

Podocyte Biology and Response to Injury

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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 α 3 β 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The bends of the actin filament arches appear to be connected directly to the microtubules of the major processes (own unpublished results).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 α 3 β 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

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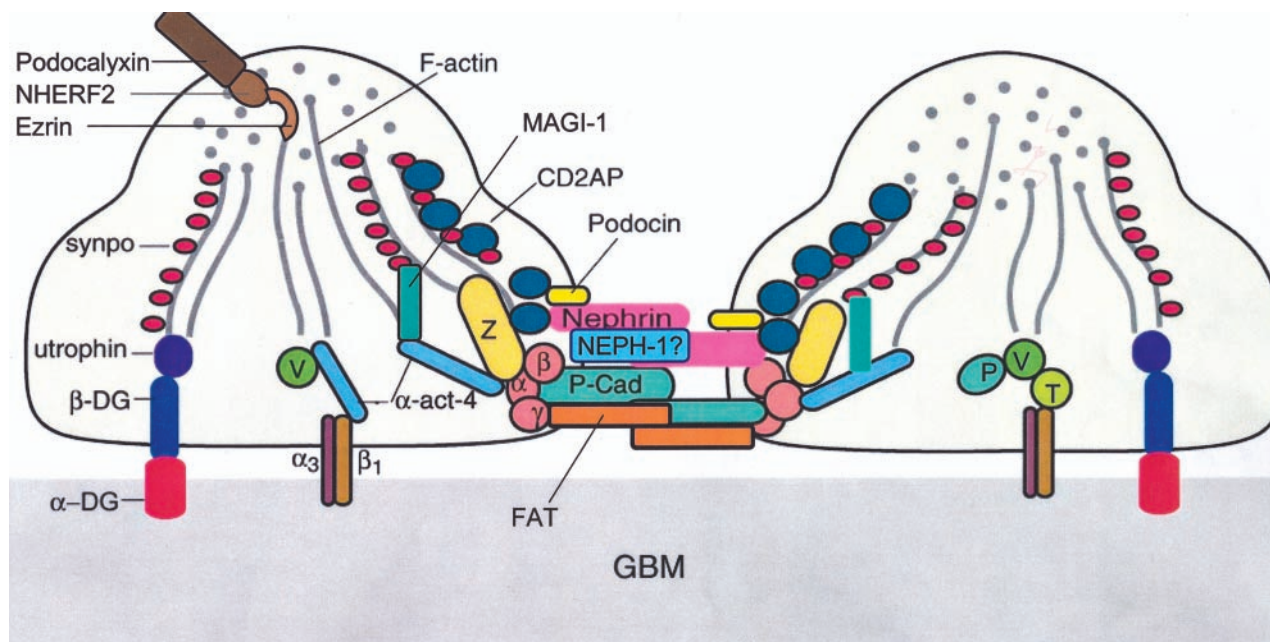


Figure 1. Molecular anatomy of the podocyte foot process (FP) actin cytoskeleton. This schematic shows two adjacent podocyte FP with the interposed slit diaphragm (SD) complex. The localization of NEPH-1 at the SD and its heterophilic interaction with nephrin remain to be established. The actin cytoskeleton is the common downstream pathway and receives input from 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; α 3 β 1, α 3 β 1 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 α 3 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 Karnovsky (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 orches-

trating 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 encoded 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 co-immunoprecipitates 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 hyalinoses 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

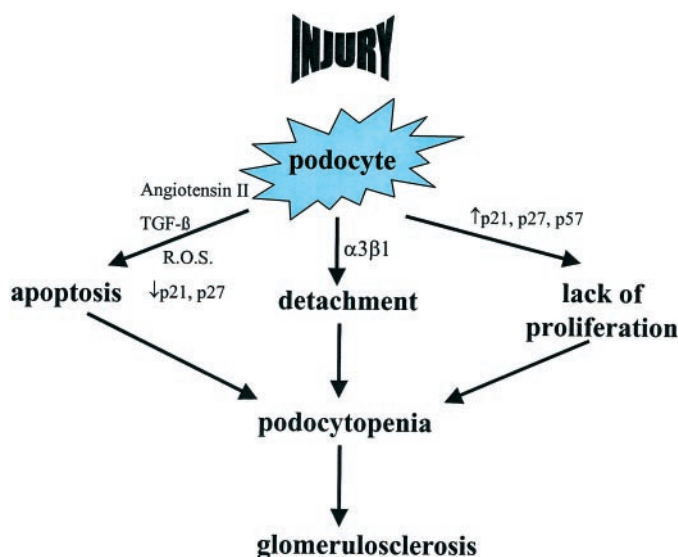


Figure 2. Causes of podocytopenia. After injury, podocytes can undergo apoptosis or detachment or fail to proliferate. These events lead to a decrease in podocyte number (podocytopenia), which contributes to the development of progressive glomerulosclerosis. The mechanisms underlying podocytopenia are 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 α 3 β 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.

