Cell Biological and biochemical characterization of drebrin complexes in mesangial cells and podocytes of renal glomeruli

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Abstract. Drebrins are actin-binding proteins (ABP) initially identified in and thought to be specific for neuronal cells, where they appear to contribute to the formation of cell processes. Recent studies have also detected the isof orm drebrin E2 in a wide range of non-neuronal cell types, notably in and near actin-rich lamellipodia and filopodia. The present study demonstrates drebrin enrichment in renal glomeruli. Immunohistochemistry and double-label confocal laser scanning microscopy have shown intense drebrin reactions in the mesangial cells of diverse mammalian species. In adult human and bovine kidneys, drebrin is, in addition, markedly enriched in the foot processes of podocytes, as also demonstrable by immunoelectron microscopy. By contrast, the podocytes of rodent glomeruli appear to contain significant drebrin concentrations only during early developmental stages. In differentiated murine podocytes cultured in vitro, however, drebrin is concentrated in the cell processes, where it partially codistributes with actin and other ABP. In biochemical analyses using protein extracts from renal cortices, large (approximately 20S) complexes ("drebrosomes") were found containing drebrin and actin. These findings confirm and extend our hypothesis that drebrin is involved in the regulation of actin dynamics also outside the nervous system. Clearly, drebrin has to be added to the ensemble of ABP regulating the actomyosin system and the dynamics of mesangial cells and foot processes in podocytes.

A central function of the renal glomerulus is plasma ultrafiltration for primary urine production. The major structure involved in this function, the glomerular filtration barrier, is composed of the glomerular basement membrane, covered on the inner surface by a fenestrated endothelial cell layer, and on the outer by podocytes, i.e., epithelioid cells of mesenchymal derivation with numerous actin filament-rich protrusions, the "foot processes" attaching to the basement membrane (1–4). This structure is supported by mesangial cells, smooth muscle-like pericytes with different functions, including the regulation of the GFR, hormon secretion, and nutrition (5–10).

At the "foot sole" of the podocytes, attachment between the actin cytoskeleton and the basement membrane is achieved by the bridging of transmembrane proteins such as the dystroglycans and integrin, which are associated with the ABP utrophin and several other ABP such as vinculin, talin, and paxillin (11–14). In their apical domain, the foot processes of neighboring podocytes are connected by the slit diaphragm, a zipper-like membrane domain (15) containing a set of specific transmembrane proteins such as nephrin and P-cadherin, which in turn are associated with cytoplasmic plaque proteins such as protein ZO-1 and catenins and thus linked to the actin filament cytoskeleton (16,17). In the most apical domain of the foot processes, the membrane glycoprotein podocalyxin (18) has been reported to interact indirectly with the ABP ezrin and, thereby, also with actin (19). Moreover, among the various ABP enriched in foot processes α-actinin and synaptopodin are particularly prominent (4,20,21).

Drebrins (developmentally regulated brain proteins) are a family of ABP originally identified in neuronal cells and tissues (for review see reference 22) that exist in three isoforms (E1, E2, and A) generated by alternative splicing and synthesized in patterns depending on the specific developmental stage. In the nervous system, the drebrins have been implicated in the regulation of the actin cytoskeleton and, thereby, in the morphogenesis of neuronal cells, particularly in the formation of neuronal cell processes (22–24), as also shown by the fact that transfection of diverse non-neuronal cells with drebrin cDNA results in the formation of neurite-like cell processes,
whereas transfection with antisense cDNA reduces the ability of cells to form processes (23–27).

While the drebrins have long been considered, by and large, to be specific for the nervous system, we (28,29) and others (27) have recently also detected the drebrin splice variant E2 in diverse non-neuronal cells. Particularly conspicuous are accumulations of drebrin in positions corresponding to cell protrusions of motile cells, notably lamellipodia and filipodia (29), and in renal glomeruli of human and bovine kidneys (28). Here, we report that drebrin is highly enriched in mesangial cells and, in some species but not in all, in the foot processes of podocytes, including those of murine podocytes growing in cell culture. Moreover, biochemical analyses of renal cortical proteins have shown novel kinds of drebrin-containing complexes.

