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Cell Biology of the Glomerular Podocyte

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Pavenstädt, Hermann, Wilhelm Kriz, and Matthias Kretzler. Cell Biology of the Glomerular Podocyte. *Physiol Rev* 83: 253–307, 2003; 10.1152/physrev.00020.2002.—Glomerular podocytes are highly specialized cells with a complex cytoarchitecture. Their most prominent features are interdigitated foot processes with filtration slits in between. These are bridged by the slit diaphragm, which plays a major role in establishing the selective permeability of the glomerular filtration barrier. Injury to podocytes leads to proteinuria, a hallmark of most glomerular diseases. New technical approaches have led to a considerable increase in our understanding of podocyte biology including protein inventory, composition and arrangement of the cytoskeleton, receptor equipment, and signaling pathways involved in the control of ultrafiltration. Moreover, disturbances of podocyte architecture resulting in the retraction of foot processes and proteinuria appear to be a common theme in the progression of acquired glomerular disease. In hereditary nephrotic syndromes identified over the last 2 years, all mutated gene products were localized in podocytes. This review integrates our recent physiological and molecular understanding of the role of podocytes during the maintenance and failure of the glomerular filtration barrier.

I. INTRODUCTION

The podocyte is a most spectacular cell type. Its location, its architecture, and its relevance are unique. Almost ignored in renal research for decades, since the mid 1990s, there has been an outset in podocyte research worldwide. However, still today not a single function defined in classic physiological terms can be solidly assigned to the podocyte. We suppose that it functions as a specific pericyte counteracting the high transmural distending forces permitting the high-pressure perfusion of glomerular capillaries, but we do not have any direct evidence. We suppose that the podocyte is crucially involved in establishing the specific permeability properties of the glomerular filter, but we do not know the details. We suppose that the podocyte is responsible for the continuous cleaning of the filter, but we know very little about how this function is carried out.

On the other hand, the evidence accumulates that failure of the podocyte decisively accounts for the initiation of progressive renal diseases, as well as for the maintenance of the progression to end-stage renal failure. With the prolongation of our life expectancy, the incidence of chronic renal failure rises dramatically along with an enormous increase of the burden to healthcare budgets worldwide to provide the expensive renal replacement therapies including dialysis and transplantation. Therefore, all research efforts are welcomed to reach a better understanding of this cell type and to develop rules on how to protect podocytes from injury. We hope that this review will stimulate research in this field.

II. DEVELOPMENTAL ASPECTS

A nephron of the permanent kidney develops from the mesenchymal metanephric blastema by induction through the ureteric bud. The tips of the branching ureteric (collecting) ducts induce the clustering of individual mesenchymal cell aggregates that convert to an epithelial phenotype. Such a cell aggregate represents a nephronanlage that undergoes many mitotic cycles and differentiation stages, connects with the duct, and subsequently generates a nephron. In the very beginning, this process centers around the development of the glomerulus and is generally divided into the following stages: vesicle, comma and S-shaped, glomerular capillary loop, and maturing glomerulus (54, 171, 396).

The vesicle is the first epithelial structure consisting of polarized cells and is surrounded by a basement membrane. On one side it joins with the ureteric bud, and a continuous lumen is formed between the vesicle and the duct. On the opposite side a cleft appears within the growing nephronanlage, producing a comma-shaped or S-shaped profile (depending on the section plane). Figure 1 shows a rat kidney S-shaped body. The lip beneath this cleft is established by a prominent crescent-shaped layer

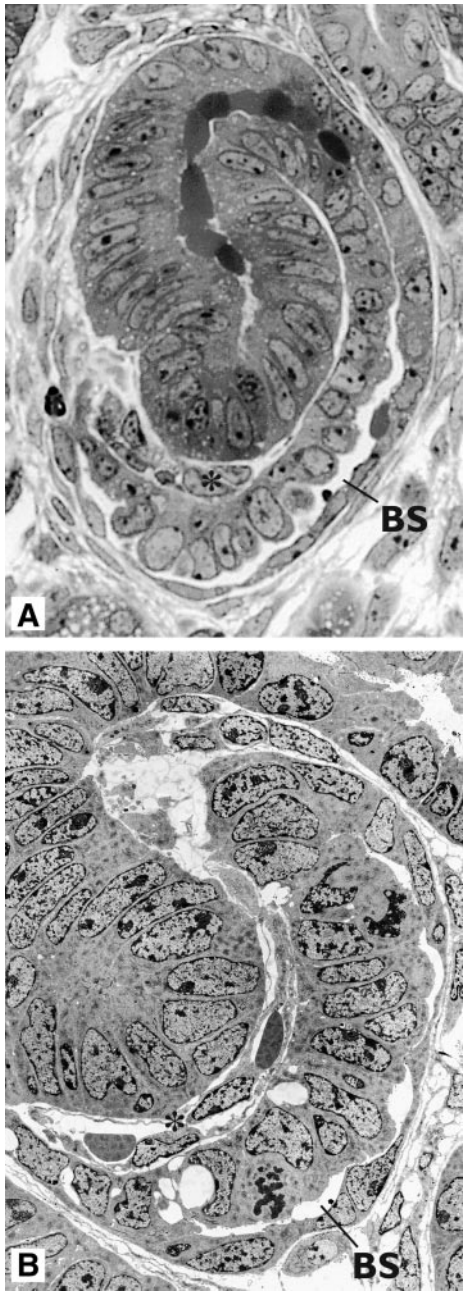

FIG. 1. Rat kidney, S-shaped body is shown. In this stage of development, the podocyte precursor cells are arranged in a crescent-shaped layer of epithelial cells. The cells broadly attached to each other laterally. Mitotic figures are frequently encountered (*B*). The basal aspect of the cells faces the vascular cleft (asterisk) that contains precursor cells of the mesangium and endothelial cells. Apically the podocytes protrude into the future Bowman's space (BS). *A*: light microscopy, magnification ×~5,000. *B*: transmission electron microscopy, ×~13,000.

of epithelial cells which ultimately differentiate into podocytes.

The podocyte precursor cells are simple, polygonal cells. They vividly multiply. During this early stage of glomerular development, the presumptive podocytes are connected by apical junctions. Structurally, these junctions resemble tight junctions, which express ZO-1 (401) but also desmosomal proteins (135). The expression of podocalyxin and of the cell junction protein ZO-1 commences at this stage (401).

As podocytes enter the subsequent capillary loop stage, they begin to establish their characteristic complex cell architecture, including the formation of foot processes and of a slit membrane. At this stage, desmosomal proteins disappear (135), ZO-1 protein migrates from its apical to a basal location where the slit membrane develops (192, 402). In conjunction with the appearance of the slit membrane-associated proteins, nephrin (192), podocin (49) and CD2AP (256) are expressed. This phenotypic conversion is associated with the loss of mitotic activity (302) (see sect. IV) and accompanied by the expression of several other specific proteins, including the actin-associated protein synaptopodin (293), the major surface protein podocalyxin (402), a podocyte-specific membrane protein tyrosine phosphatase, glomerular epithelial protein 1 (Glepp 1) (484), and the final intermediate filament protein vimentin (302).

A large number of transcription factor genes have been identified that are involved in the induction of the renal vesicle and subsequent nephrogenesis. (The following summary excludes those genes that most likely play their major role earlier in ureteric bud branching; for more comprehensive information, see Reference 54.) The *Pax-2* gene, a mammalian homeobox gene, encodes for a transcription factor that is essential for the conversion of cells of the metanephric mesenchyme to the renal vesicle. Gene knock-out experiments show that in the absence of this factor no formation of the vesicle occurs (98, 376, 465). The further differentiation of these early epithelial cells and their maturation to podocytes is then correlated with the decrease of PAX-2 and the rise of WT-1 expression, suggesting that WT-1 may negatively regulate PAX-2 expression (381). Downregulation of PAX-2 appears as a prerequisite to allow podocyte differentiation that is governed by WT-1. Podocyte precursor cells in the vesicle and subsequent stages strongly express the WT-1 protein, and this expression remains a specific marker of podocytes during the entire ontogeny and in the adult (294, 296). Dominant mutations in WT-1 are associated with the Denys-Drash and Frasier syndromes, manifested by glomerulopathy, mesangial sclerosis, and male pseudohermaphroditism (29, 273).

Lmx-1b, a Lim homeobox gene, is first expressed in podocyte precursor cells of the S-shaped body and continues to show this expression throughout nephrogenesis (59). Homozygous *Lmx-1b* mutant mice have podocyte and glomerular basement membrane (GBM) abnormalities at birth, demonstrating an essential role for glomerular development (59). Mutations of the human *Lmx-1b* gene are responsible for the nail-patella syndrome, which is frequently associated with glomerulopathy (99).

Pod-1, a basic helix-loop-helix protein like WT-1, is expressed in podocytes during glomerular development and appears to be involved in differentiation of this cell type (355).

The structural prominence of the podocyte in early nephrogenesis is emphasized by findings pointing to a central role of this cell type in regulating the development of the entire renal corpuscle. The main players in this process appear to be angiogenic factors. The development of the glomerular capillaries and mesangium starts from cells that are found within the cleft above the podocyte layer in the comma-shaped body. These cells are derived from surrounding mesenchymal cells including sprouts from existing vessels (392, 396).

There is increasing evidence that several signaling systems are involved in this recruitment and differentiation process. First the vascular epidermal growth factor (VEGF)-flk-1 axis becomes active. VEGF is expressed in the podocyte precursor cells of the comma-shaped body, and its receptor flk-1 (VEGFR2) is found on endothelial cells in the cleft and adjacent mesenchyme (211, 469), suggesting that VEGF initiates the penetration of endothelial sprouts into the cleft. Indeed, studies using genetic and immunological strategies to block VEGF in vivo (138, 211) have confirmed that VEGF is indispensable for glomerular capillary growth. Surprisingly, after completion of nephrogenesis, podocytes continue to express VEGF; here, the factor has been postulated to play a role in maintenance of endothelial fenestrae (112). Very recently, experiments blocking the VEGF expression after birth have shown that within a few weeks glomerular capillaries disappear and the tuft collapses (211).

Podocytes seem not to express VEGFR1 or VEGFR2 receptors, but they express neuropilin-I, a potential coreceptor for the VEGFR2 receptor. Thus podocytes may bind VEGF, but the functional significance of the expression of neuropilin I on podocytes is not yet clear (158).

In concert with VEGF, angiopoietins (85) play a major role in glomerular capillary development (497). Renal endothelial cells, including those within the cleft of the nephronanlage, express the TIE-1 receptor; its ligands are ANG I and ANG II. Recent evidence suggests that ANG I is derived from podocytes and ANG II from mesangial cells. Both bind to the same receptor (Tie-1), but ANG II fails to activate it; hence, ANG II is an inhibitor of ANG I (497, 511, 512).

A further important regulatory system in early glomerular capillary development is represented by the Eph/ephrin family of membrane receptors and counterreceptors (493). Ephrin-B2 expression is first prominent in the podocyte progenitor cells adjacent to the vascular cleft, and a corresponding receptor, Eph-B4, is expressed on endothelial cells (449), suggesting that cell-to-cell interactions may play an important role in glomerular microvascular assembly.

Subsequent to their recruitment, endothelial cells start to produce platelet-derived growth factor (PDGF)-BB, and the PDGF receptor β becomes expressed by mesangial precursor cells. The function of this axis is required for proliferation and assembly of glomerular capillaries and mesangium (42, 253, 440). Transforming growth factor (TGF)-β1 actions are also implicated in this process of stabilizing glomerular vasculature (258).

After establishment of a glomerular vasculature, signaling events in the opposite direction appear necessary for the final maturation of podocyte function. Production of GBM components by podocytes and its maturation are marked by the replacement of laminin-1 with laminin-11 (consisting of α-5/β-2/γ-1 chains) as well as by the replacement of α-1, α-2 chains of type IV collagen by collagen α-3, α-4, and α-5 (IV) chains characteristic for the mature GBM (282, 284). Recent work from grafting experiments (447) suggests that factors emerging from endothelial cells mediate the switch to laminin-11 [and possibly also to collagen α-3, -4, -5 (IV)] production in podocytes. As shown in several mutant mouse models (73, 160, 282, 285, 314), failure of these changes are all associated with severe structural (podocyte, GBM) and functional (protein leakage) injuries.

III. STRUCTURE OF PODOCYTES

Podocytes are highly differentiated cells. They have a voluminous cell body, which bulges into the urinary space. The cells give rise to long primary processes that extend toward the capillaries to which they affix by numerous foot processes (including the most distal portions of primary processes). The foot processes of neighboring podocytes regularly interdigitate, leaving between them meandering filtration slits that are bridged by an extracellular structure, known as the slit diaphragm. The filtration slits are the site of convective fluid flow through the visceral epithelium. Figure 2 shows the urinary side of the capillary wall, which is covered by the highly branched podocytes.

Podocytes are polarized epithelial cells with a luminal or apical and a basal cell membrane domain. The latter corresponds to the sole plates of the foot processes, which are embedded into the GBM. The border between the basal and luminal membranes is represented by the slit diaphragm. The luminal membrane and the slit diaphragm are covered by a thick surface coat that is rich in sialoglycoproteins, including podocalyxin, podoendin, and others, which are responsible for the high negative surface charge of the podocytes (173, 395) (for details, see sect. XII*B*). Frequently, the apical surface gives rise to a

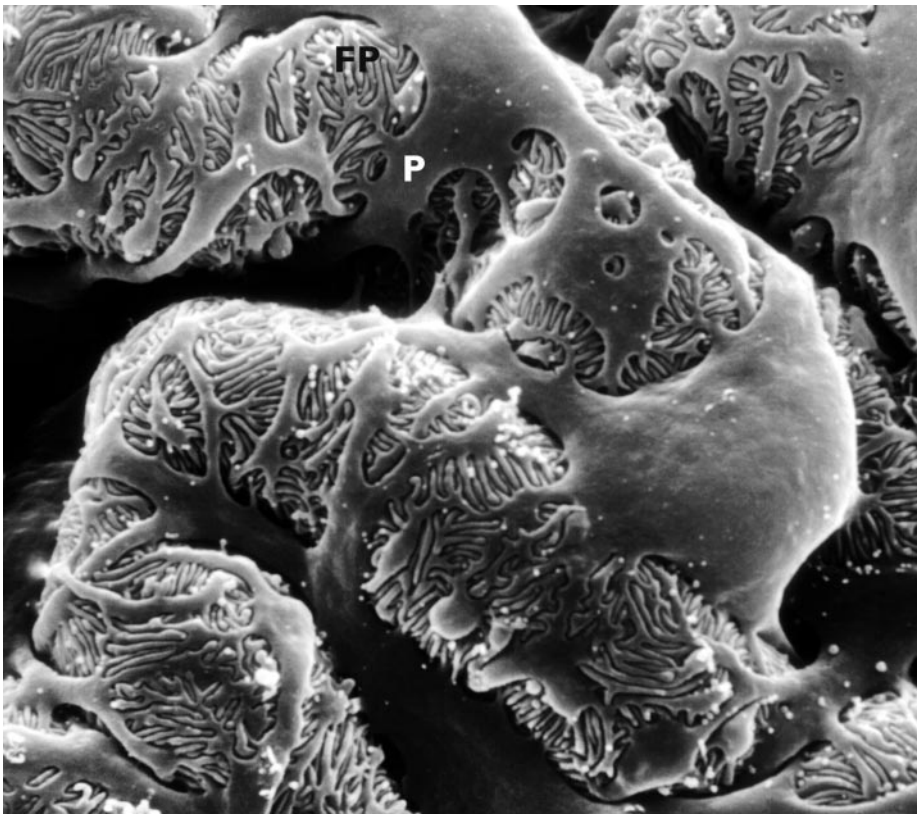

FIG. 2. Scanning electron micrograph of normal rat glomerular capillaries. The urinary side of the capillary wall is covered by the highly branched podocytes. Rat kidney, magnification ×~6,000.

few fingerlike protrusions that float within Bowman's space; under inflammatory conditions, those processes may be dramatically increased in number and length (see below). The basal cell membrane, i.e., the membrane covering the soles of the foot processes, mediates the affixation to the GBM (see below); it regularly contains coated pits (201). Both membranes, apical and basal, are heterogeneous with respect to their lipid composition; both contain densely distributed cholesterol-rich domains (331, 386), corroborating the finding that specific membrane proteins of podocytes are obviously arranged in rafts (409, 432).

The cell body contains a prominent nucleus, a well-developed Golgi system, abundant rough and smooth endoplasmic reticulum, prominent lysosomes, and many mitochondria. In contrast to the cell body, the cell processes contain only a few organelles. The density of organelles in the cell body indicates a high level of anabolic as well as catabolic activity. In addition to the work necessary to sustain the structural integrity of these complexly shaped cells in the adult, most, if not all, components of the GBM are synthesized by podocytes.

A well-developed cytoskeleton accounts for the unique shape of the cells and the maintenance of the processes. Figure 3 shows the organization of the cytoskeleton of podocyte processes. In the cell body and the primary processes, microtubules and intermediate filaments, such as vimentin and desmin, dominate, whereas microfilaments, in addition to a thin cortex of actin filaments beneath the cell membrane (97), are densely accumulated in the foot processes. Here they are part of a complex contractile apparatus (97). As seen from reconstruction studies (388), the microfilaments form loop-shaped bundles, with their limbs running along the longitudinal axis of the foot processes. The bends of these loops are located centrally at the transition to the primary processes and may readily be connected to the microtubules by the microtubule-associated protein τ (389) (Fig. 3). Peripherally, the actin bundles appear to be anchored in the dense cytoplasm associated with the cell membrane of the soles of foot processes (241) (for details, see below).

The filtration slits have a constant width of ~30–40 nm (133, 374) and are bridged by the slit diaphragm. Based on transmission electron microscopy findings in chemically fixed, tannic acid-stained material, Rodewald and Karnovsky (374) have published a model of the substructure of the slit membrane. It consists of rodlike units, connected in the center to a linear bar, together forming a zipperlike pattern. The rectangular pores have the approximate size of an albumin molecule. Thus, in an en face view, the slit membrane has the width and appearance similar to an adherent junction.

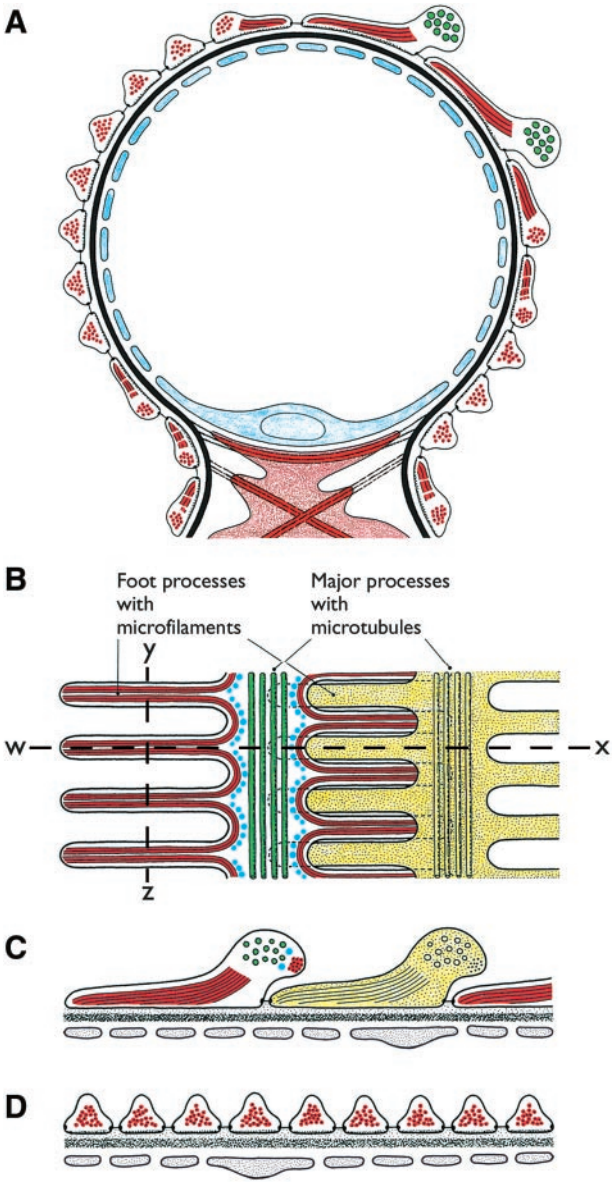

FIG. 3. Cytoskeleton of podocyte processes, shown in several schemes. *A*: cross section through a glomerular capillary loop. The foot processes, which arise from primary processes (2 are depicted in cross section) and cover the outer aspect of the glomerular basement membrane, contain a complete, actin-based contractile apparatus (shown in red). The primary processes contain longitudinally arranged bundles of microtubules (shown in green). Details of the arrangement of cytoskeletal elements in podocyte processes are seen in *B–D*. *B*: view from above. *C*: section of foot processes parallel to and, in *D*, perpendicular to, the longitudinal axis of foot processes (*C* corresponds to the *w-x* line, *D* to the *y-z* line). Two major processes (1 in white, 1 in yellow) with their foot processes are shown. The actin filaments (red) of foot processes form continuous loops that end in the foot process sole plates. Centrally, at their bend, they are in close association with microtubules (green) that run longitudinally in the major processes. The microtubule-associated protein tau (shown in blue) has been localized along the bends of the microfilament bundles and is suggested to mediate connections between the microtubules and the actin filament bundles. [Modified from Sanden (388).]

IV. PODOCYTE CELL CYCLE CONTROL

A. Introduction

As a consequence of the high degree of differentiation of podocytes, it was postulated that they, in analogy to neurons, are unable to proliferate (229). An inability to repopulate a damaged glomerulus with functional podocytes was in good agreement with the progressive ultrastructural lesions seen in podocytes during filtration barrier failure.

Systematic analysis of the cell cycle regulatory molecules in podocytes revealed indeed a tight control of cell cycle quiescence, in sharp contrast to the proliferative capacity of the neighboring mesangial cells (419). An escape of the podocyte from cell cycle blockade results in a disruption of glomerular architecture followed by a rapid decline of renal function, as demonstrated by the deleterious course of collapsing and human immunodeficiency virus (HIV) nephropathy (31, 299, 415).

In this section we discuss the origin of the proliferation block during glomerulogenesis and describe the cell cycle regulation profile in the intact glomerulus and in disease states with and without podocyte proliferation.

The deleterious consequences of uncontrolled cell proliferation or death for every multicellular organism have resulted in an evolutionary conserved, tightly regulated control mechanism for cell cycle proliferation and apoptosis. Cell proliferation is divided into discrete steps of the cell cycle, with the progression into the next step occurring in a sequential and synchronized manner. Quiescent, nondividing cells in the G_0 phase enter the cycle with the G_1 transition, progressing through the DNA duplicating step in the S phase. After the G_2 phase, mitosis occurs in the M phase. After completion of the cycle, cells can enter into the next round of duplication or into the resting G_0 phase. The cell cycle progression is regulated by cyclin and cyclin-dependent kinase (CDK) complexes. CDK activity is controlled by cyclin-dependent kinase inhibitors (CKIs). Each step in the cell cycle is initiated and controlled by a specific set of cyclins, CDKs, and CKIs. However, the p21Cip/Kip family of CKIs, including p27 and p57, can inhibit various cyclin-CDK complexes and are therefore responsible for induction and maintenance of cell cycle quiescence (for an introduction to cell cycle regulation from a nephrologists perspective, please refer to Ref. 419). Entry into the cell cycle can have three consequences for a cell: *1*) cell proliferation, if cyclin expression is followed by repression of the corresponding CKIs; *2*) cellular hypertrophy, if the cell cycle entry with increased protein synthesis is not followed by DNA synthesis and cell division is blocked as a consequence of an increase in CKIs leading to a G_1/S arrest; and *3*) cellular apoptosis as the abortive default pathway of cell cycle

progression. The balance of these three pathways determines the net effect on cell number and size in a given tissue.

An overview of the control elements of the cell cycle relevant for this review is given in Figure 4.

B. Loss of Mitotic Activity and Podocyte Differentiation Coincide During the Capillary Loop Stage in Nephrogenesis

In the S-shaped body state of glomerulogenesis, the "presumptive" podocytes still express markers of a proliferative tissue including proliferating cell nuclear antigen (PCNA) and Ki-67 together with cyclin A and B1 (300). With transition to the capillary loop state, a fundamental phenotype switch occurs with induction of mesenchymal intermediate filaments (see sect. IX), induction of a series of mature podocyte markers (see sect. II), and the development of foot-process interdigitation. In parallel to these changes, the disappearance of cell cycle promoters and a reciprocal upregulation of the cell cycle inhibitors CKI p27 and p57 occurs (32, 70, 297, 300), coinciding with the proliferation arrest of podocytes seen in mature glomerulus. These observations are consistent with the notion of cell cycle quiescence induced by an upregulation of CKI, like p27 and p57, in podocytes as a prerequisite for terminal differentiation. The knock-out of the CKI p57 mice was shown to induce a less differentiated podocyte phenotype, providing the first experimental evidence for involvement of CKIs in podocyte differentiation (513).

C. Podocyte Cell Cycle Control in the Mature Glomerulus

In the mature glomerulus, podocytes have a low level of DNA synthesis and do not readily proliferate under normal conditions nor in a wide variety of renal diseases (232, 239, 335, 371). The inability of podocytes to undergo proliferation in most adult diseases is most likely the consequence of a robust expression or even upregulation of the CKI inhibitors p21, p27, and p57 with disease progression.

Shankland et al. (416) were able to show in a Heyman nephritis model that podocytes do upregulate cyclin A and CDK2 in response to immune-mediated damage. However, a parallel induction of CKI p21 and p27 could effectively blocked entry into the next steps of the cell cycle. According to the above-stated paradigm, this should result in cellular hypertrophy, which is indeed a key finding in the response of podocytes to glomerular damage (see sect. XIII for a detailed discussion). Studies examining the response of rat podocytes to sublytic concentration of complement as an in vitro model of membranous nephropathy showed an augmentation of growth factor-induced DNA synthesis in response to C5b-9 (418). C5b-9 resulted in an increase in the S phase cyclin A and CDK2 and a decrease in CKI p27, but not p21. Furthermore, the M phase proteins cyclin B and cdc2 were repressed. This cell cycle profile led to an increased cellular DNA content without cell proliferation, confirming the above-mentioned in vivo observations of an arrest in podocytes at the G_2/M phase (418). The critical role of the CKI p21 and p27 for a quiescent podocyte phenotype could be demonstrated in p21 and p27 knock-out mice. In these mice, glomerular damage not only allowed podocytes to enter the cell cycle and increase DNA content but also to complete cell division, resulting in a increased cell number in Bowman's space (330). The cells in the Bowman's space had lost differentiated podocyte marker (WT-1 and GLEPP-1), and the animals showed an aggravated disease course (208).

D. Podocytes Are Able to Proliferate in a Defined Set of Glomerular Diseases

The alterations seen in podocyte damage in the p21 and p27 −/− mice resembled the human glomerular diseases with podocyte proliferation, namely, HIV nephropathy (31), collapsing glomerulopathy (298, 472), and the cellular variant of focal-segmental glomerulosclerosis (80). Intact human podocytes differ from murine podocytes in that they do not express p21 but show a robust signal for p27 and p57 (70, 300, 415).

In a systematic analysis of podocyte marker in HIV and collapsing nephropathy, Barisoni et al. (31) showed a severe dysregulation of the cellular phenotype in prolif-

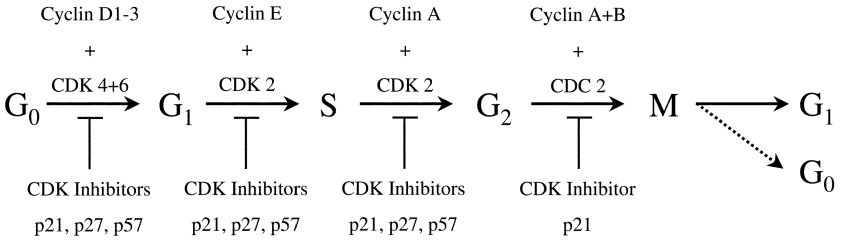

FIG. 4. Schematic diagram of cell cycle regulation. Progression from G_0 to M phase is advanced by cyclins and cyclin-dependent kinases (CDK). CDK inhibitors can block further progression, leading to podocyte hypertrophy. Only elements relevant for this review are shown.

erating podocytes with a loss of podocyte process structure accompanied by a loss of WT-1, Glepp-1, podocalyxin, CALLA, C3b receptor, and synaptopodin. In parallel, an induction of the proliferation marker Ki-67 could be observed (31). In nonproliferative minimal change or membranous nephropathy, none of the above markers was altered compared with healthy controls (32). A comparable podocyte phenotype can be induced in HIV-1 transgenic mice, directly implicating HIV in podocyte dysregulation. Conditionally immortalized podocytes from the HIV-1 transgenic mice showed increased proliferation without contact inhibition. This model system should allow a detailed functional dissection of the podocyte dysregulation by HIV (405).

In comprehensive studies of CKIs in human disease, p27 and p57 levels remained unchanged in minimal change disease and membranous nephropathy (32, 415), blocking podocyte proliferation despite DNA synthesis (25). In collapsing glomerulopathy, cellular focal segmental glomerulosclerosis (FSGS), and HIV nephropathy, p27, p57, and cyclin D1 were lost in areas of podocyte proliferation. A marked increase of the proliferation marker Ki-67 and, surprisingly, of the CKI p21 was seen in the podocyte proliferative diseases. The role of p21 under these conditions is not clear; however, it has recently been recognized that low-level p21 induction can enhance proliferation (341), and a transient p21 increase was seen during human glomerulogenesis in podocyte progenitor cells (300). That podocyte proliferation in collapsing nephropathy is a rapidly deleterious and not a regeneratory pathway has been emphasized in a recent clinicopathological follow-up study (247).

E. Growth Factors Influence Podocyte Cell Cycle Regulation

Growth factors and cell matrix interactions have been reported to influence podocyte behavior (see sects. XI and XIII). Several studies implicate an activation of the TGF-β pathway in podocyte damage in vivo and have shown its antiproliferative effects in vitro (9, 123, 131, 417, 494). A peculiar finding highlighting the unique cell cycle regulation of podocytes in the kidney is the generation of binucleated podocytes after systemic application of basic fibroblast growth factor (bFGF) in vivo. These polyploid podocytes had to go through a full cell cycle including mitosis but appear unable to undergo cytokinesis (125, 234). This cell cycle arrest could also explain contradictory conclusions reached on the ability of podocytes to proliferate in response to cytokines (125).

In analogy to neurons, one could speculate that the complex cytoskeleton effectively inhibited the completion of cell division. For reentry into the cell cycle, a deconstruction of the highly structured cytoskeletal organization in podocytes would be required. However, loss of the actin filaments in the foot processes would abolish glomerular permselectivity. Podocyte cell cycle quiescence appears therefore to be a prerequisite for a functional glomerulus.

F. Podocyte Apoptosis

The third option during cell cycle progression is the entry into the apoptotic pathway of cell removal. Loss of podocytes correlates closely with the degree of progression in type II diabetic Pima Indians, with fewer podocytes per glomerulus in the rapidly progressing group compared with slow progressors (252, 279, 336, 445). In puromycin-induced nephrosis in rats, a close correlation between the degree of glomerular damage and the reduction of podocyte number per glomerulus was observed (209). Because detachment of cells is able to induce cell death, a process termed anoikis (129), disruption of podocyte-GBM interaction could be a critical final event in podocyte damage (see sects. VIII and IX). Hara and coworkers (156, 157, 305) have indeed detected cells positive for podocyte marker in the urine of a variety of renal diseases. Interestingly, a reduction in urinary podocyte excretion was found with improved glycemic control in early diabetic nephropathy (304).

In the first study of the molecular mechanism of apoptosis induction in podocytes, Schiffer et al. (398) could unequivocally demonstrate a wave of apoptosis in the early stages of glomerulosclerosis in TGF-β1 transgenic mice. TGF-β and the downstream TGF-β signaling molecule Smad7 were able to induce apoptosis in cultured podocytes. TGF-β required p38 mitogen-activated protein (MAP) kinase and caspase-3, whereas Smad7 blocked the nuclear translocation of NF-kappaB. Involvement of CKI inhibitors in podocyte apoptosis can be concluded from the increased incidence of podocyte apoptosis in glomerulonephritis in p21 $-/-$ mice (208). As these mice exhibit significant podocyte proliferation, the activated proapoptotic pathway may be quite different from that seen in cell death in quiescent podocytes.

In summary, podocytes in the adult kidney are unable to undergo regenerative proliferation to compensate for a loss of podocytes or an increase in GBM surface area. As a consequence of the strict block in cell division, podocytes can progress to the cell cycle and can even undergo nuclear division in a variety of glomerular diseases, but not cell division. The CKIs p21, p27, and p57 appear to be responsible for this G_1/S transition block, inducing the considerable podocyte hypertrophy seen in progressive renal failure.

An escape of podocytes from the strict cell cycle control is not a regenerative, but rather a disastrous event, causing rapid glomerular destruction.

V. CELL CULTURE OF PODOCYTES

A. Culturing of Glomeruli

In the past, due to the lack of available specific morphological and immunological markers for cultured podocytes, it was uncertain whether outgrowing glomerular cells were of a visceral or parietal origin. One important question was to address whether podocytes would be seriously damaged or survive at all after the culturing of glomeruli and which structural changes of podocytes might occur in situ. By using transmission and scanning electron microscopy, Norgaard (316) investigated the effect of culturing isolated glomeruli on podocyte morphology. Just 8 h after glomerular culture, a broadening of podocyte foot processes, slendering of the primary and secondary foot processes, and the appearance of many microvilli occurred. Thereafter, slit pores became narrowed and were often occluded by a tight junction. Structures resembling slit diaphragms displaced from the basement membrane occurred. After 2 days, retraction of foot processes was completed and slit pores disappeared. Thereafter, podocytes rounded up and were in contact with each other and found in a single-layered epithelium. In addition, podocytes showed a decreased iron staining, indicating a reduction of their anionic proteins on their cell surfaces. Thus it has been suggested that the phenotype of podocytes in culture resembles the phenotype of the podocytes in the fetal glomerulus and that the morphological changes are similar to those seen in injured podocytes (316, 317). Subsequent studies have shown that the first cells to grow out of the glomerulus were of epithelial origin (224).

B. Characterization of Outgrowing Glomerular Cells

Two early outgrown glomerular cell types have been identified in several studies: a small, polyhedral ciliated cell which grows in colonies with the cells joined by junctional complexes (41, 78, 224, 276) and a second very large, often multinucleated cell (317, 506). According to the structural resemblance with glomerular cells in situ, some authors have suggested that the first cell type is derived from the parietal epithelium of Bowman's capsule, whereas the second is derived from the visceral epithelium (56, 317, 506).

However, polyhedral-shaped cultured glomerular epithelial cells have been shown to react with megalin, 27 A, and Fx1A fraction, antibodies which recognize antigens in the glomerulus that are only expressed by podocytes (65, 78, 276). In addition, in a rat podocyte cell line and a simian virus 40 (SV40)-transformed human podocyte cell line, which show a cobblestone appearance, several podocyte-specific antigens have been detected, indicating that cells were of visceral origin (19). In contrast, a lack of expression of podocyte markers has been found in early cultured glomerular epithelial cells (169). By analyzing the pattern of several podocyte-specific antigens on cultured glomerular epithelial cells, two groups came to the conclusion that most if not all glomerular-derived cells with a polygonal appearance were of parietal origin (169, 505). Some of these contrasting findings in morphology and antigen presentation of glomerular-derived cells might be due to the different techniques and conditions used for culturing glomeruli. Another explanation for the different observations is the fact that podocytes change their characteristics in cell culture. Reiser and co-workers (294) observed that glomerular-derived proliferating cells in the first cell culture passage exhibit a cobblestone appearance and that they express WT-1 and *O*-acetylated ganglisoide, specific markers of podocytes in vivo, but not synaptopodin, a marker of podocyte foot processes in vivo. Four days after reaching confluency, the cobblestone cells began to converge into large arborized cells, which developed processes. These arborized cells exhibited WT-1 and *O*-acetylated ganglisoide, and they also showed a marked expression of synaptopodin, indicating that these cells possess markers of podocyte foot processes (294). Very recently, the morphology and expression of podocyte markers from cellular outgrowths from descapsulated glomeruli, encapsulated glomeruli, and tubular fragments have been reinvestigated. Cells outgrown from decapsulated glomeruli tend to become elongated to as much as 100–200 μm and possess long thin cytoplasmatic processes, which often overgrow neighboring cells. These cells stained strongly with antibodies against WT-1, synaptopodin, and podocalyxin but not with an antibody against nephrin. In contrast, cells outgrown from encapsulated glomeruli had a cobblestone appearance, and they exhibited lamellipodia. WT-1 and synaptopodin were not detected in cells from tubular fragments, but a weak staining of WT-1 and synaptopodin was detected in pan cadherin positive parietal cells. Thus it has been suggested that parietal epithelial cells might transdifferentiate into podocytes or that alterations of parietal epithelial cells in culture may occur (503). Interestingly, 27-kDa heat shock protein (hsp27), P-31 antigen, and vimentin, proteins which are only expressed in podocytes in vivo, are also expressed in cultured parietal epithelial cells and even in cultured tubular epithelial cells. In outgrowing cells from encapsulated glomeruli, large irregularly shaped cells could be also observed after ~6 days, but their staining pattern was identical to cobblestone cells. Therefore, the authors suggested that these cells might have been converted from parietal epithelial cells (503).

C. Conditional Immortalized Podocytes

Differentiated podocytes in primary culture show little proliferative activity, and thus it is difficult to propa-

gate a cloned podocyte cell line or to perform experiments that require a large number of cells. In addition, some contamination by other glomerular cell types cannot be excluded in early cell culture passages. Therefore, a conditionally immortalized podocyte cell line has been propagated from a transgenic mouse expressing a temperature-sensitive SV40 large T antigen (295). Cells that are grown under nonpermissive conditions, i.e., at 37°C, stop growing and exhibit many morphological and immunologic properties of differentiated podocytes, i.e., they are arborized and express synaptopodin (295). Very recently, a conditionally immortalized human podocyte cell line has been established by transfection with a temperature-sensitive SV40-T gene. At the permissive temperature of 33°C, these cells grew in a cobblestone morphology. Differentiated human podocytes that were grown at 37°C expressed markers of differentiated podocytes in vivo, including nephrin; podocin; CD2AP and synaptopodin; ZO-1; α-, β-, and γ-catenin; and P-cadherin. Thus this cell model seems to be a good in vitro tool for studying human podocyte biology (387). Figure 5 shows undifferentiated and differentiated, conditionally immortalized human podocytes expressing the slit membrane proteins P-cadherin, podocin, and nephrin.

In conclusion, at this point there is no doubt that podocytes with an arborized phenotype can be propagated in primary culture and that glomerular cells that first show a cobblestone appearance can convert into arborized cells which then express markers of podocytes and even of the slit diaphragm in vivo. There is the possibility that parietal cells can convert into arborized cells. However, compared with immortalized podocytes in cell culture, these cells seem to express WT-1 and synaptopodin much weaker, and they do not express nephrin (503). Further studies have to show whether these cells, compared with podocytes in culture, may be able to express other markers of podocytes to study the process of transdifferentiation of these cells. It is easier, compared with the investigation of the podocytes in situ, to investigate biological functions of podocytes in vitro. However, it is obvious that like other cell types, podocytes in monoculture cannot mimic completely the complex in vivo characteristics of podocytes. Many cellular functions change during culturing of cells, and therefore, results obtained from podocytes in culture have to be interpreted with care.

