Two Different Cytoplasmic Tails Direct Isoforms of the Membrane Cofactor Protein (CD46) to the Basolateral Surface of Madin-Darby Canine Kidney Cells*

(Received for publication, January 11, 1996, and in revised form, May 7, 1996)

Andrea Maisner‡, M. Kathryn Liszewski‡, John P. Atkinson§, Reinhard Schwartz-Albiez†, and Georg Herrler‡

From the ‡Institut für Virologie, Philipps-Universität Marburg, Robert-Koch-Strasse 17, D-35037 Marburg, Germany, and the §Division of Rheumatology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, and the †Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.

Membrane cofactor protein (MCP; CD46), a widely distributed regulatory protein of the complement system, was analyzed for expression in polarized epithelial cells. Both a human and a simian (Vero C1008) cell line were found to contain endogenous MCP mainly on the basolateral surface. Transfected Madin-Darby canine kidney cells stably expressing human MCP delivered this protein also predominantly to the basolateral surface. A deletion mutant lacking the cytoplasmic tail was transported in a nonpolarized fashion, indicating that the targeting signal for the basolateral transport is located in the cytoplasmic domain. A characteristic feature of MCP is the presence of various isoforms that contain either of two different cytoplasmic tails as a consequence of alternative splicing. Two isoforms differing only in the cytoplasmic tail (tail 1 or 2) were analyzed for polarized expression in Madin-Darby canine kidney cells. Surface biotinylation, as well as confocal immunofluorescence microscopy, indicated that both proteins were transported to the basolateral surface. Because no sequence similarity has been observed, the two tails contain different basolateral targeting signals. A deletion mutant lacking the only tyrosine residue in tail 1 retained the polarized expression indicating that, in contrast to most basolateral sorting signals, the transport signal of the tail 1 isoform is not dependent on tyrosine. The maintenance of a targeting motif in two distinct cytoplasmic tails suggests that the basolateral expression of MCP in polarized epithelial cells is of physiological importance.

The surface of polarized epithelial cells is separated by tight junctions into an apical and a basolateral domain that differ both in composition and function. The apical membrane faces the external environment and the basolateral membrane mediates the contact with internal tissues and blood vessels. A specialized sorting apparatus exists to ensure that proteins and lipids specific for either of the two surface domains are targeted correctly. In Madin-Darby canine kidney cells, newly synthesized apical and basolateral proteins occupy the same compartment until they are sorted at the trans-Golgi network and are segregated into distinct transport vesicles for direct delivery to their respective membrane domains. In hepatocytes, all proteins are delivered to the basolateral surface, whereas the apical proteins are sorted and transported to the apical domain via transcytosis. Caco-2 (colon carcinoma) cells take an intermediate position, with some surface proteins being transported directly to the apical membrane and others being retrieved from the basolateral domain. Previously, a signal-independent transport had been proposed for the basolateral proteins. But recently, it has become evident that the cytoplasmic domain of basolateral proteins is responsible for the polarized expression (reviewed in Ref. 11). The basolateral sorting signals usually contain a critical tyrosine residue and often have a close relationship to the determinants for coated pit localization. However, there are also basolateral sorting signals that are unrelated to endocytosis signals, e.g., in the polymeric immunoglobulin receptor and the low density lipoprotein receptor. Although most of the information has been obtained using MDCK-1 cells, it appears likely that similar or identical signals are active in other epithelial cell types. Little information is available about apical sorting signals. Proteins anchored in the membrane by a glycosylphosphatidylinositol (GPI) moiety were found to be delivered to the apical surface. However, it is not clear whether the GPI anchor is responsible for the apical transport of GPI-anchored proteins. The carbohydrate portion of glycoproteins has also been proposed as a potential determinant for delivery to the apical membrane.

Membrane cofactor protein (MCP; CD46) is a widely distributed regulatory protein that inhibits complement activation on host cells. MCP is a member of the family of C3 binding membrane proteins and acts as a cofactor in concert with plasma serine protease factor I to degrade complement factors C3b and C4b deposited on self tissue. Recently, it has been shown that MCP also serves as the receptor for the measles virus. In most cells, MCP consists of a family of different isoforms that arise by alternative splicing of a single gene. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis. The NH₂ terminus of the extracellular portion is composed of four cysteine-rich repeating domains known as short consensus repeats that are sites of endocytosis.

