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Rab5a is a common component of the apical and basolateral endocytic machinery in polarized epithelial cells

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ABSTRACT **In nonpolarized cells, the small GTPase Rab5a is localized to the plasma membrane, clathrin-coated vesicles, and early endosomes. Rab5a is required for early endosome fusion *in vitro* and regulates transport between the plasma membrane and early endosomes, *in vivo*. In polarized epithelial cells endocytosis occurs from separate apical and basolateral plasma membrane domains. Internalized molecules are initially delivered to distinct apical or basolateral early endosomes. *In vitro*, apical early endosomes can readily fuse with one another but not with the basolateral endosomes and vice versa, thereby indicating that the apical and basolateral early endocytic pathways are controlled by distinct machineries. Here, we have investigated the localization and function of Rab5a in polarized epithelial cells. Confocal immunofluorescence microscopy on mouse kidney sections revealed association of the protein with the apical and basolateral plasma membrane domains and underlying structures. In polarized Madin–Darby canine kidney I cells, endogenous and overexpressed Rab5a have the same distribution. Moreover, overexpression of the protein causes a 2-fold increase in fluid-phase uptake from both domains of the cell, thus showing that Rab5a functions in apical and basolateral endocytosis. Our data indicate that the apical and basolateral endocytic machineries of epithelial cells share common regulatory components and that Rab5a *per se* is not sufficient to target endocytic vesicles to apical or basolateral early endosomes.**

The characteristic polarized surface of epithelial cells consists of apical and basolateral domains, each with distinct protein and lipid compositions (1–5). To achieve and maintain this polarity these cells rely on specialized, domain-specific, membrane transport pathways on the endocytic and exocytic routes (1–3, 6), although different cell types appear to use these pathways to varying extents (7–12). Although sorting on the exocytic pathway has been extensively studied in a number of cell lines, sorting on the endocytic pathway has only been analyzed in recent years and much remains to be learned. Endocytosis occurs to a similar extent from apical and basolateral domains (13). Initially, endocytosed molecules enter separate populations of apical and basolateral early endosomes in the cell periphery, where no mixing of their respective content occurs (14–17). Molecules internalized from separate domains and destined for degradation converge in perinuclear late endosomes and lysosomes and become inseparable. The lack of mixing of endocytic content in apical and basolateral early endosomes is not simply due to their physical separation in intact cells but rather due to their inability to fuse with each other as shown by *in vitro* studies (18). This suggests that distinct recognition molecules are responsible for regulating these interactions.

A family of proteins has emerged that may be key components of such a recognition machinery—namely, the Rab small molecular weight GTPases. The importance of these proteins in regulating specific membrane transport steps has been clearly established *in vivo* and *in vitro* (19–24). In addition, several Rab proteins are expressed only in certain tissues and differentiated cell types, where they participate in the specialized transport pathways characteristic of these cells (25–31). Taken together, the data support the view that each intracellular transport step is regulated by one or more unique Rab proteins. Whether Rab proteins prove to specify vesicle targeting to the correct acceptor membrane (21) or regulate interactions between specific components of the docking/fusion machinery (22, 24), they are likely to hold the key to understanding how the specificity of membrane transport is maintained.

Recent data have shown that epithelial cell-specific Rab proteins might be required for polarized sorting (30). Furthermore, there is evidence that ubiquitously expressed Rab proteins may be pathway-specific in polarized cells. For example, Rab8 is expressed in polarized and nonpolarized cells (32). In hippocampal neurons and Madin–Darby canine kidney (MDCK) II cells Rab8 functions exclusively in transport between the trans Golgi network and the dendritic or the basolateral plasma membrane and is excluded from the axonal and apical transport pathways (33, 34). Given the existence of apical and basolateral endocytic pathways in polarized epithelial cells, it was of interest to explore the possibility that some of the known Rab proteins may play a role in one or both of these pathways.

