# 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 cerevesiae (Segev et al., 1988; Salminen 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., 1990*b*; Morimoto et al., 1991; Elferink 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., 1990*a*; 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 $Ca^{2+}$-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-

lular environments (reviewed by Simons and Fuller, 1985; Rodriguez-Boulan and Nelson, 1989). To produce this spatial asymmetry, separate apical and basolateral exocytic and endocytic transport pathways exist (Bomsel et al., 1989; Parton et al., 1989; Wandinger-Ness et al., 1990). In addition, a direct pathway between these two surface domains serves the transepithelial transport of macromolecules and the sorting of mistargeted apical or basolateral plasma membrane components (Bartles et al., 1987; Le Bivic et al., 1989; Matter et al., 1990). This transcytotic pathway (for review see Mostov and Simister, 1985; Rodman et al., 1990) is mediated by specific vesicles which bud from the apical or basolateral early endosomes and then are targeted to the opposite plasma membrane. The differentiation into apical and basolateral transport pathways requires the presence of additional components of the trafficking machinery with respect to nonpolarized cells (for review see Simons and Fuller, 1985; Rodriguez-Boulan and Nelson, 1989; Hopkins, 1991; Mostov et al., 1992; Nelson, 1992). Which rab proteins are involved in controlling the epithelial cell-specific transport processes? We can envisage two scenarios. One possibility is that epithelial cells do not require any specific rab protein. Ubiquitous rab proteins (expressed also in nonpolarized cells, e.g., fibroblasts) could be segregated to the apical, basolateral and transcytotic pathways in epithelial cells, owing to the presence of epithelial cell-specific interacting components which would confer domain specificity (see Fig. 9 *b*). Alternatively, the epithelial cell-specific transport pathways would be controlled by specific rab proteins. The identification and characterization of rab proteins specific for epithelial cells would provide an important insight into the molecular and functional organization of the polarized transport machinery. However, despite an extensive search, no such epithelial cell-specific rab proteins have been identified so far (Chavrier, 1990*b*) and their existence has been questioned (Mostov et al., 1992). We have recently used a PCR approach to identify partial sequences of 11 hitherto undescribed rab proteins (Chavrier et al., 1992). Analysis of the mRNA expression of these novel GTPases revealed that only one of them, rab17, was specifically expressed in kidney, liver, and intestine. We provide evidence that rab17 is unique to the epithelial portions of these organs and that its expression is correlated with the polarization state of epithelial cells. In addition, localization studies in kidney proximal tubules suggest a possible involvement of rab17 in transcellular traffic.

## 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). $2 \times 10^5$ 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 $MgCl_2$, 0.01% gelatin, 200 μM of dATP, dGTP, dCTP, 130 μM of dTTP, 70 μM of dig-dUTP (Boehringer Mannheim Corp.), 20 pmol of each 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 5× SSC, 0.1% *N*-lauroylsarcosine, 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, NJ). 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 cDNA was mutagenized by PCR to introduce an EcoRI site upstream of and an NdeI cloning site at the ATG codon. The fragment was subcloned into pGEM1 (Promega Corp., Madison, WI) using the EcoRI site at the 5′ end (partial digest) and an XhoI 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 × NMRI) 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 ureter buds and placed on pieces of Nucleopore filter (nominal pore size 1.0 μm; Nucleopore Corporation, Pleasanton, CA). Fragments of spinal cord, used as inductor tissue, were cemented with agar to the lower surface of the filter (for details see Grobstein, 1956; Saxén and Saksela, 1971; Lehtonen et al., 1983). In some experiments, the mesenchymes were cultured on the filter in the absence of inductor tissue. As culture medium MEM supplemented with 10% FCS was used.

### RNA Preparation

RNA preparation from adult tissues was performed using the Guanidinium Isothiocyanate (GITC)[1] 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*-lauroylsarcosine, 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 EtOH precipitation, the RNA was resuspended in 10–25 μl of DEPC-treated $H_2O$.

