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Podocyte Biology and Response to Injury

PETER MUNDEL* and STUART J. SHANKLAND†

**Division of Nephrology, Albert Einstein College of Medicine, Bronx, New York; and †Division of Nephrology, University of Washington, Seattle, Washington.*

The visceral glomerular epithelial cell, also called podocyte, is a terminally differentiated cell that lines the outer aspect of the glomerular basement membrane (GBM). It therefore forms the final barrier to protein loss, which explains why podocyte injury is typically associated with marked proteinuria. Indeed, all forms of nephrotic syndrome are characterized by abnormalities in the podocyte. In this review, we will provide an update of the known functions of recent podocyte-specific proteins and focus on the slit diaphragm (SD) and the mechanisms underlying foot process (FP) flattening and how the podocyte responds to injury.

Molecular Anatomy of the Podocyte FP Cytoskeleton

Podocyte FP are not static, but rather contain a contractile system similar to that seen in pericytes. This contractile apparatus is composed of actin, myosin-II, α-actinin-4, talin, and vinculin (1). The actin filament bundles form arches between adjacent FP of the same podocyte (2). Figure 1 is a schema of our current understanding of the molecular composition of the cytoskeleton in podocyte FP. Importantly, the actin filaments are connected to the underlying GBM at focal contacts via an $\alpha 3\beta 1$ integrin complex (3,4). The bends of the actin filament arches appear to be connected directly to the microtubules of the major processes (own unpublished results). FP are anchored to the GBM via $\alpha 3\beta 1$ integrin (5) and dystroglycans (6,7). Neighboring FP are connected by a cell-cell junction, the glomerular SD, which represents the main size selective filter barrier in the kidney (8–10). The SD is thought to be a modified adherens junction (11) that is composed of a growing number of proteins, including nephrin (12–14), P-cadherin, CD2AP (15–18), ZO-1 (19), FAT (20), podocin (16,21), and possibly Neph1 (see reference 22 and below). In addition to the contractile proteins described above, we have reported the association of synaptopodin with the actin filaments in FP (23). Synaptopodin is the first member of a novel class of proline-rich proteins (24) and, like α-actinin-4, interacts with the tight junction protein MAGI-1 (25) that is also expressed in podocytes (26).

Four Major Causes of FP Effacement

The basolateral portion of the foot processes represents the center of podocyte function and is defined by three membrane domains: the apical membrane domain, the SD protein complex, and the basal membrane domain or sole plate (27). The submembranous regions of all compartments are connected to the FP actin cytoskeleton, *e.g.*, on the apical membrane domain, podocalyxin associates with the actin cytoskeleton through interactions with ezrin and the actin cytoskeleton via Na^+/H^+-exchanger regulatory factor 2 (NHERF2), a scaffold protein containing two PDZ (PSD-95/Dlg/ZO-1) domains and an ERM-binding region (28,29). The FP actin cytoskeleton is highly dynamic and ultimately determines the structural maintenance of the filtration slits as demonstrated in the acute PS/heparin model by several groups (30–32). Interference with one of the three domains eventually leads to changes in the actin cytoskeleton from coordinated stress fibers into a dense network (33) with fusion of podocyte FP and obliteration of filtration slit. Proteins regulating or stabilizing F-actin are therefore of critical importance for sustained function of glomerular filtration (29,33–38).

Podocyte α-actinin-4 induction precedes FP effacement in experimental nephrotic syndrome (36), and the podocalyxin/NHERF2/ezrin/actin interactions are disrupted in pathologic conditions associated with changes in FP (29). The α-actinin-4 molecule is a novel member (39) of the actin family of actin-filament cross-linking proteins and has an important function in podocytes. Mutations in the *ACTN4* gene encoding α-actinin-4 have been demonstrated by Pollak and colleagues in an autosomal dominant form of focal segmental glomerular sclerosis (FSGS) (see related article by Pollak in this issue of *JASN* [40]) and underscore the exquisite role of the actin cytoskeleton in short-term and long-term regulation of podocyte structure (41,42).

