CD2-associated protein directly interacts with the actin cytoskeleton

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Received 10 October 2001; accepted in final form 14 April 2002

Lehtonen, Sanna, Fang Zhao, and Eero Lehtonen. CD2-associated protein directly interacts with the actin cytoskeleton. Am J Physiol Renal Physiol 283: F734–F743, 2002. First published May 7, 2002; 10.1152/ajprenal.00312.2001.—CD2-associated protein (CD2AP) is an adapter protein associating with several membrane proteins, including nephrin, mutated in congenital nephrotic syndrome of the Finnish type, and polycystin-2, mutated in type 2 autosomal dominant polycystic kidney disease. Both proteins have critical roles in the maintenance of the integrity of the nephrons. Previous studies have suggested a role for CD2AP in the regulation of the organization of the actin cytoskeleton, but it has not been known whether the postulated association between CD2AP and actin is direct or mediated by other proteins. In this study, we address this question by using various cellular and biochemical approaches. We show that CD2AP and F-actin partially colocalize in cultured cells and that disruption of the actin cytoskeleton results in disorganization of endogenous CD2AP. Using cytoskeletal fractionation by differential centrifugation, we demonstrate that a proportion of CD2AP associates with the actin cytoskeleton. Furthermore, using pure actin and purified CD2AP fusion proteins in an F-actin coprecipitation assay, we show that CD2AP directly associates with filamentous actin and that this interaction is mediated by means of the COOH terminus of CD2AP. The present results suggest that CD2AP is involved in the regulation of the actin cytoskeleton and indicate that CD2AP may act as a direct adapter between the actin cytoskeleton and cell membrane proteins, such as nephrin and polycystin-2. Alterations in these interactions could explain some of the pathophysiological changes in congenital nephrotic syndrome and polycystic kidney disease.

METS-1; microfilaments; nephrotic syndrome; polycystic kidney disease; SH3 domain

IN THE SEARCH FOR NOVEL developmentally regulated genes in the developing mouse kidney, we used differential hybridization to identify a gene that is strongly upregulated during mesenchyme-to-epithelium transition (13, 18). The encoded protein, originally named METS-1 (mesenchyme-to-epithelium transition protein with multiple SH3 domains) (18), is identical to CD2-associated protein (CD2AP), which has been identified from mouse T cells as a CD2 cell adhesion protein-binding partner (6). The human homolog of CD2AP, Cas ligand with multiple SH3 domains (CMS), was identified as a focal adhesion protein p130Cas-associated protein (16).

CD2AP is an 80-kDa protein that is widely expressed in tissues (6, 16, 19). The protein includes several putative protein-protein interaction domains, suggesting that CD2AP is an adapter molecule. Thus CD2AP contains three SH3 domains in its NH2-terminal region, a proline-rich region in the midregion, and a coiled-coil domain and actin-binding sites in the COOH terminus (6, 16, 18). SH3 domains are conserved protein modules known to interact with proline-rich sequences (21, 24) that are present in a variety of signaling and cytoskeletal proteins. The proline-rich region of CD2AP provides several putative binding sites for SH3 domains, and coiled-coil domains are known to mediate protein-protein interactions.

In accord with its proposed function as an adapter molecule, CD2AP has been shown to have several interaction partners. In the kidney in vivo, CD2AP associates with polycystin-2 (18) and nephrin (21a, 27, 28), which are essential for maintaining the integrity of the nephrons (15, 20). In T cells, CD2AP binds the adhesion molecule CD2, enhancing its clustering at the T cell-antigen-presenting cell contact area, thereby affecting the cytoskeletal polarity of the cells (6). In addition, CD2AP interacts with the protocgon product c-Cbl (17), which is involved in tyrosine kinase signaling and regulation of lamellipodia formation and cell morphology (26). Collectively, CD2AP may thus act as a scaffolding protein in various signaling cascades controlling cellular adhesion, motility, and morphology, all processes depending on the actin cytoskeleton.