*This work was supported by Grant He 1168/3-2 from Deutsche Forschungsgemeinschaft (to G.H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Dedicated to Prof. Dr. R. Rott on the occasion of his 70th birthday.

¶To whom correspondence should be addressed. Tel.: 49-6421-285360; Fax: 49-6421-285482; E-mail: herrer@papin.hrz.uni-marburg.de.

1 The abbreviations used are: MDCK, Madin-Darby canine kidney; GPI, glycosylphosphatidylinositol; STP, serine, threonine, and proline; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; HRT, human rectal tumor.
lines (STP), a site of extensive O-glycosylation. The STP region may consist of three segments, designated A, B, and C. The isoforms commonly expressed on human cells contain only the C (14 amino acids) or BC (29 amino acids) segments. A short tract of unknown function connects the STP domain with a hydrophobic membrane-spanning region. The cytosolic portion of MCP comprises an intracytoplasmic anchor of 10 predominantly basic amino acids and a carboxyl-terminal cytoplasmic tail. The latter domain is subject to alternative splicing resulting in isoforms with two different tails, a shorter one with 16 amino acids (designated tail 1) or a longer one with 23 amino acids (designated tail 2).

We were interested in whether this protein is expressed on the apical and/or basolateral cell surface of epithelial cells. The distribution of MCP may be important for the physiological function of MCP, inhibiting complement activation, as well as for the function of the measles virus receptor. The transport of MCP to the cell surface was analyzed in polarized MDCK cells stably expressing different isoforms. Here we report that two isoforms with different cytoplasmic tails were both transported to the basolateral surface. In contrast, a mutant lacking the whole cytoplasmic tail was transported to the cell surface in a nonpolarized fashion. These results indicate that both cytoplasmic tails contain a signal for basolateral transport. The conservation of a basolateral sorting signal in two different cytoplasmic tails suggests an important role of the polarized expression for this regulatory protein. Evidence is presented that the basolateral sorting signal of the BC1 isoform is independent of tyrosine.

**EXPERIMENTAL PROCEDURES**

Cell Culture—Vero C1008 cells and HRT-18 cells were grown in Dulbecco’s modified essential medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 100 Units/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO2. MDCK cells (strain I) were maintained in Dulbecco’s modified essential medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum and antibiotics. Cells were passaged 1:8 twice a week. For all experiments tissue culture-treated 0.4-μm pore size Transwell polycarbonate filters (Costar Corp., Cambridge, MA) were used. Cells were plated 4–5 days before the experiments (2 × 106 cells per 24-mm unit and 2 × 106 cells per 10-mm unit). Each cell line formed an electrical tight monolayer expressing different patterns of surface proteins on the apical and basolateral membrane. The polarity was determined by measurement of the transepithelial resistance using a Millipore (Bedford, MA) ERS apparatus: 30–60 Ω·cm2 for Vero C1008 cells, 100–150 Ω·cm2 for HRT-18 cells, and 1800–3000 Ω·cm2 for MDCK cells.

Stable Expression of MCP in MDCK Cells—Three MCP forms were used in this study: isoforms BC1 and BC2 and a tail-minus form (Fig. 1). The nomenclature for MCP isoforms indicates which segments (of A, B, and C) of the STP region and which cytoplasmic tail (1 or 2) are expressed. The tail-minus form was derived from isoform BC2 by deletion of the cytoplasmic tail, making the intracytoplasmic anchor the carboxyl terminal (25). The d1–6 mutant was derived from isoform BC1 by deletion of the first 6 amino acids of the cytoplasmic tail (TYLTDE; Fig. 1; Ref. 25). All forms were cloned into the expression vector pHJAapr-1-neo (26) as described by Liszewski et al. (25). For stable expression, 1.5 × 106 MDCK cells were transfected with 6 μg of plasmid DNA using the electroporation method described by Liljestrom and Garoff (27). Transfected cells were selected in medium containing 1 mg/ml geneticin (Sigma, Deisenhofen, Germany). Resistant cells were subcloned and assayed for MCP expression by intracellular and surface immunofluorescence and Western blot.

