The EMBO Journal vol.6 no.8 pp.2241-2247, 1987

Perforated MDCK cells support intracellular transport

Kai Simons and Hilkka Virta

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Communicated by K.Simons

We have developed a method for perforating the plasma membrane of MDCK cells while retaining cellular functions. A nitrocellulose acetate filter was applied to the apical side of cells, grown on a glass coverslip, and allowed to dry. Segments of the apical plasma membrane adhered to the filter and were detached from the cell layer by shearing when the filter was peeled off. This allowed macromolecules such as antibodies and enzymes to diffuse into the cells. The cells were otherwise intact as judged by light and electron microscopy. The perforated cells maintained their capacity to support vesicular transport of proteins and lipids. Vesicular stomatitis virus infected cells readily incorporated [^{35}S]methionine into G protein following permeabilization. This G protein was core-glycosylated during assembly in the endoplasmic reticulum, and was further transported to the *trans* Golgi with high efficiency. Experiments using lipid probes demonstrated that newly synthesized fluorescent sphingolipids were transported from the Golgi complex to the basolateral cell surface in perforated cells. Our results show that perforated cells provide a convenient and efficient alternative to cell-free assays for studying the molecular mechanism of intracellular transport.

Key words: endoplasmic reticulum/epithelial cells/Golgi complex/lipid traffic/protein transport

Introduction

The unravelling of the molecular mechanisms involved in the control of membrane traffic in eukaryotic cells requires novel experimental approaches. The establishment of cell-free assays in which the vesicular transport between cellular compartments can be studied by biochemical means has been especially useful. For example, transport of a viral glycoprotein between the *cis* and medial compartments of the Golgi complex has been successfully reconstituted (Fries and Rothman, 1981; Balch *et al.*, 1984). Other assays are available for studying the transport of newly synthesized membrane proteins from the endoplasmic reticulum (ER) to the *cis* compartment of the Golgi complex (Haselbeck and Schekman, 1986; Balch *et al.*, 1987) and between the Golgi complex and the cell surface (Woodman and Edwardson, 1986). Cell-free systems have also been developed to study the fusion of secretory granules with the plasma membrane (Davis and Lazarus, 1976; Gratzl *et al.*, 1977; Konings and De Potter, 1981) and the fusion of vesicles along the endocytic pathway (Davey *et al.*, 1985; Grünberg and Howell, 1986; Braell, 1987). One problem common to all of these systems is that it is difficult and laborious to prepare the cellular components needed for reconstituting the transport event. Another problem is that subcellular organization is destroyed during homogenization and vital structures may be damaged during isolation of the donor and acceptor compartments used in these assays. For these reasons it has been difficult to gain insight into the molecular mechanisms underlying vesicular transport between cellular compartments.

Here, we introduce a complementary approach. We asked whether it is necessary to destroy intracellular organization completely by homogenization to obtain the advantage of an *in vitro* approach. Would intracellular transport still function efficiently in cells that have been permeabilized? The inside of the cell would be made accessible for manipulation as in the *in vitro* systems. If intracellular transport was maintained under such conditions, one could manipulate the composition of the cytosol and introduce macromolecules such as antibodies or inhibitors to identify components involved in intracellular transport. One could achieve this by introducing lesions in the plasma membrane which are large enough to allow free passage of macromolecules, while still leaving the subcellular structures as intact as possible. Several methods to permeabilize selectively the plasma membrane of cells have been tried. These include treatment with organic solvents and detergents, the use of osmotic shock and electropermeabilization (see Knight and Scrutton, 1986). However, none of these methods have permitted the controlled passage of macromolecules across the cell surface, and they often damaged intracellular compartments as well.

We have developed a novel method for cell permeabilization which avoids potentially harsh and damaging treatments. Nitrocellulose filters were applied to the apical surface of cell monolayers grown on glass coverslips. When the filter was peeled off, holes were introduced into the plasma membrane. The results presented here describe the features of the cells that have been perforated in this way, and demonstrate that intracellular transport continues with high efficiency after permeabilization.

Results

Preparation of perforated cells

Madin-Darby canine kidney (MDCK) cells were used for these studies. On coverslips these cells form epithelial monolayers; the plasma membranes of the cells are polarized such that the apical domain faces the medium and the basolateral domain contacts the glass surface and adjacent cells (Leighton *et al.*, 1970). We used confluent cell monolayers before they formed blisters, and covered the apical side of the monolayer with a nitrocellulose filter. The filter was dried and then carefully peeled from the coverslip. We established conditions under which the apical membrane would be stripped from the cells by adhering to the filter, leaving the perforated cell monolayer attached to the coverslip.

Conditions were optimized by using cells whose plasma membrane was first labelled with octadecyl rhodamine (R18) (Hoekstra *et al.*, 1984) and observing both the filter and the coverslip in the fluorescence microscope after 'filter-stripping'. R18 is readily introduced into the apical membrane and passes the tight junction to the basolateral side (van Meer and Simons, 1986). When the filter was dried for too long a time, the whole cell monolayer