Interestingly, mice lacking CD2AP develop nephrotic syndrome resembling the human disease. These mice show effacement of the podocyte foot processes and accumulation of mesangial deposits (28). Podocyte foot process effacement and the subsequent development of proteinuria is characterized by disaggregation and redistribution of podocyte actin microfilaments. This suggests an important role for actin and/or the associated proteins in the maintenance of
the foot process organization (29). In line with this, mutations in several actin-associated proteins have been shown to lead to glomerular disease. Thus mutations in the ACTN4 gene, encoding α-actinin-4, cause familial focal segmental glomerulosclerosis (14), and mutations in the podocin gene NPHS2 lead to autosomal recessive steroid-resistant nephrotic syndrome (3). Immunofluorescence studies on cultured podocytes showed that CD2AP colocalizes with F-actin at the leading edge of lamellipodia and in small spots, which were unevenly distributed in the cytoplasm. The spot-shaped F-actin structures were also positive for the Arp2/3 protein complex and cortactin (36). The Arp2/3 complex has been shown to be activated by cortactin and to be involved in the regulation of actin assembly (32, 35). In addition, transient transfection of COS-7 cells with CMS, the human homolog of CD2AP, has been shown to decrease actin fiber formation and lead to concentration of actin into small dots (16). CD2AP may thus have a role in the regulation of the actin cytoskeleton, and the effacement of the podocyte foot processes in CD2AP-deficient mice could result from dysregulation of the podocyte actin cytoskeleton organization.

The above studies on CD2AP and actin cytoskeleton organization rely on immunohistochemistry and do not reveal whether the postulated association between CD2AP and actin is direct or involves mediator proteins. In the present investigation, we have analyzed the association of F-actin and CD2AP at both the cellular and the biochemical levels. Our results indicate that the intracellular localization of CD2AP depends on an intact actin cytoskeleton and that CD2AP associates with the actin cytoskeleton. Most importantly, by direct coprecipitation assays utilizing pure polymerized actin and purified CD2AP fusion proteins, we show, for the first time, that the COOH terminus of CD2AP interacts directly with F-actin. These results suggest that CD2AP functions as a scaffolding protein connecting the actin cytoskeleton to plasma membrane proteins, such as CD2, nephrin, and polycystin-2.

**EXPERIMENTAL PROCEDURES**

**Cell culture.** Mouse M-1 kidney cortical collecting duct epithelial cells (CRL-2038, American Type Culture Collection) were cultured in a 1:1 mixture of Ham’s F-12 medium / Dulbecco’s modified Eagle’s medium containing 5 μM dexamethasone and supplemented with 5% fetal calf serum. 

**Production of CD2AP antisera.** The rabbit antisera (R1774) generated against amino acid residues 6–574 of CD2AP has been described (18). An independent rabbit antisera (R211) was raised against a glutathione S-transferrase (GST)-tagged fusion protein carrying amino acid residues 1–330 of CD2AP. The fusion protein was produced in *Escherichia coli* and purified as recommended by the manufacturer (Amersham Pharmacia Biotech). To show the specificity of the antibody reactivity, the antisera were diluted to working concentration and preincubated with the corresponding purified recombinant protein at 1 or 10 μg/ml at room temperature for 30 min, followed by Western blotting and immunofluorescence microscopy, respectively; this totally competed the signal (Lehtonen S, unpublished observations). Affinity-purified antibodies were obtained by incubating the antisera with the corresponding fusion protein immobilized on filter, followed by thorough washes. The antibodies were eluted using 0.2 M glycine-HCl, pH 2.9, and neutralized with unbuffered 1 M Tris. The affinity-purified antisera R211 was used for immunoelectron microscopy; all other analyses were performed with antisera R1774. The results with affinity-purified antibodies and antisera were the same.

**Immunocytochemistry.** Cells grown on glass coverslips were fixed in 3.5% paraformaldehyde in PBS for 20 min and permeabilized with 0.1% Triton X-100 in PBS for 15 min or with −20°C methanol for 10 min. Oregon green 514 phalloidin and rhodamine phalloidin for detection of F-actin were purchased from Molecular Probes, and the mouse monoclonal antibodies against actin and paxillin were from Amersham Pharmacia Biotech and Zymed, respectively. The mouse monoclonal antibodies against E-cadherin and protein disulfide isomerase (PDI) were from Transduction Laboratories and StressGen, respectively. Primary antibodies and the FITC/TRITC-conjugated secondary antibodies (Jackson Immunoresearch Laboratories) were diluted in PBS supplemented with 0.5% saponin and 5% fetal calf serum. Microscopy was performed with a Zeiss Axiohot 2 microscope and a Leica TCSSP1 confocal microscope.

