Rab17 Localizes to Recycling Endosomes and Regulates Receptor-mediated Transcytosis in Epithelial Cells*

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The small GTPase Rab17 is restricted to epithelial cells and its expression is induced during cell polarization. This observation has led to the suggestion that the protein may function in transcytosis, a pathway connecting the apical and basolateral endocytic systems. To analyze whether Rab17 plays a role in transcellular transport, we generated Madin-Darby canine kidney (MDCK) cell lines stably coexpressing wild-type or mutant Rab17 and the transcytotic polymeric immunoglobulin receptor (pIgR). Rab17 expressed in MDCK cells was found on small vesicles and tubules in the apical region of the cells. A significant fraction of the Rab17-positive structures was accessible to dimeric IgA internalized from the apical or basolateral cell surface via the pIgR. Furthermore, basolateral to apical transcytosis of dimeric IgA was impaired in MDCK cells overexpressing Rab17. Our data provides morphological and biochemical evidence for a role of Rab17 in the regulation of transcellular traffic through apical recycling endosomes in epithelial cells.

A typical feature of polarized epithelial cells is the differentiation of their plasma membrane into morphologically distinct apical and basolateral domains. The exocytic as well as the endocytic pathway play an important role in generating and maintaining the polarized phenotype (1–4). Newly synthesized proteins may be sorted in the trans-Golgi network (TGN) for direct delivery to the apical or basolateral domain. Some apical proteins, however, may reach their final apical destination indirectly via the basolateral surface and transcytosis. In hepatocytes, transport to the basolateral surface follows by transcytosis is the obligatory route for all newly synthesized apical proteins (5). Also a particular subset of endocytic proteins such as epithelial receptors for immunoglobulins A and G are transcytosed from one plasma membrane domain to the opposite (6, 7).

Initial characterization of the endocytic pathway in polarized MDCK cells indicated that fluid phase markers internalized from the apical or basolateral cell surface enter distinct apical and basolateral early endosomes before converging in late endosomes (8–11). More recently, evidence has been provided for a common endocytic compartment accessible to membrane markers internalized from the apical or basolateral surface. In intestinal Caco-2 cells, transferrin internalized from the basolateral surface reaches an apical endocytic compartment that also receives membrane proteins internalized from the apical side (12, 13). Similarly, transferrin internalized from the basolateral surface enters an apical endocytic compartment that is accessible to markers internalized from the apical surface (15, 16). Although the pIgR and the TfR have access to this apical endosomal compartment, the pIgR is efficiently transcytosed, whereas the TfR is excluded from the transcytotic pathway and recycled. Furthermore, pIgR internalized from the apical surface into apical endosomes does not transcytose to the basolateral domain but recycles. Thus, the accessibility of the apical endosomal compartment to membrane proteins internalized from the apical and basolateral surface and its ability to sort receptors into the transcytotic or recycling pathway indicate that this compartment, also termed apical recycling endosome, plays a central role in polarized sorting in the endocytic pathway of epithelial cells.

Recent evidence indicates that the organization of the intracellular transport system in epithelial cells is not unique to polarized cells. Fibroblasts possess two biocataltic routes that, based on the proteins they transport and the underlying regulatory mechanisms, correspond to the apical and basolateral pathway of epithelial cells (17, 18). Similarly, evidence has been provided for the existence of an apical recycling endosome described in polarized cells. Fibroblasts possess two biosynthetic routes that, based on the proteins they transport and the underlying regulatory mechanisms, correspond to the apical and basolateral pathway of epithelial cells (17, 18). Similarities in the organization of the endocytic system between polarized and nonpolarized cells are emerging (4). Receptors internalized by nonpolarized cells are first delivered to peripheral early endosomes before being transferred to a more perinuclear compartment (19) that shares many of the characteristics of the apical recycling endosome described in polarized cells. It is thus conceivable that the endocytic pathway of epithelial cells has been adapted for polarized sorting by the expression of specific regulatory molecules such as epithelial specific GTPases of the Rab family of proteins. Rab17 is one of the few Rab proteins restricted to epithelial cells (20). In kidney proximal tubule cells, Rab17 localizes to the basolateral membrane and apical tubules. Although it is not known if the organelles labeling for Rab17 are associated with the endosomal system, Rab17 has been implicated in regulating transcytosis.