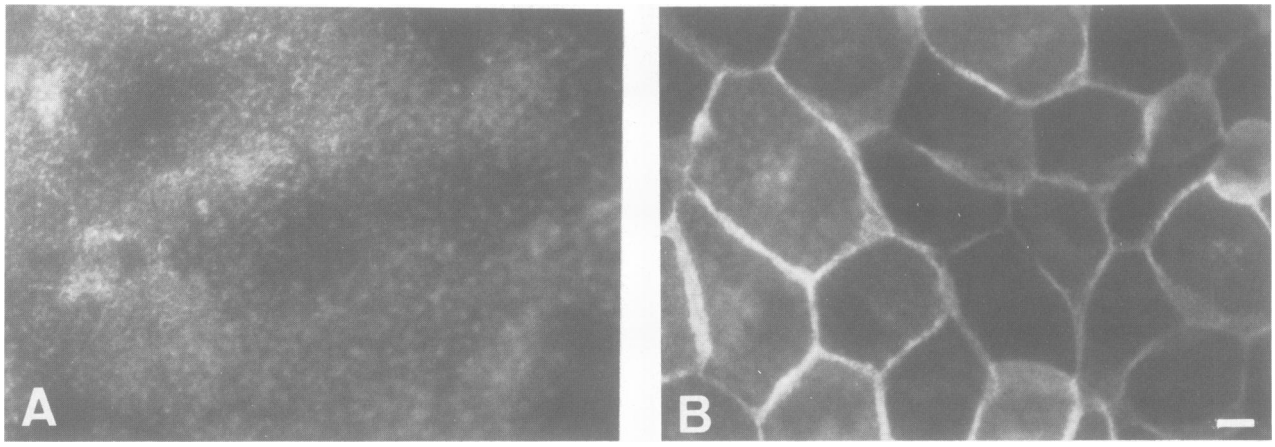

Fig. 1. Optimization of the drying time following filter application onto the cell layer. The plasma membrane was stained with R18. The monolayers were then 'filter-stripped' as described in Materials and methods, and the filter **(A)** and the cell layer on the coverslip **(B)** were viewed in the fluorescence microscope. Uniform fluorescent labelling was seen on the filter side due to the presence of apical membrane while the lateral borders of the cell layer are clearly seen on the coverslip. Bar: 10 μm.

was stripped off with the filter; when the drying time was too short the cells were left intact on the coverslip. Under optimal conditions, a uniform, diffuse fluorescent labelling of the filter was observed (Figure 1A) which reflects filter-associated plasma membrane. The cell monolayer left on the coverslip was readily visualized due to the strong fluorescent labelling of the basolateral membranes (Figure 1B). To test cellular permeability following this treatment, antibodies directed against cytokeratins were applied to the 'filter-stripped' cell monolayer. 95% of the cells were stained with the antibody and therefore permeable to exogenously added molecules (Figure 2).

We used fluorescence microscopy of specific organeller markers to evaluate the integrity of the intracellular organization in these permeabilized cells. The Golgi complex was labelled with *N*-6[7-nitro-2,1,3-benzoxadiazol-4-yl]aminocaproyl sphingosine (C6-NBD-ceramide) as described by Lipsky and Pagano (1985a, b) prior to stripping of the apical membrane (Figure 3A), endosomes were visualized by internalizing Lucifer yellow at 20°C (Figure 3B) and centrosomes were stained with specific antibodies (Figure 3C). These probes revealed no differences between control and 'filter-stripped' cell layers.

Electron microscopy confirmed that the intracellular organization of the membrane compartments was preserved in the 'filter-stripped' cell layer on the coverslip (Figure 4). The rough ER and the Golgi complex appeared normal, as did many of the junctions connecting the lateral membranes of adjacent cells. The major difference between the intact and the 'filter-stripped' monolayers was in the appearance of the apical plasma membrane. In the 'filter-stripped' cells the membrane often appeared discontinuous; 'holes' could be seen. The cytosol was also much lighter in appearance than in intact cells, suggesting partial loss of content. We conclude that the 'filter-stripping' leads to perforation of the cells, possibly by shearing microvilli from the apical membrane.

Intracellular transport of newly synthesized G protein from the ER to the Golgi complex

We studied the synthesis of G protein of vesicular stomatitis virus (VSV) to test whether the perforated cells would support intracellular transport. MDCK cells were infected with VSV, and perforated by the 'filter-stripping' method. Subsequently, the cells were pulsed-labelled with [^{35}S]methionine and chased in transport medium A containing either an ATP-regenerating or an ATP-depleting system (see Materials and methods). Intracellular transport of labelled G protein during the chase was followed by monitoring the sensitivity of its oligosaccharide chains to endoglycosidase H (endo H) digestion. The endo-H-senitive (G_{core} in Figure 5) and the endo H-resistant forms (G_{term}) of G

