated that measles virus is released from the apical plasma membrane domain of these polarized cells (1). In the present study we have analyzed the transport of measles virus glycoproteins in Madin-Darby canine kidney (MDCK) cells, because these cells have been used more often than any other cultured cell line to study the polarized transport of proteins.

Infection of confluent MDCK cells by measles virus is very inefficient. However, we found that most cells were infected when the virus was added at the time the cells were seeded on filters. When the medium containing the virus inoculum was replaced 20 h later by fresh growth medium, an electrical resistance of 400 Ω · cm² was measured, indicating that the virus infection did not prevent the formation of a confluent cell monolayer. Further incubation of the cells resulted in increases of the resistance to values of 620 Ω · cm² (44 h postinfection [p.i.]) and 700 Ω · cm² (68 h p.i.). The loss of cell polarity became evident at 92 h p.i., when the electrical resistance was reduced to 380 Ω · cm². Based on these findings the growth of measles virus was determined up to 70 h after seeding (and infecting), when the cells still retained polarity. As shown in Fig. 1A, most of the virus released from MDCK cells was detected in the apical medium. To exclude the possibility that the small amount of measles virus in the basolateral medium (about 0.01%) was due to retention of the virus by the 0.4-μm pores of the filter, we analyzed virus infection in a polarized (Vero C1008) line and in a nonpolarized (Vero) line of monkey kidney cells. With Vero C1008 cells (Fig. 1B), the proportion of virus detectable in the basal filter chamber was as low as in the case of MDCK cells. However, the amount of virus released by nonpolarized Vero cells into the basal medium was more than 1,000-fold increased, indicating that virus budding from the basolateral plasma membrane is able to pass the 0.4-μm pore. Thus, measles virus buds preferentially from the apical side of MDCK cells.

To determine the location of the viral glycoproteins, a biotin label was attached at 56 h p.i. to the surface proteins of either the apical or the basolateral plasma membrane of filter-grown MDCK cells. Following cell lysis, monoclonal antibodies were used to specifically immunoprecipitate surface glycoproteins of measles virus, the hemagglutinin (H) and the fusion (F) proteins. In the Western blot analysis (Fig. 2), labeled H protein was detected in both samples, indicating nonpolarized surface transport. The F protein was found to have a different distribution, with the majority of the protein being present in the basolateral membrane domain. The localizations of both H and F are unusual for a virus released from the apical side of polarized epithelial cells. For comparison, the distribution of the hemagglutinin (HA) protein of an influenza virus (fowl plague virus) was determined under these labeling conditions, and the protein was found to be mainly on the apical membrane domain (Fig. 2). To confirm this unexpected result, the distribution of the two measles virus glycoproteins on the surfaces of MDCK cells was determined by indirect immunofluorescence microscopy with a confocal laser scanning microscope. Filter-grown cells were infected as described above. At 56 h after infection, the cells were fixed without disruption of the plasma membrane and incubated from both the apical and basolateral sides with a monoclonal antibody directed against either H or F. As shown in Fig. 3, H protein was detected in all three horizontal sections (apical, central, and basal). The vertical section confirmed the nonpolarized distribution of this viral glycoprotein. In the case of the F protein, strong fluorescence signals were detected in the central and basal sections but not in the apical section. The vertical profile confirmed that the majority of the F protein is present at the basolateral domain of the plasma membrane.

In order to find out whether the unexpected transport behavior of the measles virus glycoproteins is dependent on other viral components, stable lines of MDCK cells expressing either H or F but no other measles virus protein were established. For this purpose, cells were transfected with the H and F protein genes cloned into the pCG vector under the control of the cytomegalovirus promoter (3). The neomycin resistance gene was used to select for cells containing foreign proteins. As a
control, a cell line that stably expressed the HA gene of fowl plague virus from the pSG5new vector (Stratagene) was isolated. The distribution of the viral proteins on the cell surface was analyzed by domain-specific biotinylation (Fig. 4). Whereas the influenza virus HA was almost exclusively present on the apical membrane domain, about 75% of the measles virus H protein and about 99% of the measles virus F protein were localized on the basolateral cell surface. Because a similar surface distribution was found in virus-infected cells (Fig. 2), we conclude that the transport of the measles virus glycoproteins to the cell surface is independent of other viral proteins.

**FIG. 2.** Distribution of the measles virus (MV) glycoproteins (H and F) and the fowl plague virus (FPV) HA on the apical (a) and basolateral (b) surfaces of virus-infected MDCK cells. Surface proteins were labeled by domain-specific surface biotinylation. Following immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the viral proteins were visualized with the enhanced chemiluminescence detection system.

**FIG. 3.** Confocal immunofluorescence microscopy of the measles virus glycoproteins H and F (MV-H and MV-F, respectively) on filter-grown MDCK cells infected by measles virus. At 56 h p.i., cells were fixed with 2% paraformaldehyde and incubated with monoclonal antibodies directed against either H or F protein. Analysis was performed with a laser scanning microscope.

**FIG. 4.** Distribution of viral glycoproteins on the apical (a) and basolateral (b) surfaces of MDCK cells stably expressing either the H or the F protein of measles virus (MV) or the HA of fowl plague virus (FPV). Surface proteins were labeled by domain-specific surface biotinylation. Following immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the viral proteins were visualized with the enhanced chemiluminescence detection system.
It should be noted that the predominant basolateral localization of F did not prevent this protein from being incorporated into virus particles. This is evident from our finding that infectious virus is released from MDCK cells. Whether the small amount of F present on the apical cell surface (1 to 5%) is by itself sufficient for incorporation into virions or whether there exists a specific mechanism remains to be shown.

Our findings imply that viral proteins other than the surface glycoproteins H and F are responsible for the apical budding of measles virus. Candidates are the matrix (M) protein or the L, N, and P proteins, which are associated with the genomic RNA. In contrast to H and F, these proteins are all located in the cytoplasms of infected cells. No information about the specific interaction of either of these proteins with the cytoplasmic face of the apical plasma membrane is available. An alternative explanation is the polarized transport by a cellular carrier, e.g., elements of the cytoskeleton. Both actin filaments and microtubules are known to be involved in transport mechanisms in polarized epithelial cells (4, 6). In this context it is interesting that in Sendai virus a mutation in the M protein was shown to result in the destruction of the microtubule network and in the bipolar budding of the virions, whereas the wild-type virus buds only from the apical side of polarized epithelial cells (11–13). Measles virus is known to interact with the cellular cytoskeleton. The vectorial growth of actin filaments is involved in the transport of the viral nucleocapsids to the cell surface (2), and destruction of actin filaments prevents virus release (10). The L protein of measles virus has been reported to interact with tubulin (5). Whether any of these interactions is important for the polarized budding of measles virus remains to be shown.

The apical budding from epithelial cells of the respiratory tract allows measles virus to spread from one organism to another. It does, however, not explain how the virus spreads from the respiratory tract to the blood. The presence of H and F on the basolateral membrane domain should allow binding of the infected cell to and fusion with uninfected neighboring cells. In this way, the infection would spread from the polarized cell of the respiratory epithelium to other cells without requiring the loss of polarity. The studies with Sendai virus have shown that the polarity of virus release is an important determinant of pathogenicity (13). However, we are well aware that factors other than virus budding may also affect the spread of virus in vivo. Therefore, more work—especially with respiratory epithelial cells—is required to analyze the importance of polarized glycoprotein transport for the pathogenesis of measles virus infection.

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