In the present study we took advantage of MDCK cells expressing a well characterized transcytotic receptor, the pIgR, to analyze whether Rab17 regulates transcellular transport. Murine Rab17 was expressed in MDCK cells, where it localized to small vesicles and tubules enriched in the apical cytoplasm of...
the cell. dIgA internalized from the apical or the basolateral cell surface colocalized to a significant extent with Rab17 positive organelles. Furthermore, basolateral to apical transcytosis of dIgA was impaired in MDCK cells overexpressing Rab17, whereas expression of a Rab17 mutant defective in membrane association did not affect transcytosis. These results implicate Rab17 in the regulation of transcytosis through the apical recycling endosome in polarized cells.

MATERIALS AND METHODS

Isolation of a Murine Rab17 cDNA—Recombinant DNA techniques were carried out according to standard procedures. Total RNA was extracted from BALB/c mouse kidney, primed with oligo-dT and reverse transcribed. Two primers covering the 5′-GGG GTA CCC CAT CGA TAG CCA TGG CGC ATG CTG GGC CTG GCG CTG CC-3′ and C-terminal 5′-TAG GTA CGG ATC CTA TCG TGC ACA CTG GC-3′ region of Rab17 (20) were used to amplify reverse transcribed RNA by PCR. The PCR product was cloned into pCB6 using ClaI and BamHI sites introduced by the PCR primers. The sequence of all PCR products was verified. The following differences to the published sequence of Rab17 (20) were found in two independent PCR products: nucleotide 55 of the coding sequence was a C (instead of G) and nucleotide 138 a T (instead of C), the first of these differences changes in a amino acid level (Val-19 to Leu).

Generation and Characterization of Antibodies to Rab17—Polyclonal rabbit antisera were custom made using keyhole limpet hemocyanin-coupled peptide derived from the hypervariable region of the murine Rab17 (amino acids QRAQDTGSSRRFQEGEAVA) and affinity purified on peptide coupled to Affi-Gel 10. The specificity of the antibody was confirmed by the following criteria: a single protein of ~25 kDa was recognized by Western blots in mouse liver, kidney, and intestine but not in other organs (not shown); b the antibody did not react with MDCK cells by immunofluorescence or Western blot, but it did recognize a ~25 kDa protein in cells transfected with the murine Rab17 cDNA (see “Results”); and c the presence of 0.1 mg/ml peptide inhibited the reactivity of the antibody in immunofluorescence or Western blot experiments (not shown).

Construction of Rab17 C211A/C212A—The Rab17 C211A/C212A mutant was constructed by PCR using a 5′ sense primer (5′-GGG GTA CCC CAT CGA TAG CCA TGG CGC AGT CTG GGC CTG GCG CTG CC-3′) and a 3′ mutagenic antisense primer (5′-GTA CGG ATC CTA TCG TGC ACG GCC CTG CGG CTG CC-3′) and the PCR product was verified by sequence analysis.

MDCK Cells Coexpressing Wild-type and Mutant Rab17 and pIgR—Cells were cultured and transfected essentially as described (21). First, MDCK cells expressing the pIgR from a cytomegalovirus-based vector encoding either a wild-type Rab17 cDNA or a Rab17 mutant in the C-terminal hypervariable region of murine Rab17 (see “Materials and Methods”) were grown on Transwell™ filters as previously outlined (21).

