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Expression of the Conjugate Export Pump Encoded by the *mrp2* Gene in the Apical Membrane of Kidney Proximal Tubules

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Abstract. A novel ATP-dependent export pump for amphiphilic anionic conjugates, which has been cloned recently from liver, was identified in rat kidney and localized to the apical membrane domain of proximal tubule epithelia. This 190-kD membrane glycoprotein (Mrp2) has been described previously as the hepatocyte canalicular isoform of the multidrug resistance protein and as the canalicular multispecific organic anion transporter. Mrp2 was identified in kidney by reverse transcription PCR followed by sequencing of the amplified 786-bp fragment and by immunoblotting, using an antibody specifically reacting with the carboxy terminus of rat Mrp2. Double immunofluorescence and confocal laser-scanning microscopy showed the presence of Mrp2 in the brush-border membrane domain of segments S_1, S_2, and S_3 of proximal tubule epithelia. Mrp2 was not detectable in other segments of the nephron. The onset of Mrp2 expression during development occurred in a very early stage of nephron development. Mrp2 represents the first cloned ATP-dependent export pump for amphiphilic organic anions identified in kidney and localized to the apical membrane domain of proximal tubule epithelia. Mrp2 may contribute to cellular detoxification and to the secretion of endogenous and xenobiotic anionic substances, most of which are conjugates, from the blood into urine. (J Am Soc Nephrol 8: 1213–1221, 1997)

Renal proximal tubule epithelia actively transport charged, potentially toxic endogenous and xenobiotic compounds from the extracellular space into the tubular lumen (1,2). Basolateral uptake into proximal tubule epithelia, intracellular transport, and active export into the lumen have long been known as essential steps for the effective elimination from the blood into urine of both conjugated and unconjugated lipophilic substrates (1–4). But until now, the molecular properties of proteins mediating the transport of these amphiphilic organic anions across the apical membrane of kidney proximal tubules into the tubular lumen were unknown. Recently, two integral membrane glycoproteins belonging to the ATP-binding cassette superfamily of transporters have been identified as primary-active export pumps for certain amphiphilic organic anions (5–10). Both the multidrug resistance protein MRP1 (11) and the distinct hepatocyte canalicular isoform MRP2 (8,9) mediate the ATP-dependent unidirectional transport of anionic conjugates of lipophilic compounds with glutathione, glucuronate, or sulfate (5–8,10,12,13). MRP2 has been cloned from human (8,14) and rat (8,9) liver and has been alternatively termed canalicular MRP (cMRP) (8,10), or canalicular multispecific organic anion transporter (9,14). In this article, we use the term MRP2 for the human homolog and Mrp2 for the rat homolog of this new apical 190-kD membrane glycoprotein. MRP2 and MRP1 share only 49% amino acid identity. The substrate specificities of MRP1 (12,13) and Mrp2 (8,10) are very similar, and this transport activity may account for at least some of the organic anion transport observed in kidney proximal tubule cells (1–4). Human MRP1 has been detected in many cell types and tissues (13,15), whereas MRP2 has been described in liver (10,14). In the rat, *mrp2* mRNA was highly expressed in liver and at low levels in kidney, duodenum, and ileum (9). High-affinity polyclonal antibodies raised against rat Mrp2 and human MRP2 served to detect this conjugate export pump in the apical membrane domain of hepatocytes (8,10).

These studies raised the question of whether Mrp2 is present in other apical membrane domains, such as the brush-border membrane of rat kidney proximal tubule cells, and functions there in the transport of amphiphilic organic anions into the lumen of proximal tubules. Before the recent cloning of Mrp2 (8,9), the function of this transporter was defined on the basis of its hereditary deficiency in two hyperbilirubinemic mutant strains of rats: the Eisai hyperbilirubinemic rat (EHBR) (16,17) and the Groningen yellow/transport-deficient Wistar rat (18–20). Both mutant strains are characterized by a reduced amount of *mrp2* mRNA in liver, a lack of Mrp2 protein in the hepatocyte canalicular membrane, and a loss of ATP-dependent membrane transport of anionic conjugates (8–10). These mu-

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tant rat strains exhibit point mutations in the *mrp2* gene (9,21) and provide useful animal models for studies on the expression and function of Mrp2 not only in hepatocytes but also in other polarized epithelial cells. In this study, we describe the expression and localization of Mrp2 in rat kidney. We localize this recently cloned transport protein for amphiphilic organic anions to the luminal (apical) plasma membrane domain of renal proximal tubule segments S_1 to S_3.

Materials and Methods

Materials

Aprotinin, leupeptin, pepstatin, fetal calf serum, agar, as well as protein standard mixtures (M_r 26,600 to 180,000), were purchased from Sigma Chemical Co. (Deisenhofen, Germany). RNase inhibitor (RNasin), StrataScript™ Moloney murine leukemia virus reverse transcriptase, Taq DNA polymerase, and β-actin primers were from Stratagene (Heidelberg, Germany). Agarose and guanidine thiocyanate were obtained from Roth (Karlsruhe, Germany), lysozyme and ampicillin were from Boehringer Mannheim (Mannheim, Germany), and CsCl was from Biomol (Hamburg, Germany). Protein A-Sepharose was from Pharmacia (Uppsala, Sweden). All other chemicals used were of analytical grade and obtained from Merck (Darmstadt, Germany).

