Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9318–9322, December 1986
Biochemistry

Polarized expression of a chimeric protein in which the transmembrane and cytoplasmic domains of the influenza virus hemagglutinin have been replaced by those of the vesicular stomatitis virus G protein

(protein transport/protein sorting/membrane protein)

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Communicated by Charles H. Sawyer, August 13, 1986

ABSTRACT **In polarized epithelial cells, influenza virus buds exclusively from the apical domain of the plasma membrane, whereas vesicular stomatitis virus (VSV) buds exclusively from the basolateral domain. In virus-infected cells, the envelope proteins, influenza hemagglutinin (HA) and vesicular stomatitis virus G (VSV G), are likewise transported to and localized in the same domain of the plasma membrane from which the viruses bud. Previous studies have shown that influenza HA and VSV G proteins, when expressed from cloned cDNAs, are accumulated preferentially on the proper domains (apical and basolateral, respectively), indicating that the signal(s) for polarized transport resides in the polypeptide backbone of the proteins. To further elucidate the structural features required for apical vs. basolateral transport, we have constructed a gene that encodes a chimeric protein (H1GA) containing the external domain of HA and the transmembrane and cytoplasmic domains of VSV G. When the chimeric protein (H1GA) is expressed in CV1 cells using a simian virus 40 late expression vector, it is transported to the cell surface with kinetics similar to that of the native HA protein. Further, the chimeric protein, when expressed in polarized MDCK cells using a vaccinia virus early expression vector, is transported only to the apical surface, suggesting that the ectodomain of HA contains a signal for apical transport.**

Studies providing the most insight into the synthesis and transport of plasma membrane proteins have utilized, as models, the infection of cells by enveloped viruses. The available evidence indicates that these viral membrane glycoproteins utilize the same biosynthetic, processing, and transport pathways as those employed by the cellular membrane proteins. Infection of polarized monolayers of MDCK cells with different enveloped viruses has shown that each virus buds asymmetrically from either the apical or the basolateral surface of the infected cells (1, 2). Influenza virus and two paramyxoviruses are found to bud only from the apical surface, whereas vesicular stomatitis virus (VSV) and several retroviruses are found to bud only from the basolateral surface (1, 2). Before the onset of budding, the viral glycoproteins [hemagglutinin (HA) and neuraminidase (NA) for influenza, and G for VSV] are found sequestered on the same membrane domain involved in the budding of virus particles (3–5).

Further studies using cloned cDNAs encoding the HA, NA, and G proteins showed that when these proteins are expressed in eukaryotic cells, they behave like authentic viral proteins. They are biologically active, glycosylated, and transported to the cell surface (6–10). In addition, HA and NA of influenza virus are preferentially expressed on the apical surface of polarized epithelial cells (11, 12), whereas the expressed G protein of VSV is selectively incorporated on the basolateral surface (13). This implies that structural features of the proteins themselves are recognized by the cellular machinery involved in both intracellular transport and polarized expression. Since influenza HA and VSV G proteins both possess a cleaved translocation signal at the NH_2 terminus and a "stop transfer" anchoring sequence at the COOH terminus but are transported to the opposite surface domains of polarized cells, chimeric constructions containing different structural domains of these two proteins would be useful in elucidating the structural features required for apical vs. basolateral transport.

Previous studies (14, 15) on chimeric integral membrane proteins have indicated that a proper three-dimensional structure plays a vital role in intracellular transport as well as in biological function. Therefore, we have now designed chimeric constructions that involve little or no alteration of the ectodomain of the proteins, by switching only the COOH-terminal transmembrane and cytoplasmic domains. Since previous studies (16, 17) have suggested a possible role for the COOH-terminal regions of the proteins for efficient transport, we have constructed a chimeric protein (H1GA), in which the external domain of HA has been fused to the transmembrane and cytoplasmic domains of G precisely at the beginning of the transmembrane domain. In this report we show that H1GA, when expressed in CV-1 cells using a simian virus 40 (SV40) late expression vector, is transported to the cell surface with kinetics similar to that of the native HA protein. Further, this chimeric protein, upon expression in polarized MDCK cells using a vaccinia virus expression vector, is selectively expressed on only the apical and not the basolateral surface.

