KIDNEY ORGANOGENESIS

Organogenesis requires the coordinated development of many different cell types and their incorporation into complex three-dimensional assemblies. The pioneering work of Clifford Grobstein in the 1950s established the kidney as a model system for studies of organogenesis. His work has also revealed that reciprocal inductive tissue interactions between intermediate-mesoderm-derived epithelial and mesenchymal cells are instrumental for kidney organogenesis (Grobstein, 1956; Saxén, 1987).

Development of the murine renal system starts at embryonic day (E) 7.5, when a subpopulation of mesenchymal cells forms an epithelial tube: the Wolffian (or pronephric) duct. The duct extends rostrocaudally and induces the formation of epithelial tubules in the adjacent nephrogenic mesenchyme. Three embryonic kidneys are formed in consecutive order: the pronephros, at around E8; the mesonephros at E9.5-E11; and the metanephros, which starts to form at about E11. Only the metanephros persists throughout the life of the individual, and it forms the functional kidney after birth. Metanephric development begins when a small epithelial bud, the ureteric bud, emerges from the Wolffian duct and grows dorsally to invade the metanephric mesenchyme (Fig. 1). This mesenchyme lies at the caudal end of the intermediate mesoderm. Growth and branching of the ureteric bud epithelium is induced by signals from the metanephric mesenchyme. Reciprocally, signals from the ureteric bud trigger differentiation of the mesenchyme, which leads to the establishment of different cell types. A subpopulation of mesenchymal cells differentiates into epithelial tubules (Fig. 1). Epithelialization starts with the formation of mesenchymal condensates, which subsequently are transformed via renal vesicles, comma-shaped bodies and S-shaped bodies into polarized epithelia that fuse with the ureter epithelium. The cycle of growth and branching of the ureter, formation of epithelial tubules from mesenchymal cells, and fusion of the two structures is then repeated many times. Finally, the many branches of the ureter epithelium mature into the collecting-duct tree of the kidney, which opens into the renal pelvis. The epithelial tubules derived from mesenchymal cells undergo a complex series of morphogenetic events that lead to the formation of excretory nephrons (reviewed by Saxén, 1987).

Our understanding of the molecular events underlying metanephric development has been facilitated by the identification of an ever-growing number of genes that have specific expression patterns in the developing kidney (Davies and Brändli, 1996; http://www.ana.ed.ac.uk/anatomy/database/kidbase/kidhome.html). Exploitation of the kidney organ culture system, studies of genetic kidney diseases and studies of mice carrying targeted mutations in genes leading to kidney defects have yielded crucial information on gene function. Although these experimental approaches are very informative, they have led to some conflicting results, which we discuss below. In this review we focus on the formation of the metanephric kidney, for which most experimental evidence is available. Several recent

SUMMARY

Functional analyses of cell-matrix interactions during kidney organogenesis have provided compelling evidence that extracellular-matrix glycoproteins and their receptors play instructive roles during kidney development. Two concepts are worthy of emphasis. First, matrix molecules appear to regulate signal transduction pathways, either by activating cell-surface receptors such as integrins directly or by modulating the activity of signaling molecules such as WNTs. Second, basement membranes are highly organized structures and have distinct molecular compositions, which are optimized for their diverse functions. The importance of these findings is highlighted by the fact that mutations affecting basement-membrane components lead to inherited forms of kidney disease.

Key words: Kidney development, Extracellular matrix, Basement membrane, Cell adhesion, Renal disease

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COMMENTARY

Cell adhesion molecules and extracellular-matrix constituents in kidney development and disease

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reviews summarize our current knowledge of the molecular mechanisms regulating kidney development (e.g. Lechner and Dressler, 1997; Vainio and Müller, 1997; Lipschutz, 1998; Davies and Bard, 1998; Perantoni, 1999; Wilson and Burrow, 1999). Here, we emphasize the role of extracellular matrix (ECM) constituents and matrix receptors in kidney development and disease. Fig. 2 and Tables 1 and 2 provide an overview of the molecules and diseases that we discuss.

