Opposite Polarity of Virus Budding and of Viral Envelope Glycoprotein Distribution in Epithelial Cells Derived from Different Tissues

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Abstract. We compared the surface envelope glycoprotein distribution and the budding polarity of four RNA viruses in Fischer rat thyroid (FRT) cells and in CaCo-2 cells derived from a human colon carcinoma. Whereas both FRT and CaCo-2 cells sort similarly influenza hemagglutinin and vesicular stomatitis virus (VSV) G protein, respectively, to apical and basolateral membrane domains, they differ in their handling of two togaviruses, Sindbis and Semliki Forest virus (SFV). By conventional EM Sindbis virus and SFV were shown to bud apically in FRT cells and basolaterally in CaCo-2 cells. Consistent with this finding, the distribution of the p62/E2 envelope glycoprotein of SFV, assayed by immunoelectronmicroscopy and by domain-selective surface biotinylation was predominantly apical on FRT cells and basolateral on CaCo-2 cells. We conclude that a given virus and its envelope glycoprotein can be delivered to opposite membrane domains in epithelial cells derived from different tissues. The tissue specificity in the polarity of virus budding and viral envelope glycoprotein distribution indicate that the sorting machinery varies considerably between different epithelial cell types.

EPITHELIAL cells in vivo are organized as selectively permeable continuous sheets that separate two different compartments. The cells within the sheets are joined by tight junctions which restrict the paracellular transit of ions and macromolecules and separate, on the plasma membrane, apical and basolateral domains. The two domains have different protein and lipid compositions (for review see Simons and Fuller, 1985; Rodriguez-Boulan and Nelson, 1989) generated and maintained by a specific sorting machinery.

The study of the molecular machinery involved in epithelial sorting has taken advantage of the use of RNA viruses as tools (Rodriguez-Boulan, 1983; Sabatini et al., 1988). These viruses bud polarly from the epithelial cell surface (Rodriguez-Boulan and Sabatini, 1978) from the same domain where their envelope glycoproteins accumulate during infection (Rodriguez-Boulan and Pendergast, 1980). The spike glycoproteins of the virus follow the same intracellular routes of endogenous plasma membrane proteins, are sorted intracellularly in the *trans*-Golgi network (TGN)[1] (Misek et al., 1984; Matlin and Simons, 1984; Rindler et al., 1984; Fuller et al., 1985*a*) and reach the appropriate membrane compartment even in the absence of other viral products (Gottlieb et al., 1986; Stephens et al., 1986). In most of these studies MDCK epithelial cells have been used (Rodriguez-Boulan, 1983; Sabatini et al., 1983; Simons and Fuller, 1985; Rodriguez-Boulan and Nelson, 1989; Caplan and Matlin, 1989).

Recent data suggest that the molecular mechanisms by which proteins are delivered to the plasma membrane can differ in cells derived from different tissues (Le Bivic et al., 1990; Matter et al., 1990). It has also has been shown that, in transgenic mice carrying the human LDL receptor gene, there is an opposite membrane domain distribution of the low-density lipoprotein (LDL) receptor protein in different tissues: apical in kidney cells and basolateral in liver and intestine (Pathak et al., 1990). These results suggest a tissue specificity in the polarity of membrane protein sorting and distribution in epithelial cells.

The rat thyroid epithelial cell line (Fischer rat thyroid cell line; FRT) (Ambesi-Impiombato and Coon, 1979) manifests morphological and functional polarity both as a monolayer (Nitsch et al., 1985) and as a suspension culture (Garbi et al., 1987). Like MDCK cells, FRT cells display basolateral budding of vesicular stomatitis virus (VSV) and basolateral location of the G protein (Nitsch et al., 1985). Moreover, FRT cells infected with Sindbis virus (SV) results in apical viral budding and apical distribution of the SV envelope glycoproteins (Nitsch et al., 1985), whereas a related virus, Semliki Forest virus (SFV) and its p62/E2 envelope glyco-

1. *Abbreviations used in this paper*: FRT, Fischer rat thyroid cell line; HA, influenza hemagglutinin; LDL, low-density lipoprotein; PFU, plaque-forming unit; SFV, Semliki Forest virus; SV, Sindbis virus; TER, transepithelial resistance; TGN, *trans*-Golgi network; VSV, vesicular stomatitis virus.

protein are basolaterally targeted in MDCK cells (Fuller et al., 1985*b*; Roman and Garoff, 1986). Although SFV and SV are highly related to each other and share large homologies at the DNA and the protein levels it cannot be established from the above mentioned experiments whether the opposite assembly site of SV in FRT cells and SFV in MDCK cells are a result of intrinsic differences between the two viruses or to cell type differences in the sorting machinery.

To answer this question it was necessary to study the sorting of both viruses in the two different epithelial cell systems. Since MDCK cells were not susceptible to SV infection, we compared the polarized budding of SV and SFV in FRT cells and in another well-established epithelial cell line CaCo-2, derived from a human colon carcinoma (Fogh and Trempe, 1975; Pinto et al., 1983). CaCo-2 cells display similar polarized budding of VSV and influenza virus as in MDCK cells (Rindler and Traber, 1988). We report here that in FRT cells the budding of SFV is apical, as shown for SV (Nitsch et al., 1985), whereas both SFV and SV bud basolaterally in CaCo-2 cells. Moreover, by both immunogold labeling and by domain-selective surface biotinylation, we show that the p62/E2 SFV envelope glycoprotein is delivered to the apical membrane domain in FRT cells and to the basolateral membrane domain in CaCo-2 cells. Our results indicate that the same virus and the same viral glycoprotein can be sorted to opposite membrane domains in different epithelial cells thus demonstrating cell type specific variations in the sorting machinery of epithelial cells.

Materials and Methods

Reagents and Antibodies

Media and reagents for cell culture were purchased from GIBCO Limited (Paisley, Scotland) and Sigma Chemical Co. (St. Louis, MO); glutaraldehyde and Poly/Bed 812 from Polysciences, Inc. (Warrington, PA); sulfo-*N*-hydroxyl-succinimido-biotin (S-NHS-Biotin) from Pierce Chemical Co. (St. Louis, MO); all other reagents were obtained from Sigma Chemical Co.

An anti-influenza hemagglutinin (HA) protein mAb was a kind gift of E. Rodriguez-Boulan (Department of Cell Biology and Anatomy, Cornell University Medical College, New York); an affinity purified anti-G protein, rabbit polyclonal antibody (Matlin et al., 1983) was donated to us by K. Matlin (Department of Anatomy and Cell Biology, Harvard Medical School, Boston, MA); the 9AB4 anti-p62/E2 mAb was kindly provided by K. Simons (European Molecular Biology Lab, Heidelberg, Germany). Rabbit anti-mouse affinity purified antibodies were purchased from Jackson ImmunoResearch (West Grove, PA).

Cell Cultures

FRT cells (Ambesi-Impiombato and Coon, 1979) were cultured in Coon's modified Ham's F12 medium containing 5% FCS. CaCo-2 cells (Fogh and Trempe, 1975) were grown in DME supplemented with 10% FCS, nonessential aminoacids (1%), penicillin (50 mU/ml), and streptomycin (50 μg/ml). Tissue culture plastics were from either Falcon Labware (Becton Dickinson & Co., Oxnard, CA) or Nunc (Roskilde, Denmark). For experiments on filters Millicell HA (0.45-μm pore size, 27-mm diam; Millipore Corp., Bedford, MA) were used when processing for transmission EM was required; Transwells (0.4-μm pore size, 24-mm diam; Costar Data Packaging Corp., Cambridge, MA) were used for biotinylation experiments. In either case 2×10^6 cells were plated on the filter and the same used after 5–7 d. Transepithelial resistance (TER) was measured in situ after culture medium change, using the Millicell ERS voltohmmeter (Millipore Corp.).