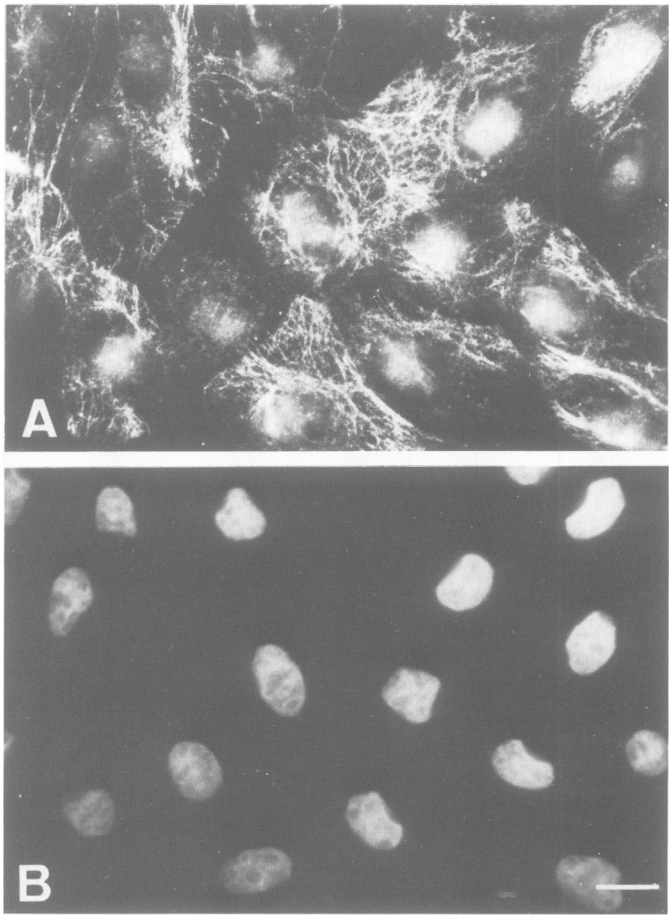

Fig. 2. Permeability of the cells to macromolecules following 'filter-stripping'. The cytokeratins were stained by specific antibodies applied to the cell layer directly after stripping (**A**). Panel **B** shows the same field seen in A with the cell nuclei stained with Hoechst dye. All the cells in this field were permeabilized. Bar: 10 μm.

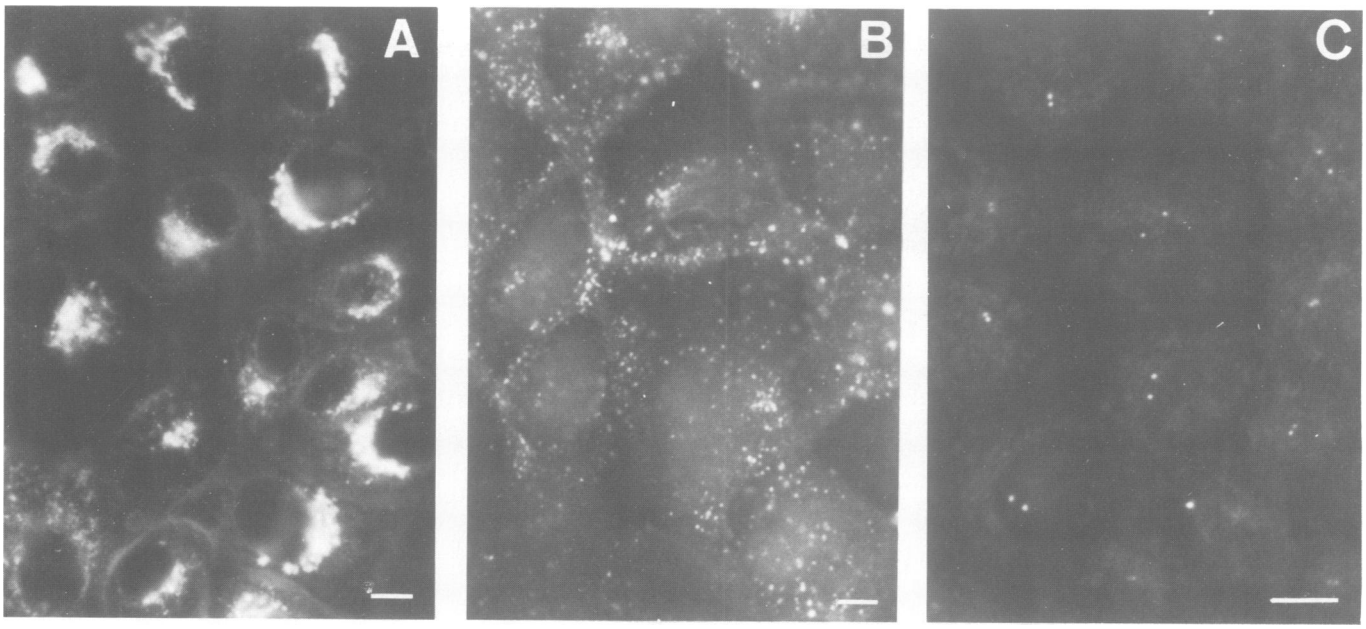

Fig. 3. Staining of the Golgi complex **(A)**, endosomes **(B)** and centrosomes **(C)** after 'filter-stripping'. For experimental details see Materials and methods.

protein can be distinguished by their mobilities in SDS−PAGE. The conversion of G protein from the endo H-sensitive to the endo H-resistant form is a convenient marker for arrival of the protein in the medial Golgi compartment (Kornfeld and Kornfeld, 1985; Dunphy *et al.*, 1985; Balch and Keller, 1986). Further movement to the *trans* Golgi compartment leads to a reduction in electrophoretic mobility due to the addition of galactose and sialic acid residues. Previous studies in MDCK cells have shown that the G_{term} form (Figure 5) contains galactose or galactose and sialic acid (Fuller *et al.*, 1985). Thus the appearance of G_{term} during the chase can be used as a measure of whether the protein has reached the *trans* compartment of the Golgi complex.

