Update in podocyte biology

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Knowledge of podocyte biology is growing rapidly. Podocytes are crucially involved in most hereditary diseases affecting the glomerulus, which all exhibit podocyte-specific defects, that is, foot process effacement and protein leakage. Efforts to understand molecular mechanisms causing these derangements are increasingly successful and will allow a better targeting of interventions to halt the progression of chronic renal disease. Curr Opin Nephrol Hypertens 10:331–340.

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Abbreviations

ACE	angiotensin converting enzyme
ANG-II	angiotensin II
CD2AP/CMS	CD2-associated protein/p130 Cas ligand with multiple SH3 domains
CDK	cyclin-dependent kinase
CNF	congenital nephrotic syndrome of the Finnish type
FAK	focal adhesion kinase
FPE	foot process effacement
FSGS	focal segmental glomerulosclerosis
GBM	glomerular basement membrane
ILK	integrin-linked kinase
LIM	Lin-11, Isl-1, Mec-3
MCN	minimal change nephrosis
PAN	puromycin aminonucleoside
PHN	passive Heymann nephritis
TGF-β	transforming growth factor β

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Introduction

The concept that the podocyte, rather than the mesangial cell, is the major culprit in the progression of glomerular diseases, has gained substantial ground. The discovery that podocyte-specific proteins cause hereditary kidney diseases, such as nephrin and the congenital nephrotic syndrome of the Finnish type, has led to the notion that podocytes are critically involved in most genetic kidney diseases affecting the glomerulus. All these diseases eventually lead to progressive renal failure.

This broadened view has greatly increased the research activities in this field and has already resulted in considerable progress in our understanding of the cell and molecular biology of this unique cell type as well as of its role in the development of glomerular diseases.

Hereditary podocyte diseases

Unlike the Alport syndrome, for which the genetic defect was elucidated some time ago, the mutated genes responsible for several other hereditary podocyte diseases have been identified only recently. The diverse nature of the proteins encoded by those genes attests to the complicated structure through which the podocyte contributes to the establishment and maintenance of the glomerular filtration barrier.

Alport syndrome

As several excellent reviews on Alport syndrome have appeared recently (e.g.Ref. [1]), only certain features of this disease will be highlighted. At a prevalence of $\sim$1 : 5000, Alport syndrome is a very frequent disease; it can be inherited in an X-chromosomal, an autosomal-recessive and an autosomal-dominant fashion. The X-chromosomal form affects $\sim$85% of all patients, whereas an autosomal-dominant inheritance can be found in only $\sim$1% of the patients. Initially, the patients present with hematuria, but eventually they develop end-stage renal failure. The ultrastructural finding of an irregular thickening and lamination of the glomerular basement membrane (GBM) is typical for Alport syndrome, and now that the mutated genes *COL4A3*, *COL4A4* and *COL4A5* have been identified, it can be understood better.

During the early stages in the differentiation of the glomerulus, the prospective GBM contains the α1 and α2 chains of collagen IV, while later on a network consisting of the α3, α4 and α5 chains of collagen IV is added by the podocyte [2–7]. The X-chromosomal form of Alport

syndrome is due to mutations in the *COL4A5* gene, which encodes the α5 chain of collagen IV, and the autosomal-recessive form has been traced back to mutations in the *COL4A3* and *COL4A4* genes on human chromosome 2 [1,8]. Apparently, autosomal-dominant Alport syndrome is caused by a splice site mutation in the *COL4A3* gene [9]. An important feature of Alport syndrome is the finding that mutations in any one of the three chains leads to the absence of the other two chains and to the continued strong expression of collagen IV α1 and α2 chains [3,10–12]. Although this phenomenon is not fully understood as yet, it has become obvious that two collagen IV networks exist in the GBM, one of them comprising $\alpha1_2\alpha2$ trimers and the other α3α4α5 trimers [13]. Recent evidence suggests that the respective NC1 domains at the COOH-terminus of the α chains discriminate among each other, such that formation of the triple helix and assembly of the network are determined by the identity of the NC1 domains [14•]. It remains to be determined, however, whether the coordinated expression of the various collagen IV chains occurs at a mRNA [12] or post-mRNA [15] level.