VI. GENE EXPRESSION ANALYSIS OF PODOCYTES

The interest in mRNA expression analysis in glomeruli has been fueled by the rapid developments in molecular biology. These novel techniques could offer information about nature and prognosis of disease processes activated in podocytes. The real-time polymerase chain reaction (PCR) allows highly accurate template quantification from minimal tissue samples (67, 119). The cDNA array technology displays gene expression patterns of thousands of mRNAs in a single reaction, but requires considerable amounts of starting material (11).

Reverse transcription (RT) PCR-based approaches have been used for some time for gene expression analysis of microdissected specimen in human and experimental renal disease (51, 351). Several technical problems including limitation in mRNA quantity, available quantification systems, and biopsy population could be recently overcome allowing for high-throughput analysis of a series of cDNAs (68). Also, laser microdissection and RNA expression analysis have been shown to be feasible on glomerular cross section of frozen or Formalin-fixed material, detecting, i.e., WT-1 and synaptopodin as podocyte-specific cDNAs (67, 68). A summary of a current protocol applicable for routine application in a multicenter setting is described in Figure 6.

The above expression analysis of microdissected glomeruli still contains glomerular endothelial and mesangial cells. The same holds true for a series of experimental systems developed to study the cellular functions of podocytes including whole animal experiments, transfilter organ culture systems, kidney cortex slices in culture, and isolated glomeruli (see above). The selective analysis of glomerular epithelium-derived cells is only possible in cell culture, but even with the latest technologies questions concerning dedifferentiation phenomena remain (see above). As a consequence of these technical limitations, our understanding of the podocyte cell biology in vivo remains incomplete. As an alternative approach, an in vivo single-cell analysis has been established (404).

The amount of RNA in a single cell is estimated to be 0.1–1 pg and is difficult to handle experimentally. A modification of the PCR was used by Lambolez et al. (245a) for single-cell RT-PCR analysis of cultured neurons. This method can only be applied to tissue allowing access to single cells. The unique glomerular anatomy with the podocytes residing on the outside of the GBM makes this cell type an ideal target for single-cell RT-PCR. Podocytes can be aspirated selectively under visual control with a micropipette preventing any contamination from nearby tissue. With the application of a modified real time RT-PCR, a quantitative approach to podocyte-specific gene expression has been demonstrated (226, 404). A schematic representation of the experimental protocol is given in Figure 7. Combinations of this technique with immunhistology and in situ hybridization should prove advantageous to investigate patterns of gene expression and elucidate the roles of specific genes in podocyte function.

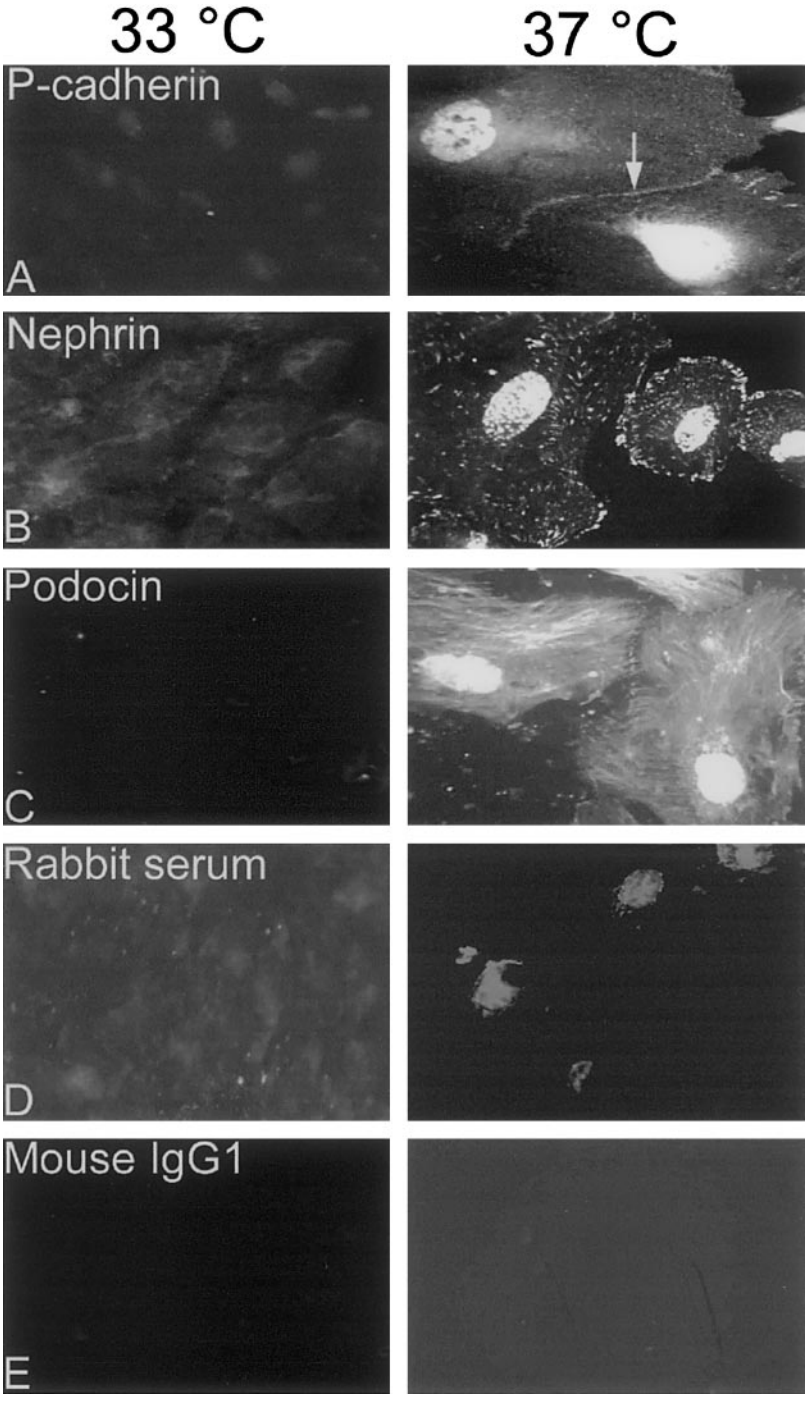

FIG. 5. Cell culture of podocytes. Conditionally immortalized human podocytes in culture are shown. *Left panels*: cells grown under permissive conditions (at 33°C). *Right panels*: cells grown under nonpermissive conditions (at 37°C). Only differentiated podocytes under permissive conditions express the slit membrane proteins P-cadherin, nephrin, and podocin (for details, see sects. V and VII). (From Saleem M. A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 13: 630–638, 2002. Copyright 2002 Lippincott Williams & Wilkins.)

VII. GLOMERULAR FILTRATION BARRIER AND SLIT MEMBRANE

Podocytes form a tight network of interdigitating cellular extensions, called foot processes, which are bridged by so-called "slit diaphragms." Figure 8*A* shows a view of a podocyte in situ, and Figure 8*B* shows the composition of the glomerular filter including the porous endothelium, the GBM, and the podocyte foot processes with the interposed slit diaphragm. The filtration barrier is freely permeable by water and small solutes, but to a large extent, the size selectivity of the filtration barrier for proteins is represented by the slit diaphragms of podocytes. There is a good body of evidence that the podocyte slit diaphragm resembles an adherens-like intercellular junction. On grazing sections the slit diaphragm has morphological features reminiscent of adherens junctions, including the presence of a wide intercellular gap and a

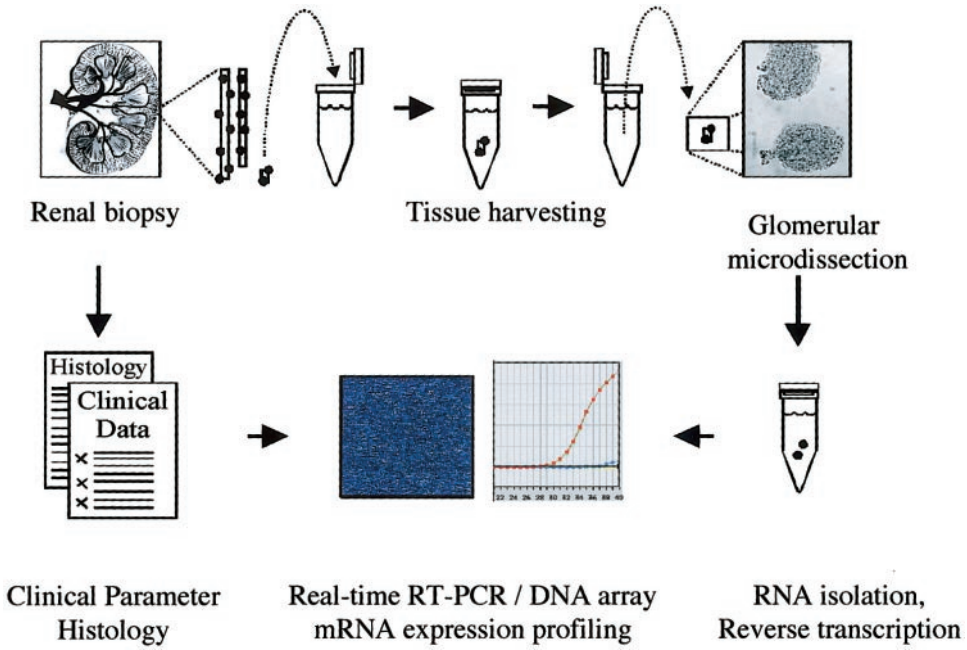

FIG. 6. Gene expression analysis in microdissected human glomeruli. From a freshly taken renal biopsy specimen, a cortical part is conserved in an RNase inhibitor solution. Glomeruli are obtained by manual or laser-assisted microdissection; RNA is isolated, reverse transcribed, and analyzed by real-time RT-PCR or cDNA array hybridization after linear amplification. Expression profiles can be correlated with clinical parameters and histological alterations. For detailed descriptions, see Cohen and coworkers (67, 68).

central dense line (374). Moreover, the slit membrane arises from a tight junction during glomerular development, and the tight junction zonula adherens-associated zonula occludens protein ZO-1 is concentrated along the cytoplasmic surface of the slit diaphragm (401). Our understanding of the molecular structure of the slit diaphragm has been greatly improved in the last few years. Several molecules, including ZO-1 (401), nephrin (379), CD2AP (424), FAT (183), and P-cadherin (368), have all been shown to be expressed within the slit diaphragm, and some of those molecules play a major role for its integrity. A schematic drawing of the molecular equipment of the podocyte foot processes is shown in Figure 9.

A. ZO-1

ZO-1 is a 225-kDa protein that is localized at the cytoplasmatic face of intercellular junctions. ZO-1 is a member of the membrane-associated guanylate kinase (MAGUK) protein family. ZO-1 may play a role in organizing signal transduction and transmembrane protein complexes. ZO-1 contains three copies of the PDZ domain. It is concentrated in slit diaphragms and has been demonstrated to interact with the components of cell-cell junctions (occludin, α-catenin, and ZO-2) and cytoskeletal networks (spectrin, F-actin) (287).

ZO-1 is localized in the podocyte foot processes

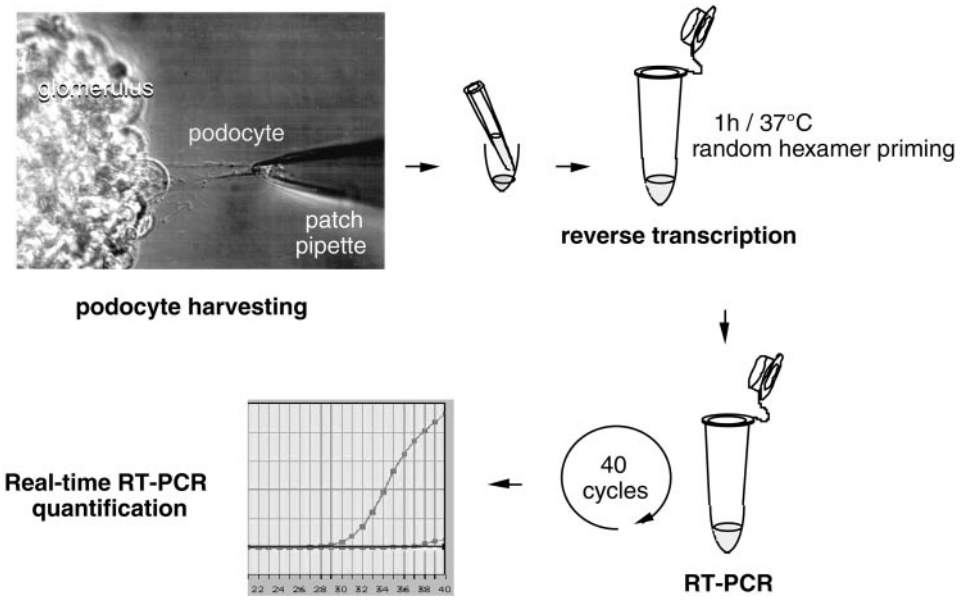

FIG. 7. Schematic of single podocyte RT-PCR. Single podocytes are aspirated by negative pressure applied to the interior of the micropipette; RNAs of a single podocyte are reverse transcribed and quantified using real-time RT-PCR technology. For detailed descriptions, see Schroeppel et al. (404) and Kretzler et al. (226).

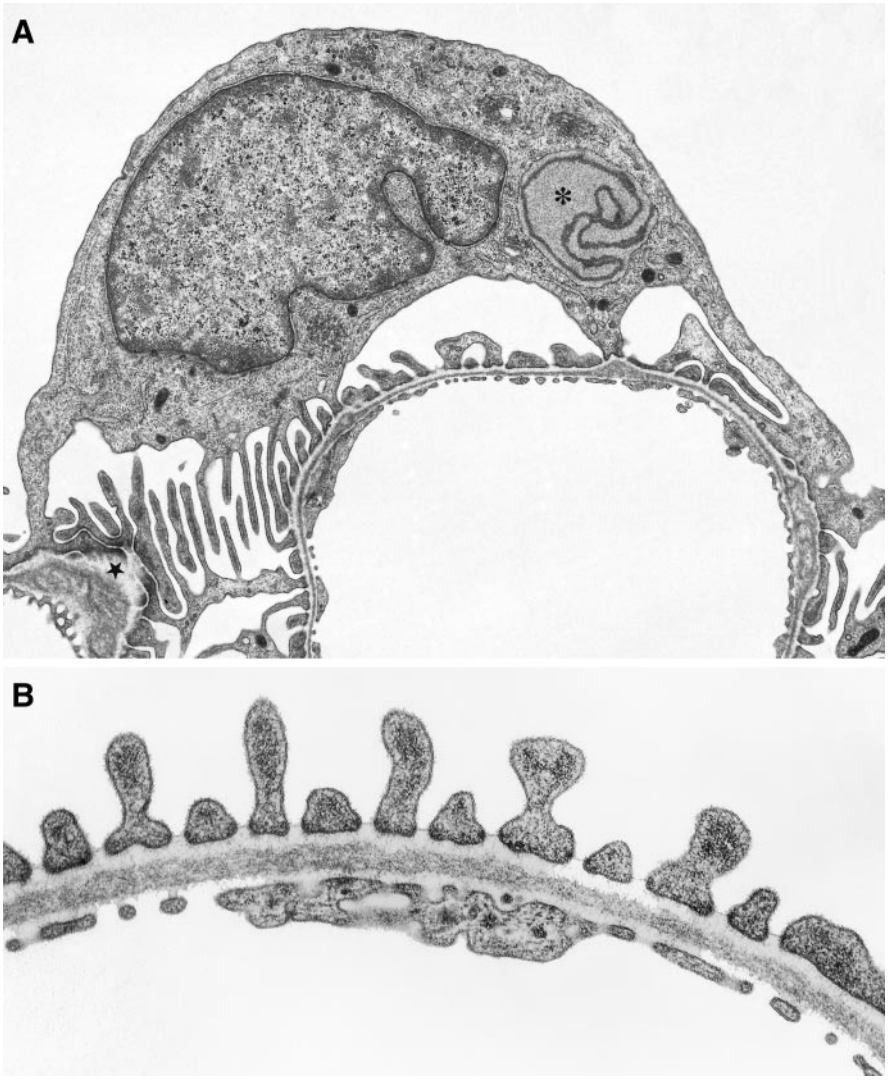

FIG. 8. *A*: low-power view of a podocyte in situ. The cell body is connected to the capillary wall by processes that finally split into the interdigitating foot processes on the outer surface of the glomerular basement membrane. In the *bottom left*, grazing section hits the slit membrane (star). Near the nucleus a characteristic membrane-bound inclusion body (asterisk) is visible; its relevance is unknown. *B*: the glomerular filter consists of three components: porous endothelium, glomerular basement membrane, and podocyte foot processes with the interposed slit membrane. The endothelial pores are not bridged by a diaphragm. Figure is transmission electron microscopy from a rat. Magnification: *A*, ×~11,000; *B*, ×~48,000.

where it is expressed precisely and continuously at the points of insertion of the slit diaphragms into the lateral cell membrane. During development, ZO-1 appears early at a time when the apical junction complexes between podocytes are composed of a kind of atypical intercellular junction. ZO-1 is connected with these junctions during the time they migrate down the lateral cell surface. These junctions disappear and are replaced by slit diaphragms. It has been suggested that the slit diaphragm is a variant of the tight junction (401). In mice, ZO-1 and nephrin are closely colocalized in the mature glomerulus, but it has been suggested that they may arrive at their final positions from opposite directions (191). In humans, ZO-1 is first expressed in the late S-shaped bodies simultaneously with nephrin. ZO-1 has been detected at the basal margin of the developing podocytes, but also on the lateral surfaces as strings and dots. Like nephrin, ZO-1 is located especially in junctions with ladderlike structures, but in contrast to nephrin, the expression of ZO-1 was more abundant at the intercellular junctions during development (380). In fetal NPHS1 kidneys with Fin-major/Fin-major genotype, expression of ZO-1 did not change, indicating that proteinuria must not ultimately result in an altered expression of ZO-1 (380). However, injection of an antibody against nephrin resulted in a progressive decrease of ZO-1 protein expression in podocytes as early as 1 h after antibody injection. After 5 days, ZO-1 could not be detected in these proteinuric rats. This suggests that this particular antibody against nephrin may induce additional alterations of the signaling pathways in podocytes, leading to a downregulation of ZO-1 protein (193).

B. Nephrin

1. Expression of nephrin

NPHS1 has recently been identified as the gene whose mutations cause congenital nephrotic syndrome of

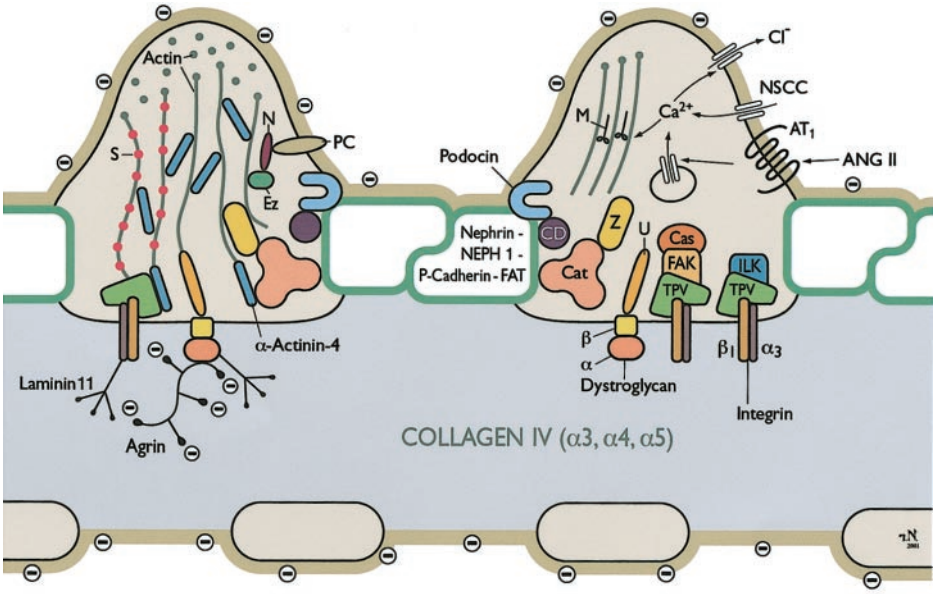

FIG. 9. Schematic drawing of the molecular equipment of the podocyte foot processes. Cas, p130Cas; Cat, catenins, CD, CD2-associated protein, Ez, ezrin, FAK, focal adhesion kinase, ILK, integrin-linked kinase; M, myosin; N, NHERF2; NSCC, nonselective cation channel; PC, podocalyxin; S, synaptopodin; TPV, talin, paxillin, vinculin; U, utrophin; z, ZO-1. See text for further explanations. [Modified from Endlich et al. (106).]

the Finnish type, an autosomal recessive disease affecting ~1:10,000 newborns in Finland (205). A total of 50 mutations have been reported so far. The Fin-major (2-bp deletion in exon 2 that results in a frameshift and introduces a stop codon within the same exon) and the Fin-minor (nonsense mutation in exon 26 resulting in a stop at Arg-1109) mutations are the two most commonly found mutations (>90% of all Finnish patients with congenital nephrotic syndrome; NPHS1) (38). The respective gene product of NPHS1, nephrin, a 180-kDa transmembrane protein, is exclusively expressed by glomerular podocytes within the kidney and predominantly localized to the glomerular slit diaphragm (205, 379). *N*-linked glycosylation has been shown to be important for nephrin folding and thereby plasma membrane localization (500). Mutations of the NPHS1 gene are characterized by massive proteinuria which starts already in utero. Patients with a congenital nephrotic syndrome of the Finish type are treated with early nephrectomy and renal transplantation, but ~20% show recurrence of nephrotic syndrome. The reason for this phenomenon seems to be an increased antibody titer against nephrin in these patients (485).

During development, beginning with early capillary loop stage glomeruli, nephrin expression in mice has been demonstrated on or near intercellular junctions between forming foot processes of podocytes (364). Nephrin could neither be detected in primitive nephric structures, such as comma- and S-shaped bodies, nor in lateral junction complexes between immature podocytes. Nephrin expression has been detected in the earliest slit diaphragm regions between adjacent cells, suggesting that localization of nephrin corresponds with the first appearance of the definitive slit diaphragm during development (96, 132, 364). In humans, nephrin mRNA was first detected in late S-shaped bodies of 13- to 23-wk human fetal kidneys. Immunoelectron microscopy studies show that nephrin is present in junctions with ladderlike structures between the differentiating podocytes. In NPHS1 mutant glomeruli, filamentous ladderlike structures and slit diaphragms are missing, whereas ZO-1 and P-cadherin, two other components of the slit diaphragm, are expressed normally. Thus formation of the ladderlike structures, as well as the slit diaphragms, seemed to be dependent on the expression of nephrin, but early junctions between the developing podocytes at the capillary loop stage were normal. Therefore, nephrin is not needed for the early development and migration of junction complexes at the S-shaped and capillary loop stages (380). On the basis of the structure of the glomerular podocyte slit diaphragm and the electron microscopic localization of nephrin, it was suggested that the NH_2-terminal six immunoglobulin repeats of nephrin form interdigitating zipperlike homophilic interactions. Whether nephrin participates in homophilic interactions remains to be established (379).

In addition to its expression in podocytes, nephrin was also detected in different regions of the brain and in the pancreas (354). In the pancreas, nephrin has been shown to be expressed in the β-cells of the islets of Langerhans (338).

Electron microscopic examination of NPHS1 kidneys reveals a thinner lamina densa of the GBM than in controls, but no other structural abnormality of the GBM has been detected so far (22). Inactivation of NPHS1 in mice leads to an immediate massive proteinuria and edema after birth, causing death within 1 day. The kidneys of NPHS1-deficient mice showed effacement of podocyte foot processes and the absence of the slit diaphragm (354).

Nephrin knock-out mice showed a normal structure of the glomerular basement membrane and a normal expression of the glomerular basement membrane proteins type IV collagen and laminin. The expression of podocyte-specific proteins, such as ZO-1, P-cadherin, and FAT, were not changed in nephrin knock-out mice. In the latter study it has been also demonstrated that α_3-collagen IV knock-out mice were normal until *week 4*, with the exception of sporadic ultrastructural defects in the glomerular basement membrane and a lack of α_3-, α_4-, and α_5-chains of type IV collagen and alterations in the laminin content of the GBM. In contrast, up until *week 4*, these mice did not show any change of the molecular structure of the slit diaphragm. However, the inception of proteinuria starting at *week 5* in α_3-collagen knock-out mice was associated with slit diaphragm alterations, podocyte effacement, and a significant reduction in the expression of nephrin. Thus a decreased expression of nephrin correlates with a loss of glomerular filter integrity (151, 354).

Injection of the monoclonal antibody (MAb) 5–1-6, which recognizes the extracellular domain of nephrin, induces proteinuria (464). Interestingly, after injection of MAb 5–1-6, focal areas of effacement occurred, suggesting that alterations of nephrin are not accompanied by structural changes of the podocytes. It may be speculated that MAb 5–1-6 leads to activation of signaling pathways, resulting in the disturbance of the cytoskeletal architecture of the podocytes (464). After injection of the antibody, the glycosyl matrix of the podocytes remained intact, but the number of anionic sides expressed by heparan sulfate as well as carboxyl groups had decreased (130).

2. Expression of nephrin in glomerular diseases

Because a decrease of nephrin expression has been suggested to be associated with proteinuria, studies have been conducted to explore whether nephrin expression is altered in glomerular diseases. With the use of RT-PCR in isolated glomeruli, it has been shown that mRNA expression of nephrin is decreased in glomeruli from patients with minimal change nephropathy and membranous nephropathy (132). Moreover, in membranous nephropathy, minimal change nephropathy, and FSGS, but not in IgA nephropathy, a reduced nephrin staining and a granular redistribution of nephrin has been reported. In membranous nephropathy, granular deposits of nephrin were colocalized with the extracellular immune deposits (96). A changed expression of nephrin has also been reported in experimental animal models such as puromycin aminonucleoside nephrosis, mercuric chloride-treated rats, as well as after injection of an antibody against nephrin, in which the pattern of nephrin IF staining shifted from epithelial/linear to granular (170, 262, 263, 464). During Heymann nephritis, nephrin dissociates from actin, and its expression is reduced in early stages of this experimental membranous nephropathy (510).

In contrast, by using in situ hybridization and immunohistochemistry, Patrakka et al. (345) found neither a change of nephrin mRNA expression nor a decrease or change of the distribution pattern of nephrin in glomeruli from pediatric patients suffering from proteinuria caused by minimal change nephrosis, FSGS, and membranous nephropathy. The authors state that immunohistochemistry and in situ hybridization may not be optimal methods for studying the role of nephrin in proteinuric renal diseases, because both methods may fail to detect small alterations in the amount or distribution of nephrin (345).

Controversial findings have been reported on the expression of nephrin in diabetic nephropathy. In the streptozotocin model of the rat and the nonobese diabetic mouse, mRNA expression of nephrin increased up to twofold during several weeks of follow-up. Glomeruli from diabetic animals showed an additional nephrin localization, i.e., a weaker reactivity at the epithelial aspect while a more intracellular localization was observed (1). In contrast, others have found a decrease in both gene and protein expression of nephrin in streptozotocin diabetic rats (48). The controversial finding might, at least in part, be explained by the fact that nephrin expression was investigated at different time points after induction of diabetes in the studies (4, 8, and 16 wk vs. 32 wk; Refs. 1, 48). In vitro studies show that preformed immune complexes, i.e., agIgG4 and other agents like membrane attack complex (MAC), tumor necrosis factor (TNF), and puromycin, which are known to change the cytoskeletal arrangements in podocytes, induce a redistribution and loss of nephrin from the cell surface of cultured human podocytes. Cytochalasin B, which disorganizes microfilaments, prevents nephrin redistribution on the surface of podocytes (96).

At this point, little is known about the signaling pathways involved in the regulation of nephrin expression. Phorbol 12-myristate 13-acetate has been reported to increase the nephrin expression in A293 cells, indicating that nephrin expression is controlled by protein kinase C (486).

3. Nephrin is a signaling molecule

The function of nephrin in slit diaphragm maintenance is poorly understood. Structural analysis suggests that nephrin is a member of the immunoglobulin superfamily (IgCAM). It has an extracellular portion containing eight Ig motifs and one type III-fibronectin domain. The intracellular domain contains eight tyrosine residues, suggesting that nephrin may act as a signaling adhesion molecule (205).

Genetic evidence indicates that CAMs of this immunoglobulin superfamily are important for activity-depen-

dent synapse formation at the neuromuscular junction in *Drosophila*. They have also been implicated in synaptic remodeling during learning in *Aplysia* (264, 268). CAMs are also signaling molecules which are involved in modulating cell-cell and cell-extracellular matrix (ECM) interactions, and they induce signal transduction events, which are crucial for cell adhesion, motility, cell growth, and survival (430). Evidence for the hypothesis that nephrin acts as a signaling molecule was raised from mutation analysis studies. A frequent point mutation, a C to T substitution at nt3325 in the NPHS1 gene, results in a nonsense mutation that leads to the deletion of the COOH-terminal 132 of 155 residues of the intracellular domain. The deletion of most of the cytoplasmic domain results in foot process effacement and proteinuria, indicating the importance of this domain (205).

It has recently been suggested that nephrin is associated in an oligomerized form with lipid rafts. Nephrin-containing rafts could be immunoisolated with a 27A antibody which stains a podocyte-specific 9-*O*-acetylated GD3 ganglioside. After injection of the antibody, foot process effacement and proteinuria occurred, and this was associated with nephrin dislocation to the apical segments of the narrowed filtration slits. In parallel, tyrosine phosphorylation of nephrin was found (432). It has been shown that nephrin and CD2AP are associated with glomerular cell actin. Nephrin seems to traverse a relatively cholesterol-poor region of the podocyte plasma membrane. In addition, a small pool of actin-associated nephrin and CD2AP resides in lipid rafts, possibly in the cholesterol-rich apical region of the podocyte foot processes (509).

Nephrin has been shown to trigger phosphorylation of p38 and c-Jun, which was augmented by podocin. Dominant-negative mutants of small G proteins (Cdc42, Rac1, RhoA) and protein kinases that activate JNK (MKK4) and p38 (MKK3, MKK6) inhibited the nephrin-mediated AP-1 activation. A dominant-negative MEK1 had no effect on the nephrin-mediated AP-1 activation. This suggests that nephrin stimulates p38 and JNK but not ERK1/ERK2. Therefore, nephrin acts as a signaling molecule that can activate MAP kinase cascades (177). Cells overexpressing podocin and nephrin showed an increase in AP-1 activation. Podocin, but not CD2AP, increased nephrin-mediated AP-1 activation to ~40-fold without affecting nephrin protein levels. Truncation of nephrin at amino acid 1160 resulted in the binding of only marginal amounts of podocin, indicating that a sufficient interaction between podocin and nephrin requires the COOH-terminal 81 amino acids of nephrin. This nephrin mutation still promotes a high level of AP-1 activation, but in contrast to wild-type nephrin, podocin failed to augment the AP-1 activation triggered by mutant nephrin. Thus interaction with podocin might stabilize nephrin or recruit nephrin to lipid rafts and thereby increase nephrin signaling in podocyte foot processes (177).

Transgenic manipulation of the podocyte might allow the study of podocyte function in vivo. The nephrin promoter has been considered a good candidate for directing the expression of transgenes in a podocyte-specific manner. A 1.25-kb DNA fragment from the human nephrin promoter and 5′-flanking region has been recently identified. The fragment, which includes the predicted initiation codon and immediate 5′-flanking region of nephrin, directs podocyte-specific expression in vivo. Using this promoter fragment could allow the study of podocyte functions in vivo and help to identify transacting factors that are required for podocyte-specific expression (496). In addition, a 8.3-kb and a 5.4-kb fragment containing the 5′-flanking promoter sequence of nephrin were recently identified and characterized, and mice transgenic for both constructs were generated. NSPH1 transgene showed an expression with a high penetrance in the podocytes and brain (288). Very recently, a glomerular-specific Cre-recombinase transgenic murine line under the control of the NPHS1 promoter was generated, and a successful Cre-mediated excision of a "floxed" transgene specifically in podocytes has been demonstrated in vivo. This murine founder line represents a very good tool for the manipulation of the expression of genes in podocytes in vivo (111).

C. NEPH1

Very recently, a transmembrane protein containing five extracellular immunoglobulin-like domains structurally related to human nephrin was identified in the mouse. The encoding protein is called NEPH1. NEPH1 is expressed widely and was found within the kidney in podocyte foot processes. Mutation of the NEPH1 locus resulted in effacement of podocyte foot processes and proteinuria, but mice showed no edema. NEPH1 knock-out mice showed a high perinatal lethality, and all mice died between 3 and 8 wk of age. Beyond podocyte foot process effacement, 3-wk-old Neph1 knock-out mice had diffuse mesangial hypercellularity and increased mesangial matrix. It has been suggested that NEPH1 plays a role in cell-cell interactions. NEPH1 may interact with other NEPH1 proteins or it may be the ligand for nephrin (95).

D. Podocin

Podocin belongs to the raft-associated stomatin family 41, whose gene NPHS2 is mutated in a subgroup of patients with autosomal-recessive steroid-resistant nephrotic syndrome. These patients show disease onset in early childhood and rapid progression to end-stage renal

failure. The disease does not reoccur after renal transplantation, and there are no extrarenal disorders.

Podocin is a ~42-kDa protein. It is predicted to form a membrane-associated hairpin-like structure with a cytosolic NH_2- and COOH-terminal domain that is typical for stomatinlike proteins and caveolins (49). Very recently, podocin has been shown to be localized on podocyte foot process membrane at the insertion site of the slit diaphragm. It accumulates in an oligomeric form in lipid rafts of the slit diaphragm, and in vivo studies demonstrate that it interacts via its COOH-terminal domain with CD2AP and with nephrin (408). In neurons of *Caenorhabditis elegans*, mutations of a stomatin homolog MEC-2, a highly homologous protein to stomatin, have been shown to link mechanosensory channels and the microtubule cytoskeleton of the touch receptor neurons, suggesting that stomatin is a molecular link in a stretch-sensitive system (172). Further studies should address whether mechanically gated ion channels are connected to the cytoskeleton of the podocytes and whether podocin links these ion channels to the cytoskeleton of podocytes.

E. CD2-Associated Protein

CD2-associated protein (CD2AP) was initially shown as an SH_3-containing protein that binds to the cytoplasmic domain of CD2 and enhances CD2 clustering. It anchors CD2, a T cell and natural killer cell membrane protein which facilitates T-cell adhesion to antigen-presenting cells. CD2AP has a molecular weight of 80 kDa. It contains an actin-binding site located at the NH_2 terminus, a proline-rich region, and three SH_3 domains, one of which interacts with CD2 (103). In developing podocytes at the capillary loop stage, CD2AP is detected slightly later than nephrin (256). Within the glomerulus, CD2AP is only expressed in the podocyte, but it is also found in collecting duct cells and some proximal tubular cells (256). CD2AP knock-out mice develop proteinuria and kidney failure. Glomeruli from 1-wk-old animals show loss of foot process integrity in some glomeruli. At 2 wk of age, almost all podocytes were affected, and in many glomeruli, mesangial matrix deposits occur. This indicates that disease progression in CD2AP knock-out mice begins with podocyte injury leading to a consecutive damage of the mesangium. By 4 wk, glomeruli are sclerotic with increased deposits and distended capillary loops and mice die at the age of 6–7 wk. Coimmunoprecipitation of CD2AP by a nephrin fusion protein indicates that both proteins are associated (424). However, in contrast, in 293T kidney cells used as an overexpression system, neither nephrin nor podocin interacted with CD2AP, which could be due to a lack of a podocyte-specific adapter in this system (177).

Developing CD2AP knock-out mice at first exhibit normal foot processes and slit diaphragms and show no alteration in nephrin expression, suggesting that CD2AP is neither necessary for the correct localization of nephrin at the slit diaphragm nor for the development of intact foot processes. On the other hand, in adult glomeruli of CD2AP knock-out mice, nephrin was not detectable in most glomeruli (256). In the laminin β_2-chain knock-out mouse model of nephrotic syndrome, extensive foot process effacement was associated with aberrant clustering of CD2AP in podocytes. This suggests that CD2AP indeed plays a role in the maintenance of the slit diaphragm (256). Mutations of the human Lmx-1b gene are responsible for the nail-patella syndrome, which is frequently associated with glomerulopathy (99). Podocytes from Lmx-1b knock-out mouse retained a cuboidal shape and did not form foot processes and slit diaphragms. These podocytes showed a reduced expression of the α_4-chain of collagen IV and the slit diaphragm proteins CD2AP and podocin. In addition, there are Lmx-1b binding sites in the putative regulatory regions of both CD2AP and NPHS2. Thus a reduced expression of proteins associated with foot processes and the glomerular slit diaphragm may contribute to the nephropathy associated with the nail-patella syndrome (283, 375).

Cas ligand with multiple SH_3 domains, the human homolog of CD2AP, interacts with p130Cas, a docking protein composed of multiple tyrosine residues, forming SH_2-binding motifs and one SH_3 domain (210). The subcellular localization of p130CAS and CD2AP has recently been investigated. In differentiated mouse podocytes, CD2AP and p130Cas target to different cytoskeletal structures. Whereas p130Cas is located at focal adhesions, CD2AP overlaps with α-actinin-4 and F-actin spots that colocalize with the actin assembly proteins ARP2/3 and cortactin, suggesting that CD2AP might play a role in dynamic actin assembly in podocytes (489).

F. FAT

FAT is a novel member of the cadherin superfamily with 34 tandem cadherin-like extracellular repeats and a molecular weight of 516 kDa (101). It has recently been shown that FAT is expressed in colocalization with nephrin along capillary walls in podocytes. On the ultrastructural level, FAT could be detected at the base of slit diaphragms and the cytoplasmic domain of FAT colocalized with ZO-1 (183). Because FAT has a huge extracellular domain, the authors speculate that slit diaphragms contain longer intercellular adhesion molecules than adherence junctions and desmosomes (183). The configuration of the huge extracellular domain of FAT within the slit diaphragm remains to be determined.

G. P-cadherin

P-cadherin is a 120-kDa transmembrane protein that consists of five cadherin domains on the extracellular part and a β-catenin binding site on the cytoplasmatic side. During development, P-cadherin was first detected during the vesicle stage, indicated by staining the apical margins of the developing epithelial cells (380, 454). In the late S-shaped bodies, P-cadherin colocalized with nephrin and ZO-1. During the capillary loop stage, staining for P-cadherin has been observed at the basal margins, and to some extent, between the developing podocytes as well. In contrast to nephrin, P-cadherin has not been detected on the lateral surfaces of developing podocytes (380).

Recently, a model was introduced in which the slit diaphragm represents a modified adherens-type junction (368). In this model, P-cadherin, via its extracellular domain, represents the core protein of the slit diaphragm. According to this model, the intracellular domain of P-cadherin is linked to β-catenin and/or plakoglobin (γ-catenin). The linkage of this complex to the actin cytoskeleton via α-actinin is mediated by α-catenin, or by interaction of α-catenin with ZO-1, which in turn can bind to actin filaments. Homophilic interactions of P-cadherin have been suggested to form the bridge between the podocytes (368). P-cadherin is normally expressed in NPHS1 kidneys, and P-cadherin knock-out mice do not develop nephrosis. Therefore, P-cadherin expression seems not to be essential for maintenance of the normal slit diaphragm (362, 380).

VIII. PODOCYE-CYTOSKELETON-GLOMERULAR BASEMENT MEMBRANE INTERACTIONS

For an intact glomerular filter podocyte-podocyte interaction generated by the unique cell contact structure of the slit diaphragm (see sect. VII), podocyte-matrix interaction of cell membrane receptors with GBM matrix proteins are essential. The two macromolecular complexes are structurally linked by the dense actin network of the podocyte foot process.