The results shown in Figure 5 demonstrated that the perforated cells supported the synthesis of ^{35}S-labelled G protein which was endo-H sensitive immediately after pulse-labelling (lanes 1 and 2). During a 40 min chase in transport medium A containing an ATP-regenerating system, the G protein became terminally glycosylated, evidenced by its slower mobility (lane 6) as compared with the core-glycosylated G protein (lane 2) and by its resistance to endo H digestion (lane 5). When the chase was performed in the presence of an ATP-depleting system the conversion of G_{core} to G_{term} was essentially blocked (lanes 3 and 4). In this experiment we used selective immunoprecipitation of G protein to demonstrate that the observed transport occurred in the perforated cells and not in residual intact ones. This was done by adding antibodies directed against the cytoplasmic domain of the G protein to the cells directly following the chase period. After washing, the cells were solubilized with detergent, and processed as described in Materials and methods (lanes 1−6). The control for this procedure is shown in lane 8. Essentially no G protein was immunoprecipitated from infected cells that had not been perforated before antibody addition.

Transport of newly synthesized sphingolipids from the Golgi complex to the basolateral cell surface

We used the fluorescent lipid probe C6-NBD-ceramide (Lipsky and Pagano, 1985a,b) to analyse the state of lipid transport in perforated cells. This lipid partitions into cellular membranes at 4°C. Upon warming the cells to 20°C, C6-NBD-ceramide is metabolically converted to C6-NBD-sphingomyelin and C6-NBD-glucosylceramide which accumulate in the Golgi complex (Figure 3A). These fluorescent products are transported to the plasma membrane in fibroblasts (Lipsky and Pagano, 1985a,b) and to both the basolateral and apical membranes in MDCK cells at 37°C (van Meer *et al.*, 1987). We incubated MDCK cells with C6-NBD-ceramide, allowing accumulation of C6-NBD-sphingomyelin and glucosylceramide in the Golgi region of the cells (Figure 3A), and then perforated the cells. The perforated cells were incubated at 37°C in transport medium B containing either an ATP-regenerating or an ATP-depleting system (see Materials and methods) and the transport of the fluorescent probes was followed by light microscopy. In the absence of ATP the fluorescent label remained in the Golgi region (Figure 6A). In the presence of ATP the fluorescent labelling of the Golgi region was greatly diminished and the label accumulated at the lateral borders of the cells (Figure 6B). Fluorescent labelling of the apical membrane could not be clearly discerned.

Our previous studies with intact cells suggested that the fluorescent sphingomyelin and glucosylceramide derivatives accumulated in the luminal leaflet of the lipid bilayer of the Golgi membrane at 20°C and then required vesicular carriers for transport to the cell surface at 37°C. The lipids could be readily extracted from the plasma membrane by the addition of serum albumin (van Meer *et al.*, 1987). The addition of 0.2% bovine serum albumin (BSA) to transport medium B had no effect on the transport of the fluorescent lipids to the cell surface. However, fluorescent labelling of the lateral membranes decreased slowly with time when incubated in BSA-containing medium. This probably results from penetration of albumin beneath the cell layer to extract the fluorescent lipids from the outer (luminal) leaflet of the basolateral plasma membrane (data not shown). These results support our previous conclusion that vesicular carriers transport the NBD-lipids from the Golgi region to the cell surface. If the lipids had been present in the cytoplasmic leaflet of the Golgi and/or they were transported into the aqueous phase, addition of albumin to the perforated cells would have blocked transport.