REGULATION OF URETER GROWTH AND BRANCHING

GDNF, WNTs and proteoglycans

Gene targeting and organ-culture experiments have shed light on the molecular identity of some of the earliest inductive signals and their receptors in the developing metanephric kidney. These results suggest that secreted signaling molecules, ECM glycoproteins and matrix receptors play important roles in the formation, growth and branching of the ureter epithelium. One of the earliest inductive signals for ureter growth is provided by the secreted signaling molecule glial-cell-line-derived neurotrophic factor (GDNF). GDNF is expressed not only in the nervous system but also in the metanephric mesenchyme. Its receptor is a heteromeric complex composed of the Ret receptor tyrosine kinase and associated glycosylphosphatidyl inositol (GPI)-linked coreceptors. The GDNF-receptor complex is expressed in the Wolffian duct and its derivative, the ureteric bud. The ureteric bud fails to develop in mice carrying targeted

<table>
<thead>
<tr>
<th>Gene product</th>
<th>Phenotype of mutant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparan sulfate-2 sulfotransferase (HS2ST)</td>
<td>Defective ureter branching; impaired condensation of the metanephric mesenchyme</td>
<td>Bullock et al., 1998</td>
</tr>
<tr>
<td>Integrin α8 subunit</td>
<td>Defective ureter growth and branching; impaired development of the metanephric mesenchyme</td>
<td>Müller et al., 1997</td>
</tr>
<tr>
<td>Integrin α3 subunit</td>
<td>Decreased branching of medullary collecting ducts; abnormal proximal tubules; multiple defects in the glomerulus</td>
<td>Kreidberg et al., 1996</td>
</tr>
<tr>
<td>Laminin β2</td>
<td>Impaired filtration function of the glomerular basement membrane; proteinuria</td>
<td>Noakes et al., 1995</td>
</tr>
<tr>
<td>Collagen α3 (IV)</td>
<td>Structural changes in the glomerular basement membrane; glomerular filtration decreases over time</td>
<td>Miner and Sanes, 1996</td>
</tr>
</tbody>
</table>
mutations in the Gdnf gene or in genes that encode for components of the GDNF-receptor complex (Schuchardt et al., 1994, 1996; Moore et al., 1996; Pichel et al., 1996; Sanchez et al., 1996; Cacalano et al., 1998; Enomoto et al., 1998). It is not clear how GDNF induces formation, growth and branching of the ureter epithelium. GDNF can serve as a chemoattractant for ureter epithelial cells in organ culture and for the renal epithelial MDCK cell line in vitro (Vega et al., 1996; Sainio et al., 1997; Pepicelli et al., 1997; Tang et al., 1998). GDNF also enhances the motility of MDCK cells, reduces cell adhesion and increases formation of lamellipodia and filopodia (Tang et al., 1998). It is tempting to speculate that GDNF modulates the activity of cell surface receptors that regulate cell migration, such as integrins.

Given that epithelial-mesenchymal interactions stimulate

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**Table 2. Human renal disorders linked to extracellular matrix deficiencies**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Gene product</th>
<th>Protein function/localization</th>
<th>Transgenic mouse model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kallmann syndrome type 1</td>
<td>X-linked</td>
<td>KAL1 (anosmin-1)</td>
<td>Extracellular matrix protein</td>
<td></td>
</tr>
<tr>
<td>Alport syndrome type I</td>
<td>Autosomal recessive</td>
<td>Collagen 3(IV)</td>
<td>GBM protein</td>
<td>Cosgrove et al., 1996;</td>
</tr>
<tr>
<td>Alport syndrome type II</td>
<td>Autosomal recessive</td>
<td>Collagen 4(IV)</td>
<td>GBM protein</td>
<td>Miner and Sanes, 1996</td>
</tr>
<tr>
<td>Congenital nephrotic syndrome (Finnish-type)</td>
<td>Autosomal recessive</td>
<td>Collagen 5(IV)</td>
<td>Integral membrane protein</td>
<td></td>
</tr>
<tr>
<td>Nail-patella syndrome</td>
<td>Autosomal dominant</td>
<td>LMX1B</td>
<td>Transcription factor</td>
<td>Chen et al., 1998</td>
</tr>
<tr>
<td>Denys-Drash syndrome</td>
<td>Autosomal dominant</td>
<td>WT1</td>
<td>Transcription factor</td>
<td>Patek et al., 1999</td>
</tr>
<tr>
<td>Frasier syndrome</td>
<td>Autosomal dominant</td>
<td>WT1</td>
<td>Transcription factor</td>
<td></td>
</tr>
<tr>
<td>Tubular disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease type 1</td>
<td>Autosomal dominant</td>
<td>Polycystin-1</td>
<td>Integral membrane protein</td>
<td>Lu et al., 1999</td>
</tr>
<tr>
<td>Polycystic kidney disease type 2</td>
<td>Autosomal dominant</td>
<td>Polycystin-2</td>
<td>Integral membrane protein</td>
<td>Lu et al., 1997</td>
</tr>
<tr>
<td>Familial juvenile nephronophthisis type 1</td>
<td>Autosomal recessive</td>
<td>NPHP-1</td>
<td>SH3 domain protein</td>
<td>Wu et al., 1998</td>
</tr>
</tbody>
</table>

