PROTEOGLYCANS (PGs) consist of a core protein and linear sugar side chains [glycosaminoglycans (GAGs)] attached to the core protein, e.g., agrin, perlecan, glypican, and syndecan. GAGs comprise repeating duplex sugar units made of an aminosugar (e.g., N-acetylgalcosamine or N-acetylgalactosamine) linked to a uronic acid (glucuronic acid or iduronic acid) that are sulfated (3). The sulfate and carboxyl groups in the GAG side chains endow the PG with negative charge. Variations in the composition of aminosugar and uronic acid give rise to defined GAGs, e.g., heparin, heparan sulfate, and chondroitin sulfate. Individual PGs demonstrate a high degree of variability among different cells and tissues. PGs can exist on the cell surface and within the cells; they are also secreted and deposited in the extracellular matrix.

Some cell surface PGs are transmembrane proteins (e.g., syndecan), whereas others are attached via a glycosylphosphatidylinositol anchor (e.g., glypican). Syndecans function as signaling molecules, facilitating cell responses to changes in their microenvironment. The cell surface PGs participate in cell anchorage in collaboration with integrins. Cell membrane PGs can cooperate with receptor tyrosine kinases and modulate their function following ligand binding. Cellular PGs can also be concentrated in secretory granules, where they may help store proteases via charge interactions. Serglycin, a secretory granule PG, is involved in heparin production by mast cells.

PGs secreted by cells into the surrounding matrix are called basement membrane and interstitial PGs. They form a complex with other basement membrane components such as collagen and laminin. Basement membrane proteoglycans, e.g., perlecan, agrin, and collagen types XV and XVIII, serve as anchors for epithelial cells and regulate their differentiation and migration. They can also regulate the filtration property of the basement membrane. Interstitial PGs include aggrecan and the small leucine-rich PGs (decorin, biglycan). The hyalectan family of PGs, e.g., aggrecan and versican, sequester water within their molecular aggregate structure and impart a gel-like consistency to the matrix, whereas other interstitial PGs such as the small leucine-rich PGs regulate collagen fibril diameter during assembly and also serve to bind growth factors.

The glomerular basement membrane (GBM) is said to regulate passage of molecules via a size- and charge-selective barrier. The anionic sites in the lamina rara externa and interna of the GBM were thought to be important in repelling albumin during filtration. Earlier studies showed that heparan sulfate (HS) GAGs contributed importantly to the negative charges in the GBM (5). However, subsequent studies employing genetic models in which glomerular heparan sulfate proteoglycan (HSPG) was deficient showed only a minor degree of albuminuria. Agrin synthesized by the podocyte is the major HSPG in the GBM. Mice with podocyte-specific expression of agrin mutants lacking HS did not develop significant albuminuria (4). However, HS in GBM may be important for preventing clogging of the GBM during filtration (9). Thus, the function of HSPG synthesized by the podocyte has remained a mystery.

HS synthesis begins with attachment of a tetrasaccharide complex xylose-galactose-galactose-glucuronic acid to the serine of the core protein (Fig. 1). HS addition starts with the loading of N-acetylgalcosamine catalyzed by Extl-3 (exostosin-like glycosyltransferase-3) and possibly Extl-2. This step is followed by the addition of glucuronic acid and N-acetylgalcosamine in a repetitive manner facilitated by a copolymerase exostosin-1 (Ext-1)/exostosin-2 (Ext-2); the sugars are provided by UDP precursors. The disaccharides are then sulfated on the glucosamine or glucuronic acid under the control of sulfotransferases, with the 3-phosphoadenyl-5′-phosphosulfate serving as a sulfate donor. N-sulfation of N-acetylgalcosamine occurs by the removal of the N-acetyl group, followed by the addition of sulfate under the control of N-deacetylas/N-sulfotransferase (Ndst). Subsequently, O-sulfation can occur at specific sites of the disaccharide catalyzed by specific sulfotransferases.

