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Molecular make-up of the glomerular filtration barrier

Jaakko Patrakka [a,b,*], Karl Tryggvason [a]

[a] Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
[b] Department of Renal Medicine, Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

The glomerular filtration barrier is composed of glomerular endothelial cells, the glomerulus basement membrane and the podocyte cell layer. The filtration barrier is a target of injury in several systemic and renal diseases, and this often leads to progressive renal disease and kidney failure. Therefore, it is essential to understand the molecular biology of the glomerulus. During the last two decades, a lot of new information about molecular components of the glomerulus filtration barrier has been generated. Many of the key discoveries have been obtained through studies on the genetic background of inherited glomerular diseases. These studies have emphasized the role of podocytes in the filtration barrier function. During the last decade, the use of knockout mouse technology has become more available and given important new insights into the functional significance of glomerular components. Large-scale approaches, such as microarray profiling, have also given data about molecules involved in the biology and pathology of the glomerulus. In the coming decade, the use of global expression profiling platforms, transgenic mouse lines, and other *in vivo* gene delivery methods will rapidly expand our understanding of biology and pathology of the glomerular filtration barrier, and hopefully expose novel target molecules for therapy in progressive renal diseases.

1. Introduction

The ultrafiltration of plasma in the kidney occurs through the capillary wall of the glomerulus. The filtration barrier is composed of glomerular endothelial cells (GECs), the glomerular basement membrane (GBM), and podocyte cells (Fig. 1). The plasma molecules are sieved through the glomerular filter based on their size and (probably) charge [1]. The size-selectivity of the renal filtration is universally accepted, whereas the charge-selectivity is still somewhat questioned [2,3]. The location of the size- and charge-selective segment of the capillary wall is a matter of debate. Present data suggest that all three layers of the glomerular capillary wall need to be intact to maintain normal filtration function. However, the studies performed during the last decade have underlined the role of the podocyte in the filtering process [4].

The filtration barrier is injured in many systemic and renal disorders leading to protein leakage to urine (proteinuria) and progressive renal disease. Dysfunction of the glomerulus is the most common primary etiological factor for renal failure, and therefore it is critical to understand the basic molecular biology of the glomerulus. In this review, we give an overview on the molecular architecture of the glomerular filtration barrier with a special emphasis on podocytes, and discuss how different components are thought to contribute to the barrier function.

2. Glomerular endothelial cells (GECs)

The endothelial cells of the glomerulus are highly fenestrated. The fenestrae are 70–100 nm in diameter and constitute 20–50% of the glomerular capillary surface area. As the fenestrations are huge compared to the size of albumin, the GECs have not been believed to play an important role in the barrier function for protein filtration. The GECs are, however, covered by an endothelial cell surface layer (mainly glycocalyx) that may hinder the passage of albumin and other plasma proteins into the GBM (Fig. 1). Major components of this surface layer are negatively charged glycoproteins, glycosaminoglycans (GAGs) and membrane associated proteoglycans, supporting primarily the idea that GECs contribute to the charge-selectivity of the filter. Indirect evidence on the role of GEC glycocalyx in the barrier function has come from many proteinuric animal models that have shown correlation between the reduced GEC glycocalyx and proteinuria (reviewed by Haraldsson et al. [1]).

More direct evidence on the role of GEC in the filtration barrier has been generated by Quaggin and colleagues through studies investigating the involvement of growth factor VEGF in the glomerulus. In the glomerulus, the blockage of VEGF signaling, either

* Corresponding author. Address: Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, 171 77 Stockholm, Sweden.
E-mail address: jaakko.patrakka@ki.se (J. Patrakka).

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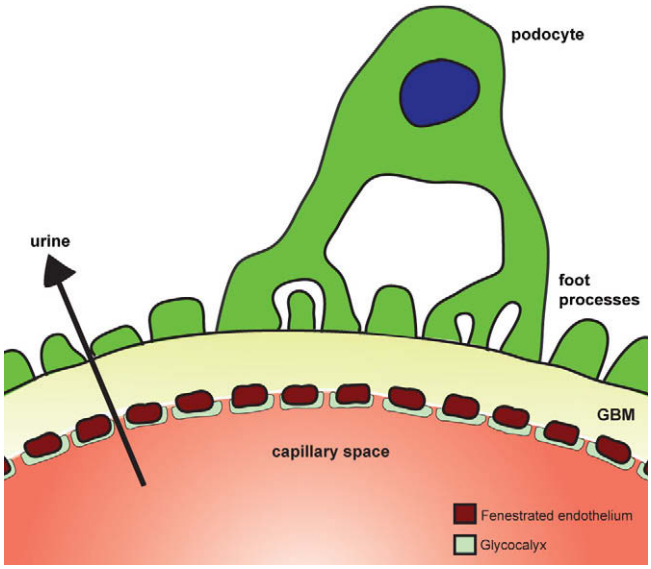

Fig. 1. Glomerular filtration occurs through the capillary wall into the urinary space. The capillary wall contains an innermost fenestrated endothelium with glycocalyx, the glomerular basement membrane (GBM), and a layer of podocytes with interdigitating foot processes connected by slit diaphragms.