**Immunoelectron microscopy.** Nickel grids placed on coverslips were covered with Formvar and coated with carbon. The grid assemblies were dipped into 1% poly-l-lysine (molecular weight 114,700; Sigma) and rinsed with PBS, followed by incubation in a moist chamber overnight to make the surface wettable. The assemblies were sterilized with ethanol and rinsed with PBS and culture medium. M-1 cells were plated on the grid assemblies at a density allowing them to reach 70% confluency in 3 days to ensure proper attachment and spreading on the grids. The cells were rinsed with 0.1 M PIPES, pH 6.9, 1 mM EGTA, 4% polyethylene glycol 8000 [microfilament stabilizing buffer (MSB)], followed by incubation with 1 mM short-arm crosslinker diethio (succinimidyl propionate) (Pierce) in MSB. Cells were extracted with 0.2% Triton X-100, 1 mM dithiobis (succinimidyl propionate) in MSB before fixation with 3.5% paraformaldehyde in PBS (2). Filamentous actin was stabilized by 20 μM phalloidin (Molecular Probes) in PBS, and the cells were stained with affinity-purified rabbit polyclonal CD2AP antibodies, a rabbit preimmune serum, and mouse monoclonal anti-actin antibodies (Sigma). Cells were first washed with PBS, followed by a rinse in 20 mM Tris-HCl, pH 7.5, 5 mM NaCl, and 0.01% gelatin [Tris-buffered saline (TBS) buffer]. Colloidal gold-conjugated secondary antibodies (5-nm gold anti-rabbit and 10-nm gold anti-mouse, Sigma) were diluted in TBS. Cells were washed with TBS and postfixed with 2.5% glutaraldehyde, followed by washing with H2O and dehydration in ascending concentrations of acetone. The samples were critical point-dried in a CPD-30 apparatus (Balzers) and observed under a JEOL JEM 1200EX electron microscope.

**Cytoskeleton disrupting treatments.** Mouse M-1 kidney cells grown on glass coverslips were treated with 2 μM cytchalasin D (Sigma) or 3 μM jasplakinolide (Calbiochem) in culture medium at 37°C for 1 h and thereafter processed for immunocytochemistry. In some experiments, the cytochalasin D-treated cells were allowed to recover in the maintenance medium for 1 h before fixation.
Cytoskeleton preparations of cells growing on coverslips. M-1 cells cultured on coverslips were washed twice with PBS and incubated in 4 M glycerol, 25 mM PIPES, 1 mM EGTA, 1 mM MgCl₂, and 0.2% Triton X-100, pH 6.9, at room temperature for 5 min (1). Immunocytochemistry was performed as above.

Preparation of cytoskeletal fractions. Mouse M-1 kidney cells were grown to 90% confluency, washed with cold PBS, and lysed in 150 μl of precooled (in mM) 100 Tris-HCl, pH 7.6, 100 NaCl, 10 EGTA, 1 MgCl₂, 1 PMSF, 10 NaF, 2 Na₃VO₄, 10 β-glycerophosphate, and 0.2 ATP (lysis buffer), as well as 2% Triton X-100, 5 μg/ml aprotinin, and 5 μg/ml leupeptin on ice. In some experiments, 2 mg/ml DNase I (preincubated with 1 mM PMSF on ice for 15 min) were added to the lysis buffer to depolymerize filamentous actin (7). After shaking in an ice bath for 15 min, the crude cytoskeleton fraction was prepared by centrifuging at 10,000 g at 4°C for 10 min. The supernatant was further centrifuged in a Beckman Airfuge at 100,000 g at 4°C for 3 h to separate the membrane skeleton and soluble fractions. The pellets were washed three times and resuspended in lysis buffer, raising their volumes to correspond to the volume of the soluble fraction. Equal volumes of the crude cytoskeleton, membrane skeleton, and soluble fractions were resolved by SDS-PAGE and Western blotted with antibodies against actin (Amersham Pharmacia Biotech) or CD2AP.