A number of Rab proteins have been localized to compartments on the endocytic pathway of nonpolarized cells (reviewed in refs. 22 and 23). In nonpolarized cells, Rab5a is localized to the early endosomes, clathrin-coated vesicles, and the cytoplasmic surface of the plasma membrane (35, 36). Further studies revealed that Rab5a is required for the fusion of early endosomes *in vitro* (37). *In vivo*, overexpression of Rab5a increases the rate of endocytosis and expands the size of early endosomes. Conversely, overexpression of a guanine nucleotide binding-defective mutant protein (Rab5a I_{133}) inhibits endocytic uptake and causes the fragmentation of early endosomes and the concomitant accumulation of endocytic vesicles (36). Owing to its importance in early endocytic traffic, Rab5a was chosen for further study in polarized epithelial cells.

In this paper we investigated the distribution of the endogenous Rab5a protein in mouse kidney using confocal immunofluorescence microscopy. To study this question in greater detail, the protein was also stably overexpressed in MDCK I cells and determined to have a distribution similar to that of

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Abbreviations: MDCK, Madin–Darby canine kidney; HRP, horseradish peroxidase.

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the endogenous protein. The MDCK I cell lines were further used to establish the involvement of Rab5a in endocytosis from the apical versus basolateral plasma membrane domains. The results were somewhat unexpected and lead us to propose that the specificity of endocytosis may be ensured through interaction between Rab5a and other domain-specific regulatory molecules.

MATERIALS AND METHODS

Cell Culture. MDCK strain I cells (38) were grown as described (14). At confluency cells were trypsinized and 5×10^5 cells were added to the apical side of a polycarbonate filter (1.2 cm^2, Transwell, 0.4-mm pore size; Costar).

Purification of Recombinant Rab5a. Rab5a was expressed in BL21 (DE3) using the pET8c expression system (39). The purification procedure was adapted from that described for p21 (40, 41). Cells were harvested, resuspended (4–5 g/20 ml) in lysis buffer [64 mM Tris, pH 8.5/10 mM $MgCl_2$/1 mM dithiothreitol (DTT)/1 mM phenylmethylsulfonyl fluoride (PMSF)/10 μM benzamidine/1 mM NaN_3/25 mg of GDP per liter], and disrupted using a French press. After removing DNA by protamine sulfate precipitation and centrifugation, the supernatant was applied to a Q-Sepharose (Pharmacia) column (1.2 × 11 cm) equilibrated with lysis buffer. Bound proteins were eluted with a linear 0–0.4 M NaCl gradient in lysis buffer. Rab5 bound loosely to the column at pH 8.5 and was recovered in the initial fractions purified to ≈90% homogeneity as judged by SDS/PAGE. This material was dialyzed against 10 mM $NaPO_4$, pH 7/0.5 mM EDTA/5 mM $MgCl_2$/0.2 mM PMSF/0.5 mM DTT and stored frozen.

Antibodies. The mouse monoclonal antibodies 6.23.3 against the 58K protein and 4.6.5 against the 114K protein were used as described (42). Two new monoclonal antibodies were generated. One (2A4) was raised against purified recombinant Rab5a and the second (4F11) was raised against a 32-amino acid peptide (KNEPQNPGANSARGRGV-DLTEPTQPTRSQCCSN) derived from the C terminus of Rab5a, which was coupled to keyhold limpet hemocyanin. Both monoclonals are of the isotype IgG2a K. The 2A4 antibody was used to detect Rab5a on Western blots, where it reacted with a single species of the expected molecular weight. Although the 2A4 epitope was not mapped, this antibody did not react in ELISA with the C-terminal peptide and therefore is presumed to recognize a different region of the protein. The 4F11 antibody was less effective on Western blots but efficiently and specifically immunoprecipitated endogenous Rab5a from MDCK cells as well as *in vitro* translated protein. By immunofluorescence microscopy, 4F11 gave a staining pattern coinciding with that of the polyclonal anti-Rab5a antibody (35). Furthermore, the antibody was monospecific for the Rab5a isoform and did not recognize overexpressed Rab5b and Rab5c isoforms (88–91% identity) by immunofluorescence staining.