Nail–patella syndrome

Nail–patella syndrome (*syn*. hereditary onycho-osteodysplasia) is an autosomal-dominant disease with an incidence of ~1 : 50 000, which usually is diagnosed because of the dysplastic nails and missing or hypoplastic patella. Between one-third and 50% of the patients also suffer from renal disease, in particular proteinuria and hematuria, which may eventually lead to chronic renal failure [16]. Ultrastructural studies of affected kidneys have demonstrated obvious defects of the podocytes and the GBM. Typically, extensive foot process effacement (FPE) can be detected, and the GBM appears thickened and contains both fibrillar inclusions and electron-lucent areas (e.g. Ref. [17]).

Recently, mutations in the *LMX1B* gene on human chromosome 9q34 were identified as being responsible for the disease [18]. An important clue for the involvement of *LMX1B* came when the corresponding gene was inactivated in mice, since they also suffer from limb and kidney defects [19]. The changes seen in glomeruli of *Lmx1b* (−/−) mice largely correspond to the renal lesions observed in patients, which places the podocyte at the center of the pathomechanism leading to proteinuria, hematuria, and chronic renal disease. Somewhat surprisingly, however, the heterozygous *Lmx1b* knockout mice show no phenotype, which contrasts with the dominant inheritance in human patients. The *LMX1B* gene encodes a transcription factor of the Lin-11, Isl-1, Mec-3 (LIM)-domain family [20]. Whereas the LIM domains, first described in Lin-11, Isl-1 and Mec-3, are cysteine-rich, zinc binding motifs, which probably mediate interaction with other proteins, homeodomains are responsible for the binding to DNA. All the mutations described so far affect those two domains, either as truncating mutations or as missense mutations [18,21–25]. Mutations in the LIM domains are predicted to cause disruption of association with interacting proteins, while mutations in the homeodomain should affect the DNA-binding properties of LMX1B. The dominant mode of inheritance could be explained by haploinsufficiency, by a gain of function and by a dominant-negative mechanism. So far only one report has addressed this question; it suggests that nail–patella syndrome is due to haploinsufficiency [26].

Congenital nephrotic syndrome of the Finnish type

Congenital nephrotic syndrome of the Finnish type (CNF) is inherited in an autosomal-recessive fashion and is characterized by the perinatal onset of severe proteinuria, which usually requires renal replacement therapy in order to save affected children. The mutated gene, *NPHS1*, is located on chromosome 19q13.1 and is organized into 29 exons; it was identified using a positional cloning strategy [27,28]. Nephrin, the protein product of *NPHS1*, is 1241 amino acids long and probably represents an integral plasma membrane protein. In the kidney it is exclusively expressed in podocytes. It consists of a large extracellular domain with eight immunoglobulin-like motifs and one fibronectin type III motif, a single transmembrane domain and a short cytoplasmic tail. Sequence comparison of human nephrin with murine [29] and rat [29–31] nephrin has revealed that the three proteins are more than 80% identical. Both the human [32••] and the rat [30] nephrin transcripts are alternatively spliced, but the functional significance of those splice products is not yet clear.

So far, no mutational hot spots have been identified, although two types of mutations account for the vast majority of cases. The most common one, Fin_{major}, represents a deletion of two nucleotides in exon 2, which is predicted to result in the synthesis of a truncated protein of only 90 amino acids, whereas in the Fin_{minor} mutation, a nonsense mutation in exon 26 should lead to a protein with 1109 amino acids. In addition to those two mutations, a number of other mutations have been described, which are distributed over the length of the protein [27,28,33,34,35•].