Antibodies

The EAG15 polyclonal antibody was raised in rabbits against the amino acid sequence EAGIENVNHTEL at the carboxy terminus of the rat Mrp2 protein as described previously (8,22). This polyclonal antiserum was affinity-purified on a protein A-Sepharose column. The polyclonal antibody B5 was obtained by immunization of rabbits against the carboxy-terminal peptide of the human MRP1 sequence (QRGLFYSMAKDAGLV) by using a method described earlier (8,22). The polyclonal antibody 6KQ had also been raised against the carboxy terminus of human MRP1 (23) and was kindly provided by Dr. Melvin Center (Kansas State University, Manhattan, KS). The monoclonal antibody De 13.4 directed against rat dipeptidyl-peptidase IV (DPPIV) (24) was kindly provided by Dr. Werner Reutter (Freie Universität Berlin, Berlin, Germany). Goat anti-rabbit secondary antibodies coupled to cyanin 3-conjugate (Cy_3) and goat anti-mouse secondary antibodies coupled to FITC were from Biotrend (Köln, Germany).

Animals and Tissues

Male Sprague-Dawley rats (200 to 300 g) and male Wistar rats (250 to 350 g) were purchased from Charles River Wiga (Sulzfeld, Germany). Newborn Sprague-Dawley rats were studied on day 1 after birth. EHBR mutants, defective in the hepatobiliary secretion of anionic conjugates (16,17), were obtained from Dr. T. Horie (Eisai Pharmaceutical Co., Ibaraki, Japan). Animals were maintained on a standard diet with free access to food and water.

RNA Isolation from Tissues

Total RNA was isolated from freeze-clamped rat kidneys by a guanidine thiocyanate lysis procedure with subsequent purification by centrifugation on cesium chloride (25). Part of the kidneys were perfused with phosphate-buffered saline (PBS), cut into longitudinal slices, and separated macroscopically into zones of cortex, outer stripe, and medulla (comprising inner stripe and inner medulla fractions). The resulting tissue sections were immediately frozen in liquid nitrogen and used for RNA isolation.

Reverse Transcription PCR and Subcloning

Total RNA was pretreated before reverse transcription (RT) with 10 U of DNase I in 50 μl of digestion buffer (100 mM sodium acetate, pH 5.0, 5 mM $MgSO_4$, and 40 U of the RNase inhibitor RNasin) at 37°C for 1 h to prevent DNA contamination. After phenol/chloroform treatment and ethanol precipitation, the RNA was reverse-transcribed with oligo$(dT)_{18}$ primer for *mrp1* and with the specific reverse primer Revc for rat *mrp2* (AGACTCTAAGATTCTGA, bases 4046-4031). Total kidney RNA (5 μg) from either healthy or mutant EHBR rats was reverse-transcribed in a total volume of 30 μl, containing transcription buffer (50 mM Tris, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM dNTP, and 40 U RNasin) and StrataScript™ Moloney murine leukemia virus reverse transcriptase at 37°C for 1 h. The resulting single-strand cDNA were purified by centrifugation through Microcon-100 columns (Amicon, Witten, Germany). First round of PCR was performed in a total volume of 20 μl of PCR buffer (10× reaction buffer as provided by the manufacturer) containing 1.5 mM $MgCl_2$, 1.25 U of Taq DNA polymerase, 0.25 μM sense and antisense primer, and 5 μl of reverse transcription mixture. The following primer pairs were used during PCR detection of *mrp1* and *mrp2* mRNA in rat kidney. For *mrp1* the sense primer was CTGCACCTAGACCTGCT (for 3337) and the antisense primer was TCCAGGCGCTTCAGCT (rev 3627). For *mrp2* the sense primer was TGAGTGCTTGGACCAG (bases 2987 to 3002) and the antisense primer was CTTCTGACGTCATCCTCAC (bases 3772 to 3754). The commercial β-actin control primer pair was obtained from Stratagene. PCR cycling conditions were chosen as follows: 94°C for 45 s; 60°C for 60 s; and 72°C for 90 s (35 and 30 cycles for Figure 1, A and B, respectively). PCR fragments were subcloned into the pCR2.1 vector (Invitrogen BV, NV Leek, The Netherlands). Transformed cells were grown in 50 ml of LB-Ampicillin medium for plasmid preparation. The cDNA clones were sequenced by the dideoxynucleotide chain termination method of Sanger using $[\alpha\text{-}^{35}S]$dATP and the sequencing kit from Pharmacia Biotech (Freiburg, Germany). Dried gels were exposed to Kodak BioMax MR-1 film obtained from Sigma.

Immunoblotting

Kidney samples (0.5 to 1.0 g) were homogenized during thawing in 10 ml of lysis buffer (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris/HCl, pH 7.4) and 0.5% (wt/vol) sodium dodecyl sulfate supplemented with the protease inhibitors phenylmethylsulfonyl fluoride (0.1 mM), leupeptin (1 μM), aprotinin (0.3 μM), and pepstatin (1 μM) at 4°C.