Podocytes are injured in many forms of human and experimental glomerular disease, including minimal change disease, FSGS, membranous glomerulopathy, diabetes mellitus, and lupus nephritis (8,27). Independent of the underlying disease, the early events are either characterized by alterations in the molecular composition of the SD without visible changes in morphology or, more obviously, by a reorganization of the FP structure with fusion of filtration slits and apical displacement of the SD (8,37,43). On the basis of recent progress in the molecular pathology of podocytes, four major causes can be

Correspondence to Dr. Stuart J. Shankland, Division of Nephrology, University of Washington, Box 356521, Seattle, WA 98195. Phone: 206-543-3792; Fax: 206-685-8661; E-mail: stuartjs@u.washington.edu

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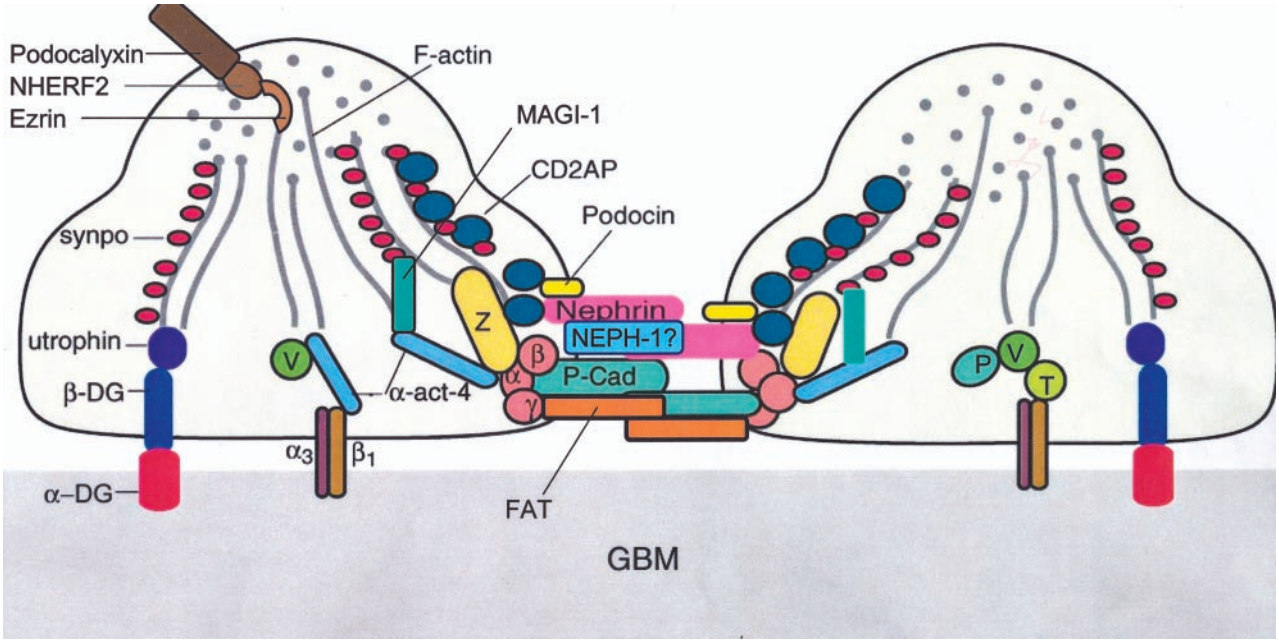

Figure 1. Molecular anatomy of the podocyte foot process (FP) actin cytoskeleton. This schematic shows two adjacent podocyte FP with the interposed slit diaphragm (SD) complex. The localization of NEPH-1 at the SD and its heterophilic interaction with nephrin remain to be established. The actin cytoskeleton is the common downstream pathway and receives input form three podocyte domains: the apical domains, the lateral SD-containing domain, and the basal domain of the FP sole plate, which links the podocyte to the GBM. Interference with any of the three domains will ultimately cause FP effacement and proteinuria/nephritic syndrome. α-act4, α-actinin-4; α3β1, α3β1 integrin; α-DG, α-dystroglycan; β-DG, β-dystroglycan; $Na^{(+)}/H^{(+)}$ERF2, $Na^{(+)}/H^{(+)}$ exchanger regulatory factor 2; P, paxillin; P-cad, P-cadherin; Synpo, synaptopodin; T, talin; V, vinculin.

identified that lead to FP effacement and proteinuria: (*1*) interference with the SD complex and its lipid rafts; (*2*) interference with the GBM or the podocyte-GBM interaction (6,7,44–49); (*3*) interference with the actin cytoskeleton and its associated protein α-actinin-4 (36,41); and (*4*) interference with the negative apical membrane domain of podocytes, *e.g.*, neutralization of negative cell surface charges (28–30,50).