The nephrin mRNA has been localized to podocytes by in-situ hybridization [27,28]. Immunogold-electron-microscopy has demonstrated that the nephrin protein is associated predominantly, although possibly not exclusively, with the slit diaphragm [32••,36••,37••], which has led to the hypothesis that nephrin forms the backbone of the slit diaphragm. This notion is supported by the finding that patients with mutations in the *NPHS1* gene typically lack a slit diaphragm (although

filtration slits are present), and that in most cases nephrin protein cannot be detected by immunohistochemistry in the glomeruli of those patients [32••,35•]. The absence of nephrin immunoreactivity is somewhat surprising, since the nephrin mRNA continues to be expressed [32••,35•]. The podocytes of one patient, however, still expressed nephrin protein; in addition slit diaphragms could be detected by electronmicroscopy. This patient had inherited the Fin_{major} mutation and a missense mutation, which resulted in the substitution of an arginine residue by a cysteine at position 743 (R743C) [35•]. Recent studies of the role of nephrin in the development of the filtration barrier indicate that nephrin is dispensable in early development of podocyte junctional complexes, but is essential for the assembly of the slit diaphragm [38•] (see also below).

Familial forms of focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) comprises several distinct disease entities, the genes for two forms of which have been identified. Steroid-resistant nephrotic syndrome is inherited in an autosomal-recessive fashion and is caused by mutations in the *NPHS2* gene located on human chromosome 1q25–q31 [39]. This disease begins with proteinuria in children and then progresses rapidly to FSGS. *NPHS2* encodes a 383 amino-acid protein named podocin, which is predicted to be an integral membrane protein. Northern blot analysis has shown that the podocin mRNA is exclusively expressed in the kidney, and using in-situ hybridization it has been localized in podocytes. So far the function of podocin is unknown; the only clue comes from its similarity to the MEC-2 protein from *Caenorhabditis elegans*, which is involved in mechanotransduction [40••]. An autosomal-dominant form of FSGS is caused by the *ACTN4* gene on human chromosome 19q13.1 [41••,42]. *ACTN4* codes for α-actinin-4, which has been localized to podocytes, where it probably serves to crosslink actin filaments [41••].

The involvement of the podocyte-specific Wilms' tumor protein in hereditary glomerular diseases such as Denys–Drash syndrome and Frasier syndrome has been reviewed extensively several times (e.g. Ref. [43]), and will not be considered here since little new information has emerged recently.

Podocyte cell biology

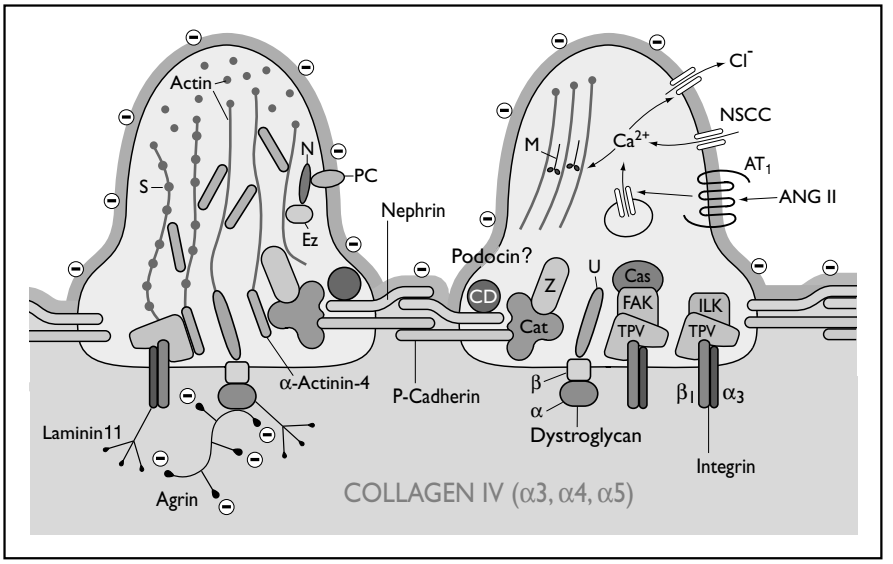
The highly specialized structure and function of podocytes is based on a complex cytoskeletal machinery that regulates the adhesion of foot processes to the GBM, the motility of foot processes on the GBM, and some kind of slit membrane dynamics, as yet poorly understood. The recent progress in understanding this machinery has emerged, to a large extent, from the analysis of structural and functional derangements; that is, loss of the interdigitating foot process pattern and loss of size-selective function. For the sake of clarity this progress will be summarized in four sections, although it is obvious that the issues are interwoven. Major points are schematically illustrated in Fig. 1.