The resulting suspension was held on ice for 15 min and was then centrifuged at 100 × *g* for 10 min at 4°C. The supernatant was withdrawn and transferred to a Beckman 50.1 tube and centrifuged at 100.000 × *g* for 60 min at 4°C. The pellet that contained the microsomal membrane proteins was resuspended in 300 μl of lysis buffer and subsequently analyzed by immunoblotting. Hepatocyte canalicular membrane vesicles (8) were used as positive controls for Mrp2 expression (5 μg of protein). Membrane fractions (25 μg of kidney protein) were loaded onto a 7.5% (wt/vol) sodium dodecyl sulfate-polyacrylamide gel, without boiling, and subjected to electrophoresis (26). After electrotransfer onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), the blots were blocked with Tris-buffered saline containing 0.05% Tween 20 and 10% (wt/vol) low-fat dry milk (Glücksklee, Frankfurt, Germany) for 1 h at room temperature and probed overnight with the polyclonal Mrp2 antibody EAG15 (dilution 1:40,000).

Antibody binding was visualized with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Bio-Rad, Munich, Germany) diluted 1:1000, followed by enhanced chemiluminescence detection

(Amersham-Buchler, Braunschweig, Germany) with exposure on Hyperfilm™-MP (Amersham-Buchler).

Fluorescence and Confocal Laser Scanning Microscopy

Rat kidneys, perfused with PBS, were removed from anesthetized animals and immediately frozen in isopentane precooled in liquid nitrogen. For single- and double-label immunofluorescence microscopy, 5-μm-thick unfixed tissue sections were prepared with a cryotome (FrigoCut 2800E; Leica, Nussloch, Germany), fixed in 100% precooled methanol at $-20°C$ for 10 min, and subsequently air-dried for 2 h at room temperature.

For double-label immunofluorescence microscopy, the primary rabbit and mouse antibodies and the secondary goat anti-rabbit and goat anti-mouse antibodies coupled to Cy_3 or FITC were incubated simultaneously. Application of the primary and secondary antibodies was for 1 h at room temperature. Unbound antibodies were removed by several washes with PBS. After a final wash with distilled water, the air-dried sections were mounted with Elvanol (Cti, Idstein/Taunus, Germany).

Confocal laser scanning fluorescence microscopy was performed with an LSM 410 apparatus (Carl Zeiss, Jena, Germany). The microscope, equipped with appropriate filter combinations, was operated with an argon ion (488 nm) and a helium/neon laser (543 nm). Prints were taken from optical sections of 0.8-μm thickness. From double-labeled kidney sections, red (EAG15/Cy_3) and green (DPPIV/FITC) fluorescent pictures were superimposed to show a yellow mixture of fluorescences in regions of colocalization of the antigens.

Results

RT-PCR Detection of mrp2 and mrp1 mRNA in Rat Kidney

The expression of the *mrp2* and *mrp1* genes in healthy and transport-deficient (EHBR) kidneys was analyzed by PCR amplification of cDNA fragments generated from reverse-transcribed rat kidney RNA. Nested PCR amplification was performed with primers corresponding to the rat liver *mrp2* and rat *mrp1* sequences (8,27). The amplification products resulted in two different fragments with the expected size of 786 bp for *mrp2* and 291 bp for *mrp1*. Amplification products corresponding to *mrp2* and *mrp1* were obtained from kidneys of healthy rats, as well as from EHBR mutants (Figure 1A). The *mrp2* and *mrp1* cDNA fragments were identified by subcloning and sequencing of nucleotides 3236 to 3772 for *mrp2*, and of 196 nucleotides in the amplified *mrp1* fragment (see Materials and Methods). The nucleotide sequences of the *mrp2* and *mrp1* cDNA fragments from kidney were identical to the corresponding sequences of rat liver *mrp2* and *mrp1* (8,27,28). As indicated by the RT-PCR, *mrp2* and *mrp1* cDNA amplification products were detectable in zones from renal cortex and medulla (comprising inner stripe and inner medulla fractions) (Figure 1B).

Immunoblot Analysis of Mrp2 and Mrp1 in Healthy and Mutant Rat Kidney

Immunoblots were probed with polyclonal antibodies raised against the different carboxy-terminal peptide sequences of Mrp2 (EAG15) and Mrp1 (B5, 6KQ). Immunoblots on membrane fractions from normal and EHBR rat kidney with the EAG15 antibody indicated the expression of a 190-kD protein only in normal kidney. This 190-kD membrane protein was not detectable in membranes from EHBR kidney (Figure 2A). As a positive control, the 190-kD glycoprotein was clearly detected in hepatocyte canalicular membranes from healthy rats, but the immunodetection of Mrp2 was negative in canalicular membranes from the EHBR mutants (8). The same membrane fractions from kidney of healthy and mutant rats were probed with the polyclonal antibodies B5 and 6KQ directed against Mrp1 (and MRP1). The kidney membranes from healthy and EHBR mutant rats both showed a positive reaction with a 190-kD protein, suggesting Mrp1 expression (Figure 2B).