MATERIALS AND METHODS

Cells, Viruses, and Plasmids. CV-1, MDCK, and human TK^-143 cells were grown as described (13). Preparation of stocks of influenza virus WSN/33 (H1N1), VSV (Indiana serotype), and vaccinia virus (strain IHD-J) have been reported (13, 18). Procedures for obtaining SV40 virus stocks expressing either influenza virus HA, VSV G protein, or chimeric H1GA proteins and helper virus (SVSal·32 or dl1055) have been described (7, 14). Vaccinia virus stocks expressing HA, G, or chimeric H1GA proteins were obtained

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Abbreviations: Endo H, endoglycosidase H; HA, hemagglutinin; NA, neuraminidase; RER, rough endoplasmic reticulum; SV40, simian virus 40; VSV, vesicular stomatitis virus; nt, nucleotide(s).

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as described (13, 19). Plasmid pGR125, containing VSV G cDNA, was obtained from J. K. Rose (Yale Univ., New Haven, CT); SVdl055 (SV40 with early gene deletion), from D. Nathans (Johns Hopkins Univ., Baltimore, MD); and vaccinia virus (strain IHD-J), pSC11 (vaccinia virus expression vector that coexpresses β-galactosidase), and VV-G (vaccinia virus expressing VSV G protein) from B. Moss (National Institutes of Health). Plasmids pHA28, pS5, and pGR7 have been described (6, 14).

Antibodies and Immunospecific Labeling. Anti-HA monoclonal antibody (H15A13-18) against A/PR/8/34 virus was obtained from W. Gerhard (Wistar Institute, Philadelphia). Anti-G monoclonal antibody (8G5F11) against VSV-Indiana was obtained from T. Sass (Washington State Univ., Pullman, WA). Fluorescein-conjugated goat anti-rabbit and goat anti-mouse immunoglobulins were bought from Cappel Laboratories (Cochranville, PA), and rhodamine-conjugated goat anti-rabbit IgG, from Miles. Polyclonal anti-influenza virus WSN and anti-VSV antibodies were prepared in rabbits. Procedures for intracellular and surface staining by indirect immunofluorescence and for indirect immunoferritin labeling have been described (7, 13).

SV40 Viruses Expressing VSV G and Chimeric H1GA Proteins. To construct pSVG, the VSV G cDNA was removed from pGR125 by *Eco*RI digestion and inserted into the *Eco*RI site of the SV40 vector pA11SVL3 (7).

To construct pSVH1GA, pHA28, which ends at nucleotide (nt) 1604 of the HA coding sequence, was cut with *Bam*HI and filled in with the Klenow fragment of DNA polymerase I to produce blunt ends. It was subsequently cut with *Mlu* I at nt 886, and the smallest fragment, encoding the COOH-terminal region of HA28, was isolated. pS5 was cut with *Mlu* I (nt 886) and *Pvu* I (nt 3737 in pBR322), and the large fragment, encoding the NH_2-terminal region of HA, was isolated. pGR7 was doubly digested with *Alu* I and *Ava* I, and the largest fragment, encoding the COOH-terminal region of G (starting at nt 1416), was isolated. This was subsequently cut with *Pvu* I (nt 3737 in pBR322) and the largest fragment was isolated. A three-way ligation using these three fragments produced a plasmid (pH1GA) containing cDNA encoding a chimeric protein containing the external domain of HA fused to the COOH-terminal anchoring and cytoplasmic domains of VSV G. The H1GA cDNA fragment was removed by partial *Eco*RI digestion and was cloned into pA11SVL3 as described (7). Virus stocks were prepared by cotransfection of CV-1 cells with SV40 recombinant DNA and SV40 helper DNA (SVSal·32 or SVdl1055) as described (20).

Vaccinia Viruses Expressing the H1GA Chimeric Protein. To construct pVV-H1GA (vaccinia insertion plasmid containing the chimeric H1GA cDNA), the H1GA cDNA fragment was isolated from pH1GA by partial *Eco*RI digestion. The overhanging ends were filled in using the Klenow fragment of DNA polymerase I and the H1GA cDNA was cloned into the *Sma* I site of the vaccinia vector pSC11 (19).