Stable Expression in MDCK Strain I Cells. Stable MDCK I cell lines expressing Rab5a were obtained using a retroviral system (43, 44). Rab5-recombinant ecotropic and amphotropic viruses were prepared as described (45). Recombinant amphotropic virus was used to infect subconfluent MDCK I cells for 12 hr in the presence of 4 μg of Polybrene per ml (Sigma). The infected cells were trypsinized and plated at different dilutions in medium containing 1 mg of G418 per ml. Colonies resistant to the antibiotic were selected and examined for overexpression of the Rab5a protein by Western blotting and by immunofluorescence microscopy, as described (14). Staining of monoclonal 4F11 antibody was visualized with tetramethylrhodamine B isothiocyanate-labeled donkey anti-mouse secondary antibody (Dianova, Hamburg, F.R.G.). Filters were mounted with spacers with 50% glycerol and then viewed with the European Molecular Biology Laboratory confocal microscope (36). For Western blot the primary monoclonal antibody 2A4 was detected by using horseradish peroxidase (HRP)-labeled goat anti-mouse antibodies (Dianova) and the bands were visualized using the enhanced chemiluminescence system (Amersham).

Estimation of Fluid-Phase Endocytosis. Estimation of HRP uptake was done as described (14, 41, 46, 53). Incubation with HRP was also carried out at 4°C for each time point and the amount of HRP adsorbed nonspecifically by the cells was always <5% of the value at 37°C. The transepithelial resistance was routinely measured before and after each experiment. We used only filters displaying a resistance of >4000 $\Omega \cdot cm^2$. The resistance measured after each experiment was >3000 $\Omega \cdot cm^2$.

RESULTS

Localization of Rab5a in Epithelial Cells. To determine the intracellular localization of Rab5a in polarized epithelial cells the monoclonal antibody 4F11 was used to stain 5-μm sections of adult mouse kidney cortex. Confocal immunofluorescence microscopy (Fig. 1*A*) revealed a very strong labeling of the kidney tubules. This labeling was specific, as no signal was obtained with the secondary antibody alone or after competition with 0.1 mg of recombinant Rab5a protein per ml (data not shown). At higher magnification (Fig. 1*B*)

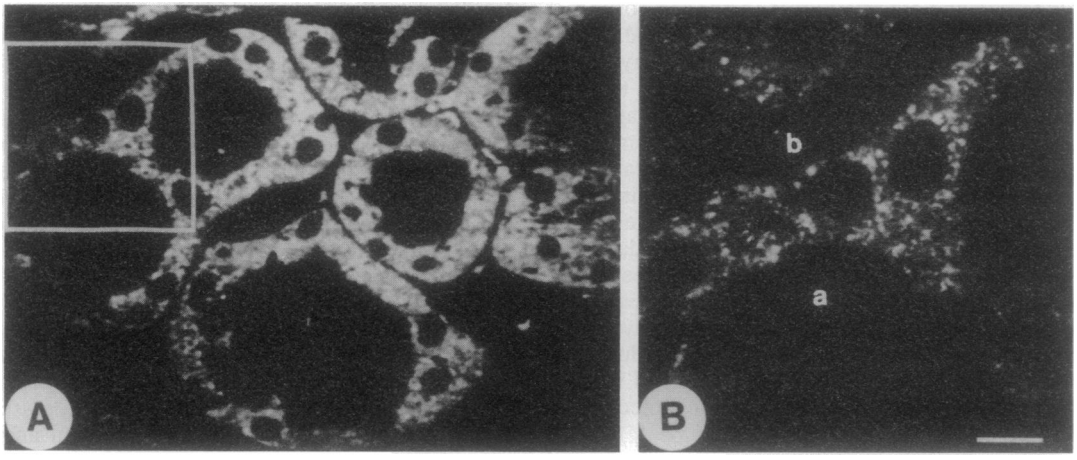

FIG. 1. Confocal immunofluorescence localization of Rab5a. The monoclonal 4F11 was used at a 1:50 dilution on 5-μm adult mouse cortex kidney cryosections. (*A*) Low-magnification view showing strong staining of tubular structures. (*B*) High-magnification view showing labeling of the Rab5a antibody on vesicles underlying the apical (a) and basolateral (b) membranes. (Bar = 10 μm.)

staining of vesicular structures throughout the cytoplasm was evident, suggesting that the protein was distributed to endocytic organelles underlying the luminal (apical) and the basolateral domains. Similar results were obtained with sections prepared from intestine (data not shown).