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).

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 α 3 β 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 G₂/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 G₂/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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References

- Drenckhahn D, Franke R-P: Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat and man. *Lab Invest* 59: 673–682, 1988
- Mundel P, Kriz W: Structure and function of podocytes: An update [review article]. *Anat Embryol* 192: 385–397, 1995
- Adler S: Characterization of glomerular epithelial cell matrix receptors. *Am J Pathol* 141: 571–578, 1992
- Kriedberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537–3547, 1996
- Adler S: Characterization of glomerular epithelial cell matrix receptors. *Am J Pathol* 141: 571–578, 1992
- Regele HM, Fillipovic E, Langer B, Poczewski H, Kraxberger I, Bittner RE, 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
- Raats CJ, van Den Born J, Baker MA, Oppers-Walgreen B, Pisa BJ, Dijkman HB, Assmann KJ, Berden JH: Expression of agrin, dystroglycan, and utrophin in normal renal tissue and in experimental glomerulopathies. *Am J Pathol* 156: 1749–1765, 2000
- Somlo S, Mundel P: Getting a foothold in nephrotic syndrome. *Nat Genet* 24: 333–335, 2000
- Tryggvason K, Wartiovaara J: Molecular basis of glomerular permselectivity. *Curr Opin Nephrol Hypertens* 10: 543–549, 2001
- Endlich K, Kriz W, Witzgall R: Update in podocyte biology. *Curr Opin Nephrol Hypertens* 10: 331–340, 2001
- Reiser J, Kriz W, Kretzler M, Mundel P: The glomerular slit diaphragm is a modified adherens junction. *J Am Soc Nephrol* 11: 1–8, 2000
- Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestil M, Jalanko H, Holmberg C, Tryggvason K: Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 96: 7962–7967, 1999
- Holthofer H, Ahola H, Solin ML, Wang S, Palmén T, Luimula P, Miettinen A, Kerjaschki D: Nephrin localizes at the podocyte filtration slit area and is characteristically spliced in the human kidney. *Am J Pathol* 155: 1681–1687, 1999
- Holzman LB, St John PL, Kovari IA, Verma R, Holthofer H, Abrahamson DR: Nephrin localizes to the slit pore of the glomerular epithelial cell. *Kidney Int* 56: 1481–1491, 1999
- Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, Lee B: Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. *J Clin Invest* 109: 1065–1072, 2002
- Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, Shaw AS, Holzman LB, Mundel P: Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 108: 1621–1629, 2001
- Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, Shaw AS: Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 286: 312–315, 1999
- Shih NY, Li J, Cotran R, Mundel P, Miner JH, Shaw AS: CD2AP localizes to the slit diaphragm and binds to nephrin via a novel C-terminal domain. *Am J Pathol* 159: 2303–2308, 2001
- Schnabel E, Anderson JM, Farquhar MG: The tight junction protein ZO-1 is concentrated along slit diaphragms of the glomerular epithelium. *J Cell Biol* 111: 1255–1263, 1990
- Inoue T, Yaoita E, Kurihara H, Shimizu F, Sakai T, Kobayashi T, Ohshiro K, Kawachi H, Okada H, Suzuki H, Kihara I, Yamamoto T: FAT is a component of glomerular slit diaphragms. *Kidney Int* 59: 1003–1012, 2001
- Roselli S, Boute N, Sich M, Benessy F, Attie T, Gubler MC, Antignac C: Podocin localizes in the kidney to the slit diaphragm area. *Am J Pathol* 160: 131–139, 2002
- Donoviel DB, Freed DD, Vogel H, Potter DG, Hawkins E, Barrish JP, Mathur BN, Turner CA, Geske R, Montgomery CA, Starbuck MBM, Gupta A, Ramirez-Solis R, Zambrowicz BP, Powell DR: Proteinuria and perinatal lethality in mice lacking