Vaccinia virus recombinants containing DNA encoding H1GA were generated by transfecting CV-1 cells with calcium phosphate-precipitated pVV-H1GA DNA and wild-type vaccinia DNA, as described (19). The resulting virus was plaqued in TK^-143 cells in the presence of 5-bromodeoxyuridine (25 μg/ml) and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (300 μg/ml). Thymidine kinase-deficient (TK^-) vaccinia virus recombinants producing blue plaques were picked and virus stocks were prepared as described (13, 19).

Other Procedures. CV-1 cells infected with SV40 recombinant viruses were labeled with L-[^{35}S]methionine (100 μCi/ml; 1 Ci = 37 GBq) at 40–48 hr postinfection as described (14). Conditions for labeling in the presence of tunicamycin (2 μg/ml), as well as for pulse–chase analysis, endoglycosidase H (Endo H) treatment, immunoprecipitation, and analysis in $NaDodSO_4$/10% polyacrylamide gels, have been described (14). The protein A binding assay for quantitation of cell surface proteins with or without EGTA treatment was done as described (13).

RESULTS

Construction of Expression Vectors Containing HA, G, and H1GA DNAs. Constructions of the SV40/G, SV40/H1GA, and vaccinia/H1GA recombinant DNAs (pSVG, pSVH1GA, and pVV-H1GA, respectively), are detailed in *Materials and Methods*. VSV G cDNA codes for a protein of 495 amino acids plus a leader sequence of 16 amino acids. In H1GA cDNA, the nucleotides encoding the 25 amino acid anchoring domain of HA plus 3 amino acids preceding the anchor, as well as the 12 amino acid cytoplasmic domain, have been removed and replaced, in phase, by nucleotides encoding the 20 amino acid anchoring and 29 amino acid cytoplasmic domains of VSV G. H1GA cDNA is expected to code for a protein of 574 amino acids plus a leader sequence of 17 amino acids. Fig. 1 shows the hydrophobic and nonhydrophobic domains of H1GA, HA, and G and the junction site of H1GA. The nucleic acid sequences at the junction of the transmembrane and ectodomains of G, HA, and H1GA were confirmed by DNA sequence analysis.

Expression of Wild-Type and Chimeric Proteins in CV-1 Cells Using SV40 Expression Vectors. Monolayers of CV-1 cells were infected with each of the SV40 recombinants and analyzed for both intracytoplasmic and cell surface expression by indirect immunofluorescence at 40 hr postinfection. Intracytoplasmic staining shows that HA, G, and H1GA are efficiently expressed in CV-1 cells, with the Golgi region showing intense fluorescence (Fig. 2 *A*, *C*, and *E*). Surface expression was also observed for all three proteins (Fig. 2 *B*, *D*, and *F*). Virtually all cells expressing intracytoplasmic G were also positive for surface fluorescence, whereas ≈50% of cells expressing intracytoplasmic HA exhibited cell surface expression. The remaining 50% showed only intracellular fluorescence typical of rough endoplasmic reticulum (RER) and/or Golgi staining (see arrow in Fig. 2*A*). Approximately 20% of the cells expressing intracellular H1GA were positive for surface fluorescence, while the remaining 80% showed only intense Golgi staining (see arrow in Fig. 2*E*).

To determine whether the HA, G, and H1GA polypeptides were processed and glycosylated, CV-1 cells were infected