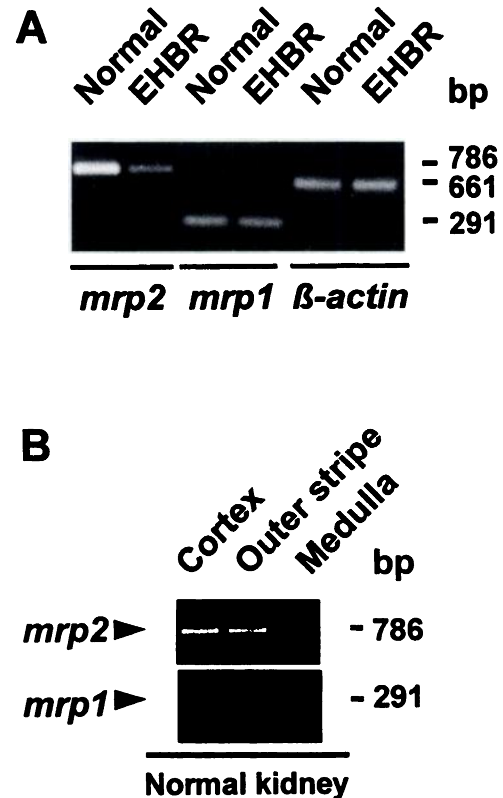

Figure 1. Analysis of *mrp2* and *mrp1* mRNA expression in kidney from normal and transport-deficient Eisai hyperbilirubinemic (EHBR) rats. Reverse transcription was performed on DNase-digested total RNA with a specific primer for *mrp2* and an oligo(dT)$_{18}$ primer for *mrp1*. For PCR analysis, two pairs of primers derived from the rat *mrp2* and *mrp1* sequences were used, yielding a 786-bp and a 291-bp fragment, respectively (8,27) (see Materials and Methods). A 661-bp fragment of β-actin, using oligo(dT)$_{18}$ primer for reverse transcription and specific primers fitting the rat sequence, was run as an internal control for the integrity of the isolated mRNA. (A) *mrp2* and *mrp1* mRNA expression in healthy rat kidney compared with the β-actin control. (B) *mrp2* and *mrp1* mRNA expression in different areas of the kidney parenchyma. "Medulla" represents the inner stripe and inner medulla fractions of rat kidney.

Immunofluorescence and Confocal Laser Scanning Microscopy of Mrp2 in Rat Kidney

The localization of Mrp2 in kidney was visualized by immunofluorescence microscopy on cryosections of the tissue.

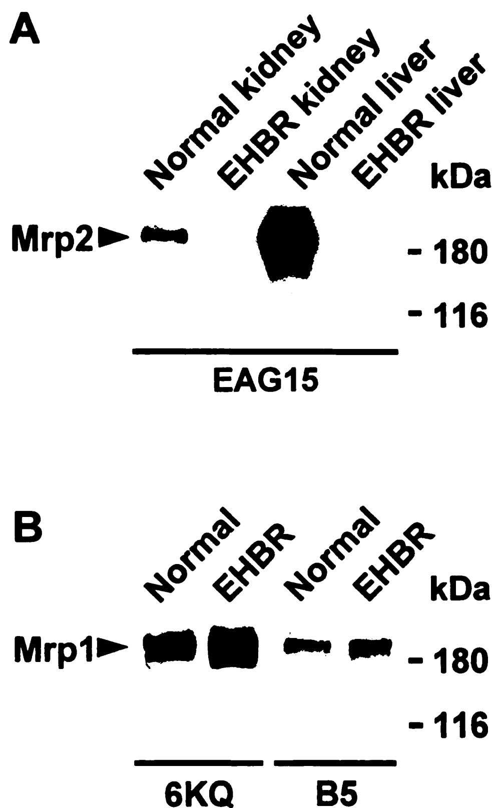

Figure 2. Immunoblot analysis of Mrp2 in the kidney membrane fractions and hepatocyte canalicular membranes from healthy and mutant (EHBR) rats. (A) Mrp2 was detected as a protein of 190 kD in healthy kidney membranes (25 μg of protein) and as a positive control in healthy hepatocyte canalicular membranes (5 μg of protein) by the EAG15 polyclonal antibody (8). Membrane preparations from EHBR kidney and EHBR liver (25 μg of protein each) did not show a detectable amount of Mrp2 protein. (B) Mrp1-positive reactions were detected in kidney microsomal membranes (25 μg of protein) from healthy and EHBR mutant rats, using the polyclonal antibodies 6KQ (23) and B5, both at a dilution of 1:10,000.

The EAG15 antibody indicated an intense fluorescence of the brush-border membrane domain of all proximal tubule segments (S_1, S_2, and S_3) (Figure 3, a through c; Figure 4, a and b). The reaction occurring in the tubular epithelial cells of the outer stripe containing the S_3 proximal tubule segments appeared more intense compared with the cortical tubule segments S_1 and S_2 (Figure 4, a and b). The very beginning of S_1 tubule brush-border staining was visible immediately from the urinary pole of Bowman's capsule.

An apical staining was missing in all lower nephron segments following the proximal tubule, *i.e.*, at the thin limbs of Henle's loop, thick ascending limbs of Henle's loop, distal tubules including the macula densa, and the collecting ducts (Figures 4b and 5). The specific apical fluorescence abruptly disappeared at the border from the outer to the inner stripe of the medulla (Figures 4b and 6d). In addition to the proximal tubules, the only additional staining with the antibody against Mrp2 was seen in the outer stripe, where the endothelia of capillary vessels surrounding proximal S_3 tubules were clearly positive. A differentiation, whether the apical, the basolateral, or both of the endothelial cell membranes reacted positively, was not possible (Figure 6d).