The slit membrane and loss of size-selective function

Major progress in this field started with the discovery of the gene that, in mutated form, causes CNF and its product nephrin [27] (see above). Cloning of the rat and

Figure 1. Schematic drawing of the molecular equipment in podocyte foot processes

Cas, p130Cas; Cat, catenins; CD, CD2-associated protein; Ez, ezrin; FAK, focal adhesion kinase; ILK, integrin-linked kinase; M, myosin; N, NHERF2; NSCC, non-selective cation channel; PC, podocalyxin; S, synaptopodin; TPV, talin, paxillin, vinculin; U, utrophin; Z, ZO-1. See text for further explanations.



mouse homologs followed quickly [36,44,45•]. The results of the corresponding immunocytochemical studies [32••,36••,37••] showed that nephrin is localized at the slit membrane. Based on the fact that nephrin is a member of the immunoglobulin family of cell–cell adhesion molecules, a model of homophilic interaction has been proposed [37••,38•]; there is, however, no experimental evidence so far to support this model.

In addition to nephrin, a second molecule, P-cadherin, has been localized to the slit diaphragm in rat and human kidneys by immunocytochemical techniques [38•,46••]. Also for this molecule a homophilic interaction at the slit membrane has been assumed, proposing that the slit membrane corresponds to a modified adherens junction [46••]. Presently, however, it is not clear to what extent nephrin and P-cadherin participate in the structural organization of the slit membrane.

There is convincing evidence that without nephrin the glomerular filter is leaky for proteins, although the underlying mechanism is a matter of debate. The nephritogenic monoclonal antibody 5-1-6, which has been known since the late 1980s to induce proteinuria in rats [47] has been shown to be directed against the extracellular domain of nephrin [31,45•]. Injection of the 5-1-6 antibody into rats led to pronounced proteinuria and to a redistribution of nephrin from a linear to a granular pattern (associated with decreased nephrin mRNA expression). Surprisingly, no structural changes of the filtration slits or of other portions of the filtration barrier were detected [31,48].

There are other findings that are difficult to reconcile with the concept that nephrin *per se* represents the major size-selective filter. In puromycin aminonucleoside (PAN) nephrosis in rat, proteinuria was accompanied by down-regulation of nephrin mRNA, dislocation of nephrin from the slit membrane, and extensive FPE [31,44]. By contrast, in passive Heymann nephritis (PHN), in anti-GBM glomerulonephritis, and in Thy1- mediated glomerulonephritis nephrin expression was not changed, though diffuse FPE and proteinuria were observed [44,49].

Taken together, there is strong evidence that nephrin is a component of the slit membrane. In the absence of nephrin a slit membrane does not develop and the filter as a whole is leaky for proteins. On the other hand, the podocytes in kidneys from patients with CNF do develop interdigitating foot processes, which are separated from each other by variably developed junctional structures that probably contain P-cadherin, are associated with ZO-1, and frequently even show a regular width [38•] (Kriz *et al.*, unpublished observations). It therefore appears that the formation of an interdigitating foot process pattern and regular spaces are independent of nephrin. The molecules underlying this arrangement are unknown [38•], but they may represent an incomplete junction (containing P-cadherin) that develops into a slit membrane when nephrin comes into play. From this point of view, nephrin is either an integral component of the slit membrane (difficult to reconcile with the maintenance of a structurally intact slit membrane after a redistribution of nephrin), or – as suggested by one group of researchers [49] – it is necessary for the assembly of the slit membrane, but not for its maintenance.