Surface Biotinylation and Immunoprecipitation—Filter-grown cells were biotinylated essentially as described by Lisanti et al. (18). MCP was immunoprecipitated from lysed cells using monoclonal antibodies (GB24 or J4/48) as described recently (28). After separation on a 12% polyacrylamide gel under nonreducing conditions, proteins were analyzed by Western blot (29). Biotinylated MCP was detected with the enhanced chemiluminescence system (Amersham Corp.) as described previously (28).

**RESULTS**

Endogenous MCP Is Predominantly Localized on the Basolateral Membrane of Polarized Vero C1008 Cells and Human Rectal Tumor (HRT-18) Cells—To study the sorting of endogenous MCP in polarized epithelial cells, we analyzed the steady state surface distribution of CD46 on the simian cell line Vero C1008. These cells have previously been described as growing in a polarized fashion (30). Simian MCP can be isolated by a monoclonal antibody, GB24, which is specific for the short consensus repeat 3 and 4 region of human MCP (28). To determine the apical/basolateral distribution of MCP, we used domain-selective biotinylation of cells cultured on permeable supports. Polarized cell monolayers grown on filters were cooled to 4°C, and either the apical or the basolateral surface proteins were labeled by adding the non-membrane-permeating reagent sulfo-N-hydroxysuccinimidyl-biotin to the respective chamber. The cells were lysed and MCP was immunoprecipitated by
were electrophoresed under nonreducing conditions and transferred labeled with sulfo-N-hydroxysuccinimidyl-biotin; cells were then lysed, and MCP was immunoprecipitated with mAb GB24. Precipitates were electrophoresed under nonreducing conditions and transferred to nitrocellulose. Surface-biotinylated MCP was visualized with streptavidin/peroxidase.

GB24. After electrophoresis on a nondenaturing sodium dodecyl sulfate-polyacrylamide gel, MCP was transferred to nitrocellulose. Biotin-labeled MCP was detected with streptavidin/peroxidase. As shown in Fig. 2, panel 1, MCP was not equally distributed on the two surface domains of Vero C1008 cells. An efficient biotinylation of MCP was obtained after labeling the cells from the basolateral side (panel 1, lane A). Only a minor band of MCP was detectable after labeling the cells from the apical side (panel 1, lane B), although in this case the labeling reagent did not have to pass through the pores of the filter. This result indicates that MCP is expressed predominantly (96%) on the basolateral surface of Vero C1008 cells. To confirm the polarized expression of MCP with human cells, the same experiment was performed with a human rectal tumor cell line, HRT-18. As shown in Fig. 2, panel 2, MCP of HRT-18 cells, which had a somewhat slower electrophoretic mobility, was also expressed predominantly (95%) on the basolateral surface. As in the case of Vero C1008 cells, apical MCP was detectable only after overexposure of the film.

Human MCP Is Predominantly Expressed on the Basolateral Surface of Polarized MDCK Cells—In contrast to simian MCP, human MCP is well characterized and different isoforms are available as cDNA clones. To determine the distribution of a distinct isoform of human MCP on a well-characterized polarized cell line, we established an MDCK cell line stably expressing the BC1 isoform. This isoform contains segments B and C of the STP region and cytoplasmic tail (Fig. 1). Expression of human MCP was analyzed by immunofluorescence microscopy using a monoclonal antibody directed against human MCP (J4/48). Although MCP was not detectable in nontransfected parental MDCK cells (Fig. 3A), bright fluorescence was observed after staining of transfected cells (Fig. 3B). The latter cells had retained the polarized phenotype of the parental cells, as indicated by measurement of the transepithelial resistance.