A. GBM-Cytoskeletal Interaction

Tightly controlled cell matrix contacts are an essential prerequisite to maintain the highly ordered foot process architecture (434). Constituents of this connection are the matrix molecules of the GBM, which serve as ligands for transmembrane adhesion receptors of the podocyte foot process. On the intracellular part of the cell-matrix, interface molecules responsible for cell-matrix signaling and transmission of mechanical forces on the cytoskeleton aggregate in a multimolecular adhesion complex.

B. GBM

During glomerulogenesis, the GBM is generated as two separate layers produced by glomerular endothelial and epithelial cells. The two sheets are fused together to form the mature GBM (4–6, 280, 391, 446). In the adult glomerulus, the podocyte continues to add and assemble matrix molecules to the GBM, maintaining a hydrated meshwork consisting of collagen IV, laminin, entactin, agrin, and perlecan (281).

The flexibility and dynamic of mature GBM requires a constant turnover. To achieve this, podocytes not only produce GBM components, but also secrete matrix-modifying enzymes. MMP-9 production by podocytes has been found in vitro and in the proteinuric phase of Heymann nephritis (267, 272). Podocytes should therefore be able to enzymatically modify the GBM they adhere to in proteinuric disease. The relevance of the GBM composition for podocyte architecture is exemplified by foot process fusion and proteinuria in the laminin β_2-chain-deficient mouse (314). α_5-/β_2-/γ_1-Laminins are assembled to form the GBM-specific heterotrimeric laminin 11 (223). The lack of a single laminin monomer is sufficient to cause severe disruption in the filtration barrier, leading to nephrotic phenotype. Interestingly, a knock-out for α_5-laminin is not able to form a functional laminin, causing an early arrest in nephrogenesis (282). Genetic modification of the collagen IV component via knock-out of collagen IV α_3 results in an Alport-like syndrome with a compensatory upregualtion of collagen IV α_1 and α_2. In the collagen IV α_3 knock-out mice, the laminin composition of the GBM is also shifted to α_2- and β_1-laminins (73), inducing podocyte foot process effacement. The podocyte damage in collagen IV α_3 knock-out mice can be ameliorated by a double knock-out of collagen IV α_3 and integrin-α_1. In the double knock-outs, a reduction of the α_2- and β_1-laminin induction could be responsible for an improved GBM-podocyte interaction (73) Integrin $\alpha_1\beta_1$ and TGF-β1 play distinct roles in Alport glomerular pathogenesis and serve as dual targets for metabolic therapy (73). The elucidation of the interdependence in the matrix composition promises to yield exciting results in the future.

C. Transmembrane Matrix Receptors

Specific matrix receptors anchor the podocyte foot processes by binding to their ligands in the GBM. Since they have considerable clinical relevance, podocyte-GBM receptors have been studied in great detail (7, 223).

Initial studies concentrated on a specific integrin heterodimer, consisting of $\alpha_3\beta_1$-integrins, found at the "sole" of podocyte foot processes facing the GBM (8, 218). Integrins are heterodimeric transmembrane molecules and are the paradigmatic matrix receptors. Each cell type

expresses a characteristic combination of α- and β-subunits. $\alpha_3\beta_1$-Integrins have been described to bind to collagen IV α_3-, α_4-, and α_5-chains, fibronectin, laminin, and entactin/nidogen, all present in the GBM (86). Ligand binding induces clustering of integrins to form focal adhesions and recruitment of intracellular cytoskeletal proteins. For intracellular signaling, associated kinase molecules are required.

A series of experimental and genetic studies indicate an involvement of integrin-mediated podocyte matrix interaction in failure of the filtration barrier. An antiserum raised against glomeruli with binding activity against β_1-integrins, a specific anti-β_1-integrin antibody or integrin-blocking RGD peptides, induced foot process fusion, proteinuria, and detachment of podocytes from the GBM (7, 8, 328a). Genetic evidence for the requirement of α_3-integrins for an intact filtration barrier was generated with the α_3-integrin knock-out mouse (222). α_3-Integrin null mice exhibit an immature GBM and foot process effacement at birth. These findings led to the hypothesis that changes in podocyte integrins could correlate with proteinuria by interfering with the dynamic of foot process anchoring in the GBM. However, several studies examining integrin expression in a variety of glomerular diseases and in vitro systems produced conflicting results concerning $\alpha_3\beta_1$-integrin distribution or levels (26, 28, 60, 184, 196, 203, 216, 228, 365, 366, 426). These data could be reconciled by a modification of integrin function without changes in the overall integrin levels in the framework of integrin inside-out signaling (see below). Alternatively, integrin function could be modified by interaction with other adhesion molecules.

A second matrix receptor in podocytes, the dystroglycan complex, is known to provide links between matrix molecules and the actin cytoskeleton in a variety of cell types (161). Starting with data on dystroglycan expression in the glomerulus (102, 260), ultrastructural analysis revealed a restricted expression of dystroglycan complexes in the basal cell membrane of podocyte foot processes (360, 365). The respective matrix ligands (laminin, agrin, and perlecan) are found in the GBM, and the intracellular binding partner utrophin is also expressed in podocytes (360, 365). Most interestingly, the expression of the dystroglycan complex is negatively correlated with disease activity in proteinuria in animal models (360) and with minimal-change disease in humans (365). However, the link between dystroglycans and the actin cytoskeleton of the podocyte and the interaction with the integrin complexes in focal adhesion needs further evaluation.

Both integrins and dystroglycans are coupled via adapter molecules to the podocyte cytoskeleton, allowing the transmission of mechanical force from the GBM to the meshwork of interdigitated foot processes and via primary processes to the cell body. Alteration of the GBM that causes foot process effacement and the loss of an intact slit membrane requires the link of the cytoskeleton as a force-transmitting unit between these two components of the foot processes. A series of studies have shown an association of altered GBM, loss of slit membranes, and proteinuria with rearranged cytoskeletal proteins. Systematic ultrastructural and immunohistochemical analyses have shown a rearrangement of cytoskeletal proteins (F-actin and α-actinin) in response to foot process effacement in anti-GBM antibody-induced Masugi nephritis (429) and in puromycin nephrosis (PAN) in rats (492). Quantification of the involved cytoskeletal molecules by immunogold transmission electron microscopy showed an increase of actin and α-actinin in the Masugi model, whereas after PAN, a cytoskeletal disaggregation of the actin cytoskeleton was described. Further functional studies are required to determine whether a compensatory hypertrophic response of the actin network in the more chronic Masugi nephritis model or a toxic effect on the cytoskeleton in the PAN model are responsible for the observed difference. A detailed description of the cytoskeletal scaffolds of the podocyte will follow below.

D. Integrin Signaling in Podocyte Damage

Integrins are more than just passive attachments of cells to their matrix (71). Ligation of integrins by the matrix induces specific cellular responses, a process referred to as outside-in signaling (400). In the framework of inside-out signaling, integrins can be downstream effectors in cell responses (117). Inside-out signaling regulates integrin-binding affinity and avidity via cytoplasmic signals, giving integrins the function of fine-tuned cellular anchors (334a). In addition, integrins appear essential for assembly of extracellular matrix molecules in a three-dimensional network (498). Integrin signaling is closely regulated by a large intracellular macromolecular complex associated with the integrin cytoplasmic domains. In the focal adhesion, integrins are connected to the actin cytoskeleton via a rapidly growing number of adapter molecules including paxillin, vinculin, and α-actinin.

1. Candidate integrin signaling molecules

Focal adhesion kinase (Fak), the prototypic integrin-associated nonreceptor tyrosine kinase, binds to the cytoplasmic domain of β-integrins. In principle, Fak is activated by integrin ligation in outside-in signaling, inducing autophosphorylation and activation of the Ras pathway. Fak activation in inside-out signaling increases adhesion to the matrix. In the context of filtration barrier failure, the role of Fak has been investigated in a variety of glomerular diseases. An increase in protein levels and hyperphosphorylation of Fak in glomeruli was found at the onset of lupus nephritis in *lpr/lpr* mice (289), in an anti-GBM disease (221), and in streptozotocin-induced

diabetic nephropathy (367). Histochemical data (289) and in vitro experiments (152) indicate that Fak induction takes place in the mesangium. Studies specifically addressing podocytes have been technically very difficult. Kurihara et al. (242) detected tyrosine hyperphosphorylation in lysates from rat glomeruli during development and after induction of proteinuria with puromycin. ZO-1 could be identified as a substrate for tyrosine phosphorylation in podocytes. The kinase responsible for the hyperphosphorylation, however, remained unclear. In examining human biopsies, no changes of Fak, talin, vinculin, paxillin, $\alpha_3\beta_1$-integrin, ZO-1, or ZO-2 protein levels could be detected using semiquantitative immunofluorescence in membranous nephropathy and minimal-change disease (26). In agreement with Kurihara's study, a modest increase in phosphorylated tyrosine residues was reported.

Topham induced reversible injury to cultured undifferentiated rat podocytes by disrupting the actin cytoskeleton with C5b-9, causing podocyte damage in vivo (463). No alterations in Fak, $\alpha_3\beta_1$-integrin, talin, vinculin, or paxillin protein levels could be found (463). Evaluating potential mechanisms of advanced glycation end products on podocytes, Krishnamurti et al. (227) showed a modest decrease in Fak tyrosine phosphorylation and MAP kinase activity in cultured human undifferentiated podocytes in response to glycated matrix molecules. However, Fak activity was not determined. Cybulsky (76) elucidated in a long-standing effort the signaling events responsible for proliferation of cultured undifferentiated podocytes. Podocyte proliferation was shown to be dependent on the activation of a receptor tyrosine kinase pathway converging into the MAP kinase pathway. A β_1-integrin-mediated induction of the inositol pathway suppressed podocyte proliferation. The cross-talk between integrin ligation and the inositol pathway in podocytes is still under investigation. Reiser et al. (369) found an increased total phosphotyrosine content in cultured differentiated mouse podocytes in response to puromycin or protamin, as observed in the in vivo model of PAN nephrosis (242). Interestingly, a phosphatase inhibitor, but not a kinase activator, produced the identical phenotype of process retraction in vitro, consistent with tyrosine dephosphorylation as a relevant regulatory mechanism in podocyte damage. In conclusion, podocyte damage appears to result in heterogeneous alterations of phosphorylation in podocyte foot process proteins. However, despite considerable efforts, no candidate signaling molecule responsible for regulating podocyte matrix interaction could be identified so far.

2. Integrin-linked kinase in podocyte damage

An expression screen conducted on glomeruli from children with the congenital nephrotic syndrome of the Finnish type (CNF) to identify relevant players in proteinuria produced an interesting novel signaling molecule. In CNF glomeruli, a mRNA induction of the integrin-linked kinase (ILK) could be identified (226). ILK, a serine threonine kinase, has become a good candidate for regulating podocyte matrix interaction in proteinuria. Since its identification in a yeast two-hybrid screen with the cytoplasmic tail of the β_1-integrin as bait (154), ILK has been shown to be involved in a wide variety of regulatory processes. It plays a key role in integrin-mediated cell adhesion and signaling. In outside-in signaling, ILK is inhibited by ligation of the corresponding integrins (154). Inside-out signaling could be demonstrated by reduced matrix adhesion in ILK-overexpressing cell lines. In addition, ILK overexpression led via Akt and GSK activation to anchorage-independent growth (361), implicating ILK not only as a mediator of integrin inside-out signaling, but also of adhesion-dependent regulation of cell cycle progression (see above). For cell phenotype regulation, ILK inhibits E-cadherin expression (498) and activates the β-catenin/LEF transcriptional complex with consecutive increase in MMP-9 levels and activity (318, 466). ILK appears downstream of phosphatidylinositol 3,4,5-trisphosphate-dependent growth factor signaling (89).

ILK induction could be demonstrated in glomeruli of three different renal diseases, all with severe alterations of the filtration barrier (226) and in diabetic nephropathy (149). In CNF, a mutation of a single molecule in the slit diaphragm nephrin causes a severe disturbance of the glomerular filtration unit (see sect. VII). In the murine model of nephrotoxic serum nephritis, the acute inflammatory insult of the anti-GBM antibodies induces the rapid onset of a severe nephrotic syndrome (397). In the chronic progressive glomerulosclerosis of mice transgenic for growth hormone (GH), glomerular hypertrophy induces slowly progressive podocyte failure (495). In all three diseases an increase in glomerular ILK mRNA is accompanied by the typical podocyte lesions of foot process effacement and denudation of the GBM (180, 429, 495). With the use of single podocyte RT-PCR (404) in combination with real-time RT-PCR, a significant increase of ILK mRNA was found in podocytes from proteinuric GH-transgenic mice compared with wild-type littermates, confirming podocyte-specific ILK induction.

To evaluate ILK in outside-in signaling, podocyte ILK activity was examined in vitro in response to different extracellular matrixes and was found to be induced by collagen I compared with collagen IV or fibronectin, matrix molecules found in the normal glomerulus. In inside-out signaling, a dose-dependent increase in ILK kinase activity in cultured podocytes in response to puromycin could be demonstrated, indicating ILK to be a downstream effector of podocyte damage. Stable overexpression of wild-type ILK in cultured podocytes led to reduced matrix adhesion, confirming the functional role of ILK in inside-out signaling. Further evidence on the involvement

of serine threonine kinases in outside-in signaling was provided by the induction of process formation with unspecific serine threonine kinase inhibitors in cultured podocytes (212).

ILK represses E-cadherin in epithelial cells via activation of the Wnt pathway with nuclear translocation of β-catenin and induction of LEF-1 (498). Because members of this pathway are expressed in podocytes (368), ILK could be involved in the cross-talk between GBM and the specialized cell-cell contact of the slit diaphragm. ILK overexpression in podocytes altered, in addition to interfering with podocyte matrix interaction, the podocyte phenotype from the differentiated arborized cell morphology to a cobblestone pattern. These changes were paralleled by a reorganization of the actin cytoskeleton, nuclear translocation of β-catenin and LEF-1, and a significant repression of the P-cadherin (456), indicating a complex downstream cascade of integrin signaling in podocytes.

IX. PODOCYTE CYTOSKELETON

The structural integrity of the foot process is crucial for establishing stability between the cell-cell and the cell-matrix contact of podocytes. This unique challenge has resulted in the development of a specialized cytoskeletal organization of podocytes in foot processes (see sect. III). The primary function of the foot process cytoskeleton is the coupling of the slit membrane complex with the podocyte-GBM contacts in their close proximity. Because the glomerular capillary wall undergoes cyclic distensions with each heart beat, a combination of mechanical strength and flexibility is also required. The cytoskeleton of the major processes has to maintain contact with the metabolic machinery of the podocyte cell body to allow vesicular transport along the process. An additional function of the podocyte cytoskeleton could be a counteraction of the distensible forces of the capillary wall (see Fig. 2).

A. Podocyte Processes Contain Defined Sets of Microfibers

The cytoskeleton, a cytoplasmic system of fibers, has to serve static and dynamic functions. It consists of three discreet sets of ultrastructural elements: microfilaments (7–9 nm in diameter), intermediate filaments (10 nm), and microtubules (24 nm). All are built in a tightly controlled process from small protein subunits.

In the podocyte, the cytoskeleton responds to the unique challenges of the filtration barrier with several levels of structural organization, represented by different molecular constituents. Podocyte foot processes contain a dense network of actin filaments connected with an array of linker proteins to the slit membrane complex and the GBM anchor proteins. Microtubules and intermediate filaments are the scaffold of podocyte major processes and the central cell body (17, 72, 97, 115, 213, 477).

The expression of the podocyte-specific microfibrils appears to be a prerequisite for foot process formation during glomerulogenesis. During the capillary loop stage, the cytoskeleton undergoes a switch from an epithelial to a mesenchymal phenotype. The expression of the intermediate microfilament vimentin is induced, followed by the development of actin-rich branched cellular processes, giving rise to the foot process interdigitation of the mature filtration barrier (168, 302). Interestingly, a similar sequence of cytoskeletal rearrangements can be observed in conditionally immortalized podocytes during in vitro differentiation (295).

B. Foot Processes Contain a Dense Network of F-actin Microfilaments

Microfilaments are the predominant cytoskeletal constituent of the foot process. F-actin is a highly dynamic structure with a polar orientation, allowing for rapid growth, branching, and disassembly. Actin filaments are bundled in closely packed parallel arrays or are associated loosely in networks. Bivalent actin cross-linking molecules from the calponin homology-domain superfamily (i.e., α-actinin and dystrophin) define the level of actin packing. Associated motor proteins like myosin allow for isometric or isotonic contraction of the bundles in muscle and nonmuscle cells (141).

The F-actin network in podocytes is modified for the specific requirement of the foot processes by a unique assembly of linker and adapter molecules. In the seminal study of Drenckhahn and Franke (97), a three-dimensional model of the foot process cytoskeletal architecture was constructed to contain loops of microfilament bundles of myosin, F-actin, and α-actinin (see also Fig. 3). The base of a microfilament loop is connected to the sole of the foot processes via matrix receptors to the GBM. The filaments form a high-arched loop between neighboring foot processes of the same podocytes.

At the bend of the loop, the actin filaments connect to the intermediate and microtubular filaments of major processes. Foot processes therefore have all elements required to generate a tensile strength to oppose the distensible forces of the capillary wall (97, 231, 241). To address the questions of whether and how podocytes react to mechanical load, Endlich et al. (107) established a biaxial cyclic stress culture system. Podocytes respond, compared with mesangial and endothelial cells, with a unique rearrangement of their cytoskeleton to mechanical stress in vitro, resulting in a reduction in cell body size and a thinning of their major processes. Microtubules and inter-

mediate filaments remain grossly unchanged, whereas a reversible reorganization of F-actin to radial stress fibers with an actin-rich center developed in a Ca^{2+}- and Rho kinase-dependent manner. Further studies of stress-activated signaling pathways should define the similarities and differences of this tissue culture system to the in vivo situation. Several actin-associated molecules have been described to be expressed in a podocyte-specific pattern. Because their functional characterization heavily relied on studies in podocyte damage, we discuss them in detail below.

C. The Slit Membrane Complex Is Coupled to the Cytoskeleton

On the lateral sides of the foot processes, the cytoskeleton is linked to the slit membrane complex. ZO-1 (243), catenins (368), and CD2AP (424) serve as adapter molecules between the transmembrane slit membrane molecules nephrin and P-cadherin and the actin filaments. CD2AP in the respective knock-out mouse caused a nephrotic syndrome most likely via its interaction with nephrin (see above). Since the first function attributed to CD2AP was an adapter molecule to the cytoskeleton in the immunological synapse (103), it was speculated early on that CD2AP serves a similar function in podocytes (424). Experimental confirmation of this hypothesis was obtained in cultured podocytes with colocalization of CD2AP and F-actin. In the same complex, the actin-associated molecules Arp2/3 and cortactin were detected (489).

D. Actin Cytoskeleton Is Connected to Integral Membrane Molecules

In addition to the actin cytoskeleton at the sole of the foot processes, a subplasmalemmal actin system is found in podocytes. This actin network is connected to transmembrane molecules generating the negative charge surface area of podocytes. The transmembrane molecule podocalyxin contributes significantly to this negative surface charge (204). It has been postulated that the negative charge of podocalyxin not only repulses proteins, but also serves as a spacer molecule between interdigitating foot processes (451). For this function, a tethering of podocalyxin to the subplasmalemmal actin network is required. In an elegant series of studies, Farquar and co-workers (332) revealed the molecular constituents of this adapter complex. First, they identified an interaction of ezrin, a member of the ezrin/radixin/moesin family of adapter molecules, with the cytoplasmic domain of podocalyxin at the apical area of podocyte foot processes (332). Ezrin was already known as a marker for activated or damaged podocytes (179). Na^+/H^+ exchanger-regulatory factor 2 (NHERF2) was found to be a second member of the podocalyxin-actin complex (452). NHERF2 binds via the NH_2-terminal ERM binding region to Tyr-567 phosphorylated ezrin. Treatment of rats with puromycin or sialic acid is associated with dephosphorylation of ezrin and uncoupling of ezrin from the actin cytoskeleton. Interestingly, neutralization of the negative podocalyxin surface charge with protamin sulfate resulted in a disruption of the intact NHERF2/ezrin complex from the cytoplasmic domain of podocalyxin (452), leaving the complex attached to actin filaments. Further studies will have to identify the signaling molecules responsible for the differential regulation of this complex in podocyte damage.

A second transmembrane molecule with plasma membrane tether function could be podocin (see sect. VII for detailed description).

Finally, the transmembrane endocytocic receptor glycoprotein 330/megalin, involved in protein uptake in epithelial cells, interacts with a molecule of the MAGUK protein family, Magi-1 (346). MAGUKs are involved in the clustering of molecules to defined membrane domains, and Magi-1 serves here as a multi-docking protein in the podocyte cell membrane to the actin cytoskeleton via α-actinin 4 and synaptopodin (347). The Magi-1 splice isoforms found in podocytes appear to confer an association with the actin cytoskeleton (346). Further studies will have to determine the molecular partners of the proposed multiprotein complex and its function in podocyte failure.

E. Major Podocyte Processes Contain Microtubles and Intermediate Filaments

Mature podocytes express with vimentin and desmin a mesenchymal intermediate filament pattern in their cell body and major processes (24, 97, 168, 329, 444, 477, 501). Desmin expression in mammals could only be found in rat podocytes. In contrast to vimentin, desmin is significantly induced in puromycin nephropathy in rats (123, 185, 244, 501, 502). Podocytes express plectin as an intermediate filament-associated protein (504). A further intermediate filament-associated molecule, a podocyte specific 250-kDa protein, was identified using a monoclonal antibody raised against rat podocytes lysates (244). Like desmin, it is induced in puromycin damage and could be involved in intermediate filament cross-linking, but molecular identification will be required before any definite functional role can be determined (244).

Microtubules are heterodimeric polymers of globular α- and β-tubulin subunits and form a stiff 24-nm-thick tubular structure. They are essential for an intact structure of major podocyte processes, since they connect the cell body with the GBM-anchored actin network in foot processes (for an in-depth review, see Ref. 213). Disrup-

tion of microtubule elongation with vinblastin resulted in severe damage to major processes in vivo (15, 17, 470) and blocked process formation in vitro (214).

Microtubules are built in a polar orientation starting from the microtubule-organizing center (MTOC), and nucleation of microtubules is initiated by γ-tubulin found in podocyte centrosomes (213). Microtubule elongation is separated into fast-growing (plus) and slow-growing (minus) poles.

In most cells, minus-end microtubules are located at the MTOC, with the fast-growing end toward the cell periphery, resulting in a "plus-end-distal" orientation (23). Process-bearing cells like neurons, glial cells, and podocytes show a mixed microtubular polarity with plus-end-distal and minus-end-distal orientation (23, 207, 213). The minus-end-distal orientation of microtubules in these cells appears to rely on the directed transport mechanism by the kinesin superfamily motor proteins (412, 413). A member of this superfamily, CHO1/MKLP1 (310, 311), has been found to be essential in formation of podocyte processes and dendrites (213, 346, 423, 508). CHO1/MKLP1 is responsible for elongation of minus-end-distal microtubles in podocyte processes (213) via antiparallel transport along plus-end-distal microtubules. Blocking of this transport mechanism, therefore causing the generation of a nonuniform microtubule polarity, effectively prevented process formation in podocytes in vitro and confirmed the critical relevance of the nonuniform microtubule polarity for podocyte function (213).

From the microtubular-associated molecules (MAPs) required for tubular elongation, MAP3 (174) and MAP4 (344) could be detected in podocytes in vivo and in vitro (214). MAP3/4 (which appear to be identical on the molecular level, Ref. 214) phosphorylation decreases the assembly of microtubules. Whether or not MAP4 is the critical molecule for process formation in podocytes is still debatable, since MAP4 phosphorylation remained unchanged after okadaic acid, which should have resulted in hyperphosphorylation of active MAPs and subsequent disassembly of the tubule (214). Furthermore, MAP4 is found in cells without processes (220). Injection of an anti-MAP4 antibody does not result in the disassembly of microtubules (488), and MAP4 overexpression is not sufficient for process formation (33). Additional members of this superfamily could cooperate with MAP4 during process induction in podocytes.

Assembly of microtubules is further regulated by protein Ser/Thr dephosphorylation of MAPs by PP2A protein phosphatase (165, 353). In podocytes in vivo, PP2A is only found in process-forming cells during glomerulogenesis (113, 448). Cultured podocytes express PP2A during process formation, and okadaic acid can suppress process formation in concentrations reported to be specific for protein phosphatase 2A (214). Unspecific inhibition of serine/threonine kinases has been shown to induce process formation in podocytes in vitro (214), further indicating a tight regulation of the microtubular machinery in podocytes.

A major role of microtubules is the trafficking of proteins from the Golgi apparatus into the cell periphery. Using vesicular stomatitis virus (VSV)-G infected podocytes, Simons et al. (431) demonstrated an active transport mechanism of VSV-G along processes of cultured podocytes. VSV-G particles were found to colocalize in the periphery of podocyte processes with the small GTPase rab8, which is relevant for membrane-directed transport in neuronal dendrites and Madin-Darby canine kidney cells (175, 350) and the membrane-docking molecule rsec6/8 (146, 195). Directed vesicular transport in podocytes was dependent on an intact Golgi apparatus. This directed membrane transport machinery should be of critical relevance for process formation and maintenance in vivo as well, securing an adequate supply of podocyte foot processes with cargo synthesized in the perinuclear Golgi complex.

Kobayashi and Mundel (213) presented in their in-depth review of microtubular function in neuronal and nonneuronal cells a compelling hypothesis on parallel processes formation between podocytes and neuronal dendrites based on similar expression and functional characteristics of the microtubular proteins.

F. Cytoskeletal Alterations in Podocyte Damage

Foot process effacement, also referred to as process simplification, retraction, or fusion, affects three aspects in the podocyte. Foot process effacement has to be initiated at the cytoskeleton of the podocyte and results in the alteration of the cell-cell contacts at the slit diaphragm and in a mobilization of the cell matrix contacts (13, 115, 197, 383, 411, 429). Foot process effacement is accompanied by an increase in microfilament density that builds a mat of intercrossing stress fibers at the sole of the foot process (128, 197, 225, 301, 328). Dense bodies, serving as cross-linkers for microfilaments in smooth muscle cells (251), have been discovered in the basal actin network of effaced foot processes (429). However, a disruption of actin filaments and a transient dispersion of the microfilament structure in podocyte foot processes was reported in puromycin nephrosis (492) and in complement-mediated injury (463). Whether these differences are a consequence of the visualization techniques employed or are a disease stage specific phenomena remains unanswered. Also whether the structural alterations could be considered to represent a compensatory response of the podocyte to counteract an increase in capillary distending forces or as a reparative mechanism of podocyte damage is still a matter of debate (see below).

In podocyte damage models, polycations such as protamine sulfate or the cytotoxic antibiotic puromycin are widely used. Both agents cause foot process effacement and proteinuria in rats in vivo (115, 189, 411) and allow the study of cytoskeletal responses in podocyte damage in vitro (369). Kerjaschki (197) addressed the role of the different cytoskeletal elements in an ex vivo system of isolated perfused rat kidneys. Blockade of actin dynamics via calcium depletion, low temperature, or cytochalasin B resulted in a 50% reduction of the protamine sulfate effect. In contrast, interference with microtubular function with colchicine or vinblastine did not alter foot process dynamics (197, 470).

G. Actin-Associated Molecules in Podocyte Damage

In podocytes, a characteristic pattern of actin-associated proteins has been identified, and their regulation in podocyte damage has highlighted the relevance of cytoskeletal dynamics for maintaining an intact filtration barrier.

Synaptopodin was the first podocyte-specific actin-associated molecule to emerge (293). The only other expression sites of synaptopodin are the spine-bearing neurons in the olfactory bulb, striatum, cerebral cortex, and hippocampus spine apparatus of telencephalic neurons (90). The exact molecular role of synaptopodin is still largely unknown. It does not appear to be essential for podocyte development, but homozygous deletion of synaptopodin in mice results in an increased sensitivity of the filtration barrier to damage (52).

α-Actinin-4 has been shown to be widely expressed in podocyte foot processes, colocalizing with actin stress fibers (245). Induction of proteinuria with puromycin in rats resulted in foot process retraction and progressive podocyte damage and was preceded by an increased α-actinin expression (429, 435). Strong genetic evidence for a critical role of α-actinin was generated by studying hereditary filtration barrier failure in humans. Mutations of ACTN4, encoding α-actinin-4, were found to cause a late-onset autosomal-dominant focal segmental glomerulosclerosis (190). In the glomerulus, α-actinin-4 is specifically expressed in podocytes, and initial functional experiments are consistent with an increased F-actin bundling by mutant α-actinin-4. In acquired proteinuric diseases, a repression of α-actinin-4 protein and an associated adapter molecule, CLP-36, have been found in immunohistochemical studies (44, 69).

Because the low-molecular-weight hsp27 binds to actin filaments in a phosphorylation-dependent manner (40, 286), Smoyer et al. (433) evaluated hsp27 regulation in podocyte failure. After puromycin-induced nephrosis, an increase both of total hsp27 and phosphorylated hsp27 was found. Because hsp27 activation is a common theme in defense against metabolic or oxidative cell damage (275, 499), the hsp27 induction could be part of a defense mechanism to protect cytoskeletal integrity of foot processes. The latter hyothesis has been supported in a recent study which demonstrates that hsp27 regulates the morphological and actin cytoskeletal response of cultured podocytes to puromycin-induced injury. In addition, hsp27 levels in podocytes correlated with resistance to puromycin-induced cell death (436).

H. Signaling Mechanism Targeting the Cytoskeleton in Podocyte Damage

Since the complex cytoskeletal organization is tightly regulated, initial studies addressing the involved signaling pathways in podocyte damage are becoming available. The cell-matrix signaling in podocytes has been described in detail in section VII.

Small GTPases regulate cytoskeletal organization. In cultured podocytes, Rho kinase blockade inhibited the stretch-induced reorganization of the actin cytoskeleton. Further evidence of the involvement of small GTPases like Rho and Rak for the regulation of podocyte cytoskeletal organization was provided by the GDP dissociation inhibitor-α (Rho GDIα) knock-out mouse (461). Rho GDI-α is a negative regulator of Rho small GTPase and retains them in their inactive, GDP-bound cytosolic form (450). Mice with homozygous deletion of the Rho GDI-α gene were born with the typical podocyte lesions of progressive glomerular failure (461). Because Rho is known to be a positive regulator of actin filament bundling, an uncontrolled Rho-induced bundling of actin filaments could be responsible for the actin-rich microfilament belt seen in process effacement (429).

Puromycin- or protamine-induced podocyte cytoskeletal reorganization in vitro was followed by an increase in overall tyrosine phosphorylation (369). These alterations could be mimicked by unspecific blockade of tyrosine dephosphorylation with vanadate. A panel of phosphatase mRNAs (SHP-2, PTP-PEST, PTP1B, and PTP-36) was detected by RT-PCR in cultured podocytes. Further studies will have to identify the specific phosphatases responsible for cytoskeletal reorganization. A further candidate for cytoskeletal regulation via dephosphorylation is the podocyte-specific receptor phosphatase GLEPP-1 (460). GLEPP-1 knock-out mice show increased sensitivity to podocyte stress with consecutive foot process alterations (490). However, defined cytoskeletal proteins have not yet been described as substrates for GLEPP-1.

Because intracellular calcium is a critical determinant for actin and tubulin polymerization, the growing number of vasoactive substances using calcium signaling in podocytes have full potential to significantly affect cytoskeletal function (421). Several growth factors are

activated in podocyte injury (153, 166, 221, 270, 476). Hepatocyte growth factor (HGF), which is secreted in the glomerulus by mesangial and endothelial cells, can protect cultured podocytes from cyclosporin-induced apoptosis (127). Initial data are consistent with the role of HGF in podocyte process formation in vitro (215).

Over the last 50 years, a considerable body of knowledge on the podocyte cytoskeleton has been accumulated. From the description of podocyte cytoskeletal alterations in glomerular damage, delineation of cell-type specific constituent of the cytoskeletal apparatus in podocytes with similarities to neuronal dendrites has been raised. Finally, the first insights into signaling mechanism allow an increasingly complex understanding of the highly specialized architecture of the mature podocyte.

Most strikingly, all molecules recently identified as critically relevant for an intact filtration barrier are cytoskeletal proteins or are closely interconnected with the cytoskeleton. This genetic evidence strongly supports the hypothesis of the cytoskeleton as the common link in podocyte failure.

X. HORMONE RECEPTORS AND SIGNALING IN PODOCYTES

A. cGMP Signaling

Guanylyl cyclases (GCs) are a family of enzymes that catalyze the conversion of GTP to cGMP. Two GC forms exist: a particulate isoform and a heme-containing soluble isoform. The particulate GC receptor family activated by natriuretic peptide has been subdivided into three types: natriuretic peptide receptor-A [NPR-A, activated by physiological concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)], natriuretic peptide receptor-B [NPR-B, activated by C-type natriuretic peptide (CNP), but not ANP or BNP], and natriuretic peptide clearance receptor (NPR-C, binds ANP, BNP, and CNP) (261). In podocytes, ANP binding sites have been mainly localized on the foot process of the podocyte (219). In addition, immunohistochemically studies detected ANP receptors in podocytes (427). Ardaillou et al. (19) showed that ANP induces a concentration-dependent increase of cGMP formation in human podocytes, which was polarized since >90% of extracellular cGMP was released in the apical medium after stimulation of ANP (19). ANP-induced cGMP formation was regulated by protein kinase A (PKA), since H-89, a PKA inhibitor, decreased ANP-stimulated cGMP formation in podocytes (248). ANP, BNP, and CNP stimulated concentration-dependent cGMP formation, and in RT-PCR studies, mRNA for NPR-A, -B, and -C receptors was detected in human podocytes (514). The functional consequences of cGMP increase on podocytes are poorly understood. Infusion of ANP in adult or neonatal rats increased the cGMP formation in podocytes, with a higher threshold for activation in immature animals, suggesting that the regulation of cGMP may play a role in podocyte development (61). In undifferentiated rat podocytes, 1 μM ANP and 1 mM SNP produced a decrease of intensity of F-actin fluorescence and a rearrangement of actin in sparse parallel bundles, suggesting that ANP might produce podocyte relaxation (421).

The soluble guanylyl cyclase (sGC) is a heme-containing heterodimer consisting of one α-subunit (73–88 kDa) and one β-subunit (70 kDa) (261). sGC expression has been suggested in podocytes in vivo (292), and sodium nitroprusside (SNP) has been shown to increase cGMP in undifferentiated rat podocytes (421). Exposure of isolated glomeruli to nitric oxide (NO) donors or a stable analog of cGMP resulted in an increase of albumin permeability of isolated glomeruli. NO donors induced tyrosine phosphorylation, and the effect of NO donors on albumin permeability could be inhibited by a tyrosine kinase inhibitor. Most of the phosphotyrosine-positive cells in glomeruli treated with NO donors corresponded to podocytes. Thus podocytes might be involved in NO-induced tyrosine phosphorylation, but a participation of endothelial and mesangial cells could not be excluded (255). With the use of a specific antibody to the β_1-subunit of sGC, sGC immunoreactivity within the glomerulus was not recognized in podocytes but instead in mesangial cells. In addition, NO donors like *S*-nitroso-*N*-acetylpenicillamine (SNAP) increased cGMP formation in mesangial cells but not in differentiated mouse podocytes, indicating that NO does not play a role in cGMP formation in podocytes (459).

B. cAMP Signaling

Adenylyl cyclases (ACs) are a family of at least nine isoforms that catalyze the formation of the second messenger cAMP (155, 264). With the use of RT-PCR mRNA, all adenylyl cyclase isoforms besides the AC VIII isoform have been found in rat glomeruli. Immunohistochemical techniques showed a very low glomerular expression of AC II, III, IV, and IX. However, within the glomerulus, type IX AC protein was only expressed in podocytes (37). The activity of AC IX can be inhibited by FK-506 and cyclosporin A (155). Cyclosporin A is a treatment choice for patients with idiopathic nephrotic syndrome, and thus there might be a link between the effects of cyclosporin A on proteinuria and a putative cyclosporin A-induced inhibition of AC IX in podocytes. An increase of cAMP concentrations in cultured differentiated mouse podocytes has been demonstrated with different agonists such as prostaglandin E_2 (35), dopamine (34), and isoproterenol (176) via so-called EP4, D1-like, and β-adrenoceptors,

respectively. It has further been shown that cAMP-inducing hormones mediate an opening of a Cl^- conductance, leading to depolarization of the podocyte (35).

A cAMP increase induced by parathyroid hormone (PTH) has been demonstrated in rat podocytes in vivo (34). The existence of a PTH/PTH-related peptide receptor mRNA in podocytes in vivo was confirmed by in situ hybridization, and a PTH/PTHrP receptor transcript was detected in cultured human undifferentiated podocytes (250). PTH-related protein is also expressed in podocytes (438). Differentiated mouse podocytes possess mRNA of the PTH-related peptide (PTHrP) and PTH/PTHrP receptors (108). The results of these studies suggest that PTH might act on podocytes in an autocrine fashion and/or regulate podocyte function. Alternatively, PTH released by podocytes can regulate phosphate reabsorption of the proximal tubule. PTHrP increased cAMP and inhibited bradykinin-induced increase of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in differentiated mouse podocytes, suggesting that it might alter the contractile status of podocytes (108). Activation of PKA by cAMP may, as in other cell types, reduce contractility and modulate the assembly of actin filaments by phosphorylation of myosin light-chain kinase (155). However, whether relaxation is induced by cAMP-increasing hormones in podocytes has not yet been confirmed. In contrast to the hypothesis that cAMP increase in podocytes may lead to relaxation, it has been proposed that increasing cAMP levels in podocytes induce a narrowing of the filtration slits, leading to a decrease in the ultrafiltration coefficient (K_f) (421).

C. Ca^{2+} Signaling

Vasoactive hormones like ANG II regulate the glomerular filtration rate by changing the tone of the glomerular arterioles and decreasing the ultrafiltration coefficient K_f. ANG II also increases the urinary protein excretion rate and induces a loss of glomerular size-selective functions. ANG II acts as a growth hormone. It stimulates proliferation of glomerular endothelial and mesangial cells and the synthesis of extracellular matrix proteins like collagen IV (181). The effects of ANG II are critical for the development of glomerulosclerosis. It has been shown that a reduction of ANG II levels by angiotensin-converting enzyme (ACE) inhibitors or AT_1 receptor blockers are renoprotective independently of their blood pressure-lowering effect in human glomerular diseases, especially in diabetic nephropathy (269, 343).