GBM, glomerular basement membrane.
renal-cell proliferation and regulate cell survival (Ekblom et al., 1983; Weller et al., 1991), the induction of local cell proliferation might represent an additional function of GDNF. GDNF-induced cell proliferation in the Wolffian duct could thereby drive ureteric-bud formation and growth (Vega et al., 1996; Pepicelli et al., 1997; Srinivas et al., 1999).

Promotion of cell proliferation could be a direct consequence of activation of the Ret signaling pathway. GDNF-mediated cell cycle control could also be achieved indirectly. For example, GDNF might activate the expression of secreted signaling molecules that then act in an autocrine manner to promote ureter proliferation. Good candidates for such signaling molecules are WNTs. The expression of one member of the Wnt gene family, Wnt-11, is induced in the Wolffian duct adjacent to the metanephric mesenchyme, which suggests that it is expressed in response to GDNF (Kispert et al., 1996). Subsequent to its initial induction, Wnt-11 expression is maintained in the tip of the growing ureter. Surprisingly, Wnt-11 expression is lost in the presence of proteoglycan-synthesis inhibitors (Kispert et al., 1996). These inhibitors also perturb growth and branching of the ureter epithelium (Davies et al., 1995), which raises the possibility that WNT-11 and proteoglycans act in a common pathway. The phenotype of mice carrying a gene-trap insertion mutation in the gene that encodes heparan sulfate 2-sulfotransferase, an enzyme involved in heparan sulfate biosynthesis, provides further support for such a hypothesis. Expression of Wnt-11, Gdnf and RET is severely affected in these mice, and ureter branching and condensation of the metanephric mesenchyme are impaired (Bullock et al., 1998). Taken together, these findings suggest a molecular loop, in which molecules such as WNT-11 and proteoglycans act downstream of GDNF to regulate ureter growth and differentiation. However, mice homozygous for a likely null mutation of Wnt-11 do not exhibit an overt kidney phenotype (Kispert et al., 1998). Other WNT molecules expressed in the ureter epithelium, such as WNT-7a (Kispert et al., 1996), might compensate for the loss of WNT-11, a model that needs to be tested.

The identity of the proteoglycans that regulate ureter morphogenesis and their precise mode of action remain to be established. Proteoglycans bind to constituents of the ECM, such as collagen and fibronectin, but also act as low affinity receptors for many growth factors, including fibroblast growth factors, WNTs, hedgehogs, bone-morphogenetic proteins and transforming growth factors (Rapraeger et al., 1991; Yayon et al., 1991; Bernfield et al., 1992; Lee et al., 1994; Reichsman et al., 1996; Ruppert et al., 1996; Binari et al., 1997; Woods et al., 1998). Studies in Drosophila provide good evidence that signaling by Wingless, a homologue of mammalian WNT-1, is dependent on the synthesis of glycosaminoglycans (GAGs). GAGs appear to be required in cells receiving the Wingless signal; this raises the possibility that GAGs are part of the Wingless signal transduction machinery (Reichsman et al., 1996; Binari et al., 1994; Hacker et al., 1997; Haerry et al., 1997). Thus, proteoglycans could modulate the activity of signaling molecules or could regulate adhesive interactions, or both.

**Basement membrane proteins and their receptors**

The first genetic data implicating matrix receptors in ureter development stem from analyses of mice carrying targeted mutations in genes that encode integrins. Integrins are a large family of heterodimeric cell-surface receptors, composed of α and β subunits, that serve as receptors for a wide variety of molecules, including ECM glycoproteins, Ig-superfamily members, ADAMS and cadherins (Sonnenberg, 1993; Wang and Springer, 1998; Evans, 1999). The expression of one member of this extended family, the integrin αβ1, is induced in mesenchymal cells adjacent to the ureter tip. Growth and branching of the ureter epithelium are severely impaired in αβ1-deficient mice, and development of the metanephric mesenchyme is perturbed. This clearly demonstrates the importance of this receptor molecule in kidney organogenesis (Müller et al., 1997).

