How Does the Kidney Filter Plasma?

The kidneys filter the plasma in special filtration units—glomeruli—and thereby excrete low-molecular-weight waste products into the urine. The mechanisms of glomerular filtration have been a matter of controversy for several decades, but recent data have revealed new details about the molecular nature of the filter and have demonstrated a central role for the podocyte slit diaphragm in the filtration process.

Glomerular Permselectivity

The main function of kidney glomeruli is to filter low-molecular-weight plasma waste products into the urine while restricting the passage of albumin and larger macromolecules that are necessary for maintaining normal homeostasis. Every day, ~180 liters of primary urine devoid of macromolecules is normally formed in the human kidneys, each of which has ~1,000,000 glomeruli. The primary urine is extensively modified in the renal tubular system in regard to composition and volume, so that the final daily excretion is normally only ~1–1.5 l/day. The glomeruli are affected in a variety of systemic and primary kidney diseases, leading to leakage of the large plasma proteins into the urine (proteinuria). Progressive proteinuria has harmful effects on the kidney and frequently leads to permanent glomerular damage and renal failure (see Ref. 31).

The unique morphology of the glomerular capillary filter has been well known for several decades, but the molecular mechanisms of filtration have been poorly understood. However, this research area has experienced a renaissance in recent years, and research in several laboratories has provided completely new insights into the nature of the glomerular filter.

The glomerulus consists of a capillary tuft located inside the Bowman's capsule that represents the most proximal part of the nephron (FIGURE 1), the distal part of which constitutes the tubular system opening into the kidney medullary calyx. The glomerular filter residing in the capillary wall consists of three quite distinct layers: a fenestrated endothelium, a glomerular basement membrane (GBM), and a slit diaphragm located between the interdigitating foot processes of the epithelial podocytes (FIGURE 1).

Numerous physiological and biochemical studies have suggested that the glomerular filter is a size- and charge-selective barrier (10, 19, 21, 32, 33), but its exact nature has been and still is the subject of debate. The fenestrae of mature glomerular endothelia do not have any visible diaphragms and are generally considered not to be a major hindrance to the passage of large plasma molecules into the porous GBM, but negative charges of the endothelial surface have been proposed to partially prevent the traversal of plasma macromolecules (see Ref. 10). The GBM was previously considered to represent the size- and charge-selective macromolecular barrier (21), but recent studies have emphasized the podocyte slit diaphragm as being the main size-selective filter (3, 6, 18, 23). Although several questions remain unanswered, recent research has created a better possibility of understanding and combining previous controversial data into reasonable hypotheses about the mechanisms of glomerular filtration.

Research into glomerular filtration was particularly lively in the 1960s and 1970s, and since then numerous elegant physiological and electron microscopic studies have been performed to elucidate the mechanisms of this important kidney function. Through the use of tracer molecules of different molecular sizes and charges, a picture of a barrier selective for size and repulsive for anionic plasma molecules emerged. In many studies, electron microscopy of kidneys perfused with cationic probes, such as ferritin (19, 32) and lysozyme (4), revealed the presence of anionic sites in the GBM. Exclusion of anionic tracers from the GBM was considered to provide support for charge selectivity of the GBM (20, 32, 43).

Electron-microscopic studies with cationic gold particles have revealed anionic sites in the GBM immediately underneath the endothelial cells and foot processes as well as in the central portion of the matrix. Negatively charged basement membrane-specific heparan sulfate proteoglycans, such as perlecan and agrin, that are components of the GBM proper (8, 9, 11) have been suggested to contribute to an anionic GBM barrier. Membrane-bound heparan sulfate proteoglycans on the surface of endothelial cells and podocytes may also form a part of a negative filtration barrier. Kidney perfusion studies with different neutral Ficolls have indicated the presence of numerous small, 45- to 50-Å-diameter pores and a lower number of 80- to 100-Å-diameter pores (see Ref. 10), but the location...
and molecular basis of such pores have never been demonstrated.

