Rab17, a Novel Small GTPase, is Specific for Epithelial Cells and is Induced During Cell Polarization

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Abstract. The Rab subfamily of small GTPases has been demonstrated to play an important role in the regulation of membrane traffic in eukaryotic cells. Compared with nonpolarized cells, epithelial cells have distinct apical and basolateral transport pathways which need to be separately regulated. This raises the question whether epithelial cells require specific Rab proteins. However, all Rab proteins identified so far were found to be equally expressed in polarized and nonpolarized cells. Here we report the identification of Rab17, the first epithelial cell-specific small GTPase. Northern blot analysis on various mouse organs revealed that the Rab17 mRNA is present in kidney, liver, and intestine but not in organs lacking epithelial cells nor in fibroblasts. To determine whether Rab17 is specific for epithelial cells we studied its expression in the developing kidney. We found that Rab17 is absent from the mesenchymal precursors but is induced upon their differentiation into epithelial cells. In situ hybridization studies on the embryonic kidney and intestine revealed that Rab17 is restricted to epithelial cells. By immunofluorescence and immunoelectron microscopy on kidney sections, Rab17 was localized to the basolateral plasma membrane and to apical tubules. Rab proteins associated with two distinct compartments have been found to regulate transport between them. Therefore, our data suggest that Rab17 might be involved in transcellular transport.

Transport of macromolecules in eukaryotic cells is largely mediated by vesicular carriers which bud from a donor membrane and fuse in a directed manner with the appropriate acceptor membrane. So far, little is known about the molecular composition of the machinery which confers specificity and directionality of vesicular transport (for review see Wilson et al., 1991; Melançon et al., 1991). The finding that two small GTPase proteins, Ypt1 and Sec4, are required at different stages of the secretory pathway in the yeast Saccharomyces cerevisiae (Segev et al., 1988; Salmonen and Novick, 1987; Goud et al., 1988) gave rise to the hypothesis that each step in vesicular traffic is regulated by a specific GTPase protein of the Ypt1/Sec4 subfamily (Bourne, 1988). The model implies that a large number of these proteins should be expressed in eukaryotic cells, and that each of them should be associated with distinct intracellular membrane compartments. In accordance with this hypothesis, ∼30 members of the Ypt1/Sec4-related Rab family have been identified in mammalian cells (Touchot et al., 1987; Bucci et al., 1988; Zahraoui et al., 1989; Vielh et al., 1989; Chavrier et al., 1990b; Morimoto et al., 1991; Efferink et al., 1992; Chavrier et al., 1992; Wilson and Wilson, 1992; Baldini et al., 1992) and some of them have been shown to display a specific location (Chavrier et al., 1990a; Goud et al., 1990; Darchen et al., 1990; Fischer von Mollard et al., 1990; Mizoguchi et al., 1990; van der Sluijs et al., 1991; Plutner et al., 1991; Wilson and Wilson, 1992; Lombardi et al., 1993). More recently, in vitro and in vivo studies have demonstrated a regulatory role for mammalian Rab proteins on vesicular traffic in the endocytic (Gorvel et al., 1991; Bucci et al., 1992; van der Sluijs et al., 1992; Lombardi et al., 1993) and secretory (Plutner et al., 1991) pathways.

The Rab protein paradigm has an important implication for specialized cells using transport pathways unique to their differentiated state. Cell-type specific steps in membrane traffic would functionally require specific Rab proteins. For instance, regulated secretion, which depends upon external stimuli, is a particular feature of highly differentiated cell types, such as neurons, exocrine and endocrine cells. So far, only two candidates for such pathway-specific Rab proteins are known. Rab3a has been postulated to regulate Ca2+-dependent secretion in neurons (Fischer von Mollard et al., 1991), neuroendocrine (Darchen et al., 1990) and some exocrine cells (Mizoguchi et al., 1989). Rab3d is thought to control the insulin-dependent exocytosis of glucose transporter-containing vesicles in adipocytes (Baldini et al., 1992).