- NEPH1, a novel protein with homology to Nephrin. *Mol Cell Biol* 21: 4829–4836, 2001
23. Mundel P, Gilbert P, Kriz W: Podocytes in glomerulus of rat kidney express a characteristic 44kD protein. *J Histochem* 39: 1047–1056, 1991
 24. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, Kriz W: Synaptopodin: An actin-associated protein in telencephalic dendrites and renal podocytes. *J Cell Biol* 193–204, 1997
 25. Patrie KM, Drescher AJ, Welihinda A, Mundel P, Margolis B: Interaction of two actin-binding proteins, synaptopodin and alpha-actinin-4, with the tight junction protein MAGI-1. *J Biol Chem* 277: 30183–30190, 2002
 26. Patrie KM, Drescher AJ, Goyal M, Wiggins RC, 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
 27. Kerjaschki D: Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis. *J Clin Invest* 108: 1583–1587, 2001
 28. Orlando RA, Takeda T, Zak B, Schmieder S, Benoit VM, McQuistan T, Furthmayr H, Farquhar MG: The glomerular epithelial cell anti-adhesion podocalyxin associates with the actin cytoskeleton through interactions with Ezrin. *J Am Soc Nephrol* 12: 1589–1598, 2001
 29. Takeda T, McQuistan T, Orlando RA, Farquhar MG: Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. *J Clin Invest* 108: 289–301, 2001
 30. Seiler MW, Venkatachalam MA, Cotran RS: Glomerular epithelium: Structural alterations induced by polycations. *Science* 189: 390–393, 1975
 31. Seiler MW, Rennke HG, Venkatachalam MA, Cotran RS: Pathogenesis of polycation-induced alterations (“fusion”) of glomerular epithelium. *Lab Invest* 36: 48–61, 1977
 32. 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
 33. Shirato I, Sakai T, Kimura K, Tomino Y, Kriz W: Cytoskeletal changes in podocytes associated with foot process effacement in masugi nephritis. *Am J Pathol* 148: 1283–1296, 1996
 34. Reiser J, Pixley FJ, Hug A, Kriz W, Smoyer WE, Stanley ER, Mundel P: Regulation of mouse podocyte process dynamics by protein tyrosin phosphatases. *Kidney Int* 57: 2035–2042, 2000
 35. Smoyer WE, Ransom RF: Hsp27 regulates podocyte cytoskeletal changes in an in vitro model of podocyte process retraction. *FASEB J* 16: 315–326, 2002
 36. Smoyer WE, Mundel P, Gupta A, Welsh MJ: Podocyte alpha-actinin induction precedes foot process effacement in experimental nephrotic syndrome. *Am J Physiol* 273:F 150–F157, 1997
 37. Smoyer WE, Mundel P: Regulation of podocyte structure during the development of nephrotic syndrome. *J Mol Med* 76: 172–183, 1998
 38. Smoyer WE, Gupta A, Mundel P, Ballew JD, Welsh MJ: Altered expression of glomerular heat shock protein 27 in experimental nephrotic syndrome. *J Clin Invest* 97: 2697–2704, 1996
 39. Honda K, Yamada T, Endo R, Ino Y, Gotoh M, Tsuda H, Yamada Y, Chiba H, Hirohaashi S: Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. *J Cell Biol* 140: 1383–1393, 1998
 40. Pollack M: Inherited podocytopathies: FSGS and nephrotic syndrome from a genetic perspective. *J Am Soc Nephrol* 13: 3016–3024, 2002
 41. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000
 42. Kaplan J, Pollak MR: Familial focal segmental glomerulosclerosis. *Curr Opin Nephrol Hypertens* 10: 183–187, 2001
 43. Reiser J, von Gersdorff G, Simons M, Schwarz K, Faul C, Giardino L, Heider T, Loos M, Mundel P: Novel concepts in understanding and management of glomerular proteinuria. *Nephrol Dial Transplant* 122: 3537–3547, 2002
 44. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Sheperd K, Jones RC, Jaenisch R: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537–3547, 1996
 45. Noakes PG, Miner JH, Gautam M, Cunningham JM, Sanes JR, Merlie JP: The renal glomerulus of mice lacking s-laminin/laminin beta 2: Nephrosis despite molecular compensation by laminin beta 1. *Nat Genet* 10: 400–406, 1995