Characterization of MDCK Cells Stably Expressing the pIgR and Wild-type or Mutant Rab17—To characterize a possible involvement of Rab17 in transcellular transport, we transfected MDCK cells stably expressing the pIgR (26) with cDNAs encoding either a wild-type Rab17 cDNA or a Rab17 mutant in which the isoprenylation site was inactivated (Rab17 C211A/C212A) and analyzed the localization of Rab17 and its effect on transcytosis. Similar approaches have been used to establish a role of other Rab proteins in different exocytic and endocytic pathways (for reviews, see Refs. 27–29).

To test whether endogenous Rab17 expressed by MDCK cells interferes with the detection of the transfected murine protein, cell extracts were subjected to Western blot analysis using an affinity purified antibody generated to a peptide derived from the C-terminal hypervariable region of murine Rab17 (see “Materials and Methods”). In Fig. 1A, no endogenous MDCK proteins were detected by the peptide antibody (lane 1). The antibody did, however, recognize a band of ~25 kDa in cells transfected with the murine Rab17 cDNA (lane 2 and see below). Reverse transcription-PCR on MDCK RNA using primers derived from the 5′- and 3′-coding region of the murine Rab17 cDNA sequence resulted in the amplification of a PCR product of ~600 base pairs, indicating the presence of an
endogenous Rab17 transcript. Thus, our anti-peptide antibody either did not cross-react with the canine Rab17 or the levels of endogenously expressed Rab17 in MDCK cells were too low to be detected by immunological methods. Because the antibody did not detect the endogenous protein, we were able to detect a transfected wild-type or a nonisoprenylated mutant murine Rab17 without the need for epitope tagging.

To characterize MDCK cells transfected with wild-type or mutant Rab17, we first analyzed the expression levels of the GTPase in selected clones by subjecting equal amounts of total protein to Western blot analysis. Two clones expressing similar levels of the wild-type or mutant protein were chosen for further experiments (Fig. 1, lanes 2 and 3). As judged by immunofluorescence, all cells in a given population expressed the GTPase as well as the pIgR (see below and Fig. 2).

Because the cysteine residues in the isoprenylation site of Rab17 C211A/C212A were substituted for alanines, the mutant was not expected to associate with membranes. To test the ability of Rab17 to associate with membranes, MDCK cells were homogenized, and membranes were pelleted from a post-nuclear fraction. The membrane pellet (P) and the supernatant (S) were then analyzed by Western blot for the presence of Rab17. Rab17 was recovered in the pellet fraction, whereas Rab17 C211A/C212A remained in the supernatant (Fig. 1B), indicating that Rab17 but not the mutant was probably isoprenylated. Similarly, endogenous Rab5 (Fig. 1C) and Rab4 (Fig. 1D) were efficiently recovered in the membrane pellet.

In conclusion, MDCK cell lines were generated that coexpressed the pIgR and similar levels of either wild-type Rab17 or a mutant unable to associate with membranes.

Rab17 Is Partly Associated with an Apical Endocytic Compartment Containing dIgA—Next we characterized the intracellular localization of transfected Rab17 by immunofluorescence microscopy. Cells expressing the wild-type or mutant GTPase were allowed to endocytose biotinylated dIgA for 60 min, and following fixation and permeabilization, Rab17 and internalized dIgA-biotin were visualized.

As shown in Fig. 2 and consistent with the Western blot results described above, no Rab17 was detected in MDCK cells expressing the pIgR alone (Fig. 2, E and F). In cells transfected with the wild-type Rab17, the protein localized to a vesicular compartment concentrated in the perinuclear region and extending into the cell periphery (Fig. 2A). The Rab17-positive compartment overlapped to a significant extent with endocytosed dIgA (Fig. 2B, compare with Fig. 2A). Confocal laser microscopy (not shown) and immunoelectron microscopy (see below) confirmed the extensive colocalization of dIgA and Rab17, although in both cases organelles exclusively staining for Rab17 or dIgA were observed also.