In addition to the SD as the major site of glomerular permselectivity (see below), glomerular filtration is also regulated on the level of the GBM (51–53). In a comprehensive and elegant study comparing mice lacking either the alpha3 chain of type IV collagen, the major constituent of glomerular basement membrane, the LMX1B transcription factor, or nephrin, Hamano *et al.* (53) showed that defects induced by proteins of GBM lead to an insidious plasma protein leak, whereas the defects induced by SD proteins lead to a precipitous plasma protein leak. Finally, there is evidence in FSGS and in idiopathic nephrotic syndrome in rats that podocyte damage may be caused by circulating albuminuric factors (54,55). As depicted in Figure 1, the α-actinin molecule can interact with components of the integrin complex at the GBM and with the β-catenin molecule of the SD complex. Hence, α-actinin may link these two different compartments of the FP together, thereby providing a molecular explanation for the observation that the actin cytoskeleton serves as the "common final pathway" organizing FP effacement independent of the original underlying site or cause of podocyte damage. From a clinical point of view, it is important to note that these early structural changes in podocyte morphology, such as substructural alterations in SD composition or FP effacement, have to be reversed within a certain period of time to prevent development of severe and progressive glomerular damage (43,56–58) (also see below).

The SD: A Dynamic Site of Glomerular Permselectivity

In mature podocytes, the SD represents the only cell-cell contact between podocytes. The SD represents a tiny membrane bridging the 30- to 40-nm-wide filtration slit. In 1974, Rodewald and Karnowsky (59) showed that the SD is made up of rodlike units connected in the center to a linear bar forming a zipper-like appearance, but its molecular composition and anchorage in the FP remained unknown. The normal SD function is crucial to maintaining the integrity of the FP (8–10). The recent discovery of several novel SD proteins and their mutation analysis, including nephrin (60), CD2AP (17,61), podocin (62), and the nephrin homologue neph1 (22), have shed light on the pathogenesis of proteinuria and emphasized the critical role of the SD in maintaining the normal function of the glomerular filtration barrier. However, the mechanisms regulating the structural changes that occur during FP effacement are still largely unknown. A fuller understanding of the molecular basis of glomerular kidney disease requires elucidation of the relationship between SD proteins and the maintenance of FP structure.

The Critical Role of Nephrin in Maintaining the Glomerular Filtration Barrier

Twenty-five years after the hallmark finding by Rodewald and Karnovsky (59), the discovery of the transmembrane protein nephrin as a major component of the SD complex by Tryggvason and others (12–14) provided a seminal progress in podocyte biology. Mutation analysis of the nephrin gene, NPHS1, by positional cloning elucidated the underlying genetic defect in congenital nephrotic syndrome of the Finnish type as causative for FP effacement in this disease (60). Similarly, injection of anti-nephrin antibody in animals induced substructural alterations of the SD with reduction of permselectivity and consecutive proteinuria (63). The inactivation of the nephrin gene in mice by homologous recombination resulted in reduction of visible SD, severe proteinuria, and partial FP effacement (53,64), Similarly, nephrin TRAP mice also lack SD and show fibrotic glomeruli as well as cystic tubular lesions (65). Nephrin is a large (1241–amino acid, 185-kD) transmembrane molecule with Ig-like domains. N-linked glycosylation is critical for the plasma membrane localization of nephrin (66). Its predicted structure and biochemical properties, as well as electron microscopy studies, suggested that nephrin may form dimers through homophilic interactions across the filtration slit (67). However, several groups have failed to show such a homophilic interaction and the nephrin homologue in drosophila, *hibris*, was found to form heterophilic interactions with a protein called *dumbfounded* but not homophilic interaction with other *hibris* molecules (68,69).

Nephrin may also contribute functional properties to the SD, perhaps by participating in a protein complex in which interference with any of the components may lead to functional destabilization of the SD and consequent FP effacement and proteinuria (see below). Although the causal role of nephrin in congenital nephrotic syndrome of the Finnish type is now well established, its functional role in acquired forms of nephrotic syndrome remains to be established. Several studies have reported a modulation or correlation of nephrin expression with levels of proteinuria, including puromycin aminonucleoside (PAN) (70), diabetes (71,72), and minimal change disease (MCD) (73). The latter study showed that MCD is associated with disruption of the SD. At this point, it is too early to conclude whether the changes are causal or secondary, but a recent study analyzing the recurrence of nephrotic syndrome in kidney grafts of patients with congenital nephrotic syndrome of the Finnish type has shed some light on this issue (74). This study showed that circulating anti-nephrin antibodies might have a pathogenic role in the development of heavy proteinuria in kidney grafts of NPHS1 patients with Fin-major/Fin-major genotype (74).