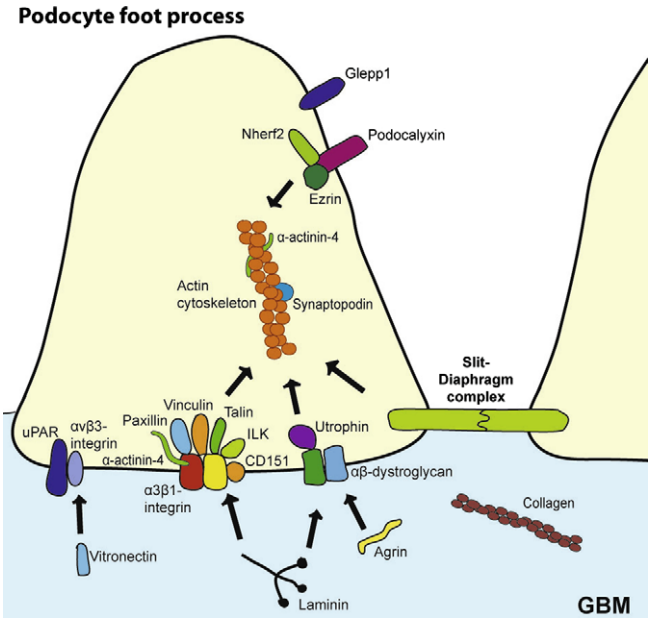

Fig. 2. Schematic picture of podocyte foot processes. Foot processes are basally anchored to the components of the GBM via α3β1 integrin, tetraspanin CD151 and αβ dystroglycan. These transmembrane proteins are linked to the actin cytoskeleton via several adaptor proteins. uPAR receptor is also found at the basal surface of foot processes in where it probably mediates its actions through αvβ3-integrin. Vitronectin, the extracellular ligand of αvβ3-integrin, is induced during proteinuria and activate uPAR signaling in podocytes. The slit diphragm protein complex is linked to actin cytoskeleton. Apical surface of podocytes contain podocalyxin and Glepp1. Podocalyxin is connected to actin via adapter proteins. The actin cytoskeleton of foot processes contains actin-associated proteins α-actinin-4 and synaptopodin, and interconnects three plasma membrane domains of foot processes together.

by the anti-VEGF antibody Avastin or by podocyte specific inactivation of VEGF in adult mice, results in GEC defects associated with proteinuria [5]. Similarly, the inactivation of one of the VEGF alleles in podocytes during development results in GEC abnormalities and proteinuria [6]. Taken together, GECs are likely to be critically involved in the formation and maintenance of the filtration barrier, and probably also act as a true barrier for the traversal of plasma macromolecules.

3. Glomerular basement membrane (GBM)

The GBM forms the structural foundation of the glomerular capillary to which endothelial and podocyte cells are anchored on both sides (Fig. 2). Similarly to all basement membranes, the main components of the GBM include type IV collagen, laminin, proteoglycans and nidogen. The triple-helical type IV collagen molecules of mature GBM are composed of α3, α4, and α5 chains. The highly cross-linked type IV collagen network provides probably tensile strength to the capillary wall, which is supported by the fact that mutations in type IV collagen genes result in Alporś syndrome, a disease that manifests as hematuria and progressive renal disease [7]. However, type IV collagens are thought to only have a minor role in the filtration selectivity of the glomerular barrier as patients with Alporś syndrome exhibit only mild proteinuria.

Laminins are heterotrimeric proteins present in all basement membranes in where they form a network that is highly cross-linked to the type IV collagen network. In the mature GBM, the main laminin is laminin-521 (composed of α5, β2, γ1 chains). Laminin-521 is essential for the glomerular filtration barrier as laminin β2 chain deficient mice exhibit massive proteinuria [8] and patients with laminin β2 gene mutations develop Piersoń syndrome [9], a rare syndrome associated with perinatal massive proteinuria. In laminin β2 knockout mice, the disorganization of the GBM precedes the onset of proteinuria, whereas podocyte foot processes and slit diaphragms appear normal at this point [10]. Furthermore, in tracer studies, laminin β2 deficient mice show increased permeability in the GBM, whereas mice with a primary podocyte defect and proteinuria show no increased permeability in the GBM [10]. Together these results suggest that the GBM contributes to the actual filtration barrier.

Heparan sulfate proteoglycans (HSPGs) provide anionic charge to the GBM. These molecules are composed of a core protein to which negatively charged GAG side chains are bound. In the GBM, three HSPGs, agrin, perlecan, and collagen XVIII have been identified [2,11]. HSPGs were earlier believed to be important for the charge-selectivity of the filtration barrier as elimination of their anionic charges after intravenous administration of heparanases was reported to result in increased permeability to ferritin [12]. Recent studies with genetically modified mice have, however, questioned the role of HSPGs in glomerular filtration as genetically engineered mice lacking podocyte-derived agrin and perlecan heparin-sulfate side chains [2] do not develop proteinuria. On the other hand, collagen XVIII deficient mice show mesangial expansion and mild renal insufficiency suggesting that this HSPG may play a role in the filter barrier structure and function [11].

4. Podocyte

The podocyte is a highly specialized epithelial cell covering the outside of the glomerular capillary [13]. The podocyte has a prominent cell body and large cytoplasmic projections (major processes) that divide into long thin processes, termed foot processes, close to the glomerular capillary (Fig. 2). The foot processes are attached firmly to the underlying GBM and arranged in a highly organized way so that they envelope the capillary wall in an interdigitating "comb-like" fashion. Foot processes from adjacent podocytes are interconnected by a specialized cell–cell junction of the podocyte, termed the slit diaphragm. The slit diaphragm divides the plasma membrane of foot processes to the basal, the apical, and the lateral (slit diaphragm) surfaces. In the cytoplasm, these three surfaces are interconnected via the actin-based cytoskeleton. Podocyte foot

processes with different plasma membrane domains have essential roles in the maintenance of the glomerular filtration barrier. In proteinuric diseases, the retractions of foot processes are often observed, a phenomenon referred to as effacement. One of the main questions is how this effacement is involved in the development of proteinuria [13]. At the present, the pathogenic role of foot process effacement in proteinuria is not well understood.