A more complex situation arises in polarized epithelial cells which have morphologically and functionally distinct apical and basolateral surfaces facing two different extracel-
Materials and Methods

pmol of each primer and 1 u of AmpliTaq (Perkin Elmer Cetus, Norwalk, Corp., Indianapolis, IN) labeled PCR fragments derived from the partial plaques were screened with digoxigenin-dUTP (Boehringer Mannheim) for 2 min, and 72°C for 1 min, with a final extension at 72°C for 2 rain.

Materials and Methods

cDNA Library Screening and Plasmid Construction

An oriented lambda cDNA library from 17-d-old embryonic mouse kidney was prepared in UNIZAP XR (Stratagene, La Jolla, CA). 2 x 10^6 phage plaques were screened with digoxigenin-dUTP (Boehringer Mannheim Corp., Indianapolis, IN) labeled PCR fragments derived from the partial sequences identified by Chavrier et al. (1992). PCR reactions were performed in a 20 µl volume in the presence of 50 mM KCl, 10 mM Tris HCl, pH 8.3, 1.5 mM MgCl2, 0.01% gelatin, 200 µM of dATP, dGTP, dCTP, 130 µM of dTTP, 70 µM of dig-dUTP (Boehringer Mannheim Corp.), 20 pmol of primer and 1 u of AmpliTaq (Perkin Elmer Cetus, Norwalk, CT). The reaction was overlaid with 20 µl of mineral oil (Sigma Immunochemicals, St. Louis, MO), transferred to a programmable thermal cycler (Perkin Elmer Cetus) and incubated for 30 cycles of 94°C for 1 min, 65°C for 2 min, and 72°C for 1 min, with a final extension at 72°C for 2 min.

The PCR products were run on a 1.2% Seaplaque agarose (FMC Corp. Bioproducts, Rockland, ME) gel and excised. After excision, the agarose was melted and the double-stranded PCR products denatured by boiling for 10 min at 100°C. Nylon membrane (Hybond N; Amersham Corp., Arlington Heights, IL) preparation, prehybridization, and hybridization were performed according to the instructions provided by Boehringer Mannheim, using a hybridization solution composed of 5x SSC, 0.1% N-laurolysarco- sine, 0.02% SDS, 1% blocking reagent (Boehringer Mannheim Corp.). In vivo excision of positive cDNA inserts was performed according to the manufacturer’s procedures (Stratagene). Phagemid DNA was prepared and used for double-stranded DNA sequencing using the T7 Sequencing Kit (Pharmacia Fine Chemicals, Piscataway, NY). One 1.6-kb clone containing the whole coding region of rab17 was found. For construction of the pGEM rab17 plasmid (see Fig. 2, upper panel), the 5’ end of the rab17 DNA was mutagenized by PCR to introduce an EcoRI site upstream of an Ndel cloning site at the ATG codon. The fragment was subcloned into pGEMI (Promega Corp., Madison, WI) using the EcoRI site at the 5’ end (partial digest) and an Xhol site at the 3’ end of the cDNA.

Tissues and Organ Culture

BALBc mice were used for RNA preparation from adult mouse tissues. Embryonic kidney anlagen were dissected from (CBA X NMR) F1 mouse embryos. The day of the vaginal plug was defined as day 0.

For the transfilter experiments, metanephric kidney rudiments and spinal cord from E11 embryos were used. The kidney rudiments were treated with 2.25% pancreatin, 0.75% trypsin in Tyrode's solution at 0°C for 1.5 min and then incubated in MEM (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FCS (Flow Cytometry Standards Corp., Res. Triangle Park, NC) at 20°C for 15 min. Subsequently, the metanephric mesenchymes were separated mechanically from the urmer buds and placed on pieces of Nucleo- pore filter (nominal pore size 1.0 µm; Nuclepore Corporation, Pleasanton, CA). Fragments of spinal cord, used as inducer tissue, were cemented with agar to the lower surface of the filter (for details see Grosboin, 1956; Saxén and Saksela, 1971; Lehtonen et al., 1983). In some experiments, the mesenchymes were cultured on the filter in the absence of inducer tissue. As culture medium MEM supplemented with 10% FCS was used.