In contrast to cells expressing wild-type Rab17, cells transfected with Rab17 C211A/C212A showed a diffuse cytosolic and nuclear staining (Fig. 2C), indicating that the mutant GTPase remained soluble in the cytosol and diffused into the nucleus.

These results show that Rab17 is associated with vesicles and tubules and that at least a subpopulation of the Rab17 compartment is accessible to internalized dIgA.

Rab17 Localizes to Small Apical Vesicles and Tubules Accessible to dIgA Internalized from the Apical or the Basolateral Surface—We next used cryo-immunogold-electronmicroscopy to determine in more detail the subcellular localization of Rab17 in polarized MDCK cell monolayers and to confirm the apparent colocalization of Rab17 and dIgA observed by light
microscopy. MDCK cells grown on Transwell™ units (see inset) were allowed to internalize biotinylated dIgA from the basolateral or apical surface for 45 min, fixed, and Rab17 and dIgA were visualized by immunogold labeling. As shown in Fig. 3A, Rab17 (arrows) was detected on a population of small vesicles and tubules highly enriched in the apical pole of the
cells. Of 900 gold particles representing Rab17 labeling, less than 3% were associated with the basolateral or apical plasma membrane. The observed localization of Rab17 to apical vesicles and tubules was consistent with the localization in proximal kidney tubules (20), but little Rab17 staining was observed on the basolateral plasma membrane. A significant fraction of the Rab17-positive vesicles and tubules contained dIgA, which had been internalized from the apical (Fig. 3B) or the basolateral (Fig. 3C) domain. Depending on the labeling density for dIgA, 30–50% of the Rab17-positive membranes also labeled for dIgA. In addition, Rab17-positive organelles that lacked dIgA as well as endocytic vesicles that carried dIgA but were devoid of Rab17 were observed.

In conclusion, murine Rab17 expressed in MDCK cells was associated with small cytoplasmic vesicles and tubules present in the apical region of polarized cells and Rab17-positive organelles were accessible to dIgA endocytosed from the apical or the basolateral cell surface.

Expression of Rab17 Impairs Basolateral to Apical Transcytosis of dIgA—The observation that Rab17 and internalized dIgA colocalize (see Figs. 2, C and D and 3, B and C) suggested that the protein might be involved in regulating polymeric immunoglobulin receptor trafficking. Therefore, we next analyzed whether transport of dIgA bound to the pIgR was altered in MDCK cells expressing wild-type or mutant Rab17. 125I-dIgA prebound to the basolateral surface of polarized cell monolayers was allowed to internalize and transcytose. After the indicated time, the fraction of dIgA released into the apical or basolateral compartment or present on the basolateral surface or inside the cells was determined.

As shown in Fig. 4A and similar to previous observations (21, 26, 30), 30–40% of the prebound dIgA was transcytosed in control MDCK cells. In cells expressing Rab17, however, the kinetics as well as the final extent of transcytosis were reduced. The inhibitory effect was not because of the transfection per se since transcytosis was not altered in MDCK clones transfected with the pIgR and the human TfR (not shown) or the mutant Rab17 (see below and Fig. 6). Furthermore, inhibition of transcytosis did not result from a block in endocytosis since dIgA was cleared from the basolateral surface with similar kinetics in control and Rab17-expressing cells (Fig. 4B). Consistent with this observation, the initial rate of intracellular dIgA...
accumulation in control and Rab17-expressing cells was similar (Fig. 4, B and C). At later times, however, less dIgA accumulated intracellularly in Rab17-expressing cells, probably reflecting a larger fraction of the ligand being recycled and released into the basolateral media (Fig. 4 D). Thus, the lack of an effect on endocytosis and the stimulation of recycling indicate that transfected Rab17 did not exert a general inhibitory effect on endocytic membrane transport.