5. Basal surface

The podocyte, as all epithelial cells, are attached to the underlying basement membrane through transmembrane cell receptors, such as integrins, tetraspanins and dystroglycans (Fig. 2). Integrins are αβ heterodimeric proteins that are differentially expressed in various cells. In podocytes, α3β1 integrin is the most abundant isoform. The α3β1 integrin is needed for the development of the glomerular capillary tuft, as α3-deficient mice exhibit defects in the glomerular capillary branching and failure to form proper foot processes [14]. On the other hand, mice in which the α3 integrin gene is turned off during the development of podocytes (by using podocin-Cre) exhibit massive proteinuria soon after birth and electron microscopy shows complete foot process effacement and widespread lamination with protrusions of the GBM [15]. In addition, the essential role of α3β1 complex has been underlined by two studies on mice lacking β1 gene in podocytes [16,17]. Mice lacking the integrin β1-chain in podocytes develop massive proteinuria, a phenotype alike to that in α3 integrin knockouts. How defects in the α3β1 integrin complex in podocytes result in proteinuria is not known. However, as α3β1 integrin is a major receptor for the GBM component laminin-521 [18], it is reasonable to speculate that disruption of the integrin–laminin complex results in a weakened podocyte–GBM interaction and the detachment of podocytes from the GBM. Loss of podocytes is, on the other hand, known to be associated with proteinuria.

The importance of integrins has been further highlighted in studies on mice lacking integrin-linked kinase (ILK) specifically in podocytes as these mice develop proteinuria and progressive proteinuric renal disease [19,20]. The mechanism behind "ILK-nephropathy" is not completely understood as two independent studies have reported somewhat different results. One study reported that the first sign of renal injury in these mice was the thickening of the GBM followed by abnormal distribution of α3-integrins [19]. Another study showed that ILK formed a complex with nephrin and α-actinin-4, and that the absence of ILK resulted in the redistribution of nephrin and α-actinin-4 [20]. In fact, it is possible that ILK mediates signaling in both the basal and the slit diaphragm surfaces, and this would explain the phenotype observed in the ILK deficient podocytes.

Tetraspanins are transmembrane proteins expressed basically in all cell types. CD151 is a member of the tetraspanin family and associates with cell–matrix adhesion complexes such as α3β1integrin. Recently, CD151 was localized to the base of the foot processes, and CD151-deficient mice were shown to develop proteinuria indicating the critical role for this protein in the glomerulus filtration barrier [15]. Electron microscopic evaluation of these mice at the onset of proteinuria revealed severe alterations of the GBM, including thickening and splitting of the GBM. These lesions seemed to precede podocyte abnormalities (including effacement of foot processes) suggesting that CD151 would be involved in the maturation and/or maintenance of the GBM structure.

Besides integrins and tetraspanins, the heterodimeric transmembrane protein αβ dystroglycan has been identified as a component of the basal surface of foot processes [21]. Dystroglycans are known to bind laminin and agrin extracellularly, whereas in the cytoplasm the dystroglycan complex is connected to the actin cytoskeleton of foot processes via utrophin [21]. The importance of dystroglycans in the adhesion of podocytes to the GBM is so far unknown.

uPAR is a proteinase receptor for urokinase that is involved in nonproteolytic pathways through interactions with other plasma membrane proteins, such as integrins. Podocytes express uPAR and this expression is upregulated in proteinuric states [22]. Induction of uPAR expression seems to have a pathogenic role in the development of proteinuria as mice lacking uPAR are protected from lipopolysaccharide (LPS)-mediated proteinuria but develop disease after expression of a constitutively active β3 integrin. Interestingly, the activation of αvβ3-integrin by itself is sufficient to induce proteinuria and conversely, the inhibition of αvβ3-integrin activation has an anti-proteinuric effect. Vitronectin, the extracellular ligand of αvβ3-integrin, is also induced during proteinuria, and vitronectin deficient mice are resistant to LPS-induced proteinuria. Taken together, uPAR, together with αvβ3-integrin and vitronectin, seem to be involved in the development of proteinuria at least in some disease processes.

6. The slit diaphragm

The slit diaphragm is a highly specialized cell–cell junction of podocytes that interconnects adjacent foot processes. In 1974, based on electron microscopic examination, Rodewald and Karnovsky proposed that the slit diaphragm has a structured, zipper-like architecture with pores that are smaller than albumin, and therefore could serve as a barrier for protein filtration [23]. Although this classic model was proposed over 35 years ago, it was first during the last decade, that the molecular architecture of the slit diaphragm started to unravel. The slit diaphragm protein complex is composed of proteins common for most cell–cell junctions, including cadherins and catenins, but importantly, also of proteins that are not generally found elsewhere in the body (Fig. 3) [4,24]. From the viewpoint of molecular structure and function, these "podocyte-specific" proteins make the slit diaphragm a unique cell–cell

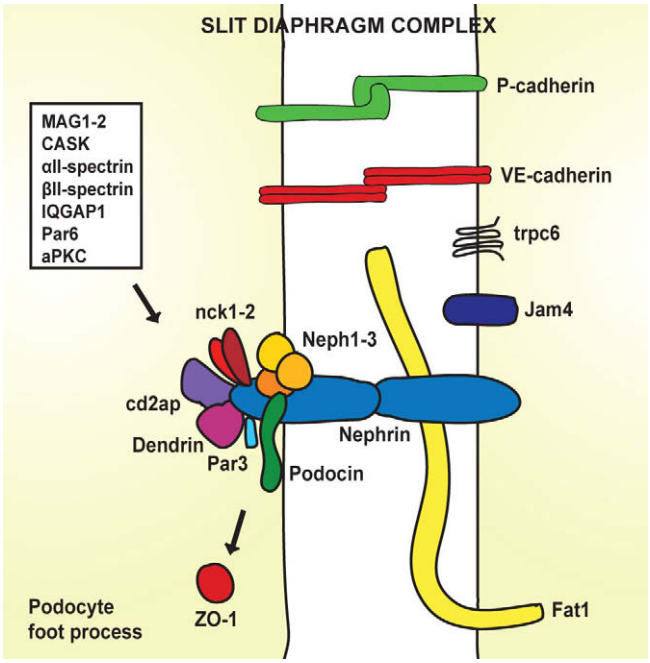

Fig. 3. Schematic picture of the slit diaphragm components. The slit diaphragm complex is extracellularly formed of at least nephrin, Neph1–3, P-cadherin, VE-cadherin, Fat1, and Jam4. Nephrin and cadherin proteins probably interact in a homophilic fashion in the center of the slit diaphragm. Intracellularly, a number of adapter proteins are involved in linking the slit diaphragm to actin.

junction that has become specialized for carrying out the renal ultrafiltration function.