 46. Miner JH, Li C: Defective glomerulogenesis in the absence of laminin alpha5 demonstrates a developmental role for the kidney glomerular basement membrane. *Dev Biol* 217: 278–289, 2000
 47. Miner JH, Sanes JR: Molecular and functional defects in kidneys of mice lacking collagen alpha 3 (IV): Implications for Alport syndrome. *J Cell Biol* 135: 1403–1413, 1996
 48. Kretzler M, Teixeira VPC, Unschuld PG, Cohen CD, Wanke R, Edenofer I, Mundel P, Schlondorff D, Holthofer H: Integrin linked kinase as a candidate downstream effector in proteinuria. *FASEB J* 15: 1843–1845, 2001
 49. Morello R, Zhou G, Dreyer SD, Harvey SJ, Nonomiya Y, Thorner PS, Miner JH, Cole W, Winterpacht A, Zabel B, Oberg KC, Lee B: Regulation of glomerular basement membrane collagen expression by LMX1B contributes to renal disease in nail patella syndrome. *Nat Genet* 27: 205–208, 2001
 50. Doyonnas R, Kershaw DB, Duhme C, Merkens H, Graf T, McNagny KM. Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. *J Exp Med* 194: 13–27, 2001
 51. Miner JH: Focusing on the glomerular slit diaphragm: Podocin enters the picture. *Am J Pathol* 160: 3–5, 2002
 52. Miner JH: Renal basement membrane components. *Kidney Int* 56: 2016–2024, 1999
 53. Hamano Y, Grunkemeyer JA, Zeisberg M, Cosgrove D, Morello R, Lee B, Sugimoto H, Kalluri R: Determinants of vascular permeability in the kidney glomerulus. *J Biol Chem* 277: 31154–31162, 2002
 54. Savin V, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, Lovell H, Waraday B, Gunwar S, Chonko AM, Artero M, Vincenti F: Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med* 334: 878–883, 1996
 55. Le Berre L, Godfrin Y, Gunther E, Buzelin F, Perretto S, Smit H, Kerjaschki D, Usal C, Cuturi C, Souillou JP, Dantal J: Extrarenal effects on the pathogenesis and relapse of idiopathic nephrotic syndrome in Buffalo/Mna rats. *J Clin Invest* 109: 491–498, 2002
 56. Kriz W, Gretz N, Lemley KV: Progression of glomerular diseases: Is the podocyte the culprit? *Kidney Int* 54: 687–697, 1998

57. Kriz W, Hartmann I, Hosser H, Hahnel B, Kranzlin B, Provoost AP, Gretz N: Tracer studies in the rat demonstrates misdirected filtration and peritubular filtrate spreading in nephrons with segmental glomerulosclerosis. *J Am Soc Nephrol* 12: 496–506, 2001
58. Kriz W, Hosser H, Hahnel B, Gretz N, 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
59. Rodewald R, Karnovsky MJ: Porous substructure of the glomerular slit diaphragm in the rat and mouse. *J Cell Biol* 60: 423–433, 1974
60. Kestila M, Ienkkeri 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, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
61. Shaw AS, Miner JH CD2-associated protein and the kidney. *Curr Opin Nephrol Hypertens* 10: 19–22, 2001
62. Boute N, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C NPHS2 encoding the glomerular proteins podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000
63. Orikasa M, Matsui K, Oite T, Shimizu F: Massive proteinuria induced in rats by a single intravenous injection of a monoclonal antibody. *J Immunol* 141: 807–814, 1988
64. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, 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
65. Rantanen M, Palmen T, Patari A, Ahola H, Lehtonen S, Astrom E, Floss T, Vauti F, Wurst W, Ruiz P, Kerjaschki D, Holthofer H: Nephrin TRAP mice lack slit diaphragms and show fibrotic glomeruli and cystic tubular lesions. *J Am Soc Nephrol* 13: 1586–1594, 2002
66. Yan K, Khoshnoodi J, Ruotsalainen V, Tryggvason K: N-linked glycosylation is critical for the plasma membrane localization of nephrin. *J Am Soc Nephrol* 13: 1385–1389, 2002