Virus Infection

Influenza virus (WSN strain) was grown and titered as described (Rodriguez-Boulan et al., 1983); VSV (Indiana serotype) was grown and titered on BHK cells (Matlin et al., 1981); a prototype of SFV was grown in BHK cells and purified as previously described (Kääriäinen et al., 1969); SV (HR strain) was plaqued, grown and titered on chick embryo fibroblasts as previously described (Pfefferkorn and Hunter, 1963). Confluent cell monolayers were infected for 1 h in PBS containing Ca^{2+} and Mg^{2+} and 1% FCS. PBS was then replaced with an appropriate volume of tissue culture medium and the infection was proceeded. VSV infection was carried out at 31°C (Van Meer et al., 1982), all other infections at 37°C. The following multiplicity of infection were used: 10 plaque forming unit (PFU)/cell for influenza; 20–30 PFU/cell for VSV; 30–40 PFU/cell for SFV; 50 PFU/cell for SV.

Electron Microscopy

Monolayer cultures on filters were fixed in situ for 10 min in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3, postfixed in 1% OsO_4 in the same buffer for 20 min, rinsed in H_2O and stained en bloc for 1 h with 1% aqueous uranyl acetate. After dehydration in a graded series of ethanols, samples were embedded in Poly/Bed 812. Thin sections were stained with uranyl acetate and lead citrate and examined in an electron microscope (400 T; Philips Electronic Instruments, Mahwah, NJ). Cell suspensions were subjected to the same treatment.

Immunoelectron Microscopy

At the end of the infection the monolayer cultures on plastic dishes were transferred to a 37°C room and were washed five times with pre-warmed PBS containing Ca^{2+} and Mg^{2+}. Cells were fixed in the same pre-warmed buffer containing 3.7% formaldehyde and 0.1% glutaraldehyde. After 5 min the monolayer cultures were transferred to room temperature, and after 30 min, were washed twice with PBS and harvested by scraping with a rubber policeman. Cells were collected by low-speed centrifugation and resuspended by gentle shaking with 100 μl of PBS containing Ca^{2+}, Mg^{2+}, 0.2% gelatin, and the appropriate antibody. Incubation was performed for 30 min with occasional agitation, cells were washed five times by repeated centrifugation and resuspended in PBS with Ca^{2+} and Mg^{2+}. The cell suspension was incubated with a second rabbit anti-mouse antibody, where needed. Finally the cells were resuspended with 100 μl of PBS containing colloidal gold (prepared by the citrate method) conjugated with protein A (Slot and Geuze, 1981), and incubated for 3 h at room temperature with occasional agitation. After five washing cycles cells were processed for EM.

Biotinylation, Immunoprecipitation, and Streptavidin Blotting

Biotinylation of confluent monolayers on Transwells with S-NHS-biotin was carried out as described (Sargiacomo et al., 1989). Infected cell monolayers were washed three times with ice-cold PBS. An aliquot of frozen (−20°C) stock of S-NHS-biotin solution was thawed just before use and diluted to a final concentration of 0.5 mg/ml. Approximately 1 ml was added to the apical or to the basolateral compartment of the filter chamber and incubated with agitation for 20 min at 4°C. The procedure was repeated twice in a row. Cells were washed with serum-free tissue culture medium and then with PBS.

Biotinylated filters were excised from the chamber, transferred to an eppendorf tube, and extracted with 1 ml of ice-cold lysis buffer (10 mM Tris, pH 7.4, 0.15 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.2% BSA). After 1 h samples were clarified by centrifugation and the supernatant was incubated for 6–12 h, at 4°C, with the specific antibody bound to protein A–Sepharose. The pellets obtained by centrifugation were resuspended in 80–100 μl of Laemli buffer, boiled for 3–5 min, and run on a SDS/6–12% PAGE under reducing conditions. After the electrophoretic separation proteins were transferred to nitrocellulose filters as described (Towbin et al., 1979) in a Trans-Blot apparatus (Bio-Rad Laboratories, Richmond, CA), at a constant voltage of 60 V for 14–16 h. The nitrocellulose filters were then incubated with ^{125}I-Streptavidin under conditions that reduce nonspecific binding (Birk and Koepsell, 1987), dried, and autoradiographed on film (XAR-5; Eastman Kodak Co., Rochester, NY). Densitometry analysis was carried out within the limits of linearity.

Results

Distribution of HA and G Proteins on the Plasma Membrane of FRT Cells

It has been established that the plasma membrane distribution of HA and G proteins corresponds to the budding polar-

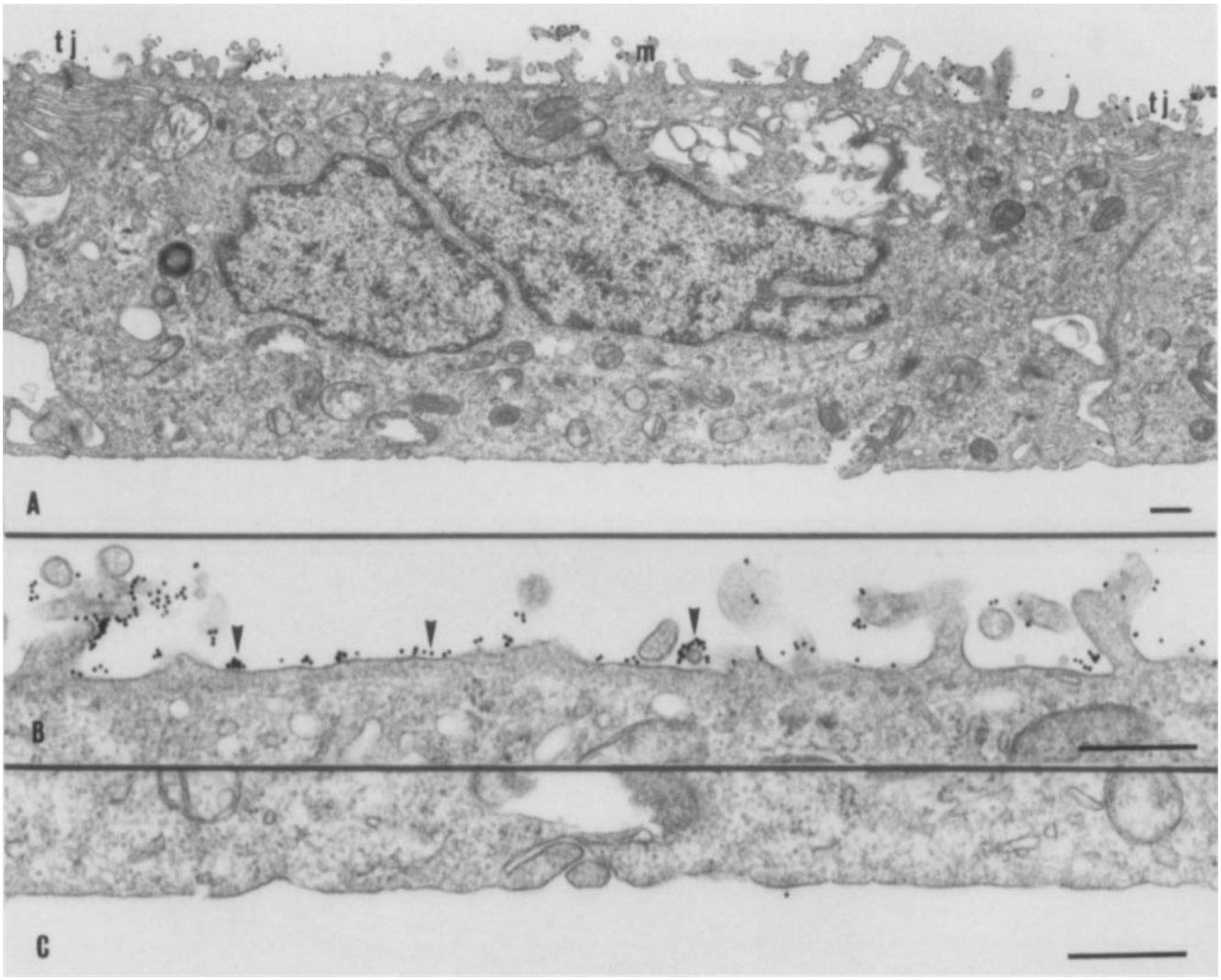


Figure 1. HA glycoprotein is localized at the apical domain of influenza virus-infected FRT monolayers. FRT cells were grown to confluency on plastic dishes, infected with influenza virus for 20 h, fixed, and scraped from the dish. HA has been localized by immunogold labeling using an anti-HA mAb. *A* shows an EM picture of a section of a labeled cell. The apical side is delimited by tight junctions (*tj*) and is covered by microvilli (*m*). *B* and *C* are details of the apical and basolateral domains, respectively. Colloidal gold particles (*arrowheads*) are present only on the apical cell side (*B*). Bar, 0.5 μm.