To determine the distribution of human MCP on the apical and basolateral membrane of polarized epithelial cells, filter-grown MDCK cells stably expressing human MCP were subjected to domain-selective biotinylation as described above for Vero C1008 cells. As shown in Fig. 4, panel 2, strong labeling of MCP was achieved by biotinylation of the basolateral proteins (lane B). Biotinylation of the apical surface yielded only a weak MCP band. This result indicates that human MCP is predominantly (98%) expressed on the basolateral membrane domain of MDCK cells, as has been determined above for simian MCP. In the parental cell line, no band was detectable at the position of MCP (panel 1), neither after apical (lane A) nor after basolateral (lane B) biotinylation.

Two Different Cytoplasmic Tails Contain a Signal for Basolateral Sorting of Human MCP in MDCK Cells—To analyze the importance of the cytoplasmic tail of MCP for the basolateral transport we established an MDCK clone expressing another isoform of MCP, BC2, which differs from BC1 only in the cytoplasmic tail. In addition, we generated an MDCK cell clone that expressed MCP and lacked the cytoplasmic tail (tail-minus). The amino acid sequence of the cytosolic portion of BC1, BC2, and the tail-minus form of MCP is shown in Fig. 1.

The polarity of the MCP isoforms and tail-minus mutant on the surface of filter-grown MDCK cells was determined by selective biotinylation. Total MCP immunoprecipitated with mAb J4/48 was divided into two aliquots. One aliquot was used for a Western blot to ensure that there was no difference in the total amount of receptor in the cells labeled from either the apical or the basolateral side, respectively (not shown). In the second aliquot, biotinylated proteins were detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transfer to nitrocellulose by incubation with streptavidin/peroxidase (Fig. 5). As already shown in Fig. 4, isoform BC1 of MCP was almost exclusively (98%) detected on the basolateral surface. By analysis of cell clones with vastly different expression levels, no difference in the polarized surface distribution was found. Isoform BC2 of MCP was also localized predominantly (97%) on the basolateral membrane (Fig. 5B) and only a minor band was detectable in the sample biotinylated from the apical side (A). This result indicates that both isoforms contain a basolateral sorting signal. In contrast, the tail-minus form of MCP was not transported in a polarized fashion. It was found almost equally distributed on both sides of the cells (40% apical; 60% basolateral). Thus, the deletion of the cytoplasmic tail
abolished the polarized transport of MCP. This result indicates that the sorting signal for the basolateral transport of MCP is located in the cytoplasmic tail. Because isoforms BC1 and BC2 differ in the cytoplasmic tail and are both transported in a polarized fashion, both tails must contain a signal for delivery to the basolateral surface.

To confirm the result from Fig. 5, the distribution of the two isoforms and the tail-mutant of MCP on the surface of MDCK cells was analyzed by indirect immunofluorescence microscopy using a confocal laser scanning microscope. Filter-grown MDCK cells were fixed without disruption of the plasma membrane. Then they were incubated from both the apical and the basolateral side with J4/48 and a FITC-conjugated second antibody. In Fig. 6, horizontal sections (parallel to the filter) are shown that represent (from top) the apical, central, and basal portions of the cells, respectively. In addition, a vertical section (side view) is shown (Fig. 6, bottom panel). For isoforms BC1 and BC2 of MCP almost no fluorescence signals were detectable in the apical section. The section through the center showed a honeycomb pattern. The brightest fluorescence was observed in the basal section. The vertical profiles displayed characteristic cup-like patterns typical for basolateral proteins. In contrast, in MDCK cells expressing the tail-minus mutant, fluorescent staining was detected on the entire plasma membrane including the apical domain. In the side view (vertical section), tail-minus MCP was found to completely encircle the cells. The confocal immunofluorescence analysis confirmed that both MCP isoforms are localized almost exclusively on the basolateral membrane of polarized MDCK cells and that deletion of the cytoplasmic tail of MCP resulted in a loss of polarized surface expression.

The Basolateral Transport Signal of MCP Is a Tyrosine-independent Motif—To determine whether tyrosine is involved in the basolateral targeting of MCP, we analyzed MDCK cells expressing a deletion mutant of the BC1 isoform. This mutant, d1–6, lacked the six NH₂-terminal amino acids of tail 1 (TYLTDE) and thus the only tyrosine residue in the cytoplasmic tail of the BC1 isoform (Fig. 1). As shown in Fig. 7, the d1–6 mutant showed the same surface distribution as the BC1 isoform. Strong labeling was detectable only after basolateral surface biotinylation; apical labeling resulted only in a weak band. This result indicates that the tyrosine is not necessary for the basolateral targeting of MCP-BC1.