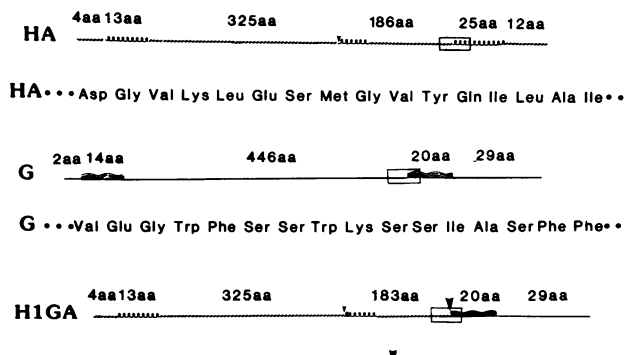

FIG. 1. Schematic representation of the primary structures of HA, G, and the chimeric protein H1GA as predicted from the DNA sequence, and the amino acid sequences at the junction site of the chimeric protein as compared to G and HA. Hydrophobic domains of HA (▪▪▪▪▪) and G (▬▬) and hydrophilic domains of HA (∿∿) and G (—) are indicated. Lengths are given in amino acids (aa). Small arrowheads indicate the site for cleavage of HA into HA1 and HA2, and large arrowheads indicate the junction site of the H1GA chimeric protein. Boxes indicate regions for which the predicted amino acid sequences are shown.

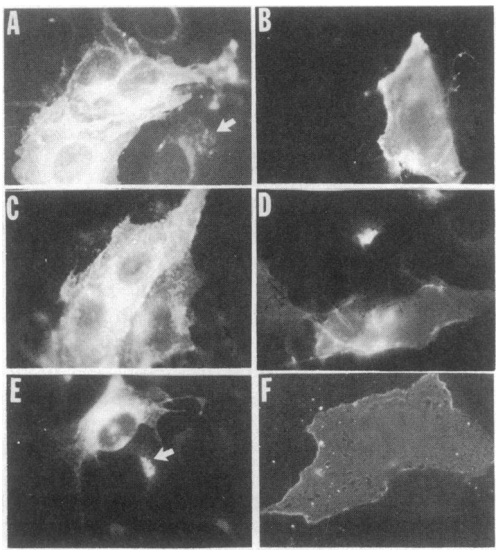

FIG. 2. Intracytoplasmic and cell surface expression of native and chimeric HA and G protein in CV-1 cells infected with SV40 recombinants. Monolayers of CV-1 cells were infected with SV40 recombinants and, at 48 hr postinfection, were stained for either intracytoplasmic expression (acetone/methanol-fixed cells) or surface expression (paraformaldehyde-fixed cells) using anti-HA monoclonal or anti-G monoclonal antibodies and fluorescein-conjugated goat anti-mouse IgG. (*A*) Intracytoplasmic HA. (*B*) Surface HA. (*C*) Intracytoplasmic G. (*D*) Surface G. (*E*) Intracytoplasmic H1GA. (*F*) Surface H1GA. (×475.)

with each of the SV40 recombinant viruses and labeled. The radiolabeled polypeptides were immunoprecipitated and analyzed by $NaDodSO_4$/PAGE. A comparison of the electrophoretic mobilities indicates that fully processed HA, G, and H1GA have molecular weights of approximately 72,000, 66,000, and 78,000, respectively. These molecular weights are expected if all three polypeptides had their signal sequences cleaved and were glycosylated. Glycosylation was further confirmed by the finding that each protein has a reduced molecular weight when isolated from cells incubated in the presence of tunicamycin (data not shown).

Since complex, Endo H-resistant sugars are added in the trans region of the Golgi complex, the acquisition of Endo H resistance was used to follow the kinetics of transport of these glycoproteins from the RER to the Golgi complex. Accordingly, monolayers of CV-1 cells were infected with each of the SV40 recombinant viruses, radiolabeled for 15 min followed by 1-, 2-, and 4-hr chases, and analyzed for Endo H resistance. Fig. 3 shows that within 1 hr of synthesis, VSV G has become completely resistant to Endo H. In contrast, both HA and H1GA exhibit a different and complex type of glycosylation pattern. As reported previously, both for WSN influenza virus-infected cells (21) and for cells expressing HA from cloned cDNA (6, 14), two major forms of glycosylated HA are found (Fig. 3). The lower molecular weight form (b, M_r 70,000) is the precursor of the higher molecular weight form (a, M_r 72,000). Form b is present in the RER and is completely Endo H-sensitive, producing b′ (M_r 64,000) upon Endo H treatment. Form a has traversed through the Golgi complex and is partially Endo H-resistant, producing a′ (M_r 71,000) upon Endo H treatment. H1GA polypeptides exhibited a similar but more heterogenous banding pattern. Again, the lower molecular weight form (d, M_r 73,000) is completely Endo H-sensitive and yields d′ (M_r 67,000) upon Endo H treatment. The higher molecular weight forms of H1GA exhibit a number of discrete bands (which possibly reflect increasing levels of glycosylation) and, unlike HA, are completely Endo H-resistant. This change in the