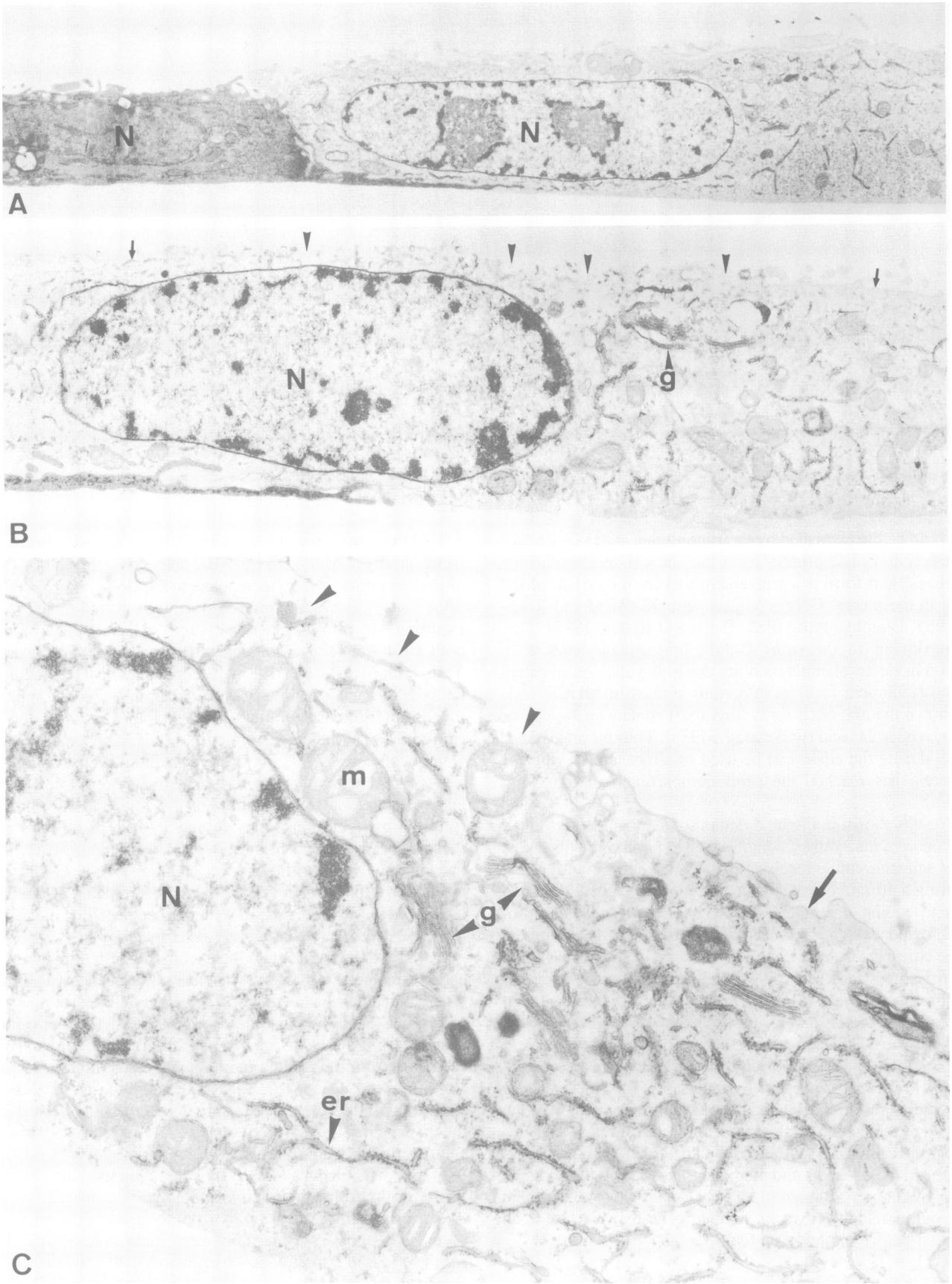

Fig. 4. Electron microscopy of the perforated cells. **Panel A** shows a low power view of two cells. The cell on the left is intact and the one on the right is perforated. The latter cell is much lighter than the intact cell. The arrowheads show sites where the apical membrane has been removed, the arrow indicates intact portions of the plasma membrane. N, nucleus; G, Golgi complex; m, mitochondrion; er, endoplasmic reticulum. Final magnifications: **A** 6400×, **B** 14300× and **C** 27300×.

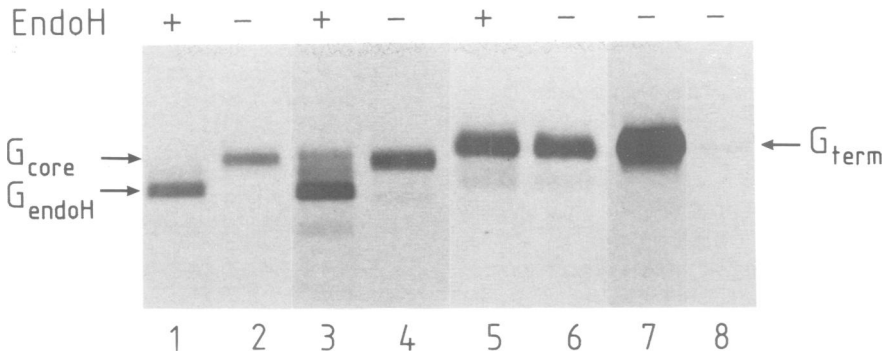

Fig. 5. Synthesis and processing of G protein in perforated cells. Cells infected with VSV were perforated by 'filter-stripping', pulse-labelled with [^{35}S]methionine for 10 min at 37°C **(lanes 1** and **2)**, and chased for 40 min at 37°C in transport medium A in the absence of ATP **(lanes 3** and **4)** or in the presence of ATP **(lanes 5** and **6)**. Intact control cells were pulse-labelled and chased in the absence of ATP **(lanes 7** and **8)**. The G protein was immunoprecipitated with antibody against the cytoplasmic tail of G, added to the cell layers before solubilization with detergent (lanes 1−6, 8). In lane 7 (control cells) the cells were solubilized before adding the antibody. The samples in lanes 1, 3 and 5 were treated with endo H. For experimental details see Materials and methods.