### 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 (Dupont Co., Wilmington, 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 $^{32}$Pα-dCTP (3,000 Ci/mmol, 10 mCi/ml). After PCR, unincorporated nucleotides were removed by a push column (NucTrap™; Stratagene). Filters were prehybridized in a solution containing 5× SSC, 50% formamide, 5× Denhardt's solution, and 0.5% SDS for 1 h at 42°C and subsequently hybridized for 18 h at 42°C in the same solution containing approximately $4 \times 10^6$ cpm/ml of the probe. Filters were washed in 0.1× SSC, 0.5% SDS at 60°C. X-ray films were exposed at −70°C with intensifying screens. Bands were quantified using the LKB Ultroscan 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 Isothiocyanate.

in 4% paraformaldehyde in PBS at 4°C for 2–24 h. After paraffin embedding, sections were cut at 5 μm and mounted on 3-aminopropyl ethoxysilane–treated glass slides. Single-stranded sense and antisense cRNA probes were prepared using $^{32}$S-labeled UTP (≥1200 Ci/mmol; Amersham Corp.) and an SP6 or T7 transcription system (Promega Corp.). The cRNA probes were transcribed from a pGEM-1 plasmid containing the last 180 nucleotides from the rab17 coding region under the control of the SP6 promoter. The probes were separated from unincorporated nucleotides on Sephadex G-50 columns (Pharmacia Fine Chemicals) and used at a final concentration of 20,000 cpm/μl. The hybridization was carried out in 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, 5 mM EDTA, pH 8.0, 10% dextran sulphate (500 kD), 1× Denhardt's solution, 0.5 mg/ml yeast tRNA at 50°C for 15–20 h. After hybridization, the sections were washed for 30 min at high stringency (50% formamide, 2× SSC, 30 mM DTT, 65°C). For autoradiography, the slides were dipped in Kodak NTB-2 nuclear track emulsion (Eastman Kodak Co., Rochester, NY) and exposed at 4°C for 12–14 d. The slides were counterstained with hematoxylin, mounted in Permount (Fisher Scientific, Fairlawn, NJ), and examined under dark- and bright-field illumination with a Zeiss Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY). Sense and antisense probes were used in parallel in all experiments. In no case, a significant signal was obtained with the sense probe.

### *Immunofluorescence and Immunoelectron Microscopy*

A polyclonal antiserum was raised against a synthetic peptide deduced from the 3′ end of the rab17 coding region (amino acids 182–199, see Fig. 2). The antiserum was affinity-purified by binding at 4°C to the peptide used for immunization coupled to an affigel 10 matrix (Bio Rad Labs, Hercules, CA) and subsequent elution in 0.1 M glycine, pH 2.8. The eluate was neutralized with 1 M Tris, pH 8.0. After affinity purification, the antibody was stored frozen in aliquots at −80°C. The specificity of the affinity-purified antibody was determined by the following criteria. In immunofluorescence, the signal was (*a*) abolished by peptide competition (0.1 mg/ml) and (*b*) increased upon overexpression of the protein. (*c*) The antibody immunoprecipitated in vitro translated rab17. Immunofluorescence was performed on cryosections of adult mouse kidneys. The kidneys were fixed by immersion in 8% paraformaldehyde in 250 mM Hepes, pH 7.35, and sectioned at −60°C. 0.5-μm sections were transferred to gelatin-coated glass slides and fixed onto the slides using 3% paraformaldehyde in PBS for 10 min. After washing with PBS, excess paraformaldehyde was quenched with 50 mM $NH_4Cl$ in PBS for 10 min. The samples were then washed three times with PBS and unspecific binding sites were blocked by incubation in 10% FCS in PBS for 20 min at room temperature. After a 20-min incubation at room temperature with the first antibody, the samples were again washed three times with PBS and incubated with a rhodamine-conjugated donkey-α-rabbit IgG (Dianova, Hamburg, Germany) for 20 min at room temperature. All antibodies were diluted in 5% FCS in PBS. After incubation with the second antibody and three washes with PBS, coverslips were mounted over the samples with moviol. They were then viewed with the EMBL confocal microscope and photographed on a Fujichrome 100D film with a Polaroid Freeze frame directly from the monitor.

Ultrathin cryosections of adult mouse kidneys were prepared as described above but sectioning was performed at −90°C. Labeling was carried out as described previously (Griffiths et al., 1984, 1985).