A Rapidly Expanding List of Proteins that Comprise the SD

At the intracellular insertion site of the SD, the adapter protein CD2AP has been localized (17,18,76), which was originally discovered as a protein interacting with the CD2 receptor in T lymphocytes (77). CD2AP is critical for orchestrating the so-called immunologic synapse between B cells and T cells (77,78) but has gained an unexpected important role in podocyte cell biology, because CD2AP knockout mice die several weeks after birth with FP effacement and nephrotic syndrome (75). CD2AP interacts with nephrin via a novel C-terminal domain (18) and is also capable of associating with the actin cytoskeleton (79). The latest putative component of the SD complex is NEPH-1, a homologue of nephrin, which was discovered using retrovirus-mediated mutagenesis (22). The homozygous knockout mice of NEPH-1 show FP effacement (22). Whether NEPH-1 interacts with nephrin is not yet known, but in the light of the absence of a nephrin-nephrin homophilic interaction and the similar phenotype of both knockouts, a heterophilic interaction between nephrin and NEPH-1 appears plausible.

The role of other molecules that are associated with the SD awaits clarification. ZO-1 has long been known to localize to the intracellular site of insertion of the SD. It interacts with the actin cytoskeleton (80) and may also participate in signaling events through tyrosine phosphorylation (81). Of note is that the redistribution of ZO-1 was associated with the development of proteinuria in spontaneously proteinuric MWF rats, although the podocyte FP were normal and SD preserved in these animals (82). P-cadherin (11) and FAT (20), which are widely expressed cadherin superfamily proteins, define the SD as a modified adherens junction and may provide structural support to this specialized cell-cell contact. Interestingly, the expression of ZO-1, P-cadherin, and FAT is not altered in nephrin null mice (53).

Podocin Interacts with CD2AP and Nephrin

Podocin is a new member of the stomatin family of hairpin-like integral membrane proteins with intracellular N- and C-termini. Podocin is encoced by the *NPHS2* gene, which is mutated in autosomal recessive, steroid-resistant nephrotic syndrome (62). Stomatin is present as high-order oligomers in erythrocyte lipid rafts, where it has a scaffolding function (83). Podocin localizes to the SD (16,21), accumulates there in an oligomeric form in lipid rafts and associates via its C-terminus with CD2AP and nephrin (16). Further studies revealed direct interaction of podocin and CD2AP (16). Hence, podocin may act as a scaffolding protein, serving in the structural organization of the slit diaphragm and the regulation of its filtration function. *In vitro* co-expression studies showed that podocin facilitated nephrin signaling via AP-1 in HEK cells (84), but the relevance of this finding for podocytes has not yet been demonstrated.

Involvement of Lipid Rafts in Functional Organization of the SD

Lipid rafts are specialized membrane domains enriched in cholesterol, glycosphingolipids, and GPI-anchored proteins (85). By compartmentalizing cell membranes, they recruit and cluster membrane proteins in a selective and dynamic fashion. Hereby, they provide molecular frameworks for numerous cell biologic processes, such as exocytosis and endocytosis, cell

adhesion, and signal transduction events (86–88). Recent work from our lab established that lipid raft microdomains are critical for the *dynamic* functional organization of the SD (89). We have shown that nephrin associates with lipid rafts and coimmunoprecipitates with a podocyte-specific 9-O-acetylated ganglioside (89). The *in vivo* injection of an antibody against this ganglioside causes morphologic changes of the filtration slits resembling FP effacement. In this model, nephrin translocated to the apical pole of the narrowed filtration slits and underwent tyrosine phosphorylation (89).

Previous studies have described a role for tyrosine phosphorylation in the assembly and disassembly of the slit diaphragm (81). So far, it is unclear which kinases are involved in regulating these events, but the genetic inactivation of the src family kinase fyn caused proteinuria in mice (90). Interestingly, as a double-acylated molecule, fyn, has a high affinity for lipid rafts (91,92). Hence, it is intriguing to speculate that fyn is involved in regulating the dynamics of the SD complex. In summary, the last 4 yr have been extremely fruitful in providing extensive information on the molecular composition of the SD and have opened up new avenues to understanding podocyte function (8,27). From a clinical perspective, it is exciting that there are novel experimental data that may link the salutary effects of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers drugs to changes in the composition of the SD (93–97).