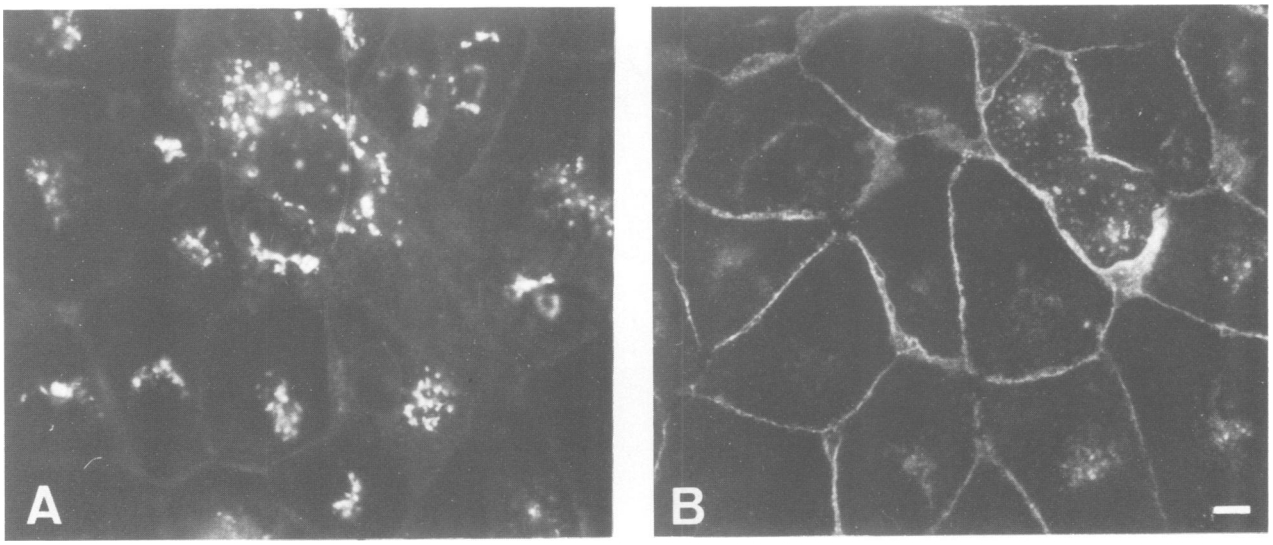

Fig. 6. Transport of C6-NBD-sphingolipids to the basolateral cell surface in perforated cells. C6-NBD-sphingomyelin and C6-NBD-glucosylceramide in the Golgi region (see Materials and methods). The cell layer was incubated in transport medium B in the absence of ATP **(A)** or in the presence of ATP **(B)** for 40 min at 37°C. Transport to the lateral membranes was only seen under the conditions used in panel B. Bar: 10 μm.

Discussion

The method presented here permits permeabilization of the plasma membrane of living cells while maintaining efficient vesicular transport between the compartments involved in biosynthetic membrane traffic. Perforated cells infected with VSV supported the synthesis of viral proteins and translocation of the viral G protein into the ER where it became core-glycosylated. Transport of G protein from the ER to the *trans* Golgi compartment could also be demonstrated by its terminal glycosylation. Transport of G protein from the Golgi to the cell surface has not yet been demonstrated due to the lack of appropriate assays. However, using a qualitative assay we showed that the transport of newly synthesized C6-NBD-sphingolipids from the Golgi region to the basolateral cell surface was maintained in the perforated cells.

The perforated cells provide an ideal experimental system for biochemical analysis of intracellular membrane traffic. The composition of the cytosol can be varied at will; chemical inhibitors, antibodies and enzymes can be introduced directly into the cell. MDCK cells were used in the studies presented here but this method can also be applied to non-epithelial cells in culture. When fibroblasts were 'filter-stripped' in the same way as MDCK cells, most of the cells became attached to the filter and were removed from the coverslip. These cells were found to be permeable to macromolecules, probably due to shearing of their plasma membranes during release from the glass surface (I.de Curtis and K. Simons, unpublished results).

Perforated cells offer several advantages over the available cell-free assays. Our results suggest that membrane traffic between all the compartments involved in the synthesis of new plasma membrane components continues with high efficiency in perforated cells. The preparation of perforated cells is simple and rapid in comparison with the time-consuming task of preparing donor and acceptor compartments for *in vitro* reconstituted systems. The most important advantage is that the cytosol can be manipulated while still maintaining some degree of intracellular organization. It may, of course, be difficult to use whole cells to study a specific transport event. Purified cell fractions have been used to reconstitute a defined stage of endocytosis (Gruenberg and

Howell, 1986) but most other cell-free assays do not use pure donor or acceptor compartments, so that the full advantage of measuring a transport process in a completely defined system has not yet been realized. The perforated cells offer a rapid means to optimize conditions and to identify components essential for intracellular membrane transport. Such information may then be used to develop more efficient cell-free assay systems. This strategy has been used with success to identify a microtubule motor protein, kinesin (Brady *et al.*, 1982; Vale *et al.*, 1986).

The perforation method also has applications in immunocytochemistry. Since cytoplasmically disposed epitopes are accessible to added antibodies in perforated cells, the antigens need not be fixed prior to antibody labelling; fixation can be performed following antigen–antibody interaction. We have used this method successfully to localize viral glycoproteins at the ultrastructural level inside the cell (E.Hughson *et al.*, in preparation). The perforated cell system may also be applied to study interactions of cytoplasmic components with the cytosolic face of cellular membranes; for instance the interactions of receptors, adhesion and junction molecules with cytoskeletal elements. Perforating the membrane converts the cell into a test tube for the study of intracellular transport and organization.

Materials and methods

The perforation method

MDCK strain I and II cells were grown on glass coverslips (18 × 18 mm) in plastic Petri dishes for 2 days until the cell layer became confluent (Fuller *et al.*, 1984). Excess moisture was removed from the cell layer by touching the edge of the coverslip to Whatman 3MM filter paper. A nitrocellulose cellulose acetate filter (HATF, 0.45 nm pore size, Millipore) was moistened in PBS containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$ blotted against Whatman 3MM paper, and carefully laid on top of the coverslip. A hair dryer (50 cm above the coverslip) blowing unheated air (22–24°C) was used to speed the drying of the Millipore filter. After drying, the filter was carefully peeled from the cells and the appropriate medium was immediately added to the cells. The optimal drying time was monitored with octadecylrhodamine B chloride (R18). Before the filter-stripping process, R18 was partitioned into the plasma membrane of the MDCK cells from an ethanolic solution at 0°C for 30 min as described by Hoekstra *et al.* (1984). After incubation with R18, the cells were washed with Eagle's minimum essential medium containing Earle's salts and 0.2% BSA (E-MEM). The R18 was incorporated into both the apical and the basolateral membranes of the cell (van Meer and Simons, 1986). A drying time of ~4 min gave optimal results: the perforated cell layer was left attached to the coverslip and uniform carpet of R18 labelling on the filter was seen after stripping. Occasional intact cells became attached to the filter and were removed from the monolayer. The success of the cell perforation depends on temperature, humidity and drying time. A chamber with controlled humidity and temperature would be needed to completely standardize the procedure. R18 labelling serves as a convenient marker for establishing the appropriate conditions.