**Material and Methods**

**Cell Cultures**

Conditionally immortalized murine podocytes were grown in culture as described (30). Briefly, podocytes were cultured in RPMI 1640 medium (Life Technologies, Karlsruhe, Germany), supplemented with 10% fetal bovine serum (Roche Diagnostics, Mannheim, Germany), 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). To allow proliferation of cells, cells were maintained at 33°C (permissive conditions), and 10 U/ml murine γ-interferon was added to the culture medium. For the induction of cell differentiation, podocytes were cultured at 38°C without γ-interferon (nonpermissive conditions) for 6 to 7 d.

In some experiments, the F-actin-arresting agent cytochalasin D (1 mM; Sigma, Deisenhofen, Germany) was applied for 20 min at 37°C before fixation and immunostaining.

**Tissues**

Tumor-free human kidney samples were obtained from tumor nephrectomies at the Department of Urology, University of Heidelberg, with the permission of the ethics committee of the Medical Faculty. Bovine kidney was freshly obtained from the local slaughterhouse. Rodent kidneys were from rats and mice kept in the animal facility of the German Cancer Research Center. Small blocks of tissue were snap-frozen in isopentane precooled in liquid nitrogen to a temperature of approximately −130°C and stored at −80°C. Frozen tissues of mouse embryos (post conceptionem, days E16.5 and E19.5) were available at the Division of Cell Biology of the German Cancer Research Center.

For some experiments, rodent kidneys were prefixed by formaldehyde perfusion, before paraffin-embedding. Renal perfusion of SD-rats was performed with solutions of 3% formaldehyde, perfusion of MRNI-mice with solutions of 5% formaldehyde. Paraffin-embedding was carried out according to standard protocols.

**Antibodies and Reagents**

Antibodies specific for the drebrin isoforms E2 and A (gp drebE2/A) were raised in guinea pigs against a synthetic peptide derived from the human drebrin amino acid (aa) sequence (aa positions 324 to 343; SHRRMAPPTIPRSPDSST; Peptide Speciality Laboratories, Heidelberg). In addition, drebrin-specific guinea pig antibodies of the serum gp dreb254.2 (29) were used. For some experiments, this serum was affinity-purified (gp dreb254.2 affin) on the iodoacetyl-immobilized peptide (aa positions 254 to 272; QSIIFG-DHRDEEEETHMKKS) as described by Mertens et al. (31). Murine monoclonal antibody against purified chicken drebrin E (MAB M2F6) was purchased from MoBiTec (Göttingen, Germany). MAB to actin (IgM, clone 2G2) reacting with cytoplasmic actin microfilaments in methanol-fixed cells was kindly provided by Prof. Dr. B. Jockusch (Technical University, Braunschweig, Germany [32]). Alternatively, actin filaments were visualized by staining with phalloidin coupled to Alexa 488 or Alexa 594 (MoBiTec). Further mAbs used were specific for synaptopodin (Progen Biotechnik, Heidelberg [21]), plakoglobin (Progen Biotechnik), β-catenin (BD Transduction Laboratories, Heidelberg, Germany), α-actinin (clone BM75-2), ezrin (clone 3C12), and vinculin (clone 11-5; all from Sigma). Rabbit antibodies to protein ZO-1 were purchased from Zymed Laboratories (San Francisco, CA) and rabbit antiserum to α- and β-catenin were from Sigma. In addition, sheep antibodies to Tamm-Horsfall glycoprotein, obtained from Biotrend (Cologne, Germany), were applied. Guinea pig preimmune and control sera and as well as guinea pig and murine antibodies against diverse unrelated antigens, including cytoskeletal and membrane proteins, were used as negative controls (33–35).

For immunofluorescence microscopy, antigen-antibody-complexes were visualized with secondary antibodies coupled to Cy-2, Cy-3 (Dianova, Hamburg, Germany), Alexa 568, or Alexa 488 (MoBiTec).