We then examined the localization of Rab5a in polarized MDCK I cells grown as polarized monolayers on filters (38). Under these experimental conditions MDCK I cells form tight junctions and develop a high transepithelial resistance. Confocal immunofluorescence analysis of the endogenous protein is shown in Fig. 2 (column MDCK I). To analyze the distribution of the protein in the cells we examined horizontal (x–y) and vertical (x–z) sections. The horizontal sections were taken along the vertical axis starting from the apical pole (Fig. 2*B*) and descending toward the basolateral one (Fig. 2*D*). x–z and x–y views confirm that the Rab5a protein is present on both plasma membrane domains and in vesicles distributed throughout the cell. Based on previous studies, we assume that these are early endocytic structures, clathrin-coated vesicles, or early endosomes (35, 36). In contrast to Rab8 (33), Rab5a was not concentrated near the junctional complexes but was uniformly distributed along the basolateral surface. Thus, in fully polarized filter-grown MDCK I cells as well as in mouse kidney tubule cells, Rab5a is associated with the apical and the basolateral plasma membrane domains.

Cell Lines Stably Expressing the Rab5a Protein. Previous studies showed that Rab5a overexpressed using the T7 RNA polymerase recombinant vaccinia virus (VT7) system was shown to be isoprenylated, properly localized (47), and able to increase the rate of fluid-phase and receptor-mediated endocytosis in nonpolarized cells (36). To perform functional studies on Rab5a in polarized epithelial cells we generated several stable MDCK I cell lines overexpressing the protein using a retroviral system (43). The clones were characterized by using monoclonal antibody 2A4. Fig. 3 shows that the 2A4 antibody recognized a single band of the expected molecular mass of Rab5a in MDCK I total cell extract. Four independent cell lines were selected and used for further studies after screening by immunofluorescence microscopy with 4F11 and Western blot analysis with 2A4. These four cell lines overexpressed Rab5a between 3- and 15-fold over the endogenous level (Fig. 3). All of these cell lines were highly polarized by the following criteria. (*i*) When grown on filters, they were able to form monolayers that had a high transepithelial resistance (>4000 $\Omega \cdot cm^2$). (*ii*) They displayed a polarized distribution of two markers: a 58-kDa basolateral protein (Fig. 4) and a 114-kDa protein (mainly apical, not shown), as previously reported for MDCK cells (42). The distribution of the overexpressed Rab5a in the stable cell lines was then analyzed by confocal microscopy using the 4F11 monoclonal antibody at a dilution of 1:300.

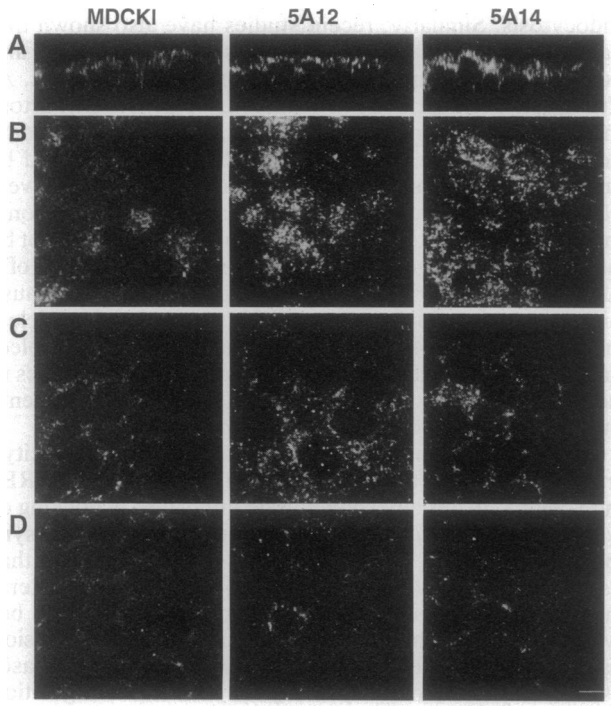

FIG. 2. (*A*) Confocal laser beam immunofluorescence localization of the Rab5a protein in filter-grown control MDCK I cells (MDCKI) and in two stable MDCK I cell lines overexpressing Rab5a (5A12 and 5A14) x–z views. (*B–D*) x–y views selected from a confocal series in which the cell layer was scanned along the vertical axis starting at the apical pole (plane *B*). Planes *C* and *D* correspond to the middle and basal region of the cells, respectively. These are images of one optical section of 0.5 μm. For detecting endogenous protein the anti-Rab5a mouse monoclonal antibody 4F11 was diluted 1:30, whereas for the overexpressed protein it was diluted 1:300. (Bar = 10 μm.)