## *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 (Olkkonen, V., A. Lütcke, P. Dupree, I. Killisch, M. Zerial, and K. Simons, manuscript in preparation. Lütcke, A., V. Olkkonen, I. Killisch, A. Valencia, P. Dupree, K. Simons, and M. Zerial, manuscript in preparation). Using specific cDNA probes, we monitored their expression by Northern blot hybridization on total RNA from adult mouse tissues and NIH3T3 fibroblasts. Most of the corresponding mRNAs, such as the 1.8-kb rab5c transcript (Fig. 1 *b*), were found to

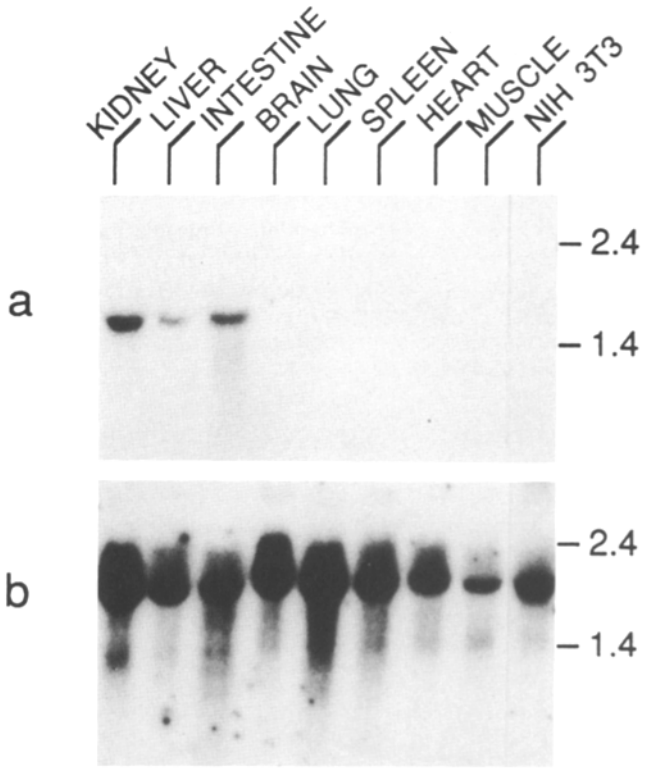


*Figure 1.* Rab17 mRNA expression in adult mouse tissues and NIH 3T3 fibroblasts. 25 μg of total RNA were probed with α$^{32}$P-labeled PCR fragments derived from the 3′ coding regions of the rab17 (*a*) or rab5c (*b*) cDNAs. Filters were washed at high stringency (see Materials and Methods) and exposed 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. 1 *a*). 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 CCXX type (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, rab17 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), rab17 shows a unique histidine at position 85 while both, rab5 and rab6, have a proline.


*Figure 2.* (*Upper panel*) Restriction map of pGEM rab17. The hatched segment corresponds to the coding region of the rab17 cDNA. (*Lower panel*) Nucleotide and deduced amino acid sequences of mouse rab17. Light grey regions correspond to the conserved GTP-binding motifs, the dark grey region represents the COOH-terminal cysteine motif. The COOH-terminal peptide used for rabbit immunization is boxed. The 3′ region used for radiolabeled PCR probes is indicated in italics. The sequence is available from the EMBL/GenBank/DDB under accession number X70804.

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,

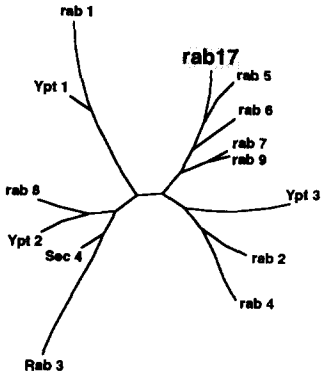


*Figure 3.* Phylogenetic tree representing the evolutionary distances between rab17 and the major groups of rab protein sequences. The tree was calculated as described by Higgins et al. (1992) over a multiple sequence alignment including all 34 rab primary structures present in the Swiss Prot data base. However, only representative sequences are included in the plot. The length of the branches is proportional to the actual distances between the sequences. Owing to the hypervariability of the $NH_2$ and COOH termini, the comparison was restricted to 166 amino acid residues of the central portion of rab proteins as described by Valencia et al. (1991). In this region, the sequence identity between rab17 and rab5 and between rab17 and rab6 is 42 and 40%, respectively.