Podocyte Number Contributes to Glomerulosclerosis

As discussed above, podocytes are the target of many forms of injury, including antibodies to podocyte membrane antigens (membranous nephropathy, minimal change disease) (98), hemodynamic injury (reduced nephron number, diabetes, metabolic diabetes) (99–101), gene mutations (nephrin, α-actinin, CD2AP; see review by Pollak in this issue [40]), protein overload states (102), toxins (NSAIDS, adriamycin) (103), infections (HIV) (see review by Ross and Klotman in this issue [104]), and unknown causes (idiopathic FSGS) (105). Moreover, in secondary forms of FSGS, such as after loss of nephron number, hypertension, and tubulointerstitial disease, podocytes are also injured (106). However, regardless of the type of renal injury, loss of podocyte number contributes to the development of glomerulosclerosis (see below).

There is a growing body of experimental and clinical literature showing that podocyte number is a critical determinant for the development of glomerulosclerosis and that a decrease in podocyte number leads to progressive renal failure. For example, Wiggins and colleagues (107) recently showed that glomerulosclerosis correlated with podocyte loss during the normal physiologic aging process in rats. A single injection of PAN, a podocyte toxin, causes a marked decrease in podocyte number in rats. Kim *et al.* (107) showed that repeated injections of PAN further augmented podocyte loss and that the regions devoid of podocytes developed glomerulosclerosis. However, glomerulosclerosis was only initiated when podocyte number decreased by 10 to 20%. Moreover, there was a significant correlation between the decrease in podocyte number and the development of glomerulosclerosis, because the authors showed that increased podocyte loss with repeated PAN injections correlated with scarring (107). Kriz and colleagues (108) showed that a decrease in podocyte number in the Masugi nephritis model also contributed significantly to the development of renal failure.

One of the first studies to show that a decrease in podocyte number also correlated with disease progression in human disease was performed by Meyer and colleagues (109). They showed that a decrease in podocyte number in type II diabetic Pima Indians correlated closely with those patients who had microalbuminuria, the earliest manifestation of diabetic nephropathy. Moreover, they showed that the decrease in podocyte number was more pronounced in patients with more advanced nephropathy (109). In contrast to the decrease in podocyte number, mesangial and glomerular endothelial cell number remained normal. More recently, Steffes *et al.* (110) showed a similar paradigm in patients with type I diabetic nephropathy. Taken together, these important studies showed that a decrease in podocyte number is a significant predictor of disease progression in diabetic nephropathy. Finally, Lemley and coworkers (111) recently showed that despite injury to the mesangial cell in IgA nephropathy, a decrease in podocyte number correlated significantly with reduced renal function and global glomerulosclerosis.

Mechanism Underlying Glomerulosclerosis after a Decrease in Podocyte Number

The mechanism(s) underlying the development of glomerulosclerosis following a decrease in podocyte number has been proposed by Kriz, Rennke, and others (56,112–115). Because podocytes are located on the outer aspect of the glomerular basement membrane, one of the functions of podocytes is to provide a tensile support to the underlying glomerular capillary loop, by opposing the hydrostatic capillary pressure (115,116). It is the belief of most authorities that there is a finite number of podocytes/glomerulus and that individual podocytes cover a specific area of GBM. Thus if podocyte number is decreased, there are insufficient podocytes to cover that specific area of basement membrane. The sequence of events in the development of glomerulosclerosis are as follows (56,113). First, podocyte loss, and the inability to replace those lost because of a lack of proliferation (see below), results in a localized "bare" or denuded GBM at that site. Second, the lack of tensile support normally provided by podocytes (117) is lost in the area of denudation and leads to the outward bulging of the capillary loop (due to hydrostatic capillary pressures). Because many forms of glomerular diseases are associated with increased intraglomerular hydrostatic capillary pressure, this process is further augmented. Third, the "expanding" capillary loop causes the denuded basement membrane to abut on Bowman's capsule, leading to synechia formation, which Schwartz and Lewis (105) have shown is the first committed step to the development of FSGS. Finally, inspissated proteins and hyalinosis develop in the capillary loops, and progressive scarring ensues.

Causes of Podocytopenia

Because a decrease in podocyte number (podocytopenia) underlies glomerulosclerosis, recent studies have focused on the causes underlying podocytopenia (Figure 2). The etiology of podocytopenia includes apoptosis, detachment, and the inability or lack of podocytes to proliferate; each will be discussed below.