Actin filament precipitation assays. Truncated forms of CD2AP encompassing the NH₂- and COOH-terminal domains of the protein (amino acids 1–330 and 331–637, respectively) were subcloned into pGEX-4T-1 vector (Amersham Pharmacia Biotech) to produce GST-tagged fusion proteins. The fusion proteins were produced in E. coli and purified as recommended by the manufacturer (Amersham Pharmacia Biotech). Rabbit skeletal muscle actin (Cytoskeleton) was diluted in G buffer ([in mM] 5 Tris-HCl, pH 8.0, 0.2 CaCl₂, 0.5 dithiothreitol, and 0.2 ATP) to produce a final concentration of 3 μM in the 50 μl polymerization reaction volume. Actin was polymerized by adding 5 μl of 10× actin polymerization initiation buffer [10× buffer (in mM) 500 KCl, 20 MgCl₂, and 10 ATP] followed by incubation at room temperature for 30 min. The CD2AP GST fusion proteins, or GST as a control, were diluted in G buffer to produce a final concentration of 1 μM in the 50 μl polymerization volume. The concentration of the intact COOH-terminal fusion protein was estimated from Coomassie blue-stained gels. The fusion proteins were combined with polymerized actin and incubated at room temperature for 30 min. The samples were centrifuged in a Beckman TL100 ultracentrifuge at 100,000 g at 4°C for 1 h. The pellets were resuspended in SDS sample buffer, and equal amounts of the supernatant and pellet fractions were resolved by SDS-PAGE. The Coomassie blue-stained gels were scanned, and the intensities of actin and the fusion protein bands were quantified by using the NIH Image 1.62 program.

RESULTS

Distribution of endogenous CD2AP in cultured cells. The sequence of CD2AP predicts a cytosolic protein with no apparent membrane spanning regions (6, 16, 18). Accordingly, the predominant distribution pattern of CD2AP in various cultured cells is cytosolic, concentrating around the nucleus in a fine, reticular pattern (Fig. 1, A–D). Double staining for CD2AP and PDI revealed colocalization of the two proteins, suggesting that cytosolic CD2AP localizes to endoplasmic reticulum (data not shown). In addition, accumulation of CD2AP to certain subcellular locations could be seen. In sparsely plated, well-spread cells, CD2AP accumulations were seen along the leading edge formations (Fig. 1A) colocalizing with F-actin (Fig. 1, D–F). Accumulations of CD2AP were also detected in intercellular contact regions (Fig. 1B) and in plaque-like structures often localizing close to cell-cell contacts (Fig. 1C). These plaque-like accumulations of CD2AP were found in solitary cells, subconfluent cells, and confluent cells. The distribution of these structures differed from that of paxillin-positive focal adhesion plaques, although double staining for CD2AP and paxillin revealed partial overlap of the specific signals on rare occasions (Fig. 1, G–I). In subconfluent cells, paxillin-positive focal adhesions were frequently seen in the leading edge regions but rarely in cell-cell contact regions (data not shown). The latter, however, regularly showed concentrations of CD2AP (Fig. 1, A–C).

To analyze whether the CD2AP concentrations colocalize with proteins involved in cell contact formation, we performed double staining for CD2AP and E-cadherin, a component of the cell-cell adherens junction. Analysis of sparsely plated cells revealed both CD2AP and E-cadherin in cellular projections extending from one cell to another on cell contact formation (Fig. 2, A–C). In subconfluent cultures, CD2AP partially colocalized with E-cadherin in cell-cell contact sites (Fig. 2, D–F), suggesting that CD2AP may associate with adherens junctions. Collectively, the majority of CD2AP appears in the cytosol colocalizing with a marker (PDI) for endoplasmic reticulum, but a fraction of the protein accumulates in motile cellular structures and cell-cell contacts, suggesting that CD2AP could be involved in controlling cell movement and adhesion.