RNA Preparation

RNA preparation from adult tissues was performed using the Guanidinium Isothioyanate (GITC) method and an SW27 rotor (Sambrook et al., 1989). RNA from embryonic tissues and transfilter samples was prepared using the same solutions in a small-scale protocol. Tissue samples were pooled into 3.5 ml of GITC solution (4 M GITC, 25 mM Na-citrate, 100 mM β-mercaptoethanol, 0.5% N-laurolysarcosine, 0.1% Antifoam A) (Sigma Immunochemicals) sheared through a 22-gauge needle and loaded onto a 1.3-ml CsCl cushion in an SW50.1 rotor tube. The RNA was pelleted for 24 h at 42,000 rpm. After extraction and DEPC precipitation, the RNA was resuspended in 10-25 µl of DEPC-treated H2O.

Northern Blot Analysis

Total RNA (25 µg of adult tissue RNA, 10 µg of embryonic kidney and transfilter tissue RNA) was separated on a 1% agarose gel and transferred in 10× SSC to Gene Screen Plus membranes (Du Pont Co., Wilmingon, DE). Probes were synthesized by PCR using primers derived from the 3’ coding region of the rab17 cDNA (nucleotides 464-642; Fig. 2) in a standard PCR protocol as described above, in the presence of 200 µM dATP, dTTP, dGTP, 50 µM dCTP, and 7.5 µl 32P-dCTP (3,000 Ci/mmol, 10 mCi/ml). After PCR, unincorporated nucleotides were removed by a pulse column (NucTrapr; Stratagene). Filters were prehybridized in a solution containing 5x SSC, 50% formamide, 5× Denhardt’s solution, and 0.5% SDS. After hybridization, filters were washed in 0.1x SSC, 0.5% SDS at 60°C. X-ray films were exposed at -70°C with intensifying screens. Bands were quantified using the LKB Ultrascan XL laser densitometer.

In Situ Hybridization

In situ hybridization experiments were essentially performed as described (Wilkinson and Green, 1990). Whole embryos or isolated organs were fixed 1. Abbreviation used in this paper: GITC, Guanidinium Isothioyanate.
Results

Tissue-specific Expression of Rab17

We have previously reported 11 novel rab protein sequences spanning the first and second conserved regions involved in GTP binding (Chavrier et al., 1992). 10 corresponding full-length cDNA clones were obtained using the RACE protocol (Frohman et al., 1988) and cDNA library screening (Olkko

Figure 1. Rab17 mRNA expression in adult mouse tissues and NIH 3T3 fibroblasts. 25 µg of total RNA were probed with 32P-labeled PCR fragments derived from the 3' coding regions of the rabbit (a) or rabbit (b) cDNAs. Filters were washed at high stringency (see Materials and Methods) and exposed for 3 d at -70°C with intensifying screens. Longer exposure times (10 d) gave an identical pattern (not shown).

be present at similar levels in all organs examined (not shown). In contrast, a 1.6-kb mRNA, corresponding to rab17, was only detected in kidney, liver, and intestine (Fig. 1a). These organs are characterized by a high proportion of epithelial cells. Most importantly, the rab17 transcript was neither detected in nonepithelial organs such as spleen and skeletal muscle nor in NIH3T3 fibroblasts. The primary structure of rab17 deduced from the nucleotide sequence (Fig. 2) reveals that this protein is a typical member of the rab sub-family. The 214-amino acid-long protein contains the four highly conserved regions forming the GTP-binding site and a COOH-terminal isoprenylation motif of the CCX motif (Peter et al., 1992). As expected, the protein was shown to bind radiolabeled GTP by in vitro ligand blot experiments (not shown).