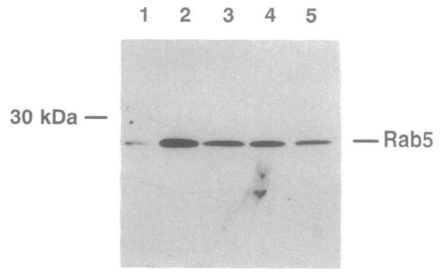

FIG. 3. Immunoblot analysis with monoclonal antibody 2A4 of total proteins from MDCK I cells (lane 1) and four different stable cell lines overexpressing rab5a: 5A12 (lane 2), 5A13 (lane 3), 5A14 (lane 4), and 5A15 (lane 5).

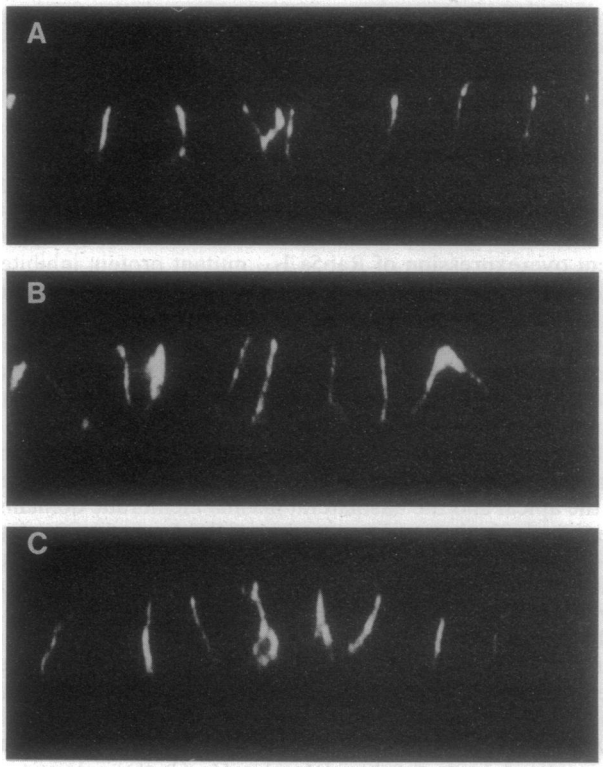

FIG. 4. Confocal laser beam immunofluorescence localization of the 58-kDa antigen in control MDCK I cells (*A*) and in two stable MDCK I cell lines, 5A12 (*B*) and 5A14 (*C*) overexpressing Rab5a. These x–z views show the basolateral distribution of the protein in the control cells and in the stable cell lines.

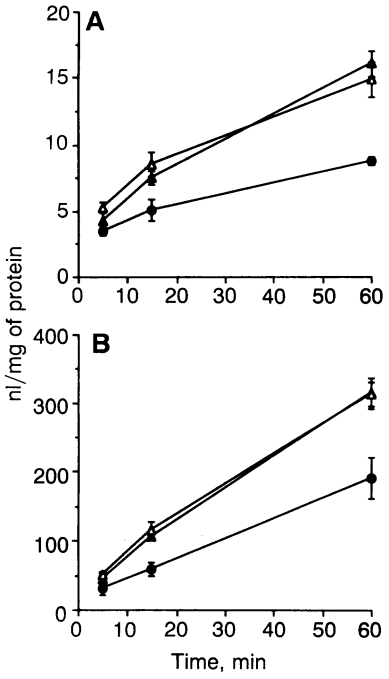

FIG. 5. HRP internalization from the apical side (*A*) and from the basolateral side (*B*) in control MDCK I cells (●) and in the 5A12 (▲) and 5A14 (△) cell lines overexpressing Rab5a. HRP (10 mg/ml) was added to the cells from the apical (*A*) or from the basolateral (*B*) side and the cells were incubated for 5, 15, and 60 min at 37°C. After extensive washes the cells were lysed and the content of HRP was determined (14). Here a typical experiment is shown in which each time point represents the mean of triplicate samples and the bar is the standard error of the mean when larger then the size of the symbol. The experiments were repeated four times with similar results.