DISCUSSION

Our work shows that MCP is transported to the basolateral surface of polarized epithelial cells. In recent years evidence has been presented that clearly indicates that the delivery to the basolateral membrane is signal mediated with the critical determinant being located in the cytoplasmic tail (reviewed in Ref. 11). This is true also for MCP, because deletion of the cytoplasmic tail abolished the polarized transport. A unique feature of MCP is that there exist several isoforms with either of two different cytoplasmic tails. Computer-assisted alignments did not reveal any sequence similarity between tail 1 and tail 2. As the isoforms BC1 and BC2 were both found to be transported to the basolateral membrane, both tails have to contain their own transport signal. Such a situation has not been described for other basolateral proteins.

A tyrosine residue was found to be essential for the polarized transport of most basolateral proteins analyzed so far. The basolateral sorting signal may overlap with the coated pit localization signal of a protein (14, 16, 31–33). However, in all cases that have been examined it was found by mutating neighboring amino acids that the two determinants are not identical (see Ref. 31). Basolateral sorting signals that are unrelated to coated pit localization signals have been found in the low density lipoprotein receptor (34) and the polymeric immunoglobulin receptor (35). Sequence comparison suggested the motif YXX-aliphatic as common denominator of most basolateral sig-
nals identified so far (31). In the case of the Fc receptor, a critical role has been assigned to a di-leucine motif (36). The latter motif is not present in either of the two alternative tails of MCP. Tyrosine residues are present in both tails. Neither the tyrosine in tail 1 (Y(2)TD) nor the two tyrosine residues in tail 2 (YATQYTK) fit the motif mentioned above. These considerations argue against a major role of a tyrosine for the basolateral targeting of MCP. Experimental support for this conclusion was obtained by analysis of the d1–6 mutant that lacked a tyrosine in the cytoplasmic tail and, nevertheless, was predominantly transported to the basolateral surface.

Secretory glycoproteins are usually released from the apical surface of simple epithelial cells, whereas nonglycosylated proteins are released in a nonpolarized fashion (reviewed in Ref. 19). Several basolateral glycoproteins have been reported to be transported to the apical surface after deletion of the cytoplasmic tail. Based on these data, it has been speculated that the carbohydrate portion may contain a determinant for apical sorting (41, 19). In the case of basolateral glycoproteins, this would imply that the basolateral signal in the cytosolic domain has a higher binding affinity to its sorting machinery than the putative glycan signal in the luminal domain to the receptor for apical sorting. MCP is heavily glycosylated, containing both N- and O-glycans. Removal of the cytoplasmic tail abolished the basolateral transport of MCP, but did not result in the apical delivery of the truncated protein. The tail-minus mutant was rather transported in a nonpolarized fashion. If glycans in general serve as apical sorting signals, one would have to assume that the tail-minus mutant has retained a weak signal for basolateral transport. This weak basolateral sorting signal and the apical sorting signal of the glycans would have the same affinity for their respective receptors, resulting in a nonpolarized expression on the cell surface. As to the location of such a signal, one might consider the intracytoplasmic anchor of 10 amino acids that has been retained on the cytosolic domain of the tail-minus mutant. However, analysis of the basolateral targeting signal in the cytoplasmic domain of glycoprotein G from the vesicular stomatitis virus indicated that a certain minimal distance (≥5 residues) between the transmembrane domain and the transport signal is required for optimal function of the targeting determinant (31). Moreover, the 6 carboxyl-terminal amino acids of the intracytoplasmic anchor consist mainly of basic amino acids (RRKKG), which have so far been described only as having a modulating effect, not as being major determinants for the basolateral transport of proteins. Deletion of this anchor will provide a definitive answer, but it should be noted that nonpolarized expression was also observed with the asialoglycoprotein receptor after inactivation of the basolateral sorting signal (32) and with the G protein of vesicular stomatitis virus after deletion of the cytoplasmic tail (31). Therefore, the view of glycans as general apical sorting signals is still open to debate. For a final conclusion about apical targeting signals one has to await the identification of the respective receptor.