A staining pattern identical to the tubular staining of Mrp2 in kidney (Figure 6b) was observed with the monoclonal antibody De 13.4 raised against DPPIV (24). This ectoenzyme served as a marker for the apical membrane domain (24,27) (Figure 6, a and b). Double-labeling experiments using both the EAG15 and the DPPIV antibody and concomitant superimposition of the two different primary colors resulted in a yellow mixture of colors, indicating colocalization of both antigens in the brush-border membrane of proximal tubules (Figure 6, c and d). Control experiments using the EAG15 preimmune serum and preabsorption of the EAG15 polyclonal antiserum with the 12-amino acid immunization peptide coupled to keyhole limpet hemocyanin did not produce fluorescent membrane structures in kidney (data not shown). Neonatal rat kidney, obtained on day 1 after birth, was used for double-labeling experiments with the EAG15 and De 13.4 (anti-DPPIV) antibodies to study the onset of Mrp2 expression in the cortex during maturation. Mrp2 was detected on the apical membrane of proximal tubule segments at an early stage of development, *i.e.*, as soon as a tubular structure was discernible as a proximal tubule, it was positive for Mrp2 (Figure 3, d and e).

Discussion

In this study, we have identified an ATP-dependent export pump for anionic amphiphilic conjugates in the apical membrane of kidney proximal tubule epithelia (Figures 1 through 5). Mrp2, previously described as a hepatocyte canalicular multidrug resistance protein (cMrp) (8) or as a hepatocyte canalicular multispecific organic anion transporter (cMoat) (9), has a broad substrate specificity (8,10,20) that can account for some of the known transport processes of organic anions into the luminal space of renal proximal tubules (1–4). The substrate specificity of Mrp2 has been defined by measurements of ATP-dependent substrate transport into inside-out membrane vesicles from hepatocyte canalicular (apical) membranes in comparison with ATP-dependent transport by membrane vesicles from mutant rat livers lacking the Mrp2 protein (8,10,20,27–29). A ranking of some Mrp2 substrates based on the V_{max}/K_m ratio is as follows (10): Leukotriene C_4 (a lipophilic glutathione conjugate) > leukotriene D_4 (lipophilic cysteinylglycine conjugate) > *S*-(2,4-dinitrophenyl)glutathione > 17β-glucuronosyl-estradiol > 3α-sulfolithocholyltaurine > glutathione disulfide. It is of interest that MRP1, the human multidrug resistance protein cloned by Cole *et al.* (11), has a very similar substrate specificity as Mrp2 (5–7,10,12,13). However, MRP1 has been localized to basolateral membrane domains (30) and was detected in many different cell types (15), whereas rat Mrp2 and human MRP2 have an apical localization in polarized cells, such as hepatocytes (8–10,31) and proximal tubule epithelia (Figures 3 through 6). Previous studies on *mrp2* mRNA expression in different rat tissues indicated a low level expression in kidney, duodenum, and ileum, in addition to the predominant expression in hepatocytes (9,21). Demonstration and localization of