As depicted in Fig. 3, a comparison of all known rab protein sequences shows that rab17 is most similar to the rab5-rab6 group (Valencia et al., 1991). However, the evolutionary distances between rab5, rab6, and rab17 are at least as large as those between other groups of rab protein sequences. The notion of rab17 belonging to a distinct branch of the rab subfamily is corroborated by the finding that some of the structural differences are located in functional regions. For instance, in the effector loop, rab7 has a cysteine in position 53 whereas in rab5 and rab6, an alanine and isoleucine are found, respectively. Similarly, in the switch II region (Milburn et al., 1990), rab7 shows a unique histidine at position 85 while both, rab5 and rab6, have a proline.
Collectively, these data suggest that rab17 is a novel rab protein and might represent the first epithelial cell-specific GTPase protein to be identified.

**Rab17 Is Specific for the Polarized State of Epithelial Cells**

The finding that rab17 transcripts are detected in organs enriched in epithelial cells but not in fibroblasts prompted us to investigate whether the expression of rab17 is correlated with the degree of epithelial cell polarization. To this end, we studied the expression of rab17 during the embryonic differentiation of kidney tubule epithelial cells. These cells derive from the nonpolarized cells of the metanephric mesenchyme. The differentiation process is triggered by the interaction with the ureter bud which, in turn, is induced by the mesenchyme to branch (reviewed in Ekblom, 1984). Induction, which takes place starting from embryonic day 11 (E11) in the mouse, leads to condensation of the mesenchymal cells and to epithelial differentiation. Thus, nephric tubules begin to form. Tubule development comprises morphologically characterized intermediate stages (vesicles, comma- and S-shaped bodies) and is completed by an elongation process. The developmental events leading to the formation of fully differentiated kidney tubules have been reconstituted in vitro using the transfilter organ culture technique (Grobstein, 1956; Saxén et al., 1968). Uninduced metanephric mesenchymes at E11 can be induced to undergo nephric differentiation by cocultivation with the normal inducer, the ureter bud, or with the more potent embryonic spinal cord, separated by a filter support. After one day in culture, the induction process has been completed and the inducer tissue can be removed. At this point, the mesenchymal cells are committed to differentiate into epithelial cells and tubule morphogenesis takes place with similar kinetics as in vivo. The embryonic kidney at all stages of development contains undifferentiated mesenchyme, mesenchyme-derived tubules, ureter bud-derived structures, and invading endothelium. In contrast, the transfilter cultures consist exclusively of undifferentiated mesenchymal and differentiated stromal and epithelial cells. Thus, the in vitro system provides the possibility to monitor gene expression during differentiation of a particular cell type.

Expression of rab17 during kidney development in vivo (Fig. 4, a and b) and in vitro (Fig. 4, c and d) was investigated by Northern blot analysis. We also studied the expression of rab5c since this transcript appears to be ubiquitously expressed (Fig. 1 b). While the rab5c mRNA is present at a constant level throughout in vivo and in vitro development, rab17 is clearly absent from the nonpolarized mesenchyme and is detected only 3 d after induction (Fig. 4, a and c), that is, shortly before the onset of apical marker expression (Ek-
Rabl7 Expression is Limited to Epithelial Cells