In MDCK cells, newly synthesized plgR is directly delivered from the TGN to the basolateral cell surface (see also Fig. 6B) and the receptors present on the apical surface reflect molecules that have transcytosed (31). If Rab17 interferes with transcytosis of the plgR, the number of receptor molecules on the apical plasma membrane at steady state may be reduced. Thus, we determined the polarized surface distribution of the plgR in control cells and cells expressing wild-type or mutant Rab17 by measuring the relative binding of 125I-dIgA to the apical or basolateral compartments on ice. As shown in Fig. 5, 65–70% of the total surface plgR localized to the apical domain of control cells or cells expressing Rab17 C211A/C212A, whereas in Rab17-expressing cells the plgR was predominantly on the basolateral surface. The effect of Rab17 on the surface distribution of the plgR is thus consistent with the observed inhibition of transcytosis.

To confirm that overexpression of Rab17 specifically affected the endocytic transport of the plgR, we analyzed the apical secretion of newly synthesized gp80 (32) and the basolateral delivery of newly synthesized plgR (31) in control and Rab17-overexpressing cells. MDCK cells grown on permeable filters were pulse-labeled with [35S]methionine/cysteine and labeled proteins were then chased for 30 min to the cell surface. Equal volumes of apical and basolateral media were precipitated with trichloroacetic acid and analyzed by SDS-PAGE and autoradiography. Western blot analysis showed that the major 80-kDa protein secreted by MDCK cells was gp80 (not shown). A, apical; B, basolateral. B, basolateral transport of the plgR. The apical or basolateral cell surface of the cell monolayer was biotinylated, cells were lysed, and the plgR was immunoprecipitated. An aliquot of the immunoprecipitate was directly analyzed by SDS-PAGE and autoradiography (Total), from the remainder biotinylated surface receptors were isolated using immobilized streptavidin and analyzed by SDS-PAGE autoradiography (Surface). A, apical; B, basolateral.

FIG. 6. Polarized cell surface delivery of gp80 and the plgR. A, gp80 secretion. MDCK cells overexpressing Rab17 or control cells were metabolically labeled for 15 min and labeled proteins were then chased for 30 min to the cell surface. Equal volumes of apical and basolateral media were precipitated with trichloroacetic acid and analyzed by SDS-PAGE and autoradiography. Western blot analysis showed that the major 80-kDa protein secreted by MDCK cells was gp80 (not shown). A, apical; B, basolateral. B, basolateral transport of the plgR. The apical or basolateral cell surface of the cell monolayer was biotinylated, cells were lysed, and the plgR was immunoprecipitated. An aliquot of the immunoprecipitate was directly analyzed by SDS-PAGE and autoradiography (Total), from the remainder biotinylated surface receptors were isolated using immobilized streptavidin and analyzed by SDS-PAGE autoradiography (Surface). A, apical; B, basolateral.

FIG. 7. Inhibition of transcytosis requires membrane association of Rab17. 125I-dIgA prebound to the basolateral surface of control cells or cells expressing Rab17 or Rab17 C211A/C212A was internalized for 10 min at 37 °C. Cells were washed on ice with acid to remove noninternalized dIgA and then returned to 37 °C for the indicated time. dIgA that was transcytosed (released into the apical media; A), intracellular (acid resistant; B), or released into the basolateral medium (basolateral release; C) was determined after the indicated periods of time and plotted as percent (%) of initially bound dIgA. Values were determined in triplicate, and data from a representative experiment are shown.
transcytosis at a post-endocytic step, we compared the kinetics of basolateral to apical transport of a preinternalized pool of dIgA in control and Rab17-transfected cells. In addition, we analyzed whether transcytosis was affected in cells expressing the soluble Rab17 C211A/C212A mutant. For these experiments, dIgA prebound to the basolateral surface of cells expressing wild-type or mutant Rab17 was allowed to internalize for 10 min, and noninternalized ligand was then removed by washing at acidic pH (21). After warming the cells for the indicated time, the fraction of dIgA that was released into the apical media by control cells. As observed for prebound dIgA (Fig. 4), transport of preinternalized ligand was impaired in cells expressing Rab17 (Fig. 7A) and instead accumulated inside the cells (Fig. 7B) or was recycled and released into the basolateral chamber (Fig. 7C). In contrast, cells expressing the Rab17 mutant were not significantly affected in either the kinetics or the extent of transcytosis (Fig. 7A).