The integrin αβ1 is expressed in the mesenchyme, but its expression is required in a non-cell-autonomous manner for development of the ureter epithelium. Therefore, it seems likely that the epithelium expresses ligands or counter-receptors mediating the effects of the integrin αβ1. We used a soluble recombinant αβ1 heterodimer containing an alkaline-phosphatase tag at the C terminus of the β1 subunit as a ligand detection tool on tissue section, where it strongly stains developing kidney tubules (Müller et al., 1997; Denda et al., 1998a,b). At least two integrin αβ1 ligands are expressed in ureter epithelial cells: the ECM molecule osteopontin (Denda et al., 1998a) and a previously unidentified ECM constituent (R. Brandenberger and L. F. Reichardt, University of California at San Francisco, personal communication). Antibodies against osteopontin disrupt kidney morphogenesis in organ culture (Rogers et al., 1997). However, no obvious defects have been detected in the kidneys of mice that lack osteopontin (Liaw et al., 1998). This suggests that in vivo compensatory mechanisms mask a requirement for osteopontin. The identification of a second integrin αβ1 ligand in the ureter epithelium opens the door to address this issue. Mutant mouse strains lacking this second ligand, alone or in combination with osteopontin, should clarify whether these molecules mediate integrin αβ1 functions in the kidney. It can not at present be excluded that additional ligands for the integrin αβ1 are expressed in the ureter epithelium.

In the future, it will be important to determine whether the primary function of the integrin αβ1 is to establish adhesive contact between the ureter epithelium and the surrounding mesenchyme. Alternatively, αβ1 could have instructive roles. For example, the integrin might be involved in the assembly of a specialized matrix between the ureter epithelium and the mesenchyme that helps to localize signaling molecules close to their site of action. The differentiation defect seen in the metanephric mesenchyme of mice that lack integrin αβ could also indicate that integrin αβ1 has additional functions, such as transmission of extracellular signals into mesenchymal cells (Müller et al., 1997).

**TUBULOGENESIS**

**WNTs and proteoglycans**

The original organ-culture experiments described by Grobstein provided evidence that direct cell-cell contact between the ureter epithelium and the metanephric mesenchyme is required for the transformation of mesenchymal cell into epithelial tubules (Grobstein, 1956; Saxén, 1987). Although the molecular nature of the inducer molecules is still elusive,
current evidence is consistent with the model that multiple signals regulate tubulogenesis. Upon contact with the ureter, initial signals appear to induce in the metanephric mesenchyme the differentiation of multiple cell lineages. Some metanephric mesenchymal cells become committed to the epithelial cell lineage that gives rise to kidney tubules; other cells differentiate into stromal cells. The subsequent differentiation of cells of the epithelial cell lineage into tubular structures seems to require signals not only from stromal cells but also from the cells that are already committed to the epithelial lineage.

Evidence for signals provided by stromal cells comes from analysis of mice carrying targeted mutations in a gene encoding a stromal-cell-specific transcription factor, BF2. In BF2-deficient mice, formation of stromal cells and development of epithelial tubules is defective (Hatini et al., 1996). The data suggest that BF2 controls the expression of a stromal signaling molecule that acts on epithelial cells to regulate their further differentiation. Evidence for signals exchanged between cells committed to the epithelial cell lineage was obtained from the analysis of WNT-4 functions in kidney organogenesis (Stark et al., 1994). WNT-4 expression is induced upon contact with the ureter in those mesenchymal cells that will subsequently epithelialize. In Wnt-4-deficient mice the epithelialization process is initiated, but cannot progress; this suggests that WNT-4 is a mesenchymal autoducer of tubulogenesis (Stark et al., 1994). The fact that recombinant WNT-4 can trigger tubulogenesis in isolated metanephric mesenchyme supports this model (Kispert et al., 1998).

Strikingly, addition of NaClO₃, a competitive inhibitor of sulfation of glycosaminoglycans side chains, blocks tubule induction in organ culture (Davies et al., 1995; Kispert et al., 1998). Proteoglycans might therefore modulate the signaling events that regulate the formation of epithelial tubules from mesenchymal cells, much as they appear to regulate the signaling events regulating growth and branching of the ureter (see above). In analogy to the potential functional interactions between proteoglycans and WNT-11 in the formation of the ureter, it is tempting to speculate that proteoglycans and WNT-4 functionally interact during tubulogenesis.