Tubule morphogenesis occurs asynchronously throughout the organ. To determine at which morphological stage of tubule formation rabl7 is induced, we performed in situ hybridization on embryonic kidneys (Fig. 5). No specific signal was detected in E13 kidneys (Fig. 4, A and B) where tubule formation had only reached the stage of early S-shaped bodies (Se). The undifferentiated mesenchyme (M), the mesenchymal condensates (C) and tubules at the vesicle stage (V) were clearly not labeled. In addition, both the branching ureter bud (Ub) in the central part and the tips of the ureter tree (Ut) in the periphery were negative for rabl7 expression. In E16 (Fig. 5, C and D) and E18 (Fig. 5, E-H) kidneys, rabl7 transcript was detected in fully developed proximal tubules (P) and late S-shaped (SI) bodies whereas earlier structures (comma-shaped and early S-shaped bodies) were negative. In addition, no expression of rabl7 was detected in glomeruli (G). Finally, at this stage, branches of the ureter tree (Ub in Fig. 5 D) which develop into collecting ducts were clearly labeled by the rabl7 probe whereas the peripheral tips (Ut) were negative. Due to branching of the ureter tree and to proliferation of the mesenchyme in the periphery, developing tubules are gradually displaced towards the center (Osathanondh and Potter, 1963). In accordance with the finding that expression of rabl7 is restricted to later stages of tubule development, the signal was present both in the cortical and medullary region of the organ. However, the most peripheral region of the nephrogenic zone, which contains undifferentiated mesenchyme (M) and early stages of epithelial differentiation (C and Se) was devoid of rabl7. The distribution of the rabl7 transcript was similar to the expression pattern of epithelial cell markers like cytokeratin (data not shown). Taken together, these results indicate that rabl7 is induced during epithelial cell differentiation and does not appear until a late stage of the polarization process.

Since rabl7 mRNA was also found in intestine (Fig. 1 a) we investigated its expression by in situ hybridization in the developing small intestine at stage E18 (Fig. 6). Rabl7 expression was confined to the columnar epithelium covering the plicae and villi projecting into the intestinal lumen. In contrast, the sub-epithelial mesenchymal part of these projections as well as the layers of lamina propria mucosae, muscularis mucosae, submucosa (Sm), tunica muscularis externa (M) and the tunica serosa (Se) were negative for rabl7 expression. These data indicate that, as in the developing kidney, rabl7 mRNA is restricted to the polarized epithelial cells in the developing intestine.

Rabl7 Is Expressed at Different Levels in Adult Kidney Tubules

As a first step to elucidate the nature of the transport pathway regulated by rabl7, we studied the subcellular localization of the protein in the adult mouse kidney. A polyclonal antiserum was raised in rabbits against a COOH-terminal peptide (amino acids 182-199, Fig. 2). Since this region of the protein doesn't show sequence homology to any other rab protein identified so far, a cross-reactivity with other rab proteins is very unlikely. The specificity of affinity-purified antibodies was established as described in the Materials and Methods section according to Zerial et al. (1992). This antibody was used for immunofluorescence confocal microscopy on 0.5-μm cryosections of adult mouse kidney cortices. In accordance with the mRNA expression in the developing kidney, rabl7 was detected in kidney tubules (Fig. 7 A), but not in blood vessel endothelia (E) and glomeruli (not shown). The capsule of Bowman, which is also a mesenchyme-derived structure, was also not labeled (not shown). Strongly (T) and weakly (t) positive tubules could be discriminated, reflecting differences in the expression between proximal and distal tubules (see below). Higher magnification (Fig. 7 B) of a strongly positive tubule revealed a vesicular staining throughout the cell (arrows) and an intense staining of the basolateral plasma membrane. The accumulation of signal in the basal region of the cell is due to the numerous invaginations of the basal plasma membrane characteristic of kidney epithelial...
Figure 5. Expression of rabl7 mRNA in the developing kidney. Sections were hybridized with rabl7 antisense cRNA and photographed under brightfield illumination (A, C, E, and G) and by double exposure, under darkfield and brightfield illumination (B, D, F, and H). (A and B) A 13-d embryonic kidney with no detectable labeling. Ub, central branch of the ureter tree; Ut, tip of the ureter tree; M, undifferentiated mesenchyme; C, condensate of mesenchymal cells; V, vesicle stage of tubulogenesis; and S_e, early S-shaped body. (C and D) A 16-d embryonic kidney. The central parts of the branching ureter tree (Ub) are labeled while the peripheral tips (Ut) are not. The undifferentiated and stromal mesenchyme and early stages of tubulogenesis (C, condensates) are unlabeled. Late S-shaped bodies (S_l) show some labeling. (E-H) An 18-d kidney. (G and H) Higher magnification of the insets in E. The stromal mesenchyme in the central part of the kidney as well as the undifferentiated mesenchyme (M) and early stages of tubulogenesis (V, S_e) in the periphery of the nephrogenic zone remain unlabeled. The central branches (Ub), forming the collecting tubule system, but not the tips (Ut) of the ureter tree are labeled. Tubules at advanced stage of development (P, proximal tubule) show clear labeling whereas glomeruli (G) are negative. Bars: (A-F) 100 μm; (G-H) 50 μm.
lar distribution of the rabl7 protein as observed at the ultrastructural level is in agreement with the immunofluorescence pattern (Fig. 8, A and B correspond to apical and basolateral regions indicated by frames in Fig. 7 C). The basolateral plasma membrane (Fig. 8, B, arrowheads) was strongly labeled. In addition, we observed labeling of tubular structures below the apical brush border (Fig. 8 A, arrowheads) but little labeling of the brush border (bb) itself. The labeled structures may correspond to the so-called apical dense tubules, endocytic structures which have been implicated in membrane recycling to the apical surface and in transcytosis (Christensen, 1982; Nielsen et al., 1985).