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 inductor, 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-

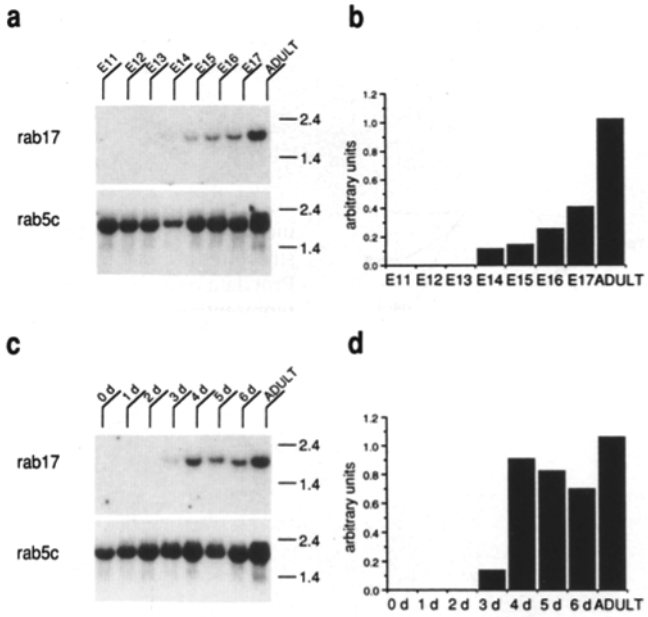


*Figure 4.* Northern blot analysis of rab17 mRNA expression during kidney development. 10 μg of total RNA from in vivo (*a*) or vitro (*c*) developing kidneys were sequentially probed for rab17 and rab5c. In vivo developing kidneys were from embryonic days E11–17, transfilter culture samples (see Materials and Methods) were taken at 0–6 d in culture. Adult kidney RNA was included as a positive control. The rab17 mRNA expression in relation to the rab5c expression in vivo (*b*) and in vitro (*d*) was quantified by scanning the autoradiographs.

blom et al., 1980). Quantification of rab17 expression in relation to the level of rab5c transcripts revealed a gradual increase during in vivo development (Fig. 4 *b*). In contrast, in the transfilter culture system, the level of rab17 mRNA increased dramatically after 3 d and decreased slowly after 4 d in culture (Fig. 4 *d*). The quantitative difference between in vivo and in vitro development suggests that rab17 is enriched in the mesenchyme-derived tubule epithelial cells compared to other cell types of the developing kidney. The slight decrease of rab17 transcript after 4 d in culture might reflect a limited lifetime of the in vitro system. The induction of rab17 during kidney development was also confirmed by 2-dimensional gel GTP-binding mapping using rab17 transiently expressed in BHK cells as a standard (data not shown).

### *Rab17 Expression Is Limited to Epithelial Cells*

Tubule morphogenesis occurs asynchronously throughout the organ. To determine at which morphological stage of tubule formation rab17 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 rab17 expression. In E16 (Fig. 5, *C* and *D*) and E18 (Fig. 5, *E–H*) kidneys, rab17 transcript was detected in fully developed proximal tubules (*P*) and late S-shaped (*Sl*) bodies whereas earlier structures (comma-shaped and early S-shaped bodies) were negative. In addition, no expression of rab17 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 rab17 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 rab17 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 rab17. The distribution of the rab17 transcript was similar to the expression pattern of epithelial cell markers like cytokeratin (data not shown). Taken together, these results indicate that rab17 is induced during epithelial cell differentiation and does not appear until a late stage of the polarization process.

Since rab17 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). Rab17 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 rab17 expression. These data indicate that, as in the developing kidney, rab17 mRNA is restricted to the polarized epithelial cells in the developing intestine.

### *Rab17 Is Expressed at Different Levels in Adult Kidney Tubules*

As a first step to elucidate the nature of the transport pathway regulated by rab17, 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, rab17 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