One attractive candidate for the regulation of nephrin's function is CD2AP/CMS (CD2-associated protein/p130Cas ligand with multiple SH3 domains). CD2AP/CMS has been cloned independently by virtue of its interaction with the T-cell protein CD2 [50] and the docking protein p130Cas (crk-associated substrate) [51]. CD2AP/CMS possesses multiple motifs for protein–protein interaction. CD2AP/CMS-deficient mice exhibit a congenital nephrotic syndrome and die at 6–7 weeks of age from renal failure [52•,53••]. In HeLa cells CD2AP/CMS and nephrin can be co-immunoprecipitated, suggesting a direct interaction of both proteins also in podocyte foot processes [53••]. CD2AP/CMS therefore is a candidate for an adaptor linking the cytoskeleton to nephrin and the targeting of signaling complexes to nephrin. However, it is also clear that the absence of CD2AP/CMS in knockout mice interferes neither with the formation of interdigitating foot processes and a slit diaphragm nor with the expression of nephrin in foot processes [52•,53••]; FPE in those mice develops secondarily.

Finally, two further aspects of nephrin should be mentioned. First, several splice variants of nephrin have been found in humans and rats [30]. The relevance of these alternative splice forms, especially of those lacking a transmembrane domain, is not yet clear, but it should be mentioned that nephrin lacking the transmembrane domain is excreted in the urine in PAN nephritis [44]. Second, constructs of the nephrin promoter have very recently been used to faithfully target expression of a transgene to podocytes [54•,55•] These constructs will be extremely useful to study podocyte function in transgenic animals.

The cytoskeleton: its connections to the glomerular basement membrane and foot process effacement

The cytoskeleton of major processes of podocytes consists mainly of microtubules. These microtubules are of mixed polarity, which appears to be essential for process formation of podocytes [56]. The nonuniform microtubular polarity is generated by the motor protein CHO1/MKLP1, which is thought to transport fragments of microtubules in a minus end-distal fashion along preexisting plus end-distal microtubules [56].

The podocyte foot processes contain an actin-based cytoskeleton. The microfilaments form loop-shaped bundles, with their limbs running in the longitudinal axis of the foot processes. The bends of these loops are located centrally at the transition to the major processes and are probably connected to the microtubular skeleton [57]. The microfilament bundles are densely interconnected by α-actinin. Recently, it has been discovered that podocytes specifically express α-actinin-4 [41••]. So far, α-actinin-4 has been localized to membrane ruffles and implicated in cell motility in non-renal cells [58,59]. The specific function of α-actinin-4 in podocytes is not known; however, the mutations in α-actinin-4, which cause familial FSGS, result in enhanced F-actin bundling [41••].

The relevance of a further podocyte-specific actin-associated protein, synaptopodin [60], is so far unknown. Furthermore, podocytes express ezrin, which probably attaches the actin cytoskeleton to podocalyxin via the linker protein NHERF2 (Na^+/H^+-exchanger regulatory factor 2) [61,62].

Peripherally the actin bundles are linked to the slit membrane by the adaptor proteins ZO-1, catenins and probably CD2AP [46••,52], and to the GBM by integrins and dystroglycans. The integrin complex consists of vinculin, paxillin and talin, and of α3β1 integrin dimers, which bind to collagen IV α3, α4 and α5 chains as well as to laminin 11, a heterotrimer composed of α5/β2/γ1 laminin chains [63•]. The dystroglycan complex [64,65•,66•] consists of the cytoplasmic adaptor protein utrophin, of the transmembranous β-dystroglycan, and of the extracellular matrix-binding α-dystroglycan which is a receptor for agrin and laminin α5 chains.

On the one hand, dystroglycans and integrins are thought to coordinate the formation of a polygonal network of laminin in basement membranes [67]. Thus, cell matrix contacts in podocyte foot processes may be crucially involved in maintenance of GBM substructure and hence of barrier function. On the other hand, outside-in signaling via these systems quite obviously influences the function of the cytoskeleton which, in cases of derangements of the GBM, may lead to FPE (and to protein leakage).