Redistribution of CD2AP after cytochalasin D and jasplakinolide treatments. The NH₂ terminus of CD2AP contains three SH3 domains (6, 16, 18). Many SH3 domain proteins are known to associate with cytoskeletal components and to direct the proteins to specific subcellular regions (1). The COOH terminus of CD2AP contains sequences implicated in binding actin (33, 34). To investigate whether CD2AP associates with the actin cytoskeleton, M-1 cells growing on coverslips were subjected to cytochalasin D and jasplakinolide treatments (Fig. 3, A–I). Double staining of nontreated cells with CD2AP antibodies and phalloidin revealed cytosolic distribution of CD2AP, including perinuclear accumulation, but also a distinct signal in the cell periphery rich in F-actin (Fig. 3, A–C). Cytochalasin D, which disrupts the actin cytoskeleton, had a dramatic effect on CD2AP organization. In treated cells, CD2AP appeared as aggregates largely colocalizing with the collapsed actin along the cell periphery (Fig. 3, D–F). On recovery in the maintenance medium, the cytoplasmic and filamentous staining patterns, respectively, of CD2AP and F-actin were restored (not shown). Jasplakinolide stabilizes F-actin and induces actin polymerization (4). Jasplakinolide treatment caused a redistribution of CD2AP into large aggregates, partially colocalizing with masses of actin, which...
was visualized using anti-actin antibodies (Fig. 3, G–I). The results indicate that the organization of CD2AP depends on intact actin cytoskeleton.

**CD2AP is partially retained in detergent-extracted cells.** To further investigate the association between CD2AP and the actin cytoskeleton, we used detergent extraction to produce cytoskeleton preparations of adherent M-1 cells for immuno-fluorescence microscopy. In these preparations, proteins not connected to the cytoskeleton are extracted. In the detergent-extracted M-1 cells, most of the CD2AP-specific fluorescence was lost (Fig. 3J). However, some CD2AP was retained along the cell periphery in the cell contact regions and as diffusely distributed small granular structures (Fig. 3J). The extraction did not alter the organization of the actin cytoskeleton (Fig. 3J). The cytoskeleton preparations exhibited regions positive for both F-actin and CD2AP, but also regions showing either F-actin or CD2AP alone (Fig. 3, J and K). The cells also showed distinct focal adhesion plaques positive for paxillin (not shown). The results indicate that a fraction of CD2AP is resistant to detergent extraction, suggesting that CD2AP may associate with the actin cytoskeleton.

**CD2AP and actin colocalize in cortical actin cytoskeleton.** To further confirm the close association of CD2AP and actin, detergent-extracted whole-mount cells were studied by immunoelectron microscopy. Actin and actin-associated proteins were stabilized by a crosslinker before detergent extraction, and after the extraction, actin was further stabilized by treatment with phalloidin. As a result, the actin cytoskeleton was well preserved, and the motile structures of the cell, such as lamellipodia, leading edges, membrane ruffles, and microspikes, were clearly discernible (Fig. 4A). In immunoelectron microscopy, distinct CD2AP-specific staining could be detected in the cell periphery, especially in leading edges (Fig. 4B). The CD2AP-specific label often associated with the cortical cytoskeleton.
focally as small patches of several gold particles (Fig. 4, B and D), a pattern consistent with the granular staining observed by immunofluorescence microscopy (Fig. 3J). Controls using rabbit preimmune serum showed negligible labeling (Fig. 4C). After double staining for CD2AP and actin, colabeling of the same structures was seen, suggesting a close association of the two proteins (Fig. 4D).

A fraction of CD2AP pellets with membrane skeleton. Highly cross-linked actin filaments, representing the majority of filamentous actin, are pelleted at low-force centrifugation, and free or loosely cross-linked actin filaments, representing the submembranous actin filaments, require high-speed centrifugation for sedimentation (7, 30). To analyze the partition of CD2AP in these two cytoskeletal structures, we prepared M-1 cell extracts and analyzed equal volumes of the 10,000 g crude cytoskeleton fraction, the 100,000-g membrane skeleton fraction, and the remaining Triton X-100-soluble fraction by Western blotting (Fig. 5, lanes marked DNase I–). Most of the CD2AP protein was found in the Triton X-100-soluble fraction (100,000 S, DNase I–). Of the cytoskeleton-associated CD2AP, most was found in the high-speed membrane skeleton fraction (100,000 pellet (P), DNase I–) and a negligible part in the crude cytoskeleton fraction (10,000 P, DNase I–). As expected, most of the actin pelleted in the crude cytoskeleton fraction (10,000 P, DNase I–).