Nephrin was the first transmembrane protein identified in the slit diaphragm (reviewed in Ref. [25]). It was discovered through positional cloning, as the nephrin-coding gene (*NPHS1*) was found to be mutated in the congenital nephrotic syndrome of the Finnish type [26]. This disease manifests as massive proteinuria already at the perinatal stage. The crucial importance of nephrin for the filtration barrier has been confirmed in nephrin knockout mice as these animals lack slit diaphragms and die perinatally due to massive proteinuria [25]. Nephrin has a short intracellular domain, a transmembrane domain, and an extracellular domain with eight IgG-like motifs and a fibronectin motif. Nephrin molecules probably interact in the slit diaphragm with one another in a homophilic fashion. Studies performed with a high-resolution electron-tomography method have given us some insights how nephrin contributes to the formation of the slit diaphragm structure [27]. With this method, convoluted strands, resembling the zipper-like model, are detected in the slit diaphragm, and in solution, nephrin molecules resemble these convoluted strands. Moreover, the N-terminal part of nephrin seems to be in the central region of the slit diaphragm as detected by combination of immunoelectron microscopy and electron-tomography. Taken together, the data promote the idea that nephrin could form the porous zipper-like structure of the slit diaphragm proposed earlier by Rodewald and Karnovsky. However, the relationship of nephrin to other molecular components of the slit diaphragm (see below), and how these molecules together contribute to the zipper-like structure, remains to be seen.

Intracellularly, nephrin and the slit diaphragm are connected to the actin cytoskeleton through linker proteins, such as Cd2-associated protein (Cd2ap) and Nck proteins (Fig. 3) [28–30]. Cd2ap binds directly to nephrin and actin, and serves as a direct link between the slit diaphragm and the actin cytoskeleton. This link is essential for the functional renal filtration as Cd2ap deficient mice die due to massive proteinuria and exhibit foot process effacement [30]. The Nck proteins (Nck1 and Nck2) are composed of an SH2 domain, which can interact with phosphotyrosines, and of SH3 domains, which can recruit several other proteins involved in the regulation of actin assembly. In podocytes, Nck has been shown to interact with tyrosine phosphorylated nephrin [28,29]. This interaction is required for the development of normal filtration barrier as mice lacking both Nck proteins in podocytes develop massive proteinuria [29]. Also, Nck proteins are needed in the maintenance of the mature filtration barrier as inactivation of Nck proteins in adult mouse podocytes result in proteinuria and foot process effacement [31]. Taken together, both Cd2ap and Nck proteins are crucial for linking the slit diaphragm to actin. These interactions mediate the actin polymerization and the cytoskeletal reorganization in foot processes that is required for normal functional filtration barrier.

Neph1, Neph2, and Neph3 have been located to the slit diaphragm [4]. Neph–proteins are structurally related to nephrin with five extracellular IgG-like motifs. Neph–proteins interact with nephrin, and Neph1 seems to cooperate with nephrin in actin recruitment via Nck proteins [32]. The importance of Neph1 is highlighted by the fact that Neph1 deficient mice die perinatally due to massive proteinuria [33].

Podocin is another central component of the slit diaphragm. Podocin was discovered through studies on hereditary proteinuria syndromes, as mutations in the podocin coding gene *NPHS2* were identified in families with a progressive proteinuric disease [34]. Podocin is a hairpin-shaped integral membrane protein with both ends directed into the intracellular space. Podocin interacts directly with nephrin, Neph1 and Cd2ad [35,36], and podocin seems to be essential for the recruitment of nephrin to the slit diaphragm [36]. The essential role of podocin in the glomerulus barrier has been shown in podocin deficient mice which lack slit diaphragms and die perinatally due to massive proteinuria [37].

Trpc6 is a member of a family of nonselective cation channels that are involved in the regulation of intracellular calcium concentration in response to the activation of G-protein-coupled receptors and receptor tyrosine kinases. Dominant mutations in the *Trpc6* gene have been identified in families with a progressive proteinuric renal disease [38,39]. Trpc6 is found in the slit diaphragm and some *Trpc6* mutations found in patients result in increased amplitude and duration of calcium influx after stimulation. Together, these results suggest that impaired slit diaphragm signaling is involved in the development of "Trpc6 nephropathy". However, Trpc6 knockout mice do not show any obvious renal phenotype indicating that this protein is not critical for the normal function of the filtration barrier [4].

Three cadherin proteins, a large protocadherin Fat1, P-cadherin, and vascular endothelial cadherin (VE-cadherin) have been localized to the slit diaphragm [4]. Fat1 is a huge protein with 34 tandem cadherin-like repeats, and it is essential for normal filtration barrier as Fat1 knockout mice lack slit diaphragms and exhibit proteinuria [40]. P-cadherin, on the other hand, does not seem to be essential for the functional renal filtration barrier, whereas the role of VE-cadherin in the podocyte is so far unknown [4]. What is the relation of these cadherin proteins to the other molecular components of the slit diaphragm remains to be determined.