Within the glomerulus, ANG II was thought to influence preferentially mesangial cell function (21). However, it has been shown that ACE inhibitors ameliorate glomerular function and, in contrast to other antihypertensive agents, reduce podocyte hypertrophy in rat kidneys after subtotal nephrectomy, suggesting that podocyte morphology may be directly influenced by ANG II (12). Especially in the rat, there is a considerable body of evidence that ANG II directly modulates podocyte function. By using a technique which allows the examination of electrophysiological properties of podocytes in the intact glomerulus, we have demonstrated that ANG II depolarized podocytes in the isolated rat glomerulus. ANG II increased the inward current of podocytes, and the depolarizing effect of ANG II was augmented by the presence of a reduced extracellular Cl^- concentration, suggesting that ANG II activates a Cl^- conductance in podocytes (139). A 1 nM threshold concentration of ANG II was required to induce a depolarization of podocytes in the glomerulus. A half-maximal response was observed at 10 nM ANG II (139). The estimated ANG II concentration in the glomerular filtrate is ~0.2 nM, but it is increased under various circumstances such as a low-NaCl diet. In addition, a considerable higher concentration of ANG II has been detected in the proximal tubule fluid, in the range of 10 nM (308). Therefore, it is likely that ANG II modulates podocyte functions under physiological conditions as well. Recently, we introduced a new method allowing for the measurement of $[Ca^{2+}]_i$ in single podocytes in the intact glomeruli. Fluorescence measurements with a video-imaging approach or a laser-scanning microscope indicate that podocytes in the glomerulus responded to ANG II with a reversible increase of $[Ca^{2+}]_i$. Similar to the patch-clamp experiments, the threshold concentration for eliciting a $[Ca^{2+}]_I$ response was 1 nM ANG II. A second ANG II-mediated $[Ca^{2+}]_i$ response could only be elicited after ~10 min, indicating a desensitization of the receptor involved. In contrast to patch-clamp experiments, where every podocyte responded to ANG II, ANG II did not always elicit a $[Ca^{2+}]_i$ increase in all cells identified by morphological criteria as podocytes. This lack of ANG II response might have technical reasons or, alternatively, a small increase in $[Ca^{2+}]_i$ may not be detectable, especially since all cells cannot be focused in the same plane (312). In ~50% of the experiments, the Ca^{2+} transients induced by ANG II showed a small plateau phase after the $[Ca^{2+}]_i$ peak. The ANG II-mediated $[Ca^{2+}]_i$ plateau, but not the peak, was inhibited in the presence of a low extracellular Ca^{2+} concentration, indicating that the ANG II-induced increase of $[Ca^{2+}]_i$ was due to both a Ca^{2+} release from the intracellular space and a Ca^{2+} influx from the extracellular space.

$[Ca^{2+}]_i$ in unstimulated glomeruli did not increase by a high extracellular K^+ concentration. The L-type Ca^{2+} channel antagonist nicardipine did not inhibit the ANG II-mediated $[Ca^{2+}]_i$ peak and plateau response. Both results suggest that L-type Ca^{2+} channels are not activated by ANG II (312). Similar results have been found in differentiated rat podocytes in culture, but the Ca^{2+} influx in cultured cells dominates the signal, whereas Ca^{2+} store release is dominant in podocytes in the intact glomerulus

(162). In differentiated podocytes in culture flufenamate, an inhibitor of nonselective ion channels, inhibited ANG II-mediated increase of $[Ca^{2+}]_i$ with an IC_{50} of ~20 μM, whereas the L-type Ca^{2+} channel blocker nicardipine, even in high concentrations of >1 μM, only had a small inhibitory effect (162). The AT_1 receptor antagonist losartan reversibly and completely inhibited the ANG II-stimulated depolarization and the increase of $[Ca^{2+}]_i$ in podocytes in the glomerulus and in culture, suggesting that the effect of ANG II was mediated by an AT_1 receptor.

However, a lack of expression of ANG II receptors in undifferentiated human and mouse podocytes has been reported in several studies (278, 475). In the latter study, no mRNA for AT_1 or AT_2 receptors, besides mRNA expression for ANG II degrading hydrolase aminopeptidase A and angiotensinogen, was detected in cultured undifferentiated mouse podocytes (278).

In some studies in addition of the expression of AT_1 receptors, a AT_2 receptor signaling pathway has been suggested. In undifferentiated rat podocytes, ANG II increased cAMP and inositol trisphosphate. After 1 h of incubation, ANG II induced a maximal increase of $[Ca^{2+}]_i$ with a threshold concentration of 100 nM ANG II. ANG II-mediated cAMP and $[Ca^{2+}]_i$ increase could only be partially inhibited by losartan, an AT_1 receptor antagonist, or PD-123,319, an AT_2 receptor antagonist. Only the simultaneous addition of both antagonists completely inhibited the effect of ANG II on cAMP and $[Ca^{2+}]_i$ increase in undifferentiated rat podocytes, suggesting that it was mediated by both ANG II receptors (408, 420). In the latter studies, podocytes with a cobblestone appearance in long-term culture were used. The controversial findings might be explained by loss or changes of AT_1 and AT_2 receptor expression and function of the highly differentiated podocytes in culture.

Several other agonists have been reported to modulate $[Ca^{2+}]_i$ in podocytes (19, 35, 121, 159, 176, 313, 348, 349, 363, 422, 441–443). Table 1 summarizes the respective hormones and their receptors, which have been detected in podocytes in vitro and in vivo. In addition, it has been reported that the damage of podocytes mediated by complement C5b-9 complex is associated with $[Ca^{2+}]_i$ increase and an activation of phospholipase C. The activation of $[Ca^{2+}]_i$ and phospholipase resulted in an inhibition of complement C5b-9 complex-mediated podocyte injury (77).

Whereas many of the receptors shown in Table 1 are also expressed within various cell types within the glomerulus, the muscarinic M5 receptor has recently been found to be expressed only in podocytes. Immunofluorescence studies indicate that M5 receptors are expressed in glomerular podocytes, and two-photon laser-scanning microscopy showed that single podocytes in glomeruli increased $[Ca^{2+}]_i$ in response to ACh. Figure 10 shows the ACh-induced $[Ca^{2+}]_i$ increase in podocytes. Interestingly, in contrast to ANG II-mediated strong desensitization of the AT_1 receptor, a repetitive addition of high concentrations of ACh did not inhibit the $[Ca^{2+}]_i$ increase induced by ACh, indicating a lack of receptor desensitization. This suggests that AT_1 and M_5 receptors are activated in different ways by their respective ligands (313). The role of

TABLE 1. *Ca^{2+}-stimulating hormones in undifferentiated and differentiated podocytes*

Hormone/Type of Receptor	Species	In Situ/In Vitro	Cellular Event	Reference Nos.
Angiotensin II	Rat	In situ	Cl^- channel ↑	139, 312
AT_1/AT_2		In vitro[u+d]	cAMP ↑, $[Ca^{2+}]_i$ ↑	162, 420, 422
Acetylcholine	Rat	In situ	$[Ca^{2+}]_i$ ↑	313
M5			Cl^- channel ↑	
Prostaglandin	Mouse	In vitro[d]	$[Ca^{2+}]_i$ ↑	35
E_2			Cl^- channel ↑	
EP1				
PGF_2	Mouse	In vitro[d]	$[Ca^{2+}]_i$ ↑	35
FP			Cl^- channel ↑	
Thromboxane	Mouse	In vitro[d]	$[Ca^{2+}]_i$ ↑	35
TP				
AVP, oxytocin	Human	In vitro[u]	cAMP ↑, $[Ca^{2+}]_i$ ↑	442
V_{1a}, unknown				
Bradykinin	Human	In vitro[u+d]	$[Ca^{2+}]_i$ ↑, IP_3 ↑	19, 348
BK_2	Mouse		cAMP ↓	108
ATP	Human	In vitro[u+d]	$[Ca^{2+}]_i$ ↑, IP_3 ↑	121
P2Y1, P2Y2, P2Y6, P2X7	Mouse			349
Endothelin	Human	In vitro[u]	$[Ca^{2+}]_i$ ↑, IP_3 ↑	363, 441
ET_A				
Histamine	Human	In vitro[o]	$[Ca^{2+}]_i$ ↑, IP_3 ↑	443
H_1				
Norepinephrine α_1	Mouse	In vitro[d]	$[Ca^{2+}]_i$ ↑	176
Thrombin unknown	Human	In vitro[u]	$[Ca^{2+}]_i$ ↑, IP_3 ↑	159

$[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; IP_3, inositol 1,4,5-trisphosphate; AVP, arginine vasopressin. [u]Undifferentiated podocytes; [d]differentiated podocytes.

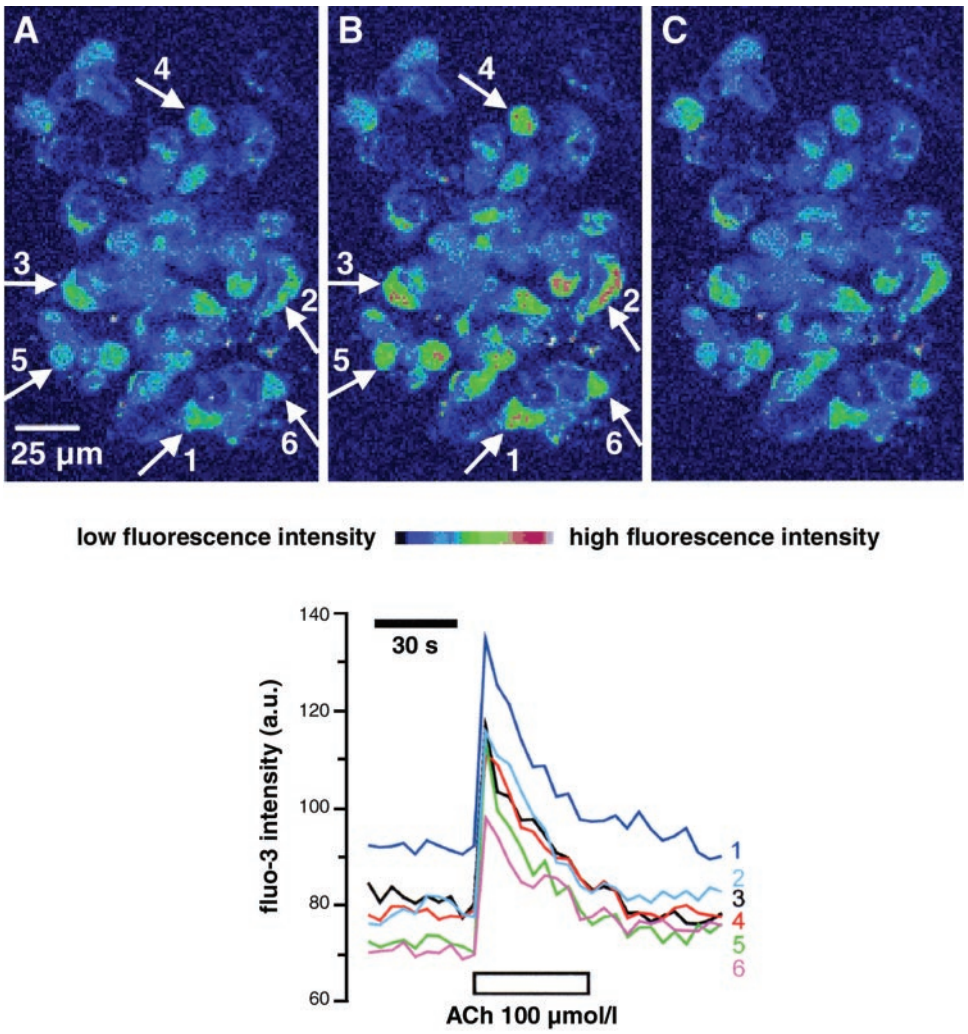

FIG. 10. Acetylcholine (ACh) increases the cytosolic calcium concentration ($[Ca^{2+}]_i$) in podocytes. Confocal two-photon fluo 3 fluorescence imaging is shown. ACh increased fluo 3 fluorescence intensity as a measure of $[Ca^{2+}]_i$ (arrows). *Bottom panel*: original trace of fluorescence intensity as a measure of $[Ca^{2+}]_i$ recorded from the cells marked in the confocal images. [From Nitschke et al. (313). Copyright 2001 Lippincott Williams & Wilkins.]

the M_5 receptor for podocytes as well as for other cell types is poorly understood. The M_5 receptor has been reported to possess unique properties, as the second intracellular loop has been demonstrated as an ordered cluster of residues where diverse substitutions cause constitutive activation (55).

What may be the consequences of a stimulation of podocytes with Ca^{2+}-mobilizing hormones?

1) Hormone-induced cellular signaling might modify the contractile structures of podocyte foot processes, resulting in an alteration of the ultrafiltration coefficient K_f.

It has been proposed that an increase in $[Ca^{2+}]_i$ induces a narrowing of the filtration slits, leading to a decrease in K_f, but direct evidence for this hypothesis is missing (97, 233, 421).

2) It has recently been demonstrated that thrombin stimulates TGF-β gene expression in undifferentiated rat podocytes in culture, resulting in an increased stimulation of the production of type IV collagen and fibronectin. In addition, TGF-β inhibited podocyte proliferation. Therefore, thrombin and perhaps other vasoactive hormones may play a role in the progression of glomerulosclerosis through the upregulation of TGF-β production in podocytes (468). However, nothing is known about possible effects of ANG II on MAP kinases, phospholipase D, and phospholipase A_2; induction of protooncogene expression; cross-talk with tyrosine kinase receptors; stimulation of nuclear signaling cascades; and production of other growth factors, which might modulate long-term functions of podocytes.

3) Reactive oxygen species (ROS) are important mediators in pathophysiological events occurring in Heyman nephritis, a model for human membranous nephropathy characterized by subepithelial immune complex deposition and proteinuria. Although not particularly shown for ANG II, extracellular ATP, which increases $[Ca^{2+}]_i$ in podocytes (349), stimulates the production of superoxide in human cultured podocytes, suggesting that vasoactive hormones might influence the properties of the glomerular filtration barrier via the release of ROS (144).

4) In several experimental models of chronic renal failure, podocyte injury initiates and maintains the progression of glomerulosclerosis. The activation of podocyte signaling systems by ANG II and other vasoactive hormones probably contributes to podocyte injury in chronic renal failure (12). Inhibition or antagonism of angiotensin inhibits proteinuria by preserving the size-selective function of the glomerular capillary (370). Results from recent studies suggest that ACE inhibitors or AT_1 receptor antagonists affect the expression of proteins of the slit membrane. In proteinuric Wistar rats, alterations in glomerular basement membrane permeability to water and macromolecules in vitro did not occur, but the pattern of ZO-1 distribution changed, i.e., compared with controls, the labeling distribution of ZO-1 appeared more heterogeneous and discontinuous. ZO-1 redistribution was not accompanied by ultrastructural change of the foot processes and the epithelial slit diaphragms. Treatment of Wistar rats with an ACE inhibitor prevented proteinuria and prevented the glomerular redistribution of ZO-1 without changing the amount of glomerular ZO-1 protein expression, suggesting that preservation of glomerular distribution of ZO-1 may be important for the maintenance of normal properties of the glomerular capillary wall (265). In an animal model of progressive renal injury, downregulation of nephrin mRNA and nephrin protein has been demonstrated. Downregulation of nephrin expression was prevented by an ACE inhibitor and ANG II receptor blocker, indicating that inhibition of the function of ANG II may result in a change of nephrin expression (39). Podocytes have been shown to be the primary cellular target in early diabetic nephropathy and ACE inhibition or blockade of the AT1 receptor reduce proteinuria and delay the progression of renal injury in diabetic nephropathy (269, 343). AT_1 receptor blockade normalizes a reduced nephrin expression in diabetic rats, suggesting that inhibition of the AT_1 receptor may directly or indirectly influence the expression of nephrin in vivo (48).

The links between inhibition of the AT_1 receptor and nephrin expression are unclear. ANG II is known to induce tyrosine phosphorylation of proteins such as paxillin (249). Increased tyrosine phosphorylation of proteins at cell-cell junctions including ZO-1 protein may be responsible for the reorganization of the junctional complex and increase in paracellular permeability, but until now, this has not been demonstrated to be induced by vasoactive hormones in podocytes (242).

Major extrusion pathways for Ca^{2+} are the plasma membrane Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger. Recently, it has been shown that podocytes express the Na^+/Ca^{2+} exchanger. The activity of the the Na^+/Ca^{2+} exchanger in podocytes was inhibited by stimulation of protein kinase C and by a toxic injury induced by puromycin. Thus it has been proposed that inhibition of Na^+/Ca^{2+} exchanger in podocytes may contribute to podocyte injury (120).

XI. DAMAGING MECHANISMS OF PODOCYTES

Several toxins, antibodies, complement factors, and forms of mechanical stress are able to induce damage of the podocytes.

A. ROS

The reactive oxygen product hydrogen peroxide (H_2O_2) is known to be a mediator of cellular injury. It acts either directly or serves as a source of hypochloride, which is formed in the presence of chloride and myeloperoxidase, an enzyme secreted by polymorphonuclear leukocytes (186). The glomerular toxicity of H_2O_2 has been directly demonstrated in studies where intra-arterial infusion of H_2O_2 caused derangements in glomerular permselectivity without change of glomerular filtration rate (GFR), renal plasma flow (RPF), or structural changes of the glomerular filtration barrier. H_2O_2-induced proteinuria was inhibited by pretreatment with catalase or deferroxamine, suggesting that iron-dependent metabolites of hydrogen peroxide mediate the effects of H_2O_2 (507). Overproduction of ROS has been detected in several glomerular diseases, including puromycin nephrosis, a model of minimal change disease (373), Heyman nephritis, a model of membranous nephropathy (309, 414), and the Mpv 17 (−/−) mouse, a model for steroid-resistant focal segmental sclerosis (43). In these glomerular diseases, pretreatment of animals with ROS scavengers prevented foot process effacement and proteinuria (43, 148, 373, 414). In puromycin nephrosis, phosphatidylcholine-bound superoxide dismutase, which has a higher affinity to the cell membrane than recombinant human superoxide dismutase, decreased proteinuria to the control level and improved podocyte density. Puromycin induced a decrease of α_3-integrin expression and a change of polarity of its site of expression, which was preserved by phosphatidylcholine-bound superoxide dismutase (216). In biopsies of patients with membranous nephropathy, Grone et al. (148) demonstrated oxidatively modified proteins in podocytes, mesangial cells, and basal membranes (148). In addition, an increase in glomerular XO activity due to a conversion of xanthine dehydrogenase to the oxidase form has been shown to be responsible for ROS production in this model of glomerular damage (150). Podocytes seem to be not only the target but also the source of ROS in membranous nephropathy. In Heymann nephritis, proteinuria is dependent on antibody-induced formation of the complement C5b-9 membrane attack complex. It has been demonstrated that sublytic C5b-9 attack on podocytes causes upregulation of expression of

the NADPH oxidoreductase enzyme complex by podocytes, which is translocated to their cell surfaces. Subsequently, ROS are produced locally and reach the GBM matrix. ROS initiate lipid peroxidation and subsequent degradation of GBM collagen IV, leading to proteinuria (202, 309). Despite the evidence of the roles of ROS in podocyte injury, the clinical benefit derived from these insights has yet to come. One reason for the lacking efficiency of ROS scavengers in reversing established glomerular injury may be that ROS itself changes several signaling cascades of podocytes, which then maintain podocyte injury by mechanisms distinct from ROS. In this regard, we recently demonstrated that exogenous ROS causes a marked increase in the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA as well as GM-CSF protein release in cultured differentiated mouse podocytes (143). The time course of GM-CSF mRNA increase in response to even short-term stimulation with ROS was prolonged, suggesting that a short-lived exposure of podocytes to ROS induces a relatively long-lived sequel. GM-CSF release by podocytes was also stimulated by lipopolysaccharide, interleukin-1, and phorbol ester and was inhibited by ROS scavengers. Interestingly, activation of the transcription factor NF-κB, but not AP-1, was involved in the upregulation of ROS-induced GM-CSF production (143). ROS-induced release of GM-CSF in podocytes may therefore play a prolonged role in the inflammatory events by functionally activating mature leukocytes on the inflammatory side, by inhibiting their migration away from the focus, and also by enhancing the proliferation and differentiation of progenitor cells. In addition, GM-CSF release by podocytes may not only alter functions of macrophages, but might also modulate cellular properties of glomerular endothelial cells in a paracrine fashion. We are about to study the role of GM-CSF and other genes that are upregulated by ROS for podocyte function.

B. Puromycin Aminonucleoside-Induced Nephrosis

Puromycin aminonucleoside-induced nephrosis (PAN) is one of the best described animal models of proteinuria. It was thought to mimic human idiopathic nephrotic syndrome because after the injection of PAN, there was a flattening of podocyte foot processes, which is associated with the development of palmlike domains, and a reduction in anionic charge, and proteinuria occurred (182, 383, 479). Glomerular injury induced by PAN has also been demonstrated to progress to focal glomerulosclerosis (93). However, damage of proximal tubules, including the loss of brush border, dilated lumina, abnormally thin walls, and accumulation of periodic acid-Schiff positive electron-dense luminal casts and cytoplasmic protein absorption droplets (14) has also been found in PAN rats, and some authors believe that albuminuria occurring in PAN is not of glomerular, but of tubular origin (333).

Podocyte depletion is a crucial hallmark of glomerulosclerosis and has been considered a central problem in the progression of renal diseases (240). Therefore, it is of major interest to investigate the mechanisms that lead to podocyte depletion. In PAN, apoptosis in podocytes can be partially inhibited by ROS scavengers and by actinomycin D. In addition, necrosis of podocytes was found when high concentrations of puromycin aminonucleoside (PA) were used (118, 390). During PAN-induced FSGS, apoptosis of podocytes was accompanied by an increase of Bcl-2 expression. In contrast, no expression of p53, Fas antigen, or Fas ligand could be detected in podocytes (425). Very recently, it was shown that sequential administration of PA can reduce glomerular podocytes. The part of the glomerulus lacking podocytes developed glomerulosclerosis, and this part progressively increased as podocytes progressively depleted (209). But conflicting data exist. In a recent study using the PAN model of FSGS, apoptosis could not be found in glomerular cells. Competitive RT-PCR performed in this study showed that Bax, Bcl-2, Fas, and Fas ligand mRNA were not changed in glomeruli in PAN. In situ hybridization studies indicated that Bax and Bcl-2 mRNA are expressed in podocytes and parietal epithelial cells. An increased expression of Bcl-2 occurred only segmentally in glomeruli in some PAN animals (487). The different findings of the rate of apoptosis of podocytes in PAN may be due to different time points of apoptosis determination or to different frequencies of PAN injections and PAN concentrations used. Several studies suggest that antioxidants provide protection against PAN-induced podocyte injury. Antioxidants reduce proteinuria in PAN and inhibit reduced foot process effacement but do not change the damage that occurs in podocyte cell bodies and major processes (92, 373, 458, 487). Furthermore, antioxidants did not prevent interstitial inflammation or fibrosis occurring in PAN (100). An increase of bleomycin-detectable iron, which is capable of catalyzing free radical reactions, has been observed in glomeruli of PAN, whereas bleomycin-detectable iron was not changed in tubules. The iron chelator deferroxamine prevented the increase in the bleomycin-detectable iron in glomeruli and completely inhibited proteinuria in PAN (471). During PAN, cytochrome *P*-450 could not be found in glomeruli. Inhibitors of cytochrome *P*-450 prevented an increase in the catalytic iron in the glomeruli and decreased proteinuria, suggesting that cytochrome *P*-450 may serve as a source of catalytic iron in PAN (259).

Expression levels of several proteins, which are assumed to be involved in the foot process effacement of podocytes, changed in PAN. During PAN, the glomerular expression of α-actinin is increased in podocytes. Increased α-actinin expression preceded podocyte foot pro-

cess effacement and proteinuria, suggesting that it might have a pathogenic role in foot process effacement. The authors also observed an induction of glomerular α_3-integrin in PAN, but this was not limited to podocytes. No changes in glomerular vinculin, talin, β_1-integrin, or total actin expression have been detected in PAN (435). An increased expression of heparanase, which may mediate loss of glomerular charge selectivity, has been reported in PAN (254). In PAN, an upregulation of heparin-binding EGF-like growth factor has been demonstrated (337). Injection of a monoclonal antibody against heparin-binding EGF-like growth factor did not induce proteinuria, but it augmented proteinuria observed in PAN. Adhesion of the human podocytes to laminin and fibronectin was decreased by the antibody, suggesting that heparin-binding EGF-like growth factor may influence adhesion of the podocyte (206). Proteinuria in PAN is accompanied by downregulation of nephrin mRNA and protein expression (262). Likewise, the expression of podoplanin, a 43-kDa glycoprotein, is also markedly reduced in PAN (50). It has recently been shown that podocalyxin, the major sialoprotein of podocytes, is linked to ezrin and the actin cytoskeleton via Na^+/H^+ exchanger regulatory factor (NHERF2). NHERF2 and ezrin form a multimeric complex with podocalyxin. This complex interacts with the actin cytoskeleton, and this interaction is disrupted in PAN (452).

In vitro studies show that PA causes podocyte blebbing and rounding and reduces adhesion to plastic (122). It has been suggested that PA might alter the organization of cytoskeletal and extracellular matrix proteins with loss of podocyte adhesion. In cultured undifferentiated podocytes, PA and adriamycin caused a decreased protein expression of several cytoskeletal proteins including actin, vimentin, keratin, and β-tubulin along with a decreased expression of laminin and heparan sulfate. In addition, PA induced a loss of the β_1-integrin focal adhesions. The PA-mediated effects were not accompanied by a decrease of overall protein synthesis (64). It has recently been shown that treatment of human podocytes with 5 μg/ml PA resulted in a reduction of cell numbers, without affecting cell viability or DNA synthesis (228). PAN decreased mRNA and protein expression of α_3- and β_1-integrin, which was accompanied by a decrease in the adhesion of podocytes, suggesting that PAN-induced detachment might be, at least in part, due to an inhibition of the expression of β_1-integrin in podocytes. In contrast, mRNA and protein expression of podocalyxin was slightly increased by PAN, whereas no change of ZO-1 protein expression could be detected (228).

Formation of podocyte processes is highly dependent on a constant source of fresh lipids and proteins in differentiated mouse podocytes in culture (431). In mouse glomeruli and in differentiated mouse podocytes in culture, mRNA expression for several amino acid uptake transporters has been demonstrated, such as the neutral AAT systems ASCT1, ASCT2, IAT, and B0/+, the cationic AAT systems CAT1 and CAT3, and the anionic AAT systems EAAT2 and EAAT3. Pretreatment of podocytes with PA decreased their membrane voltage slightly, indicating that PA did not significantly change resting ion currents in podocytes. However, after PA treatment, amino acid-induced depolarization and conductance increase were markedly inhibited, suggesting that PA-induced injury of podocytes is associated with a decrease in amino acid transport (140).

C. Protamine Sulfate

Injection of the polycation protamine sulfate (PS) causes structural alterations of the podocyte similar to those caused by PAN nephropathy, i.e., after PS injection, junction complexes form between adjacent podocyte processes and the processes flatten and retract (16). During PS nephropathy, ZO-1, which is normally located along the cytoplasmic surfaces of the slit diaphragms, is replaced along both the newly formed occluding-type junctions and the remaining slit diaphragms (243). The PS-mediated changes of podocyte morphology were accompanied by an increase in phosphotyrosine expression in podocytes. Newly phosphorylated proteins were concentrated along newly formed tight junctions and the basal membrane of the foot processes. Furthermore, ZO-1 was found to be a target for tyrosine phosphorylation after PS treatment, suggesting that phosphorylation of tight junctions occurs in PAN (243). In vitro experiments also indicate that the effects of PS on podocyte structure may be controlled by tyrosine phosphatases (369). Treatment of cultured podocytes with 600 μg/ml PS resulted in process retraction and cell rounding after 6 h, which was accompanied by a loss of actin filament bundles. PS-induced detachment of podocyte processes requires an intact actin cytoskeleton, since depolymerization of the actin filaments with cytochalasin B inhibits the effects of PS. RT-PCR studies show that podocytes possess mRNA for the protein tyrosine phosphatases SHP-2, PTP-PEST, PTP1B, and PTP-36. Within PS-treated podocytes, increased staining for tyrosine-phosphorylated proteins could be detected in the cytoplasm. In addition, vanadate, an inhibitor of protein tyrosine phosphatases, mimicked the response to PS, whereas an inhibitor of protein tyrosine kinases neither influenced podocyte morphology nor inhibited PS-induced process retraction (369). Early cellular responses of PS in podocytes have been recently reported; PS or positively charged DEAE-dextrans caused a increase in $[Ca^{2+}]_i$ in podocytes. The effects of PS and DEAE-dextran were not completely reversible, suggesting that PS and DEAE-dextran may have induced an impairment of the $[Ca^{2+}]_i$ regulation. Compared with PS, DEAE-

dextran was ~75 times more potent, and the Hill coefficients for PS and DEAE-dextran were ~0.9 and 2.0, respectively. These differences may be explained by the different amounts of positive charges of the agents: PS possesses 21 positive charges/mol and DEAE-dextran 1,140. Therefore, there seems to be a good correlation between the amount of positive charges and the efficiency of PS and DEAE-dextran to increase $[Ca^{2+}]_i$ (377). Perfusion of kidneys with PS in a solution with a reduced extracellular Ca^{2+} concentration inhibited PS-mediated podocyte foot process injury by ~50%, indicating that an influx of Ca^{2+} is crucial for the PS-mediated damage of podocyte foot processes (197). In vitro reduction of extracellular Ca^{2+} also inhibited the PS-mediated increase of $[Ca^{2+}]_i$, indicating that PS induced a Ca^{2+} influx in addition to the release of $[Ca^{2+}]_i$ from intracellular stores. Interestingly, after emptying inositol trisphosphate-sensitive Ca^{2+} stores with thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase, PS still increased $[Ca^{2+}]_i$ in podocytes, indicating that it increases $[Ca^{2+}]_i$ by a release of Ca^{2+} from thapsigargin-insensitive stores. Neutralization of positive charges by heparin prevented the effect of PS, whereas inhibitors of tyrosine kinase, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, and protein phosphatases were not affected (377).

Podocyte injury, proteinuria, and renal failure can be induced by the systemic application of *Vibrio cholerae* sialidase. In this experimental model of proteinuric disease, sialidase primarily removes $\alpha 2 \rightarrow 6$-linked sialic acid from the podocyte, and a subsequent transient loss of negative charge from endothelial cells and podocytes has been documented. The loss of anionic charge was accompanied by retraction of foot processes and formation of tight junctions between adjacent foot processes. Although the loss of anionic charge after application of sialidase was reversible, foot process retraction was irreversible. The experiments support the idea that a transient loss of sialic acid leads to foot process injury and proteinuria (137).

D. Cyclosporin A

Twenty-four-hour treatment with cyclosporin A (CsA) has recently been shown to induce apoptosis in podocytes in culture with a threshold concentration of 0.5 μg/ml CsA. Pretreatment of podocytes with hepatocyte growth factor reverses CsA-induced apoptosis in podocytes. CsA did not change Fas or Fas-ligand protein levels but reduced Bcl-xL protein levels, the latter reversed by the pretreatment of cells with hepatocyte growth factor. The authors also show evidence that Bcl-xL in podocytes is regulated in a phosphatidylinositol 3′-kinase-dependent but MEK-1-independent manner (127).

E. TGF-β

There is strong evidence that local production of TGF-β contributes to the pathogenesis of glomerular diseases. Podocytes have been shown to secrete TGF-β in response to several agents such as high glucose (475), low-density lipoprotein (94), or thrombin (468). Mice that are transgenic for an active form of TGF-β1 develop progressive glomerulosclerosis with mesangial expansion and thickened capillary loops after 3 wk of age. Mice with the highest levels of TGF-β1 developed proteinuria and subsequent nephrotic syndrome (217). In 2-wk-old transgenic kidneys, local expression of TGF-β1, -β2, and -β3 protein did not change, whereas in 5-wk-old transgenic mice, TGF-β1 and -β2 protein expression increased, suggesting that elevated concentrations of circulating TGF-β1 induce primary glomerular injury (290). It has recently been shown that within this model of glomerulosclerosis, TGF-β1 and Smad7, intracellular mediators of TGF-β signaling, might be important mediators of injury in podocytes (398). In TGF-β transgenic mice, the number of podocytes per glomerulus was reduced and the rate of apoptosis increased. This was accompanied by an increase of Smad7. In podocytes in culture, 1 ng/ml TGF-β1 induced apoptosis, which was accompanied by an increased Bax protein synthesis and caspase-3 activity. Overexpression of Smad7 in podocytes also induced an increase of apoptosis in podocytes. The proapoptotic effects of Smad7 overexpression and of TGF-β were additive. However, only TGF-β, but not Smad7 overexpression, activated p38 MAP kinase. Inhibition of p38 MAP kinase reversed TGF-β- but not Smad7-induced apoptosis, suggesting that p38 MAP kinase is required for induction of apoptosis by TGF-β, but not by Smad7. In contrast to the apoptosis induced by TGF-β, Smad-7 overexpression had no effect on Bax and procaspase-3 protein levels (398).

F. Fibroblast Growth Factor-2

Fibroblast growth factor-2 (FGF-2) belongs to a family of signaling molecules that regulate basic biological processes such as proliferation, survival, and differentiation in several cell types (324). Recent studies indicate that FGF-2 signaling is also important for the regulation of basic functions of podocytes.

In vivo and in vitro studies show that expression of FGF-2 proteins is increased in podocytes with mitotic arrest (83). Podocytes derived from FGF-2 knock-out mice showed marked changes in cell morphology, i.e., in contrast to wild-type podocytes, FGF-2 mutant podocytes grow tightly associated to one another and fail to form cellular processes. These changes in podocyte morphology were associated with a disorganization of actin fila-

ments and a decreased expression of synaptopodin and WT1. The authors also show evidence that the molecular steps, which mediate the induction of mesenchymal differentiation in podocytes, are inhibited in podocytes with deficient FGF signaling. In FGF-2 mutant podocytes, expression of vimentin and slug, markers of mesenchymal cells, decreased, whereas the expression of the epithelial markers cytokeratin and desmocollin type 2 increased. In addition, podocytes with deficient FGF-2 showed a changed ZO-1 expression profile, which is reminiscent of epithelial cells. Therefore, FGF-2 signaling plays an important role in regulating differentiation of podocytes (83). Other studies show that FGF-2 stimulates proliferation in rat podocytes and that it may therefore contribute to podocyte injury (453). Indeed, in vivo experiments demonstrated that rats receiving long-term FGF-2 treatment developed albuminuria, severe podocyte injury leading to FSGS, and chronic renal failure. Because mitotic figures have been observed in podocytes without an increase of cell number of podocytes in FGF-2-treated rats, it has been suggested that FGF-2 may stimulate podocytes to undergo mitosis (234). Also, in passive Heymann nephritis, bFGF increased proteinuria and augmented podocyte injury without altering antibody or complement deposition or glomerular leukocyte influx, suggesting that bFGF may enhance podocyte damage (126). Similar results have been reported in PAN, where FGF-2 increased PA-induced podocyte injury and proteinuria, whereas injection of FGF-2 neutralizing antibody reduced podocyte injury and proteinuria (393). In the latter model of glomerular injury, FGF-2 also increased the number of bromo-deoxyuridine and proliferating cell nuclear antigen positive cells, suggesting that FGF-2 might influence the cell cycle regulation of podocytes (393).

G. Complement Activation

Complement activation can proceed via the classical or alternative pathway. Central to both pathways is the cleavage of C3 and C5, generation of proinflammatory complement factors such as C3a and C5a, as well as formation of the cell-damaging C5b-9 complex. C5a receptor expression was recently reported to be upregulated in human podocytes and other glomerular cells in membranous nephropathy (2), and complement receptor 1 (CR1, CD35, C3b/C4b receptor) is expressed on podocytes in the healthy glomerulus (315). CR1 binds to the activated complement components C3b and C4b, and thereby induces several functions, including clearance of immune complexes from the circulation, enhancement of phagocytosis, and regulation of complement activation by means of decay-accelerating activity for C3 convertases and cofactor activity for factor I (10). CR1 protein is first expressed in developing podocytes. Interestingly, although CR1 receptor mRNA has been detected in early stages of glomerular development, its expression decreases with glomerular maturation, and it became undetectable in mature podocytes, suggesting that CR1 receptor mRNA amounts are below the detection level or that turnover of CR1 receptor mRNA is very slow (18). Downregulation of the CR1 receptor in podocytes has been shown in rapidly progressive glomerulonephritis, in severe proliferative nephritis of systemic lupus erythematosis, in collapsing idiopathic FSGS, and in HIV-associated nephropathy (31, 194). Pretreatment of rats with soluble human CR1 that lacked transmembrane and cytoplasmic domains and therefore inhibited C3 and C5 convertase activity by preferentially binding C4b and C3b, proved to reduce proteinuria in passive Heymann nephritis and two other forms of complement-mediated glomerular injury. Thus soluble human CR1 may serve as a therapeutic agent in complement-mediated glomerular disease (74).

In vivo and in vitro studies have demonstrated that complement activation is a crucial step in the development of complement-mediated podocyte injury. In active Heymann nephritis (AHN), a rat model of human membranous nephropathy, immunization of rats with fraction 1A (Fx1A), a crude renal tubular preparation, induced the production of IgG autoantibodies after the formation of large, subepithelial immune deposits, which included IgG, C3, and membrane attack (5b-9) components of complement (163, 202). Within 8 wk of immunization with Fx1A, most of the animals developed proteinuria. The target autoantigen of AHN is a transmembrane renal glycoprotein with a molecular mass of ~600 kDa, variously named megalin, a protein belonging to the low-density lipoprotein-receptor family (200, 385). In addition, highly purified megalin alone and a recently identified 60-kDa proteolytic fragment of megalin can induce AHN (325). The passive form of Heymann nephritis (PHN) is induced by injecting heterologous antibodies against rat megalin or anti-Fx1A antibodies into rats and is therefore, unlike AHN, not an autoimmune disease. Progressive staining of heterologous IgG in subpodocyte immune deposits accompanied by podocyte effacement is observed. C3 and the C5b-9 membrane attack complex are also present in a distribution similar to heterologous IgG, suggesting that IgG in the immune complexes may activate complement (202).

Complement activation on podocytes results in their injury and proteinuria. Depletion of complement completely inhibited proteinuria, strongly suggesting an important role of complement activation in PHN (79, 394). The precise mechanisms by which complement activation causes proteinuria are unclear. C5b-9 is incorporated into vesicles and transported by podocytes into the urinary space. Although podocytes seem to be resistant to cell lysis by C5b-9, C5b-9 induces podocytes to produce reactive oxygen radicals. This leads to an alteration of the properties of the glomerular filtration barrier (202). In

addition, an upregulation of the matrix metalloproteinase-9 (272) and growth factors like TGF-β2 and -β3 and their receptors TGF-β receptor type I and type II (417) has been detected in podocytes in PHN. Upregulation of matrix metalloproteinase-9 and TGF-β may play a role in the breakdown of the ultrafiltration barrier (272) and overproduction of matrix in membranous nephropathy, respectively (417). Not long ago, an increased NF-κB binding activity was demonstrated in PHN. Treatment with pyrrolidonethiocarbamate (PDTC), an inhibitor of NF-κB, reduced NF-κB binding activity and albuminuria in PHN (291). In addition, PDTC treatment decreased matrix metalloproteinase-9 mRNA. Thus NF-κB activity seems to contribute to the development of proteinuria in PHN and may regulate genes relevant for podocyte injury (291).

Three cell-associated complement regulators are expressed in mouse kidneys that function to protect renal cells from autologous complement-mediated injury. These regulators are complement receptor-related protein-Y (Crry), the membrane inhibitor of reactive lysis (CD59), and decay-accelerating factor (DAF or CD55) (306). Complement regulatory proteins are important for limiting complement activation in podocytes. Crry and CD59 are regulators of the complement system and inhibit C3 convertases and assembly of C5b-9, respectively (358, 482). Crry and CD59 are expressed by renal podocytes and have been shown to protect against antibody-directed complement-mediated injury in vitro (75).

During nephrotoxic serum nephritis, Crry transgenic mice showed a diminished proteinuria, suggesting that complement inhibition at the C3 convertase step is effective in complement-mediated injury states (357). It has also been shown that after inducing PHN with antimegalin, simultaneous neutralization of Crry and CD59 resulted in proteinuria. Therefore, inhibition of Crry and CD59 can induce podocyte injury and proteinuria in PHN, and normal complement regulation can restrain complement-mediated glomerular injury (399). In AHN however, rats immunized with Fx1A lacking Crry do not develop proteinuria or C3 deposition in glomeruli, although anti-Fx1A Abs deposits are found in glomeruli (356). The authors discuss that normal glomerular complement regulation by Crry is effective to limit antibody-directed complement-mediated damage in AHN but not in PHN, in which a more sudden antibody binding to the podocyte occurs (399). These studies indicate that soluble complement regulators or targeted site-specific complement inhibitors may be a future treatment option in complement-mediated glomerular injury (399).