Apoptosis

Cell number reflects the balance between an increase in cell number due to proliferation, and a decrease in cell number due to apoptosis (programmed cell death). Although earlier studies failed to document significant podocyte apoptosis (118), recent studies have shown that podocytes undergo apoptosis in glomerular disease (119). One explanation for the earlier difficulty in detecting podocyte apoptosis is that apoptotic podocytes are likely flushed out in the urine, making it technically difficult to detect these cells. However, apoptosis has recently been shown in podocytes with human glomerular disease (V. D'Agati, personal communication). Moreover, Wiggins and colleagues (107) have clearly demonstrated podocyte apoptosis after toxic injury in the PAN model. Bottinger and colleagues (119) recently showed that apoptosis is increased in TGF-β transgenic mice, which leads to a decrease in podocyte number and glomerulosclerosis. Follow-up studies by Bottinger's group (120) showed that TGF-β induced podocyte apoptosis was mediated by specific Smad pathways, and we have recently shown that TGF-β induced podocyte apoptosis is augmented in the absence of the CDK-inhibitors p21 and p27 (unpublished data).

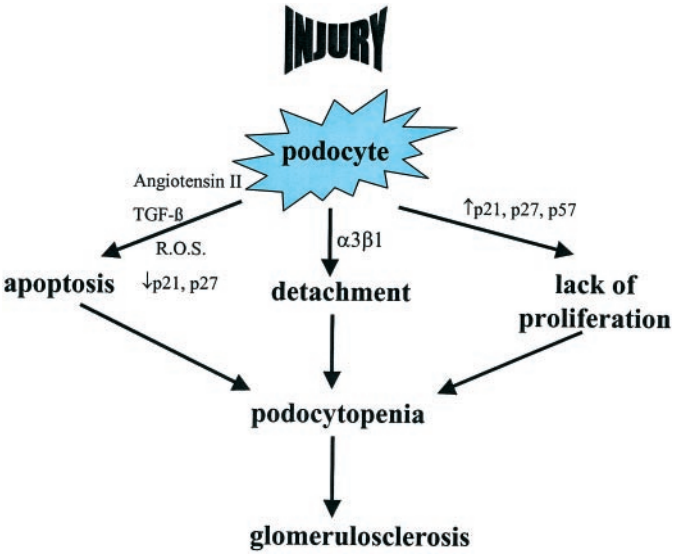

Figure 2. Causes of podocytopenia. After injury, podocytes can undergo apoptosis or detachment or fail to proliferate. These events lead to a decrease in podocyte number (podocytopenia), which contributes to the development of progressive glomerulosclerosis. The mechanisms underlying podocytopenia are being elucidated. Apoptosis results from increased transforming growth factor–β (TGF-β), angiotensin II, reactive oxygen species (ROS), and a decrease in the cyclin-dependent kinase (CDK) inhibitors p21 and p27. The $\alpha 3\beta 1$ integrin is most likely to be critical in podocyte detachment from the underlying glomerular basement membrane (GBM). In contrast to other glomerular cells, podocytes do not typically proliferate in response to injury and cannot replace those lost by apoptosis and detachment. The inability to proliferate is secondary to increased levels of the CDK-inhibitors p21, p27, and/or p57.

Recent studies have further examined the mechanisms underlying podocyte apoptosis. Singhal and colleagues (121) showed that angiotensin II (AngII) induces apoptosis in cultured rat podocytes. This effect was dose- and time-dependent. AngII-induced apoptosis was reduced by blocking either the subtype I or II receptors and was completely prevented when both receptors were inhibited. AngII-induced apoptosis was in part TGF-β–dependent. The Smad signaling pathways underlying TGF-β–induced podocyte apoptosis have recently been delineated by Bottinger and coworkers (119). Other mediators of podocyte apoptosis have been shown. For example, puromycin induces podocyte apoptosis in culture, which is mediated through reactive oxygen species (122). Taken together, these studies show that apoptosis increases in podocytes under certain circumstances and contributes to the loss of cell number. Future studies are now focusing on understanding the pathways mediating this process.