McCarthy et al. (1) have systematically studied the consequences of interrupting this sequence of events in HS synthesis in the podocyte. Podocyte-specific Ext-1 deletion (PExtKO) in mice reduced the deposition of HS GAGs in the GBM, resulting in reduction in anionic sites, effacement of podocyte foot processes, and splitting and outpocketing of the GBM; glomerular and renal hypertrophy were also seen. Interestingly, agrin was increased in the mesangium of the PExtKO mice, suggesting cues to mesangial cells from HS-deficient podocytes to augment agrin synthesis. Albuminuria showed a nonsignificant minor increment at 8 mo of age, confirming previous observations that podocyte-associated and podocyte-derived GBM HSPG may not play a major regulatory role in glomerular permselectivity (4). Given the podocyte foot process effacement in PExtKO mice, the podocyte interaction with matrix was explored, focusing on cell surface HSPG syndecan-4 (2). HS-deficient podocytes generated by Ext-1 deletion showed reduced attachment to fibrinogen matrix, impaired spreading, and migration. HS cells expressed a greater amount of syndecan-4 core protein on the cell surface, and distribution of protein kinase-Cα (PKCα) to the cell membrane was increased relative to cytosol. These data suggest that HS GAGs in syndecan-4 may constitutively inhibit core protein expression in a negative feedback manner.

Having shown the importance of HS syndecan-4 in the podocyte-matrix interaction, further molecular refinement was sought by focusing on N-sulfation of HS side chains. Thus, mice with specific deficiency of Ndst-1 in the podocyte were generated, which showed the aforementioned phenotype of podocyte foot process effacement and glomerular hypertrophy (7). Age-related increase in albuminuria was amplified in the
podocyte Ndst-1 knockout mice. These data identified $N$-sulfation in HS as a critical determinant of podocyte-matrix interaction. Additionally, there was a surprising suggestion that $N$-sulfation of a podocyte cell surface HS could regulate the overall glomerular size. In contrast to the PXeKO mice lacking HS chains with their $N$- and $O$-sulfation, the anionic site density in the GBM in the podocyte-specific Ndst-1 knockout mice was unaffected probably because of intact $O$-sulfation of HS.

Sugar et al. (8) have extended their studies on the effect of the absence of HS $N$-sulfation by Ndst-1 on podocyte biology. The investigators generated mice by crossing the immortal mouse with the floxed Ndst-1 mouse. The immortalized podocytes were grown from isolated glomeruli, and then the Ndst-1 gene was deleted using adeno viral-mediated delivery of GFP-Cre recombinase. Lack of $N$-sulfation of HS impaired the association of syndecan-4 with $\alpha$-actinin-4, and attachment and spreading of podocytes on fibronectin matrix. However, since over time the attachment of Ndst-1-deficient podocytes to fibronectin appears to catch up with the control cells, some compensation may occur via $O$-sulfation. Migration following wounding in a scratch assay was also less efficient in Ndst-1-deficient podocytes. The interaction of integrins and syndecan $\alpha$-integrin, activation of focal adhesion kinase (FAK) were all impaired in Ndst-1-deficient podocytes. The interaction of integrins and syndecan facilitates cell matrix interaction; lack of $N$-sulfation of HS side chains resulted in decreased engagement of $\alpha$-integrin at the cell surface with a matrix component without reduction in the $\alpha$-integrin content. These studies demonstrate that $S$-sulfation of extracellular HS chains in syndecan-4 regulates extracellular events, e.g., cell matrix interaction, as well as intracellular colocalization of the core protein with signaling molecules such as PKC$\alpha$.

These are exciting findings that lay the groundwork for future exploration. 1) The manner in which cell surface HS chains of syndecan-4 affect intracellular processes remains to be addressed. In podocyte injury, FAK activation by phosphorylation is implicated in foot process effacement (6), a feature that is also present in podocytes from PXeKO and Ndst-1$^{-/-}$ mice but with reduced FAK phosphorylation. The interaction between syndecan-4 and FAK in the podocyte injury would be interesting. 2) Disruption of podocyte HSPG is associated with glomerular hypertrophy, suggesting that it may normally inhibit general protein synthesis in not only the parent cell but perhaps in other glomerular cells. The mechanisms by which podocyte syndecan-4 regulates signaling pathways that govern protein synthesis, such as mechanistic target of rapamycin, and how it facilitates cross-talk among glomerular cells would also be of great interest.

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Fig. 1. A schematic showing important steps in heparan sulfate (HS) synthesis. The process begins with the addition of a xylose (Xyl), galactose (Gal), and glucuronic acid (GlcA) to the serine residue of the core protein. HS synthesis occurs with the loading of N-acetylgalactosamine (GlcNAc) catalyzed by ExtI-3 (exostosin-like glycosyltransferase-3), followed by the addition of GlcA and N-acetylgalactosamine (GlcNAc) in a repetitive manner facilitated by a copolymerase exostosin-1 (ExtI-1)/exostosin-2 (Ext-2). $N$-sulfation of GlcNAc is catalyzed by N-deacetylase N-sulfotransferase-1 (NDST-1).