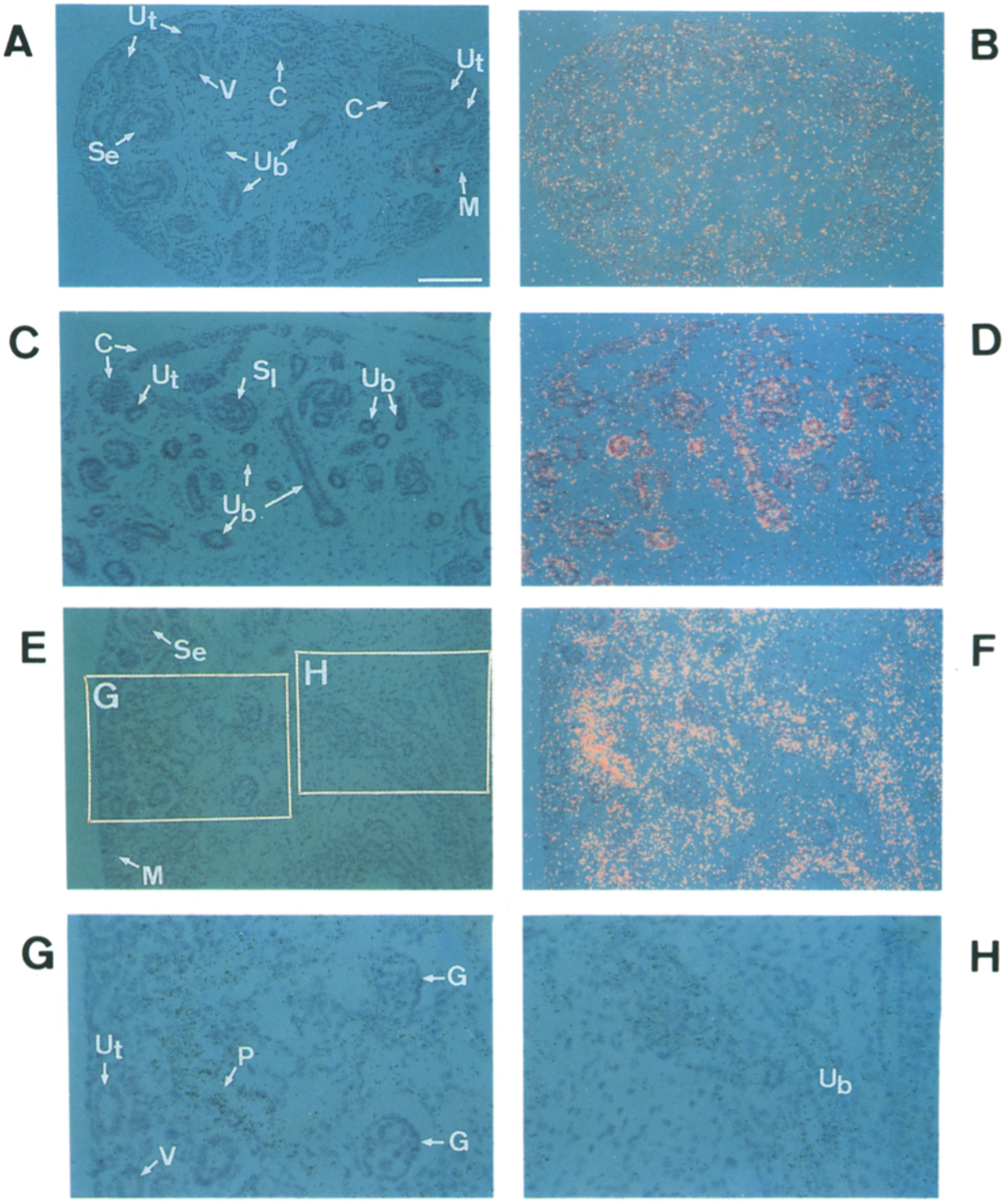


*Figure 5.* Expression of rab17 mRNA in the developing kidney. Sections were hybridized with rab17 antisense cRNA and photographed under brightfield illumination (*A*, *C*, *E*, and *G*) and by double exposure, under darkfield and brightfield illumination (*B*, *D*, *F*, and *H*). (*A* and *B*) A 13-d embryonic kidney with no detectable labeling. $U_b$, central branch of the ureter tree; $U_t$, 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 ($U_b$) are labeled while the peripheral tips ($U_t$) 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 ($U_b$), forming the collecting tubule system, but not the tips ($U_t$) 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.