Detachment

A second mechanism underlying a decrease in podocyte number is detachment of cells from the underlying GBM (Figure 2). Indeed, studies by Hara and colleagues (123–125) showed that cells obtained in the urine of patients with various glomerular diseases stained positive for the podocyte marker, podocalyxin. Similar results have been shown in PAN model of podocyte injury in rats (107). We have recently asked if podocytes detaching are viable or only apoptotic, as has been discussed above. Our data show that in the passive Heymann nephritis model of membranous nephropathy and in the streptozotocin model of diabetic nephropathy in rats, podocytes were readily detected in the urine, identified by immunostaining with podocyte-specific antibodies, such as nephrin, podocin, and Glepp-1. When these cells obtained in the urine were resuspended in tissue culture media and plated onto tissue culture dishes and grown under cell culture conditions, they adhered to tissue culture plates (75). The vast majority of adherent cells were podocytes. Moreover, there was an increase in podocyte cell number during the first days in culture. These results suggest that a fraction of podocytes detaching from the GBM in experimental membranous and diabetic nephropathy are viable and that they may have proliferative potential under these conditions. Future studies need to be directed toward better understanding the mechanisms of podocyte detachment, especially the role of specific integrins, such as the $\alpha 3\beta 1$ integrin (126) or dystroglycans (6).

Lack of Proliferation

A decrease in podocyte number has also been shown to be consequent to a lack of appropriate proliferation after injury in this cell type (113). As a result, after cell loss (by detachment and/or apoptosis), the inability to proliferate prevents the res-

toration of normal podocyte number (118). This contrasts with mesangial and glomerular endothelial cells, which readily proliferate in response to many forms of injury (127). There is a large body of literature showing that podocyte proliferation correlates closely with its state of differentiation, which may provide important clues into the mechanisms underlying the lack of proliferation (128). During glomerulogenesis, presumptive and immature podocytes proliferate and are actively engaged in the cell cycle (129). However, during the critical S-phase of kidney development, podocytes exit the cell cycle to take on a terminally differentiated and quiescent phenotype, which is required for their highly specialized function.

Proliferation is governed at the level of the cell cycle by cell cycle regulatory proteins (130). To proliferate, cyclins must bind to and activate partner cyclin-dependent kinases (CDK). In contrast, CDK are inactivated by CDK-inhibitors, including p21, p27, and p57 (131). Thus the balance of cyclin-CDK complexes and CDK-inhibitors determines if cells proliferate or are quiescent. In both mice and humans, immunostainings for p27 and p57 are absent in immature proliferating podocytes during the S-shaped stage of glomerular development. However, podocyte differentiation coincides with a marked increase in the expression of the CDK-inhibitors p27 and p57 in podocytes (132,133). This differential expression of CDK-inhibitors persists in normal podocytes. However, the CDK-inhibitors p21, p27, and p57 alone are not required for normal glomerular development, because the kidneys from these null mice are histologically normal (134–136).

The passive Heyman nephritis (PHN) model has many similarities to human membranous nephropathy, and it is induced by the administration of an antibody directed against the Fx1A antigen on the rat podocyte (137). We began by asking if podocytes are capable of increasing cyclins and CDK required for proliferation. After C5b-9–induced injury in PHN rats, protein levels for cyclin A and CDK2 increase (118), suggesting that the lack of podocyte proliferation may be due to a cell cycle inhibitor(s), rather than a failure to engage the cell cycle *per se*. Indeed, the levels of the CDK-inhibitors p21 and p27 increase specifically in podocytes after complement-dependent injury in PHN rats (118). Furthermore, the CDK-inhibitors limit podocyte proliferation by binding to and inhibiting specific cyclin-CDK complexes.

A key role for p21 and p27 in limiting the proliferative response of podocytes has been confirmed in studies utilizing specific CDK-inhibitor null mice. The administration of an anti-glomerular antibody to induce experimental podocyte injury caused marked podocyte de-differentiation in p21−/− (135) and p27−/− (134) mice compared with control wild-type mice receiving the same antibody, and this was accompanied by increased podocyte proliferation. Glomerular extracellular matrix protein accumulation was also increased in diseased p21 and p27−/− mice, and this was accompanied by a significant decrease in renal function (134,135). The role of the CDK-inhibitor p57 remains enigmatic due to the lack of a viable knockout mouse (138). However, podocyte protein levels for p57 are decreased in PHN, and in anti-glomerular antibody disease in the mouse, loss of expression localizes predominantly in proliferating podocytes (136).

Although the vast majority of human podocyte diseases are not associated with proliferation, podocyte proliferation does occur in idiopathic collapsing glomerulopathy and HIV-associated nephropathy (see review by Ross and Klotman in this issue [104]). In these diseases, there is increased expression of cyclin A and Ki-67 and a reduction in p27 and p57 in cells that are proliferating (139,140). In contrast, CDK-inhibitors do not decrease in human diseases characterized by the absence of podocyte proliferation (membranous nephropathy, MCD and FSGS). Taken together, these studies show that the CDK-inhibitors p21, p27, and p57 have a critical role in determining the outcome of diseases of podocytes and limit proliferation by reducing DNA synthesis (Figure 2).

