Commentary

Focusing on the Glomerular Slit Diaphragm

Podocin Enters the Picture

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The nature of the glomerular filtration barrier is currently one of the most intensely studied and exciting problems in nephrology research, primarily because of the relatively recent discoveries that two novel and two previously known genes are intimately involved in glomerular filtration. The two novel genes, *NPHS1* and *NPHS2* (encoding nephrin and podocin, respectively) were identified by positional cloning. *NPHS1* is mutated in congenital nephrotic syndrome of the Finnish type,¹ and *NPHS2* is mutated in steroid resistant nephrotic syndrome.² The other two genes, previously characterized but not known to be involved in filtration, are *ACTN4*, encoding α -actinin-4, and *Cd2ap*, encoding CD2-associated protein (CD2AP). *ACTN4* was found to be mutated in several families exhibiting inherited focal segmental glomerulosclerosis,³ and knockout mice lacking CD2AP exhibit congenital nephrotic syndrome.⁴ Several recent reviews have addressed the potential roles of these molecules in glomerular filtration.^{5–10}

All four of these genes have been shown to be expressed by podocytes, the specialized epithelial cells that lie atop the glomerular basement membrane (GBM) in the urinary space. Podocytes make up the final cell layer across which the glomerular ultrafiltrate must pass before flowing down the tubular portions of the nephron toward the ureter and bladder. Podocytes elaborate long, regularly spaced, interdigitated foot processes that envelop the glomerular capillaries. Near the GBM, the foot processes are connected by a thin structure termed the glomerular slit diaphragm. The existence of the slit has been known at an ultrastructural level for decades,¹¹ but until recently its molecular composition and role in glomerular filtration had been a mystery.

Several groups have shown by immunoelectron microscopy (IEM) that nephrin is found at the slit diaphragm.^{12–15} Nephrin is a transmembrane protein with a

substantial extracellular domain, and both humans and mice lacking nephrin are born without typical slit diaphragms and exhibit massive proteinuria.^{1,16} Together, these data suggest that nephrin is an actual component of the slit and lend support to the hypothesis that the slit has a crucial role in glomerular filtration. The localization of CD2AP and α -actinin-4 proteins has also been investigated by immunohistological methods. CD2AP is an adapter molecule¹⁷ that localizes to podocytes and is capable of binding to the cytoplasmic tail of nephrin, perhaps linking the slit diaphragm to the podocyte cytoskeleton and providing stability to what might otherwise be a fragile structure.⁴ Our more recent studies demonstrate by IEM that CD2AP is present at the slit and binds to nephrin via its carboxyl terminal domain.¹⁸ Other IEM studies are consistent with these results but suggest that CD2AP is more widely distributed in the foot process,¹⁹ in agreement with our original immunofluorescence data.⁴ α -actinin-4 is a member of a family of proteins that cross-link and anchor actin filaments, and it was shown to be associated with the actin cytoskeleton in podocyte foot processes.^{3,20} The fact that mutations in *ACTN4* cause glomerulosclerosis suggests that α -actinin-4 plays a crucial role in maintaining the structure of podocyte foot processes and/or the slit diaphragm via the actin cytoskeleton.³

But what about the localization of podocin, the fourth member of this genetically defined quartet? The article by Corinne Antignac and colleagues²¹ published in this issue of *The American Journal of Pathology* clearly confirms that podocin, like nephrin, is present in the podocyte plasma membrane in the area of the slit diaphragm. The authors made several antisera that specifically recognize podocin and showed by both high-resolution confocal microscopy and IEM that podocin is found in foot processes and concentrated at the slit diaphragms. Thus, this entire quartet of molecules localizes to podocyte foot processes, with nephrin, CD2AP, and podocin being

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Table 1. Proteins that Have Been Localized to the Glomerular Slit Diaphragm

Protein	Protein class	References
ZO-1	PDZ domain-containing	22
Nephrin	Ig superfamily	12–15
P-cadherin	Classical cadherin	23
FAT	Cadherin superfamily	26
CD2AP	SH3 domain-containing adapter	18, 19
Podocin	Stomatin	21, 31

present at slit diaphragms. The fact that mutations in any of the four genes causes proteinuria and renal failure indicates that podocytes, their foot processes, and the intervening slits are likely the most critical components of the glomerular filtration barrier.