**Basement membrane proteins and their receptors**

A basement membrane that contains matrix molecules including laminin, collagen IV, entactin/nidogen and perlecan forms around the developing kidney tubules as a result of epithelial-mesenchymal interactions. Laminins are a family of αβγ heterotrimers. Multiple genes encode several α, β and γ subunits, and the expression of these subunits changes dramatically during the induction, formation and maturation of polarized kidney tubules (Eklblom et al., 1998; Miner, 1998). Two laminin receptors, the integrin α6β1 and α-dystroglycan, are expressed on forming epithelial cells (Fig. 3) (Korhonen et al., 1990a,b; Sorokin et al., 1990; Durbeej et al., 1995). Splice variants of the integrin α6 subunit, differing only in the cytoplasmic domain, the α6A and α6B isoforms, have been identified, and it is the α6B variant that is predominantly expressed in the kidney (Falk et al., 1996). Antibodies that were generated against the E8 and E3 proteolytic fragments of one laminin isoform, laminin-1, and might recognize other laminin isoforms as well, perturb kidney-tubule formation in organ culture (Klein et al., 1988; Sorokin et al., 1992). Antibodies directed against the integrin α6 subunit and against α-dystroglycan also disrupt kidney tubulogenesis (Sorokin et al., 1990; Durbeej et al., 1995; Falk et al., 1996). Given that the integrin α6β1 binds to the E8 fragment of laminin and α-dystroglycan binds to the E3 fragment, both receptors might cooperate to mediate laminin functions during kidney tubulogenesis (Gullberg and Ekblom, 1995; Ekblom, 1996).

Surprisingly, no apparent defects have been observed in kidneys of mice carrying a targeted mutation in the gene that encodes the integrin α6 subunit (Georges-Labouesse et al., 1996). Although more-detailed analysis might reveal some defects in the structure or function of kidneys in these mice, other members of the integrin family or α-dystroglycan could compensate for the loss of α6 function. Unfortunately, mice that lack α-dystroglycan die too early for assessment of its role in kidney morphogenesis (Williamson et al., 1997). It will therefore be necessary to establish mouse strains carrying alleles suitable for the tissue-specific gene inactivation of α-dystroglycan to elucidate its functions during kidney tubulogenesis.

A still-open question is at which step during tubulogenesis integrins and α-dystroglycan are required. Both receptors can regulate matrix assembly in vivo, which suggests that their major function is the assembly of a basement membrane around forming epithelial tubules (Kreidberg et al., 1996; Bloch et al., 1997; DiPersio et al., 1997; Williamson et al., 1997; Fleischmajer et al., 1998; Sasaki et al., 1998; Henry and Campbell, 1998). Indeed, an additional line of evidence suggests that matrix assembly is important for kidney tubulogenesis. On the basis of biochemical evidence, a model has been proposed in which laminin and collagen IV are crosslinked during basement-membrane assembly by another component of the basement membrane, entactin/nidogen-I (Yurchenco, 1994; Eklblom et al., 1996; Timpl and Brown, 1996). Antibodies directed against the entactin/nidogen-I binding site on the laminin γ1 chain perturb kidney tubulogenesis in organ culture (Eklblom et al., 1994), which is consistent with the interpretation that disruption of matrix assembly impairs kidney tubulogenesis (Fig. 3).

Taken together, the data support a model in which matrix receptors such as integrins and α-dystroglycan expressed on forming epithelial cells interact with basement-membrane components secreted from epithelial and mesenchymal cells. These interactions lead to the assembly of an organized matrix around forming epithelial tubules, which is essential for the development of polarized epithelia. The scenario is reminiscent of the mammary gland, in which formation of polarized epithelia is similarly dependent on the assembly of a basal lamina. Matrix receptors in the mammary gland cooperate with growth factor receptors to activate signal transduction cascades in differentiating epithelial cells (Roskelly et al., 1995; Boudreau and Bissell, 1998). This suggests that cooperative signaling also plays a role during kidney tubulogenesis.

**DEVELOPMENT OF EXCRETORY NEPHRONS**

**Cadherins**

Once tubule formation is initiated, mesenchymal cells undergo a complex series of transformations that leads to the development of excretory nephrons. The molecular mechanisms governing tubule morphogenesis are poorly
understood, but the renal expression patterns of cell adhesion molecules suggest that they play important roles in the process. Of particular interest are the cadherins, calcium-dependent cell-adhesion molecules that are implicated in regulating epithelialization processes elsewhere in the embryo and allow selective cell aggregation, thereby defining boundaries between groups of cells (Gumbiner, 1996; Suzuki, 1996). Various cadherins are expressed in restricted segments of the developing nephrons (Vestweber et al., 1985; Xiang et al., 1994; Thomson et al., 1995; Piepenhagen and Nelson, 1995; Goto et al., 1998; Cho et al., 1998). Antibodies directed against cadherin-6 disrupt formation of epithelial kidney tubules in organ culture (Cho et al., 1998); definitive functional studies for other cadherin molecules have not been performed. Thus, the precise functions of individual cadherins during kidney development remain to be established.