The cell polarity complex composed of the partitioning defective 3 (Par3), partitioning defective 6 (Par6) and atypical protein kinase C (aPKC) is located at the cytoplasmic side of the slit diaphragm (reviewed by Simons et al. [41]). Par3 can bind directly to nephrin and Neph1, and recruit Par6/aPKC to the slit diaphragm area. The deletion of an aPKC isoform in podocytes results in proteinuria indicating that this cell polarity complex is essential for the glomerular filtration barrier. Dendrin, a cytosolic protein originally identified in telencephalic dendrites, is also found at the cytoplasmic face of the slit diaphragm where it interacts directly with nephrin and Cd2ap [42,43]. In experimental proteinuric model, dendrin relocates to nucleus and enhances TGF-β1 mediated apoptotic signaling suggesting that dendrin may provide a link whereby changes in the slit diaphragm could translate into alterations of podocyte survival. However, dendrin knockout mice have normal functional glomerulus filtration barrier (Patrakka et al. unpublished data) indicating that this protein is not critical for normal podocyte biology. ZO-1, junctional adhesion molecule 4 (Jam4), densin, MAGI-1/2, CASK, IQGAP1, αII spectrin, and βII spectrin, have also been reported to be associated with the slit diaphragm [44]. The role of these molecules in podocytes remains to be established.

7. Apical surface

Podocalyxin is the main molecular components of the apical plasma membrane of podocyte foot processes (Fig. 2, reviewed by Nielsen et al. [45]). Podocalyxin is an extensively *O*-glycosylated and sialylated transmembrane protein that is responsible for the negative charge of the apical membrane domain. This negative charge has been shown to be neutralized in animal models, which results in disruption of the ordered foot process architecture and proteinuria. Podocalyxin-deficient mice lack foot processes and slit diaphragms, and it seems that these mice are unable to filter primary urine [46]. Thus, podocalyxin is required for the development of the normal podocyte architecture and the functional filtration barrier. Intracellularly, podocalyxin is connected to the actin cytoskeleton via ezrin and Na+/H+ exchanger regulatory factor 2 (NHERF2). The podocalyxin/ezrin/NHERF/actin association is dis-

rupted in some animal models with foot process effacement and proteinuria suggesting podocalyxin may be involved in the regulation of podocyte morphology in some disease processes [47].

Glomerular epithelial protein 1 (GLEPP1) is a receptor tyrosine phosphatase found in the kidney exclusively on the apical cell surface of the podocyte (Fig. 2) [48]. Glepp1 has a large ectodomain consisting of multiple fibronectin type III repeats, a transmembrane domain, and a single cytoplasmic phosphatase active site sequence. Glepp1 deficient mice exhibit abnormal podocyte morphology in electron microscopic examination with an amoeboid rather than the typical octopoid structure [49]. These mice do not show proteinuria but have reduced glomerular filtration rate, and are prone to develop high blood pressure. Thus, Glepp1 seems to be essential for the regulation of glomerular filtration rate and normal podocyte structure. However, the ligand of Glepp1, and how Glepp1 associates with other molecular components of foot processes, is not known.

8. Actin cytoskeleton of foot processes

In foot processes, the cytoskeleton is formed of actin-based highly ordered bundles that run parallel to the longitudinal axis of foot processes [13]. The cytoskeleton is connected to three plasma membrane domains (basal, lateral, and apical) of foot processes through several linker proteins (Fig. 2, discussed above), and therefore brings together signals from the extracellular environment. Interference with any of the three plasma membrane domains can cause parallel actin bundles to reorganize to dense network. This is observed as foot process effacement, which, on the other hand, associates generally with proteinuria. Many actin-associated proteins, some of which are very specific to podocytes, are involved in the dynamic regulation of actin assembly in foot processes.

α-Actinin-4 is an actin-filament crosslinking protein of podocyte foot processes (Fig. 2). It was discovered through genetic studies as point mutations in the α-actinin-4 coding gene *ACTN4* were identified to be responsible for a dominantly inherited proteinuric disease [50]. The pathogenic role of one of mutations has been confirmed in a transgenic mouse line carrying the mutation as these mice develop progressive proteinuric disease [51]. These point mutations are in the actin-binding domain of α-actinin-4, and mutated α-actinin-4 molecules show increased binding affinity to actin [52]. This altered binding to actin somehow impairs the dynamics of cytoskeleton resulting in foot process effacement and proteinuria. On the other hand, α-actinin-4 has also a critical role in the basal surface of foot processes, as α-actinin-4 knockout podocytes show impaired adhesion to the GBM that causes shedding of podocytes to the urine and progressive proteinuric disease [53].

Synaptopodin is a proline-rich actin-associated protein found in podocyte foot processes (Fig. 2) and dendritic spines of the brain (reviewed by Faul et al. [13]). In podocytes, synaptopodin binds to α-actinin-4 and regulates its actin-bundling activity [54]. Synaptopodin is involved in the regulation of foot process structure as synaptopodin deficient mice show impaired recovery after the induction of foot process effacement [54]. It is intriguing that synaptopodin has been linked to the anti-proteinuric effects of calcineurin inhibitor cyclosporine A (CsA) [55]. CsA is a commonly used immunosuppressive drug that has been thought cause its effects via inhibition of NFAT signaling in T cells. However, CsA seems to have a direct anti-proteinuric effect on podocytes through phosphorylation of synaptopodin [55]. This phosphorylation protects synaptopodin from cathepsin L mediated proteolysis and results in stabilization of the foot process cytoskeleton and resistance to proteinuria. This discovery can have significant clinical applications, as it exposes new possibilities to develop more specific anti-proteinuric drugs that lack unwanted side effects of the NFAT inhibition.