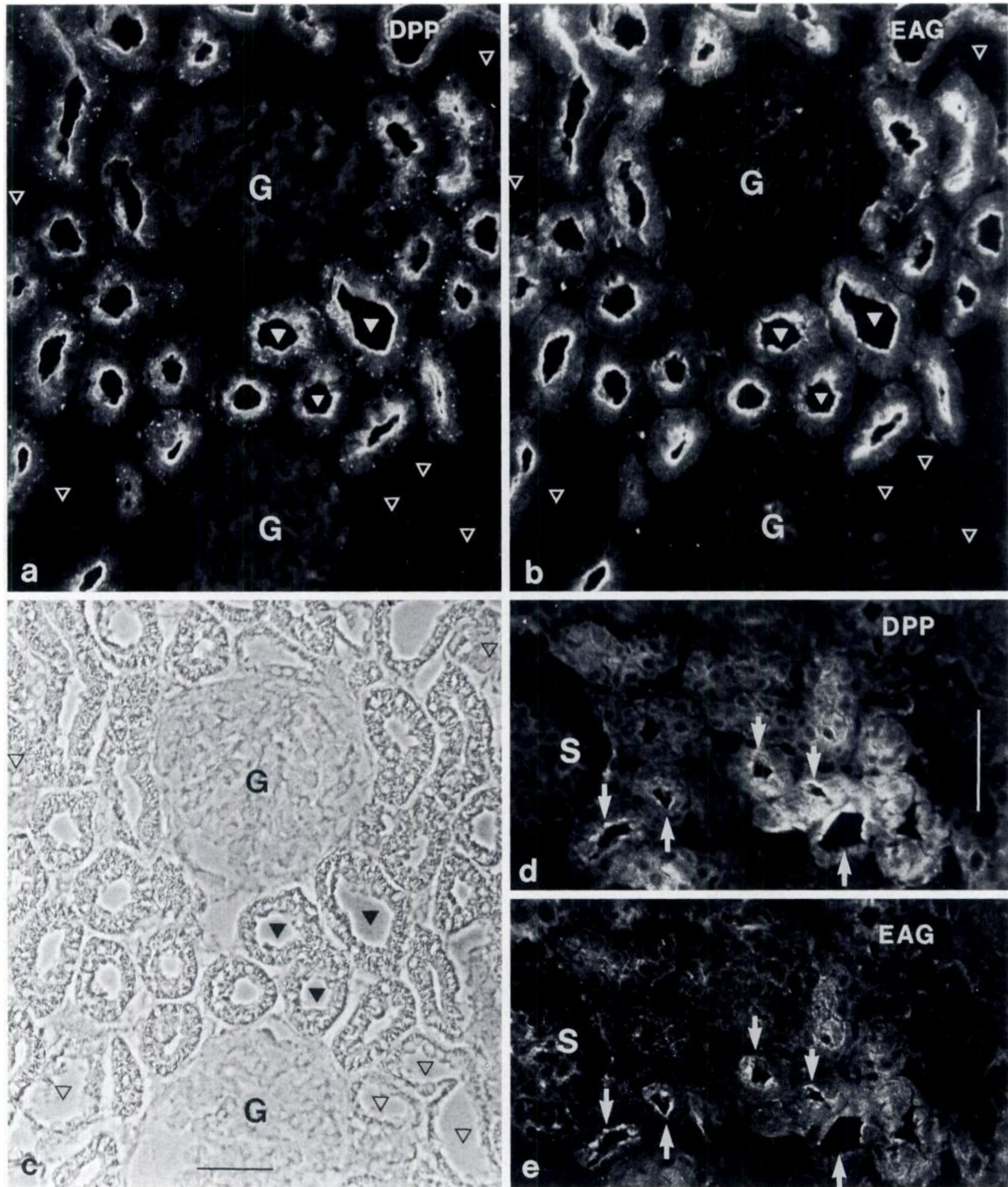

Figure 3. Immunofluorescence micrographs of adult (a through c) and neonatal (d and e) rat kidney. Frozen sections were examined after double-labeling with the affinity-purified Mrp2 antibody EAG15 (8) and the monoclonal antibody De 13.4 directed against rat dipeptidyl-peptidase IV (DPPIV) (24) as a colocalization marker for the apical membrane domain. The brush-border membrane of proximal tubule segments is strongly fluorescent with the EAG15 (EAG) antibody (b) and colocalizes with DPPIV (DPP; a). Phase-contrast microscopy of the identical tissue slice (c) indicates an intact morphology. In the developing cortex of neonatal rat kidney, the Mrp2-positive immunofluorescence is detected on day 1 after birth (e). Colocalization for DPPIV in neonatal rat kidney cortex with Mrp2 is shown in proximal tubule segments (arrows; d and e). Filled triangles show proximal tubule brush-border staining for Mrp2 (b) and DPPIV (a), and open triangles point to nonimmunoreactive regions in the more distal nephron segments (a through c). G, glomeruli; S, S-shaped body. Bars, 50 μm.

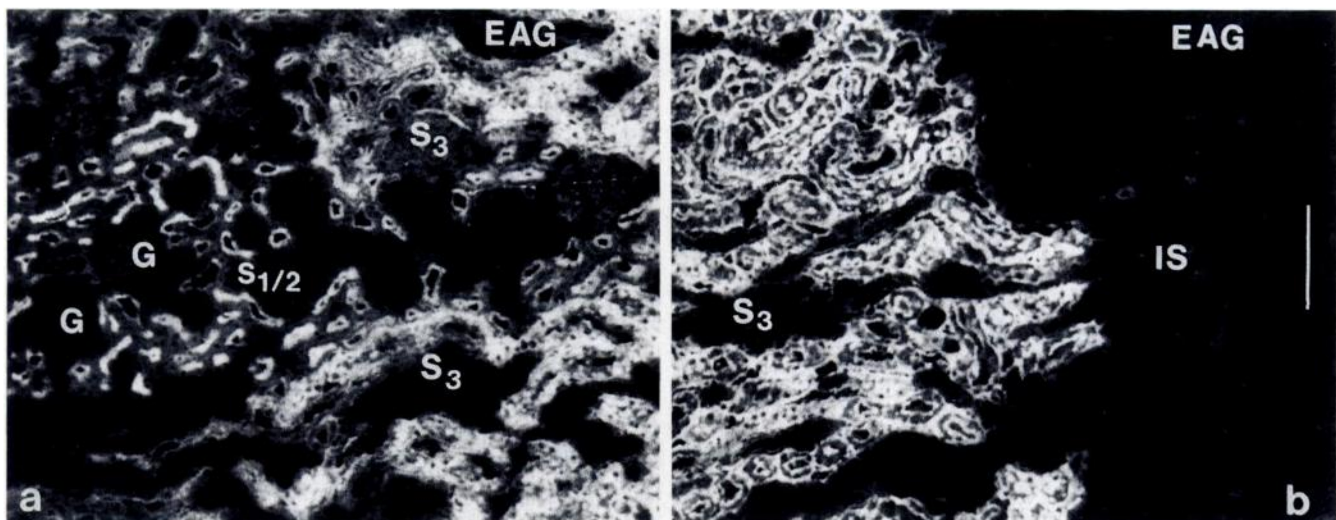

Figure 4. Immunofluorescence micrographs of adult rat kidney. Frozen sections were stained with the Mrp2 antibody EAG15 (EAG). The micrographs are at a lower magnification than in Figure 3. Panel a shows the reactivity of the antibody in the S_1 and S_2 segments of the proximal tubules (S_1/S_2), and the adjoining S_3 segments (S_3). In Panel b, the intense staining of the S_3 segments (S_3) is not observed in the adjacent areas of the inner stripe (IS). The glomeruli are indicated (G). Same magnification in Panels a and b. Bar, 250 μm.

the Mrp2 protein in kidney (Figures 2 through 6) was achieved by means of a high-affinity antibody selectively recognizing the carboxy terminus of rat Mrp2 (8). Mrp2 colocalized with DPPIV (Figure 6), which is an ectoenzyme characteristically expressed in apical membrane domains of polarized cells (24). Within rat kidney, Mrp2 staining was visible predominantly in all segments (S_1, S_2, and S_3) of the proximal tubules (Figures 3 through 6). This localization of the export pump encoded by the *mrp2* gene is consistent with *in vivo* and *in vitro* transport studies demonstrating the accumulation of organic anions (including carboxy-fluorescein, phenol red, and bimane conjugates) in the luminal space of proximal tubules (1–4).