FPE is the stereotypical structural response of podocytes to a great variety of challenges. It is a process in which the foot processes of two adjacent podocytes retract in a strictly coordinated way, thus leading to the simplification of the interdigitating pattern, a total rearrangement of the cytoskeleton [68,69], a marked shortening of the slit membrane and a variable appearance of other types of junctions [70]. The classic in-vivo experiments to induce FPE are treatment with polycations [71] such as protamine sulfate, which neutralize the surface charges of podocytes and PAN treatment [70], a toxic process to which the cells respond with FPE.

Treatment of cultured podocytes with protamine sulfate and PAN was used to obtain further insights into the cellular mechanisms underlying FPE [72••,73]. After both treatments, profound alterations in cell shape, a loss of actin bundles and a reduction and redistribution of focal contacts were consistently observed [72•,74]. These structural changes were accompanied by complex changes in the cellular phosphorylation pattern. The increase in cytoplasmic tyrosine phosphorylation contrasts with a reduction in tyrosine phosphorylation at focal contacts. An important role in this process appears to be played by cytosolic protein tyrosine phosphatases, which target proteins at focal contacts such as paxillin [72••]. Although the initial damaging mechanism in complement-mediated injury is certainly different, dephosphorylation of focal contact proteins including paxillin appears to be common in situations terminating in FPE [73]. The deletion of the receptor tyrosine phosphatase GLEPP1 also suggests a participation of tyrosine dephosphorylation in the formation of foot processes [75•]. Previous immunofluorescence studies of a variety of adhesion and cytoplasmic proteins in normal and nephritic human kidneys are in good agreement with these experimental results [76].

There are exciting new pieces of evidence regarding the role of the cytoskeleton and the GBM in initiating FPE, both experimentally and in human cases. Kidney development in the laminin α5 chain knockout mouse is arrested at a very early stage; a proper GBM does not form and podocytes do not assemble in a correct pattern [77•]. In the GBM, the α5 laminin chain, which together with β2 and γ1 chains is assembled into the GBM-specific laminin 11, is the cognate binding partner for α3β1 integrins as well as for dystroglycan α. In the laminin β2 chain mutant mouse a correct assembly of α, β and γ chains to laminin 11 is not possible [78]. This mouse develops FPE and proteinuria soon after birth, suggesting that the lack of the interaction between GBM components and integrins in the podocytes severely impairs the ability of the cytoskeleton to maintain the interdigitating pattern of foot processes [52,78].

Important observations were also made in podocytes of the integrin α3 mutant mouse. This mouse never develops foot processes and dies in the perinatal period [79]. Cultured podocytes from this mouse (lacking the α3 integrin gene) surprisingly displayed increased adhesion to the substrate together with considerable changes in the expression pattern of cytoskeletal proteins [80]. Thus, the α3 partner in the α3β1 integrin dimer appears to modify the strength of the adhesion, a mechanism that

may play an important role in permitting motility of podocytes on the GBM.

The *Col4α3−/−* mouse develops an Alport syndrome and shows again the great relevance of outside-in signaling for the proper development (and dynamics) of podocyte foot processes [81,82]. The GBM of this mouse contains collagen IV composed of α1 and α2 chains instead of α3, α4 and α5 chains; for unknown reasons it also contains laminin composed of α2 and α4 laminin chains, which are usually restricted to the mesangium [83,84•] In a double knockout of collagen α3 and integrin α1 chains (α1β1is the mesangial integrin) the content of α2 and α4 laminin chains in the GBM was significantly decreased and FPE was markedly improved [83]. Again, these observations underline the great importance of the correct integrin binding partners in the GBM.

An important player in inside-out as well as outside-in signaling may be an integrin-linked kinase (ILK), a β-integrin coupled serine/threonine kinase, which was found to be upregulated in children with CNF as well as in two murine models of nephrotic syndrome [85]. ILK overexpression in cultured podocytes reduced their ability to adhere to the extracellular matrix [86]. Focal adhesion kinase (FAK) and p130Cas are generally involved in integrin signaling [87]. Though expression of FAK and p130Cas has been demonstrated in mesangial cells and podocytes in human biopsies [76], the specific function of these proteins in foot processes is unknown.