Although most earlier studies favored the GBM to be the major size- and charge-selective barrier, investigators such as Karnovsky (22) showed, based on perfusion studies, strong evidence for the podocyte slit diaphragm being the ultimate macromolecular barrier. On the basis of electron microscopy of perfusion-fixed kidneys, his group even proposed an isoporous, zipper-like filter structure model for the slit diaphragm (34). This model was later questioned, particularly following freeze-etching results with unfixed tissue using deep etching of quick-frozen samples, which suggested a sheet-like, rather than a zipper-like, substructure for the slit diaphragm (13). However, as described below, the slit diaphragm and Karnovsky’s filter model have again obtained a central position in today’s glomerulus research.

**Does the Fenestrated Endothelium Contribute to the Macromolecular Barrier?**

The glomerular endothelial cells, with their numerous fenestrae of 50–100 nm in diameter, have generally not been considered to hinder passage of plasma macromolecules into the GBM matrix. However, the glycosaminoglycan (GAG)-rich glyocalyx of vascular endothelia has been implicated in microvascular permeability. Thus injections of infused GAG-degrading enzymes have been shown to increase blood flow and permeation of capillaries in many organs, including glomeruli. Glomerular endothelial cells have been shown to contain on their surface negatively charged sialoglycoproteins and proteoglycans (1), most of the GAG being heparan sulfate but also hyaluronate (1, 12). Therefore, the endothelial glyocalyx could potentially contribute to a primary glomerular anionic barrier that diminishes the load of macromolecules being passed into the GBM and up to the slit diaphragm. However, direct evidence for such a role of the endothelial glyocalyx remains to be presented.

Various studies with hyaluronidase, heparanase, and chondroitinase have revealed that intravenous injection or kidney perfusion with these enzymes leads to proteinuria, suggesting a crucial role for the GAG in the glomerular permselectivity (10, 12, 19). However, such experiments do not demonstrate a more significant role for endothelial GAGs over that of others within the GBM or on the podocytes, as these enzymes do get into the GBM. Whereas the endothelial glyocalyx may have a significant role in glomerular filtration, more sophisticated and specific methods that specifically ablate certain GAGs from the endothelial cells need to be tested before one can make definite conclusions in this particular matter.

**The GBM**

The GBM is an amorphous, 300- to 350-nm-thick extracellular structure that previously has been considered to be the primary size- and charge-selective macromolecular filter. Main components of the GBM are triple-helical type IV collagen, proteoglycans, laminin, and nidogen (entactin) (16, 17). Collagen IV forms a highly cross-linked three-dimensional structural meshwork to which other components are attached. In the embryo and post-
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charge selectivity of the GBM because they contain negatively charged heparan sulfate side chains (9). Also, several earlier studies reported decreased amounts of heparan sulfate in the GBM of human patients and animals with proteinuria (45). To test this hypothesis, we recently used gene targeting in embryonic stem cells to generate mice containing heparan sulfate-deficient perlecan. To our surprise, the mice had no visible GBM defects and exhibited no signs of proteinuria (35). However, they did have cataracts due to abnormal development of the lens capsule basement membrane, which demonstrates that the heparan sulfate in perlecan does have some important functions. These results cast some doubt on the role of GBM heparan sulfate in glomerular charge selectivity, but it cannot be excluded that other proteoglycans, such as agrin, can compensate for the loss of anionic charges from perlecan. Further studies need to be carried out to more exactly determine the role of GBM proteoglycans in the filter function.

Laminin is a large heterotrimeric glycoprotein that has an important role in the GBM. Laminins are considered important for cellular differentiation and adhesion, but they also have a structural function and self-assemble into a laminin network. As with type IV collagen, there is a fetal laminin-10 form (α5β1γ1) in the GBM, and this is replaced postnatally by laminin-11 (α5β2γ1) (26). Mutations in mouse laminin-11 (β2-chain gene) cause a nephrotic syndrome-like phenotype (27), which implies that this particular GBM laminin is important for the integrity of the filtration barrier.