In conclusion, our morphological data provide evidence that, in the kidney, rabl7 is mainly expressed in proximal tubules. The protein is located on the plasma membrane of the basolateral domain and vesicular structures in the apical domain.

**Discussion**

Over the last few years, evidence has accumulated for a function of small GTPases in the regulation of vesicular traffic (reviewed in Pfeffer, 1992). These findings have raised the question of whether specific small GTPases serve as regulators of specialized membrane transport pathways in polarized epithelial cells. This paper reports the identification of rab17, a novel small GTPase which is exclusively expressed in polarized epithelial cells. In situ hybridization studies revealed that rab17 mRNA is present in the polarized epithelial cells of mouse embryonic kidney and intestine. In the developing kidney, the rab17 transcript is not detected in the nonpolarized nephrogenic mesenchymal cells. In contrast, expression of rab17 is induced upon conversion of the mesenchymal cells into polarized epithelial cells. These findings strongly suggest that regulation of membrane traffic in epithelial cells does require the presence of specific GTPases in addition to those ubiquitously expressed.

**Rabl7 Is Specifically Expressed in Simple Polarized Epithelia**

By Northern blot hybridization, rab17 mRNA is found in kidney, liver and intestine, whereas it is undetectable in fibroblasts and muscle cells. Within the kidney, proximal tubules contained a higher level of rab17 protein compared with distal tubules, indicating that rab17 expression varies between different epithelial cell types. In contrast, rab17 was neither detected in the glomeruli nor in the cells of the Bowman capsule. Similarly, rab17 was detected in the epithelial ureter bud only upon differentiation of this tissue. No rab17 transcript was detected in lung or in brain, the latter indicating that neurons, which exhibit several properties of polarized epithelial cells (Dotti and Simons, 1990; Dotti et al., 1991), might not express the protein. Thus, rab17 is detected in some but not all epithelial cells. For which type of epithelial cell is rab17 specific? Several categories of epithelia are classically defined according to morphological criteria such as number of cell layers and cell shape. The limited number of biochemical and genetic markers has hindered a classification of epithelial cells based on molecular criteria. The pattern of expression we observed suggests that rab17 might be restricted to simple epithelia of the cuboidal and