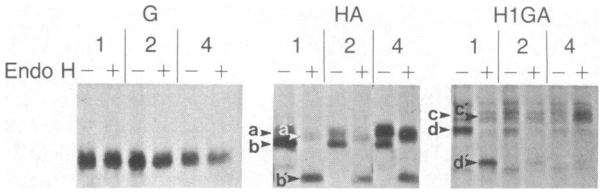

FIG. 3. Pulse–chase and Endo H treatment of G, HA, and H1GA proteins in CV-1 cells infected with SV40 recombinants. Monolayers of CV-1 cells were infected with either SVG, SVHA or SVH1GA. At 48 hr postinfection, cells were preincubated for 1 hr in methionine-free Dulbecco's modified Eagle's medium and then labeled for 15 min by incubation with L-[^{35}S]methionine. After two washes, cells were incubated in maintenance medium (Dulbecco's modified Eagle's medium containing 2% fetal bovine serum) without labeled methionine for 1, 2, or 4 hr ("chase" period) and then lysed in RIPA buffer [0.05 M Tris Cl, pH 7.4/0.15 M NaCl/1% (vol/vol) Triton X-100/1% sodium deoxycholate/0.1% $NaDodSO_4$] and used for immunoprecipitation with either monoclonal anti-HA or monoclonal anti-G antibodies. Aliquots of the immunoprecipitates were treated either with (+) or without (−) Endo H before analysis by $NaDodSO_4$/10% PAGE followed by fluorography. See text for discussion of bands indicated by arrowheads.

glycosylation pattern of H1GA may occur because of its prolonged stay in the Golgi complex (Fig. 2*E*). A similar change was observed (22) in the glycosylation pattern of a WSN NA mutant in which the transport is blocked in the Golgi complex. Further, the kinetics data also show that after a 1-hr chase, the majority of both HA and H1GA, unlike VSV G, are still Endo H-sensitive. Therefore, this indicates that the rate of the transport of H1GA from the RER to the Golgi complex is more similar to that of the native WSN HA than that of the VSV G.

Expression of the Wild-Type and Chimeric Proteins in MDCK Cells Using Vaccinia Expression Vectors. MDCK cells, which have been used extensively for studying polarized expression, are not permissive for SV40. To examine the site of surface expression of the native and chimeric proteins, we used recombinant vaccinia virus vectors. Accordingly, confluent monolayers of MDCK cells were infected with the recombinant vaccinia viruses and analyzed for surface expression by indirect immunofluorescence of both intact and EGTA-treated cells. Proteins that are localized on the apical surface can be detected by immunofluorescence of intact monolayers, whereas cells expressing basolateral proteins are negative. However, when intact monolayers are treated with EGTA, the tight junctions between cells are disrupted, enabling antibodies to gain access to the basolateral surfaces. Such EGTA-treated cells exhibit peripheral immunofluorescence when stained for basolaterally expressed proteins (13). Infection of MDCK cells with the recombinant vaccinia virus expressing the native influenza HA or the chimeric H1GA produced cell surface immunofluorescence without EGTA treatment, a characteristic of apically expressed proteins (Fig. 4 *A* and *B*). In contrast, MDCK cells infected with the vaccinia recombinant expressing the VSV G protein showed no fluorescence with intact monolayers but exhibited, following treatment with EGTA, intense peripheral fluorescence, which is a characteristic of basolaterally expressed proteins (Fig. 4 *C* and *D*).