For immunoblot analysis, horseradish peroxidase-conjugated secondary antibodies (Dianova) were used in combination with the enhanced chemiluminescence system (ECL, Amersham Buchler, Braunschweig, Germany).

**Immunofluorescence and Confocal Laser Scanning Microscopy**

Murine podocytes were grown on glass coverslips at 38°C for 6 to 7 d to induce differentiation. The coverslips were briefly rinsed in PBS, and cells were fixed either in 2% formaldehyde in PBS (10 min, room temperature [RT]), followed by permeabilization in 0.3% Triton X-100 for 8 min or, alternatively, in methanol (−20°C, 20 min). Before immunostaining, cells were blocked with 2% fetal bovine serum for 1 h.

Frozen tissues were sectioned (4 to 5 μm), using a Leica cryomicrotome (Vienna, Austria). After air-drying for at least 1 h, sections were fixed in acetone (−20°C, 10 min) and blocked with 5% goat serum for 20 min. Immunostaining of cells and tissue sections was performed as described (29). Primary antibodies were applied for 1 h, secondary antibodies for 30 min. For visualization and photography, an Axiphot II photomicroscope (Carl Zeiss, Jena and Oberkochen, Germany) was used. Confocal laser scanning microscopy was performed with a Zeiss LSM 510 UV microscope, operating with an argon ion laser (488 nm) and a HeNe laser (543 nm [28]).

Immunolabeling of paraffin-embedded tissues was performed using the avidin-biotin-complex technique with peroxidase. Briefly, 4-μm-thick sections of kidney tissue were deparaffinized according to standard techniques and, thereafter, pretreated by microwaving in 10 mM sodium citrate buffer to retrieve masked antigens (5 × 5 min, 600 W). This was followed by incubation in 0.1 M NH₄Cl for blocking of free aldehydes and by avidin-biotin blocking (Vector blocking kit; Linaris, Wertheim, Germany). Endogenous peroxidase activity was blocked with 3% H₂O₂. Before immunolabeling, the sections were permeabilized with 0.3% Triton X-100 for 5 min. Immunoreactions were performed with the Vecastatin Elite ABC peroxidase kit (Linaris) according to the manufacturer’s instructions, with the modification that for immunostaining with primary guinea pig antibodies to drebrin, biotinylated goat anti-guinea pig IgG antibodies (also from Linaris) were used as secondary antibody. The sections were visualized and photographed with a Leica Polyvar 2-microscope.
**Electron and Immuno-electron Microscopy**

For conventional electron microscopy, cells and tissue sections were briefly rinsed in PBS containing 1 mM MgCl₂, fixed in 2.5% glutaraldehyde in cacodylate buffer (50 mM sodium cacodylate, pH 7.2) for 30 min and washed thrice in cacodylate buffer. Postfixation was performed with 2% OsO₄ in cacodylate buffer for 2 h on ice, followed by several washes in distilled water and by heavy metal staining (0.5% uranylacetate) overnight. After three washes in distilled water, samples were dehydrated in an ethanol series and in propyleneoxide and embedded in Epon. Ultrathin sections of 50 nm thickness were made with a Reichert ultracut cryotome (Leica). For contrast enhancement, the sections were stained with 2% uranylacetate in methanol for 15 min and with lead citrate for 5 min.

For immuno-electron microscopy, podocytes grown on glass coverslips and frozen sections of kidney tissues were fixed in 2% formaldehyde in PBS (7 min, RT), followed by incubation in 50 mM NH₄Cl (5 min), two washes in PBS (5 min each), and permeabilization in 0.1% Triton X-100 for 3 min. Primary antibodies were applied for at least 2 h. After three washes in PBS, samples were incubated overnight with the secondary antibodies, anti-mouse, or anti-guinea pig immunoglobulins conjugated with gold particles of 1.4 nm diameter (Nanogold, Biotrend). Secondary antibodies not stably bound were removed by washing in PBS. Samples were then postfixed with 2.5% glutaraldehyde in cacodylate buffer (15 min, RT), briefly rinsed in the same buffer, and twice incubated in a solution of 200 mM sucrose in 50 mM HEPES buffer (pH 5.8) for 10 min. This was followed by silver enhancement (Nanoprobes; Stony Brook, New York, NY) for 3 to 8 min, two washes in 250 mM sodium thiosulfate buffered with 50 mM Hepes (pH 5.8; 8 min each), and eight to ten washes in distilled water. After fixation with 0.2% OsO₄ in cacodylate buffer for 30 min on ice, samples were dehydrated, embedded in Epon, sectioned, and stained as described for conventional electron microscopy. Electron micrographs were taken at 80 kV, using an EM 900 electron microscope (LEO, Oberkochen, Germany; for immuno-electron microscopy with silver enhancement see also reference 29 and literature cited therein).