ity of the respective viruses (Rodriguez-Boulan and Pendergast, 1980). The notion that HA is transported to the apical domain and G to the basolateral domain directly from the Golgi complex has been largely substantiated (Rodriguez-Boulan et al., 1984; Misek et al., 1984; Matlin and Simons, 1984). HA and G have been, in fact, extensively used as representative models for apical and basolateral targeted proteins in many studies on the biosynthetic protein sorting in epithelial cells (Simons and Fuller, 1985; Sabatini et al., 1988; Rodriguez-Boulan and Nelson, 1989).

To further establish that FRT cells acquire and maintain the polarized phenotype both as confluent monolayers on plastic and on filters, we determined the membrane distribution of HA and G proteins by immunogold labeling and by domain-selective surface biotinylation. By immunogold labeling of virus-infected plastic-grown cell monolayers we found that HA was predominantly apical (Fig. 1) while G was predominantly basolateral (Fig. 2). Table I summarizes the number of gold particles per cell profile and the average particle density per micrometer in either case. No labeling was observed on uninfected cells. The same result was obtained by domain-selective surface biotinylation of virus-infected cells that had been grown to confluency on filters (Fig. 3). The data indicate that HA is mostly expressed on the apical domain and G on the basolateral domain of virus-infected FRT cells. Quantitative scanning densitometry of two independent experiments indicated that >85% of HA was apical and >80% of G was basolateral. We conclude that FRT cells are able to sort viral proteins to both the apical and the basolateral membrane compartments and that HA and G are sorted to the same membrane compartments as was previously reported for MDCK cells (Rodriguez-Boulan and Pen-

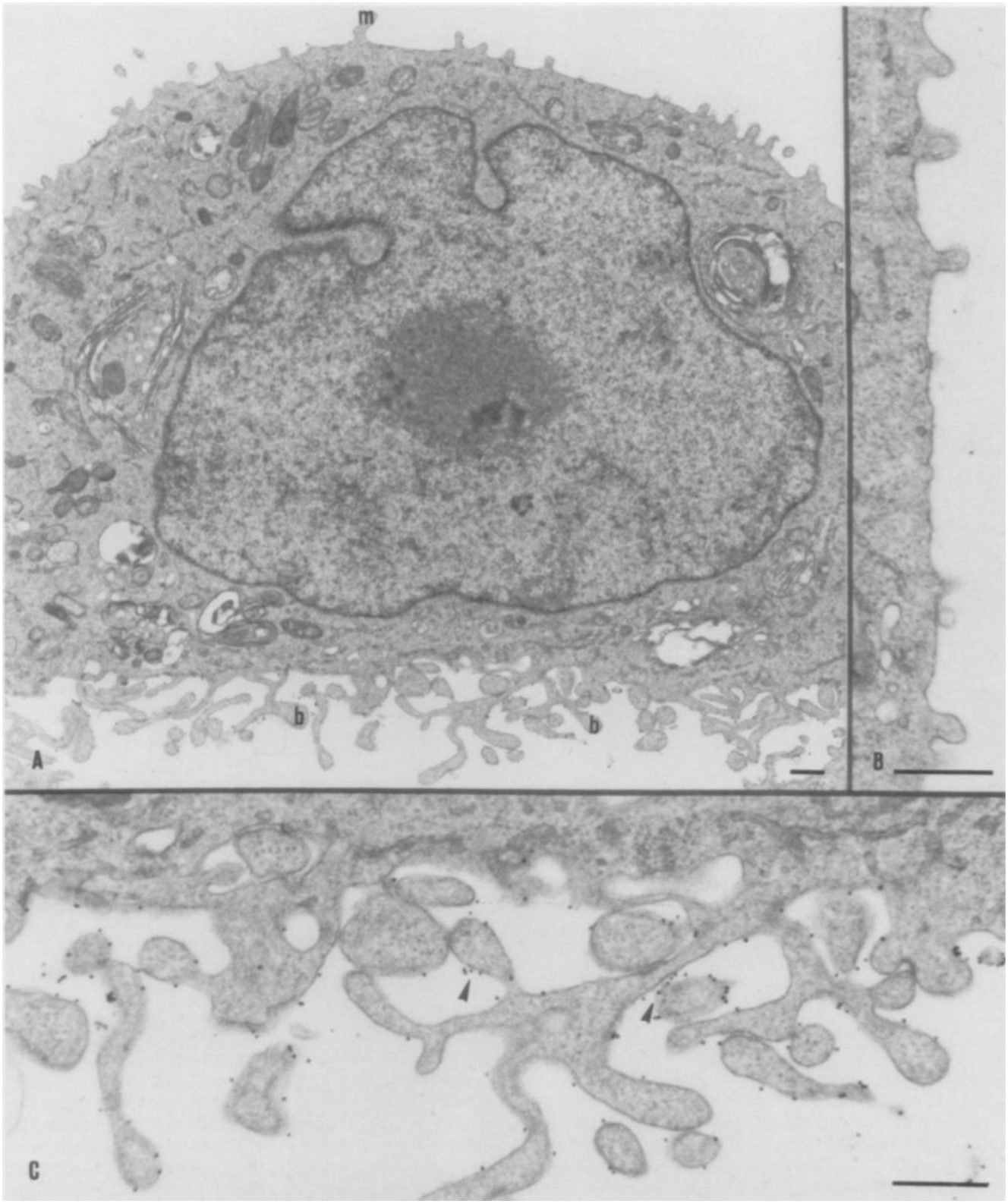


Figure 2. G protein is localized at the basolateral domain of VSV-infected FRT cells. Confluent monolayers on plastic dishes were infected at 31°C with VSV, for 7 h, fixed, and scraped from the dish. For immunogold localization an affinity purified polyclonal antibody was used. The apical side of the cell in the EM picture (*A*) has several microvilli (*m*) on its apical side. The basolateral side is characterized by several blebs (*b*), possibly do to the virus cytopathic effect. Few gold particles are seen at the apical border of the cell, shown in *B* at higher magnification. Many gold particles (*arrowheads*) are instead present at the basal side of the cell (*C*). Bar, 0.5 μm.

Table I. Immunogold Labeling on the Plasma Membrane of FRT Cells Infected with Influenza or VSV and Stained with the anti-HA or the anti-G Antibody, Respectively

	*Average number of gold particles per cell profile (± SD)		‡Average density of gold particles per μm (± SD)	
	Apical	Basolateral	Apical	Basolateral
HA	408 ± 113	22 ± 11	14.6 ± 4	0.5 ± 0.25
G	64 ± 23	441 ± 153	2.2 ± 0.8	10 ± 3.5

* Colloidal gold particles were counted on the apical and basolateral profile of each cell. At least 30 different cells were counted in each case.
‡ The number of gold particles counted on the apical and basolateral cell domain was divided by the length of the corresponding cell profile.

dergast, 1980). Polarized sorting of the two viral glycoproteins is observed in plastic-grown cells as well as in filter-grown cells.

Infection of FRT and CaCo-2 Cells with SFV and SV

The susceptibility of FRT and CaCo-2 cells to SFV and SV infection was determined by indirect immunofluorescence (data not shown).