Surprisingly, in addition to proximal tubule epithelia, the endothelia of capillaries located exclusively in the outer stripe of the outer medulla showed positive staining with the antibody against Mrp2. It is known that the capillaries in the outer stripe are distinct from those in the cortex, because most of the capillaries in the outer stripe do not represent proper capillaries derived from efferent arterioles but do represent ascending *vasa recta*, *i.e.*, capillary vessels draining the renal medulla (32). These vessels and their specific arrangement among proximal tubules in the outer stripe have been suggested to be responsible for specific metabolic functions (32,33).

We have included in this study as a negative control the EHBR mutant, which lacks the Mrp2 protein in the apical membrane of hepatocytes (8) and kidney proximal tubules (Figure 2A). The EHBR mutant rats have a point mutation in the coding sequence of the *mrp2* gene (21) and exhibit renal abnormalities (17), in addition to their impairment of the hepatobiliary elimination of amphiphilic anionic conjugates (16). It will be of interest to define in more detail the functional abnormalities in renal transport of the EHBR mutant and to examine how these viable mutant rats compensate for the defect of this ATP-dependent export pump. One may consider the possibility that the conjugate export pump encoded by the

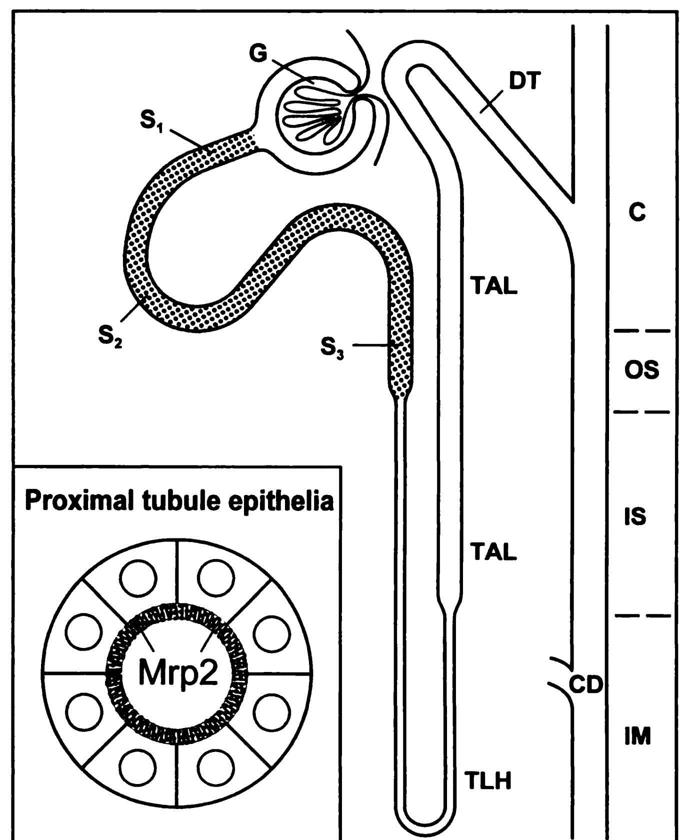

Figure 5. Scheme of the localization of Mrp2 in the proximal tubule brush-border membrane. G, glomerulus; S_1, S_2, and S_3, segments of the proximal tubule; TLH, thin limb of Henle's loop; TAL, thick ascending limb of Henle's loop; DT, distal tubule; CD, collecting duct; C, cortex; OS, outer stripe; IS, inner stripe; IM, inner medulla. Dotted areas point to the site of expression of Mrp2.