Decay-accelerating factor (DAF) inhibits the C3 convertase of both the classical and alternative pathways. Human and rat cultured podocytes express DAF (359, 403), and DAF mRNA has been found in podocytes of human kidney biopsy specimens (3). DAF knock-out mice showed increased proteinuria, glomerular volume, and cellularity during nephrotoxic serum nephritis (437). In addition, nephrotoxic serum nephritis in DAF-deficient mice caused severe podocyte fusion, whereas only mild focal changes were observed in the controls. This was accompanied by a marked increase of deposition of autologous murine C3 in DAF-deficient mice. Thus the studies indicate that DAF seems to play a critical role for protecting glomeruli in complement-mediated glomerular injury (257, 437).

In vitro studies in undifferentiated rat podocytes show that complement-mediated injury induced by fresh serum as a source of complement and anti-Fx1A sensitization is cytotoxic for podocytes. Cytotoxicity induced under these conditions was Mg^{2+} and factor B dependent but Ca^{2+} independent, indicating that anti-Fx1A activates the complement alternative pathway. In the presence of Mg^{2+} and factor B, anti-Fx1A increased C3b deposition on podocytes and complement C alternative pathways. C3 and C5 convertases were inactivated and stabilized in podocytes over time, suggesting that anti-Fx1A inhibits complement regulation in podocytes (75). In undifferentiated podocytes, complement activation leads to an activation of different cellular events, i.e., it increases intracellular calcium concentrations, activates phospholipases A_2 and C, induces ATP depletion, and leads to a reversible disruption of actin microfilaments and focal contacts (77, 339, 356, 399, 463).

H. Cytokines and Chemokines

Development of nephrotic syndrome may also follow allergic reactions in some cases (274). In atopy, Th2-mediated inflammation plays a central role. Interleukin (IL)-4 and IL-13, two Th-2 cytokines, decreased transepithelial electrical resistance in undifferentiated rat podocytes (474). Both cytokines also activate basolateral proton secretion and redistribution of the small GTPases Rab5b and Rab7. In addition, they activate basolateral secretion of the lysosomal proteinase procathepsin L, suggesting that IL-4 and IL-13 modulate intracellular trafficking of proteins and promote proteolysis at the basolateral surface of podocytes (473). Podocytes from patients with minimal change nephropathy and undifferentiated rat podocytes express IL-4Rα and IL-13R2 receptors, suggesting that activation of these receptors plays a role in podocyte injury in minimal change nephropathy (474). IL-4 has been shown to decrease the viability of undifferentiated podocytes. IL-4 and interferon-γ also change the immunoreactive pattern of the tight junction protein ZO-1 in undifferentiated rat podocytes (66). Human undifferentiated podocytes have been shown to express mRNA of the IL-4Rα, IL-10R1 and -2, and IL-13Rα1 and -α2. These cytokines inhibited the release of VEGF from podocytes, whereas TGF-β and IL-1β had the opposite effect (342).

Glomerular-produced chemokines have been implicated not only to induce recruitment of inflamatory cells, but also to alter functions of resident glomerular cells. Chemokines and their receptors are expressed by intrinsic renal cells as well as by infiltrating cells during glomerular inflammation (for review, see Ref. 410). Recently, the expression of functional active chemokine receptors CCR4, CCR8, CCR9, CCR10, CXCR1, CXCR3, CXCR4, and CXCR5 have been detected in human differentiated podocytes. Ligands of these chemokine receptors increased $[Ca^{2+}]_i$ and stimulated the generation of superoxide anion in podocytes, suggesting that activation of these receptors may be involved in the pathogenesis of podocyte injury (178). In the latter study it has been also demonstrated that podocytes are able to produce IL-8, a ligand for the CXCR1/CXCR2 receptor. Thus the CXCR1 receptor in poocytes may be activated in an autocrine fashion (178). In minimal change nephropathy, overproduction of IL-8 by peripheral blood mononuclear cells has been proposed to play a role in the pathogenesis of proteinuria (134). In addition, IL-8 has been shown to be involved in the pathogenesis of complement-mediated injury. Wada et al. (481) showed that a neutralizing antibody against IL-8 decreased the number of glomerular neutrophils, prevented the fusion of podocytes foot processes, and abolished proteinuria in acute immune complex-mediated glomerulonephritis. IL-8 release of podocytes during glomerular diseases may therefore, in an autocrine fashion, participate in the development of proteinuria in glomerular diseases.

Interestingly, within the glomerulus, only podocytes express the CCR7 receptor ligand SLC/CCL21. Mesangial cells express the respective CCR7 receptor. Activation of this receptor resulted in migration, proliferation, and inhibition of Fas/CD95-mediated apoptosis of mesangial cells. Thus functions of mesangial cells might be controlled by the local synthesis of SLC/CCL21 by podocytes (27).

Besides IL-8 and SLC/CCL21, it has been demonstrated that undifferentiated rat podocytes in culture release monocyte chemoattractant protein-1, suggesting that they may actively participate in local inflammatory events during glomerulonephritis (307).

I. Mechanical Stress

The foot processes of podocytes probably experience capillary wall distension, but little is known about the effects of mechanical forces on podocyte structure and function. Endlich et al. (107) recently showed that biaxial cyclic mechanical stress induces a change in cultured mouse podocyte morphology as well as reorganization of the actin cytoskeleton. In mechanically stressed podocytes, the processes of podocytes get thinner and more elongated, whereas the cell body size decreases. No apoptosis occurred in mechanically stressed podocytes, but radial stress fibers were formed, which converged to a focus, the actin-rich center. A Rho kinase inhibitor initially prevented the formation of radial stress fibers and the formation of the actin-rich center but had no effect after the actin-rich center had been formed. F-actin reorganization in podocytes in response to mechanical stress was inhibited by high concentrations of Ni^{2+}, suggesting that Ca^{2+} influx may play a role in the mechanical stress-induced cytoskeletal reorganization (107). A recent study showed that mechanical stress decreases the growth of podocytes and modulates the expression of cyclins and cyclin-dependent kinase inhibitors (352).

XII. PODOCYTE INJURY LEADS TO PROTEINURIA

The glomerular filtration barrier prevents all but the smallest plasma proteins from entering the urinary space and at the same time allows an extraordinarily high permeability to water and small molecules. Podocytes maintain a large filtration surface through the slit membranes and are responsible for ~40% of the hydraulic resistance of the glomerular filtration barrier. During proteinuric disease, the ultrafiltration coefficient K_f and thus the GFR are often lowered. A detailed review of the way of filtration of water and small molecules by the glomerular filtration barrier has been recently given by Deen et al. (87). The ability of the kidney to retain plasma proteins is essential for life. Proteinuria is a hallmark of glomerular injury and also an independent predictor of progression of renal disease, i.e., a higher level of urine protein is associated with a faster rate of progression, and a reduction in proteinuria is associated with slowing progression of the glomerular diseases (378).

The mechanisms that determine how the glomerular filtration barrier excludes the entry of proteins into the urine space and which structures of the glomerular filtration barrier are disturbed in proteinuric states are still controversial and poorly understood. In principle, proteinuria may be caused by defects of the podocytes, the GBM, the endothelial cells, and/or by alterations of the negatively charged proteins present on all three components of the glomerular filtration barrier.

It has been proposed that neutral dextrans and nonmetabolized organic molecules are excluded from urine on the basis of size and shape but not of charge. Anionic molecules have been suggested to be restricted more than neutral or cationic proteins because of an interaction with the glomerular filtration barrier (467). However, whether the size selectivity of the glomerular filtration barrier is disturbed in proteinuria and whether the glomerular filtration barrier interacts with proteins by virtue of charge characteristics is still a matter of debate. The following

sections discuss the different aspects of the filtration of proteins through the glomerular filtration barrier under physiological conditions and during proteinuric diseases.

A. Size Selectivity of the Glomerular Filtration Barrier

Two different models of the functional properties of the glomerular barrier have been introduced.

1. The glomerular barrier restricts albumin

By using a fractional micropuncture method which excludes contamination of extratubular proteins, Tojo and Endou (462) found that the glomerular-filtrated albumin was 22.9 μg/ml, suggesting an albumin sieving coefficient of 6.2×10^{-4}. According to a serum albumin concentration of 37 g/l and a GFR of 1.05 ml/min, daily glomerular albumin filtration would be in the range of 35 mg/day (GFR $\times$ plasma albumin $\times$ 0.00062 = $1.05 \times 1440 \times 37 \times 0.00062$).

Measurements of the glomerular sieving coefficients also showed that small dextran molecules are freely filtered, whereas the fractional clearance for dextran molecules with Stokes-Einstein radius >42 Å approaches zero (58). It has been suggested, however, that the conformation of dextran is unfolded during glomerular permeation and that its transport through the glomerular filtration barrier is therefore facilitated (46). Ficoll, a branched, spherical, rigid, and neutral polysaccharide, has been proposed to be a more suitable probe molecule compared with dextran for determining size selectivity of the glomerular filtration barrier (326). Fractional clearances for Ficoll of 0.2 at Stokes-Einstein radius (rs) = 21 Å, 6×10^{-3} at rs = 37 Å, and 7.1×10^{-4} at rs = 65 Å have been found in rats. (326). Similar values have been found in isolated perfused rat kidneys (321). In rats and in isolated perfused rat kidneys, fractional clearances for Ficoll were ~30 and 90 times greater than for albumin (321). In humans, a fractional clearance of 0.1 has been reported for 36-Å Ficoll; however, the fractional clearance of albumin was only in the range of 10^{-6}. The results suggest a very low glomerular filtration of albumin in the range of ~9 mg/day (GFR $\times$ [Alb]P $\times 10^{-6}$ = 180 l/day $\times$ 50 g/l $\times 10^{-6}$ = 9 mg/day). In a recent study, the effect of the filtration rate on fractional clearances of four molecules with similar Stokes-Einstein radii, but with different shapes and charges, has been investigated in isolated rat kidneys. Compared with albumin, neutral Ficoll and two other elongated and negative charged molecules, hyaloran and bikunin, had >100 times higher fractional clearances despite their similar size and charges, indicating that the elongated shape of a molecule increases the transglomerular passage. For albumin and large Ficoll molecules >45 Å, the fractional clearance values increased with an increase in the glomerular filtration rate. However, for 36-Å Ficoll, hyaloran, and bikunin, the fractional clearance values decreased with an increase in the glomerular filtration rate, suggesting that the glomerular filtration barrier is a dynamic structure that does not have the properties of an artificial membrane (323). Ficoll sieving data have also been obtained in isolated glomerular basement membranes. Here, a decline in the sieving coefficient with an increase in molecular size from 0.6 at rs = 20 Å and ~0.03 at rs = 50 Å were found.

The question as to which slit diaphragm of the podocyte or the GBM is important for normal glomerular permselectivity is still a matter of debate. In early studies, Farquhar et al. (116) reported that injected ferritin freely passes the endothelial cells and accumulated beween the endothelium and the GBM. Small amounts of ferritin (rs = 61 Å) that did cross through the GBM were detected in lysosmes of podocytes. The authors therefore propose that the GBM is the main filtration barrier to proteins whereas the endothelium is freely permeable and that podocytes partly recover proteins that passes the GBM (116). During PAN nephritis, ferritin was still restricted by the GBM, but the GBM became more leaky and uptake of ferritin by podocytes was increased (114). Like ferritin, dextran molecules with similar size and charge than albumin did not cross the GBM, suggesting that albumin is restricted by the GBM (57).

Other studies suggest that the slit diaphragm serves as the most selective filter for albumin.

To test whether smaller proteins than ferritin might pass throught the GBM, horseradish peroxidase (rs = 30 Å), myeloperoxidase (rs = 36 Å), and catalase (rs = 52 Å) have been used as tracers. Horseradish peroxidase passed through the GBM and the slit diaphragm, whereas myeloperoxidase and catalase crossed the GBM but were restricted from entering the urine, probably at the level of the slit diaphragm. Therefore, it has been proposed that large molecules like ferritin are restricted by the GBM, whereas the slit diaphragm is the final barrier for smaller proteins such as albumin (142, 478). Ryan and Karnovsky and co-workers (382, 384) reported that under normal hemodynamic conditions albumin and catalase were restricted at the endothelial and GBM levels. However, after reduction of blood flow, albumin passed across the GBM and slit diaphragm, whereas catalase was restricted by the slit diaphragm. Thus changes in hemodynamics seem to affect permeability properties of the glomerular filtration barrier.

An electron microscopy study performed by Rodewald and Karnovsky (374) also supports the idea that the slit diaphragm is a selective filter for albumin. The study shows that the slit diaphragm is composed of rodlike structures connected to a central bar providing a zipperlike structure. The central bar, the rodlike extensions, and the plasma membranes of the foot processes seem to

outline rectangular spaces of 4 × 14 nm, which is about the size of an albumin molecule (374). It has been proposed that nephrin may fit well into a zipperlike isoporous filter structure similar to that presented by Rodewald and Karnovsky (374).

To investigate the relative resistance of the GBM and cell layers to the movement of uncharged macromolecules, the diffusional permeabilities of intact and cell-free capillaries to narrow fractions of Ficoll were measured. The diffusional permeability of cell-free capillaries to Ficoll was shown to be 10–20 times that of intact capillaries. Thus the contribution of the GBM to the diffusional resistance of the intact glomerular filtration barrier has been estimated to be ~13–26% of the total, increasing with molecular size. It has been proposed that the slit diaphragm of the podocytes is most likely responsible for the cellular part of the diffusional resistance (104). In a theoretical model, the size selectivity of the glomerular barrier has been related to the structural characteristics of the individual layers of the capillary wall. The slit diaphragm has been considered a row of cylindrical fibers with variable spacing. With the use of this model, the local sieving coefficient has been calculated for by four hypothetical barriers (bare GBM, GBM with endothelial cells, GBM with both endothelial cells and podocytes but without a slit diaphragm, and the complete glomerular filtration barrier). As each barrier was added, the sieving coefficient for any given molecular size was reduced, but the addition of the slit diaphragm caused a sizable reduction of the sieving coefficient of large macromolecules by some two orders of magnitude. A twofold increase in the thickness of the GBM and/or a threefold decrease in the filtration slit frequency had little effect on the predicted sieving curves. The limitation of such biophysical models, however, comes from molecular structures that are not yet fully understood and unknown molecular interactions of the yet identified proteins of the slit diaphragm (105).

In favor for the hypothesis that podocyte injury leads to proteinuria, it has been also demonstrated that big molecules like ferritin are able to permeate the GBM at sites of podocytes detachment (189). In addition, a selective damage of the podocyte in vivo leads to albuminuria, and deletion of several molecules of the podocyte or the slit diaphragm results in severe proteinuria (246). These data thus indicate that the slit diaphragm represents a crucial filter for albumin.

2. The glomerular barrier is leaky for albumin

In contrast to the above proposed model, which assumes that the glomerular filtration barrier is highly selective, the "albumin retrieval hypothesis" suggests that charge interactions of albumin with the glomerular filtration barrier are negligible and that albumin in Bowman's space is ~8% of plasma albumin. In the studies supporting this idea, a glomerular sieving coefficient for albumin was found in the range of 0.068–0.079. This means that the flux of albumin across the glomerular filtration barrier is of the order of ~2,000 μg/min (53, 109). If the rat data were applicable to humans, ~600 g/day of albumin would be filtered and reabsorbed (GFR × plasma albumin × 0.07 = 180 l/day × 50 g/l × 0.07 = 630 g/day). By analyzing the decrease of radioactive labeled albumin in the venous effluent after its injection into the renal artery, it has been subsequently demonstrated that postglomerular filtered albumin is returned intact to the blood at a rate of 1,830 μg/min (109). Albumin transport could be inhibited by albumin peptides and ammonium chloride, which inhibits tubular protein uptake, but do not alter glomerular size selectivity. The authors propose the existence of a high-capacity retrieval pathway for albumin, which is most likely located in proximal tubule cells. A disturbance of this pathway could be accounted for in most albuminuric states without affecting glomerular permselectivity (53, 334). Beyond this major retrieval pathway, a small amount of filtered albumin that escapes this pathway has been suggested to undergo tubular uptake, where it is degraded by lysosomal enzymes and where degradation products are regurgitated back into the tubular lumen (334). Several arguments against the "albumin retrieval hypothesis" have been raised.

1) Although it has been shown that albumin can be taken up by the proximal tubule, there is no evidence that labeled albumin injected into single proximal tubules crosses the wall of the tubules by passing between the cells or that intracellular located albumin, which has been mostly found in lysosomes, is transported into the peritubular space (271).

2) It has been suggested that at least some of the absorbed albumin is degraded within lysosomes and that almost all albumin transported by the cubulin-megalin complex is degraded (63).

3) So far, no evidence for the transcellular transport of protein ligands, including carrier proteins, has been published (63, 340).

4) By using a fractional micropuncture method, which excludes contamination of extratubular proteins, Tojo and Endou (462) found that the glomerular filtrated albumin was only 22.9 μg/ml, suggesting an albumin sieving coefficient of 6.2×10^{-4} (462).

5) Fractional clearance studies with albumin in isolated perfused rat kidneys in which the tubular activity was inhibited by low temperature found a fractional albumin clearance of only 0.19% (321).

6) Electron microscopy studies showed that molecules like ferritin and dextran did not cross the GBM, suggesting that they are restricted by the GBM (57, 116).

7) A decreased expression of the slit diaphragm protein nephrin correlates with a loss of glomerular filter integrity (354).

B. Charge Characteristics of the Glomerular Filtration Barrier

Negatively charged molecules are found in all layers of the filtration barrier. The charge-selective filter has been thought to be located in the basement membrane, a cross-linked meshwork of type IV collagen, laminin, fibronectin, entactin, and heparan sulfate proteoglycan (147). The basement membrane of podocytes is endowed with sulfated glycosaminoglycans, mainly heparan sulfate. The slit membrane and the foot processes of podocytes are covered by a glycocalyx containing heavily sialyated glycoconjugates as well as sulfated molecules. The major sialoprotein of the podocyte foot process glycocalyx is podocalyxin, a 140-kDa sialoprotein. Podocalyxin is highly glycosylated with 20% hexose, 4.5% sialic acid, and additional *N*-acetylglucosamine. Podocalyxin is synthesized in the podocyte, and its insertion into the apical membrane is closely correlated with the development of podocyte foot processes and filtration slits (198).

The polyanionic sites of the GBM are sensitive to heparitinase treatment (187). Heparitinase treatment also increases the permeability of the GBM by anionic macromolecules such as native ferritin (188), suggesting that heparan sulfate proteoglycans (HSPGs) are crucial structures of the charge-selective permeability of the GBM. However, recent studies have doubted the functional relevance of the GBM for charge selectivity. Treatment of isolated GBM with heparanase to remove heparan sulfate proteoglycan anionic side chains or protamine to effect charge neutralization had no effect on the albumin sieving coefficient. In contrast, in intact glomeruli, both agents increased albumin permeability (82). The results from the latter study suggest that the charge selectivity of the glomerular filtration barrier requires the presence of glomerular cells (82).

Ferritin molecules of the same size but with varying charge have been shown to cross differentially through the glomerular flitration barrier. Anionic ferritin was almost completely excluded from entering the GBM, whereas the most cationic ferritin reached the level of the slit diaphragm (372). Studies in which fractional clearances of neutral and negative charged dextrans were compared came to the conclusion that there is a functional relevant charge selectivity of the glomerular filtration barrier with a charge density of ~120–170 meq/l (88). However, it has since been discussed that dextran sulfate can bind to proteins and that it is taken up by cells from isolated glomeruli (455) and by glomerular endothelial cells (480), which would reduce the fractional clearance of negatively charged dextran relative to uncharged dextran.

By using four anionic proteins and neutral Ficoll, Sorensson et al. (439) recently showed that all anionic proteins used had lower fractional clearances than size-matched neutral Ficolls, and a wall charge density of ~30–40 meq/l has been estimated (439). In a recently introduced model of glomerular size and charge selectivity, Ohlson et al. (322) proposed that the glomerular filtration barrier is composed of a dynamic gel and a more static membrane layer. According to this model, the glomerular filtration barrier size and charge selectivity are connected in series. The size-selective structures possess many small pores with a radius of 45–50 Å and a few large pores with a radius of 75–115 Å. It is probably located at the slit diaphragm of the podocyte (322). The estimated charge density was calculated to be only 35–40 meq/l, and it has been assumed that it may be confined to the glomerular endothelium (322). Low ionic strength increased the size selectivity, whereas it reduced the charge density, indicating that size and charge selectivity are influenced by ionic strength in an opposite manner (439). In contrast to the data reported in these studies, Greive et al. (145) did not find a different fractional clearance of negatively charged carboxymethyl Ficoll versus uncharged Ficoll in rats, indicating a complete lack of charge selectivity of the glomerular filtration barrier. In agreement with the latter study, it has been shown that the sieving coefficients of Ficoll sulfate of the isolated GBM were not different from those of Ficoll at physiological ionic strength, although the values for Ficoll sulfate were depressed at low ionic strength (47). The results of both studies indicate that although the GBM possesses fixed negative charges, it does not contribute to charge selectivity under physiological conditions. The conclusion of the studies on isolated GBM is based, however, on the assumption that isolated GBM is not functionally different from that in vivo. Although several studies suggest that isolated GBM is relatively intact, possible interaction of podocytes and endothelial cells with the GBM, which may affect its functional properties, could have been overlooked in these studies.

Whether glomerular size and/or charge selectivity is altered in proteinuric diseases has been studied in human glomerular diseases as well as in experimental animal models of glomerulonephritis. In human membranous nephropathy, the fractional clearance for 36 Å Ficoll did not differ between the nephrotic and healthy groups, suggesting that almost all albuminuria in nephrotics was due to a charge-related effect. In contrast, fractional clearance for 54–56 Å Ficoll, which has an equivalent radius to IgG, was enhanced by ~20-fold in nephrotic patients, suggesting that immunoglobulinuria is due to an impairment of size selectivity in the glomerular filtration barrier.

In uninephrectomized fawn-hooded rats, which develop an accelerated proteinuria, fractional clearances for >42 Å Ficoll were increased by ~5- to 13-fold compared with controls. They remained unchanged for smaller Ficoll molecules <42 Å. The increase of albuminuria and the lack of changes of the fractional clearance of 36 Å Ficoll suggest that the size selectivity is not disturbed in

uninephrectomized fawn-hooded rats. Other mechanisms, i.e., alterations of the charge selectivity or impaired albumin reabsorption by tubules, could mediate albuminuria (327). Proteinuria in PAN nephrosis is associated with a loss of podocyte pedicels and podocyte major processes, an increase in pinocytotic activity, and an accumulation of cytoplasmic vacuoles and granules of variable size, shape, and electron density (14). The charge density within the GBM has been reported to show early and significant reductions in puromycin nephropathy (266). On the other hand, less or no alterations of charge density within the glomerulus were found (491). Very recently, the effect of PAN on glomerular size selectivity has been reinvestigated in two independent studies. The fractional clearance of albumin and 35.5 Å Ficoll in isolated kidneys perfused at 8°C were 17×10^{-4} and 0.15, respectively. Treatment of rats with 7.5 mg/100 g body wt PAN did not increase fractional clearance of 35.5 Å Ficoll in vivo and in isolated perfused kidneys. Higher concentrations of puromycin (15 mg/100 g body wt) caused a twofold increase of fractional clearance of 35.5 Å Ficoll, indicating that PAN affects the glomerular size barrier. By estimating the charge density in perfused isolated kidneys by calculating the ratio of albumin to 35.5 Å Ficoll, a small but significant decrease of charge density was found in PAN (167). In contrast to the concept that proteinuria is due to alterations of the slit diaphragm, one study showed a small increase of the fractional clearance for IgG but a lack of an increase of the fractional clearance of 36 Å Ficoll during PAN (10 mg/100 mg body wt) and anti-GBM glomerulonephritis. This suggests that the glomerular permeability of albumin is not disturbed in glomerulonephritis, but the albumin processing by tubular cells is altered in glomerulonephritis. In addition, negatively charged carboxymethyl Ficoll had the same fractional clearance as uncharged Ficoll, indicating a lack of charge selectivity in the glomerular filtration barrier (145). Thus it is still a matter of debate whether the glomerular filtration barrier possesses a functional glomerular charge selectivity.

XIII. PODOCYTE INJURIES AND THEIR PROGRESSION TO NEPHRON LOSS

The decline in renal function in chronic renal disease is underlaid by the progressive loss of viable nephrons. In the majority of cases, the loss of nephrons most likely starts with the injury of podocytes. The damaging factors acting on podocytes as well as the immediate pathophysiological responses of any podocyte injury, i.e., loss of barrier function for macromolecules leading to proteinuria, were summarized in the previous sections. Here we inquire about the structural changes and their possible progression to nephron loss. At present, three different patterns of changes initiated by podocyte injury may be distinguished: *1*) degenerative changes prone to progress to "classic FSGS," *2*) inflammatory changes prone to progress to crescent formation, and *3*) changes indicating dedifferentiation leading to collapsing FSGS. In a given case of chronic renal failure, these patterns and the subsequent pathways to nephron degeneration may be mixed up.

A. Degenerative Changes and the Development of "Classic" FSGS

Degenerative changes of podocytes are typically seen in models of glomerular hypertension and hyperfiltration including subtotal renal ablation (301, 457), deoxycorticosterone trimethyl acetate (DOCA)-salt hypertension (225), in the Milan normotensive rat (124), in the hypertensive Fawn-hooded rat (237), and in the *fa/fa* Zucker rat (136), as well as in models of toxic injury to podocytes including PAN nephrosis and after long-term mitogenic stimulation by exogenous FGF-2 (234). Exposed to the challenges in these models, podocytes are unable to maintain their normal cell shape but change in appearance in a fairly stereotyped manner (199, 230, 238). These changes comprise cell hypertrophy, foot process effacement, cell body attenuation, pseudocyst formation, cytoplasmic overload with reabsorption droplets, and, finally, detachment from the GBM. Figure 11 shows an injured podocyte with foot process effacement and apically "microvillous transformation." Foot-process effacement has been interpreted to represent an adaptive change in cell shape, accompanied by hypertrophy of the contractile apparatus which reinforces the supportive role of podocytes (429). Cell-body attenuation and pseudocyst formation have been shown to result directly from mechanical overextension (301). Plausible explanations are available for cell hypertrophy, such as hyperfiltration, and for accumulation of absorption droplets, such as increased lysosomal uptake and degradation of filtered proteins, most dramatically seen in protein overload proteinuria (84), and for cell detachments, such as the impairment of connections with the GBM (62) (see above).

B. The FSGS Pattern of Nephron Degeneration

This pathway to nephron degeneration may be subdivided into three essential steps (235, 237). *1*) The first step is loss of podocytes. As discussed above, podocytes in the adult are incapable of regenerative cell replication. Thus, when podocytes are lost for any reason, they cannot be replaced by new podocytes. The only way remaining podocytes may compensate for the loss of podocytes is by cell hypertrophy taking over the increased work load. *2*) The second step is formation of a tuft adhesion. If the remaining podocytes fail to cover the defect, naked GBM

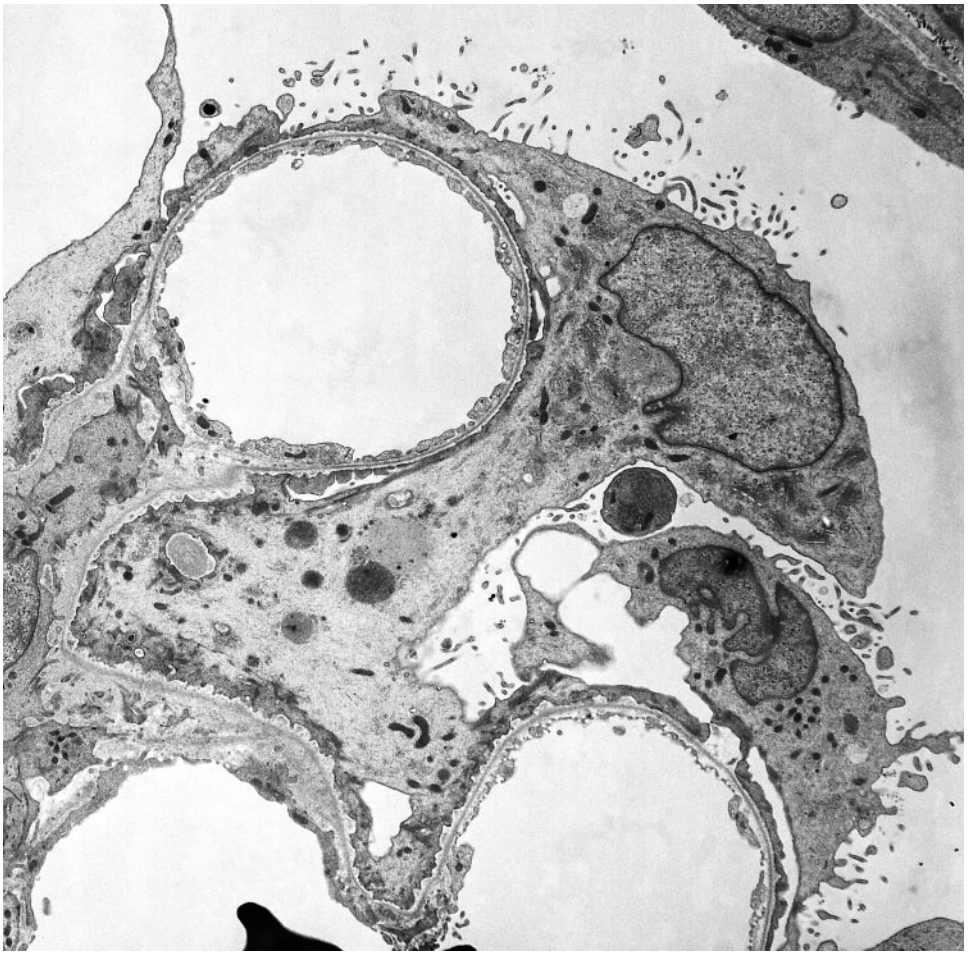

FIG. 11. Injured podocyte with foot process effacement and, apically, "microvillous transformation." In the basal cytoplasm of the podocyte adhering to the glomerular basement membrane, a darkly staining cytoskeletal belt is seen. Figure is transmission electron micrograph of masugi nephritis. Magnification ×~3,800.

areas will result, allowing access to the GBM by parietal cells of Bowman's capsule. A gap in the parietal epithelium then develops, through which the injured glomerular tuft portions come into direct contact with the interstitium. Such a circumscribed tuft adhesion represents the initial "committed" lesion in the development of segmental glomerulosclerosis. *3*) The third step is misdirected filtration. Once established, a tuft adhesion spreads and eventually encompasses the entire lobule (Fig. 12). The sequence is quite uniform; it roots in the progressive loss of podocytes from the flanks of a tuft adhesion, allowing the encroachment of parietal epithelial cells onto adjacent capillaries (replacing the podocytes). A variety of mechanisms for the further loss of podocytes have been discussed (232, 237, 428). The most important mechanism appears to be "misdirected filtration" onto the outer aspect of the parietal epithelium (235, 237). Capillaries contained in the tuft adhesion may deliver their filtrate into the space between the parietal epithelium and its basement membrane instead of into Bowman's space. In response, interstitial fibroblasts establish a dense cover of sheetlike processes that encloses the focus of misdirected filtration, preventing the dissipation of the filtrate into the surrounding interstitium. This leads to the formation of crescent-shaped paraglomerular spaces which, due to ongoing filtration, have the tendency to enlarge by separating the parietal epithelium from its basement membrane in all directions. As a consequence, the gap in the parietal epithelium increases and the adherent sclerotic tuft portions become progressively herniated into the enlarging paraglomerular space. This process may stop after an entire lobule has been engulfed, leading to a segmental synechia.

On the other hand, this is not an altogether stable situation. Via the vascular pole, this process may progressively involve the other lobules and eventually lead to global sclerosis. Via the urinary pole, the misdirected filtrate may extend onto the outer aspect of the tubule and lead to the formation of peritubular spaces as an equivalent of paraglomerular spaces (235–237). Again, these spaces become delimited from the interstitium by a layer of fibroblast processes. Therefore, the spreading of the filtrate is confined to the outer aspect of the affected tubule, eventually leading to its degeneration, generally starting with the initial segment of the tubule traveling downstream.

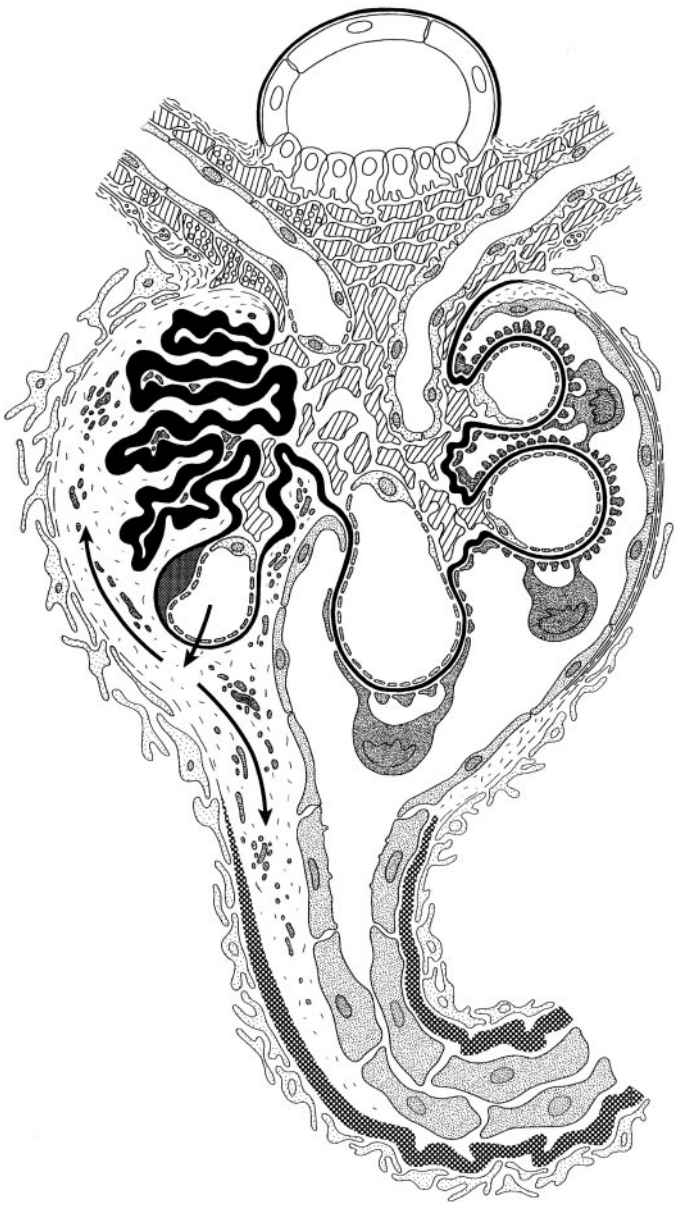

FIG. 12. Scheme to illustrate nephron degeneration sustained by misdirected filtration. An intact glomerular lobule protrudes into Bowman's space, which is outlined by the parietal epithelium passing over at the urinary pole into the tubular epithelium (parietal and tubular epithelia are densely stippled). The sclerotic glomerular lobule consists of collapsed (shown in black) and hyalinized (shown in dark gray) former capillaries and mesangial portions herniated into a paraglomerular space that is separated from the interstitium by a layer of fibroblast processes (loosely stippled). In addition to collapsed capillaries, the sclerotic tuft portion contains a patent capillary (partially hyalinized) which delivers its filtrate into the paraglomerular space (arrow), accounting for the expansion of the paraglomerular space. This space extends toward the vascular pole and via the urinary pole onto the outer aspect of the tubule separating the expanded tubular basement membrane (shown in light gray) from its epithelium. The peritubular spaces created by filtrate spreading (expansion of the glomerular basement membrane, separation of the expanded glomerular basement membrane from the epithelium) are separated from the interstitium by a layer of fibroblast processes. [Modified from Kriz et al. (230).]

Two patterns of final nephron degeneration have been identified (237). In the first pattern, the degeneration of the glomerulus and the tubule occurs concurrently and leads finally to their replacement by fibrous tissue. In the second pattern, presumably as an early consequence of peritubular filtrate spreading, the tubule obstructs, which leads to tubular atrophy downstream and upstream to the development of atubular glomerular remnants that may expand to glomerular cysts (236). In both instances, the striking consequence of this mechanism of nephron destruction is that the destructive process remains confined to the injured nephron.

C. Inflammatory Changes and the Development of Glomerular Crescents

An inflammation of the glomerulus generally starts within the endocapillary compartment, i.e., inside the GBM. Thus, at the very beginning of the disease, podocytes are not affected. However, by unknown mechanisms, mediators of the inflammatory process may reach the podocytes and may stimulate a hyperactive pattern of response. Podocytes develop abundant tiny fingerlike processes by outgrowth from the apical cell surface (164). These changes have long been known as "microvillous transformation" of the apical cell membrane (277, 303, 483). Basally, this process may be associated with the retraction of foot processes, i.e., with foot process effacement. The newly formed apical processes extend into all directions and may come to touch the parietal epithelium, to pierce through the epithelium in between to parietal epithelial cells (PECs), and finally to fix to the parietal basement membrane (PBM). At the PBM those processes may behave like lamellipodia, spreading on the PBM and displacing PECs. This event appears to trigger PEC proliferation and, consequently, formation of a cellular crescent (164).

In the case that the inflammatory process dies down and comes to a stop, it appears that the cellular crescents may likely be resolved, leading to full reconstitution or to a segmental scar. In the case that the inflammatory process continues to flourish, crescents may enlarge and overgrow the urinary orifice, leading to tubular obstruction. Similar to what has been described above concerning the degenerative pattern of nephron destruction, this event may lead downstream to tubular atrophy and finally degeneration and upstream to the development of atubular glomerular remnants. The role of the podocyte in this pathway to nephron loss is not well understood, but the establishment of podocyte bridges between the tuft and Bowman's capsule appears to be the crucial event in the progression of an inflammatory process from the tuft to Bowman's capsule and to the periglomerular interstitium. Detailed knowledge of the cytoskeletal changes that lead

to the switch to a "migratory phenotype" of podocytes is urgently needed.

D. Proliferation of Podocytes and the Collapse of the Glomerular Tuft

In HIV-infected patients, a kind of glomerulopathy was encountered that was associated with vivid proliferation of podocytes and subsequently the collapse of the glomerular tuft; it was termed collapsing glomerulosclerosis. Since then, collapsing FSGS has been appreciated more broadly, possibly as a consequence of other viral infections (45, 81, 91). The observation of podocyte proliferation under these circumstances appeared to disprove the rule that podocytes in the adult are unable to carry out cell replication. However, it became obvious that this kind of podocyte cell multiplication never led to new differentiated podocytes that might functionally replace lost podocytes. The phenotype of podocytes that proliferate under these conditions is highly dedifferentiated (30, 31). This dedifferentiation includes loss of cell shape with loss of all processes; compared with foot process effacement seen in the usual podocyte phenotype, the dedifferentiated podocytes also lose typical components of the cytoskeleton, most dramatically an actin-based cytoskeleton in the cell portions adhering to the GBM. These changes may readily be expected to be the consequence of the loss of the expression of several podocyte-specific proteins including WT-1, synaptopodin, and others (31). So far, no evidence has been presented that such podocytes may enter development to redifferentiation.