**Sucrose Gradient Centrifugation and Protein Biochemistry**

To prepare lysates of renal cortices, pieces of frozen tissues with a diameter of approximately 5 mm were homogenized on ice in digitonin buffer (0.005% digitonin, 20 mM Hepes, 150 mM NaCl, pH 7.4) containing an EDTA-free protease inhibitor cocktail (Roche Diagnostics), using a Polytron homogenizer (Ika-Ultra-Turrax T25; Janke and Kunkel, Staufen, Germany). After treatment with a Dounce homogenizer (30 strokes) and centrifugation at 2500 rpm (5 min, 4°C), supernatants were loaded on top of the sucrose gradients.

Briefly, 10 to 40% sucrose gradients were prepared from solutions of 10% and 40% sucrose in 20 mM Hepes and 150 mM NaCl (pH 7.4), using a gradient mixer (Heidolph, Mannheim, Germany), with a volume of 11 ml for each gradient. Centrifugation was performed in a SW40 rotor (Beckman Instruments, Munich, Germany) for 16 h at 23,000 rpm at 4°C. Bovine serum albumin (BSA, 66 kD, 4.3S), catalase (232 kD, 11.3S), and thyroglobulin (669 kD, 16.5S; all from Sigma), serving as marker proteins, were dissolved in digitonin buffer, and fractionated in parallel gradients. In addition, the 40S and 60S subunits of cytoplasmic ribosomes were used as high molecular weight markers. Cytoplasmic ribosomes were prepared from the oocytes of Xenopus laevis toads and dissociated into their subunits as described by Hügge et al. (36).

After sucrose gradient centrifugation, fractions of 400 µl each (30 to 33) were collected from top to bottom of the gradients. The pellets of the gradients were solubilized in 400 µl of digitonin buffer. An aliquot of each fraction was supplemented with thrice-concentrated sample buffer (325 mM Tris-HCl, 30% (vol/vol) glycerol, 15% (wt/vol) SDS, 60 mM DTT, 0.3% (wt/vol) Bromphenol Blue; pH 7.6). The samples were then heated (95°C, 3 min), briefly centrifuged (13,000 rpm, 2 min) and subjected to SDS-PAGE, followed by immunoblotting as described (28).

To prepare lysates of podocytes in culture, cells grown in 10-mm dishes or flasks were induced to differentiate for 6 to 7 d as described and rinsed twice with PBS precooled to 4°C. Cells were then scraped off in thrice-concentrated sample buffer, using a rubber policeman. After addition of 0.1% Benzonase (Merck, Darmstadt, Germany), cells were homogenized in a Dounce homogenizer and the lysate was heated to 95°C for 3 min. Proteins were precipitated with methanol and chloroform, air-dried, and re-dissolved in a small volume of thrice-concentrated sample buffer, before SDS-PAGE and immunoblotting.

**Results**

**Immunofluorescence Microscopy of Drebrin in Kidneys of Different Species**

Using immunofluorescence microscopy on cyrostat sections, we had previously noted in human and bovine kidney strong immunoreactions in the glomeruli (28). To further characterize the immunoreactive cells in the glomeruli, we performed double-label immunofluorescence microscopy, using antibodies to drebrin and to synaptopodin, a marker protein for podocytes enriched in foot processes (21). In human and bovine glomeruli, both drebrin and synaptopodin antibodies reacted intensely in podocytes (Figure 1, a-a' and b-b'). Immunoreactions for drebrin were also consistently observed in mesangial cells (Figure 1b, arrow; see also below) and in the epithelia of certain tubules (asterisks in Figure 1b), confirming the report by Keon et al. (27). Double-staining with antibodies to Tamm-Horsfall glycoprotein ascertained these drebrin-positive tubules as distal tubules (data not shown). We also noted faint immunostaining in interstitial as well as in vascular smooth muscle cells (28).

