CHAPTER 18

Enveloped Virus Maturation at Restricted Membrane Domains

RICHARD W. COMPANS*

Synthesis and assembly of the envelope proteins of lipid-containing viruses require the biosynthetic and transport processes involved in cellular membrane biogenesis, and such viruses have therefore been used extensively for investigation of these processes. With the exception of poxviruses, which assemble their membranes *de novo* in the cytoplasm, the assembly of enveloped viruses takes place on preformed cellular membranes. The precise location at which assembly occurs is a distinctive characteristic that is highly conserved among structurally similar viruses. Herpesvirus maturation occurs by budding at the inner nuclear membrane. Coronaviruses are assembled at the rough endoplasmic reticulum, and bunyaviruses bud at membranes of the Golgi complex. Virus particles of several other families are formed by budding at the plasma membrane. Polarized epithelial cells exhibit distinct apical and basolateral surface domains separated by tight junctions, and it has been observed that assembly of enveloped viruses occurs at one or the other of these membrane domains. Thus, with the exception of mitochondrial membranes, any membrane of the cell is known to be capable of serving as a virus maturation site.

What determines the cellular membrane selected for maturation by a particular enveloped virus? In those cases that have been investigated, viral envelope proteins accumulate at the membrane where virus budding occurs. The mechanisms by which viral envelope proteins become localized at specific membrane sites are likely to involve normal cellular processes for sorting proteins into different compartments. Current research in this area is centered around several key questions: (1) What is the precise nature of the information that determines sorting of viral proteins into distinct membrane

* University of Alabama in Birmingham, Alabama.

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compartments? (2) Where do the sorting events occur, and what are the cellular mechanisms for sorting of membrane proteins? (3) What are the biological consequences of virus maturation at restricted membrane domains?

Polarized Expression of Viral Glycoproteins

In the polarized line of Madin-Darby canine kidney cells (MDCK), which resemble differentiated cells of kidney distal tubule epithelia, influenza and parainfluenza viruses have been observed to assemble at the free apical surface, whereas vesicular stomatitis virus (VSV) and several retroviruses are assembled at basolateral membranes [1,2]. In each case, the viral surface glycoproteins are localized at the membrane domain from which the virus buds [2,3], suggesting that the site of glycoprotein insertion determines the viral maturation site. An alternate possibility would involve directional transport of viral matrix proteins (internal, nonglycosylated envelope proteins), and subsequent localization of glycoproteins at the same site by specific transmembrane interactions with the matrix proteins. This possibility is unlikely, however, since glycoprotein expression was shown to be polarized in the absence of any other viral gene products. Cloned DNA copies of influenza virus HA glycoprotein genes inserted into SV40 vectors yield fully glycosylated HA molecules, which are expressed at cell surfaces. When such recombinant viruses were used to infect primary African Green monkey kidney epithelial cells, in which influenza virus maturation is polarized at apical surfaces, the expression of the HA glycoprotein was found to be restricted to apical cell surfaces [4]. These observations indicate that the information for directional transport (sorting) of the HA glycoprotein is contained in the HA molecule itself, and does not depend on any other viral component. Further, polarized virus maturation and glycoprotein insertion continue under conditions in which glycosylation of HA is completely inhibited by the drug tunicamycin [5,6], demonstrating that the information for glycoprotein sorting resides in the polypeptide portion of the molecule, and not the carbohydrate portion.

Although similar information is not yet available for other viral glycoproteins, it is likely that the site of accumulation of viral glycoproteins will prove to be of general importance as a determinant of budding sites of enveloped viruses. In the case of a virus that forms at an intracellular membrane, the E1 glycoprotein of mouse hepatitis virus, a coronavirus, has been observed to be restricted to the perinuclear region of infected cells [7]. At least two exceptional cases must be considered, however, in which enveloped viruses appear to assemble in the absence of the viral glycoproteins. These include avian retroviruses as well as temperature-sensitive glycoprotein mutants of vesicular stomatitis virus, both of which yield virus particles that appear to be devoid of surface glycoproteins [8,9]. The assembly processes of such virus particles should be investigated further.