This kind of podocyte proliferation may lead to obstruction of Bowman's capsule simply by filling it with cells that finally penetrate into the tubular lumen; such cases are seen in HIV-nephropathy. In less comprehensive cases, the proliferation may be restricted, leading to what is called a "cellular lesion" (406, 407). A very serious consequence of podocyte dedifferentiation in this context is the collapse of the glomerular tuft, therefore the name "collapsing" glomerulosclerosis. Capillaries, as well as mesangial cells, are progressively lost from the endocapillary compartment, leading to the local or universal collapse of the tuft which finally consists of little more than matrix, wrinkled remnants of thickened GBM, and some mesangial matrix.

XIV. CLOSE AND OUTLOOK

Since the first description of a heavily branched cell type within the renal glomerulus in 1929 by Karl Zimmermann (515), there have been episodes of intensive podocyte research (most of them centered around Marilyn Gist Farquar), but the broad interest in podocyte biology among nephrologists did not develop until the 1990s. At that time the podocyte came into the visor of renal pathologists as a candidate accounting for the progressive nature of chronic renal failure. Fries et al. in 1989 (128) were the first to suggest that an inability of podocytes for cell replication in the adult represents the major fact underlying nephron degeneration. In the subsequent years the mechanisms were elucidated how podocyte injury leads to nephron degeneration. The real kick-off in podocyte research was switched on by the discovery of the crucial involvement of podocyte failure in the development of three hereditary diseases leading to FSGS, i.e., in the congenital nephrotic syndrome of the Finnish type (205), in the steroid-resistant nephrotic syndrome (49), and in an autosomal dominant familiar form of FSGS (190). In these diseases a gene encoding for a "more or less" podocyte-specific protein is mutated leading to massive proteinuria and nephron degeneration according to a pattern nowadays termed "classic" FSGS. Many efforts are now necessary to identify the precise interaction and function of these proteins that are crucial for the maintenance of podocyte structure. In addition, transgene technology in mice allows a podocyte-specific overexpression of these genes and their effect on podocyte injury and proteinuria in glomerular diseases. Meanwhile, there is no longer any doubt that the podocyte is the culprit in collapsing FSGS and a major player in diabetic nephropathy. Moreover, very recent studies suggest that the transition of an inflammatory glomerular disease into a chronic course is decisively mediated by the podocyte. These findings together launch the podocyte into the center stage of glomerular diseases, i.e., of glomerular diseases that potentially will proceed to end-stage renal failure. The knowledge about the mediators that contribute to podocyte injury in chronic glomerular diseases has increased, but the intracellular signaling mechanisms leading to podocyte injury under these circumstances are poorly understood. The discovery of the precise signaling mechanisms leading to podocyte foot process effacement, to podocyte hypertrophy, and to loss of the podocyte in chronic glomerular diseases will hopefully be a first step in the development of more specific therapeutic tools for preserving the functions of the podocyte.

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REFERENCES

1. Aaltonen P, Luimula P, Astrom E, Palmen T, Gronholm T, Palojoki E, Jaakkola I, Ahola H, Tikkanen I, and Holthofer H. Changes in the expression of nephrin gene and protein in experimental diabetic nephropathy. *Lab Invest* 81: 1185–1190, 2001.
2. Abe K, Miyazaki M, Koji T, Furusu A, Nakamura-Kurashige T, Nishino T, Ozono Y, Harada T, Sakai H, and Kohno S. Enhanced

expression of complement C5a receptor mRNA in human diseased kidney assessed by in situ hybridization. *Kidney Int* 60: 137–146, 2001.
3. ABE K, MIYAZAKI M, KOJI T, FURUSU A, OZONO Y, HARADA T, SAKAI H, NAKANE PK, AND KOHNO S. Expression of decay accelerating factor mRNA and complement C3 mRNA in human diseased kidney. *Kidney Int* 54: 120–130, 1998.
4. ABRAHAMSON DR. Origin of the glomerular basement membrane visualized after in vivo labeling of laminin in newborn rat kidneys. *J Cell Biol* 100: 1988–2000, 1985.
5. ABRAHAMSON DR. Structure and development of the glomerular capillary wall and basement membrane. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F783–F794, 1987.
6. ADLER S. Characterization of glomerular epithelial cell matrix receptors. *Am J Pathol* 141: 571–578, 1992.
7. ADLER S. Integrin receptors in the glomerulus: potential role in glomerular injury. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F697–F704, 1992.
8. ADLER S AND CHEN X. Anti-Fx1A antibody recognizes a β 1-integrin on glomerular epithelial cells and inhibits adhesion and growth. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F770–F776, 1992.
9. ADLER S, CHEN X, AND ENG B. Control of rat glomerular epithelial cell growth in vitro. *Kidney Int* 37: 1048–1054, 1990.
10. AHEARN JM AND FEARON DT. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv Immunol* 46: 183–219, 1989.
11. ALCORTA DA, PRAKASH K, WAGA I, SASAI H, MUNGER W, JENNETTE JC, AND FALK RJ. Future molecular approaches to the diagnosis and treatment of glomerular disease. *Semin Nephrol* 20: 20–31, 2000.
12. AMANN K, NICHOLS C, TORNIG J, SCHWARZ U, ZEIER M, MALL G, AND RITZ E. Effect of ramipril, nifedipine, and moxonidine on glomerular morphology and podocyte structure in experimental renal failure. *Nephrol Dial Transplant* 11: 1003–1011, 1996.
13. ANDREWS PM. Scanning electron microscopy of the nephrotic kidney. *Virchows Arch B Cell Pathol* 17: 195–211, 1975.
14. ANDREWS PM. A scanning and transmission electron microscopic comparison of puromycin aminonucleoside-induced nephrosis to hyperalbuminemia-induced proteinuria with emphasis on kidney podocyte pedicel loss. *Lab Invest* 36: 183–197, 1977.
15. ANDREWS PM. The effect of vinblastine-induced microtubule loss on kidney podocyte morphology. *Am J Anat* 150: 53–61, 1977.
16. ANDREWS PM. Scanning electron microscopy of the kidney glomerular epithelium after treatment with polycations in situ and in vitro. *Am J Anat* 153: 291–303, 1978.
17. ANDREWS PM. Investigations of cytoplasmic contractile and cytoskeletal elements in the kidney glomerulus. *Kidney Int* 20: 549–562, 1981.
18. APPAY MD, KAZATCHKINE MD, LEVI-STRAUSS M, HINGLAIS N, AND BARIETY J. Expression of CR1 (CD35) mRNA in podocytes from adult and fetal human kidneys. *Kidney Int* 38: 289–293, 1990.
19. ARDAILLOU N, LELONGT B, TURNER N, PIEDAGNEL R, BAUDOUIN B, ESTRADE S, CASSINGENA R, AND RONCO PM. Characterization of a simian virus 40-transformed human podocyte cell line producing type IV collagen and exhibiting polarized response to atrial natriuretic peptide. *J Cell Physiol* 152: 599–616, 1992.
21. ARDAILLOU R, CHANSEL D, CHATZIANTONIOU C, AND DUSSAULE JC. Mesangial AT1 receptors: expression, signaling, and regulation. *J Am Soc Nephrol* 10 *Suppl* 11: S40–S46, 1999.
22. AUTIO-HARMAINEN H AND RAPOLA J. The thickness of the glomerular basement membrane in congenital nephrotic syndrome of the Finnish type. *Nephron* 34: 48–50, 1983.
23. BAAS PW, BLACK MM, AND BANKER GA. Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. *J Cell Biol* 109: 3085–3094, 1989.
24. BACHMANN S, KRIZ W, KUHN C, AND FRANKE WW. Differentiation of cell types in the mammalian kidney by immunofluorescence microscopy using antibodies to intermediate filament proteins and desmoplakins. *Histochemistry* 77: 365–394, 1983.
25. BAILEY E, HARPER SJ, PRINGLE JH, BAKER F, FURNESS PN, SALANT DJ, AND FEEHALLY J. Visceral glomerular epithelial cell DNA synthesis in experimental and human membranous disease. *Exp Nephrol* 6: 352–358, 1998.
26. BAINS R, FURNESS PN, AND CRITCHLEY DR. A quantitative immunofluorescence study of glomerular cell adhesion proteins in proteinuric states. *J Pathol* 183: 272–280, 1997.
27. BANAS B, WORNLE M, BERGER T, NELSON PJ, COHEN CD, KRETZLER M, PFIRSTINGER J, MACK M, LIPP M, GRONE HJ, AND SCHLONDORFF D. Roles of SLC/CCL21 and CCR7 in human kidney for mesangial proliferation, migration, apoptosis, and tissue homeostasis. *J Immunol* 168: 4301–4307, 2002.
28. BARALDI A, FURCI L, ZAMBRUNO G, RUBBIANI E, ANNESSI G, AND LUSVARGHI E. Very late activation-3 integrin is the dominant β 1-integrin on the glomerular capillary wall: an immunofluorescence study in nephrotic syndrome. *Nephron* 62: 382–388, 1992.
29. BARBAUX S, NIAUDET P, GUBLER MC, GRUNFELD JP, JAUBERT F, KUTTENN F, FEKETE CN, SOULEYREAU-THERVILLE N, THIBAUD E, FELLOUS M, AND MCELREAVEY K. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. *Nat Genet* 17: 467–470, 1997.
30. BARIETY J, NOCHY D, MANDET C, JACQUOT C, GLOTZ D, AND MEYRIER A. Podocytes undergo phenotypic changes and express macrophagic-associated markers in idiopathic collapsing glomerulopathy. *Kidney Int* 53: 918–925, 1998.
31. BARISONI L, KRIZ W, MUNDEL P, AND D'AGATI V. The dysregulated podocyte phenotype: a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 10: 51–61, 1999.
32. BARISONI L, MOKRZYCKI M, SABLAY L, NAGATA M, YAMASE H, AND MUNDEL P. Podocyte cell cycle regulation and proliferation in collapsing glomerulopathies. *Kidney Int* 58: 137–143, 2000.
33. BARLOW S, GONZALEZ-GARAY ML, WEST RR, OLMSTED JB, AND CABRAL F. Stable expression of heterologous microtubule-associated proteins (MAPs) in Chinese hamster ovary cells: evidence for differing roles of MAPs in microtubule organization. *J Cell Biol* 126: 1017–1029, 1994.
34. BEK M, FISCHR KG, GREIBER S, HUPFER C, MUNDEL P, AND PAVENSTADT H. Dopamine depolarizes podocytes via a D1-like receptor. *Nephrol Dial Transplant* 14: 581–587, 1999.
35. BEK M, NUSING R, KOWARK P, HENGER A, MUNDEL P, AND PAVENSTADT H. Characterization of prostanoid receptors in podocytes. *J Am Soc Nephrol* 10: 2084–2093, 1999.
37. BEK MJ, ZHENG S, XU J, YAMAGUCHI I, ASICO LD, SUN XG, AND JOSE PA. Differential expression of adenylyl cyclases in the rat nephron. *Kidney Int* 60: 890–899, 2001.
38. BELTCHEVA O, MARTIN P, LENKKERI U, AND TRYGGVASON K. Mutation spectrum in the nephrin gene (*NPHS1*) in congenital nephrotic syndrome. *Hum Mutat* 17: 368–373, 2001.
39. BENIGNI A, TOMASONI S, GAGLIARDINI E, ZOJA C, GRUNKEMEYER JA, KALLURI R, AND REMUZZI G. Blocking angiotensin II synthesis/activity preserves glomerular nephrin in rats with severe nephrosis. *J Am Soc Nephrol* 12: 941–948, 2001.
40. BENNDORF R, HAYESS K, RYAZANTSEV S, WIESKE M, BEHLKE J, AND LUTSCH G. Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. *J Biol Chem* 269: 20780–20784, 1994.
41. BERNIK MB. Contractile activity of human glomeruli in culture. *Nephron* 6: 1–10, 1969.
42. BETSHOLTZ C AND RAINES EW. Platelet-derived growth factor: a key regulator of connective tissue cells in embryogenesis and pathogenesis. *Kidney Int* 51: 1361–1369, 1997.
43. BINDER CJ, WEIHER H, EXNER M, AND KERJASCHKI D. Glomerular overproduction of oxygen radicals in Mpv17 gene-inactivated mice causes podocyte foot process flattening and proteinuria: a model of steroid-resistant nephrosis sensitive to radical scavenger therapy. *Am J Pathol* 154: 1067–1075, 1999.
44. BLATTNER SM, TEIXEIRA VP, RASTALDI MP, COHEN CD, SCHLONDORFF D, AND KRETZLER M. Integrin linked kinase associates with a-actinin and CLP-36 in glomerular epithelial cells. *J Am Soc Nephrol* 14: 211–219, 2001.
45. BLOUCH K, DEEN WM, FAUVEL JP, BIALEK J, DERBY G, AND MYERS BD. Molecular configuration and glomerular size selectivity in healthy and nephrotic humans. *Am J Physiol Renal Physiol* 273: F430–F437, 1997.
46. BOHRER MP, DEEN WM, ROBERTSON CR, TROY JL, AND BRENNER BM. Influence of molecular configuration on the passage of macromol-

ecules across the glomerular capillary wall. *J Gen Physiol* 74: 583–593, 1979.
47. Bolton GR, Deen WM, and Daniels BS. Assessment of the charge selectivity of glomerular basement membrane using Ficoll sulfate. *Am J Physiol Renal Physiol* 274: F889–F896, 1998.
48. Bonnet F, Cooper ME, Kawachi H, Allen TJ, Boner G, and Cao Z. Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. *Diabetologia* 44: 874–877, 2001.
49. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, and Antignac C. Nphs2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000.
50. Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, Schaffner G, and Kerjaschki D. Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. *Am J Pathol* 151: 1141–1152, 1997.
51. Briggs JP, Todd-Turla K, Schnermann JB, and Killen PD. Approach to the molecular basis of nephron heterogeneity: application of reverse transcription-polymerase chain reaction to dissected tubule segments. *Semin Nephrol* 13: 2–12, 1993.
52. Brogan M, Chabanis S, Schwarz SJ, Shankland SJ, and Mundel P. Synaptopodin limits podocyte damage in experimental glomerulonephritis (Abstract). *J Am Soc Nephol* 12: 672A, 2001.
53. Burne MJ, Osicka TM, and Comper WD. Fractional clearance of high molecular weight proteins in conscious rats using a continuous infusion method. *Kidney Int* 55: 261–270, 1999.
54. Burrow CR. Regulatory molecules in kidney development. *Pediatr Nephrol* 14: 240–253, 2000.
55. Burstein ES, Spalding TA, and Brann MR. The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. *J Biol Chem* 273: 24322–24327, 1998.
56. Camussi G, Brentjens Noble B Jr, Kerjaschki D, Malavasi F, Roholt OA, Farquhar MG, and Andres G. Antibody-induced redistribution of Heymann antigen on the surface of cultured glomerular visceral epithelial cells: possible role in the pathogenesis of Heymann glomerulonephritis. *J Immunol* 135: 2409–2416, 1985.
57. Caulfield JP and Farquhar MG. The permeability of glomerular capillaries to graded dextrans. Identification of the basement membrane as the primary filtration barrier. *J Cell Biol* 63: 883–903, 1974.
58. Chang RL, Deen WM, Robertson CR, and Brenner BM. Permselectivity of the glomerular capillary wall. III. Restricted transport of polyanions. *Kidney Int* 8: 212–218, 1975.
59. Chen H, Lun Y, Ovchinnikov D, Kokubo H, Oberg KC, Pepicelli CV, Gan L, Lee B, and Johnson RL. Limb and kidney defects in Lmx1b mutant mice suggest an involvement of LMX1B in human nail patella syndrome. *Nat Genet* 19: 51–55, 1998.
60. Chen HC, Chen CA, Guh JY, Chang JM, Shin SJ, and Lai YH. Altering expression of $\alpha 3\beta 1$ integrin on podocytes of human and rats with diabetes. *Life Sci* 67: 2345–2353, 2000.
61. Chevalier RL. Atrial natriuretic peptide in renal development. *Pediatr Nephrol* 7: 652–656, 1993.
62. Cho CR, Lumsden CJ, and Whiteside CI. Epithelial cell detachment in the nephrotic glomerulus: a receptor co-operativity model. *J Theor Biol* 160: 407–426, 1993.
63. Christensen EI and Birn H. Megalin and cubilin: synergistic endocytic receptors in renal proximal tubule. *Am J Physiol Renal Physiol* 280: F562–F573, 2001.
64. Coers W, Huitema S, Van Der Horst ML, and Weening JJ. Puromycin aminonucleoside and adriamycin disturb cytoskeletal and extracellular matrix protein organization, but not protein synthesis of cultured glomerular epithelial cells. *Exp Nephrol* 2: 40–50, 1994.
65. Coers W, Reivinen J, Miettinen A, Huitema S, Vos JT, Salant DJ, and Weening JJ. Characterization of a rat glomerular visceral epithelial cell line. *Exp Nephrol* 4: 184–192, 1996.
66. Coers W, Vos JT, Van Der Meide PH, Van Der Horst ML, Huitema S, and Weening JJ. Interferon-γ (IFN-γ) and IL-4 expressed during mercury-induced membranous nephropathy are toxic for cultured podocytes. *Clin Exp Immunol* 102: 297–307, 1995.
67. Cohen CD, Gröne HJ, Gröne E, Schlöndorff D, Nelson PJ, and Kretzler M. Laser microdissection and gene expression analysis on formaldehyde-fixed archival tissue: IP-10 and RANTES in renal allograft rejection. *Kidney Int* 357: 282–283, 2002.
68. Cohen CD, Gröne HJ, Schlöndorff D, Neslson PJSB, Gross M, and Kretzler M. Quantitative gene expression analysis in renal biopsies: a novel protocol for a high throughput multicenter application. *Kidney Int* 357: 282–283, 2002.
69. Cohen CD, Rastaldi MPKC, Delarue F, Moenks D, Giardino L, Sraer JD, Ferrario F, Schlondorff D, and Kretzler M. α-Actinin-4 protein and mRNA expression in acquired proteinuric diseases. *J Am Soc Nephrol* 357: 282–283, 2001.
70. Combs HL, Shankland SJ, Setzer SV, Hudkins KL, and Alpers CE. Expression of the cyclin kinase inhibitor, p27kip1, in developing and mature human kidney. *Kidney Int* 53: 892–896, 1998.
71. Coppolino MG and Dedhar S. Bi-directional signal transduction by integrin receptors. *Int J Biochem Cell Biol* 32: 171–188, 2000.
72. Cortes P, Mendez M, Riser BL, Guerin CJ, Rodriguez-Barbero A, Hassett C, and Yee J. F-actin fiber distribution in glomerular cells: structural and functional implications. *Kidney Int* 58: 2452–2461, 2000.
73. Cosgrove D, Rodgers K, Meehan D, Miller C, Bovard K, Gilroy A, Gardner H, Kotelianski V, Gotwals P, Amatucci A, and Kalluri R. Integrin $\alpha 1\beta 1$ and transforming growth factor-$\beta 1$ play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy. *Am J Pathol* 157: 1649–1659, 2000.
74. Couser WG, Johnson RJ, Young BA, Yeh CG, Toth CA, and Rudolph AR. The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. *J Am Soc Nephrol* 5: 1888–1894, 1995.
75. Cunningham PN, Hack BK, Ren G, Minto AW, Morgan BP, and Quigg RJ. Glomerular complement regulation is overwhelmed in passive Heymann nephritis. *Kidney Int* 60: 900–909, 2001.
76. Cybulsky AV. Extracellular matrix as a determinant of signaling responses in glomerular epithelial cells. *Kidney Int* 56: 1242–1246, 1999.
77. Cybulsky AV, Bonventre JV, Quigg RJ, Lieberthal W, and Salant DJ. Cytosolic calcium and protein kinase C reduce complement-mediated glomerular epithelial injury. *Kidney Int* 38: 803–811, 1990.
78. Cybulsky AV, Quigg RJ, Badalamenti J, and Salant DJ. Anti-Fx1A induces association of Heymann nephritis antigens with microfilaments of cultured glomerular visceral epithelial cells. *Am J Pathol* 129: 373–384, 1987.
79. Cybulsky AV, Quigg RJ, and Salant DJ. The membrane attack complex in complement-mediated glomerular epithelial cell injury: formation and stability of C5b-9 and C5b-7 in rat membranous nephropathy. *J Immunol* 137: 1511–1516, 1986.
80. D'agati V. The many masks of focal segmental glomerulosclerosis. *Kidney Int* 46: 1223–1241, 1994.
81. D'agati V and Appel GB. Renal pathology of human immunodeficiency virus infection. *Semin Nephrol* 18: 406–421, 1998.
82. Daniels BS. Increased albumin permeability in vitro following alterations of glomerular charge is mediated by the cells of the filtration barrier. *J Lab Clin Med* 124: 224–230, 1994.
83. Davidson G, Dono R, and Zeller R. FGF signalling is required for differentiation-induced cytoskeletal reorganisation and formation of actin-based processes by podocytes. *J Cell Sci* 114: 3359–3366, 2001.
84. Davies DJ, Brewer DB, and Hardwicke J. Urinary proteins and glomerular morphometry in protein overload proteinuria. *Lab Invest* 38: 232–243, 1978.
85. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, and Yancopoulos GD. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* 87: 1161–1169, 1996.
86. Dedhar S, Jewell K, Rojiani M, and Gray V. The receptor for the basement membrane glycoprotein entactin is the integrin α 3/β 1. *J Biol Chem* 267: 18908–18914, 1992.
87. Deen WM, Lazzara MJ, and Myers BD. Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol* 281: F579–F596, 2001.
88. Deen WM, Satvat B, and Jamieson JM. Theoretical model for glomerular filtration of charged solutes. *Am J Physiol Renal Fluid Electrolyte Physiol* 238: F126–F139, 1980.
89. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, and Dedhar S. Phosphoinositide-3-OH kinase-dependent regulation of glycogen

synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci USA* 95: 11211–11216, 1998.
90. DELLER T, MUNDEL P, AND FROTSCHER M. Potential role of synaptopodin in spine motility by coupling actin to the spine apparatus. *Hippocampus* 10: 569–581, 2000.
91. DETWILER RK, FALK RJ, HOGAN SL, AND JENNETTE JC. Collapsing glomerulopathy: a clinically and pathologically distinct variant of focal segmental glomerulosclerosis. *Kidney Int* 45: 1416–1424, 1994.
92. DIAMOND JR, BONVENTRE JV, AND KARNOVSKY MJ. A role for oxygen free radicals in aminonucleoside nephrosis. *Kidney Int* 29: 478–483, 1986.
93. DIAMOND JR AND KARNOVSKY MJ. Focal and segmental glomerulosclerosis following a single intravenous dose of puromycin aminonucleoside. *Am J Pathol* 122: 481–487, 1986.
94. DING G, VAN GOOR H, RICARDO SD, ORLOWSKI JM, AND DIAMOND JR. Oxidized LDL stimulates the expression of TGF-β and fibronectin in human glomerular epithelial cells. *Kidney Int* 51: 147–154, 1997.
95. DONOVIEL DB, FREED DD, VOGEL H, POTTER DG, HAWKINS E, BARRISH JP, MATHUR BN, TURNER CA, GESKE R, MONTGOMERY CA, STARBUCK M, BRANDT M, GUPTA A, RAMIREZ-SOLIS R, ZAMBROWICZ BP, AND POWELL DR. Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to nephrin. *Mol Cell Biol* 21: 4829–4836, 2001.
96. DOUBLIER S, RUOTSALAINEN V, SALVIDIO G, LUPIA E, BIANCONE L, CONALDI PG, REPONEN P, TRYGGVASON K, AND CAMUSSI G. Nephrin redistribution on podocytes is a potential mechanism for proteinuria in patients with primary acquired nephrotic syndrome. *Am J Pathol* 158: 1723–1731, 2001.
97. DRENCKHAHN D AND FRANKE RP. Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. *Lab Invest* 59: 673–682, 1988.
98. DRESSLER GR. Pax-2, kidney development, and oncogenesis. *Med Pediatr Oncol* 27: 440–444, 1996.
99. DREYER SD, ZHOU G, BALDINI A, WINTERPACHT A, ZABEL B, COLE W, JOHNSON RL, AND LEE B. Mutations in LMX1B cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. *Nat Genet* 19: 47–50, 1998.
100. DRUKKER A AND EDDY AA. Failure of antioxidant therapy to attenuate interstitial disease in rats with reversible nephrotic syndrome. *J Am Soc Nephrol* 9: 243–251, 1998.
101. DUNNE J, HANBY AM, POULSOM R, JONES TA, SHEER D, CHIN WG, DA SM, ZHAO Q, BEVERLEY PC, AND OWEN MJ. Molecular cloning and tissue expression of FAT, the human homologue of the *Drosophila* fat gene that is located on chromosome 4q34–q35 and encodes a putative adhesion molecule. *Genomics* 30: 207–223, 1995.
102. DURBEEJ M, HENRY MD, FERLETTA M, CAMPBELL KP, AND EKBLOM P. Distribution of dystroglycan in normal adult mouse tissues. *J Histochem Cytochem* 46: 449–457, 1998.
103. DUSTIN ML, OLSZOWY MW, HOLDORF AD, LI J, BROMLEY S, DESAI N, WIDDER P, ROSENBERGER F, VAN DER MERWE PA, ALLEN PM, AND SHAW AS. A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 94: 667–677, 1998.
104. EDWARDS A, DANIELS BS, AND DEEN WM. Hindered transport of macromolecules in isolated glomeruli. II. Convection and pressure effects in basement membrane. *Biophys J* 72: 214–222, 1997.
105. EDWARDS A, DANIELS BS, AND DEEN WM. Ultrastructural model for size selectivity in glomerular filtration. *Am J Physiol Renal Physiol* 276: F892–F902, 1999.
106. ENDLICH K, KRIZ W, AND WITZGALL R. Update in podocyte biology. *Curr Opin Nephrol Hypertens* 10: 331–340, 2001.
107. ENDLICH N, KRESS KR, REISER J, UTTENWEILER D, KRIZ W, MUNDEL P, AND ENDLICH K. Podocytes respond to mechanical stress in vitro. *J Am Soc Nephrol* 12: 413–422, 2001.
108. ENDLICH N, NOBILING R, KRIZ W, AND ENDLICH K. Expression and signaling of parathyroid hormone-related protein in cultured podocytes. *Exp Nephrol* 9: 436–443, 2001.
109. EPPEL GA, OSICKA TM, PRATT LM, JABLONSKI P, HOWDEN BO, GLASGOW EF, AND COMPER WD. The return of glomerular-filtered albumin to the rat renal vein. *Kidney Int* 55: 1861–1870, 1999.
111. EREMINA V, WONG MA, CUI S, SCHWARTZ L, AND QUAGGIN SE. Glomerular-specific gene excision in vivo. *J Am Soc Nephrol* 13: 788–793, 2002.
112. ESSER S, WOLBURG K, WOLBURG H, BREIER G, KURZCHALIA T, AND RISAU W. Vascular endothelial growth factor induces endothelial fenestrations in vitro. *J Cell Biol* 140: 947–959, 1998.
113. EVERETT AD, XUE C, AND STOOPS T. Developmental expression of protein phosphatase 2A in the kidney. *J Am Soc Nephrol* 10: 1737–1745, 1999.
114. FARQUHAR MG AND PALADE GE. Glomerular permeability. II. Ferritin transfer across the glomerular capillary wall in nephrotic rats. *J Exp Med* 114: 699–716, 1965.
115. FARQUHAR MG, VERNIER RL, AND GOOD RA. An electron microscopic study of the glomerulus in nephrosis, glomerulonephritis and lupus erythematosus. *J Exp Med* 106: 649–660, 1957.
116. FARQUHAR MG, WISSIG SL, AND PALADE GE. Glomerular permeability. I. Ferritin transfer across the normal capillary wall. *J Exp Med* 113: 47–66, 1961.
117. FAULL RJ AND GINSBERG MH. Inside-out signaling through integrins. *J Am Soc Nephrol* 7: 1091–1097, 1996.
118. FERNANDEZ L, ROMERO M, SOTO H, AND MOSQUERA J. Increased apoptosis in acute puromycin aminonucleoside nephrosis. *Exp Nephrol* 9: 99–108, 2001.
119. FINK L, SEEGER W, ERMERT L, HANZE J, STAHL U, GRIMMINGER F, KUMMER W, AND BOHLE RM. Real-time quantitative RT-PCR after laser-assisted cell picking. *Nat Med* 4: 1329–1333, 1998.
120. FISCHER KG, JONAS N, POSCHENRIEDER F, COHEN C, KRETZLER M, GREIBER S, AND PAVENSTÄDT H. Characterization of a Na^+-Ca^{2+} exchanger in podocytes. *Nephrol Dial Transplant* 281: F1075–F1081, 2002.
121. FISCHER KG, SAUERESSIG U, JACOBSHAGEN C, WICHELMANN A, AND PAVENSTADT H. Extracellular nucleotides regulate cellular functions of podocytes in culture. *Am J Physiol Renal Physiol* 281: F1075–F1081, 2001.
122. FISHMAN JA AND KARNOVSKY MJ. Effects of the aminonucleoside of puromycin on glomerular epithelial cells in vitro. *Am J Pathol* 118: 398–407, 1985.
123. FLOEGE J, ALPERS CE, SAGE EH, PRITZL P, GORDON K, JOHNSON RJ, AND COUSER WG. Markers of complement-dependent and complement-independent glomerular visceral epithelial cell injury in vivo. Expression of antiadhesive proteins and cytoskeletal changes. *Lab Invest* 67: 486–497, 1992.
124. FLOEGE J, HACKMANN B, KLIEM V, KRIZ W, ALPERS CE, JOHNSON RJ, KUHN KW, KOCH KM, AND BRUNKHORST R. Age-related glomerulosclerosis and interstitial fibrosis in Milan normotensive rats: a podocyte disease. *Kidney Int* 51: 230–243, 1997.
125. FLOEGE J, JOHNSON RJ, ALPERS CE, FATEMI-NAINIE S, RICHARDSON CA, GORDON K, AND COUSER WG. Visceral glomerular epithelial cells can proliferate in vivo and synthesize platelet-derived growth factor B-chain. *Am J Pathol* 142: 637–650, 1993.
126. FLOEGE J, KRIZ W, SCHULZE M, SUSANI M, KERJASCHKI D, MOONEY A, COUSER WG, AND KOCH KM. Basic fibroblast growth factor augments podocyte injury and induces glomerulosclerosis in rats with experimental membranous nephropathy. *J Clin Invest* 96: 2809–2819, 1995.
127. FORNONI A, LI H, FOSCHI A, STRIKER GE, AND STRIKER LJ. Hepatocyte growth factor, but not insulin-like growth factor I, protects podocytes against cyclosporin A-induced apoptosis. *Am J Pathol* 158: 275–280, 2001.
128. FRIES JW, SANDSTROM DJ, MEYER TW, AND RENNKE HG. Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest* 60: 205–218, 1989.
129. FRISCH SM AND FRANCIS H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 124: 619–626, 1994.
130. FUJIGAKI Y, NAGASE M, HIDAKA S, MATSUI K, SHIRAI M, NOSAKA H, KAWACHI H, SHIMIZU F, AND HISHIDA A. Altered anionic GBM components in monoclonal antibody against slit diaphragm-injected proteinuric rats. *Kidney Int* 54: 1491–1500, 1998.
131. FUJIGAKI Y, WATANABE T, IKEGAYA N, YONEMURA K, SUN DF, HISHIDA A, YAMAMOTO T, KOJIMA K, AND NAGASE M. Immunoelectron microscopic study on type I, II and III TGF-β receptors on visceral glomerular epithelial cells in relation to glomerular basement membrane alterations in proteinuric rats. *Nephrol Dial Transplant* 15: 191–199, 2000.
132. FURNESS PN, HALL LL, SHAW JA, AND PRINGLE JH. Glomerular expres-

sion of nephrin is decreased in acquired human nephrotic syndrome. *Nephrol Dial Transplant* 14: 1234–1237, 1999.
133. Furukawa T, Ohno S, Oguchi H, Hora K, Tokunaga S, and Furuta S. Morphometric study of glomerular slit diaphragms fixed by rapid-freezing and freeze-substitution. *Kidney Int* 40: 621–624, 1991.
134. Garin EH, Blanchard DK, Matsushima K, and Djeu JY. IL-8 production by peripheral blood mononuclear cells in nephrotic patients. *Kidney Int* 45: 1311–1317, 1994.
135. Garrod DR and Fleming S. Early expression of desmosomal components during kidney tubule morphogenesis in human and murine embryos. *Development* 108: 313–321, 1990.
136. Gassler N, Elger M, Kranzlin B, Kriz W, Gretz N, Hahnel B, Hosser H, and Hartmann I. Podocyte injury underlies the progression of focal segmental glomerulosclerosis in the fa/fa Zucker rat. *Kidney Int* 60: 106–116, 2001.
137. Gelberg H, Healy L, Whiteley H, Miller LA, and Vimr E. In vivo enzymatic removal of α 2→6-linked sialic acid from the glomerular filtration barrier results in podocyte charge alteration and glomerular injury. *Lab Invest* 74: 907–920, 1996.
138. Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, Wright BD, Radtke F, Aguet M, and Ferrara N. VEGF is required for growth and survival in neonatal mice. *Development* 126: 1149–1159, 1999.
139. Gloy J, Henger A, Fischer KG, Nitschke R, Mundel P, Bleich M, Schollmeyer P, Greger R, and Pavenstadt H. Angiotensin II depolarizes podocytes in the intact glomerulus of the Rat. *J Clin Invest* 99: 2772–2781, 1997.
140. Gloy J, Reitinger S, Fischer KG, Schreiber R, Boucherot A, Kunzelmann K, Mundel P, and Pavenstadt H. Amino acid transport in podocytes. *Am J Physiol Renal Physiol* 278: F999–F1005, 2000.
141. Gordon AM, Homsher E, and Regnier M. Regulation of contraction in striated muscle. *Physiol Rev* 80: 853–924, 2000.
142. Graham RC Jr and Karnovsky MJ. Glomerular permeability. Ultrastructural cytochemical studies using peroxidases as protein tracers. *J Exp Med* 124: 1123–1134, 1966.
143. Greiber S, Müller B, Daemisch P, and Pavenstädt H. Reactive oxygen species alter gene expression in podocytes: induction of granulocyte. macrophage-colony-stimulating factor. *J Am Soc Nephrol* 13: 86–95, 1996.
144. Greiber S, Munzel T, Kastner S, Muller B, Schollmeyer P, and Pavenstadt H. Nad(P)H oxidase activity in cultured human podocytes: effects of adenosine triphosphate. *Kidney Int* 53: 654–663, 1998.
145. Greive KA, Nikolic-Paterson DJ, Guimaraes MA, Nikolovski J, Pratt LM, Mu W, Atkins RC, and Comper WD. Glomerular permselectivity factors are not responsible for the increase in fractional clearance of albumin in rat glomerulonephritis. *Am J Pathol* 159: 1159–1170, 2001.
146. Grindstaff KK, Yeaman C, Anandasabapathy N, Hsu SC, Rodriguez-Boulan E, Scheller RH, and Nelson WJ. Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* 93: 731–740, 1998.
147. Groffen AJ, Veerkamp JH, Monnens LA, and Van Den Heuvel LP. Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. *Nephrol Dial Transplant* 14: 2119–2129, 1999.
148. Grone HJ, Walli AK, and Grone EF. The role of oxidatively modified lipoproteins in lipid nephropathy. *Contrib Nephrol* 120: 160–175, 1997.
149. Guo L, Sanders PW, Woods A, and Wu C. The distribution and regulation of integrin-linked kinase in normal and diabetic kidneys. *Am J Pathol* 159: 1735–1742, 2001.
150. Gwinner W, Plasger J, Brandes RP, Kubat B, Schulze M, Regele H, Kerjaschki D, Olbricht CJ, and Koch KM. Role of xanthine oxidase in passive Heymann nephritis in rats. *J Am Soc Nephrol* 10: 538–544, 1999.
151. Hamano Y, Grunkemeyer JA, Sudhakar A, Zeisberg M, Cosgrove D, Morello R, Lee B, Sugimoto H, and Kalluri R. Determinants of vascular permeability in the kidney glomerulus. *J Biol Chem* 277: 31154–31162, 2002.
152. Hamasaki K, Mimura T, Furuya H, Morino N, Yamazaki T, Komuro I, Yazaki Y, and Nojima Y. Stretching mesangial cells stimulates tyrosine phosphorylation of focal adhesion kinase pp125FAK. *Biochem Biophys Res Commun* 212: 544–549, 1995.
153. Hammerman MR. Growth factors in renal development. *Semin Nephrol* 15: 291–299, 1995.
154. Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC, and Dedhar S. Regulation of cell adhesion and anchorage-dependent growth by a new β 1-integrin-linked protein kinase. *Nature* 379: 91–96, 1996.
155. Hanoune J and Defer N. Regulation and role of adenylyl cyclase isoforms. *Annu Rev Pharmacol Toxicol* 41: 145–174, 2001.
156. Hara M, Yanagihara T, Itoh M, Matsuno M, and Kihara I. Immunohistochemical and urinary markers of podocyte injury. *Pediatr Nephrol* 12: 43–48, 1998.
157. Hara M, Yanagihara T, Takada T, Itoh M, Matsuno M, Yamamoto T, and Kihara I. Urinary excretion of podocytes reflects disease activity in children with glomerulonephritis. *Am J Nephrol* 18: 35–41, 1998.
158. Harper SJ, Xing CY, Whittle C, Parry R, Gillatt D, Peat D, and Mathieson PW. Expression of neuropilin-1 by human glomerular epithelial cells in vitro and in vivo. *Clin Sci* 101: 439–446, 2001.
159. He CJ, Peraldi MN, Adida C, Rebibou JM, Meulders Q, Sraer JD, and Rondeau E. Thrombin signal transduction mechanisms in human glomerular epithelial cells. *J Cell Physiol* 150: 475–483, 1992.
160. Heikkila P, Tryggvason K, and Thorner P. Animal models of Alport syndrome: advancing the prospects for effective human gene therapy. *Exp Nephrol* 8: 1–7, 2000.
161. Hemler ME. Dystroglycan versatility. *Cell* 97: 543–546, 1999.
162. Henger A, Huber T, Fischer KG, Nitschke R, Mundel P, Schollmeyer P, Greger R, and Pavenstadt H. Angiotensin II increases the cytosolic calcium activity in rat podocytes in culture. *Kidney Int* 52: 687–693, 1997.
163. Heymann H, Manniello JM, and Barkulis SS. Structure of streptococcal cell walls. V. Phosphate esters in the walls of group A *Streptococcus pyogenes*. *Biochem Biophys Res Commun* 26: 486–491, 1967.
164. Hir ML, Keller C, Eschmann V, Hahnel B, Hosser H, and Kriz W. Podocyte bridges between the tuft and Bowman's capsule: an early event in experimental crescentic glomerulonephritis. *J Am Soc Nephrol* 12: 2060–2071, 2001.
165. Hiraga A and Tamura S. Protein phosphatase 2A is associated in an inactive state with microtubules through 2A1-specific interaction with tubulin. *Biochem J* 346: 433–439, 2000.
166. Hirschberg R and Ding H. Growth factors and acute renal failure. *Semin Nephrol* 18: 191–207, 1998.
167. Hjalmarsson C, Ohlson M, and Haraldsson B. Puromycin aminonucleoside damages the glomerular size barrier with minimal effects on charge density. *Am J Physiol Renal Physiol* 281: F503–F512, 2001.
168. Holthofer H, Miettinen A, Lehto VP, Lehtonen E, and Virtanen I. Expression of vimentin and cytokeratin types of intermediate filament proteins in developing and adult human kidneys. *Lab Invest* 50: 552–559, 1984.
169. Holthofer H, Sainio K, and Miettinen A. Rat glomerular cells do not express podocytic markers when cultured in vitro. *Lab Invest* 65: 548–557, 1991.
170. Holzman LB, St John PL, Kovari IA, Verma R, Holthofer H, and Abrahamson DR. Nephrin localizes to the slit pore of the glomerular epithelial cell. *Kidney Int* 56: 1481–1491, 1999.
171. Horster MF, Braun GS, and Huber SM. Embryonic renal epithelia: induction, nephrogenesis, and cell differentiation. *Physiol Rev* 79: 1157–1191, 1999.
172. Huang M, Gu G, Ferguson EL, and Chalfie M. A stomatin-like protein necessary for mechanosensation in *C. elegans*. *Nature* 378: 292–295, 1995.
173. Huang TW and Langlois JC. Podoendin. A new cell surface protein of the podocyte and endothelium. *J Exp Med* 162: 245–267, 1985.
174. Huber G and Matus A. Microtubule-associated protein 3 (MAP3) expression in non-neuronal tissues. *J Cell Sci* 95: 237–246, 1990.
175. Huber LA, Pimplikar S, Parton RG, Virta H, Zerial M, and Simons K. Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. *J Cell Biol* 123: 35–45, 1993.
176. Huber TB, Gloy J, Henger A, Schollmeyer P, Greger R, Mundel P,