**Basement membrane proteins and integrins**

ECM receptors such as integrins, and matrix components such as laminins and collagens, are also expressed in complex patterns during tubule morphogenesis (Korhonen et al., 1990a,b; Sorokin et al., 1990; Patey et al., 1994; Sterk et al., 1998; Miner, 1998; Ekblo, 1998). The available data support the view that these molecules regulate formation and function of the glomerulus, which develops at the proximal end of the nephrogenic tubules. The process involves the invasion of endothelial cells into the glomerular cleft, a space between the lower and middle limb of the late-S-shaped tubule. Lining the lower limb of the S-shaped tubule are the visceral epithelial cells that differentiate into the glomerular podocytes. As the podocytes mature, their basal surfaces develop into foot processes. These foot processes form a meshwork scaffold that supports the capillary loops that are formed by the endothelial cells (Potter, 1965; Osathanondh and Potter, 1966a,b). During development of the glomerulus, an extensive and highly specialized glomerular basement membrane (GBM) is assembled. The GBM consists of a crosslinked meshwork of collagen (mostly trimers of type IV α chains), laminin, polyanionic proteoglycans (mostly heparan sulfate), fibronectin, entactin/nidogen and several other glycoproteins (Kasinath and Kanwar, 1993). Strikingly, the composition of the renal basement membranes changes during development (McCarthy, 1997; Miner, 1998). The basement membranes of pretubular aggregates, and S-shaped and comma-shaped bodies contain the laminin β1 chain, and the collagen α1 and α2 type IV subunits, but there is a switch in subunit composition around the stage when capillary loops form. Expression of laminin β1, as well as of collagens α1(IV) and α2(IV), is downregulated. Instead, laminin β2 and the collagen α3(IV)-α5(IV) chains are expressed (Miner and Sanes, 1994; Sterk et al., 1998). Mice carrying targeted mutations in the genes that encode laminin β2 and collagen α3(IV) have been generated (Noakes et al., 1995; Miner and Sanes, 1996; Cosgrove et al., 1996). In mice lacking laminin β2, laminin β1 expression persists. Similarly, in mice lacking collagen α3(IV), expression of the fetal α1(IV) and α2(IV) chains persists. Mutations in either gene lead eventually to kidney malfunction and death of the mice; this supports the view that the composition of the basement membrane is essential for kidney function.

Analyses of the expression of several GBM components, such as collagen IV, agrin and perlecan, and electron-microscopic studies indicate that the structural integrity of the GBM appears to be maintained in mice lacking laminin β2. However, functional deficits are obvious: filtration is affected, which leads to proteinuria (Noakes et al., 1995). In mice lacking collagen α3(IV), there are dramatic changes in the structure of the basement membrane prior to detectable functional defects, including ectopic deposition of basement membrane components such as collagen α1(IV), α2(IV), perlecan and fibronectin. Although these changes are profound, they are also selective: expression of laminin and subtype switching from laminin β1 to laminin β2 takes place. In mice that lack collagen α3(IV), glomerular filtration decreases over time, and the integrity of the glomerular structure is compromised (Miner and Sanes, 1996; Cosgrove et al., 1996). This suggests that the functions of collagens and laminins as components of the GBM differ. Collagens appear to play a fundamental structural role in the GBM, whereas laminins could have additional functions. Indeed, the podocyte foot processes are abnormal and fuse in animals lacking laminin β2, which suggests that the differentiation or function of these cells is affected by the loss of laminin β2 (Noakes et al., 1995).

Prominent defects in the foot processes of podocytes are also observed in mice lacking the integrin α3 subunit (Kreidberg et al., 1996). These mice have pleiotropic defects in kidney morphogenesis, which lead to decreased branching of...
medullary collecting duct, and malformed proximal tubules; however, the most-prominent defects are found in the glomerulus. Not only are the foot processes of podocytes abnormal, but the GBM is also disorganized, and glomerular capillary loops show defective branching. This suggests that this matrix receptor mediates some effects of matrix molecules on the development of the glomerulus and its basement membrane.