Under these conditions overexpressed but not endogenous Rab5a protein could be detected. The protein appeared to be associated with apical and basolateral plasma membrane domains and in cytoplasmic vesicles (Fig. 2, columns 5A12 and 5A14). Identical results were obtained with all cell lines, although only two of the four cell lines analyzed are shown in Figs. 2 and 4. These data indicate that, similar to endogenous Rab5a, overexpressed Rab5a is targeted to the apical and the basolateral plasma membrane domains. Since transient overexpression of Rab5a I_{133} mutant protein inhibited endocytosis in baby hamster kidney cells, we tried to generate stable MDCK I lines expressing this protein. Unfortunately, we could not select clones expressing the mutant protein, suggesting that, similar to analogous mutants of Ypt1 and Sec4 in yeast, expression of Rab5a I_{133} is toxic to mammalian cells.

Rab5a Is Functional on Both Membrane Domains in the Stable Cell Lines. Having established that Rab5a is localized to the apical and the basolateral plasma membrane domains, we tested whether overexpression of Rab5a could stimulate endocytosis from both sides by measuring the kinetics of HRP uptake. The results for two of the four stable cell lines (5A12 and 5A14) are shown in Fig. 5. In control MDCK I cells, the amount of intracellular HRP measured after different times of continuous internalization at 37°C was in agreement with previous results (14). Consistent with these data, HRP internalized from the apical side resulted in >30-fold lower accumulation as compared to samples where HRP was internalized from the basolateral side. Overexpression of Rab5a led to an ≈2-fold increase in intracellular HRP when internalized either from the apical (*A*) or the basolateral (*B*) side relative to control MDCK I cells. All three cell lines exhibiting at least a 5-fold increase over endogenous Rab5a levels displayed this marked increase in HRP internalization. The cell line 5A15, which overexpressed Rab5a only 2- to 3-fold, showed a more limited increase in the internalization of HRP (20–30% more than the control MDCK I cells), but again this increase was observed for both sides (not shown).

DISCUSSION

The results obtained in this study indicate that Rab5a is a common component of the apical and basolateral endocytic machineries of polarized epithelial cells. Previous data showed that Rab5a is a rate-limiting factor in transport from the plasma membrane to the early endosomes in nonpolarized cells (36). Here, we have shown that in kidney epithelial cells and in polarized MDCK I cells Rab5a is associated with the apical and basolateral plasma membrane domains and underlying endocytic structures. Upon overexpression, the protein is targeted to both surfaces, resulting in stimulation of apical and basolateral endocytic uptake. These data raise a number of questions concerning the regulation of membrane traffic in polarized epithelial cells.

Several *in vitro* and *in vivo* studies have shown that distinct machineries regulate the apical and basolateral endocytic pathways of epithelial cells (15–19). Since Rab proteins regulate specific intracellular transport events (19–24) this family of proteins could contribute to the specificity of membrane traffic in polarized cells. Consistent with this hypothesis, Rab8, which is also expressed in nonpolarized cells, regulates transport from the trans Golgi network specifically to the basolateral plasma membrane in epithelial cells or to the dendritic plasma membrane in neurons (33, 34) and it appears to be excluded from apical or axonal transport. However, unlike Rab8, Rab5a is not restricted to the basolateral transport circuit but regulates basolateral and apical endocytosis. Similarly, recent studies have also shown that Rab5a is found on early endosomes of the dendritic and axonal domains in hippocampal neurons (M. de Hoop, L. A. Huber, H. Stenmark, E. Williamson, M.Z., R. G. Parton, and C. G. Dotti, unpublished data). Therefore, these data clearly rule out the possibility that Rab5a *per se* would be sufficient to target plasma membrane-derived endocytic vesicles to either apical or basolateral endosomes. Mutational analysis has suggested that also Ypt1p and Sec4p may not be the sole vesicle targeting molecules. In fact, expression of a bifunctional chimeric Ypt1p/Sec4p protein did not cause missorting of endoplasmic reticulum and Golgi vesicles, suggesting that Sec4p and Ypt1p alone do not act as molecular "tags" to specify the destination of transport vesicles in the yeast secretory pathway (48). Additional components must be present to confer targeting specificity.