With regard to other molecules at the slit, it has been known for a long time that ZO-1, a protein normally associated with tight junctions, is localized to the cytoplasmic face of the slit.²² This led to the conclusion that the slit diaphragm is a modified tight junction. However, it was shown by IEM that P-cadherin localizes to the extracellular portion of the slit diaphragm and co-localizes with ZO-1 on the cytoplasmic face of the slit, suggesting that the slit has features of adherens junctions, which can also contain ZO-1.²³ However, the importance of P-cadherin at the slit remains to be demonstrated. The facts that 1) knockout mice lacking P-cadherin are viable and fertile with no reported kidney defects,²⁴ and 2) humans with a mutation in *CDH3*, the gene encoding P-cadherin, have hair and retinal but not kidney defects²⁵ suggests that any role P-cadherin plays in glomerular filtration is either subtle or at least partially redundant. Indeed, another member of the cadherin superfamily, FAT, has also been localized to slit diaphragms.²⁶ However, a role for FAT in kidney function has not yet been addressed. Finally, *Neph1*, the gene encoding NEPH1, a protein with weak homology to nephrin, is transcribed by podocytes, and knockout mice lacking NEPH1 develop proteinuria. Most *Neph1*^{−/−} mice die between 1 and 12 days after birth, but a few live up to 8 weeks of age.²⁷ It will be very interesting to determine the subcellular localization of NEPH1 and the mechanisms leading to the onset of proteinuria. Unlike *Nphs1*, *Neph1* is also expressed by mesangial, proximal tubular, and collecting duct cells (and in many other tissues besides kidney).²⁷ Thus, NEPH1 may have functions in the kidney quite distinct from those attributed to nephrin.

The notion that the glomerular slit diaphragm merely serves as a filter to keep albumin and other plasma proteins from entering the urinary space is being challenged by evidence that slit-associated molecules are involved in signaling events. For example, Huber and colleagues have shown that transfection of nephrin into 293T cells initiates activation of AP-1 transcriptional activity and of stress-activated p38 and c-Jun N-terminal protein kinases.²⁸ Interestingly, co-transfection of podocin synergistically increased nephrin-initiated AP-1 activation,²⁸ suggesting that nephrin and podocin may cooperate at the slit to mediate signaling. Furthermore, two groups have shown that nephrin is associated with lipid

rafts, specialized cholesterol-rich membrane domains associated with signaling molecules and signaling events.^{29,30} Podocin is also associated with lipid rafts and interacts directly with nephrin and CD2AP at the slit diaphragm.³¹

What of the forgotten role of the GBM in glomerular filtration? With all of the focus on podocytes and glomerular slit diaphragms, less attention is being paid to the GBM. Although the GBM may not be the major size-selective filter, its negative charge—imparted by the presence of heparan sulfate proteoglycans such as agrin—is likely important in establishing a charge-selective barrier to negatively charged plasma proteins. In addition, the unique molecular structure of the GBM seems to be crucial for maintaining podocyte homeostasis. Mutations that alter the composition of the GBM and that result in filtration defects may affect podocytes directly and filtration indirectly. The GBM is normally composed of laminin-11 ($\alpha 5\beta 2\gamma 1$), the collagen $\alpha 3$, $\alpha 4$, and $\alpha 5(IV)$ chains, agrin, and nidogen, as well as other matrix molecules.³² Knockout mice lacking the laminin $\beta 2$ chain have no laminin-11 in their GBMs and develop massive proteinuria at 7 days of age.³³ Laminin-11 may therefore be necessary for proper podocyte adhesion and for maintenance of foot process architecture. Similarly, mice, humans, and dogs with collagen IV gene mutations exhibit Alport syndrome, which is characterized by later onset filtration defects.^{34,35} The GBM becomes thickened and split, and novel laminin chains, such as $\alpha 2$ and $\beta 1$, accumulate within it.^{36,37} These ectopic laminins may provide atypical signals to the overlying podocytes that disrupt their behavior, leading to foot process effacement and proteinuria. Indeed, reduced levels of laminin $\alpha 2$ in Alport GBM was associated with a reduction in podocyte damage.³⁶

In conclusion, it is clear that the glomerular slit diaphragm is a major, if not the most important, component of the kidney's ultrafiltration barrier. The finding that podocin is concentrated there reinforces this notion, because mutations in *NPHS2*, the gene encoding podocin, cause nephrotic syndrome and glomerulosclerosis. Determining the function of podocin will be challenging, given that podocin is a member of the stomatin family of proteins, about which little is known. Nevertheless, the generation of knockout mice lacking podocin, which is presumably underway, will allow for those lines of experimentation that are not possible with human patients, but that will hopefully lead to a better understanding of podocin and the structure and function of the glomerular slit diaphragm.

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