Immunoelectron microscopy was used to confirm the site of surface expression in intact MDCK monolayers. Confluent monolayers of MDCK cells, grown on permeable supports, were infected with the H1GA recombinant vaccinia virus and cells were examined at 4 hr postinfection. MDCK cells infected with the recombinant vaccinia virus expressing the H1GA chimera demonstrated ferritin labeling predominantly on the apical surface (Fig. 5*A*) with little or no label bound on the basolateral surface (Fig. 5*B*). The basolateral surfaces of

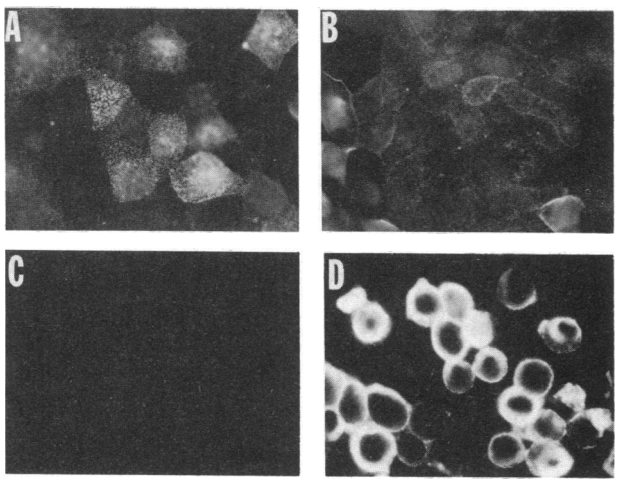

FIG. 4. Surface immunofluorescence of native and chimeric HA and G proteins in MDCK cells. Confluent monolayers of MDCK cells were infected with recombinant vaccinia viruses (multiplicity of infection 5–10) for 4 hr. Intact or EGTA-treated cells were treated with either rabbit anti-influenza virus WSN antibody or rabbit anti-VSV antibodies followed by rhodamine-conjugated goat anti-rabbit antibodies. (*A*) VV-HA-infected cells showing apical fluorescence on intact cells. (*B*) VV-H1Ga-infected cells showing apical fluorescence on intact cells. (*C*) VV-G-infected cells showing no apical fluorescence. (*D*) VV-G-infected-cells showing basolateral fluorescence after EGTA treatment. (×720.)

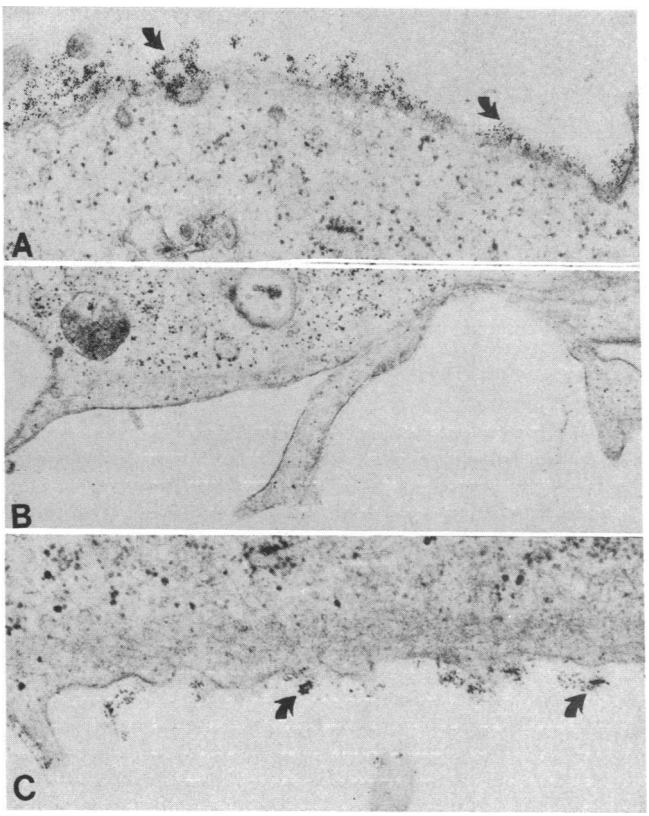

FIG. 5. Polarized expression of the H1GA chimeric protein in MDCK cells observed by immunoelectron microscopy. Confluent monolayers of MDCK cells on cellulose nitrate/acetate filters were infected with the recombinant vaccinia viruses expressing the H1GA protein (multiplicity of infection 5) or G protein (multiplicity of infection 10). At 4 hr postinfection, the infected cells were treated with rabbit antibodies to either influenza virus or VSV virus followed by ferritin-conjugated goat anti-rabbit antibodies and prepared for electron microscopy. (*A*) Apical surface of VV-H1GA-infected MDCK cells showing intense ferritin labeling. (*B*) Basal surface of cells in *A*, with no ferritin labeling. (*C*) Basal surface of VV-G-infected MDCK cells showing ferritin labeling. (*A*, ×29,500; *B*, ×21,000; *C*, ×31,500.)