What are the components responsible for this specificity? Present candidates are members of a family of v-SNAREs and t-SNAREs initially shown to be important in docking of synaptic vesicles with the plasma membrane in the presynaptic terminal (49, 50). One would have to assume that distinct pairs of SNAREs exist on the apical and basolateral endocytic pathways. Rab5a may regulate interactions between these or other components of the docking/fusion machinery present on the vesicles and on apical and basolateral early endosomes (22, 24). In this case, regulation would be effected by the same molecule interacting with two distinct machineries. In this respect, it is noteworthy that, while in MDCK I cells the rate of fluid internalization from the basolateral surface is 3.6 times higher than at the apical surface, fluid is internalized at the same rate from both surface domains per unit area (14, 15). In addition, the size of the apical and basolateral early endosomal compartments is proportional to the endocytic uptake from each surface (15). Thus, the finding that endocytosis from both surface domains is controlled by Rab5a raises the possibility that this GTPase could be responsible for keeping the apical and basolateral

endocytic traffic balanced. It is also important to consider that, beside Rab5a, several other Rab proteins have been localized to the early endosomes—e.g., Rab4a (51), Rab4b, Rab5b, Rab5c (C.B., A.L., and M.Z., unpublished data), Rab18, Rab20 (unpublished data), and Rab22 (52). Functional data on these GTPases are now required to test their role in the endocytic pathways of polarized cells.