As shown in Fig. 7A, 50–60% of the preinternalized ligand was released into the apical media by control cells. As observed for prebound dIgA (Fig. 4), transport of preinternalized ligand was impaired in cells expressing Rab17 (Fig. 7A) and instead accumulated inside the cells (Fig. 7B) or was recycled and released into the basolateral chamber (Fig. 7C). In contrast, cells expressing the Rab17 mutant were not significantly affected in either the kinetics or the extent of transcytosis (Fig. 7A).

In conclusion, these experiments confirm that Rab17 interferes with transcytosis at a postendocytic step and indicate that this effect requires a functional Rab17 protein.

**DISCUSSION**

Among Rab GTPases, Rab17 is one of few members of this protein family whose expression is restricted to epithelial cells. Furthermore, expression of Rab17 is selectively induced during the differentiation of mesenchymal precursors into polarized epithelial cells in the kidney (20). Rab17 may therefore provide regulatory mechanisms to allow epithelial cells to mediate vectorial intracellular transport events. Rab17 has been proposed to play a role in transcytosis based on its localization to the basolateral membrane and apical tubules in epithelial cells of the proximal kidney tubule (20). To test this hypothesis, we analyzed the intracellular routing of the pIgR in MDCK cells coexpressing Rab17. Based on the localization of the Rab17 and its effect on transcytosis of the pIgR, our results implicate Rab17 in the regulation of polarized receptor trafficking through recycling endosomes.

In nonpolarized cells, internalized transferrin first localizes to peripheral “sorting” endosomes and is then transferred to a morphologically and biochemically distinct perinuclear “recycling” endosomal compartment (19). Recycling endosomes carry recycling receptors, whereas fluid phase markers or membrane proteins that enter the degradative pathway are excluded. Morphologically, the recycling endosome consists of tubules and vesicles clustered around the centrosome (33–37). The pericentriolar organization of the compartment is maintained by microtubules. In transfected MDCK cells grown on plastic, Rab17 associated with a perinuclear vesicular compartment very similar to the endocytic compartment labeled after internalization of dIgA or transferrin (see Fig. 2 and Ref. 30).

The recycling endosome characterized in nonpolarized cells shares several morphological and functional similarities with the “apical” recycling endosome described in polarized epithelial cells. The compartment consists of tubules and vesicles and is located in the apical region in the cell reflecting the apical localization of the centrosome in polarized MDCK cells (38). As in nonpolarized cells, recycling receptors (i.e., TR and pIgR) enter the apical recycling endosome, whereas fluid phase markers are excluded (15, 16). Reminiscent to the Rab17-positive compartment observed in proximal kidney tubule cells (20), Rab17 was associated with small vesicles and tubules concentrated to the apical pole of polarized MDCK monolayers. The Rab17 labeling on the basolateral plasma membrane of MDCK cells, however, represented less than 3% of the total Rab17 labeling and may reflect differences in cell type or expression levels. For example, the invaginations of the basolateral plasma membrane in proximal kidney tubule cells that labeled for Rab17 are not observed in MDCK cells and a significant heterogeneity in Rab17 expression has been observed in the kidney, with high Rab17 levels in proximal tubule and barely detectable expression in the more distal part (20). We cannot estimate how much endogenous Rab17 is expressed in MDCK cells because our antibody does not detect the canine protein. Although we cannot completely rule out that structural differences in the murine Rab17 affect its ability to associate with the basolateral plasma membrane when expressed in canine cells, this is unlikely because neither the endogenous nor an overexpressed transfected murine Rab17 are present on the basolateral plasma membrane of murine EpH4 epithelial cells.2,3