Abnormalities in Podocyte Mitosis. Studies have unequivocally shown podocyte polyploidy in experimental membranous nephropathy (141,142). Polyploidy is defined as an increase in DNA content and is seen histologically as multinucleated cells. These observations suggest that podocytes can undergo mitosis but that there is either an abnormality in the completion of mitosis and/or in cytokinesis (cell division). When cultured podocytes are exposed to sublytic C5b-9 attack, a variety of signaling pathways are activated, including JNK, phospholipases, calcium, and MAPK cascades (143–145). Sublytic C5b-9 attack also causes cells to engage the cell cycle *in vitro* and *in vivo*. However, our data suggested a delay and/or inhibition of podocytes entering mitosis (146). To test the possibility that this observation could be due to a defect in the G_2/M checkpoint, cultured podocytes were exposed to antibody with and without a complement source. Sublytic C5b-9 injury caused a marked increase in the cell cycle inhibitor p53, and this was also accompanied by an increase in p21. This was accompanied by a delayed entry into mitosis. An increase in p53 and p21 was also shown *in vivo* in the PHN model of C5b-9 induced podocyte injury.

Follow-up studies showed that sublytic C5b-9 induced DNA damage in podocytes *in vitro* and *in vivo*. Moreover, C5b-9 increased the levels of checkpoint kinase-1 and -2 protein levels, which have been shown to arrest cells at G_2/M. Taken together, these results suggest that the reduction in podocyte mitosis after sublytic C5b-9 induced injury is due to DNA damage.

Mechanical Stretch Reduces Podocyte Proliferation. Glomerular disease is initiated by specific types of injury to individual glomerular cell types. However, regardless of the inciting injury, studies have shown that the common pathway to progressive glomerular scarring is an increase in intraglomerular capillary pressure, also known as glomerular hypertension (147). Indeed, lowering intraglomerular pressure with ACE inhibitors and/or angiotensin receptor blockers reduces the progression of glomerular diseases, including diabetic nephropathy (94). One of the consequences of increased intraglomerular pressure is increasing mechanical stretch on resident glomerular cell types (148). Studies have shown that applying mechanical stretch to glomerular cells is a useful model to study the effect of stretch on these cell types. Apply-

ing mechanical stretch to cultured mesangial cells activates a variety of signaling pathways and leads to increased proliferation (149). In contrast, mechanical stretch decreases podocyte proliferation, and the decrease in cell number was not due to apoptosis (150).

Recent studies have shown that when podocyte were grown in serum (a source of growth factors), stretch decreased the levels of cyclins D1, A and B1 and cdc2 in cultured podocytes (150). Moreover, in cultured mouse podocytes, stretch also increases the levels of specific CDK-inhibitors. Stretch caused an early increase in p21, followed by an increase in p27 at 24 h and a delayed increase in p57 at 72 h (150). In contrast to the growth arrest seen in wild-type cells exposed to stretch, $p21^{Cip1}$ $-/-$ podocytes exposed to stretch continued to proliferate. These results show that a role for CDK-inhibitors in limiting the podocyte's proliferative capacity after stretch, and may explain in part why podocytes do not proliferate in states of increased intraglomerular pressure.

The studies discussed above show that podocytes typically try to maintain their differentiated, specialized, and quiescent phenotype at all costs, even to the detriment of renal function. The inability to readily proliferate and replace those lost due to apoptosis or detachment results in a "nude" basement membrane, which leads to glomerulosclerosis. The notion that podocytes undergo "compensatory" hypertrophy to cover the "nude" areas has been proposed. However, with time, podocyte hypertrophy is detrimental, and this uncompensated state leads to scarring. Nagata and coworkers (151) recently showed that podocyte hypertrophy was mediated by specific CDK-inhibitors.

In summary, our understanding of podocyte biology has increased significantly in the past few years, and we are learning about new proteins that are specifically expressed in this cell type and may underlie certain diseases that we previously classified as "idiopathic." The molecular mechanisms leading to podocyte effacement are now better understood, as is the response to injury. As more investigators continue to focus on podocytes, it is likely that future therapeutic targets will be identified, which will improve the renal survival of patients with podocyte diseases.

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