Recent developments have also begun to shed light on the dystroglycan system. In adriamycin nephropathy in rats, which is accompanied by bulk leakage of proteins through the glomerular barrier, a significant overall decrease in the glomerular wall staining for dystroglycan α and β (with segmentally enhanced expression) was observed at sites with extensive FPE [65•]. In a study of kidney specimens from patients with minimal change nephrosis (MCN) and FSGS it was shown that the density of α- and β-dystroglycan was significantly reduced in MCN but not in FSGS compared with normal kidneys. The decreased levels of dystroglycan expression returned to normal in MCN after steroid treatment [66•]. The underlying mechanisms for these observations are obscure but probably also involve changes in outside-in signaling.

Angiotensin II and the podocyte as a target of proteinuria treatment

It has long been known that angiotensin II (ANG-II), in addition to its effects on glomerular hemodynamics, also affects the properties of the glomerular filtration barrier. Importantly, the effects of ANG-II on macromolecular permeability were found to occur in the absence of detectable hemodynamic changes and to be mediated via the AT_1 receptor subtype [88]. Podocytes express AT_1 receptors as demonstrated by immunohistochemistry and functional studies [89,90•,91]. ANG-II stimulation of podocytes *in situ* in isolated glomeruli triggers a membrane depolarization and an increase in intracellular Ca^{2+} [90•,91]. Podocytes can retain ANG-II receptors in culture and also respond to ANG-II stimulation by an increase in intracellular Ca^{2+} concentration [92]. Interestingly, Ca^{2+} influx in podocytes appears to be independent of voltage-operated channels and may rely on non-selective cation channels [90•,91,93]. Whether ANG-II signaling in podocytes further involves the cAMP pathway or AT_2 receptors cannot be clearly answered at present [94].

Although foot processes are equipped with a contractile apparatus, ANG-II has no discernable effect on foot process width, spacing, or total filtration slit length [95]. Alternatively, ANG-II could change the coordinated motility of foot processes on the GBM or alter the permeability of the slit pore by signaling events that ultimately induce conformational changes of slit pore proteins. Furthermore, ANG-II could affect the structure of the GBM via inside-out signaling through dystroglycans and integrins, and via altered matrix production of podocytes.

Recent work has supported the long-standing observations that in experimental models as well as in human cases of proteinuric diseases, angiotensin converting enzyme (ACE) inhibitors have renoprotective properties beyond systemic blood pressure reduction [96,97••, 98•,99•,100,101]. There is increasing evidence that these additional beneficial effects of ACE inhibitors stem from the improvement of the glomerular filter, thus lowering the protein leakage through the filter. The site and the mechanism by which these effects are brought about are a matter of debate. First, there is evidence that it is indeed the blockage of ANG-II and not of bradykinin that accounts for the positive effects [101,102•]. Moreover, there is increasing evidence that these positive effects result from preservation of podocyte function. In passive Heymann nephritis (PHN) [98•], ACE inhibitors prevented structural changes of podocytes (decrease in filtration slit frequency). Moreover, aging male MWF rats develop spontaneous proteinuria, which is associated with cellular changes in podocytes such as the redistribution of ZO-1 from its usual location at the slit membrane into the cytoplasm [97••]. This redistribution, as well as proteinuria and further sclerosis development, was inhibited by treatment with lisinopril. In addition, beneficial effects of ACE inhibitors were noted in adriamycin nephropathy, where they prevent the loss of

heparan sulfate proteoglycans from the GBM [102•]. Taken together, these observations suggest that the renoprotective effects of ACE inhibitors are mediated directly at the podocyte. Further support for this hypothesis comes from the finding that dihydropyridine-type calcium-channel blockers, which act on voltage-operated channels, have in general not had antiproteinuric effects [103,104].