The presence of CD2AP in the membrane skeleton fraction could be due to either Triton X-100 insolubility of the protein or its association with actin filaments. To distinguish between these two possibilities, we depolymerized filamentous actin by using DNase I treatment (Fig. 5, lanes marked DNase I+) as described earlier (7). The depolymerization of F-actin was confirmed by Western blots showing the absence of the free or loosely cross-linked actin in the membrane skeleton fraction after DNase I treatment (Fig. 5, 100,000 P, DNase I+). The depolymerization of F-actin led to a partitioning of CD2AP exclusively in the Triton X-100-soluble fraction (Fig. 5, 100,000 S, DNase I+), suggesting that the protein, indeed, is actin associated.

The COOH terminus of CD2AP directly associates with F-actin. To investigate whether CD2AP and F-actin interact directly, we performed an F-actin precipitation assay using COOH- and NH2-terminal GST-fusion proteins of CD2AP. For this, pure, polymerized actin was mixed with the purified CD2AP fusion proteins. The samples were then centrifuged to pellet filamentous actin and the possible bound fusion proteins. Analysis of equal amounts of the soluble and pelleted fractions revealed that under the conditions used, about one-half of the COOH-terminal fusion pro-
tein pelleted in the presence of F-actin (Fig. 6A, lanes marked actin+). In the absence of actin, the COOH-termin al fusion protein pelleted at a negligible level, suggesting that it does not precipitate by itself (Fig. 6A, lanes marked actin–). Densitometric analysis showed that ~60% of the COOH-termin al CD2AP used in the assay cosedimented with F-actin (Fig. 6B, C+actin). In contrast, the NH2-termin al fusion protein pelleted at a similar level in the absence (Fig. 6C, lanes marked actin–) and presence (Fig. 6C, lanes marked actin+) of F-actin. This suggests that the NH2 terminus of CD2AP does not bind actin but that a part of the
In the present study, we show that endogenous CD2AP and F-actin partially colocalize in cultured epithelial M-1 cells and that disruption of the actin cytoskeleton results in disorganization of CD2AP. Using cytoskeletal fractionation combined with actin depolymerization, we show that a fraction of CD2AP associates with the membrane skeleton. Finally, using an F-actin coprecipitation assay, we show that the COOH terminus of CD2AP directly binds F-actin.

Our immunofluorescence and immunoelectron microscopy analysis showed that the distribution of CD2AP is related to the locomotory status of the cells. The prevailing localization of CD2AP was cytosolic, colocalizing with a marker (PDI) for endoplasmic reticulum. However, in motile cells, CD2AP accumulations were also found along the leading edge, colocalizing with F-actin, and in plaque-like structures, which were distinct from paxillin-positive focal adhesion plaques. CD2AP has previously been shown to associate with the protoncogene product c-Cbl (17), which is involved in tyrosine kinase signaling and regulation of lamellipodia formation and cell morphology (26). Localization of CD2AP in the leading edge of lamellipodia (this study and Refs. 16 and 36) suggests that CD2AP might also have a role in these processes. Furthermore, targeting of c-Cbl to the actin cytoskeleton of NIH3T3 fibroblasts has been shown to require interaction with an actin-associated SH3 domain-containing protein (26), such as CD2AP. CD2AP could thus act as a scaffolding protein in specific signaling cascades controlling cellular motility and morphology.

The above immunofluorescence colocalization studies suggest a close association between CD2AP and a fraction of cellular F-actin. In the present study, we also obtained findings suggesting that CD2AP is associated with a distinct cytoskeletal fraction, the membrane skeleton. First, double staining of detergent-extracted cells revealed that although the majority of CD2AP was extracted from these cytoskeleton preparations, a distinct fraction of the protein was retained.
along the cell periphery, partially overlapping with F-actin in the vicinity of the intercellular contacts. Second, cytoskeletal fractionation confirmed that the majority of CD2AP is soluble but that a fraction of the protein pellets with the loosely cross-linked actin filaments or the membrane skeleton. The crude cytoskeleton fraction, which includes highly cross-linked actin filaments, representing the majority of filamentous actin, contained scarcely any CD2AP.