To assess the kinetics of viral infection on filters the TER of confluent cell monolayers was monitored for several hours of viral infection. A dramatic drop in TER occurred, both in FRT and in CaCo-2 cells, during virus infection (Fig. 4)

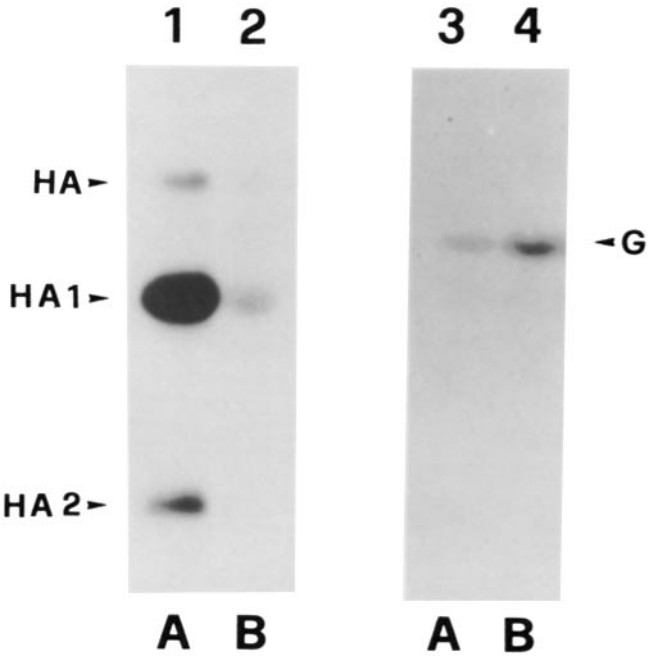
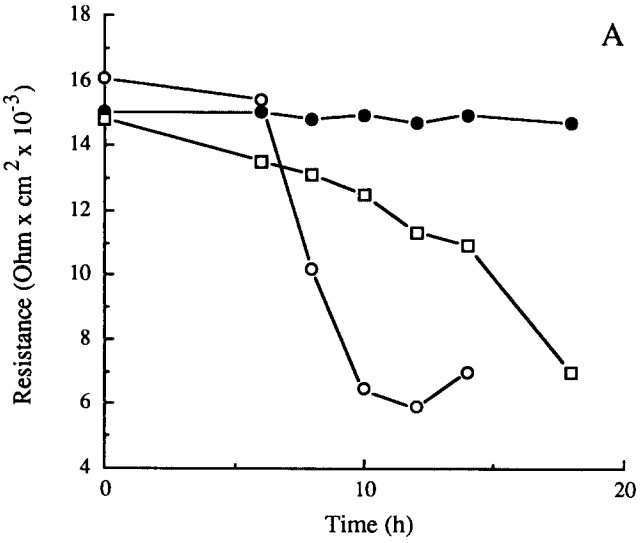


Figure 3. Membrane domain distribution of HA and G proteins in filter-grown FRT cells. Confluent monolayers of FRT cells were grown for 5 d on Costar Transwells and infected with influenza virus for 20 h (*1* and *2*) or with VSV for 7 h (*3* and *4*). After surface labeling of the apical (*A*) or basal (*B*) side of the monolayer with S-NHS-biotin, HA and G were immunoprecipitated, separated on SDS/PAGE, and transferred to nitrocellulose. Biotinylated proteins were revealed by ^{125}I-streptavidin blotting. HA is mostly labeled from the apical side (*1–A*); G is mainly labeled from the basolateral side (*4–B*).



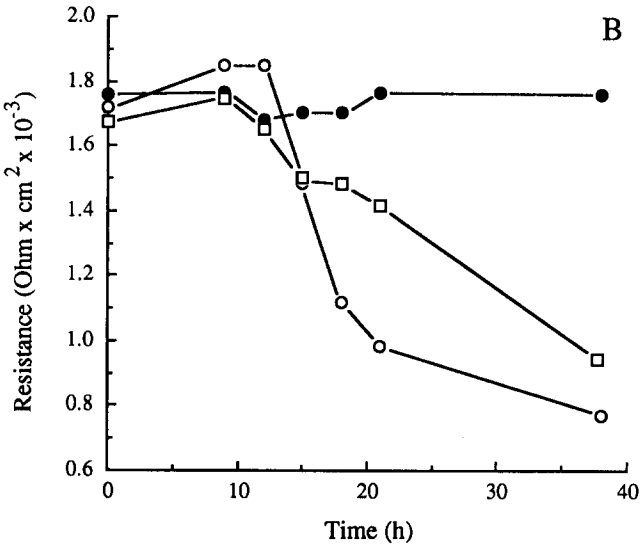
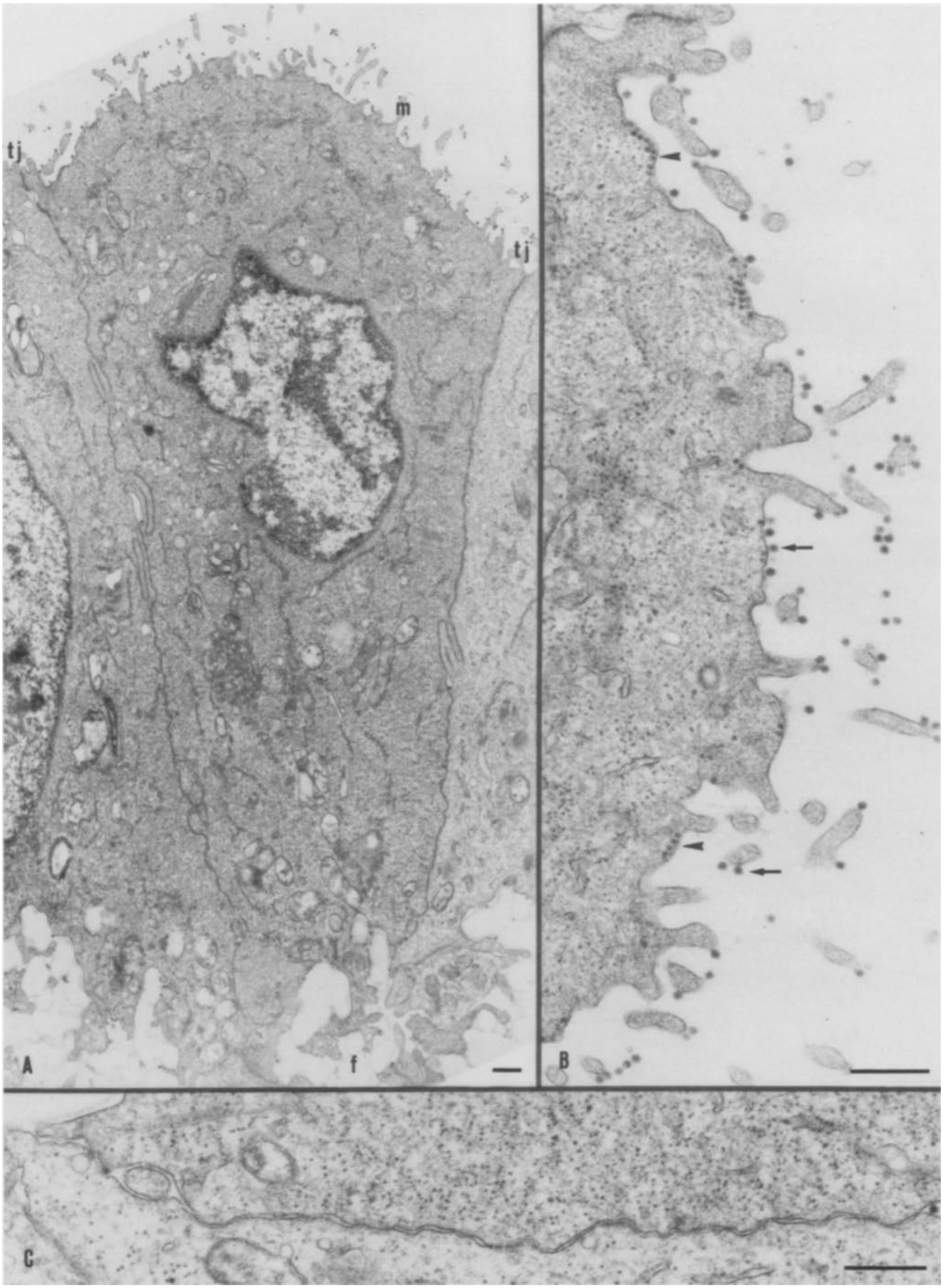