cells grown on permeable supports were accessible to antibody: cells infected with the vaccinia recombinant expressing the VSV G protein were shown to be labeled extensively on the basal surface (Fig. 5*C*), as reported (13).

To quantitate the relative amount of the H1GA chimeric protein on apical and basolateral cell surfaces, we performed ^{125}I-labeled protein A binding assays on intact and EGTA-treated monolayers of MDCK cells infected with the vaccinia recombinants containing either HA, G, or H1GA cDNA. MDCK cells infected with the vaccinia virus/HA and/H1GA recombinants showed similar levels of antibody binding in both the intact and the EGTA-treated monolayers at 4 hr postinfection (Fig. 6). We conclude that the majority of the expressed HA and H1GA molecules were localized on the apical membrane. In contrast, infection with the vaccinia virus/G recombinant resulted in little antibody binding to the apical surface of MDCK cells. However, EGTA treatment resulted in a marked increase in the amount of antibody binding, reflecting the expression of the G protein on the basolateral membrane (Fig. 6).

DISCUSSION

Viral glycoproteins such as VSV G protein and influenza HA and NA are membrane-spanning glycoproteins that follow the same transport pathway as the cellular plasma membrane proteins. They are synthesized on membrane-bound polyribosomes and are cotranslationally translocated across the RER (23, 24). In the lumen of the RER, asparigine-linked oligosaccharides are added to the polypeptide chains (25). Subsequently, these viral glycoproteins move in a rate-determining step from the RER to the Golgi complex, where these proteins are acylated and the oligosaccharides are processed and become Endo H-resistant (25, 26). Further, it has been reported that the glycoproteins of both influenza virus and VSV traverse the same Golgi apparatus (27) and that the VSV G protein is in physical contact with NA through the terminal steps of Golgi processing (28). Thus, up to the stage of processing in the trans-Golgi complex, the three proteins G, HA, and NA follow the same transport pathway. When the HA, NA, and G proteins leave the Golgi complex, the HA and NA proteins are transported directly to the apical domain, whereas G is transported directly to the basolateral domain (4, 5, 11–13). Thus, the sorting of these proteins probably occurs either at the stage of exit from the trans-Golgi complex or in some post-Golgi compartment. Polarized expression of glycoproteins in the presence of tunicamycin shows that oligosaccharides do not play a specific role in protein sorting (29), indicating that structural features of the proteins are recognized by the cellular sorting machinery.

To determine the location of sorting signal(s) for polarized transport of the HA and G glycoproteins, we have made chimeric constructions of these two genes. In the H1GA chimera reported here, we directly fused the nucleotides encoding the external domain of HA to those encoding the COOH terminus of G, precisely at the beginning of the transmembrane domain. Thus, we maintained the entire ectodomain of HA unperturbed. Upon expression in CV-1 cells by means of the SV40 expression vectors, the chimeric H1GA protein as well as the wild-type viral proteins were found both intracellularly and on the cell surface. However, as discussed in *Results*, there were some differences in the levels of surface expression and in the intracellular distributions of G, HA, and H1GA. Rose and Bergmann (16), who have observed a similar heterogeneity in the distribution of