The finding that viral glycoproteins are localized in distinct plasma membrane domains of epithelial cells indicates that cellular transport processes result in their vectorial transport and insertion into a particular membrane site. Once inserted, barriers must exist to prevent lateral diffusion in the plane of the membrane, which would otherwise be expected to result in a randomized distribution around the cell surface. The junctional complexes that are present in regions of contact between adjacent epithelial cells probably form such a barrier. However, virus maturation was also found to be polarized at apical or basal surfaces even in individual MDCK cells in contact with a substrate, in which no tight junctions could exist [10]. How the mobility of surface components is restricted in such cells remains to be determined.

Because of the extensive structural information available about many viral glycoproteins, it is of particular interest to consider where, within the amino acid sequence, the signals for intracellular sorting and directional movement reside. Since the movement of glycoprotein molecules occurs relative to cellular membranes in which they are embedded, such "sorting signals" might be found in proximity to the hydrophobic membrane-anchoring sequences, either within the hydrophobic domain itself or in the adjacent cytoplasmic or external regions. The sites at which such information resides can probably be determined by introducing specific alterations into the amino acid sequences of glycoproteins by modifying genes at the level of cloned DNA. This approach has shown that modification of the cytoplasmic domain of the VSV G protein affects the rate of its transport to the cell surface [11].

Intracellular Pathways for Membrane Glycoprotein Transport

As discussed above, it is likely that maturation sites of enveloped viruses are determined, at least in the majority of cases, by the sites of insertion and accumulation of their envelope proteins. The accumulated evidence indicates that the intracellular pathway followed by viral and other membrane glycoproteins resembles the pathway of exocytosis of secretory proteins: synthesis on membrane-bound polyribosomes in the endoplasmic reticulum, movement through the Golgi complex, and transport to the cell surface in vesicles that fuse with the plasma membrane [12,13]. Glycosylation is initiated by transfer of lipid-linked oligosaccharides to nascent polypeptide chains in the rough endoplasmic reticulum, and many of the subsequent processing events in the formation of complex carbohydrate chains occur in the various compartments of the Golgi complex. In the case of viral glycoproteins synthesized in doubly infected MDCK cells, proteins destined for opposite membrane domains share a similar intracellular pathway of transport until they reach the Golgi complex [14]. Several observations suggest that such proteins are sorted into distinct sets of transport vesicles within the Golgi complex, which are then targeted to apical or basolateral cell surfaces.

The sodium ionophore monensin blocks the exit from the Golgi complex

of secretory proteins as well as most membrane glycoproteins [15]; however, the ionophore differentially affects the transport of viral glycoproteins destined for apical versus basolateral membranes of MDCK cells [16,17]. Influenza virus glycoprotein transport, and virus maturation at apical cell surfaces, are unaffected by the ionophore, whereas transport of the G protein of VSV to basolateral membranes is completely inhibited. The terminal stages of glycosylation of both viruses are inhibited [17], indicating that the glycoproteins may not reach the distal cisternae of the Golgi complex. Nevertheless, the influenza viral glycoproteins are able to reach the cell surface efficiently, suggesting that exit from the Golgi complex may occur by distinct pathways: a monensin-resistant pathway utilized by influenza virus, and a monensin-sensitive pathway utilized by VSV.

Simultaneous infection of MDCK cells with influenza virus and VSV results in continued polarity of virus maturation, at least during early stages of infection [18]. Influenza virus budding is found at the free apical surfaces, and VSV is assembled at basolateral membranes. VSV readily incorporates heterologous envelope proteins to form phenotypically mixed virus particles and pseudotypes, but such particles are not detected in doubly infected MDCK cells at early times after infection, indicating that viral glycoproteins are segregated into different cell surface domains [18]. These observations also support the conclusion that the viral glycoproteins are sorted into distinct sets of transport vesicles, since vesicles containing mixtures of glycoproteins would be expected to result in simultaneous insertion of both types of viral glycoproteins into apical or basolateral membranes.