Figure 4. Drop in TER after virus infection of FRT and CaCo-2 cell monolayers. Confluent monolayers of FRT cells (*A*) and CaCo-2 cells (*B*) were infected with SFV (○) or SV (□). The TER of uninfected cells (●) is stable over several days. After infection a drop in TER is observed in both cell lines, with both viruses. The drop occurs earlier in FRT than in CaCo-2 cells and is more dramatic in SFV- than in SV-infected cells. Note that in FRT cells the TER is almost 10 times higher than in CaCo-2 cells.

indicating that both viruses were able to infect the cells and to produce a cytopathic effect. The drop in TER occurred between the 6th and 10th hour in FRT cells and between the 12th and the 20th hour in CaCo-2 cell monolayers after SFV infection. During SV infection the resistance fell down much more slowly and gradually in both cell types with a more dramatic drop after the 14th hour in FRT cells and after the 20th hour in CaCo-2 cells. All subsequent experiments were performed at a time after infection when no significant drop in TER had occurred yet.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Budding Polarity of SFV and SV in FRT and CaCo-2 Cells

We compared the budding polarity of SFV and SV in FRT and CaCo-2 cell lines. Budding polarity of SFV in FRT cells and of SFV and SV in CaCo-2 cells was determined by conventional EM of confluent monolayers of cells grown on filters. We found that SFV budding in FRT cells occurred predominantly from the apical side (Fig. 5), i.e., the same as SV. In a small percentage of cells (<15%) budding appeared to be non-polarized. In the vast majority of infected cells (>85%) virus particle budding from the apical membrane domain exceeded 83% of total budding viruses. These data refer to the apical versus basolateral domain of individual cell profiles irrespective of the surface area of each membrane domain.

On the other hand, when CaCo-2 confluent monolayers were infected with SFV it was found that the virus budded basolaterally (Fig. 6), i.e., opposite to FRT. A similar result was obtained with SV (Fig. 7). The number of both SFV and SV particles detected on the basolateral domain of CaCo-2 cells exceeded 90% of the total budding viruses. A small number of cells (<5%) displayed non-polarized budding of the two viruses.

Budding polarity was also determined on confluent monolayers grown on plastic with similar results (data not shown).

Distribution of p62/E2 on the Plasma Membrane of FRT and CaCo-2 Cells

The membrane domain of MDCK cells where budding occurs is the same where virus envelope glycoproteins are localized (Rodriguez-Boulan and Pendergast, 1980). We demonstrated that this is indeed the case also for SFV-infected FRT and CaCo-2 cells. We focused our attention on SFV since, in both cell lines, it infected with higher efficiency and shorter latency. We determined the distribution on the plasma membrane of SFV-infected cells of p62/E2, one of the virus envelope glycoproteins. The p62/E2 glycoprotein was detected by colloidal gold localization in plastic-grown monolayers of FRT (Fig. 8) and CaCo-2 cells (Fig. 9). Table II summarizes the number of gold particles per cell profile and the average particle density per micron, in either case. Labeling on uninfected cells was almost undetectable. The data clearly show that p62/E2 is predominantly apical in FRT cells and predominantly basolateral in CaCo-2 cells. The apical location of SFV envelope glycoprotein in infected FRT cells was confirmed by the use of an anti-SV glycoprotein polyclonal antibody that cross-reacts with SFV spike glycoproteins (data not shown).

A similar result was also obtained by domain-selective surface biotinylation (Fig. 10) of filter-grown FRT and CaCo-2 cells infected with SFV. A higher fraction of immunoprecipitated p62/E2 was labeled when FRT cell monolayer was biotinylated from the apical side. The opposite was true for CaCo-2 cells; immunoprecipitated p62/E2 was labeled almost exclusively when cells were biotinylated from the basolateral side. Quantitation by scanning densitometry of two independent experiments indicated that >80% of p62/E2 was apical in FRT cells and basolateral in CaCo-2 cells.

Discussion

We have compared the budding polarity of two RNA viruses and the viral envelope glycoprotein distribution in two epithelial cell lines: FRT, derived from rat thyroid (Ambesi-Impiombato and Coon, 1979) and CaCo-2, derived from a human colon carcinoma (Fogh and Trempe, 1975). The purpose of this work was to determine whether the molecular mechanisms responsible for the acquisition/maintenance of membrane protein polarity are identical, or not, in cells derived from different tissues.

We have demonstrated an opposite budding polarity of SV and SFV in FRT and CaCo-2 cells: both viruses, in fact, bud apically in FRT cells and basolaterally in CaCo-2 cells. We have also shown that the viral envelope glycoprotein p62/E2 of SFV is delivered to the apical domain of FRT cells and to the basolateral domain of CaCo-2 cells. It has been previously demonstrated that the p62/E2 glycoprotein is expressed on the basolateral surface of MDCK cells (Roman and Garoff, 1986).

This is the first time that evidence is presented for opposite budding polarity of the same virus and for opposite domain distribution of the same transmembrane viral glycoprotein in epithelial cell lines derived from different tissues. The observation does not appear to be limited to viral envelope proteins.

It has been recently found that GPI anchor proteins, which are delivered to the apical domain of several epithelial cell lines, including CaCo-2 and MDCK (Lisanti and Rodriguez-Boulan, 1990), are either unpolarized or basolateral in FRT cells (Zurzolo et al., manuscript in preparation). Moreover, preliminary data indicate that CD8 lymphocyte antigen, which is delivered to the apical domain of transfected MDCK cells (Migliaccio et al., 1990), is found predominantly on the basolateral domain of transfected FRT cells (Nitsch et al., unpublished results).

Tissue-specificity or Somatic Cell Mutation?

Taken together the data obtained suggest the existence of tissue specificity in the sorting polarity of viruses, viral proteins, and perhaps endogenous proteins, in thyroid-, intestine-, and kidney-derived cells. The evidence obtained so far indicates that intestinal and kidney cells have similar sorting polarity while thyroid cells manifest more differences. This may reflect the fact that the thyroid is a rather unusual endocrine organ in which the apical domain of follicular cells faces a closed storage compartment. The tissue specificity

Figure 5. Budding of SFV in FRT cells. Confluent monolayers of FRT cells on Millipore filters were infected with SFV for 7 h and processed for transmission EM. (*A*) The preservation of the polarized phenotype was judged by the presence of tight junctions (*tj*) and microvilli (*m*) on the apical border, where most of the virus budding occurs. The cell surface in contact with the filter (*f*) is irregular and devoid of virus particles. *B* is a magnification of a portion of the apical cell domain of the cell in *A*. Budding viruses (*arrows*) and nucleocapsids adhering to the cytoplasmic side of the apical cell membrane (*arrowheads*) can be seen. *C* is a magnification of a portion of the basolateral domain of the same cell. Bar, 0.5 μm.