67. Tryggvason K, Ruotsalainen V, Wartiovaara J: Discovery of the congenital nephrotic syndrome gene discloses the structure of the mysterious molecular sieve of the kidney. *Int J Dev Biol* 43: 445–451, 1999
68. Artero RD, Castanon I, Baylies MK: The immunoglobulin-like protein Hibris functions as a dose-dependent regulator of myoblast fusion and is differentially controlled by Ras and Notch signaling. *Development* 128: 4251–4264, 2001
69. Dworak HA, Charles MA, Pellerano LB, Sink H: Characterization of Drosophila hibris, a gene related to human nephrin. *Development* 128: 4265–4276, 2001
70. Luimula P, Sandstrom N, Novikov D, Holthofer H: Podocyte-associated molecules in puromycin aminonucleoside nephrosis of the rat. *Lab Invest* 82: 713–718, 2002
71. Forbes JM, Bonnet F, Russo LM, Burns WC, Cao Z, Candido R, Kawachi H, Allen TJ, Coor ME, Jerums G, Osicka TM: Modulation of nephrin in the diabetic kidney: association with systemic hypertension and increasing albuminuria. *J Hypertens* 20: 985–992, 2002
72. Kelly DJ, Aaltonen P, Cox AJ, Rumble JR, Langham R, Panagiotopoulos S, Jerums G, Holthofer H, Gilbert RE: Expression of the slit-diaphragm protein, nephrin, in experimental diabetic nephropathy: differing effects of anti-proteinuric therapies. *Nephrol Dial Transplant* 17: 1327–1332, 2002
73. Patrakka J, Lahdenkari AT, Koskimies O, Holmberg C, Wartiovaara J, Jalanko H: The number of podocyte slit diaphragms is decreased in minimal change nephrotic syndrome. *Pediatr Res* 52: 349–355, 2002
74. Patrakka J, Ruotsalainen V, Reponen P, Qvist E, Laine J, Holmberg C, Tryggvason K, Jalanko H: Recurrence of nephrotic syndrome in kidney grafts of patients with congenital nephrotic syndrome of the Finnish type: Role of nephrin. *Transplantation* 73: 394–403, 2002
75. Petermann AT, Hiromura K, Pippin J, Krofft R, Durvasula R, Shauckland SJ: Podocytes that detach in vivo are viable: Novel insights into cell loss [Abstract]. *J Am Soc Nephrol* 13: 44A, 2002
76. Li C, Ruotsalainen V, Tryggvason K, Shaw AS, 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
77. Dustin ML, Olszowy MW, Holdorf AD, Li J, Bromley S, Desai N, Widder P, Rosenberger F, van der Merwe PA, Allen PM, Shaw AS: A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 94: 667–677, 1998
78. Bromley SK, Burack WR, Johnson KG, Somersalo K, Sims TN, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML: The immunological synapse. *Annu Rev Immunol* 19: 375–396, 2001
79. Yuan H, Takeuchi E, Taylor GA, McLaughlin M, Brown D, Salant DJ: Nephrin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. *J Am Soc Nephrol* 13: 946–956, 2002
80. Yuan H, Takeuchi E, Salant DJ: Podocyte slit-diaphragm protein nephrin is linked to the actin cytoskeleton. *Am J Physiol Renal Physiol* 282: F585–F591, 2002
81. Kurihara H, Anderson JM, Farquhar MG: Increased tyrosine phosphorylation of ZO-1 during modification of tight junctions between glomerular foot processes. *Am J Physiol* 268: F514–F524, 1995
82. Macconi D, Ghilardi M, Bonassi ME, Mohamed EI, Abbate M, Colombi F, Remuzzi G, 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
83. Snyers L, Umlauf E, Prohaska R: Oligomeric nature of the integral membrane protein stomatin. *J Biol Chem* 273: 17221–17226, 1998
84. Huber TB, Kottgen M, Schilling B, Walz G, Benzing T: Interaction with podocin facilitates nephrin signaling. *J Biol Chem* 276: 4153–4156, 2001
85. Simons K, Ikonen E: Functional rafts in cell membranes. *Nature* 387: 569–572, 1997
86. Simons K, Toomre D: Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 1: 31–39, 2000