AND PAVENSTADT H. Catecholamines modulate podocyte function. *J Am Soc Nephrol* 9: 335–345, 1998.
177. HUBER TB, KOTTGEN M, SCHILLING B, WALZ G, AND BENZING T. Interaction with podocin facilitates nephrin signaling. *J Biol Chem* 276: 41543–41546, 2001.
178. HUBER TB, REINHARDT HC, EXNER M, BURGER JA, KERJASCHKI D, SALEEM MA, AND PAVENSTADT H. Expression of functional CCR and CXCR chemokine receptors in podocytes. *J Immunol* 168: 6244–6252, 2002.
179. HUGO C, NANGAKU M, SHANKLAND SJ, PICHLER R, GORDON K, AMIEVA MR, COUSER WG, FURTHMAYR H, AND JOHNSON RJ. The plasma membrane-actin linking protein, ezrin, is a glomerular epithelial cell marker in glomerulogenesis, in the adult kidney and in glomerular injury. *Kidney Int* 54: 1934–1944, 1998.
180. HUTTUNEN NP, HALLMAN N, AND RAPOLA J. Glomerular basement membrane antigens in congenital and acquired nephrotic syndrome in childhood. *Nephron* 16: 401–414, 1976.
181. INAGAMI T. Molecular biology and signaling of angiotensin receptors: an overview. *J Am Soc Nephrol* 10 *Suppl* 11: S2–S7, 1999.
182. INOKUCHI S, SHIRATO I, KOBAYASHI N, KOIDE H, TOMINO Y, AND SAKAI T. Re-evaluation of foot process effacement in acute puromycin aminonucleoside nephrosis. *Kidney Int* 50: 1278–1287, 1996.
183. INOUE T, YAOITA E, KURIHARA H, SHIMIZU F, SAKAI T, KOBAYASHI T, OHSHIRO K, KAWACHI H, OKADA H, SUZUKI H, KIHARA I, AND YAMAMOTO T. Fat is a component of glomerular slit diaphragms. *Kidney Int* 59: 1003–1012, 2001.
184. JIN DK, FISH AJ, WAYNER EA, MAUER M, SETTY S, TSILIBARY E, AND KIM Y. Distribution of integrin subunits in human diabetic kidneys. *J Am Soc Nephrol* 7: 2636–2645, 1996.
185. JOHNSON RJ, ALPERS CE, YOSHIMURA A, LOMBARDI D, PRITZL P, FLOEGE J, AND SCHWARTZ SM. Renal injury from angiotensin II-mediated hypertension. *Hypertension* 19: 464–474, 1992.
186. JOHNSON RJ, LOVETT D, LEHRER RI, COUSER WG, AND KLEBANOFF SJ. Role of oxidants and proteases in glomerular injury. *Kidney Int* 45: 352–359, 1994.
187. KANWAR YS. Biophysiology of glomerular filtration and proteinuria. *Lab Invest* 51: 7–21, 1984.
188. KANWAR YS, LINKER A, AND FARQUHAR MG. Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion. *J Cell Biol* 86: 688–693, 1980.
189. KANWAR YS AND ROSENZWEIG LJ. Altered glomerular permeability as a result of focal detachment of the visceral epithelium. *Kidney Int* 21: 565–574, 1982.
190. KAPLAN JM, KIM SH, NORTH KN, RENNKE H, CORREIA LA, TONG HQ, MATHIS BJ, RODRIGUEZ-PEREZ JC, ALLEN PG, BEGGS AH, AND POLLAK MR. Mutations in ACTN4, encoding α-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000.
191. KAWACHI H, ABRAHAMSON DR, ST JOHN PL, GOLDSTEIN DJ, SHIA MA, MATSUI K, SHIMIZU F, AND SALANT DJ. Developmental expression of the nephritogenic antigen of monoclonal antibody 5–1-6. *Am J Pathol* 147: 823–833, 1995.
192. KAWACHI H, KOIKE H, KURIHARA H, YAOITA E, ORIKASA M, SHIA MA, SAKAI T, YAMAMOTO T, SALANT DJ, AND SHIMIZU F. Cloning of rat nephrin: expression in developing glomeruli and in proteinuric states. *Kidney Int* 57: 1949–1961, 2000.
193. KAWACHI H, KURIHARA H, TOPHAM PS, BROWN D, SHIA MA, ORIKASA M, SHIMIZU F, AND SALANT DJ. Slit diaphragm-reactive nephritogenic MAb 5–1-6 alters expression of ZO-1 in rat podocytes. *Am J Physiol Renal Physiol* 273: F984–F993, 1997.
194. KAZATCHKINE MD, FEARON DT, APPAY MD, MANDET C, AND BARIETY J. Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in seventy-five cases of renal diseases: loss of C3b receptor antigen in focal hyalinosis and in proliferative nephritis of systemic lupus erythematosus. *J Clin Invest* 69: 900–912, 1982.
195. KEE Y, YOO JS, HAZUKA CD, PETERSON KE, HSU SC, AND SCHELLER RH. Subunit structure of the mammalian exocyst complex. *Proc Natl Acad Sci USA* 94: 14438–14443, 1997.
196. KEMENY E, MIHATSCH MJ, DURMULLER U, AND GUDAT F. Podocytes loose their adhesive phenotype in focal segmental glomerulosclerosis. *Clin Nephrol* 43: 71–83, 1995.
197. KERJASCHKI D. Polycation-induced dislocation of slit diaphragms and formation of cell junctions in rat kidney glomeruli: the effects of low temperature, divalent cations, colchicine, and cytochalasin B. *Lab Invest* 39: 430–440, 1978.
198. KERJASCHKI D. Dysfunctions of cell biological mechanisms of visceral epithelial cell (podocytes) in glomerular diseases. *Kidney Int* 45: 300–313, 1994.
199. KERJASCHKI D. Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis. *J Clin Invest* 108: 1583–1587, 2001.
200. KERJASCHKI D AND FARQUHAR MG. The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *Proc Natl Acad Sci USA* 79: 5557–5581, 1982.
201. KERJASCHKI D AND FARQUHAR MG. Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. *J Exp Med* 157: 667–686, 1983.
202. KERJASCHKI D AND NEALE TJ. Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis). *J Am Soc Nephrol* 7: 2518–2526, 1996.
203. KERJASCHKI D, OJHA PP, SUSANI M, HORVAT R, BINDER S, HOVORKA A, HILLEMANNS P, AND PYTELA R. A β 1-integrin receptor for fibronectin in human kidney glomeruli. *Am J Pathol* 134: 481–489, 1989.
204. KERJASCHKI D, SHARKEY DJ, AND FARQUHAR MG. Identification and characterization of podocalyxin—the major sialoprotein of the renal glomerular epithelial cell. *J Cell Biol* 98: 1591–1596, 1984.
205. KESTILA M, LENKKERI U, MANNIKKO M, LAMERDIN J, MCCREADY P, PUTAALA H, RUOTSALAINEN V, MORITA T, NISSINEN M, HERVA R, KASHTAN CE, PELTONEN L, HOLMBERG C, OLSEN A, AND TRYGGVASON K. Positionally cloned gene for a novel glomerular protein–nephri–is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998.
206. KHONG TF, FRASER S, KATERELOS M, PAIZIS K, HILL PA, AND POWER DA. Inhibition of heparin-binding epidermal growth factor-like growth factor increases albuminuria in puromycin aminonucleoside nephrosis. *Kidney Int* 58: 1098–1107, 2000.
207. KIDD GJ, ANDREWS SB, AND TRAPP BD. Organization of microtubules in myelinating Schwann cells. *J Neurocytol* 23: 801–810, 1994.
208. KIM YG, ALPERS CE, BRUGAROLAS J, JOHNSON RJ, COUSER WG, AND SHANKLAND SJ. The cyclin kinase inhibitor p21CIP1/WAF1 limits glomerular epithelial cell proliferation in experimental glomerulonephritis. *Kidney Int* 55: 2349–2361, 1999.
209. KIM YH, GOYAL M, KURNIT D, WHARRAM B, WIGGINS J, HOLZMAN L, KERSHAW D, AND WIGGINS R. Podocyte depletion and glomerulosclerosis have a direct relationship in the PAN-treated rat. *Kidney Int* 60: 957–968, 2001.
210. KIRSCH KH, GEORGESCU MM, ISHIMARU S, AND HANAFUSA H. CMS: an adapter molecule involved in cytoskeletal rearrangements. *Proc Natl Acad Sci USA* 96: 6211–6216, 1999.
211. KITAMOTO Y, TOKUNAGA H, AND TOMITA K. Vascular endothelial growth factor is an essential molecule for mouse kidney development: glomerulogenesis and nephrogenesis. *J Clin Invest* 99: 2351–2357, 1997.
212. KOBAYASHI N, MOMINOKI K, WAKISAKA H, SHIMAZAKI Y, AND MATSUDA S. Morphogenetic activity of extracellular matrices on cultured podocytes. Laminin accelerates podocyte process formation in vitro. *Ital J Anat Embryol* 106: 423–430, 2001.
213. KOBAYASHI N AND MUNDEL P. A role of microtubules during the formation of cell processes in neuronal and non-neuronal cells. *Cell Tissue Res* 291: 163–174, 1998.
214. KOBAYASHI N, REISER J, SCHWARZ K, SAKAI T, KRIZ W, AND MUNDEL P. Process formation of podocytes: morphogenetic activity of microtubules and regulation by protein serine/threonine phosphatase PP2A. *Histochem Cell Biol* 115: 255–266, 2001.
215. KOBAYASHI N, SAITO S, WAKISAKA H, MIYAWAKI K, SAITO K, MATSUDA S, NAKAMURA T, AND MUNDEL P. Hepatocyte growth factor enhances process formation of cultured podocytes. *J Am Soc Nephol* 47: 476–480, 2001.
216. KOJIMA K, MATSUI K, AND NAGASE M. Protection of α(3) integrin-mediated podocyte shape by superoxide dismutase in the puromycin aminonucleoside nephrosis rat. *Am J Kidney Dis* 35: 1175–1185, 2000.
217. KOPP JB, FACTOR VM, MOZES M, NAGY P, SANDERSON N, BOTTINGER EP, KLOTMAN PE, AND THORGEIRSSON SS. Transgenic mice with increased

plasma levels of TGF-β 1 develop progressive renal disease. *Lab Invest* 74: 991–1003, 1996.
218. KORHONEN M, YLANNE J, LAITINEN L, AND VIRTANEN I. The α 1-α 6 subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. *J Cell Biol* 111: 1245–1254, 1990.
219. KOSEKI C, KANAI Y, HAYASHI Y, OHNUMA N, AND IMAI M. Intrarenal localization of receptors for α-rat atrial natriuretic polypeptide: an autoradiographic study with [^{125}I]-labeled ligand injected in vivo into the rat aorta. *Jpn J Pharmacol* 42: 27–33, 1986.
220. KOTANI S, MUROFUSHI H, MAEKAWA S, AIZAWA H, AND SAKAI H. Isolation of rat liver microtubule-associated proteins. Evidence for a family of microtubule-associated proteins with molecular mass of around 200,000 which distribute widely among mammalian cells. *J Biol Chem* 263: 5385–5389, 1988.
221. KOUKOURITAKI SB, TAMIZUDDIN A, AND LIANOS EA. Enhanced expression of the cytoskeleton-associated proteins paxillin and focal adhesion kinase in glomerular immune injury. *J Lab Clin Med* 134: 173–179, 1999.
222. KREIDBERG JA, DONOVAN MJ, GOLDSTEIN SL, RENNKE H, SHEPHERD K, JONES RC, AND JAENISCH R. α 3 β 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537–3547, 1996.
223. KREIDBERG JA AND SYMONS JM. Integrins in kidney development, function, and disease. *Am J Physiol Renal Physiol* 279: F233–F242, 2000.
224. KREISBERG JI, HOOVER RL, AND KARNOVSKY MJ. Isolation and characterization of rat glomerular epithelial cells in vitro. *Kidney Int* 14: 21–30, 1978.
225. KRETZLER M, KOEPPEN-HAGEMANN I, AND KRIZ W. Podocyte damage is a critical step in the development of glomerulosclerosis in the uninephrectomised-desoxycorticosterone hypertensive rat. *Virchows Arch* 425: 181–193, 1994.
226. KRETZLER M, TEIXEIRA VP, UNSCHULD PG, COHEN CD, WANKE R, EDENHOFER I, MUNDEL P, SCHLONDORFF D, AND HOLTHOFER H. Integrin-linked kinase as a candidate downstream effector in proteinuria. *FASEB J* 15: 1843–1845, 2001.
227. KRISHNAMURTI U, RONDEAU E, SRAER JD, MICHAEL AF, AND TSILIBARY EC. Alterations in human glomerular epithelial cells interacting with nonenzymatically glycosylated matrix. *J Biol Chem* 272: 27966–27970, 1997.
228. KRISHNAMURTI U, ZHOU B, FAN WW, TSILIBARY E, WAYNER E, KIM Y, KASHTAN CE, AND MICHAEL A. Puromycin aminonucleoside suppresses integrin expression in cultured glomerular epithelial cells. *J Am Soc Nephrol* 12: 758–766, 2001.
229. KRIZ W. Progressive renal failure—inability of podocytes to replicate and the consequences for development of glomerulosclerosis. *Nephrol Dial Transplant* 11: 1738–1742, 1996.
230. KRIZ W, ELGER M, HOSSER H, HAHNEL B, PROVOOST A, KRANZLIN B, AND GRETZ N. How does podocyte damage result in tubular damage? *Kidney Blood Press Res* 22: 26–36, 1999.
231. KRIZ W, ELGER M, MUNDEL P, AND LEMLEY KV. Structure-stabilizing forces in the glomerular tuft. *J Am Soc Nephrol* 5: 1731–1739, 1995.
232. KRIZ W, GRETZ N, AND LEMLEY KV. Progression of glomerular diseases: is the podocyte the culprit? *Kidney Int* 54: 687–697, 1998.
233. KRIZ W, HACKENTHAL E, NOBILING R, SAKAI T, ELGER M, AND HAHNEL B. A role for podocytes to counteract capillary wall distension. *Kidney Int* 45: 369–376, 1994.
234. KRIZ W, HAHNEL B, ROSENER S, AND ELGER M. Long-term treatment of rats with FGF-2 results in focal segmental glomerulosclerosis. *Kidney Int* 48: 1435–1450, 1995.
235. KRIZ W, HARTMANN I, HOSSER H, HAHNEL B, KRANZLIN B, PROVOOST A, AND GRETZ N. Tracer studies in the rat demonstrate misdirected filtration and peritubular filtrate spreading in nephrons with segmental glomerulosclerosis. *J Am Soc Nephrol* 12: 496–506, 2001.
236. KRIZ W, HOSSER H, HAHNEL B, GRETZ N, AND PROVOOST AP. From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. *Nephrol Dial Transplant* 13: 2781–2798, 1998.
237. KRIZ W, HOSSER H, HAHNEL B, SIMONS JL, AND PROVOOST AP. Development of vascular pole-associated glomerulosclerosis in the Fawn-hooded rat. *J Am Soc Nephrol* 9: 381–396, 1998.
238. KRIZ W, KRETZLER M, NAGATA M, PROVOOST AP, SHIRATO I, UIKER S, SAKAI T, AND LEMLEY KV. A frequent pathway to glomerulosclerosis: deterioration of tuft architecture-podocyte damage-segmental sclerosis. *Kidney Blood Press Res* 19: 245–253, 1996.
239. KRIZ W, KRETZLER M, PROVOOST AP, AND SHIRATO I. Stability and leakiness: opposing challenges to the glomerulus. *Kidney Int* 49: 1570–1574, 1996.
240. KRIZ W AND LEMLEY KV. The role of the podocyte in glomerulosclerosis. *Curr Opin Nephrol Hypertens* 8: 489–497, 1999.
241. KRIZ W, MUNDEL P, AND ELGER M. The contractile apparatus of podocytes is arranged to counteract GBM expansion. *Contrib Nephrol* 107: 1–9, 1994.
242. KURIHARA H, ANDERSON JM, AND FARQUHAR MG. Increased Tyr phosphorylation of ZO-1 during modification of tight junctions between glomerular foot processes. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F514–F524, 1995.
243. KURIHARA H, ANDERSON JM, KERJASCHKI D, AND FARQUHAR MG. The altered glomerular filtration slits seen in puromycin aminonucleoside nephrosis and protamine sulfate-treated rats contain the tight junction protein ZO-1. *Am J Pathol* 141: 805–816, 1992.
244. KURIHARA H, SUNAGAWA N, KOBAYASHI T, KIMURA K, TAKASU N, AND SHIKE T. Monoclonal antibody P-31 recognizes a novel intermediate filament-associated protein (p250) in rat podocytes. *Am J Physiol Renal Physiol* 274: F986–F997, 1998.
245. LACHAPELLE M AND BENDAYAN M. Contractile proteins in podocytes: immunocytochemical localization of actin and α-actinin in normal and nephrotic rat kidneys. *Virchows Arch B Cell Pathol Incl Mol Pathol* 60: 105–111, 1991.
245a. LAMBOLEZ B, AUDINAT E, BOCHET P, CREPEL F, AND ROSSIER J. AMPA receptor subunits expressed by single Purkinje cells. *Neuron* 9: 247–258, 1992.
246. LAURENS W, BATTAGLIA C, FOGLIENI C, DE VOS R, MALANCHINI B, VAN DAMME B, VANRENTERGHEM Y, REMUZZI G, AND REMUZZI A. Direct podocyte damage in the single nephron leads to albuminuria in vivo. *Kidney Int* 47: 1078–1086, 1995.
247. LAURINAVICIUS A, HURWITZ S, AND RENNKE HG. Collapsing glomerulopathy in HIV and non-HIV patients: a clinicopathological and follow-up study. *Kidney Int* 56: 2203–2213, 1999.
248. LEDOUX S, DUSSAULE JC, CHATZIANTONIOU C, ARDAILLOU N, VANDERMEERSCH S, AND ARDAILLOU R. Protein kinase A activity modulates natriuretic peptide-dependent cGMP accumulation in renal cells. *Am J Physiol Cell Physiol* 272: C82–C89, 1997.
249. LEDUC I AND MELOCHE S. Angiotensin II stimulates tyrosine phosphorylation of the focal adhesion-associated protein paxillin in aortic smooth muscle cells. *J Biol Chem* 270: 4401–4404, 1995.
250. LEE K, BROWN D, URENA P, ARDAILLOU N, ARDAILLOU R, DEEDS J, AND SEGRE GV. Localization of parathyroid hormone/parathyroid hormone-related peptide receptor mRNA in kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F186–F191, 1996.
251. LEMANSKI LF, PAULSON DJ, HILL CS, DAVIS LA, RILES LC, AND LIM SS. Immunoelectron microscopic localization of α-actinin on Lowicryl-embedded thin-sectioned tissues. *J Histochem Cytochem* 33: 515–522, 1985.
252. LEMLEY KV, BLOUCH K, ABDULLAH I, BOOTHROYD DB, BENNETT PH, MYERS BD, AND NELSON RG. Glomerular permselectivity at the onset of nephropathy in type 2 diabetes mellitus. *J Am Soc Nephrol* 11: 2095–2105, 2000.
253. LEVEEN P, PEKNY M, GEBRE-MEDHIN S, SWOLIN B, LARSSON E, AND BETSHOLTZ C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev* 8: 1875–1887, 1994.
254. LEVIDIOTIS V, KANELLIS J, IERINO FL, AND POWER DA. Increased expression of heparanase in puromycin aminonucleoside nephrosis. *Kidney Int* 60: 1287–1296, 2001.
255. LI B, YAO J, MORIOKA T, AND OITE T. Nitric oxide increases albumin permeability of isolated rat glomeruli via a phosphorylation-dependent mechanism. *J Am Soc Nephrol* 12: 2616–2624, 2001.
256. LI C, RUOTSALAINEN V, TRYGGVASON K, SHAW AS, AND MINER JH. Cd2ap is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. *Am J Physiol Renal Physiol* 279: F785–F792, 2000.
257. LIN F, EMANCIPATOR SN, SALANT DJ, AND MEDOF ME. Decay-accelerating factor confers protection against complement-mediated

podocyte injury in acute nephrotoxic nephritis. *Lab Invest* 82: 563–569, 2002.
258. Liu A, Dardik A, and Ballermann BJ. Neutralizing TGF-β1 antibody infusion in neonatal rat delays in vivo glomerular capillary formation 1. *Kidney Int* 56: 1334–1348, 1999.
259. Liu H, Shah SV, and Baliga R. Cytochrome *P*-450 as a source of catalytic iron in minimal change nephrotic syndrome in rats. *Am J Physiol Renal Physiol* 280: F88–F94, 2001.
260. Loh NY, Newey SE, Davies KE, and Blake DJ. Assembly of multiple dystrobrevin-containing complexes in the kidney. *J Cell Sci* 113: 2715–2724, 2000.
261. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, and Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol Rev* 52: 375–414, 2000.
262. Luimula P, Ahola H, Wang SX, Solin ML, Aaltonen P, Tikkanen I, Kerjaschki D, and Holthofer H. Nephrin in experimental glomerular disease. *Kidney Int* 58: 1461–1468, 2000.
263. Luimula P, Sandstrom N, Novikov D, and Holthofer H. Podocyte-associated molecules in puromycin aminonucleoside nephrosis of the rat. *Lab Invest* 82: 713–718, 2002.
264. Luthl A, Laurent JP, Figurov A, Muller D, and Schachner M. Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. *Nature* 372: 777–779, 1994.
265. Macconi D, Ghilardi M, Bonassi ME, Mohamed EI, Abbate M, Colombi F, Remuzzi G, and Remuzzi A. Effect of angiotensin-converting enzyme inhibition on glomerular basement membrane permeability and distribution of zonula occludens-1 in MWF rats. *J Am Soc Nephrol* 11: 477–489, 2000.
266. Mahan JD, Sisson-Ross S, and Vernier RL. Glomerular basement membrane anionic charge site changes early in aminonucleoside nephrosis. *Am J Pathol* 125: 393–401, 1986.
267. Martin J, Steadman R, Knowlden J, Williams J, and Davies M. Differential regulation of matrix metalloproteinases and their inhibitors in human glomerular epithelial cells in vitro. *J Am Soc Nephrol* 9: 1629–1637, 1998.
268. Martin KC and Kandel ER. Cell adhesion molecules, CREB, and the formation of new synaptic connections. *Neuron* 17: 567–570, 1996.
269. Maschio G, Alberti D, Janin G, Locatelli F, Mann JF, Motolese M, Ponticelli C, Ritz E, and Zucchelli P. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The angiotensin-converting-enzyme inhibition in progressive renal insufficiency study group. *N Engl J Med* 334: 939–945, 1996.
270. Matsumoto K and Kanmatsuse K. Elevated interleukin-18 levels in the urine of nephrotic patients. *Nephron* 88: 334–339, 2001.
271. Maunsbach AB. Absorption of I-125-labeled homologous albumin by rat kidney proximal tubule cells. A study of microperfused single proximal tubules by electron microscopic autoradiography and histochemistry. *J Ultrastruct Res* 15: 197–241, 1966.
272. McMillan JI, Riordan JW, Couser WG, Pollock AS, and Lovett DH. Characterization of a glomerular epithelial cell metalloproteinase as matrix metalloproteinase-9 with enhanced expression in a model of membranous nephropathy. *J Clin Invest* 97: 1094–1101, 1996.
273. Mctaggart SJ, Algar E, Chow CW, Powell HR, and Jones CL. Clinical spectrum of Denys-Drash and Frasier syndrome. *Pediatr Nephrol* 16: 335–339, 2001.
274. Meadow SR and Sarsfield JK. Steroid-responsive and nephrotic syndrome and allergy: clinical studies. *Arch Dis Child* 56: 509–516, 1981.
275. Mehlen P, Briolay J, Smith L, Diaz-Latoud C, Fabre N, Pauli D, and Arrigo AP. Analysis of the resistance to heat and hydrogen peroxide stresses in COS cells transiently expressing wild type or deletion mutants of the *Drosophila* 27-kDa heat-shock protein. *Eur J Biochem* 215: 277–284, 1993.
276. Mendrick DL, Kelly DM, and Rennke HG. Antigen processing and presentation by glomerular visceral epithelium in vitro. *Kidney Int* 39: 71–78, 1991.
277. Mendrick DL and Rennke HG. Induction of proteinuria in the rat by a monoclonal antibody against SGP-115/107. *Kidney Int* 33: 818–830, 1988.
278. Mentzel S, Van Son JP, De Jong AS, Dijkman HB, Koene RA, Wetzels JF, and Assmann KJ. Mouse glomerular epithelial cells in culture with features of podocytes in vivo express aminopeptidase A and angiotensinogen but not other components of the renin-angiotensin system. *J Am Soc Nephrol* 8: 706–719, 1997.
279. Meyer TW, Bennett PH, and Nelson RG. Podocyte number predicts long-term urinary albumin excretion in Pima Indians with Type II diabetes and microalbuminuria. *Diabetologia* 42: 1341–1344, 1999.
280. Miner JH. Developmental biology of glomerular basement membrane components. *Curr Opin Nephrol Hypertens* 7: 13–19, 1998.
281. Miner JH. Renal basement membrane components. *Kidney Int* 56: 2016–2024, 1999.
282. Miner JH and Li C. Defective glomerulogenesis in the absence of laminin α5 demonstrates a developmental role for the kidney glomerular basement membrane. *Dev Biol* 217: 278–289, 2000.
283. Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, and Lee B. Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. *J Clin Invest* 109: 1065–1072, 2002.
284. Miner JH and Sanes JR. Collagen IV α 3, α 4, and α 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. *J Cell Biol* 127: 879–891, 1994.
285. Miner JH and Sanes JR. Molecular and functional defects in kidneys of mice lacking collagen α 3(IV): implications for Alport syndrome. *J Cell Biol* 135: 1403–1413, 1996.
286. Miron T, Wilchek M, and Geiger B. Characterization of an inhibitor of actin polymerization in vinculin-rich fraction of turkey gizzard smooth muscle. *Eur J Biochem* 178: 543–553, 1988.
287. Mitic LL and Anderson JM. Molecular architecture of tight junctions. *Annu Rev Physiol* 60: 121–142, 1998.
288. Moeller MJ, Kovari IA, and Holzman LB. Evaluation of a new tool for exploring podocyte biology: mouse Nphs1 5′ flanking region drives LacZ expression in podocytes. *J Am Soc Nephrol* 11: 2306–2314, 2000.
289. Morino N, Matsumoto T, Ueki K, Mimura T, Hamasaki K, Kanda H, Naruse T, Yazaki Y, and Nojima Y. Glomerular overexpression and increased tyrosine phosphorylation of focal adhesion kinase p125FAK in lupus-prone MRL/MP-lpr/lpr mice. *Immunology* 97: 634–640, 1999.
290. Mozes MM, Bottinger EP, Jacot TA, and Kopp JB. Renal expression of fibrotic matrix proteins and of transforming growth factor-β (TGF-β) isoforms in TGF-β transgenic mice. *J Am Soc Nephrol* 10: 271–280, 1999.
291. Mudge SJ, Paizis K, Auwardt RB, Thomas RJ, and Power DA. Activation of nuclear factor-kappa B by podocytes in the autologous phase of passive Heymann nephritis. *Kidney Int* 59: 923–931, 2001.
292. Mundel P, Gambaryan S, Bachmann S, Koesling D, and Kriz W. Immunolocalization of soluble guanylyl cyclase subunits in rat kidney. *Histochem Cell Biol* 103: 75–79, 1995.
293. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, and Kriz W. Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. *J Cell Biol* 139: 193–204, 1997.
294. Mundel P, Reiser J, and Kriz W. Induction of differentiation in cultured rat and human podocytes. *J Am Soc Nephrol* 8: 697–705, 1997.
295. Mundel P, Reiser J, Zuniga Mejia BA, Pavenstadt H, Davidson GR, Kriz W, and Zeller R. Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. *Exp Cell Res* 236: 248–258, 1997.
296. Mundlos S, Pelletier J, Darveau A, Bachmann M, Winterpacht A, and Zabel B. Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. *Development* 119: 1329–1341, 1993.
297. Nagahama H, Hatakeyama S, Nakayama K, Nagata M, Tomita K, and Nakayama K. Spatial and temporal expression patterns of the cyclin-dependent kinase (CDK) inhibitors p27Kip1 and p57Kip2 during mouse development. *Anat Embryol* 203: 77–87, 2001.
298. Nagata M, Hattori M, Hamano Y, Ito K, Saitoh K, and Watanabe T. Origin and phenotypic features of hyperplastic epithelial cells in collapsing glomerulopathy. *Am J Kidney Dis* 32: 962–969, 1998.
299. Nagata M, Horita S, Shu Y, Shibata S, Hattori M, Ito K, and Watanabe T. Phenotypic characteristics and cyclin-dependent kinase inhibitors repression in hyperplastic epithelial pathology in

idiopathic focal segmental glomerulosclerosis. *Lab Invest* 80: 869–880, 2000.
300. Nagata M, Nakayama K, Terada Y, Hoshi S, and Watanabe T. Cell cycle regulation and differentiation in the human podocyte lineage. *Am J Pathol* 153: 1511–1520, 1998.
301. Nagata M, Scharer K, and Kriz W. Glomerular damage after uninephrectomy in young rats. I. Hypertrophy and distortion of capillary architecture. *Kidney Int* 42: 136–147, 1992.
302. Nagata M, Yamaguchi Y, and Ito K. Loss of mitotic activity and the expression of vimentin in glomerular epithelial cells of developing human kidneys. *Anat Embryol* 187: 275–279, 1993.
303. Nakahama H, Obata K, Matsuyama T, Sugita M, Horio M, Oka K, and Moriyama T. Effect of a novel immunosuppressant, FK 506, on autoimmune glomerulonephritis in Brown Norway rats. *Nephron* 81: 215–220, 1999.
304. Nakamura T, Ushiyama C, Osada S, Hara M, Shimada N, and Koide H. Pioglitazone reduces urinary podocyte excretion in type 2 diabetes patients with microalbuminuria. *Metabolism* 50: 1193–1196, 2001.
305. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, and Koide H. The urinary podocyte as a marker for the differential diagnosis of idiopathic focal glomerulosclerosis and minimal-change nephrotic syndrome. *Am J Nephrol* 20: 175–179, 2000.
306. Nangaku M. Complement regulatory proteins in glomerular diseases. *Kidney Int* 54: 1419–1428, 1998.
307. Natori Y, Natori Y, Nishimura T, Yamabe H, Iyonaga K, Takeya M, and Kawakami M. Production of monocyte chemoattractant protein-1 by cultured glomerular epithelial cells: inhibition by dexamethasone. *Exp Nephrol* 5: 318–322, 1997.
308. Navar LG, Harrison-Bernard LM, Imig JD, Wang CT, Cervenka L, and Mitchell KD. Intrarenal angiotensin II generation and renal effects of AT1 receptor blockade. *J Am Soc Nephrol* 10 *Suppl* 12: S266–S272, 1999.
309. Neale TJ, Ullrich R, Ojha P, Poczewski H, Verhoeven AJ, and Kerjaschki D. Reactive oxygen species and neutrophil respiratory burst cytochrome b_{558} are produced by kidney glomerular cells in passive Heymann nephritis. *Proc Natl Acad Sci USA* 90: 3645–3649, 1993.
310. Nislow C, Lombillo VA, Kuriyama R, and McIntosh JR. A plus-end-directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. *Nature* 359: 543–547, 1992.
311. Nislow C, Sellitto C, Kuriyama R, and McIntosh JR. A monoclonal antibody to a mitotic microtubule-associated protein blocks mitotic progression. *J Cell Biol* 111: 511–522, 1990.
312. Nitschke R, Henger A, Ricken S, Gloy J, Muller V, Greger R, and Pavenstadt H. Angiotensin II increases the intracellular calcium activity in podocytes of the intact glomerulus. *Kidney Int* 57: 41–49, 2000.
313. Nitschke R, Henger A, Ricken S, Muller V, Kottgen M, Bek M, and Pavenstadt H. Acetylcholine increases the free intracellular calcium concentration in podocytes in intact rat glomeruli via muscarinic M(5) receptors. *J Am Soc Nephrol* 12: 678–687, 2001.
314. Noakes PG, Miner JH, Gautam M, Cunningham JM, Sanes JR, and Merlie JP. The renal glomerulus of mice lacking s-laminin/laminin β 2: nephrosis despite molecular compensation by laminin β 1. *Nat Genet* 10: 400–406, 1995.
315. Nolasco FE, Cameron JS, Hartley B, Coelho RA, Hildredth G, and Reuben R. Abnormal podocyte CR-1 expression in glomerular diseases: association with glomerular cell proliferation and monocyte infiltration. *Nephrol Dial Transplant* 2: 304–312, 1987.
316. Norgaard JO. Retraction of epithelial foot processes during culture of isolated glomeruli. *Lab Invest* 38: 320–329, 1978.
317. Norgaard JO. Rat glomerular epithelial cells in culture. Parietal or visceral epithelial origin? *Lab Invest* 57: 277–290, 1987.
318. Novak A, Hsu SC, Leung-Hagesteijn C, Radeva G, Papkoff J, Montesano R, Roskelley C, Grosschedl R, and Dedhar S. Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signaling pathways. *Proc Natl Acad Sci USA* 95: 4374–4379, 1998.
321. Ohlson M, Sorensson J, and Haraldsson B. Glomerular size and charge selectivity in the rat as revealed by FITC-Ficoll and albumin. *Am J Physiol Renal Physiol* 279: F84–F91, 2000.
322. Ohlson M, Sorensson J, and Haraldsson B. A gel-membrane model of glomerular charge and size selectivity in series. *Am J Physiol Renal Physiol* 280: F396–F405, 2001.
323. Ohlson M, Sorensson J, Lindstrom K, Blom AM, Fries E, and Haraldsson B. Effects of filtration rate on the glomerular barrier and clearance of four differently shaped molecules. *Am J Physiol Renal Physiol* 281: F103–F113, 2001.
324. Okada-Ban M, Thiery JP, and Jouanneau J. Fibroblast growth factor-2. *Int J Biochem Cell Biol* 32: 263–267, 2000.
325. Oleinikov AV, Feliz BJ, and Makker SP. A small N-terminal 60-kD fragment of gp600 (megalin), the major autoantigen of active Heymann nephritis, can induce a full-blown disease. *J Am Soc Nephrol* 11: 57–64, 2000.
326. Oliver JD III, Anderson S, Troy JL, Brenner BM, and Deen WH. Determination of glomerular size-selectivity in the normal rat with Ficoll. *J Am Soc Nephrol* 3: 214–228, 1992.
327. Oliver JD III, Simons JL, Troy JL, Provoost AP, Brenner BM, and Deen WM. Proteinuria and impaired glomerular permselectivity in uninephrectomized fawn-hooded rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F917–F925, 1994.
328. Olson JL and Heptinstall RH. Nonimmunologic mechanisms of glomerular injury. *Lab Invest* 59: 564–578, 1988.
328a. O'Meara YM, Natori Y, Minto AW, Goldstein DJ, Manning EC, and Salant DJ. Nephrotoxic antiserum identifies a β 1-integrin on rat glomerular epithelial cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F1083–F1091, 1992.
329. Oosterwijk E, Van Muijen GN, Oosterwijk-Wakka JC, and Warnaar SO. Expression of intermediate-sized filaments in developing and adult human kidney and in renal cell carcinoma. *J Histochem Cytochem* 38: 385–392, 1990.
330. Ophascharoensuk V, Fero ML, Hughes J, Roberts JM, and Shankland SJ. The cyclin-dependent kinase inhibitor p27Kip1 safeguards against inflammatory injury. *Nat Med* 4: 575–580, 1998.
331. Orci L, Brown D, Amherdt M, and Perrelet A. Distribution of intramembrane particles and filipin-sterol complexes in plasma membranes of kidney. I. Corpuscle of Malpighi. *Lab Invest* 46: 545–553, 1982.
332. Orlando RA, Takeda T, Zak B, Schmieder S, Benoit VM, McQuistan T, Furthmayr H, and Farquhar MG. The glomerular epithelial cell anti-adhesin podocalyxin associates with the actin cytoskeleton through interactions with ezrin. *J Am Soc Nephrol* 12: 1589–1598, 2001.
333. Osicka TM, Hankin AR, and Comper WD. Puromycin aminonucleoside nephrosis results in a marked increase in fractional clearance of albumin. *Am J Physiol Renal Physiol* 277: F139–F145, 1999.
334. Osicka TM, Panagiotopoulos S, Jerums G, and Comper WD. Fractional clearance of albumin is influenced by its degradation during renal passage. *Clin Sci* 93: 557–564, 1997.
334a. O'Toole TE, Katagiri Y, Faull RJ, Peter K, Tamura R, Quaranta V, Loftus JC, Shattil SJ, and Ginsberg MH. Integrin cytoplasmic domains mediate inside-out signal transduction. *J Cell Biol* 124: 1047–1059, 1994.
335. Pabst R and Sterzel RB. Cell renewal of glomerular cell types in normal rats. An autoradiographic analysis. *Kidney Int* 24: 626–631, 1983.
336. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, and Meyer TW. Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest* 99: 342–348, 1997.
337. Paizis K, Kirkland G, Khong T, Katerelos M, Fraser S, Kanellis J, and Power DA. Heparin-binding epidermal growth factor-like growth factor is expressed in the adhesive lesions of experimental focal glomerular sclerosis. *Kidney Int* 55: 2310–2321, 1999.
338. Palmen T, Ahola H, Palgi J, Aaltonen P, Luimula P, Wang S, Jaakkola I, Knip M, Otonkoski T, and Holthofer H. Nephrin is expressed in the pancreatic β cells. *Diabetologia* 44: 1274–1280, 2001.
339. Panesar M, Papillon J, McTavish AJ, and Cybulsky AV. Activation of phospholipase A_2 by complement C5b-9 in glomerular epithelial cells. *J Immunol* 159: 3584–3594, 1997.
340. Park CH and Maack T. Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of the rabbit. *J Clin Invest* 73: 767–777, 1984.
341. Parry D, Mahony D, Wills K, and Lees E. Cyclin D-CDK subunit