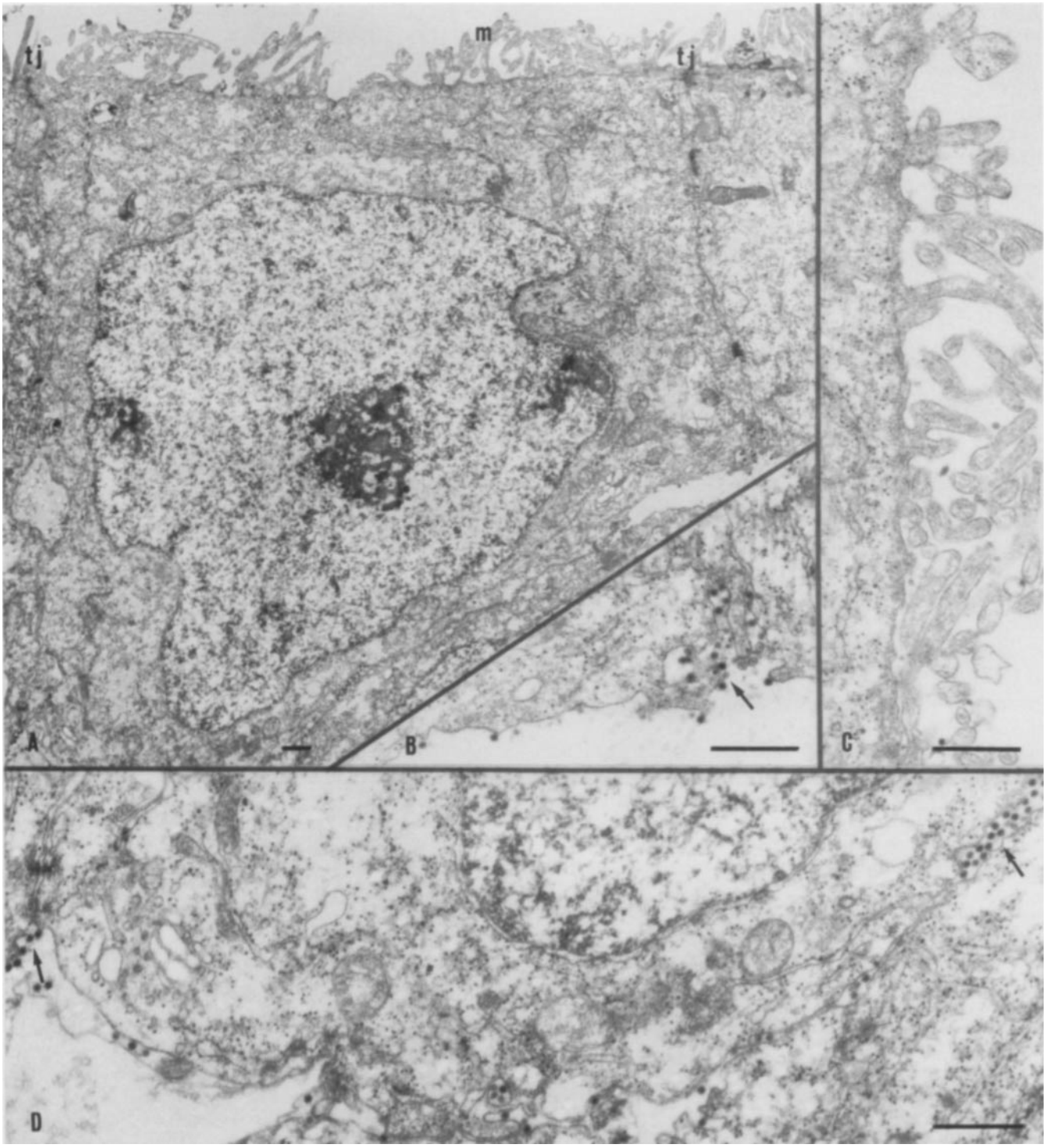
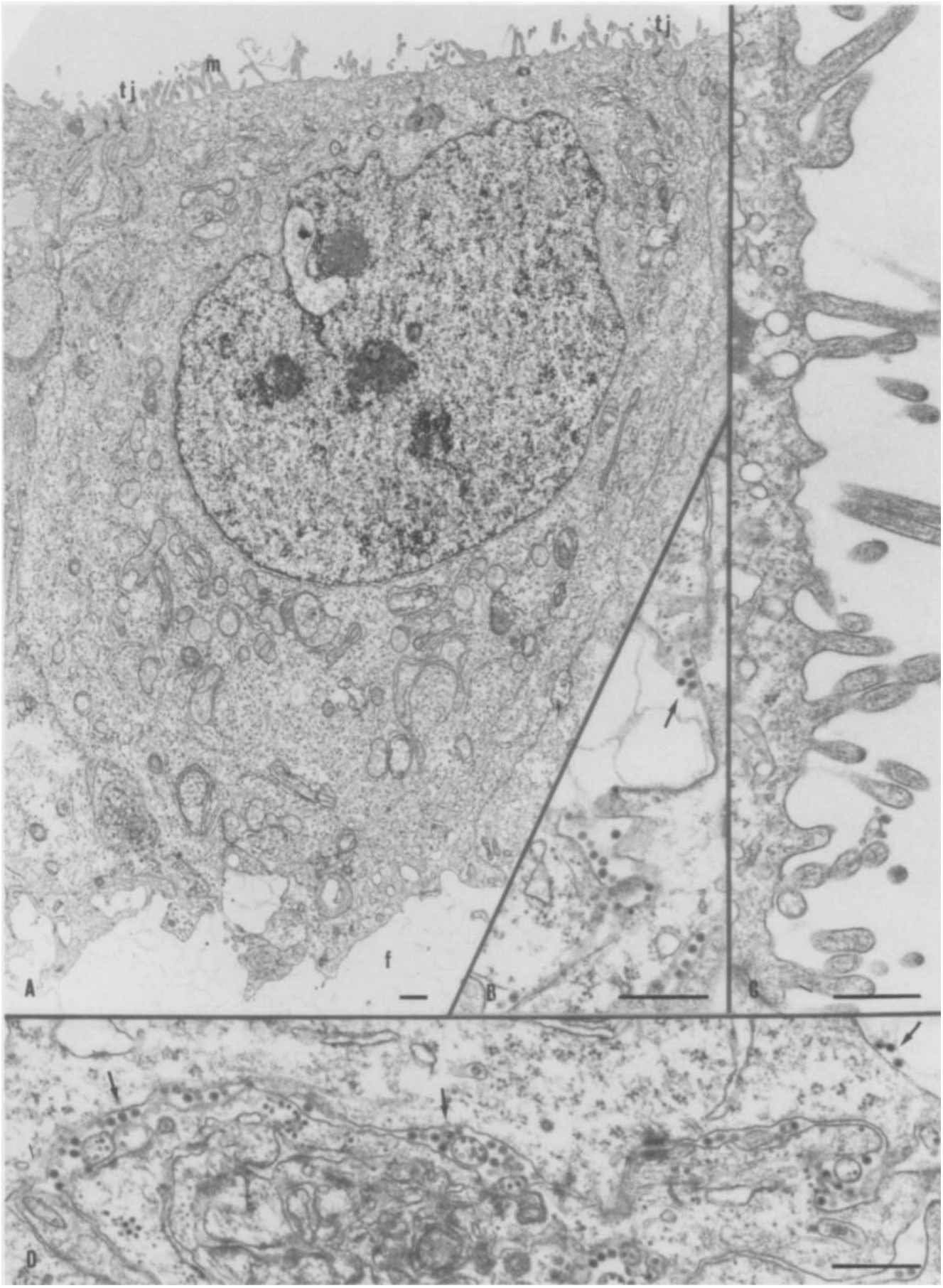


Figure 6. Budding of SFV in CaCo-2 cells. Confluent monolayers on Millipore filters were infected with SFV for 12 h and processed for EM (*A*). The cell apical surface, delimited by tight junctions (*tj*) and microvilli (*m*), is devoid of virus particles. This is best seen in *C*, a higher power picture of the apical border of the cell in *A*. *B* and *D* are details of the basolateral surface of the cell shown in *A*. Several virus particles (*arrows*) can be seen. Bar, 0.5 μm.