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**Figure 6.** In situ hybridization analysis of the rabl7 mRNA expression in embryonic intestine. Sections of embryonic day 18 (E18) mouse small intestine were hybridized and analyzed as described in Fig. 5. (A) Darkfield illumination; (B and C) darkfield and brightfield illumination superimposed. The rabl7 signal is strongly present in the intestinal epithelium (arrowheads in C), but absent from the nonepithelial tissues of the Tunica Submucosa (Sm), Tunica Muscularis (M) and Tunica Serosa (Se). Bars: (A and B) 100 μm; (C) 50 μm.
Figure 7. Confocal immunofluorescence localization of rab17. The affinity-purified α-rab17 antiserum was used at a 1:10 dilution on 0.5-μm adult mouse kidney cryosections. The Rhodamine-labeled secondary antibody was diluted 1:300. Specificity of the signal was determined by peptide competition (not shown). (A) Low magnification (field size 240 μm) showing strongly (T) and weakly (t) labeled kidney tubules. Endothelia of blood vessels (E) are negative. (B) High magnification (field size 24 μm) revealing an intense labeling of the basolateral plasma membrane and a diffuse labeling of vesicular structures (arrows). Arrowheads indicate invaginations of the basolateral plasma membrane. (C) Schematic diagram of a kidney tubule epithelial cell illustrating the invaginations of the basolateral plasma membrane. Arrowheads indicate invaginations of the basolateral plasma membrane (arrowheads) typical for this cell type. a and b indicate apical and basolateral regions analyzed by immunoelectron microscopy (see Fig. 8). Bars: (A) 20 μm; (B) 2 μm.

columnar type (Fawcett, 1986) and further indicates that this GTPase belongs to a transport machinery which is particular for these two epithelial cell types. Cells of simple squamous epithelia such as endothelial cells are negative for rab17 expression. This is also the case for lung which contains squamous alveolar epithelial cells. According to this tentative classification, it is likely that simple polarized epithelial cells other than hepatocytes, kidney, and intestinal epithelial cells express rab17. The availability of DNA probes and antisera will allow us to test this hypothesis. If expression of rab17 and similar proteins could be used as epithelial markers, then classification of epithelia at a molecular level could be further refined.

Rab17 Is Induced during Epithelial Cell Polarization
In the developing kidney, the first stages of nephrogenic mesenchyme differentiation are evident morphologically as cell condensation and adhesion. These events depend on the synthesis of transcription factors (for review see Bard,
extracellular matrix proteins and receptors (Aufderheide et al., 1987; Klein et al., 1988; Vainio et al., 1989; Ekblo et al., 1990; Korhonen et al., 1990; Sorokin et al., 1990; Weller et al., 1991), as well as cell adhesion molecules (Vestweber et al., 1985; Schnabel et al., 1990) 24–48 h after induction. The spatial distribution and kinetics of expression of rab17 during in vitro and in vivo murine kidney development suggest that this GTPase is probably not involved in signaling epithelial cell differentiation. Rab17 was detected by Northern blot hybridization three days following mesenchyme induction both in vivo and in vitro. The kinetics of induction of rab17 closely resemble those of the appearance of apical markers. Apical brush border antigens appear only 3–5 d after induction (Ekblo et al., 1980, 1981; Lehtonen et al., 1983). However, while apical brush border antigens are not expressed in S-shaped bodies (Ekblo et al., 1980), our in situ hybridization studies indicated that rab17 is already detectable at that stage. Thus, rab17 is induced prior
Figure 9. Different models for the organization of rab proteins in (a) nonpolarized and b-d) polarized epithelial cells. Grey arrows indicate transport routes governed by rab proteins common to nonpolarized cells, black arrows to routes controlled by epithelial cell-specific rab proteins. Short arrows schematically represent the exocytic and endocytic pathways, long arrows represent the transcytotic routes. (b) Epithelial cells could use the the same rab proteins as nonpolarized cells. In this case, the specificity for apical, basolateral, or transcytotic pathways would have to be mediated by specific interacting components x, y, z. (c) In contrast to apical and basolateral exo- and endocytosis, transcytosis is a transport pathway unique to polarized epithelial cells. Thus, only transcytosis might require specific rab proteins. (d) The rab proteins found in nonpolarized cells might be exclusively used in one domain whereas a set of specific rab proteins would control transport in the opposite domain. Accordingly, the domain using specific rab proteins would be epithelial cell-specific (bold lining of the plasma membrane), whereas the other would be fibroblast-like (plain lining).

to the apical markers. This temporal difference in expression might suggest that rabl7 is involved in generating the apical-basolateral polarity of epithelial cells.