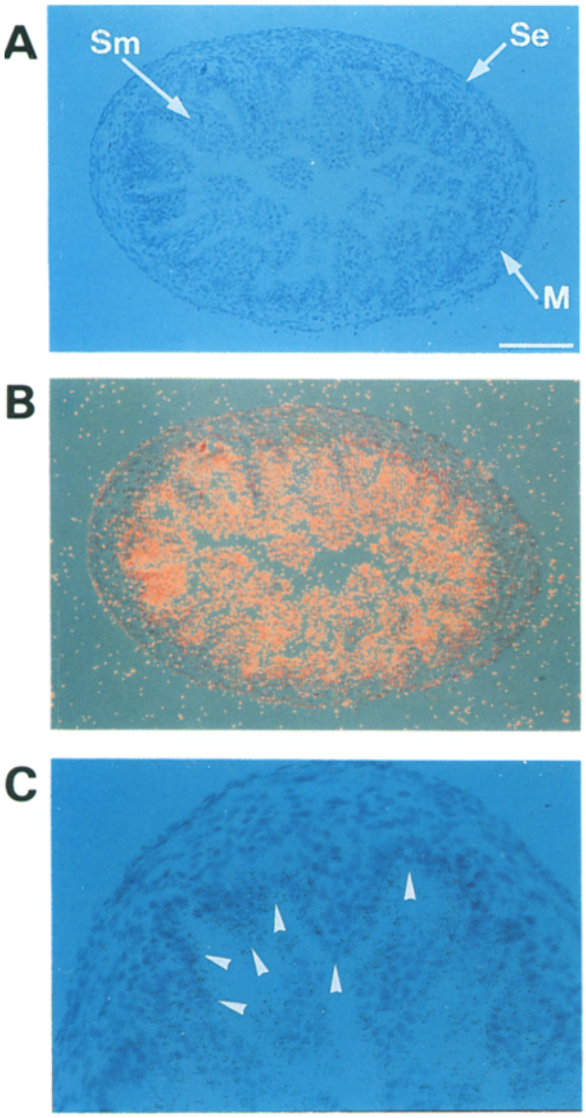


*Figure 6.* In situ hybridization analysis of the rab17 mRNA expression in embryonic intestine. Sections of embryonic day 18 (E18) mouse small intestine were hybridized and analyzed as described in Fig. 5. (*A*) Darkfield illumination; (*B* and *C*) darkfield and brightfield illumination superimposed. The rab17 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.

cells (Fig. 7, *B* and *C*, *arrowheads*). These results indicate that rab17 protein is associated with the basolateral plasma membrane and with cytoplasmic vesicular structures of kidney epithelial cells.

### *Rab17 Is Predominantly Present in Proximal Tubules and Associated with the Basolateral Plasma Membrane and Apical-Dense Tubules*

To extend the localization studies on rab17 we next performed immunoelectron microscopy analysis on cryosections. Rab17 protein was found to be expressed at a relatively high level in proximal tubules (Fig. 8), whereas distal tubules contained only little rab17 (not shown). The subcellular distribution of the rab17 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, rab17 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.

### *Rab17 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 cells 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