Figure 7. Budding of SV in CaCo-2 cells. Confluent monolayers on Millipore filters were infected with SV for 18 h and processed for EM. (*A*) The cell apical surface, delimited by tight junctions (*tj*) and microvilli (*m*), is devoid of virus particles. Viruses are present on the basal cell surface facing the filter (*f*). This is best seen in *B* and *D*, that show details of the basolateral surface of the same cell. *C* is a higher power picture of the apical border of the same cell. Bar, 0.5 μm.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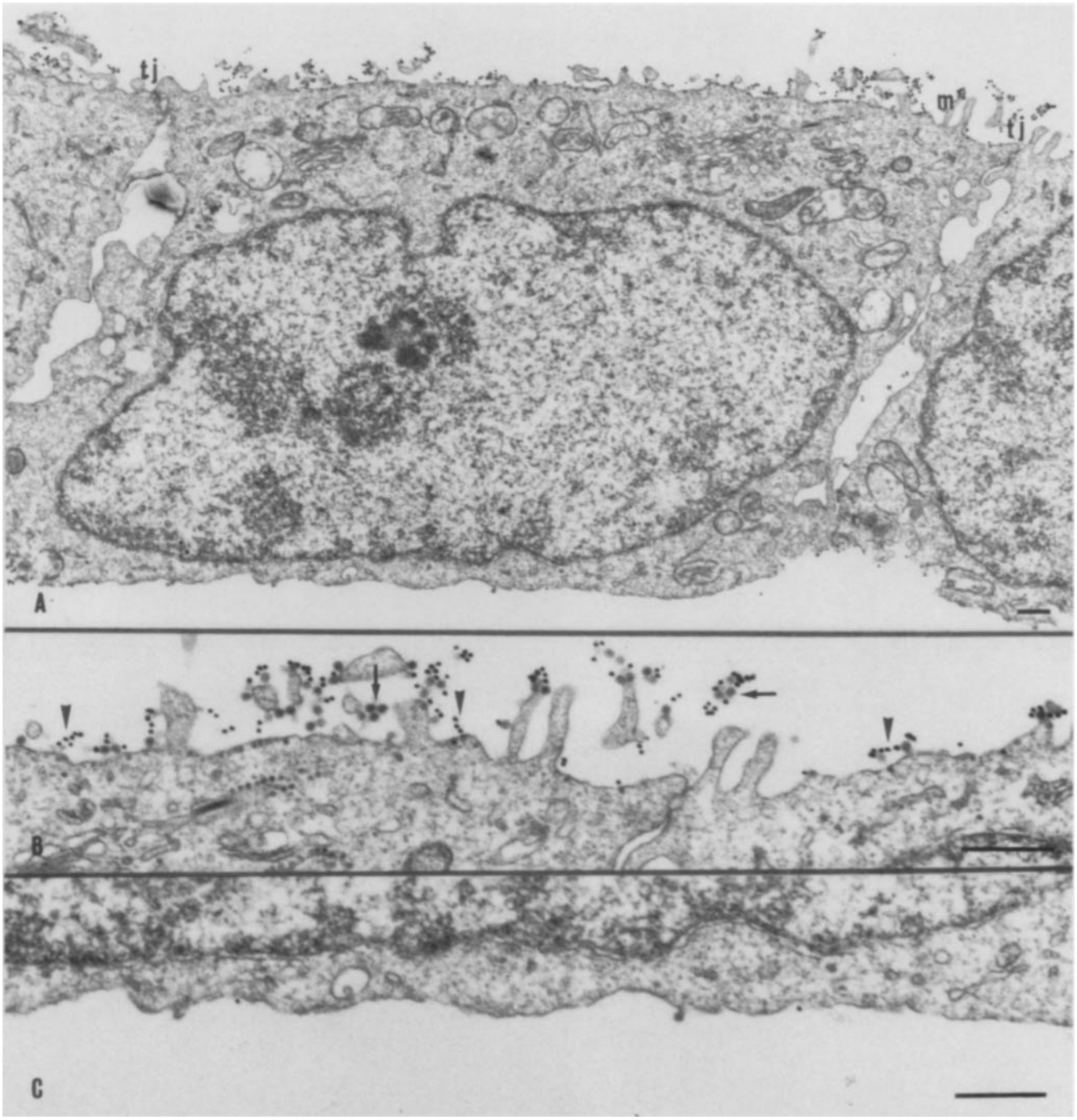

Figure 8. p62/E2 glycoprotein is localized at the apical side of SFV-infected FRT cells. Confluent monolayers on plastic dishes were infected with SFV for 4 h, fixed, and scraped from the dish. For immunogold localization the mAb 9AB4 was used. The polarity of the cell in *A* can be easily determined by the presence of tight junctions (*tj*) and microvilli (*m*). *B* and *C* are respectively a magnification of the apical and basal side of the same cell shown in *A*. Gold particles (*arrowheads*) and budding viruses (*arrows*) are predominantly on the cell apex (*B*). Bar, 0.5 μm.

Figure 9. p62/E2 glycoprotein is localized at the basolateral side of SFV-infected CaCo-2 cells. Confluent monolayers on plastic dishes were infected with SFV for 10 h, fixed, and scraped from the dish. For immunogold localization the mAb 9AB4 was used. Although few microvilli (*m*) are seen in this specific section, the cell apex can be easily identified by the presence of tight junctions (*tj*). It is almost completely devoid of gold particles, as also shown at higher power in *B*. Gold particles (*arrowheads*) are mainly present on the basolateral side of the cell as best seen, at higher power, in *C*. Bar, 0.5 μm.

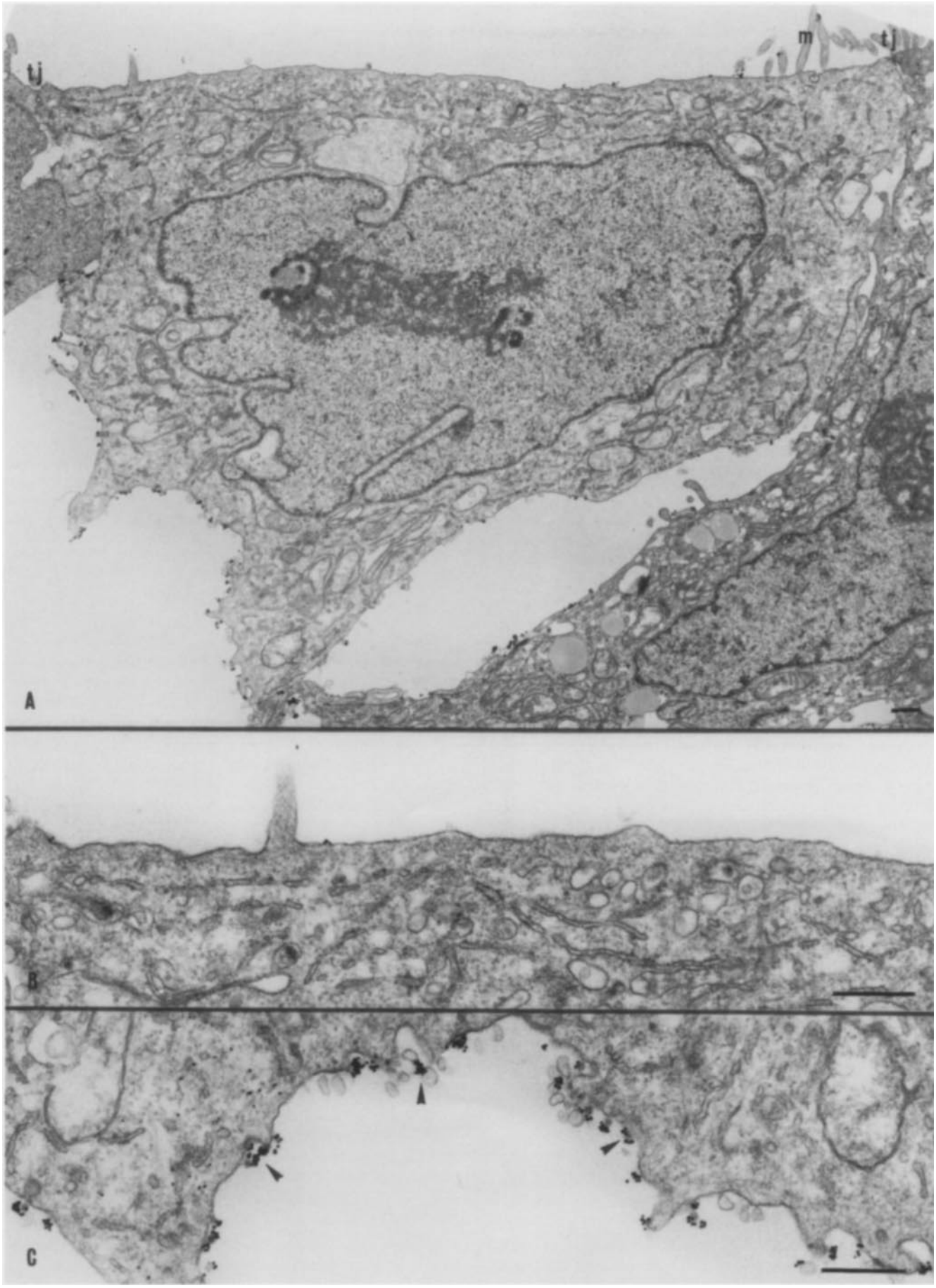
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Table II. Immunogold Labeling on the Plasma Membrane of FRT Cells and CaCo-2 Cells Infected with SFV and Stained with the anti-p62/E2 Protein Antibody