The percentage of permeabilized cells was assessed by adding a rabbit antiserum against cytokeratins (T.Kreis, EMBL) to the cells after 'filter-stripping'. The antiserum was diluted 1/50 with PBS–0.2% gelatin before addition to the cells. The antigen–antibody complexes were visualized by indirect immunofluorescence using rhodamine-labelled anti-rabbit IgG (Louvard, 1980). The total cell number (permeabilized and unpermeabilized) was determined from the same microscope fields by staining the cell nuclei with Hoechst dye 33258 (Serva). The cytokeratins were not labelled in unpermeabilized cells.

Staining for the Golgi complex, endosomes and centrosomes

Several fluorescent markers which specifically label different intracellular organelles were used to evaluate the condition of the perforated cells. C6-NBD-ceramide was used to stain the Golgi complex. The C6-NBD-ceramide was synthesized according to Kishimoto (1975), and allowed to partition into the cells from liposomes by incubating at 0°C for 30 min as described previously (Lipsky and Pagano, 1985a,b; van Meer *et al.*, 1987). Liposomes containing the C6-NBD-ceramide were washed away and accumulation of the C6-NBD metabolites in the Golgi region was achieved by raising the temperature to 20°C for 60 min.

Lucifer yellow (specially purified by J.Davoust, EMBL), a fluid phase endocytosis marker, was used to stain the endosomes at 20°C (Dunn *et al.*, 1980; Riezmann, 1985). The cells were incubated in the presence of Lucifer yellow (1 mg/ml in E-MEM) for 60 min at 20°C.

The centrosomes were stained as follows. The cell layer was fixed in methanol at −20°C for 5 min immediately after 'filter-stripping' and then stained by indirect immunofluorescence with a human anticentrosome antiserum (E.Karsenti, EMBL; Tuffanelli *et al.*, 1983).

Electron microscopy was performed on cell layers which had been fixed *in situ* with 1% glutaraldehyde in 200 mM Pipes pH 7.0 for 60 min. The cells were rinsed with the same buffer, post fixed with 1% osmium tetroxide in cacodylate buffer for 30 min and contrasted in 0.5% uranyl acetate in water for 60 min prior to dehydration in ethanol. The monolayers were removed by dissolving a thin layer of the plastic of the coverslip with propylene oxide as described by Griffiths *et al.* (1984). Pieces of monolayer were centrifuged and embedded in Epon. Thin sections were contrasted with lead citrate and examined in a Philips 400 electron microscope.

Intracellular transport of newly synthesized G protein

MDCK strain II cells, grown on coverslips, were infected with VSV as described previously (Fuller *et al.*, 1985). After infection for 3.5 h, the cell layer was perforated by 'filter-stripping' and immediately overlaid with 80 μCi [^{35}S]methionine in 250 μl E-MEM containing 1/10 the normal concentration of methionine. After 10 min at 37°C the coverslip was washed with transport medium A [78 mM KCl, 50 mM Hepes–KOH (pH 7.0), 4.0 mM $MgCl_2$, 10 mM EGTA, 8.37 mM $CaCl_2$, 1 μM DTT; Burke and Gerace, 1986] at 0°C. The chase was carried out for different periods of time at 37°C in 500 μl of this medium containing 0.1 M methionine and either an ATP-regenerating system (Davey *et al.*, 1985; 0.5 mM ATP, 4 mM creatine phosphate and 20 μg/ml creatine phosphokinase 800 U/mg, all from Boehringer) or an ATP-depleting system [either 50 U/ml of potato apyrase (grade VIII, Sigma) or 2 mg/ml hexokinase in 5 mM D-glucose (Pfanner and Neupert, 1986; Davey *et al.*, 1985]. After the pulse–chase the ^{35}S-labelled G protein was immunoprecipitated with a monoclonal antibody (P5D4) against the cytoplasmic domain of the G protein as described by Kreis (1986), the only difference being that the antibody was added directly to the perforated cells to allow the antigen–antibody reaction to occur (30 min at 0°C) before washing (3× PBS – 0.2% gelatin) and solubilizing the cells with 1% Triton lysis buffer (Kreis, 1986). Intact MDCK cells infected with VSV were pulse-labelled and chased in transport medium A containing an ATP-depleting system as above. The cells were then treated with the anti G protein antibody either before (Figure 5, lane 8) or after (Figure 5, lane 7) cell solubilization to assess whether the G protein in unpermeabilized cells was accessible to the antibody.

After immunoprecipitation and binding of the antigen–antibody complex to protein A–Sepharose 4B (Pharmacia), the washed beads (Kreis, 1986) were resuspended in 200 μl of 0.2 M sodium citrate (pH 5.5). The samples divided into two 100 μl aliquots to which 1 μl of protease inhibitor mixture (chymostatin, leupeptin, antipain, pepstatin, all from Sigma, each in 10 mg/ml dimethylsulphoxide) was added. One of these aliquots was further treated with 5 μl endo H (1 U/ml; Seikagaku). The aliquots were incubated overnight at 37°C. One μl of 20% SDS was then added and the samples were heated for 3 min at 95°C to release the proteins from the beads. Equal volumes of 40% TCA were added for 2 h at 0°C. The TCA precipitates were collected, washed, and dissolved in sample buffer. The proteins were resolved by SDS–PAGE (10% polyacrylamide gels) as described before (Pfeiffer *et al.*, 1985).