According to the current understanding of basement membrane structure, two main independent networks formed by type IV collagen and laminin are interconnected through nidogen (entactin) and some other basement membrane components to form a tight, highly cross-linked mesh (47). This structure is porous but does not contain pores of defined sizes.

Slit Diaphragm: A Size-Selective Porous Membrane, Defects of Which Lead to Proteinuria

As early as 1972, Karnovsky and Ainsworth (22) presented evidence for the podocyte slit diaphragm being the size-selective molecular sieve, as demonstrated by using large tracer markers. Furthermore, based on electron microscopic findings, Rodewald and Karnovsky (34) proposed that the slit diaphragm has an ordered structure with pores smaller than albumin. However, direct evidence for a filter function of the slit diaphragm was not shown, and knowledge about its molecular composition remained obscure until recently. In 1998, Kestila et al. (23) isolated the gene mutated in congenital nephrotic syndrome of the Finnish type (CNF, NPHS1), a rare autosomal recessive disease characterized by massive nonselective proteinuria at birth and lack of a podocyte slit diaphragm. The disease gene was shown to encode a novel protein named "nephrin," which in the kidney is solely located in glomerular podocytes. Nephrin was the first known protein to be localized, via immunoelectron microscopy, to the slit diaphragm (36). Inactivation of the gene in mice leads to massive proteinuria, absence of a slit diaphragm, and neonatal death (28).

Recently, a few other proteins have been localized to the slit diaphragm, and furthermore, some intracellular proteins have been shown to be crucial for a functional slit diaphragm structure and function. Podocin is an integral membrane protein of podocyte foot processes that is mutated in steroid-resistant congenital nephrotic syndrome (NPHS2) (3). Two transmembrane proteins, Nep1 and a large cadherin-like protein FAT, have also been localized to the slit diaphragm area (18, 25) as well as the intracellular protein zonula occludens (ZO-1) (38), which binds to Nep family proteins (15). The CD2 adapter protein (CD2AP) is an intracellular protein that possibly interacts with the intracellular domains of nephrin and podocin (39, 41). In vitro studies have indicated that nephrin and Nep1 that colocalize in the slit diaphragm can form homo- and heterodimers through their extracellular domains (6, 7, 24), and the intracellular domains of both proteins react with podocin (14, 40). Importantly, mutations in all of these four proteins that seem to be crucial for a functional slit diaphragm protein complex have been associated

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with a nephrotic syndrome-like phenotype (3, 6, 23, 41). These data demonstrate a crucial role for the slit diaphragm in glomerular permselectivity.

Some important milestones have recently been reached in studies on glomerular filtration. The mysterious podocyte slit diaphragm, previously mostly known as a fine, dark line in electron microscopy cross-sections of the glomerular capillary wall, is beginning to show a defined composition of highly specific molecules that somehow form an ultrathin membrane structure connecting two adjacent foot processes. We know of at least four proteins that are located at this membrane, but preliminary data have shown that there are some more to come (unpublished results). However, there are several important questions that await an answer. One key question is whether or not the slit diaphragm truly is a size-selective macromolecular filter. Also, how are the proteins assembled into the slit diaphragm? Is it a rigid and possibly cross-linked structure or a flexible membrane that can relatively easily open up to allow for passage of large proteins, e.g., due to increased blood pressure or glomerular filtration? And if the slit diaphragm is the main size-selective barrier, why does it not clog? Although we do not, as yet, have good answers to these questions, we have recently taken some big steps forward.