9. Large-scale profiling approaches disclose novel components of the glomerular filtration barrier

Glomerular profilings using microarray analysis have revealed numerous highly glomerulus-associated transcripts. How the protein products of these transcripts participate to the filtration barrier is not known. In our profiling approach, we identified >300 highly glomerulus-enriched transcripts [56]. Together with our collaborators we have taken our global approach further by generating antibodies against the protein products of these transcripts. This approach is generating a large amount of information, and we have already identified >30 novel molecular components of podocyte foot processes (Patrakka et al., unpublished). The next enormous task is to characterize signaling pathways and protein–protein networks in which these proteins are participating, and to identify functional roles of these proteins in the filtration barrier.

Acknowledgments

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References

[1] B. Haraldsson, J. Nystrom, W.M. Deen, Properties of the glomerular barrier and mechanisms of proteinuria, Physiol. Rev. 88 (2008) 451–487.
[2] S. Goldberg, S.J. Harvey, J. Cunningham, K. Tryggvason, J.H. Miner, Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane, Nephrol. Dial. Transplant. 24 (2009) 2044–2051.
[3] S.J. Harvey, G. Jarad, J. Cunningham, A.L. Rops, J. van der Vlag, J.H. Berden, M.J. Moeller, L.B. Holzman, R.W. Burgess, J.H. Miner, Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity, Am. J. Pathol. 171 (2007) 139–152.
[4] J. Patrakka, K. Tryggvason, New insights into the role of podocytes in proteinuria, Nat. Rev. Nephrol. 5 (2009) 463–468.
[5] V. Eremina, J.A. Jefferson, J. Kowalewska, H. Hochster, M. Haas, J. Weisstuch, C. Richardson, J.B. Kopp, M.G. Kabir, P.H. Backx, H.P. Gerber, N. Ferrara, L. Barisoni, C.E. Alpers, S.E. Quaggin, VEGF inhibition and renal thrombotic microangiopathy, N. Engl. J. Med. 358 (2008) 1129–1136.
[6] V. Eremina, M. Sood, J. Haigh, A. Nagy, G. Lajoie, N. Ferrara, H.P. Gerber, Y. Kikkawa, J.H. Miner, S.E. Quaggin, Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases, J. Clin. Invest. 111 (2003) 707–716.
[7] B.G. Hudson, K. Tryggvason, M. Sundaramoorthy, E.G. Neilson, Alport's syndrome, Goodpasture's syndrome, and type IV collagen, N. Engl. J. Med. 348 (2003) 2543–2556.
[8] P.G. Noakes, J.H. Miner, M. Gautam, J.M. Cunningham, J.R. Sanes, J.P. Merlie, The renal glomerulus of mice lacking s-laminin/laminin beta 2: nephrosis despite molecular compensation by laminin beta 1, Nat. Genet. 10 (1995) 400–406.
[9] M. Zenker, T. Aigner, O. Wendler, T. Tralau, H. Muntefering, R. Fenski, S. Pitz, V. Schumacher, B. Royer-Pokora, E. Wuhl, P. Cochat, R. Bouvier, C. Kraus, K. Mark, H. Madlon, J. Dotsch, W. Rascher, I. Maruniak-Chudek, T. Lennert, L.M. Neumann, A. Reis, Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities, Hum. Mol. Genet. 13 (2004) 2625–2632.
[10] G. Jarad, J. Cunningham, A.S. Shaw, J.H. Miner, Proteinuria precedes podocyte abnormalities inLamb2−/− mice, implicating the glomerular basement membrane as an albumin barrier, J. Clin. Invest. 116 (2006) 2272–2279.
[11] A. Utriainen, R. Sormunen, M. Kettunen, L.S. Carvalhaes, E. Sajanti, L. Eklund, R. Kauppinen, G.T. Kitten, T. Pihlajaniemi, Structurally altered basement membranes and hydrocephalus in a type XVIII collagen deficient mouse line, Hum. Mol. Genet. 13 (2004) 2089–2099.
[12] Y.S. Kanwar, A. Linker, M.G. Farquhar, Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion, J. Cell Biol. 86 (1980) 688–693.
[13] C. Faul, K. Asanuma, E. Yanagida-Asanuma, K. Kim, P. Mundel, Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton, Trends Cell Biol. 17 (2007) 428–437.