A recent study examining the role of MCP as the measles virus receptor reported that Caco-2 cells express MCP predominantly on the apical surface (37). The differential polarized expression of a protein in two different cell types suggests that the basolateral targeting signal of MCP is not recognized by all epithelial cells. Differential expression of surface proteins by different epithelial cells has been demonstrated so far only for apical proteins. Contrary to the transport in MDCK cells, all apical proteins in hepatocytes and some of them in Caco-2 cells are transiently expressed on the basolateral surface from where they are transported via transcytosis to the apical membrane. GPI-anchored proteins, which are usually delivered to the apical surface of polarized cells, have been localized on the basolateral plasma membrane of Fischer rat thyroid epithelial cells (38). Differences in the polarized expression between MDCK cells and Caco-2 cells have not been described for basolateral proteins. We found that MCP is transported to the basolateral surface of MDCK cells, Vero C1008 cells, and HRT-18 cells, indicating that the basolateral sorting signal is functional in these cells. Histochemical analysis of cells from the human colon also showed that MCP is located on the basolateral surface of epithelial cells (39). Therefore, the basolateral transport of MCP in polarized cells may be the rule and the lack of polarized transport as in the case of Caco-2 cells may be the exception.

The biological importance of the two alternative cytoplasmic tails for MCP is not clear. Isoforms containing cytoplasmic tail 1 are transported to the cell surface more rapidly than their tail 2 counterparts (25). These and other differences between isoforms may allow the cell to use MCP more effectively. The fact that both tails contain a basolateral targeting signal suggests that the location of MCP on the basolateral membrane domain is an important feature. Because the basolateral surface of epithelial cells faces the serosal compartment, it appears to be at an appropriate place for a regulatory protein of the complement system. In this context it is interesting to note that another regulatory protein, the decay accelerating factor (CD55), is an apical protein. Like other glycoproteins that are inserted into the membrane via a GPI-anchor, decay accelerating factor is transported to the apical plasma membrane of polarized cells (40). The targeting signal of MCP may ensure that regulatory proteins are also present on the basolateral surface of polarized epithelial cells.

Acknowledgments—The technical assistance of A. Heiner is gratefully acknowledged. We sincerely thank Dr. Herbert Spring, Biomedical Structure Analysis Group, German Cancer Research Center, Heidelberg, Germany, for performing laser scanning microscopy.