87. Cherukuri A, Dykstra M, Pierce SK: Floating the raft hypothesis: Lipid rafts play a role in immune cell activation. *Immunity* 14: 657–660, 2001
88. Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A: T lymphocyte costimulation mediated by reorganization of membrane microdomains. *Science* 283: 680–682, 1999
89. Simons M, Schwarz K, Kriz W, Miettinen A, Reiser J, Mundel P, Holthofer H: Involvement of lipid rafts in nephrin phosphor-

- ylation and organization of the glomerular slit diaphragm. *Am J Pathol* 159: 1069–1077, 2001
90. Yu CC, Yen TS, Lowell CA, deFranco AL: Lupus-like kidney disease in mice deficient in the Src family tyrosine kinases Lyn and Fyn. *Curr Biol* 11: 34–38, 2001
 91. Liang X, Nazarian A, Erdjument-Bromage H, Bornmann W, Tempst P, Resh MD: Heterogeneous fatty acylation of Src family kinases with polyunsaturated fatty acids regulates raft localization and signal transduction. *J Biol Chem* 276: 30987–30994, 2001
 92. Kramer EM, Klein C, Koch T, Boytinck M, Trotter J: Compartmentation of Fyn kinase with glycosylphosphatidylinositol-anchored molecules in oligodendrocytes facilitates kinase activation during myelination. *J Biol Chem* 274: 29042–29049, 1999
 93. Investigators HHOPE: Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: Results of the HOPE study and MICRO-HOPE study. *Lancet* 355: 253–259, 2000
 94. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345: 851–860, 2001
 95. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, P HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
 96. Parving HH, Hommel E, Jensen BR, Hansen HP: Long-term beneficial effect of ACE inhibition on diabetic nephropathy in normotensive type I diabetic patients. *Kidney Int* 60: 228–234, 2001
 97. Parving HH, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, 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
 98. Couser WG, Abrass CK: Pathogenesis of membranous nephropathy. *Ann Rev Med* 39: 517–530, 1988
 99. Brenner BM: Nephron adaptation to renal injury or ablation. *Am J Physiol* 249: F324–F337, 1985
 100. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19: 410–415, 1981
 101. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *J Am Soc Nephrol* 12: 1315–1325, 2001
 102. Eddy A: Interstitial nephritis induced by protein-overload proteinuria. *Am J Pathol* 135: 719–733, 1989
 103. Whiteside C, Prutis K, Cameron R, Thomson J: Glomerular epithelial detachment, not reduced charge density correlates with proteinuria in adriamycin and puromycin nephrosis. *Lab Invest* 61: 650–660, 1989
 104. Ross MD, Klotman P: Recent progress in HIV nephropathy. *J Am Soc Nephrol* 13: 2997–3004, 2002
 105. Schartz MM, Lewis EJ: Focal segmental glomerulosclerosis: The cellular lesion. *Kidney Int* 28: 968–974, 1985
 106. Schwartz M, Bidani A, Lewis E: Glomerular epithelial cell function and pathology following extreme ablation of Renal mass. *Am J Pathol* 126: 315–324, 1987
 107. Kim Y-H, Goyal M, Kurmit D, Wharram B, Wiggins J, Holzman L, Kershaw D, Wiggins R: Podocyte depletion and glomerulosclerosis have a direct relationship in the PAN-treated rat. *Kidney Int* 60: 957–968, 2001
 108. Shirata I, Hosser H, Kimura K, Sakai T, Tomino Y, Kriz W: The development of focal segmental glomerulosclerosis in Masugi nephritis is based on progressive podocyte damage. *Virchows Arch* 429: 255–273, 1996
 109. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW: Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest* 99: 342–348, 1997
 110. Steffes MW, Schmidt D, Mccrery R, Basgen JM: Glomerular cell number in normal subjects and type I diabetic patients. *Kidney Int* 59: 2104–2113, 2001
 111. Vogelmann SU, Nelson WJ, Myers BD, Lemley KV: Urinary excretion of viable podocytes (P) in health and renal disease [Abstract]. *J Am Soc Nephrol* 12: 129A, 2001