Transport of newly synthesized C6-NBD-sphingolipids to the basolateral cell surface

C6-NBD-ceramide was used to assess the ability of the perforated cells to transport lipids, C6-NBD-ceramide was allowed to partition into the MDCK cells from liposomes at 0°C for 30 min. The metabolic products; C6-NBD-sphingomyelin and C6-NBD-glucosylceramide, were accumulated in the Golgi region by incubating at 20°C for 1 h. After perforation, the cells were transferred to transport medium B [25 mM Hepes–KOH (pH 7.0), 25 mM KCl, 2.5 mM Mg acetate, 0.25 M sucrose: Balch *et al.*, 1984] containing either an ATP-regenerating or an ATP-depleting system (see above). The transport of the fluorescent lipids to the cell surface was monitored by fluorescence microscopy using a 40× water immersion objective (van Meer and Simons, 1986).

Acknowledgements

We would like to thank Ruth Back and Gareth Griffiths for doing the electron microscopy for us, Mark Bennett, David Meyer and Angela Wandinger-Ness for a critical reading of the manuscript, and Anne Walter for typing the manuscript. We are also grateful to Ruud Brands for communicating unpublished results concerning the use of nitrocellulose filters to remove plasma membranes from tissue culture cells.

References

Balch,W.E., Dunphy,W.G., Braell,W.A. and Rothman,J.E. (1984) *Cell*, **39**, 405–416.

Balch,W.E. and Keller,D.S. (1986) *J. Biol. Chem.*, **261**, 14690–14696.

Balch,W.E., Wagner,K.R. and Keller,D.S. (1987) *J. Cell Biol.*, **104**, 749–760.
Brady,S.T., Lasek,R.J. and Allen,R.D. (1982) *Science*, **218**, 1129–1131.
Braell,W.A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1137–1141.
Burke,B. and Gerace,L. (1986) *Cell*, **44**, 639–652.
Davey,J., Hurtley,S.M. and Warren,G. (1985) *Cell*, **43**, 643–652.
Davis,B. and Lazarus,N.R. (1976) *J. Physiol.*, **256**, 709–729.
Dunn,W.A., Hubbard,A.L. and Aronson, N.N., Jr (1980) *J. Biol. Chem.*, **255**, 5971–5978.
Dunphy,W.G., Brands,R. and Rothman,J.E. (1985) *Cell*, **40**, 463–472.
Fries,E. and Rothman,J.E. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3870–3874.
Fuller,S., von Bonsdorff,C.-H. and Simons,K. (1984) *Cell*, **38**, 65–77.
Fuller,S., Bravo,R. and Simons,K. (1985) *EMBO J.*, **4**, 297–307.
Gratzl,M., Dahl,G., Russel,J.T. and Thorn,N.A. (1977) *Biochim. Biophys. Acta*, **470**, 45–47.
Griffiths,G., Warren,G., Quinn,P., Mathieu-Costello,O. and Hoppeler,H. (1984) *J. Cell Biol.*, **98**, 2133–2141.
Gruenberg,J.E. and Howell,K.E. (1986) *EMBO J.*, **5**, 3091–3101.
Haselbeck,A. and Schekman,R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2018–2021.
Hoekstra,D., de Boer,T., Klappe,K. and Wilschut,J. (1984) *Biochemistry*, **23**, 5675–5681.
Kishimoto,Y. (1975) *Chem. Phys. Lipids*, **15**, 33–36.
Knight,D.E. and Scrutton,M.C. (1986) *Biochem. J.*, **234**, 497–506.
Konings,F. and de Potter,W. (1981) *FEBS Lett.*, **126**, 103–106.
Kornfeld,R. and Kornfeld,S. (1985) *Annu. Rev. Biochem.*, **54**, 631–664.
Kreis,T.E. (1986) *EMBO J.*, **5**, 931–941.
Leighton,J., Estes,L.W., Mansukhani,S. and Brada,Z. (1970) *Cancer*, **26**, 1022–1028.
Lipsky,N.G. and Pagano,R.E. (1985a) *J. Cell Biol.*, **100**, 27–34.
Lipsky,N.G. and Pagano,R.E. (1985b) *Science*, **228**, 745–747.
Louvard,D. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4132–4136.
Pfanner,N. and Neupert,W. (1986) *FEBS Lett.*, **209**, 152–156.
Pfeiffer,S., Fuller,S.D. and Simons,K. (1985) *J. Cell Biol.*, **101**, 470–476.
Riezman,H. (1984) *Cell*, **40**, 1001–1009.
Tuffanelli,D.L., McKeon,F., Kleinsmith,D.K., Burnham,T.K. and Kirschner,M. (1983) *Arch. Derm.*, **119**, 560–566.
Vale,R.D., Scholey,J.M. and Sheetz,M.P. (1986) *Trends Biochem. Sci.*, **11**, 464–486.
van Meer,G. and Simons,K. (1986) *EMBO J.*, **5**, 1455–1464.
van Meer,G., Stelzer,E.H.K., Wijnaendts-van-Resandt,R.W. and Simons,K. (1987) *J. Cell Biol.*, in press.
Woodman,P.G. and Edwardson,J.M. (1986) *J. Cell Biol.*, **103**, 1829–1835.

Received on May 22, 1987; revised on May 29, 1987