Taken together, these studies suggest that integrins and matrix molecules play a fundamental role in the formation and function of the glomerulus. Integrins might regulate the appropriate assembly of basement membranes and anchor foot processes of podocytes to the GBM. The basement membrane in turn might regulate the differentiation of podocytes and other cell types in the glomerulus; the developmentally regulated composition of the GBM appears essential for the execution of specialized functions. This model is reinforced by the finding that mutations in basement-membrane proteins lead to inherited forms of kidney disease (see below).

**MATRIX MOLECULES AND RECEPTORS IN KIDNEY DISEASE**

**Manifestation of renal diseases**

The past decade has seen significant advances in our understanding of the molecular defects associated with kidney diseases (Goodyer and Kashtan, 1998; Sariola and Philipson, 1999; Woolf and Winyard, 1998). In particular, the elucidation of the genetic lesions underlying inherited renal diseases, which manifest in abnormal ECM organization, has provided a valuable resource for the identification of new genes involved in renal cell-matrix interactions. Inherited renal diseases can affect one or several of the four basic morphological components of the kidney: glomeruli, tubules, interstitium and blood vessels (Coltran et al., 1999). They include renal-malformation diseases, a group of disorders characterized by anatomical kidney defects that arise during embryonic development and range from hypoplasia (failure of the kidney to develop to a normal size) and dysplasia (abnormal kidney differentiation) to renal agenesis (absence of kidneys) (Goodyer and Kashtan, 1998; Woolf and Winyard, 1998). Several genetic loci linked to renal diseases affect the maintenance rather than the development of renal structures (Goodyer and Kashtan, 1998; Smeets et al., 1996). Finally, the genetic basis of disorders affecting renal tubular physiology, such as cystinuria, Bartter syndrome and nephrogenic diabetes insipidus, have been defined (van’t Hoff, 1996). Here, we consider only the subset of human renal diseases where a link to the ECM is apparent (Table 2).

**Renal malformation disorders: Kallmann syndrome**

Most genes causing renal malformation syndromes identified to date are transcriptional regulators (Goodyer and Kashtan, 1998; Woolf and Winyard, 1998). The gene affected in individuals that have X-linked Kallmann syndrome (XLKS), however, encodes a secreted protein KAL1 that shares homology with adhesion molecules (Franco et al., 1991; Legouis et al., 1991). The defining features of XLKS are hypogonadotropic hypogonadism with anosmia (lack of smell) resulting from defects in the migration of gonadotropin-releasing-hormone-synthesizing neurons and olfactory axons (Rugarli and Ballabio, 1993). Up to 40% of patients that have XLKS have unilateral renal agenesis, although bilateral renal agenesis has also been described (Colquhoun-Kerr et al., 1999; Hardelin et al., 1992, 1993; Kirk et al., 1994). The KAL1 gene therefore plays a role not only in neuronal targeting but also in kidney organogenesis. KAL1 transcripts have been detected in the Wolffian duct, mesonephric tubules and the outer cortex of the metanephric kidney (Duke et al., 1995; Legouis et al., 1994). The principal structural features of the KAL1 protein (fibronectin type III repeats and a putative protease-inhibitor domain) are found in several cell- and substrate-adhesion molecules. Indeed, the KAL protein is a secreted glycoprotein localizing to the cell surface (Rugarli et al., 1996; Soussi-Yanicostas et al., 1996). Recent in vitro studies indicate that the KAL1 protein promotes proteoglycan-dependent adhesion and that it can modulate neurite growth and fasciculation (Soussi-Yanicostas et al., 1998). These properties are consistent with a role for KAL1 in mediating adhesive cell-matrix interactions.

The function of KAL1 during kidney development is currently unknown. The renal agenesis phenotype observed in XLKS patients suggests that KAL1 is required at an early stage of kidney development. Strikingly, proteoglycan-carrying heparan sulfate chains play an important role in mediating the binding of the KAL1 protein to cell surfaces (Soussi-Yanicostas et al., 1996). As described above, disruption of proper heparan sulfate synthesis causes renal agenesis (Bullock et al., 1998). This raises the possibility that KAL1 interacts with proteoglycans during kidney morphogenesis. KAL1 could provide a permissive substrate for elongation of the Wolffian duct or promote adhesive interactions between the ureteric bud epithelium and the metanephric mesenchyme. Further analysis of KAL1 function in kidney development would benefit greatly if a mouse model of XLKS were available. Although KAL1 genes have been identified in several vertebrate species, rodent KAL1 homologues have not been reported to date (Legouis et al., 1993).