 112. Kriz W: Podocyte is the major culprit accounting for the progression of chronic renal disease. *Microsc Res Tech* 57: 189–195, 2002
 113. Kriz W: Progressive renal failure-inability of podocytes to replicate and the consequences for development of glomerulosclerosis. *Nephrol Dial Transplant* 11: 1738–1742, 1996
 114. Kriz W, Kretzler M, Nagata M, Provoost AP, Shirata I, Uiker S, Sakai T, Lemley KV: A frequent pathway to glomerulosclerosis: deterioration of tuft architecture-podocyte damage-segmental sclerosis. *Kidney Blood Press Res* 19: 245–253, 1996
 115. Rennke HG: How does glomerular epithelial cell injury contribute to progressive glomerular damage? *Kidney Int* 45: S58–S63, 1994
 116. Salant DJ: The structural biology of glomerular epithelial cells in proteinuric diseases [Commentary]. *Curr Opin Nephrol Hypertens* 3: 1–6, 1994
 117. Kriz W, Hackenthal E, Nobiling R, Sakai T, Elger M: A role for podocytes to counteract capillary wall distention. *Kid Int* 45: 369–376, 1994
 118. Shankland SJ, Floege J, Thomas SE, Nangaku M, Hugo C, Pippin J, Henne K, Hockenberry DM, Johnson RJ, Couser WG: Cyclin kinase inhibitors are increased during experimental membranous nephropathy: Potential role in limiting glomerular epithelial cell proliferation in vivo. *Kidney Int* 52: 404–413, 1997
 119. Schiffer M, Bitzer m, Roberts IS, Kopp JB, ten Dijke P, Mundel P, Bottinger EP: Apoptosis in podocytes induced by TGF-beta and Smad7. *J Clin Invest* 108: 807–816, 2001
 120. Schiffer M, Schiffer LE, Gupta A, Shaw AS, Roberts IS, Mundel M, Böttinger EP: Inhibitory Smads and TGF-β signaling in glomerular cells. *J Am Soc Nephrol* 13: 2657–2666, 2002
 121. Ding G, Reddy K, Kapasi AA, Franki N, Gibbons N, Kasinath BS, Singhal PC: Angiotensin II induces apoptosis in rat glomerular epithelial cells. *Am J Renal Physiol* 283: F173–180, 2002
 122. Sanwal V, Pandya M, Bhaskaran M, Franki N, Reddy H, Ding G, Kapasi A, Valderrama E, Singhal PC: Puromycin aminonucleoside induces glomerular epithelial cell apoptosis. *Expt Mol Path* 70: 54–64, 2001
 123. Hara M, Yanagihar T, Kihara I: Urinary podocytes in primary focal segmental glomerulosclerosis. *Nephron* 89: 342–347, 2001
 124. Nakamura T, Ushiyama C, Osada S, Hara M, Shimada N, Koide H: Pioglitazone reduces urinary podocyte excretion in type 2 diabetes patients with microalbuminuria. *Metabolism* 50: 1193–1196, 2001
 125. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Sekizuka K, Ebihara I, Koide H: Effects of angiotensin-converting enzyme inhibitor, angiotensin II receptor antagonist and

- calcium antagonist on urinary podocytes in patients with IgA nephropathy. *Am J Nephrol* 20: 373–379, 2000
126. Cybulsky AV, Carbonetto S, Huang Q, McTavish A, Cyr MD: Adhesion of rat glomerular epithelial cells to extracellular matrices: Role of β_1 integrins. *Kidney Int* 42: 1099–1106, 1992
 127. Shankland SJ, Hugo C, Coats SR, Nangaku M, Pichler RH, Gordon KL, Pippin J, Roberts JM, Couser WG, Johnson RJ: Changes in cell cycle protein expression during experimental mesangial proliferative glomerulonephritis. *Kidney Int* 50: 1230–1239, 1996
 128. Barisoni L, Kriz W, Mundel P, 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
 129. Saxen L: *Organogenesis of the Kidney*. Cambridge: Cambridge University Press, 1997
 130. Shankland SJ, Wolf G: Cell cycle proteins and the kidney: role in proliferation, growth and apoptosis [Invited Review]. *Am J Physiol* 278: F515–F529, 2000
 131. Sherr CJ, Roberts J M: CDK-inhibitors: positive and negative regulators of G 1-phase progression. *Genes and Development* 13: 1501–1512, 1999