Our immunofluorescence microscopic studies of rodent kidneys confirmed observations made in cow and humans but also showed some remarkable differences (Figure 1, c-f'). In rodents, drebrin-positive reactions were also observed in distal tubules of both rat (asterisks in Figure 1c) and mouse (asterisks in Figure 1, e and f). In addition, the straight segments of proximal tubules were immunoreactive in mice (not shown). Surprisingly, however, when glomeruli of rats and mice were double-labeled for drebrin and synaptopodin, the antibodies appeared to react with different cell types (Figure 1, c and c': rat; Figure 1, e and e': mouse). Drebrin-positive cells were mostly located in the center of the glomeruli, whereas the cells in the periphery were immunoreactive for synaptopodin but not for drebrin. To examine whether the centrally located, drebrin-positive cells belong to the mesangium, we performed double-labeling for drebrin and for desmin, an intermediate filament protein known to occur in mesangial cells (8,33,35). In the glomeruli of both rats (Figure 1, d and d') and mice (Figure 1, d and d'), the nuclei showed some remarkable differences (Figure 1, e-f'). For immunoelectron microscopy, podocytes grown on glass coverslips and frozen sections of kidney tissues were fixed in 2% formaldehyde in PBS (7 min, RT), followed by incubation in 50 mM HEPES buffer (pH 5.8) for 10 min. This was followed by silver enhancement (Nanoprobes; Stony Brook, New York, NY) for 3 to 8 min, two washes in 250 mM sodium thiosulfate buffered with 50 mM Hepes (pH 5.8; 8 min each), and eight to ten washes in distilled water. After fixation with 0.2% OsO₄ in cacodylate buffer for 30 min on ice, samples were dehydrated, embedded in Epon, sectioned, and stained as described for conventional electron microscopy. Electron micrographs were taken at 80 kV, using an EM 900 electron microscope (LEO, Oberkochen, Germany; for immuno-electron microscopy with silver enhancement see also reference 29 and literature cited therein).
f and f') the staining pattern of drebrin and desmin antibodies was very similar and far-reaching colocalization of these proteins was directly demonstrable by confocal laser scanning microscopy (Figure 2).

To determine the spatial relationship between drebrin and synaptopodin in detail, cryostat sections through kidneys of different species where double-immunostained and observed by confocal laser scanning microscopy (Figure 3). In human kidney (Figure 3, a-c), drebrin and synaptopodin both reacted with podocytes, showing colocalization in the cell periphery, notably the foot processes. In addition, intense drebrin immunoreaction was consistently observed in the synaptopodin-negative mesangial cells (asterisk in Figure 3c). Confocal laser scanning microscopy of sections through bovine kidney gave essentially identical results (data not shown). By contrast, however, when renal glomeruli of rats were labeled for drebrin and synaptopodin (Figure 3, d-f), the staining patterns of both antibodies appeared mutually exclusive, indicating that here drebrin is practically specific for mesangial cells, and this was seen with two different polyclonal antibodies and with mAb M2F6. The significance of weak and variable immunoreactions sometimes observed in podocytes on sections of aldehyde-fixed, paraffin-embedded rodent kidneys (see Material and Methods) remains to be seen.

Localization of Drebrin in Developing Murine Glomeruli

As the complement of cytoskeletal proteins in mesangial cells and podocytes can vary remarkably between species as well as in different developmental stages and under different environmental conditions (6–8,11,35,37) we have decided to study drebrin synthesis in developing rodent glomeruli. During embryonic development, presumptive glomeruli pass through four stages, the renal vesicle, the S-shaped body, the capillary loop stage and, finally, the maturing glomerulus (11,38).