An important feature of the recycling endosome in polarized cells is that it is accessible to receptor ligands internalized from either the apical or the basolateral cell surface (12–16). Recycling endosomes may be distinct from basolateral and apical early endosomes but connected via vesicular traffic (8–10). Alternatively, apically and basolaterally internalized receptors may enter a continuous system of interconnected tubules from where receptors would be sorted for transcytosis or recycling (12–14). dIgA internalized from the basolateral or the apical cell surface of MDCK monolayers has access to the Rab17 positive tubules and vesicles, indicating that the GTPase is indeed associated with recycling endosomes. The colocalization of internalized dIgA and Rab17 is also in line with the presence of Rab17 on transcytotic vesicles immunosolated from rat liver using an antibody to the cytosolic domain of the pIgR (39). Even though Rab17 colocalized to a significant extent with internalized dIgA, not all Rab17 positive structures carried internalized dIgA, indicating that ligand internalized from the apical or basolateral domain was either not able to reach a subset of Rab17 positive organelles or that the protein levels were below the detection limit of the immunogold labeling. Alternatively, Rab17 could be associated with discrete domains or regions of an interconnected system of endosomal tubules. It therefore remains to be determined whether dIgA internalized from the apical or basolateral surface enters the same Rab17 positive compartment or whether different subcompartments are labeled. The observed dIgA-containing vesicles lacking the GTPase could represent endocytic elements involved in the initial uptake of dIgA or in the recycling from endosomes back to the basolateral or the apical plasma membrane.

Because recycling endosomes can receive receptors internalized from the apical and basolateral plasma membrane and subsequently sort them for recycling or transcytosis, this compartment is considered to be an important site for vectorial sorting in the endocytic pathway of epithelial cells. It is thus not surprising that overexpressing Rab17 affects transcytosis. The observed inhibition of transcytosis was specific because neither basolateral internalization or recycling of dIgA nor apical and basolateral biosynthetic transport were affected in cells overexpressing Rab17 and because it required a functional Rab17 able to associate with membranes. Many other studies confirm that overexpression of Rab proteins interferes with specific pathways and does not lead to nonspecific effects because of competition for common regulatory factors (for reviews, see Refs. 27–29). Overexpression of most Rab proteins in their wild-type form results in the stimulation of specific trans-

3 W. Hunziker, unpublished observations.
2 M. Zerial, personal communication.
port steps. However, inhibitory effects are not unprecedented and have been observed for Rab3a (40, 41) and Rab6 (42). The inhibitory effect of Rab17 on transcytosis may result from a stimulatory effect on basolateral recycling thereby reducing the pool of dIgA available for transcytosis. Although we observed an increase in basolateral dIgA recycling in cells expressing Rab17, this most likely reflected dIgA prevented from transcytosis because recycling of transferrin was not stimulated in these cells (data not shown). However, if only a small fraction of the total transferrin recycling occurs from recycling endosomes, it is conceivable that a stimulatory effect of Rab17 on this minor pathway for the TIR may go undetected. Finally, we cannot completely rule out the possibility that the murine Rab17 acts as a dominant negative mutant when expressed in canine cells. In any case, it is clear that Rab17 plays a key regulatory role in transcytosis.

Our data provide morphological and biochemical evidence that Rab17 is associated with recycling endosomes in epithelial cells and plays a role in regulating polarized traffic through this compartment. Further work will be required to determine the precise step in transcytosis regulated by Rab17 and its mode of action. The analysis of constitutively active or inactive Rab17 mutants will require inducible expression systems because we have not been able to stably express such mutants. Interestingly, we have not found any effect of overexpressing Rab17 on transcytosis of lipids or bulk membrane markers such as ricin.4 This observation suggests that Rab17 might be involved in regulating specific sorting events at the level of recycling endosomes rather than to play a general role in vesicular targeting.

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