 132. Nagata M, Nakayama K-I, Terada Y, Hoshi S, Watanabe T: Cell cycle regulation and differentiation in the human podocyte lineage. *Am J Pathol* 153: 1511–1520, 1998
 133. Coombs HL, Shankland SJ, Setzer SV, Hudkins KL, Alpers CE: Expression of the cyclin kinase inhibitor, p27^{kip1}, in developing and mature human kidney. *Kidney Int* 53: 892–896, 1998
 134. Ophascharoensuk V, Fero ML, Hughes J, Roberts JM, Shankland SJ: The cyclin-dependent kinase inhibitor p27Kip1 safeguards against inflammatory injury. *Nat Med* 4: 575–80, 1998
 135. Kim Y-G, Pippin JW, Johnson RJ, Alpers CE, Couser WG, Shankland SJ: The cyclin kinase inhibitor p21Cip1/WAF1 limits visceral glomerular epithelial cell proliferation in experimental glomerulonephritis. *Kidney Int* 55: 2349–2361, 1999
 136. Hiromura K, Haseley LA, Zhang P, Monkawa T, Alpers CE, Mundel P, Shankland SJ: Podocyte expression of the CDK-inhibitor p57 during development and disease. *Kidney Int* 2001, in press
 137. Salant DJ, Darby C, Couser WG: Experimental membranous glomerulonephritis in rats. *J Clin Invest* 66: 71–81, 1990
 138. Zhang P, Liegeois NJ, Wong C, Finegold M, Hou H, Thompson JC, Silverman A, Harper JW, DePinho RA, Elledge SJ: Altered cell differentiation and proliferation in mice lacking p57Kip2 indicates a role in Beckwith-Wiedemann syndrome. *Nature* 387: 151–158, 1997
 139. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'Agati V, Alpers CE: Differential expression of CDK-inhibitors in human glomerular disease: Role in podocyte proliferation and differentiation. *Kidney Int* 58: 674–683, 2000
 140. Nagata M, Horita S, Shu Y, Shibata S, Hattori M, Ito K, 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
 141. Floege J, Johnson RJ, Alpers CE, Fatemi-Nainie S, Richardson CA, Gordon K, Couser WG: Visceral glomerular epithelial cells can proliferate in vivo and synthesize platelet-derived growth factor β -chain. *Am J Pathol* 142: 637–650, 1993
 142. Kriz W, Hahnel B, Rosener S, Elger M: Long-term treatment of rats with FGF-2 results in focal segmental glomerulosclerosis. *Kidney Int* 48: 1435–1450, 1995
 143. Cybulsky AV, Monge JC, Papillon J, McTavish AJ: Complement C5b-9 activates cytosolic phospholipase A2 in glomerular epithelial cells. *Am J Physiol* 269: F739–F749, 1995
 144. Cybulsky AV, Papillon J, McTavish AJ: Complement activates phospholipases and protein kinases in glomerular epithelial cells. *Kidney Int* 54: 360–372, 1998
 145. Cybulsky AV, Takano T, Papillon J, McTavish AJ: Complement-induced phospholipase A₂ activation in glomerular epithelial cells in culture and experimental membranous nephropathy. *Kidney Int* 57: 1052–1062, 2000
 146. Shankland SJ, Pippin JW, Couser WG: Complement (C5b-9) induces glomerular epithelial cell DNA synthesis but not proliferation in vitro. *Kidney Int* 56: 538–548, 1999
 147. Anderson S, Meyer TW, Rennke HG, Brenner B: Control of glomerular hypertension limits glomerular injury in rats with reduced mass. *J Clin Invest* 76: 612–619, 1985
 148. Ingram AJ, Scholey JW: Stress-responsive signal transduction mechanisms in glomerular cells. *Curr Opin Nephrol Hypertens* 9: 49–55, 2000
 149. Akai Y, Homma T, Burns KD, Yasuda T, Badr KF, Harris RC: Mechanical stretch/relaxation of cultured rat mesangial cells induces protooncogenes and cyclooxygenase. *Am J Physiol* 267: C482–C490, 1994
 150. Petermann A, Hiromura K, Blonski M, Pippin J, Monkawa T, Durvasula R, Couser WG, Shankland SJ: Mechanical stress reduces podocyte proliferation in vitro. *Kidney Int* 2001, in press
 151. Hoshi S, Shu Y, Yoshida F, Inagaki T, Sonoda J, Watanabe T, Nomoto K, Nagata M: Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats. *Lab Invest* 82: 25–35, 2002