When cryostat sections through kidneys of embryonic mice at days E16.5 and E19.5 were immunostained (Figure 4), drebrin immunoreactions were seen along intercellular contacts of renal vesicles (not shown). Analyses of S-shaped bodies (Figure 4a) and of developing glomeruli in the capillary loop stage (Figure 4, b and c) revealed positive cell border immunostaining of the presumptive podocytes as well as immunoreactions in the forming mesangial and endothelial cells of capillary loop stage. When maturing glomeruli were immunoreacted, however, the differentiating podocytes were mostly negative for drebrin, and labeling was almost exclusively observed in the mesangium and the capillaries (Figure 4d).

Developing mouse kidneys were also labeled for drebrin in combination with synaptopodin, which is first synthesized in presumptive podocytes in the capillary loop stage (11,39). In a number of glomeruli, notably those of the capillary loop stage, antibodies to drebrin (Figure 4c) and to synaptopodin (Figure 4c') both reacted in presumptive podocytes. In addition, drebrin reactions were seen in mesangial cells. In more mature glomeruli, however, drebrin was exclusively found in the center of the glomeruli, i.e., the mesangial and the endothelial cells (Figure 4d), whereas synaptopodin antibodies at this stage...
strongly and exclusively reacted in peripherally located cells corresponding to presumptive podocytes (Figure 4d'). Thus, drebrin seemed to be synthesized in presumptive podocytes of rodent kidneys in early developmental stages but rapidly diminished during podocyte maturation.

**Immunoelectron Microscopic Localization of Drebrin in Human and Bovine Kidneys**

To further determine the subcellular localization of drebrin in renal glomeruli, we performed immunoelectron microscopy of human (Figure 5) and bovine (not shown) renal glomeruli, using the silver enhancement technique. Silver-enhanced immunogold labeling showed a marked accumulation of drebrin in the foot processes of podocytes, compared with the cell bodies (Figures 5, c and d), similar to synaptopodin studied in parallel (Figure 5b). One might get the impression that drebrin was associated within the foot processes with the masses of actin microfilament bundles as it is also known for other ABP (4,20,21). Drebrin immunogold labeling of mesangial cells extended through almost the entire cell known to be rich in actin filaments (data not shown; 40–42).

**Detection and Subcellular Localization of Drebrin in Cultured Murine Podocytes**

The distribution of drebrin was also studied in cultured murine podocytes induced to differentiate and to form cell processes. Here we observed prominent drebrin staining in the cell processes and partly also along the plasma membrane (Figure 6). In some cells, drebrin antibodies additionally stained small dot-like cytoplasmic structures. Again, essentially identical observations were made with different monoclonal and polyclonal drebrin antibodies (see above). Immunoelectron microscopy of such podocytes showed significant enrichment of drebrin in the cortical cytoplasm, notably along the plasma membrane and in filopodia-like cell protrusions (Figure 7), confirming our immunofluorescence microscopic observations.