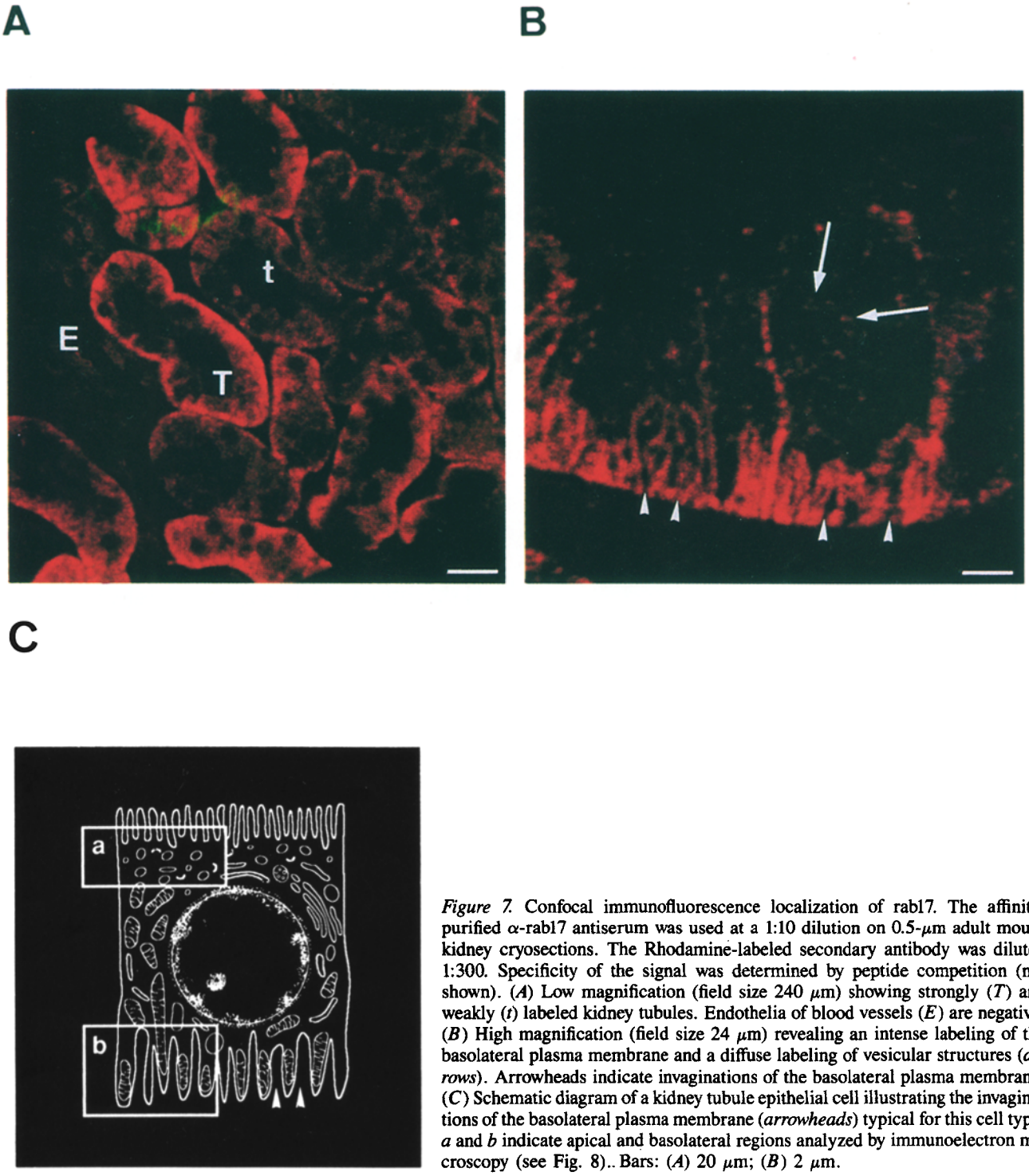


*Figure 7.* Confocal immunofluorescence localization of rab17. The affinity-purified α-rab17 antiserum was used at a 1:10 dilution on 0.5-μm adult mouse kidney cryosections. The Rhodamine-labeled secondary antibody was diluted 1:300. Specificity of the signal was determined by peptide competition (not 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*) 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,