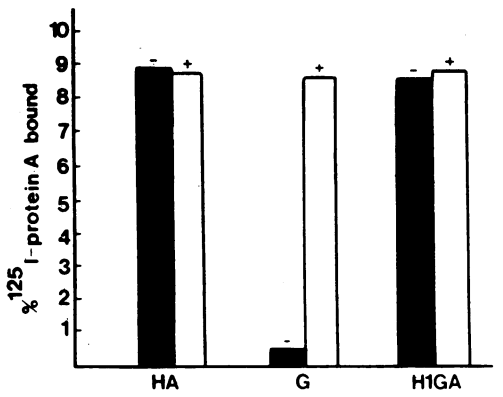

FIG. 6. ^{125}I-labeled protein A binding assays of MDCK cells infected with the vaccinia virus HA, G, and H1GA recombinants. Confluent monolayers of MDCK cells were infected with each vaccinia recombinant or wild-type vaccinia virus (strain IHD-J). At 4 hr postinfection intact (−) or EGTA-treated (+) monolayers were used in ^{125}I-labeled protein A binding assays with 125,000 cpm added to each assay mixture (17). Infection with vaccinia virus (strain IHD-J) yielded background levels of protein A binding that were <0.3% that for each antiserum; background values have been subtracted from all values plotted.

mutant VSV G glycoproteins, have proposed that since cells may begin expressing proteins asynchronously, cells fixed in different stages of the cycle would exhibit a different distribution of the protein in cases where intracellular transport of protein between different organelles is slow.

Our results also show that the WSN HA as well as the chimeric H1GA are transported slowly when compared to VSV G (Fig. 3). It has been reported that, within 20–30 min of synthesis, VSV G, influenza virus A/Udorn HA (H3), and A/Japan HA (H2) are transported from the RER to the Golgi as indicated by their acquisition of Endo H resistance (8–10), whereas WSN HA is transported slowly from the RER to the Golgi complex. Even 3 hr after synthesis, a substantial fraction of the WSN HA remains Endo H-sensitive (A. K. Pattnaik and D.P.N., unpublished data). Factors responsible for slower transport of WSN HA or H1GA are unknown. Perhaps WSN HA has a lower affinity for a receptor that is responsible for transporting nascent glycoproteins from the RER to the Golgi complex. Alternatively, WSN HA may be slow to form a stable trimer, which, in turn, may be a prerequisite for efficient transport. In any case, kinetic studies of the acquisition of Endo H resistance show that the chimeric H1GA is transported with kinetics essentially similar to that of the wild-type HA protein. Earlier studies (refs. 16 and 17; T. J. Bos and D.P.N., unpublished data) have shown that, although anchorless HA or G proteins are capable of being transported to and secreted from the cell surface, they move at a much slower rate from the RER to the Golgi complex and their terminal glycosylation pattern differs from that of native proteins. The data presented in this paper show that the addition of the heterologous G anchor and cytoplasmic domains to an anchorless HA protein restores but does not otherwise affect the rate of transport of the native HA. Puddington *et al.* (30) have recently shown that heterologous cytoplasmic domains may affect the kinetics of intracellular transport of chimeric VSV G proteins.

The present data show that, like the wild-type HA glycoprotein, the chimeric protein (H1GA) containing the external domain of HA and the transmembrane and cytoplasmic domains of G is transported to and localized on the apical membrane of polarized MDCK cells. VSV G protein, on the other hand, is localized on the basolateral membrane of MDCK cells (13). Three types of analyses—indirect surface immunofluorescence, binding of ^{125}I-labeled protein A, and immunoelectron microscopy—support this conclusion. These results indicate that a signal for apical transport of HA may reside in the HA ectodomain. Alternatively, it is possible that there is no specific signal for apical transport. Membrane proteins may be normally delivered to the apical domain unless they have a specific signal directing them to the basolateral domain. Recent results (E.B.S. and R.W.C., unpublished) have shown that a chimeric protein (MCFHA) containing the ectodomain of a retroviral (Friend murine leukemia virus) glycoprotein fused to the transmembrane and cytoplasmic domains of WSN HA is expressed on the apical surface of MDCK cells. These data, unlike those in the present report, suggest the presence of a targeting signal on the transmembrane and cytoplasmic domain of HA. Taken together, these results indicate that HA may contain multiple sorting signals in different domains of the polypeptide and that either each signal may function independently or one signal may override another.

This research was supported by Public Health Service Grants AI16348 and AI12680.

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