REFERENCES

1. Rinder, M. J., Ivanov, I. E., Plesken, H., Rodriguez-Boulan, E., and Sabatini, D. D. (1984) J. Cell Biol. 98, 1304–1319.
2. Fuller, S. D., Bravo, R., and Simons, K. (1985) EMBO J. 4, 297–307.
3. Griffiths, G., and Simons, K. (1986) Science 234, 438–443.
4. Wandinger-Ness, A., Bennett, M. K., Antony, C., and Simons, K. (1990) J. Cell Biol. 111, 987–1000.
5. Bartle, J. R., Faerci, H. M., Stieger, B., and Hubbard, A. L. (1987) J. Cell Biol. 105, 1241–1251.
6. Matter, K., Brauchbier, M., Bucher, K., and Hauri, H. P. (1990) Cell 60, 429–437.
7. Le-Bivic, A., Quaroni, A., Nichols, B., and Rodriguez-Boulan, E. (1990) J. Cell Biol. 111, 1351–1361.
8. Mostov, K., Apodaca, G., Aroeti, B., and Okamoto, C. (1992) J. Cell Biol. 116, 577–583.
9. Caplan, M., and Matlin, K. S. (1989) in Functional Epithelial Cells in Culture (Matlin, K. S., and Valentinich, D., eds) pp. 71–127, Allan Liss, New York.
10. Simons, K., and Wandinger-Ness, A. (1990) Cell 62, 207–210.
11. Matter, K., and Melman, I. (1994) Curr. Opin. Cell Biol. 6, 545–554.
12. Le-Bivic, A., Saney, L., Patzak, A., Pabst, N., Chao, M., and Rodriguez-Boulan, E. (1991) J. Cell Biol. 115, 607–618.
13. Fuhrer, C., Geffen, I., and Spiess, M. (1991) J. Cell Biol. 114, 423–431.
14. Prill, V., Lehmann, L., van-Figura, K., and Peters, C. (1993) EMBO J. 12, 2181–2193.
15. Canasova, J. E., Apodaca, G., and Mostov, K. E. (1991) Cell 66, 65–75.
16. Hunziker, W., Harter, C., Matter, K., and Melman, I. (1991) Cell 66, 907–920.
17. Yokode, M., Pathak, R. K., Taniyama, K., et al., Brown, M. S., Goldstein, J. L., and Anderson, R. G. (1992) J. Cell Biol. 117, 39–46.
18. Lisanti, M. P., Sargiacomo, M., Greave, L., Saltiel, A. R., and Rodriguez-Boulan, E. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 9575–9581.
19. Fiedler, K., and Simons, K. (1992) Cell 71, 109–120.
20. Liszewski, M. K., and Atkinson, J. P. (1991) Curr. Top. Microbiol. Immunol. 178, 45–60.
21. Nance, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Combe, C., and Gerlier, D. (1993) J. Virol. 67, 6025–6032.
22. Dörig, R. E., Mardel, A., Chopra, A., and Richardson, C. D. (1993) Cell 75, 295–305.
23. Manche, M., Liszewski, M. K., Atkinson, J. P., and Oldstone, M. B. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 2161–2165.
24. Post, T. W., Liszewski, M. K., Adams, E. M., Tiedja, I., Miller, E. A., and Atkinson, J. P. (1993) J. Exp. Med. 174, 93–102.
25. Liszewski, M. K., Tiedja, I., and Atkinson, J. P. (1994) J. Biol. Chem. 269, 18857.
Different Basolateral Targeting Signals in MCP Isoforms

10776–10779

26. Gunning, P., Leavitt, J., Muscat, G., Ng, S. Y., and Kedes, L. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4831–4835
27. Liljestro¨m, P., and Garoff, H. (1991) Bio/Technology 9, 1356–1361
28. Maisner, A., Schneider-Schaulies, J., Liszewski, M. K., Atkinson, J. P., and Herrler, G. (1994) J. Virol. 65, 6232–6237
29. Schultze, B., Gross, H. J., Brossmer, R., and Herrler, G. (1991) J. Virol. 65, 6299–6304
30. Srinivas, R. V., Balachandran, N., Alonso-Caplen, F. V., and Comans, R. W. (1986) J. Virol. 58, 689–693
31. Thomas, D. C., and Roth, M. G. (1994) J. Biol. Chem. 269, 15732–15739
32. Geffen, I., Fuhrer, C., Leitinger, B., Weiss, M., Huggel, K., Griffiths, G., and Spiess, M. (1993) J. Biol. Chem. 268, 20772–20777
33. Brewer, C. B., and Roth, M. G. (1991) J. Cell Biol. 114, 413–421
34. Matter, K., Hunziker, W., and Mellman, I. (1992) Cell 71, 741–753
35. Aroeti, B., Kosen, P. A., Kuntz, I. D., Cohen, F. E., and Mostov, K. E. (1993) J. Cell Biol. 123, 1149–1160
36. Matter, K., Yamamoto, E. M., and Mellman, I. (1994) J. Cell Biol. 126, 991-1004
37. Blau, D. M., and Comans, R. W. (1995) Virology 210, 91–99
38. Zurzolo, C., Lisanti, M. P., Caras, I. W., Nitsch, L., and Rodriguez-Boulan, E. (1993) J. Cell Biol. 121, 1031–1039
39. Inoue, H., Mizuno, M., Uesu, T., Ueki, T., and Tsuji, T. (1994) Acta Med. Okayama 48, 271–277
40. Lisanti, M. P., Caras, I. W., Davitz, M. A., and Rodriguez-Boulan, E. (1989) J. Cell Biol. 109, 2145–2156