Our results from actin filament coprecipitation assay, using pure actin and purified CD2AP fusion proteins, demonstrate that the association between CD2AP and F-actin is direct and does not depend on mediator proteins. Furthermore, the results indicate that the interaction between CD2AP and F-actin is mediated by the COOH-terminus of CD2AP. These results agree with the observation that the formation of the specialized cell contact between the T cell and the antigen presenting cell, a process involving adhesion molecules and the actin cytoskeleton (9), is disturbed by overexpression of a CD2AP construct lacking the COOH terminus (6).

The studies reported here provide strong evidence that CD2AP may link the actin cytoskeleton to nephrin, earlier shown to interact with CD2AP (21a, 27, 28).

Nephrin has been suggested to form the framework of the slit diaphragm, the ultrafiltration unit of the glomerulus (11, 12, 25). In congenital nephrotic syndrome of the Finnish type (NPHS1 or CNF) (10), nephrin is mutated (15), leading to loss of slit diaphragms and effacement of the podocyte foot processes (15, 23). As CD2AP−/− mice develop nephrotic syndrome resembling the human disease, CD2AP apparently has a role in maintaining the integrity of the slit diaphragm, supposedly as a protein anchoring nephrin to the cytoskeleton (28). Our present results support this proposal and show that CD2AP may act as a direct adapter between nephrin and the actin cytoskeleton. In line with this, while the present manuscript was under review, it was demonstrated that nephrin anchors the slit diaphragm to the actin cytoskeleton, possibly by linkage to CD2AP (37). Collectively, the data suggest that disturbances in the protein complex, including nephrin, CD2AP, and actin, could account for the alterations observed on foot process effacement in CNF and in CD2AP−/− mice.

Our present and earlier (18) findings suggest that CD2AP may have an important role in the signaling cascade involved in the pathogenesis of the type 2 autosomal dominant polycystic kidney disease (AD-
PKD2) phenotype. In polycystic kidney disease, the kidney tubular cells lose their differentiated function and morphology, and the kidney develops cystic structures lined by flattened epithelial cells, a process apparently involving changes in cytoskeletal organization (5). We have shown earlier that CD2AP interacts in vivo with polycystin-2 (18), mutated in ADPKD2 (20). Mutations in ADPKD2 typically result in the production of truncated forms of polycystin-2 (see Ref. 31), which might be unable to associate with CD2AP, because the interaction involves the COOH-terminal domain of polycystin-2 (18). The data would thus be compatible with a molecular mechanism, in which a mutation in ADPKD2 results in loss of connection, mediated by CD2AP, between polycystin-2 and the actin cytoskeleton. This would then lead to the development of the morphological and pathophysiological changes observed in ADPKD2. The significance of intact cell membrane-actin cytoskeleton interactions for protein complexes containing polycystin-2 is further emphasized by the observation that cytochalasin D disrupts polycystin-1/polycystin-2-containing macromolecular complexes (8). In line with the proposed role of CD2AP in the polycystin-2 protein complex, we also show here that CD2AP partially colocalizes with E-cadherin, a component of this complex (8).

In conclusion, the present findings demonstrate a direct association between CD2AP and the actin cytoskeleton and indicate that CD2AP may act as a direct adapter between the actin cytoskeleton and cell membrane proteins, such as nephrin and polycystin-2. Both nephrin and polycystin-2 have been shown to interact with CD2AP in vivo, thus associating CD2AP with CNF and ADPKD2, respectively. Collectively, the data suggest that disturbances in CD2AP-mediated interactions between membrane protein complexes and the actin cytoskeleton may have an important role in the pathogenesis of the changes characteristic to congenital nephrotic syndrome and polycystic kidney disease.

We thank U. Kiiski for technical assistance and M. G. Farquhar, P. Lappalainen, V.-P. Lehto, V. M. Olkkonen, and I. Virtanen for a critical reading of the manuscript. P. Salmikangas is acknowledged for her advice concerning the F-actin cosedimentation assay.

The work was supported by Academy of Finland Grants 68290 and 71234 (E. Lehtonen), the Emil Aaltonen Foundation (S. Lehtonen), the Wihuri Foundation (E. Lehtonen), the Paulo Foundation (S. Lehtonen), the Finnish Cultural Foundation (S. Lehtonen), the Sigrid Jusélius Foundation (P. Zhao), and the Clinical Research Fund of Helsinki University Central Hospital, Finland (E. Lehtonen).

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