[14] J.A. Kreidberg, M.J. Donovan, S.L. Goldstein, H. Rennke, K. Shepherd, R.C. Jones, R. Jaenisch, Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis, Development 122 (1996) 3537–3547.
[15] N. Sachs, M. Kreft, M.A. van den Bergh Weerman, A.J. Beynon, T.A. Peters, J.J. Weening, A. Sonnenberg, Kidney failure in mice lacking the tetraspanin CD151, J. Cell Biol. 175 (2006) 33–39.
[16] A. Pozzi, G. Jarad, G.W. Moeckel, S. Coffa, X. Zhang, L. Gewin, V. Eremina, B.G. Hudson, D.B. Borza, R.C. Harris, L.B. Holzman, C.L. Phillips, R. Fassler, S.E. Quaggin, J.H. Miner, R. Zent, Beta1 integrin expression by podocytes is required to maintain glomerular structural integrity, Dev. Biol. 316 (2008) 288–301.
[17] K. Kanasaki, Y. Kanda, K. Palmsten, H. Tanjore, S.B. Lee, V.S. Lebleu, V.H. Gattone Jr., R. Kalluri, Integrin beta1-mediated matrix assembly and signaling are critical for the normal development and function of the kidney glomerulus, Dev. Biol. 313 (2008) 584–593.
[18] M. Doi, J. Thyboll, J. Kortesmaa, K. Jansson, A. Iivanainen, M. Parvardeh, R. Timpl, U. Hedin, J. Swedenborg, K. Tryggvason, Recombinant human laminin-10 (alpha5beta1gamma1). Production, purification, and migration-promoting activity on vascular endothelial cells, J. Biol. Chem. 277 (2002) 12741–12748.
[19] C. El-Aouni, N. Herbach, S.M. Blattner, A. Henger, M.P. Rastaldi, G. Jarad, J.H. Miner, M.J. Moeller, R. St-Arnaud, S. Dedhar, L.B. Holzman, R. Wanke, M. Kretzler, Podocyte-specific deletion of integrin-linked kinase results in severe glomerular basement membrane alterations and progressive glomerulosclerosis, J. Am. Soc. Nephrol. 17 (2006) 1334–1344.
[20] C. Dai, D.B. Stolz, S.I. Bastacky, R. St-Arnaud, C. Wu, S. Dedhar, Y. Liu, Essential role of integrin-linked kinase in podocyte biology: bridging the integrin and slit diaphragm signaling, J. Am. Soc. Nephrol. 17 (2006) 2164–2175.
[21] H.M. Regele, E. Fillipovic, B. Langer, H. Poczewki, I. Kraxberger, R.E. Bittner, D. Kerjaschki, Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis, J. Am. Soc. Nephrol. 11 (2000) 403–412.
[22] C. Wei, C.C. Moller, M.M. Altintas, J. Li, K. Schwarz, S. Zacchigna, L. Xie, A. Henger, H. Schmid, M.P. Rastaldi, P. Cowan, M. Kretzler, R. Parrilla, M. Bendayan, V. Gupta, B. Nikolic, R. Kalluri, P. Carmeliet, P. Mundel, J. Reiser, Modification of kidney barrier function by the urokinase receptor, Nat. Med. 14 (2008) 55–63.
[23] R. Rodewald, M.J. Karnovsky, Porous substructure of the glomerular slit diaphragm in the rat and mouse, J. Cell Biol. 60 (1974) 423–433.
[24] S. Lehtonen, J.J. Ryan, K. Kudlicka, N. Iino, H. Zhou, M.G. Farquhar, Cell junction-associated proteins IQGAP1, MAGI-2, CASK, spectrins, and alpha-actinin are components of the nephrin multiprotein complex, Proc. Natl. Acad. Sci. USA 102 (2005) 9814–9819.
[25] J. Patrakka, K. Tryggvason, Nephrin—a unique structural and signaling protein of the kidney filter, Trends Mol. Med. 13 (2007) 396–403.
[26] M. Kestila, U. Lenkkeri, M. Mannikko, J. Lamerdin, P. McCready, H. Putaala, V. Ruotsalainen, T. Morita, M. Nissinen, R. Herva, C.E. Kashtan, L. Peltonen, C. Holmberg, A. Olsen, K. Tryggvason, Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome, Mol. Cell 1 (1998) 575–582.
[27] J. Wartiovaara, L.G. Ofverstedt, J. Khoshnoodi, J. Zhang, E. Makela, S. Sandin, V. Ruotsalainen, R.H. Cheng, H. Jalanko, U. Skoglund, K. Tryggvason, Nephrin strands contribute to a porous slit diaphragm scaffold as revealed by electron tomography, J. Clin. Invest. 114 (2004) 1475–1483.
[28] R. Verma, I. Kovari, A. Soofi, D. Nihalani, K. Patrie, L.B. Holzman, Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization, J. Clin. Invest. 116 (2006) 1346–1359.
[29] N. Jones, I.M. Blasutig, V. Eremina, J.M. Ruston, F. Bladt, H. Li, H. Huang, L. Larose, S.S. Li, T. Takano, S.E. Quaggin, T. Pawson, Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes, Nature 440 (2006) 818–823.
[30] N.Y. Shih, J. Li, V. Karpitskii, A. Nguyen, M.L. Dustin, O. Kanagawa, J.H. Miner, A.S. Shaw, Congenital nephrotic syndrome in mice lacking CD2-associated protein, Science 286 (1999) 312–315.
[31] N. Jones, L.A. New, M.A. Fortino, V. Eremina, J. Ruston, I.M. Blasutig, L. Aoudjit, Y. Zou, X. Liu, G.L. Yu, T. Takano, S.E. Quaggin, T. Pawson, Nck proteins maintain the adult glomerular filtration barrier, J. Am. Soc. Nephrol. 20 (2009) 1533–1543.
[32] P. Garg, R. Verma, D. Nihalani, D.B. Johnstone, L.B. Holzman, Neph1 cooperates with nephrin to transduce a signal that induces actin polymerization, Mol. Cell Biol. 27 (2007) 8698–8712.
[33] D.B. Donoviel, D.D. Freed, H. Vogel, D.G. Potter, E. Hawkins, J.P. Barrish, B.N. Mathur, C.A. Turner, R. Geske, C.A. Montgomery, M. Starbuck, M. Brandt, A. Gupta, R. Ramirez-Solis, B.P. Zambrowicz, D.R. Powell, Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to NEPHRIN, Mol. Cell. Biol. 21 (2001) 4829–4836.