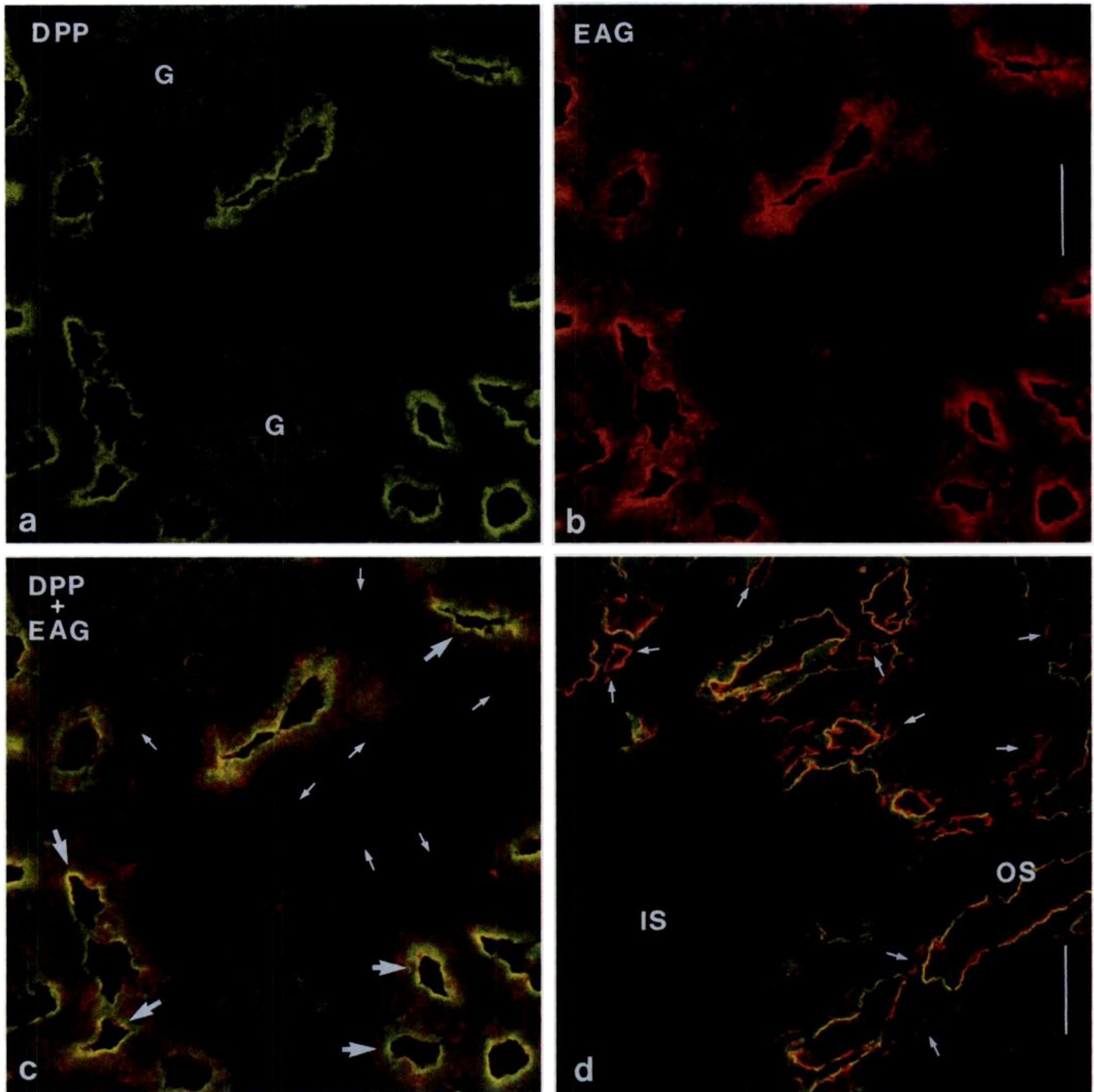

Figure 6. Confocal laser scanning micrographs of adult rat kidney. Frozen sections of kidney after reaction with antibody directed against DPPIV (green; DPP; a) and after reaction with the EAG15 antibody directed against Mrp2 (red; EAG; b) show positive reactions in brush-border membranes of proximal tubule epithelial cells. Superimposition of both fluorescences demonstrates colocalization of both antigens in the apical membrane domain of the proximal tubule epithelia (yellow mixture; EAG + DPP; c). Large arrows point to the positive staining of proximal tubule epithelia, and small arrows point to nonimmunoreactive regions in more distal tubule epithelia (c). Double-immunofluorescence, using the EAG15 and the De 13.4 (anti-DPPIV) antibodies, indicate in the outer medulla (OS and IS as explained in Figure 5) colocalization of both antigens (yellow fluorescence) only in S_3 proximal tubules (d). Controls, using the preimmune serum and EAG15 antibodies preabsorbed with the immunization antigen, did not show any fluorescence. Small arrows point to capillary endothelial cells between S_3 proximal tubules in the outer stripe of the outer medulla (d). G, glomerulus; OS, outer stripe (positive S_3 proximal tubule segments); IS, inner stripe (without immunoreactivity). Bar, 50 μm.

mrp1 gene in part compensates for the defect in the mutant. Immunoblotting with the 6KQ (23) and the B5 polyclonal antibodies resulted in the detection of a 190-kD membrane protein in EHBR mutant and normal kidney membranes (Figure 2B). These antibodies recognize the carboxy terminus of Mrp1 (8,27), suggesting that Mrp1 is equally expressed in

mutant EHBR and in normal rat kidney. After the localization of the MDR1 P-glycoprotein in kidney (34), Mrp2 represents the second cloned ATP-binding cassette transporter to be localized to the proximal tubule brush-border membrane (Figures 3 through 6). MDR1 P-glycoprotein has a very different function and sequence compared with MRP1, MRP2, and their rat homologs (8–11). MDR1 P-glycoprotein functions in the ATP-dependent export of a broad spectrum of lipophilic, mostly cationic, substances (35), whereas MRP1 (12) and Mrp2 (8,10) mediate ATP-dependent export of many anionic lipophilic substances and conjugates across membranes. Both ATP-binding cassette transporters may complement each other in terms of substrate specificity and may contribute to cellular detoxification. Mrp2 contributes to the apical secretion of endogenous and xenobiotic substances from the blood into urine.

Acknowledgments

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