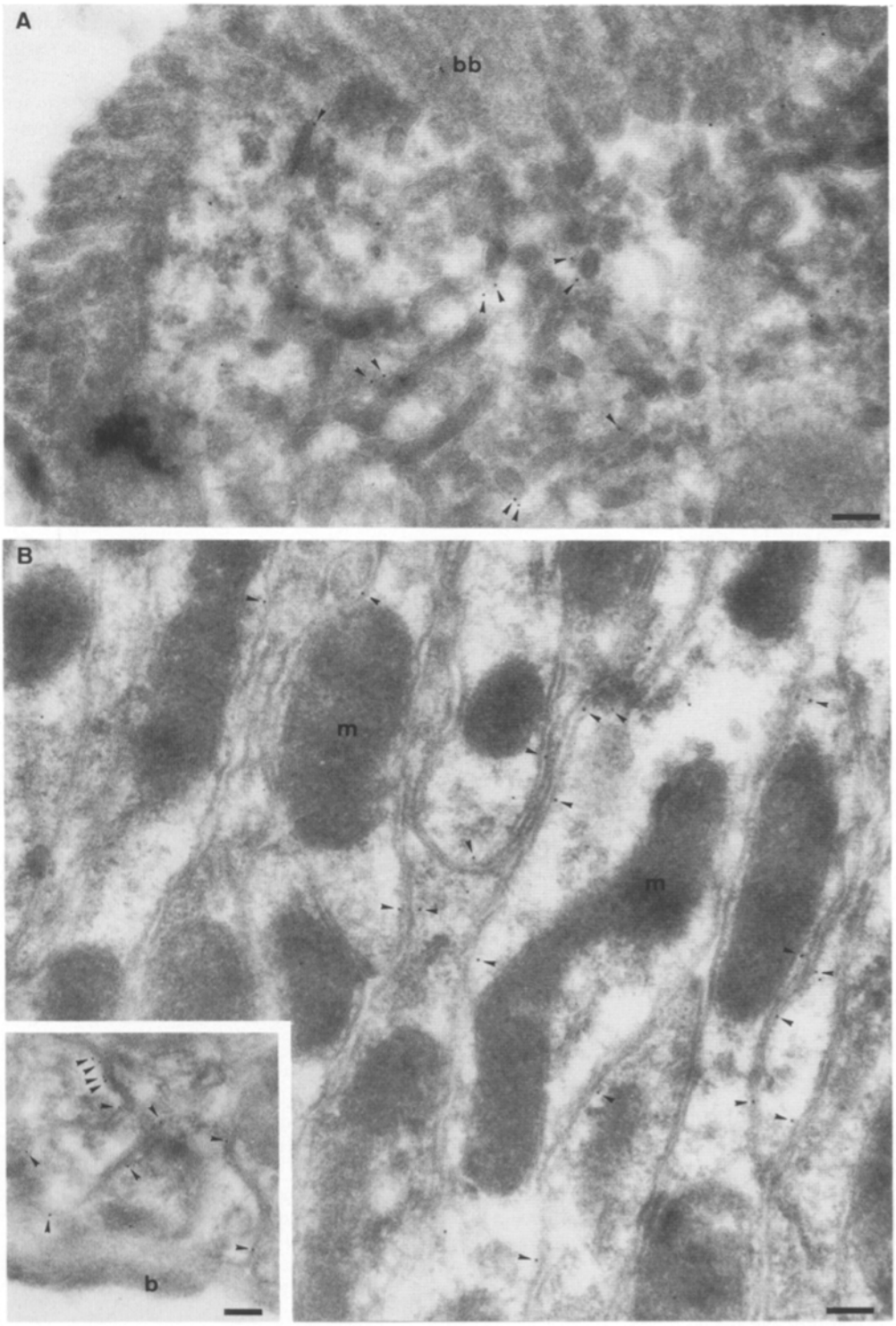


*Figure 8.* Immunoelectron microscopic localization of rab17 in mouse kidney. Thin frozen sections of mouse kidney were incubated with anti-peptide antibodies to rab17 followed by 9 nm protein A–gold. *A* shows the apical region of a proximal tubule cell (Fig. 7 *C*, *a*) whereas *B* (Fig. 7 *C*, *b*) and the inset show basolateral regions of the same cell type. (*A*) In the apical region of the cell labeling is evident on electron-dense tubules (*arrowheads*) underlying the brush border (*bb*). (*B*) In the basal portion of the cells labeling is evident on the basolateral membrane. Gold particles close to the lateral plasma membranes are indicated by arrowheads. Negligible labeling is present on the adjacent mitochondria (*m*). Higher labeling is evident close to the basal surface of the cell (*b*, *inset*). Bars, 200 nm.

1992), extracellular matrix proteins and receptors (Aufderheide et al., 1987; Klein et al., 1988; Vainio et al., 1989; Ekblom 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 (Ekblom et al., 1980, 1981; Lehtonen et al., 1983). However, while apical brush border antigens are not expressed in S-shaped bodies (Ekblom et al., 1980), our in situ hybridization studies indicated that rab17 is already detectable at that stage. Thus, rab17 is induced prior

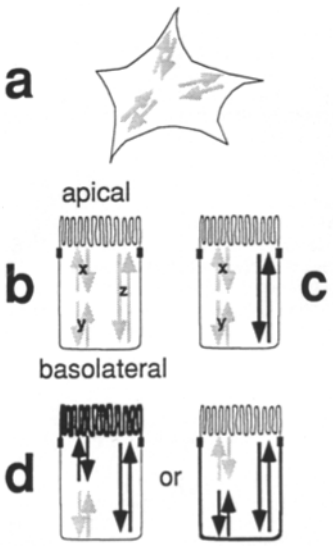


*Figure 9.* Different models for the organization of rab proteins in (*a*) nonpolarized and *b–d*) polarized epithelial cells. Grey arrows indicate transport routes governed by rab proteins common to nonpolarized cells, black arrows to routes controlled by epithelial cell-specific rab proteins. Short 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 rab17 is involved in generating the apical-basolateral polarity of epithelial cells.

### *Rab17 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 rab17 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 rab17 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 rab17. Immunofluorescence and immunoelectron microscopic studies gave a first clue into the putative site of function of rab17. Since no labeling was detected on organelles of the secretory pathway it seems unlikely that rab17 could function in exocytosis. Interestingly, in the phylogenetic tree shown in Fig. 3, rab17 is clustered together with most of the rab proteins localized to the endocytic pathway. Rab17 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 rab17 might be involved in the regulation of transcytotic trafficking. This hypothesis is supported by the presence of rab17 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 rab17 or dominant interfering mutants (Bucci et al., 1992) affects transcellular transport in polarized epithelial cells.

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