[34] N. Boute, O. Gribouval, S. Roselli, F. Benessy, H. Lee, A. Fuchshuber, K. Dahan, M.C. Gubler, P. Niaudet, C. Antignac, NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome, Nat. Genet. 24 (2000) 349–354.
[35] L. Sellin, T.B. Huber, P. Gerke, I. Quack, H. Pavenstadt, G. Walz, NEPH1 defines a novel family of podocin interacting proteins, FASEB J. 17 (2003) 115–117.
[36] K. Schwarz, M. Simons, J. Reiser, M.A. Saleem, C. Faul, W. Kriz, A.S. Shaw, L.B. Holzman, P. Mundel, Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin, J. Clin. Invest. 108 (2001) 1621–1629.
[37] S. Roselli, L. Heidet, M. Sich, A. Henger, M. Kretzler, M.C. Gubler, C. Antignac, Early glomerular filtration defect and severe renal disease in podocin-deficient mice, Mol. Cell. Biol. 24 (2004) 550–560.
[38] J. Reiser, K.R. Polu, C.C. Moller, P. Kenlan, M.M. Altintas, C. Wei, C. Faul, S. Herbert, I. Villegas, C. Avila-Casado, M. McGee, H. Sugimoto, D. Brown, R. Kalluri, P. Mundel, P.L. Smith, D.E. Clapham, M.R. Pollak, TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function, Nat. Genet. 37 (2005) 739–744.
[39] M.P. Winn, P.J. Conlon, K.L. Lynn, M.K. Farrington, T. Creazzo, A.F. Hawkins, N. Daskalakis, S.Y. Kwan, S. Ebersviller, J.L. Burchette, M.A. Pericak-Vance, D.N. Howell, J.M. Vance, P.B. Rosenberg, A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis, Science 308 (2005) 1801–1804.
[40] L. Ciani, A. Patel, N.D. Allen, C. ffrench-Constant, Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype, Mol. Cell. Biol. 23 (2003) 3575–3582.
[41] M. Simons, B. Hartleben, T.B. Huber, Podocyte polarity signalling, Curr. Opin. Nephrol. Hypertens. 18 (2009) 324–330.
[42] J. Patrakka, Z. Xiao, M. Nukui, M. Takemoto, L. He, A. Oddsson, L. Perisic, A. Kaukinen, C. Al-Khaliliszigyarto, M. Uhlen, H. Jalanko, C. Betsholtz, K. Tryggvason, Expression and subcellular distribution of novel glomerulus-associated proteins dendrin, Ehd3, Sh2d4a, Plekhh2, and 2310066E14Rik, J. Am. Soc. Nephrol. 18 (2007) 689–697.
[43] K. Asanuma, K.N. Campbell, K. Kim, C. Faul, P. Mundel, Nuclear relocation of the nephrin and CD2AP-binding protein dendrin promotes apoptosis of podocytes, Proc. Natl. Acad. Sci. USA 104 (2007) 10134–10139.
[44] K. Tryggvason, J. Patrakka, J. Wartiovaara, Hereditary proteinuria syndromes and mechanisms of proteinuria, N. Engl. J. Med. 354 (2006) 1387–1401.
[45] J.S. Nielsen, K.M. McNagny, The role of podocalyxin in health and disease, J. Am. Soc. Nephrol. 20 (2009) 1669–1676.
[46] R. Doyonnas, D.B. Kershaw, C. Duhme, H. Merkens, S. Chelliah, T. Graf, K.M. McNagny, Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin, J. Exp. Med. 194 (2001) 13–27.
[47] T. Takeda, T. McQuistan, R.A. Orlando, M.G. Farquhar, Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton, J. Clin. Invest. 108 (2001) 289–301.
[48] P.E. Thomas, B.L. Wharram, M. Goyal, J.E. Wiggins, L.B. Holzman, R.C. Wiggins, GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit, J. Biol. Chem. 269 (1994) 19953–19962.
[49] B.L. Wharram, M. Goyal, P.J. Gillespie, J.E. Wiggins, D.B. Kershaw, L.B. Holzman, R.C. Dysko, T.L. Saunders, L.C. Samuelson, R.C. Wiggins, Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate, J. Clin. Invest. 106 (2000) 1281–1290.
[50] J.M. Kaplan, S.H. Kim, K.N. North, H. Rennke, L.A. Correia, H.Q. Tong, B.J. Mathis, J.C. Rodriguez-Perez, P.G. Allen, A.H. Beggs, M.R. Pollak, Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis, Nat. Genet. 24 (2000) 251–256.
[51] J.L. Michaud, L.I. Lemieux, M. Dube, B.C. Vanderhyden, S.J. Robertson, C.R. Kennedy, Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant alpha-actinin-4, J. Am. Soc. Nephrol. 14 (2003) 1200–1211.
[52] A. Weins, J.S. Schlondorff, F. Nakamura, B.M. Denker, J.H. Hartwig, T.P. Stossel, M.R. Pollak, Disease-associated mutant alpha-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity, Proc. Natl. Acad. Sci. USA 104 (2007) 16080–16085.
[53] S.V. Dandapani, H. Sugimoto, B.D. Matthews, R.J. Kolb, S. Sinha, R.E. Gerszten, J. Zhou, D.E. Ingber, R. Kalluri, M.R. Pollak, Alpha-actinin-4 is required for normal podocyte adhesion, J. Biol. Chem. 282 (2007) 467–477.
[54] K. Asanuma, K. Kim, J. Oh, L. Giardino, S. Chabanis, C. Faul, J. Reiser, P. Mundel, Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner, J. Clin. Invest. 115 (2005) 1188–1198.
[55] C. Faul, M. Donnelly, S. Merscher-Gomez, Y.H. Chang, S. Franz, J. Delfgaauw, J.M. Chang, H.Y. Choi, K.N. Campbell, K. Kim, J. Reiser, P. Mundel, The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A, Nat. Med. 14 (2008) 931–938.
[56] M. Takemoto, L. He, J. Norlin, J. Patrakka, Z. Xiao, T. Petrova, C. Bondjers, J. Asp, E. Wallgard, Y. Sun, T. Samuelsson, P. Mostad, S. Lundin, N. Miura, Y. Sado, K. Alitalo, S.E. Quaggin, K. Tryggvason, C. Betsholtz, Large-scale identification of genes implicated in kidney glomerulus development and function, EMBO J. 25 (2006) 1160–1174.