Very little information is available on the exact nature of the vesicles involved in transport from the Golgi complex to the cell surface, or how the sorting of proteins into different vesicle populations might occur. Coated vesicles have been implicated in various stages of membrane traffic within the cell, including the movement of viral envelope proteins [19,20]. One attractive possibility is that different subpopulations of coated vesicles could form at the Golgi complex, possessing receptors that recognize the sorting signals in viral glycoproteins. Such vesicles could effect the subsequent movement of membrane proteins to various compartments. Further information on transport pathways of viral glycoproteins should provide considerable insight into the process of sorting of cellular membrane proteins in general.

Consequences of Restricted Viral Maturation Sites

Virus maturation at restricted membrane domains, or at intracellular sites, limits the availability of virions or viral antigens for interaction with components of the immune system. This has been demonstrated for VSV infection of MDCK cells, in which antibody was unable to prevent the spread of virus from cell to cell and the resultant formation of plaques [21]. In contrast,

influenza virus plaque formation in MDCK cells, or VSV plaque formation in nonpolarized cell lines such as BHK21, was completely prevented by the respective antisera. Since VSV maturation in MDCK cells occurs beneath tight junctions, which prevent the passage of large molecules such as antibodies into the intercellular spaces, the progeny virions are able to infect adjacent cells without exposure to antibody. It will be of interest to investigate the extent to which viral maturation sites may play a role in limiting the accessibility of viral components to antibody or cytotoxic cells in natural disease processes.

The site of insertion of viral glycoproteins at cell surfaces may also affect the type of cytopathology resulting from virus–cell interaction. Striking differences have been observed in the responses of various cell types to infection by paramyxoviruses [22]. Many cell types are highly susceptible to virus-induced cell fusion, whereas other cell types are resistant to fusion and the cells continue to appear morphologically normal. The latter are cells of epithelial morphology, in which parainfluenza virus assembly occurs at free apical surfaces. Paramyxovirus-induced cell fusion probably involves viral fusion glycoproteins acting to form a bridging structure between two adjacent cells, and the ability of virions or surface glycoproteins to form contacts between adjacent cells in monolayers of epithelial cell types is prevented by restriction of viral glycoproteins to apical surfaces above tight junctions. The role of intact junctions in resistance to cell fusion was demonstrated by treatment of MDCK cells with EGTA, which causes disruption of the junctional complexes [23]. Intact MDCK monolayers exposed to concentrated Sendai virus showed no fusion, whereas cells pretreated with EGTA showed formation of large syncytia. In the EGTA-treated cells, the virus was presumably able to penetrate into spaces between cells and induce fusion between adjacent cell membranes.

There is little information available on the possible influence that the sites of virus assembly in infected tissues may exert on the pathogenesis of viral diseases. Numerous examples exist where structurally similar viruses cause distinctly different natural infections. Parainfluenza viruses cause infections restricted to the respiratory tract, whereas measles virus, which is structurally similar, causes a generalized infection. Human influenza viruses are also restricted to the respiratory tract, whereas in avian species, influenza virus multiplies to high titers in the intestinal tract as well. In MDCK cells, all of these agents were found to be assembled at apical cell surfaces [24,23], indicating that the differences in disease processes are not reflected by a difference in viral maturation sites in this cell system. The biological properties of avian influenza viruses may be explained by their stability to low pH, enabling the virions to pass through the digestive tract and retain infectivity [25]. Since virus maturation sites in the MDCK cell system may not reflect the process of infection *in vivo*, it will be of interest to further investigate the possible consequences of restricted virus maturation sites on disease processes.

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