arrangement is dependent on the availability of competing INK4 and p21 class inhibitors. *Mol Cell Biol* 19: 1775–1783, 1999.
342. PARRY RG, GILLESPIE KM, AND MATHIESON PW. Effects of type 2 cytokines on glomerular epithelial cells. *Exp Nephrol* 9: 275–283, 2001.
343. PARVING HH, LEHNERT H, BROCHNER-MORTENSEN J, GOMIS R, ANDERSEN S, AND ARNER P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345: 870–878, 2001.
344. PARYSEK LM, WOLOSEWICK JJ, AND OLMSTED JB. Map 4: a microtubule-associated protein specific for a subset of tissue microtubules. *J Cell Biol* 99: 2287–2296, 1984.
345. PATRAKKA J, RUOTSALAINEN V, KETOLA I, HOLMBERG C, HEIKINHEIMO M, TRYGGVASON K, AND JALANKO H. Expression of nephrin in pediatric kidney diseases. *J Am Soc Nephrol* 12: 289–296, 2001.
346. PATRIE KM, DRESCHER AJ, GOYAL M, WIGGINS RC, AND MARGOLIS B. The membrane-associated guanylate kinase protein MAGI-1 binds megalin and is present in glomerular podocytes. *J Am Soc Nephrol* 12: 667–677, 2001.
347. PATRIE KM, DRESCHER AJ, WELIHINDA A, MUNDEL P, AND MARGOLIS B. Interaction of two actin-binding proteins, synaptopodin and α-actinin-4, with the tight junction protein MAGI-1. *J Biol Chem* 277: 30183–30190, 2002.
348. PAVENSTADT H, SPATH M, FIEDLER C, FISCHER R, SCHLUNCK G, WANNER C, AND SCHOLLMEYER P. Effect of bradykinin on the cytosolic free calcium activity and phosphoinositol turnover in human glomerular epithelial cells. *Renal Physiol Biochem* 15: 277–288, 1992.
349. PAVENSTADT H, SPATH M, SCHLUNCK G, NAUCK M, FISCHER R, WANNER C, AND SCHOLLMEYER P. Effect of nucleotides on the cytosolic free calcium activity and inositol phosphate formation in human glomerular epithelial cells. *Br J Pharmacol* 107: 189–195, 1992.
350. PERANEN J, AUVINEN P, VIRTA H, WEPF R, AND SIMONS K. Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts. *J Cell Biol* 135: 153–167, 1996.
351. PETEN EP, STRIKER LJ, CAROME MA, ELLIOTT SJ, YANG CW, AND STRIKER GE. The contribution of increased collagen synthesis to human glomerulosclerosis: a quantitative analysis of α 2IV collagen mRNA expression by competitive polymerase chain reaction. *J Exp Med* 176: 1571–1576, 1992.
352. PETERMANN AT, HIROMURA K, BLONSKI M, PIPPIN J, MONKAWA T, DURVASULA R, COUSER WG, AND SHANKLAND SJ. Mechanical stress reduces podocyte proliferation in vitro. *Kidney Int* 61: 40–50, 2002.
353. PRICE NE, WADZINSKI B, AND MUMBY MC. An anchoring factor targets protein phosphatase 2A to brain microtubules. *Brain Res* 73: 68–77, 1999.
354. PUTAALA H, SOININEN R, KILPELAINEN P, WARTIOVAARA J, AND TRYGGVASON K. The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death. *Hum Mol Genet* 10: 1–8, 2001.
355. QUAGGIN SE, VANDEN HEUVEL GB, AND IGARASH P. Pod-1, a mesoderm-specific basic-helix-loop-helix protein expressed in mesenchymal and glomerular epithelial cells in the developing kidney. *Mech Dev* 71: 37–48, 1998.
356. QUIGG RJ, CYBULSKY AV, JACOBS JB, AND SALANT DJ. Anti-Fx1A produces complement-dependent cytotoxicity of glomerular epithelial cells. *Kidney Int* 34: 43–52, 1988.
357. QUIGG RJ, HE C, LIM A, BERTHIAUME D, ALEXANDER JJ, KRAUS D, AND HOLERS VM. Transgenic mice overexpressing the complement inhibitor crry as a soluble protein are protected from antibody-induced glomerular injury. *J Exp Med* 188: 1321–1331, 1998.
358. QUIGG RJ, HOLERS VM, MORGAN BP, AND SNEED AE III. Crry and CD59 regulate complement in rat glomerular epithelial cells and are inhibited by the nephritogenic antibody of passive Heymann nephritis. *J Immunol* 154: 3437–3443, 1995.
359. QUIGG RJ, NICHOLSON-WELLER A, CYBULSKY AV, BADALAMENTI J, AND SALANT DJ. Decay accelerating factor regulates complement activation on glomerular epithelial cells. *J Immunol* 142: 877–882, 1989.
360. RAATS CJ, VAN DEN BJ, BAKKER MA, OPPERS-WALGREEN B, PISA BJ, DIJKMAN HB, ASSMANN KJ, AND BERDEN JH. Expression of agrin, dystroglycan, and utrophin in normal renal tissue and in experimental glomerulopathies. *Am J Pathol* 156: 1749–1765, 2000.
361. RADEVA G, PETROCELLI T, BEHREND E, LEUNG-HAGESTEIJN C, FILMUS J, SLINGERLAND J, AND DEDHAR S. Overexpression of the integrin-linked kinase promotes anchorage-independent cell cycle progression. *J Biol Chem* 272: 13937–13944, 1997.
362. RADICE GL, FERREIRA-CORNWELL MC, ROBINSON SD, RAYBURN H, CHODOSH LA, TAKEICHI M, AND HYNES RO. Precocious mammary gland development in P-cadherin-deficient mice. *J Cell Biol* 139: 1025–1032, 1997.
363. REBIBOU JM, HE CJ, DELARUE F, PERALDI MN, ADIDA C, RONDEAU E, AND SRAER JD. Functional endothelin 1 receptors on human glomerular podocytes and mesangial cells. *Nephrol Dial Transplant* 7: 288–292, 1992.
364. REEVES W, CAULFIELD JP, AND FARQUHAR MG. Differentiation of epithelial foot processes and filtration slits: sequential appearance of occluding junctions, epithelial polyanion, and slit membranes in developing glomeruli. *Lab Invest* 39: 90–100, 1978.
365. REGELE HM, FILLIPOVIC E, LANGER B, POCZEWKI H, KRAXBERGER I, BITTNER RE, AND KERJASCHKI D. Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. *J Am Soc Nephrol* 11: 403–412, 2000.
366. REGOLI M AND BENDAYAN M. Alterations in the expression of the α 3 β 1 integrin in certain membrane domains of the glomerular epithelial cells (podocytes) in diabetes mellitus. *Diabetologia* 40: 15–22, 1997.
367. REGOLI M AND BENDAYAN M. Expression of β1 integrins in glomerular tissue of streptozotocin-induced diabetic rats. *Biochem Cell Biol* 77: 71–78, 1999.
368. REISER J, KRIZ W, KRETZLER M, AND MUNDEL P. The glomerular slit diaphragm is a modified adherens junction. *J Am Soc Nephrol* 11: 1–8, 2000.
369. REISER J, PIXLEY FJ, HUG A, KRIZ W, SMOYER WE, STANLEY ER, AND MUNDEL P. Regulation of mouse podocyte process dynamics by protein tyrosine phosphatases rapid communication. *Kidney Int* 57: 2035–2042, 2000.
370. REMUZZI A, PUNTORIERI S, BATTAGLIA C, BERTANI T, AND REMUZZI G. Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *J Clin Invest* 85: 541–549, 1990.
371. RENNKE HG. How does glomerular epithelial cell injury contribute to progressive glomerular damage? *Kidney Int Suppl* 45: S58–S63, 1994.
372. RENNKE HG, COTRAN RS, AND VENKATACHALAM MA. Role of molecular charge in glomerular permeability tracer studies with cationized ferritins. *J Cell Biol* 67: 638–646, 1975.
373. RICARDO SD, BERTRAM JF, AND RYAN GB. Antioxidants protect podocyte foot processes in puromycin aminonucleoside-treated rats. *J Am Soc Nephrol* 4: 1974–1986, 1994.
374. RODEWALD R AND KARNOVSKY MJ. Porous substructure of the glomerular slit diaphragm in the rat and mouse. *J Cell Biol* 60: 423–433, 1974.
375. ROHR C, PRESTEL J, HEIDET L, HOSSER H, KRIZ W, JOHNSON RL, ANTIGNAC C, AND WITZGALL R. The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. *J Clin Invest* 109: 1073–1082, 2002.
376. ROTHENPIELER UW AND DRESSLER GR. Pax-2 is required for mesenchyme-to-epithelium conversion during kidney development. *Development* 119: 711–720, 1993.
377. RUDIGER F, GREGER R, NITSCHKE R, HENGER A, MUNDEL P, AND PAVENSTADT H. Polycations induce calcium signaling in glomerular podocytes. *Kidney Int* 56: 1700–1709, 1999.
378. RUGGENENTI P, SCHIEPPATI A, AND REMUZZI G. Progression, remission, regression of chronic renal diseases. *Lancet* 357: 1601–1608, 2001.
379. RUOTSALAINEN V, LJUNGBERG P, WARTIOVAARA J, LENKKERI U, KESTILA M, JALANKO H, HOLMBERG C, AND TRYGGVASON K. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 96: 7962–7967, 1999.
380. RUOTSALAINEN V, PATRAKKA J, TISSARI P, REPONEN P, HESS M, KESTILA M, HOLMBERG C, SALONEN R, HEIKINHEIMO M, WARTIOVAARA J, TRYGGVASON K, AND JALANKO H. Role of nephrin in cell junction formation in human nephrogenesis. *Am J Pathol* 157: 1905–1916, 2000.
381. RYAN G, STEELE-PERKINS V, MORRIS JF, RAUSCHER FJ III, AND DRESSLER GR. Repression of Pax-2 by WT1 during normal kidney development. *Development* 121: 867–875, 1995.
382. RYAN GB, HEIN SJ, AND KARNOVSKY MJ. Glomerular permeability to proteins. Effects of hemodynamic factors on the distribution of

endogenous immunoglobulin G and exogenous catalase in the rat glomerulus. *Lab Invest* 34: 415–427, 1976.
383. Ryan GB and Karnovsky MJ. An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int* 8: 219–232, 1975.
384. Ryan GB and Karnovsky MJ. Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. *Kidney Int* 9: 36–45, 1976.
385. Saito A, Pietromonaco S, Loo AK, and Farquhar MG. Complete cloning and sequencing of rat gp330/"megalin," a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci USA* 91: 9725–9729, 1994.
386. Sakai T, Sabanovic S, Hosser H, and Kriz W. Heterogeneity of the podocyte membrane in rat kidney as revealed by ethanol dehydration of unosmicated specimens. *Cell Tissue Res* 246: 145–151, 1986.
387. Saleem MA, Iser J, Ward R, Ward C, Rren T, Ng Cy L, Thieson P, and Mundel P. A conditionally immortalized human podocyte cell line, demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 54: 1029–1033, 2002.
388. Sanden S. Das Aktinskelett der Fußfortsätze und seine Beziehungen zum Tubulinskelett der Primärfortsätze—morphologische und immunzytochemische Untersuchungen an den Podozyten der Rattenniere. Dissertation 2001 Heidelberg, 1–77. *J Am Soc Nephrol* 27: 1265–1270, 2000.
389. Sanden S, Elger M, Mundel P, and Kriz W. The architecture of podocyte cytoskeleton suggests a role in glomerular filtration dynamics. *Ann Anat* 177: 44–45, 1995.
390. Sanwal V, Pandya M, Bhaskaran M, Franki N, Reddy K, Ding G, Kapasi A, Valderrama E, and Singhal PC. Puromycin aminonucleoside induces glomerular epithelial cell apoptosis. *Exp Mol Pathol* 70: 54–64, 2001.
391. Sariola H. Incomplete fusion of the epithelial and endothelial basement membranes in interspecies hybrid glomeruli. *Cell Differ* 14: 189–195, 1984.
392. Sariola H, Ekblom P, Lehtonen E, and Saxen L. Differentiation and vascularization of the metanephric kidney grafted on the chorioallantoic membrane. *Dev Biol* 96: 427–435, 1983.
393. Sasaki T, Jyo Y, Tanda N, Kawakami Y, Nohno T, Tamai H, and Osawa G. Changes in glomerular epithelial cells induced by FGF2 and FGF2 neutralizing antibody in puromycin aminonucleoside nephropathy. *Kidney Int* 51: 301–309, 1997.
394. Savin VJ, Johnson RJ, and Couser WG. C5b-9 increases albumin permeability of isolated glomeruli in vitro. *Kidney Int* 46: 382–387, 1994.
395. Sawada H, Stukenbrok H, Kerjaschki D, and Farquhar MG. Epithelial polyanion (podocalyxin) is found on the sides but not the soles of the foot processes of the glomerular epithelium. *Am J Pathol* 125: 309–318, 1986.
396. Saxen L and Sariola H. Early organogenesis of the kidney. *Pediatr Nephrol* 1: 385–392, 1987.
397. Schadde E, Kretzler M, Banas B, Luckow B, Assmann K, and Schlondorff D. Expression of chemokines and their receptors in nephrotoxic serum nephritis. *Nephrol Dial Transplant* 15: 1046–1053, 2000.
398. Schiffer M, Bitzer M, Roberts IS, Kopp JB, Ten Dijke P, Mundel P, and Bottinger EP. Apoptosis in podocytes induced by TGF-β and Smad7. *J Clin Invest* 108: 807–816, 2001.
399. Schiller B, He C, Salant DJ, Lim A, Alexander JJ, and Quigg RJ. Inhibition of complement regulation is key to the pathogenesis of active Heymann nephritis. *J Exp Med* 188: 1353–1358, 1998.
400. Schlaepfer DD and Hunter T. Integrin signalling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol* 8: 151–157, 1998.
401. Schnabel E, Anderson JM, and Farquhar MG. The tight junction protein ZO-1 is concentrated along slit diaphragms of the glomerular epithelium. *J Cell Biol* 111: 1255–1263, 1990.
402. Schnabel E, Dekan G, Miettinen A, and Farquhar MG. Biogenesis of podocalyxin–the major glomerular sialoglycoprotein–in the newborn rat kidney. *Eur J Cell Biol* 48: 313–326, 1989.
403. Schrijver G, Assmann KJ, Bogman MJ, Robben JC, De Waal RM, and Koene RS. Antiglomerular basement membrane nephritis in the mouse. Study on the role of complement in the heterologous phase. *Lab Invest* 59: 484–491, 1988.
404. Schroppel B, Huber S, Horster M, Schlondorff D, and Kretzler M. Analysis of mouse glomerular podocyte mRNA by single-cell reverse transcription-polymerase chain reaction. *Kidney Int* 53: 119–124, 1998.
405. Schwartz EJ, Cara A, Snoeck H, Ross MD, Sunamoto M, Reiser J, Mundel P, and Klotman PE. Human immunodeficiency virus-1 induces loss of contact inhibition in podocytes. *J Am Soc Nephrol* 12: 1677–1684, 2001.
406. Schwartz MM, Evans J, Bain R, and Korbet SM. Focal segmental glomerulosclerosis: prognostic implications of the cellular lesion. *J Am Soc Nephrol* 10: 1900–1907, 1999.
407. Schwartz MM and Lewis EJ. Focal segmental glomerular sclerosis: the cellular lesion. *Kidney Int* 28: 968–974, 1985.
408. Schwarz K. Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 108: 1621–1629, 2001.
409. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, Shaw AS, Holzman LB, and Mundel P. Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 108: 1621–1629, 2001.
410. Segerer S, Nelson PJ, and Schlondorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. *J Am Soc Nephrol* 11: 152–176, 2000.
411. Seiler MW, Venkatachalam MA, and Cotran RS. Glomerular epithelium: structural alterations induced by polycations. *Science* 189: 390–393, 1975.
412. Setou M, Nakagawa T, Seog DH, and Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 288: 1796–1802, 2000.
413. Severt WL, Biber TU, Wu X, Hecht NB, Delorenzo RJ, and Jakoi ER. The suppression of testis-brain RNA binding protein and kinesin heavy chain disrupts mRNA sorting in dendrites. *J Cell Sci* 112: 3691–3702, 1999.
414. Shah SV. Evidence suggesting a role for hydroxyl radical in passive Heymann nephritis in rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 254: F337–F344, 1988.
415. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'agati V, and Alpers CE. Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. *Kidney Int* 58: 674–683, 2000.
416. Shankland SJ, Hugo C, Coats SR, Nangaku M, Pichler RH, Gordon KL, Pippin J, Roberts JM, Couser WG, and Johnson RJ. Changes in cell-cycle protein expression during experimental mesangial proliferative glomerulonephritis. *Kidney Int* 50: 1230–1239, 1996.
417. Shankland SJ, Pippin J, Pichler RH, Gordon KL, Friedman S, Gold LI, Johnson RJ, and Couser WG. Differential expression of transforming growth factor-β isoforms and receptors in experimental membranous nephropathy. *Kidney Int* 50: 116–124, 1996.
418. Shankland SJ, Pippin JW, and Couser WG. Complement (C5b-9) induces glomerular epithelial cell DNA synthesis but not proliferation in vitro. *Kidney Int* 56: 538–548, 1999.
419. Shankland SJ and Wolf G. Cell cycle regulatory proteins in renal disease: role in hypertrophy, proliferation, and apoptosis. *Am J Physiol Renal Physiol* 278: F515–F529, 2000.
420. Sharma M, Sharma R, Greene AS, McCarthy ET, and Savin VJ. Documentation of angiotensin II receptors in glomerular epithelial cells. *Am J Physiol Renal Physiol* 274: F623–F627, 1998.
421. Sharma R, Lovell HB, Wiegmann TB, and Savin VJ. Vasoactive substances induce cytoskeletal changes in cultured rat glomerular epithelial cells. *J Am Soc Nephrol* 3: 1131–1138, 1992.
422. Sharma R, Sharma M, Vamos S, Savin VJ, and Wiegmann TB. Both subtype 1 and 2 receptors of angiotensin II participate in regulation of intracellular calcium in glomerular epithelial cells. *J Lab Clin Med* 138: 40–49, 2001.
423. Sharp DJ, Yu W, Ferhat L, Kuriyama R, Rueger DC, and Baas PW. Identification of a microtubule-associated motor protein essential for dendritic differentiation. *J Cell Biol* 138: 833–843, 1997.
424. Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, and Shaw AS. Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 286: 312–315, 1999.
425. Shiiki H, Sasaki Y, Nishino T, Kimura T, Kurioka H, Fujimoto S, and Dohi K. Cell proliferation and apoptosis of the glomerular epithelial

cells in rats with puromycin aminonucleoside nephrosis. *Pathobiology* 66: 221–229, 1998.
426. SHIKATA K, MAKINO H, MORIOKA S, KASHITANI T, HIRATA K, OTA Z, WADA J, AND KANWAR YS. Distribution of extracellular matrix receptors in various forms of glomerulonephritis. *Am J Kidney Dis* 25: 680–688, 1995.
427. SHIMONAKA M, SAHEKI T, HAGIWARA H, HAGIWARA Y, SONO H, AND HIROSE S. Visualization of ANP receptor on glomeruli of bovine kidney by use of a specific antiserum. *Am J Physiol Renal Fluid Electrolyte Physiol* 253: F1058–F1062, 1987.
428. SHIRATO I, HOSSER H, KIMURA K, SAKAI T, TOMINO Y, AND KRIZ W. The development of focal segmental glomerulosclerosis in masugi nephritis is based on progressive podocyte damage. *Virchows Arch* 429: 255–273, 1996.
429. SHIRATO I, SAKAI T, KIMURA K, TOMINO Y, AND KRIZ W. Cytoskeletal changes in podocytes associated with foot process effacement in Masugi nephritis. *Am J Pathol* 148: 1283–1296, 1996.
430. SIMMONS PJ, LEVESQUE JP, AND HAYLOCK DN. Mucin-like molecules as modulators of the survival and proliferation of primitive hematopoietic cells. *Ann NY Acad Sci* 938: 196–206, 2001.
431. SIMONS M, SAFFRICH R, REISER J, AND MUNDEL P. Directed membrane transport is involved in process formation in cultured podocytes. *J Am Soc Nephrol* 10: 1633–1639, 1999.
432. SIMONS M, SCHWARZ K, KRIZ W, MIETTINEN A, REISER J, MUNDEL P, AND HOLTHOFER H. Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. *Am J Pathol* 159: 1069–1077, 2001.
433. SMOYER WE, GUPTA A, MUNDEL P, BALLEW JD, AND WELSH MJ. Altered expression of glomerular heat shock protein 27 in experimental nephrotic syndrome. *J Clin Invest* 97: 2697–2704, 1996.
434. SMOYER WE AND MUNDEL P. Regulation of podocyte structure during the development of nephrotic syndrome. *J Mol Med* 76: 172–183, 1998.
435. SMOYER WE, MUNDEL P, GUPTA A, AND WELSH MJ. Podocyte α-actinin induction precedes foot process effacement in experimental nephrotic syndrome. *Am J Physiol Renal Physiol* 273: F150–F157, 1997.
436. SMOYER WE AND RANSOM RF. Hsp27 regulates podocyte cytoskeletal changes in an in vitro model of podocyte process retraction. *FASEB J* 16: 315–326, 2002.
437. SOGABE H, NANGAKU M, ISHIBASHI Y, WADA T, FUJITA T, SUN X, MIWA T, MADAIO MP, AND SONG WC. Increased susceptibility of decay-accelerating factor deficient mice to anti-glomerular basement membrane glomerulonephritis. *J Immunol* 167: 2791–2797, 2001.
438. SOIFER NE, VAN WHY SK, GANZ MB, KASHGARIAN M, SIEGEL NJ, AND STEWART AF. Expression of parathyroid hormone-related protein in the rat glomerulus and tubule during recovery from renal ischemia. *J Clin Invest* 92: 2850–2857, 1993.
439. SORENSSON J, OHLSON M, AND HARALDSSON B. A quantitative analysis of the glomerular charge barrier in the rat. *Am J Physiol Renal Physiol* 280: F646–F656, 2001.
440. SORIANO P. Abnormal kidney development and hematological disorders in PDGF β-receptor mutant mice. *Genes Dev* 8: 1888–1896, 1994.
441. SPATH M, PAVENSTADT H, MULLER C, PETERSEN J, WANNER C, AND SCHOLLMEYER P. Regulation of phosphoinositide hydrolysis and cytosolic free calcium induced by endothelin in human glomerular epithelial cells. *Nephrol Dial Transplant* 10: 1299–1304, 1995.
442. SPATH M, PAVENSTADT H, PETERSEN J, AND SCHOLLMEYER P. Arginine vasopressin and oxytocin increase intracellular calcium and cAMP in human glomerular epithelial cells in culture. *Kidney Blood Press Res* 19: 81–86, 1996.
443. SPATH MM, PAVENSTADT H, FISCHER R, SCHLUNCK G, WANNER C, AND SCHOLLMEYER P. Effects of histamine on inositol phosphates and intracellular Ca^{2+} in human glomerular epithelial cells. *Nephrol Dial Transplant* 9: 758–763, 1994.
444. STAMENKOVIC I, SKALLI O, AND GABBIANI G. Distribution of intermediate filament proteins in normal and diseased human glomeruli. *Am J Pathol* 125: 465–475, 1986.
445. STEFFES MW, SCHMIDT D, MCCRERY R, AND BASGEN JM. Glomerular cell number in normal subjects and in type 1 diabetic patients. *Kidney Int* 59: 2104–2113, 2001.
446. ST JOHN PL AND ABRAHAMSON DR. Glomerular endothelial cells and podocytes jointly synthesize laminin-1 and -11 chains. *Kidney Int* 60: 1037–1046, 2001.
447. ST JOHN PL, WANG R, YIN Y, MINER JH, ROBERT B, AND ABRAHAMSON DR. Glomerular laminin isoform transitions: errors in metanephric culture are corrected by grafting. *Am J Physiol Renal Physiol* 280: F695–F705, 2001.
448. SVENNILSON J, SANDBERG-NORDQVIST A, AND APERIA A. Age-dependent expression of protein phosphatase 2A in the developing rat kidney. *Pediatr Nephrol* 13: 800–805, 1999.
449. TAKAHASHI T, TAKAHASHI K, GERETY S, WANG H, ANDERSON DJ, AND DANIEL TO. Temporally compartmentalized expression of ephrin-B2 during renal glomerular development. *J Am Soc Nephrol* 12: 2673–2682, 2001.
450. TAKAI Y, SASAKI T, AND MATOZAKI T. Small GTP-binding proteins. *Physiol Rev* 81: 153–208, 2001.
451. TAKEDA T, GO WY, ORLANDO RA, AND FARQUHAR MG. Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. *Mol Biol Cell* 11: 3219–3232, 2000.
452. TAKEDA T, MCQUISTAN T, ORLANDO RA, AND FARQUHAR MG. Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. *J Clin Invest* 108: 289–301, 2001.
453. TAKEUCHI A, YOSHIZAWA N, YAMAMOTO M, SAWASAKI Y, ODA T, SENOO A, NIWA H, AND FUSE Y. Basic fibroblast growth factor promotes proliferation of rat glomerular visceral epithelial cells in vitro. *Am J Pathol* 141: 107–116, 1992.
454. TASSIN MT, BEZIAU A, GUBLER MC, AND BOYER B. Spatiotemporal expression of molecules associated with junctional complexes during the in vivo maturation of renal podocytes. *Int J Dev Biol* 38: 45–54, 1994.
455. TAY M, COMPER WD, AND SINGH AK. Charge selectivity in kidney ultrafiltration is associated with glomerular uptake of transport probes. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F549–F554, 1991.
456. TEIXEIRA VP, SEGRETO HR, BOIM MA, RAZVICKAS CV, AND SCHOR N. Effects of ionizing radiation on progressive experimental renal disease: a hemodynamic approach. *Nephron* 87: 58–65, 2001.
457. TENSCHERT S, ELGER M, AND LEMLEY KV. Glomerular hypertrophy after subtotal nephrectomy: relationship to early glomerular injury. *Virchows Arch* 426: 509–517, 1995.
458. THAKUR V, WALKER PD, AND SHAH SV. Evidence suggesting a role for hydroxyl radical in puromycin aminonucleoside-induced proteinuria. *Kidney Int* 34: 494–499, 1988.
459. THEILIG F, BOSTANJOGLO M, PAVENSTADT H, GRUPP C, HOLLAND G, SLOSAREK I, GRESSNER AM, RUSSWURM M, KOESLING D, AND BACHMANN S. Cellular distribution and function of soluble guanylyl cyclase in rat kidney and liver. *J Am Soc Nephrol* 12: 2209–2220, 2001.
460. THOMAS PE, WHARRAM BL, GOYAL M, WIGGINS JE, HOLZMAN LB, AND WIGGINS RC. Glepp1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J Biol Chem* 269: 19953–19962, 1994.
461. TOGAWA A, MIYOSHI J, ISHIZAKI H, TANAKA M, TAKAKURA A, NISHIOKA H, YOSHIDA H, DOI T, MIZOGUCHI A, MATSUURA N, NIHO Y, NISHIMUNE Y, NISHIKAWA S, AND TAKAI Y. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIα. *Oncogene* 18: 5373–5380, 1999.
462. TOJO A AND ENDO UH. Intrarenal handling of proteins in rats using fractional micropuncture technique. *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F601–F606, 1992.
463. TOPHAM PS, HAYDAR SA, KUPHAL R, LIGHTFOOT JD, AND SALANT DJ. Complement-mediated injury reversibly disrupts glomerular epithelial cell actin microfilaments and focal adhesions. *Kidney Int* 55: 1763–1775, 1999.
464. TOPHAM PS, KAWACHI H, HAYDAR SA, CHUGH S, ADDONA TA, CHARRON KB, HOLZMAN LB, SHIA M, SHIMIZU F, AND SALANT DJ. Nephritogenic mAb 5–1-6 is directed at the extracellular domain of rat nephrin. *J Clin Invest* 104: 1559–1566, 1999.
465. TORRES M, GOMEZ-PARDO E, DRESSLER GR, AND GRUSS P. Pax-2 controls multiple steps of urogenital development. *Development* 121: 4057–4065, 1995.
466. TROUSSARD AA, COSTELLO P, YOGANATHAN TN, KUMAGAI S, ROSKELLEY

CD, AND DEDHAR S. The integrin linked kinase (ILK) induces an invasive phenotype via AP-1 transcription factor-dependent upregulation of matrix metalloproteinase 9 (MMP-9). *Oncogene* 19: 5444–5452, 2000.
467. TRYGGVASON K AND WARTIOVAAR AJ. Molecular basis of glomerular permselectivity. *Curr Opin Nephrol Hypertens* 10: 543–549, 2001.
468. TSUNODA S, YAMABE H, OSAWA H, KAIZUKA M, SHIRATO K, AND OKUMURA K. Cultured rat glomerular epithelial cells show gene expression and production of transforming growth factor-β: expression is enhanced by thrombin. *Nephrol Dial Transplant* 16: 1776–1782, 2001.
469. TUFRO A, NORWOOD VF, CAREY RM, AND GOMEZ RA. Vascular endothelial growth factor induces nephrogenesis and vasculogenesis. *J Am Soc Nephrol* 10: 2125–2134, 1999.
470. TYSON GE. Scanning electron microscopic study of the effect of vinblastine on podocytes of rat kidney. *Virchows Arch B Cell Pathol* 25: 105–115, 1977.
471. UEDA N, BALIGA R, AND SHAH SV. Role of "catalytic" iron in an animal model of minimal change nephrotic syndrome. *Kidney Int* 49: 370–373, 1996.
472. VALERI A, BARISONI L, APPEL GB, SEIGLE R, AND D'AGATI V. Idiopathic collapsing focal segmental glomerulosclerosis: a clinicopathologic study. *Kidney Int* 50: 1734–1746, 1996.
473. VAN DEN BERG JG, ATEN J, ANNINK C, RAVESLOOT JH, WEBER E, AND WEENING JJ. Interleukin-4 and -13 promote basolateral secretion of H^+ and cathepsin L by glomerular epithelial cells. *Am J Physiol Renal Physiol* 282: F26–F33, 2002.
474. VAN DEN BERG JG, ATEN J, CHAND MA, CLAESSEN N, DIJKINK L, WIJDENES J, LAKKIS FG, AND WEENING JJ. Interleukin-4 and interleukin-13 act on glomerular visceral epithelial cells. *J Am Soc Nephrol* 11: 413–422, 2000.
475. VAN DET NF, VERHAGEN NA, TAMSMA JT, BERDEN JH, BRUIJN JA, DAHA MR, AND VAN DER WOUDE FJ. Regulation of glomerular epithelial cell production of fibronectin and transforming growth factor-β by high glucose, not by angiotensin II. *Diabetes* 46: 834–840, 1997.
476. VARGAS GA, HOEFLICH A, AND JEHLE PM. Hepatocyte growth factor in renal failure: promise and reality. *Kidney Int* 57: 1426–1436, 2000.
477. VASMANT D, MAURICE M, AND FELDMANN G. Cytoskeleton ultrastructure of podocytes and glomerular endothelial cells in man and in the rat. *Anat Rec* 210: 17–24, 1984.
478. VENKATACHALAM MA, COTRAN RS, AND KARNOVSKY MJ. An ultrastructural study of glomerular permeability in aminonucleoside nephrosis using catalase as a tracer protein. *J Exp Med* 132: 1168–1180, 1970.
479. VENKATACHALAM MA, KARNOVSKY MJ, AND COTRAN RS. Glomerular permeability. Ultrastructural studies in experimental nephrosis using horseradish peroxidase as a tracer. *J Exp Med* 130: 381–399, 1969.
480. VYAS SV, PARKER JA, AND COMPER WD. Uptake of dextran sulphate by glomerular intracellular vesicles during kidney ultrafiltration. *Kidney Int* 47: 945–950, 1995.
481. WADA T, TOMOSUGI N, NAITO T, YOKOYAMA H, KOBAYASHI K, HARADA A, MUKAIDA N, AND MATSUSHIMA K. Prevention of proteinuria by the administration of anti-interleukin 8 antibody in experimental acute immune complex-induced glomerulonephritis. *J Exp Med* 180: 1135–1140, 1994.
482. WALPORT MJ. Complement. First of two parts. *N Engl J Med* 344: 1058–1066, 2001.
483. WANG JS, YANG AH, CHEN SM, YOUNG TK, CHIANG H, AND LIU HC. Amelioration of antioxidant enzyme suppression and proteinuria in cyclosporin-treated puromycin nephrosis. *Nephron* 65: 418–425, 1993.
484. WANG R, ST JOHN PL, KRETZLER M, WIGGINS RC, AND ABRAHAMSON DR. Molecular cloning, expression, and distribution of glomerular epithelial protein 1 in developing mouse kidney. *Kidney Int* 57: 1847–1859, 2000.
485. WANG SX, AHOLA H, PALMEN T, SOLIN ML, LUIMULA P, AND HOLTHOFER H. Recurrence of nephrotic syndrome after transplantation in CNF is due to autoantibodies to nephrin. *Exp Nephrol* 9: 327–331, 2001.
486. WANG SX, MENE P, AND HOLTHOFER H. Nephrin mRNA regulation by protein kinase C. *J Nephrol* 14: 98–103, 2001.
487. WANG W, TZANIDIS A, DIVJAK M, THOMSON NM, AND STEIN-OAKLEY AN. Altered signaling and regulatory mechanisms of apoptosis in focal and segmental glomerulosclerosis. *J Am Soc Nephrol* 12: 1422–1433, 2001.
488. WANG XM, PELOQUIN JG, ZHAI Y, BULINSKI JC, AND BORISY GG. Removal of MAP4 from microtubules in vivo produces no observable phenotype at the cellular level. *J Cell Biol* 132: 345–357, 1996.
489. WELSCH T, ENDLICH N, KRIZ W, AND ENDLICH K. Cd2ap and p130Cas localize to different F-actin structures in podocytes. *Am J Physiol Renal Physiol* 281: F769–F777, 2001.
490. WHARRAM BL, GOYAL M, GILLESPIE PJ, WIGGINS JE, KERSHAW DB, HOLZMAN LB, DYSKO RC, SAUNDERS TL, SAMUELSON LC, AND WIGGINS RC. Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. *J Clin Invest* 106: 1281–1290, 2000.
491. WHITESIDE C, PRUTIS K, CAMERON R, AND THOMPSON J. Glomerular epithelial detachment, not reduced charge density, correlates with proteinuria in adriamycin and puromycin nephrosis. *Lab Invest* 61: 650–660, 1989.
492. WHITESIDE CI, CAMERON R, MUNK S, AND LEVY J. Podocytic cytoskeletal disaggregation and basement-membrane detachment in puromycin aminonucleoside nephrosis. *Am J Pathol* 142: 1641–1653, 1993.
493. WILKINSON DG. Eph receptors and ephrins: regulators of guidance and assembly. *Int Rev Cytol* 196: 177–244, 2000.
494. WILSON HM, MINTO AW, BROWN PA, ERWIG LP, AND REES AJ. Transforming growth factor-β isoforms and glomerular injury in nephrotoxic nephritis. *Kidney Int* 57: 2434–2444, 2000.
495. WOLF E, KAHNT E, EHRLEIN J, HERMANNS W, BREM G, AND WANKE R. Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: lessons from transgenic animal models. *Mech Ageing Dev* 68: 71–87, 1993.
496. WONG MA, CUI S, AND QUAGGIN SE. Identification and characterization of a glomerular-specific promoter from the human nephrin gene. *Am J Physiol Renal Physiol* 279: F1027–F1032, 2000.
497. WOOLF AS AND YUAN HT. Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. *Pediatr Nephrol* 16: 177–184, 2001.
498. WU C, KEIGHTLEY SY, LEUNG-HAGESTEIJN C, RADEVA G, COPPOLINO M, GOICOECHEA S, MCDONALD JA, AND DEDHAR S. Integrin-linked protein kinase regulates fibronectin matrix assembly, E-cadherin expression, and tumorigenicity. *J Biol Chem* 273: 528–536, 1998.
499. WU W AND WELSH MJ. Expression of the 25-kDa heat-shock protein (HSP27) correlates with resistance to the toxicity of cadmium chloride, mercuric chloride, cis-platinum(II)-diammine dichloride, or sodium arsenite in mouse embryonic stem cells transfected with sense or antisense HSP27 cDNA. *Toxicol Appl Pharmacol* 141: 330–339, 1996.
500. YAN K, KHOSHNOODI J, RUOTSALAINEN V, AND TRYGGVASON K. *N*-linked glycosylation is critical for the plasma membrane localization of nephrin. *J Am Soc Nephrol* 13: 1385–1389, 2002.
501. YAOITA E, FRANKE WW, YAMAMOTO T, KAWASAKI K, AND KIHARA I. Identification of renal podocytes in multiple species: higher vertebrates are vimentin positive/lower vertebrates are desmin positive. *Histochem Cell Biol* 111: 107–115, 1999.
502. YAOITA E, KAWASAKI K, YAMAMOTO T, AND KIHARA I. Variable expression of desmin in rat glomerular epithelial cells. *Am J Pathol* 136: 899–908, 1990.
503. YAOITA E, KURIHARA H, SAKAI T, OHSHIRO K, AND YAMAMOTO T. Phenotypic modulation of parietal epithelial cells of Bowman's capsule in culture. *Cell Tissue Res* 304: 339–349, 2001.
504. YAOITA E, WICHE G, YAMAMOTO T, KAWASAKI K, AND KIHARA I. Perinuclear distribution of plectin characterizes visceral epithelial cells of rat glomeruli. *Am J Pathol* 149: 319–327, 1996.
505. YAOITA E, YAMAMOTO T, SAITO M, KAWASAKI K, AND KIHARA I. Desmin-positive epithelial cells outgrowing from rat encapsulated glomeruli. *Eur J Cell Biol* 54: 140–149, 1991.
506. YAOITA E, YAMAMOTO T, TAKASHIMA N, KAWASAKI K, KAWACHI H, SHIMIZU F, AND KIHARA I. Visceral epithelial cells in rat glomerular cell culture. *Eur J Cell Biol* 67: 136–144, 1995.
507. YOSHIOKA T, ICHIKAWA I, AND FOGO A. Reactive oxygen metabolites cause massive, reversible proteinuria and glomerular sieving defect without apparent ultrastructural abnormality. *J Am Soc Nephrol* 2: 902–912, 1991.
508. YU W, SHARP DJ, KURIYAMA R, MALLIK P, AND BAAS PW. Inhibition of

a mitotic motor compromises the formation of dendrite-like processes from neuroblastoma cells. *J Cell Biol* 136: 659–668, 1997.
509. YUAN H, TAKEUCHI E, AND SALANT DJ. Podocyte slit-diaphragm protein nephrin is linked to the actin cytoskeleton. *Am J Physiol Renal Physiol* 282: F585–F591, 2002.
510. YUAN H, TAKEUCHI E, TAYLOR GA, MCLAUGHLIN M, BROWN D, AND SALANT DJ. Nephrin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. *J Am Soc Nephrol* 13: 946–956, 2002.
511. YUAN HT, SURI C, LANDON DN, YANCOPOULOS GD, AND WOOLF AS. Angiopoietin-2 is a site-specific factor in differentiation of mouse renal vasculature. *J Am Soc Nephrol* 11: 1055–1066, 2000.
512. YUAN HT, SURI C, YANCOPOULOS GD, AND WOOLF AS. Expression of angiopoietin-1, angiopoietin-2, and the Tie-2 receptor tyrosine kinase during mouse kidney maturation. *J Am Soc Nephrol* 10: 1722–1736, 1999.
513. ZHANG P, LIEGEOIS NJ, WONG C, FINEGOLD M, HOU H, THOMPSON JC, SILVERMAN A, HARPER JW, DEPINHO RA, AND ELLEDGE SJ. Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature* 387: 151–158, 1997.
514. ZHAO J, ARDAILLOU N, LU CY, PLACIER S, PHAM P, BADRE L, CAMBAR J, AND ARDAILLOU R. Characterization of C-type natriuretic peptide receptors in human mesangial cells. *Kidney Int* 46: 717–725, 1994.
515. ZIMMERMANN KW. Über den Bau des Glomerulus der menschlichen Niere. *Z Mikr Anat Forsch* 18: 520–552, 1929.