	*Average number of gold particles per cell profile (± SD)		‡Average density of gold particles per μm (± SD)	
	Apical	Basolateral	Apical	Basolateral
FRT	145 ± 38	35 ± 16	5.6 ± 1.5	0.7 ± 0.32
CaCo-2	73 ± 24	390 ± 121	2.8 ± 0.9	8.6 ± 2.7

* Colloidal gold particles were counted on the apical and basolateral profile of each cell. At least 30 different cells were counted in each case.
‡ The number of gold particles counted on the apical and basolateral cell domain was divided by the length of the corresponding cell profile.

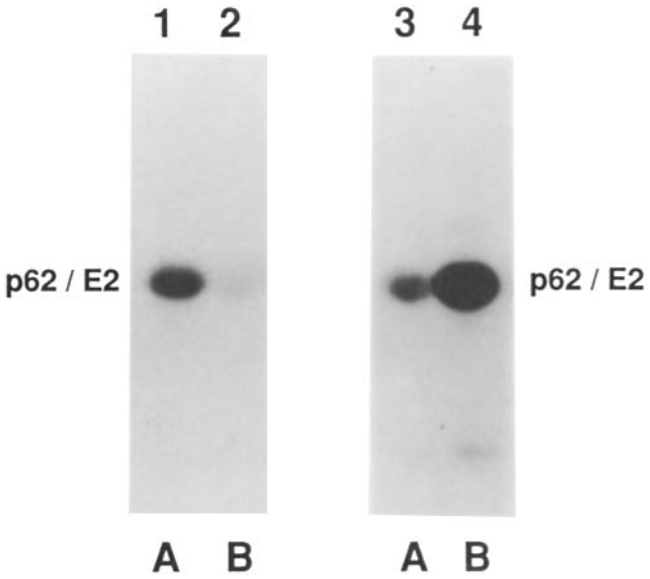

Figure 10. Membrane domain distribution of the p62/E2 glycoprotein in filter-grown SFV-infected cells. Confluent monolayers of FRT and CaCo-2 cells were infected with SFV for 4 h and 10 h, respectively. They were then surface labeled from the apical (*A*) or basal (*B*) side of the filter chamber with S-NHS-biotin. The p62/E2 protein was immunoprecipitated, separated on SDS-PAGE, and transferred to nitrocellulose. Biotinylated proteins were revealed by ^{125}I-streptavidin blotting. In FRT cells (*1* and *2*) labeling of p62/E2 is predominant from the apical side (*A*); in CaCo-2 cells (*3* and *4*) it is predominant from the basolateral side (*B*).

is however limited to some proteins, or to some classes of proteins: it is known, in fact, that several endogenous membrane proteins have identical distribution in different tissues (Simons and Fuller, 1985). FRT cells are not an exception: it has been recently proven that several FRT endogenous proteins, such as Na,K-ATPase, uvomorulin, transferrin receptor, 35/40-kD antigen, and dipeptidylpeptidase IV, have the same membrane distribution as in other cell lines (Zurzolo et al., manuscript in preparation). We also show here that the HA and G viral proteins, commonly used as markers of the apical and basolateral membrane domains, respectively (Rodriguez-Boulan and Pendergast, 1980), are confined to these same domains in FRT cells.

An alternative explanation for our results, other than tissue specificity, is that FRT cells are indeed mutants in their ability to properly sort some membrane proteins. FRT cells have been in culture, in fact, for many years (Ambesi-Impiombato and Coon, 1979) and they could have accumulated many mutations. However, we do not favor this possibility. First of all, preliminary evidence indicates that SV budding polarity is apical also in rat thyroid follicles in primary suspension culture (Nitsch and Wollman, 1980) where somatic mutations should not be suspected. Moreover, to explain the differences in membrane domain distribution of several different proteins, such as the endogenous GPI-anchored proteins, the transfected CD8 and the viral SV and SFV envelope proteins, one should possibly invoke more than a single mutation and, more important, a selective mechanism that would have favored them all. This is unlikely: no such mutations have ever been described in the often used MDCK cell line! Finally, in favor of the hypothesis of tissue specificity in the polarized distribution of some membrane proteins is the finding that, in transgenic mice, LDL receptor protein is segregated at the apical domain of kidney cells and at the basolateral domain of liver and intestinal cells (Pathak et al., 1990).

Hypothetical Mechanisms for Different Membrane Distribution of p62/E2

Concerning the molecular mechanisms that may determine a different distribution of the p62/E2 glycoprotein in FRT cells with respect to CaCo-2 and MDCK cells, two main possibilities can be considered: first, the protein undergoes different posttranslational modification(s) in FRT cells: a new modification, or the lack of a modification, could cause a change in the sorting signal of the protein thus altering its membrane distribution. The modification could consist in the introduction of a new signal or in the abolition or alteration of a pre-existing signal. We do not know whether the p62/E2 protein synthesized in FRT cells is identical to, or different from, that synthesized by CaCo-2 and MDCK cells. By now, we can only say that their apparent molecular weight is the same.

A second possibility is that the cell machinery decodes differently the same sorting signal on the protein. Sorting of plasma membrane proteins is thought to occur by two different pathways: either they are immediately addressed to their final destination, as they emerge from the TGN (for review see Griffith and Simons, 1986; Klausner, 1989), by inclusion into distinct exocytic vesicles that are directly routed to either plasma membrane domain (Wandinger-Ness et al., 1990), or segregation is delayed, taking place at the level of plasma membrane. In this indirect route of sorting membrane proteins are thought to be delivered by transcytotic vesicular carriers (Casanova et al., 1990; Brändli et al., 1990; Le Bivic et al., 1990; Matter et al., 1990). The difference between FRT and the two other cell lines could reside at either one of the two sorting stations: TGN and/or plasma membrane. To discriminate between these two alternatives it should be determined whether, in each cell line, the p62/E2 glycoprotein follows a direct or an indirect route to the plasma membrane.

Budding Polarity and Spreading of Virus Infections

Polarity of virus budding has been demonstrated in vitro and in vivo in many cases (Rodriguez-Boulan, 1983; Sabatini et

al., 1983; Sabatini et al., 1988). Together with polarity of virus entry (Fuller et al., 1984; Fuller et al., 1985*b*; Clayson and Compans, 1988; Basak and Compans, 1989; Rodriguez et al., 1991), it may represent a relevant factor in the spreading of virus infections in intact organisms. A virus could easily cross an intact epithelium if polarity of entry and polarity of budding are opposite. On the contrary, an epithelium could represent a barrier, at least temporarily, to virus spreading if budding occurs at the same domain as virus entry. Tissues that manifest different budding polarity of the same virus may then play different roles in the spreading of a viral infection. The potential relevance of these considerations will become clear only when enough data on budding polarity in vivo will become available. With respect to SV and SFV it should be considered that they are mainly neurotropic viruses. The role played by budding polarity in epithelial cells with respect to the spreading of SV and SFV infections is then difficult to evaluate. It might be, however, of interest to investigate which is their budding polarity in endothelial cells in different tissues. Recent data indicate, in fact, that infection of endothelium may be important in the maintenance of viremia and neuroinvasion (Dropuli and Masters, 1990).

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