Rabl7 May Function in Transcytosis

As shown in Fig. 9, different models can account for the regulation of intracellular traffic by rab proteins in nonepithelial and epithelial cells. In principle, the same rab proteins present in nonpolarized cells (Fig. 9 a) could regulate membrane traffic in polarized epithelial cells (Fig. 9 b). The specificity would instead be mediated by a set of interacting components peculiar to epithelial cells. Our results argue against this possibility. The finding that rabl7 is restricted to polarized epithelial cells suggests that this protein functions in a transport process exclusively occurring in these cells. Which transport step would be expected to require a specific rab protein? The exocytic and endocytic routes could be regulated by the same rab proteins present in nonepithelial cells, both on the apical and basolateral pathways (Fig. 9 c). However, transcytosis, which can be regarded as a transport pathway unique to polarized cells, might require epithelial cell-specific rab proteins. Alternatively (Fig. 9 d), in addition to the transcytotic pathway, also transport to and from the apical or basolateral plasma membrane might be controlled by specific rab proteins. The latter model takes into account the possibility that either the apical or the basolateral route depend on epithelial cell-specific transport machineries and rab proteins (reviewed in Simons and Fuller, 1985; Simons and Wandinger-Ness, 1990; Mostov et al., 1992).

The development of the transcytotic route is a crucial step in organizing the polarized distribution of membrane proteins and lipids in epithelial cells. In a hierarchical order, transcytosis would follow immediately after changes in the substratum and reorganization of the cytoskeleton (for review see Schoenenberger and Matlin, 1991). Recent studies on a polarized thyroid cell line have indicated that the transcytotic route may function as a salvage pathway for missorted proteins during the establishment of the polarized phenotype (Zurzolo et al., 1992). Therefore, transcytotic proteins would be expected to appear once the factors involved in cell-substratum and cell-cell interaction have been produced. The kinetics of rabl7 expression during kidney development fulfill this criterion.

Rab proteins have been shown to regulate transport between the subcellular compartments where they have been localized (Gorvel et al., 1991; Plutner et al., 1991; Bucci et al., 1992; van der Sluijs et al., 1992; Lombardi et al., 1993). Thus, we investigated the localization of rabl7. Immunofluorescence and immunoelectron microscopic studies gave a first clue into the putative site of function of rabl7. Since no labeling was detected on organelles of the secretory pathway it seems unlikely that rabl7 could function in exocytosis. Interestingly, in the phylogenetic tree shown in Fig. 3, rabl7 is clustered together with most of the rab proteins localized to the endocytic pathway. Rabl7 was found on the basolateral plasma membrane and on apical structures probably corresponding to the dense apical tubules described previously (Maunsbach and Christensen, 1991; van Deurs and Christensen, 1984; Christensen, 1983). It may be significant that HRP internalized from the basolateral surface appeared in similar apical structures in studies using isolated perfused proximal tubules (Nielsen et al., 1985). Since these studies have led to the proposal that dense apical tubules are involved in transcytosis, one possibility is that rabl7 might be involved in the regulation of transcytotic trafficking. This hypothesis is supported by the presence of rabl7 in the epithelium of kidney, liver, and intestine which all display a high transcytotic activity (for review see Kraehenbuhl and Neutra, 1992). It will now be possible to experimentally address this question by examining whether the overexpression of wild type rabl7 or dominant interfering mutants (Bucci et al., 1992) affects transcellular transport in polarized epithelial cells.

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