Overwhelming recent data support the notion that the slit diaphragm is a true macromolecular barrier. Thus abnormal function or absence of most of the proteins that have been localized to the slit diaphragm protein complex, i.e., nephrin (23), Nep1 (6), FAT (5), podocin (3), or CD2AP (41), lead to proteinuria. However, P-cadherin, which has been localized to the slit diaphragm (30), does not appear to be crucial for glomerular development or function (29). We still do not know in any detail how the slit diaphragm proteins assemble, but it has been proposed that nephrin molecules from two neighboring foot processes might interact in the center of the slit to form a zipper-like structure of a certain width (FIGURE 1; Ref. 44). It has been shown that nephrin molecules can interact with each other in vitro (7, 14, 24). To address this question further, we recently applied novel electron tomography techniques (46). With this method, it is possible to visualize single macromolecules in solution and in native tissues at a 3- to 5-nm resolution (37). Using this approach, one can detect in the slit diaphragm (FIGURE 2) winding molecular strands crossing the filtration slit, frequently forming a zipper-like pattern, with pores of the size of albumin or smaller, which are located on both sides of a central density (46). The cross strands contacting each other in the slit center frequently have free distal endings. The strands have a similar appearance to that of single nephrin molecules in vitrified solution visualized with the same technique. In immunoelectron tomography, one can visualize the location of the distal extracellular nephrin IgG-like motifs close to the center of the slit (46). Kidneys deficient for nephrin (CNF patients or knockout mice) only have a narrow slit lacking a slit diaphragm. These data support the hypothesis that nephrin molecules can interact with each other in the slit and contribute to a slit diaphragm skeleton of a constant width. But how are other molecules, such as Nep1 and FAT, located in the slit diaphragm? It was apparent from the electron tomography studies that the slit diaphragm is not a single molecular layer but that it frequently can be observed to have two or even three layers. These layers are interconnected by molecular strands (46), and it is feasible to propose that, for example, the huge cadherin-like FAT protein is located in such structures. Nep1, with its estimated maximum extracellular length of 15–20 nm, is too short to be able to reach deep into the ~40-nm-wide filtration slit. The location of these proteins and their different domains need to be addressed by using immunoelectron tomography. It seems plausible to believe that a combination of protein crystallographic data and results from high-resolution electron tomography combined with immunoelectron microscopy can in the near future provide quite a detailed understanding of the three-dimensional structure of slit diaphragm molecules.

Although the presently available data have demonstrated a significant role for the slit diaphragm as the major physical barrier for albumin and other plasma macromolecules, it is still an open question whether or not the slit diaphragm is a true size-selective macromolecular barrier. Thus, we can ask the same questions as before: Does the slit diaphragm really prevent the passage of proteins and other plasma macromolecules? And if so, why? Although we do not yet have good answers to these questions, we have recently taken some big steps forward.

By combining immunoelectron microscopy with novel electron tomography techniques, we have shown that nephrin molecules can interact with each other in the slit and contribute to a slit diaphragm skeleton of a constant width. But how are other molecules, such as Nep1 and FAT, located in the slit diaphragm? It was apparent from the electron tomography studies that the slit diaphragm is not a single molecular layer but that it frequently can be observed to have two or even three layers. These layers are interconnected by molecular strands (46), and it is feasible to propose that, for example, the huge cadherin-like FAT protein is located in such structures. Nep1, with its estimated maximum extracellular length of 15–20 nm, is too short to be able to reach deep into the ~40-nm-wide filtration slit. The location of these proteins and their different domains need to be addressed by using immunoelectron tomography. It seems plausible to believe that a combination of protein crystallographic data and results from high-resolution electron tomography combined with immunoelectron microscopy can in the near future provide quite a detailed understanding of the three-dimensional structure of slit diaphragm molecules.
open question as to why its small pores or channels do not clog. It is possible that the negative charges of the GBM and podocyte cell surfaces, a gel exclusion effect (42), or some other as yet unidentified mechanisms are necessary for hindering such clogging.

Conclusion

During the past six years, kidney research has facilitated new insights into molecular aspects of the glomerular barrier and mechanisms of proteinuria. This work has particularly elucidated the molecular composition of the podocyte slit diaphragm and has demonstrated its crucial role in macromolecular filtration. However, it is also clear that the GBM somehow contributes to the filtration process, and a role for the endothelial cell layer and its glycocalyx is not excluded. Glomerular research is currently in a very active phase, and this work can, in the near future, be expected to generate extensive new knowledge that will explain the mechanisms of glomerular filtration and development of disease.

References