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1. Simons, K. & Fuller, S. D. (1985) *Annu. Rev. Cell Biol.* **1**, 243–288.
2. Rodriguez-Boulan, E. & Nelson, J. (1989) *Science* **245**, 718–725.
3. Mostov, K., Apodaca, G., Aroeti, B. & Okamoto, C. (1992) *J. Cell Biol.* **116**, 577–583.
4. Nelson, J. (1992) *Science* **258**, 948–955.
5. Gumbiner, B. M. (1992) *Cell* **69**, 385–387.
6. Simons, K. & Wandinger-Ness, A. (1990) *Cell* **62**, 207–210.
7. Geuze, H. J., Slot, J. W., Strous, G. J. A. M., Peppard, J., von Figura, K., Hasilik, A. & Schwartz, A. L. (1984) *Cell* **37**, 195–204.
8. Griffiths, G. & Simons, K. (1986) *Science* **234**, 438–443.
9. Bartles, J. R., Feracci, H. M., Stieger, B. & Hubbard, A. L. (1987) *J. Cell Biol.* **105**, 1241–1251.
10. Hughson, E. J., Wandinger-Ness, A., Gausepohl, H., Griffiths, G. & Simons, K. (1988) in *Centenary Symposium of the Pasteur Institute*, ed. Schwartz, M. (Elsevier, Paris), pp. 75–89.
11. Hubbard, A. L. & Stieger, B. (1989) *Annu. Rev. Physiol.* **51**, 755–770.
12. Wandinger-Ness, A., Bennet, M. K., Antony, C. & Simons, K. (1990) *J. Cell Biol.* **111**, 987–1000.
13. Parton, R. G. (1991) *Semin. Cell Biol.* **2**, 387–395.
14. Bomsel, M., Prydz, K., Parton, R. G., Gruenberg, J. & Simons, K. (1989) *J. Cell. Biol.* **109**, 3243–3258.
15. Parton, R. G., Prydz, K., Bomsel, M., Simons, K. & Griffiths, G. (1989) *J. Cell Biol.* **109**, 3259–3272.
16. Hughson, E. J. & Hopkins, C. R. (1990) *J. Cell Biol.* **110**, 337–348.
17. Fujita, M., Reinhart, F. & Neutra, M. (1990) *J. Cell Sci.* **97**, 385–394.
18. Bomsel, M., Parton, R. G., Kunetsov, S. A., Schroer, T. A. & Gruenberg, J. (1990) *Cell* **62**, 719–731.
19. Bourne, H. R. (1988) *Cell* **53**, 669–671.
20. Goud, B. & McCaffrey, M. (1991) *Curr. Op. Cell Biol.* **3**, 626–633.
21. Pfeffer, S. R. (1992) *Trends Cell Biol.* **2**, 41–45.
22. Zerial, M. & Stenmark, H. (1993) *Curr. Opin. Cell Biol.* **5**, 613–620.
23. Simons, K. & Zerial, M. (1993) *Neuron* **11**, 789–799.
24. Novick, P. & Brennwald, P. (1993) *Cell* **75**, 597–601.
25. Padfield, P. J., Balch, W. E. & Jamieson, J. D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1656–1660.
26. Senyshyn, J., Balch, W. E. & Holz, R. W. (1992) *FEBS Lett.* **309**, 41–46.
27. Oberhauser, A. F., Monck, J. R., Balch, W. E. & Fernandez, J. M. (1992) *Nature (London)* **360**, 270–273.
28. Baldini, G., Hohl, T., Lin, H. Y. & Lodish, H. F. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5049–5052.
29. Elferink, L. A., Anzai, K. & Scheller, R. H. (1992) *J. Biol. Chem.* **267**, 1–8.
30. Lütcke, A., Jansson, S., Parton, R. G., Chavrier, P., Valencia, A., Huber, L., Lehtonen, E. & Zerial, M. (1993) *J. Cell Biol.* **121**, 553–564.
31. Olkkonen, V. M., Peterson, J. R., Dupree, P., Lütcke, A., Zerial, M. & Simons, K. (1993) *Gene* **138**, 207–211.
32. Chavrier, P., Vingron, M., Sander, C., Simons, K. & Zerial, M. (1990) *Mol. Cell. Biol.* **10**, 6578–6585.
33. Huber, L. A., Pimplikar, S., Parton, R. G., Virta, H., Zerial, M. & Simons, K. (1993) *J. Cell Biol.* **123**, 34–45.
34. Huber, L. A., de Hoop, M., Dupree, P., Zerial, M., Simons, K. & Dotti, C. (1993) *J. Cell Biol.* **123**, 47–55.
35. Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. & Zerial, M. (1990) *Cell* **62**, 317–329.
36. Bucci, C., Parton, R. G., Mather, I. H., Stunnenberg, H., Hoflack, B. & Zerial, M. (1992) *Cell* **70**, 715–728.
37. Gorvel, J. P., Chavrier, P., Zerial, M. & Gruenberg, J. (1991) *Cell* **64**, 915–925.
38. Fuller, S. D., von Bonsdorff, C.-H. & Simons, K. (1984) *Cell* **38**, 65–77.
39. Studier, F., Rosenberg, A., Dunn, J. & Dubendorf, J. (1990) *Methods Enzymol.* **185**, 60–86.
40. Tucker, J., Sczakiel, G., Feuerstein, J., John, J., Goody, R. S. & Wittionghofer, A. (1986) *EMBO J.* **5**, 1351–1358.
41. John, J., Frech, M. & Wittinghofer, A. (1988) *J. Biol. Chem.* **263**, 11792–11799.
42. Balcarova-Ständer, J., Pfeiffer, S. E., Fuller, S. D. & Simons, K. (1984) *EMBO J.* **3**, 2687–2694.
43. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**, 980–990.
44. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**, 2895–2902.
45. Stenmark, H., Parton, R. G., Steele-Mortimer, O., Lütcke, A., Gruenberg, J. & Zerial, M. (1994) *EMBO J.* **13**, 1287–1296.
46. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
47. Chavrier, P., Gorvel, J.-P., Stelzer, E., Simons, K., Gruenberg, J. & Zerial, M. (1991) *Nature (London)* **353**, 769–772.
48. Brennwald, P. & Novick, P. (1993) *Nature (London)* **362**, 560–563.
49. Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E. (1993) *Nature (London)* **362**, 318–324.
50. Whiteheart, S. W., Griff, J. C., Brunner, M., Clary, D. O., Mayer, T., Buhrow, S. A. & Rothman, J. E. (1993) *Nature (London)* **362**, 353–355.
51. van der Sluijs, P., Hull, M., Zahraoui, A., Tavitian, A., Goud, B. & Mellman, I. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6313–6317.
52. Olkkonen, V. M., Dupree, P., Killisch, I., Lütcke, A., Zerial, M. & Simons, K. (1993) *J. Cell Sci.* **106**, 1249–1261.
53. Marsh, M., Schmid, S., Kern, H., Harms, E., Male, P., Mellman, I. & Helenius, A. (1987) *J. Cell Biol.* **104**, 875–886.