The deleterious effects of transforming growth factor β (TGF-β) production induced by ANG-II on the progression of chronic renal failure [105] may also involve the podocyte. First there is evidence that transactivation of the EGF receptor by ANG-II through a Ca^{2+}-dependent tyrosine kinase may be responsible for ANG-II-induced TGF-β production as shown in fibroblasts [106]. Second, podocytes upregulate TGF-β and all three types of TGF-β receptors under disease conditions [107•,108•]. Podocyte injury was shown to be an early event in transgenic mice with elevated levels of circulating TGF-β [109], and TGF-β can exert apoptotic effects on podocytes *in vitro* [110].

Cell cycle of podocytes

Mature podocytes are highly differentiated cells. In response to most forms of injury they may undergo mitosis but not cell division. Exceptions to this rule are collapsing glomerulopathies such as HIV-associated nephropathy and idiopathic forms of collapsing glomerulopathies where podocytes undergo a dysregulation of their differentiated phenotype and proliferate [111]. Proliferation is regulated by cell cycle proteins such as cyclins, cyclin-dependent kinases (CDK) and their inhibitors [112•] Normal quiescent podocytes strongly express the CDK inhibitors p27 and p57, but not p21 [113•].

Two studies elucidating the pattern of cell cycle regulatory proteins in collapsing glomerulopathies [113•,114•] show that, in contrast to normal and to non-proliferating glomerular diseases, there is a marked and uniform decrease in the expression of the CDK inhibitors p27 and p57; p21, on the other hand, shows de-novo expression in podocytes in the collapsing glomerulopathies. This, together with the results of a further study [115] indicates that p21 is not required by podocytes to reach their characteristic differentiated phenotype. However, in disease states the loss of p21 is associated with podocyte re-entry into the cell cycle and development of a de-differentiated proliferative phenotype. As to non-proliferating diseases, in-vitro studies [116•] showed that C5b-9-induced injury to cultured podocytes led to a decrease in p27 and to S-phase DNA synthesis but subsequently the mitotic proteins (cyclin B and cdc-2) were decreased preventing mitosis and cytokinesis.

Causes of podocyte injury and progression to segmental glomerulosclerosis

The potential causes of podocyte injury are legion; an overview together with the pathways leading to FSGS was given two years ago in this journal [117]. Since then there is increasing evidence pointing to a crucial involvement of podocytes in rat models of type II diabetes and/or hyperlipidemia, which eventually lead to FSGS [118•,119•,120]. In the obese Zucker rat (fa/fa rat) it was clearly shown that early podocyte damage clearly preceded the development of FSGS [119•]; mesangial cell proliferation and matrix production was absent throughout the observation period. The pronounced accumulation of lipids and other macromolecules in podocytes was made responsible for a gradual loss of podocytes. Studies of further advanced stages of the disease demonstrated that the degeneration of nephrons occurred via loss of podocytes, which in turn resulted in tuft adhesions and misdirected filtration towards the interstitium [120]. An involvement of the mesangium was not obvious. In an experimental study comparing the effects of hypercholesterolemia and hypertriglyceridemia after uninephrectomy it was shown that under both circumstances sclerosis development occurred via podocyte rather than via mesangial cell damage [118•]. Two studies in humans with type II diabetic nephropathy show a crucial involvement of podocytes in disease development. First, the onset of the disease was shown to correlate with the appearance of FPE and of defects in size permselectivity presenting as 'macromolecular shunts' through the glomerular barrier [121•]. Second, the number of podocytes per glomerulus was the strongest predictor of disease progressions with fewer cells predicting more rapid progression [122•].

Conclusion

The growing knowledge of podocyte biology has placed the podocyte into the center of mechanisms underlying the permselectivity of the glomerular filter. Failure of this function, that is, loss of permselectivity, is a major player in most glomerular diseases, becoming the decisive defect that leads to disease progression. Progressive renal failure appears to be a podocyte-based disease.

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- • of special interest
- •• of outstanding interest

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