Moreover, the distribution of drebrin in cultured murine podocytes was compared with those of actin and of different ABP, using double-labeling and confocal laser scanning microscopy (Figure 8). When such experiments were performed with antibodies to drebrin (Figure 8, a, a', b, and b') and synaptopodin (Figure 8, a', a", b', and b''), drebrin reacted in cell processes, whereas synaptopodin localized to the cytoplasmic actin microfilament bundles (stress fibers). These stress fiber cables were negative for drebrin, corresponding to our previous observations in diverse other cell types (28,29). The ABP α-actinin (Figure 8, c' and c") was detected along actin microfilament bundles, often in linear punctate arrays, and also in cell protrusions, mostly colocalizing with drebrin in the latter (Figure 8, c and c'). Similarly, when podocytes were labeled for drebrin in combination with actin antibodies, actin localized to the stress fibers as well as to cell processes, here showing far-reaching colocalization with drebrin (data not shown). In addition, in podocytes double-labeled for drebrin and ezrin, an ABP known for its close association with the plasma membrane (43), we observed marked ezrin enrichment in cell protrusions, sometimes at their tips, and colocalization with drebrin (data not shown). By contrast, double-staining for drebrin (Figure 8, d and d") and the ABP vinculin, a marker of focal contacts (Figure 8, d' and d"), showed differential distribution patterns and confirmed the absence of drebrin from the vinculin-positive focal adhesions, as previously reported for other cells (28). Differentiated cultured podocytes were also reacted with antibodies to α- and β-catenin, plakoglobin and protein ZO-1, a component of the plaques of tight and adhering junctions, in combination with drebrin. Both catenins, plako-
Figure 3. Double-label confocal laser scanning immunomicroscopy of sections through glomeruli of human (a-c) and rat (d-f) kidneys, comparing drebrin (a and c, serum gp dreb254.2; d and f, serum gp drebE2/A, red) to synaptopodin (b, e, and f, green). In human glomeruli, the drebrin reaction is prominent in many glomerular cells (a), including a number that are also positive for synaptopodin (b), i.e., podocytes. The merge picture shows colocalization of drebrin and synaptopodin at sites often corresponding to peripheral portions of podocytes (c, yellow). Immunoreactions for drebrin are additionally noted in the synaptopodin-negative mesangial cells (asterisk). In rat glomeruli, by contrast, antibodies to drebrin (d) and synaptopodin (e) react with different cell types. Drebrin-positive reactions are seen in the centrally located mesangial cells whereas synaptopodin antibodies intensely stain cells located in the periphery of the glomerulus, i.e., podocytes. Here, the merge picture (f) demonstrates mutually exclusive distribution of both proteins. Scale bars, 50 μm.
globin and protein ZO-1 were accumulated at contact sites between two podocytes (data not shown).

To examine whether the localization of drebrin was dependent on the actin cytoskeleton, cultured podocytes were first treated with the actin-arresting agent cytochalasin D and then double-labeled for drebrin and actin. Confocal laser scanning microscopic analysis showed that most of the drebrin was redistributed into aggregates and smaller punctate and linear structures in the cytoplasm, some of which were strongly positive for actin, suggesting that the distribution pattern of drebrin depended on the actin cytoskeleton (data not shown).

The occurrence of drebrin in cultured murine podocytes was also confirmed by immunoblot analysis. When total proteins of differentiated podocytes were separated by SDS-PAGE and immunoreacted with mAb M2F6 or with polyclonal antibodies for drebrin, a distinct single band appeared at a molecular weight of approximately 125 kD.
mesangial cell as a vascular smooth muscle derivative cytes (28), and are in agreement with the general concept of the brin reactions in vascular smooth muscle cells, including peri-
angial cells corresponds to our previous results showing dre-
cytes of some species (human, bovine) as well as in fetal
noted marked drebrin enrichment in foot processes of podo-
ings of the ABP drebrin in renal glomeruli (28). The protein is
Discussion
plexes, we modified the extraction protocol, using a
EDTA. To search for possible larger drebrin-containing com-
concentrations such as 1% Triton X-100, 0.2% SDS, and 2 mM
extracts of bovine kidney, obtained with rather high detergent

Identification of Drebrin-Containing Complexes of Approximately 20S (Drebrosomes)
Using sucrose gradient centrifugation, we have previously
described drebrin-containing particles of approximately 13S in
extracts of bovine kidney, obtained with rather high detergent
concentrations such as 1% Triton X-100, 0.2% SDS, and 2 mM
EDTA. To search for possible larger drebrin-containing com-
plexes, we modified the extraction protocol, using a “milder”,
digitonin-containing buffer (see Material and Methods). When

Figure 7. Immunoelectron microscopic localization of drebrin (gp dreb254.2) in cultured murine podocytes induced to differentiate and form processes. Using the silver-enhanced immunogold labeling tech-
nique, drebrin is preferentially detected along the plasma membrane and in cell processes (insert, higher magnification). Scale bars, 1 μm.

our finding of consistently intense drebrin reactions in mes-
angial cells corresponds to our previous results showing dre-
brin reactions in vascular smooth muscle cells, including peri-
cytes (28), and are in agreement with the general concept of the mesangial cell as a vascular smooth muscle derivative
(5,7,8,10,44,45). As changes of the mesangial cytoskeleton, notably the disassembly of actin microfilaments, have been reported as characteristic for the pathogenesis of a number of glomerular diseases, including diabetic glomerulopathy (42,46,47), it will be important to elucidate the mechanisms and the ABP involved in these processes.

Drebrin has also been found as a prominent protein of podocytes in situ and in cell culture, although with remarkable interspecies differences; on cryostat sections, the podocytes of human and bovine kidneys are strongly immunoreactive, whereas those of rodent kidneys are not. However, drebrin is also detected in the primordial podocytes of early and intermediate stages of glomerular development in murine embryos, i.e., in the S-shaped body and in the capillary loop stage (11,38). At present, we have no functional explanation for the reduction of drebrin contents in podocytes of adult rodents.

Interestingly, drebrin is well demonstrable both by immuno-
localization and by immunoblot on gel electrophoretically
separated proteins in murine podocytes in cell culture. Drebrin
probably represents another example of the cases in which cells in culture recapitulate gene expression patterns characteristic of embryonic development or regeneration (7,48–50). The observed enrichment of drebrin in foot processes of podocytes as well as in the mesangial cells characterized by stellate processes (40,51,52) now provide renal examples of the dre-
brin accumulations in cell protrusions observed in diverse other
cell types (29 and references therein), including neurons
(26,53,54). This adds another feature of similarity between podocytes and neurons (55), and it is also remarkable that both
types of cell processes contain the ABP synaptopodin (21).

It is widely accepted that actin and ABP are functionally
important in the formation and maintenance of the processes of
tocytobs and mesangial cells, regulating their shape and
supporting their stability. Thus, the possibility of an involve-
ment of drebrin complexes will have to be examined for the
various situations of actin filament–membrane interactions (see
Introduction); notably, in the region of the slit diaphragm of podocyte foot processes the transmembrane protein nephrin
(16) is connected to actin filaments via the CD2-associated
protein (CD2AP [56,57]) and possibly also via podocin (58),
and P-cadherin is also associated with actin and several ABP
(17). In the course of the present study, we have also identified
partial colocalizations of drebrin and further ABP such as
α-actinin and ezrin in cell processes of cultured murine
podocytes.

Recently, it has been reported that mutations in some ABP
are causes of certain hereditary kidney diseases and that abro-
gations of some ABP genes result in diverse nephropathies
(59–62). It is mandatory that the potential role of drebrin in
kidney pathogenesis will now also have to be studied using
 genetic approaches. Moreover, causes for nephrotic syndromes
that appear to involve damages of the podocyte actin cytoskel-
eton are the collapsing glomerulopathies, often accompanied
by the disappearance of synaptopodin (63).

Similarly, disruption of the actin cytoskeleton of mesangial
cells essentially contributes to different kinds of glomerulopa-
thies, including diabetic glomerulopathy (42,46,47,64,65). Re-
Double-label confocal laser scanning microscopy of drebrin (a, b, c, and d) and different ABP (a', b', c', and d') on cultured murine podocytes. (a-a' and b-b') Drebrin (red in a, green in b) is enriched in the cell protrusions of the podocytes, whereas antibodies to synaptopodin (green in a', red in b') decorate cytoplasmic actin microfilament bundles (stress fibers), which appear negative for drebrin (a', b', merge). Note the significance of this specific and mutually exclusive localization of two ABP. (c-c') Double-staining with antibodies to drebrin (c, red) and α-actinin (c', green; c', merge) reveals partial colocalization of both proteins in cell processes and regionally along the plasma membrane (yellow). Drebrin is again absent from the α-actinin-positive stress fibers. (d-d') When podocytes are double-stained for drebrin (d, red) in combination with vinculin (d', green), a general absence of drebrin from the vinculin-positive focal adhesions is observed (d', merge). Scale bars, 25 μm.
Acknowledgments

Markedly, expressions of a number of ABP genes in mesangial cells appear to be altered in various kidney diseases (66–68), and here again it will have to be examined whether concentrations and functions of drebrin are also affected.

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