**Disorders of glomerular structure: Alport syndrome, congenital nephrotic syndrome and other related disorders**

The GBM has a highly specialized composition and represents the central structure of the glomerular filtration apparatus. Both fenestrated endothelial cells and podocytes secrete components of the GBM and matrix-degrading enzymes that maintain the integrity of the GBM (Kasinath and Kanwar, 1993). Multiple diseases impair the integrity of the GBM, leading in general to enhanced permeability of the GBM, which is accompanied by significant hematuria and/or proteinuria (Coltran et al., 1999). Many conditions causing glomerular-filtration-barrier defects are immunologically mediated and will not be discussed here. Several inherited renal diseases manifesting in hematuria and proteinuria, such as Alport syndrome (ATS) and congenital nephrotic syndrome, are also characterized by alterations in the balance and/or structural composition of the GBM. The elucidation of the genetic lesions underlying these diseases has led to the identification of important components of the glomerular filtration barrier.

Collagen type IV, a trimer composed of three α chains, is the major collagenous constituent of the GBM (Kasinath and
Collagen type IV that contains only the \( \alpha \) chains has been proposed to form, because of its high cysteine functionally for \( \alpha \) chains alone can initially substitute structurally and composed of (Leinonen et al., 1994; Gunwar et al., 1998). Collagen trimers known. Collagen type IV that contains the progressively deteriorates. Why this is the case is currently not reproduced in mice that lack collagen thinning, multilamination and splitting of the GBM, which can frequently observed (Kashtan and Michael, 1996). The most characteristic morphological findings in ATS are focal thinning, multilamination and splitting of the GBM, which can be seen by electron microscopy.

The pathological and ultrastructural changes associated with the disease progression in ATS patients are accurately reproduced in mice that lack collagen \( \alpha3(IV) \) (Cosgrove et al., 1996; Miner and Sanes, 1996). Surprisingly, however, mutations in any of the three genes that encode collagen \( \alpha3(IV) \)-\( \alpha5(IV) \) in humans result in the absence of all \( \alpha3(IV) \)-\( \alpha5(IV) \) chains from the mature GBM (Kashtan and Michael, 1996). Although this provides a plausible explanation for the similarity in the phenotypes observed in ATS patients, the mechanisms coordinating the expression of the \( \alpha3(IV) \), \( \alpha4(IV) \) and \( \alpha5(IV) \) chains in the GBM remain poorly understood. The mRNA levels of the unaffected \( \alpha \) chains remain normal, which probably reflects regulation at the post-transcriptional level. The absence of any of one of the \( \alpha3(IV) \)-\( \alpha5(IV) \) chains might result in misfolding, abnormal trimer formation and/or defective supramolecular assembly of collagen IV trimers, and lead to degradation of these chains (Prockop, 1992). Developmental regulation of collagen IV chain expression during human nephrogenesis is similar to that in rodents. As the fetal glomerulus matures, the \( \alpha1(IV) \) and \( \alpha2(IV) \) chains are selectively eliminated from the GBM, leaving only the \( \alpha3(IV) \)-\( \alpha5(IV) \) chains in the mature GBM (Kalluri et al., 1997; Lohi et al., 1997). Preventing the switch from \( \alpha1(IV)/\alpha2(IV) \) to \( \alpha3(IV) \)-\( \alpha5(IV) \) collagen composition of the GBM is detrimental to long-term renal function. The late onset of the disease indicating that a GBM composed of \( \alpha1(IV) \) and \( \alpha2(IV) \) chains alone can initially substitute structurally and functionally for \( \alpha3(IV) \)-\( \alpha5(IV) \), but such a fetal GBM progressively deteriorates. Why this is the case is currently not known. Collagen type IV that contains the \( \alpha3(IV) \)-\( \alpha5(IV) \) chains has been proposed to form, because of its high cysteine content, a tougher and more protease-resistant GBM than does collagen type IV that contains only the \( \alpha1 \) and \( \alpha2 \) chains (Leinonen et al., 1994; Gunwar et al., 1998). Collagen trimers composed of \( \alpha1(IV) \) and \( \alpha2(IV) \) chains appear to be inherently more susceptible to proteolysis than those containing \( \alpha3(IV) \)-\( \alpha5(IV) \) chains (Kalluri et al., 1997). In summary, renal morphogenesis can take place in the absence of collagen \( \alpha3(IV) \)-\( \alpha5(IV) \) chains, but there is a significant change in the molecular architecture, which eventually leads to the breakdown of the GBM.

Congenital nephrotic syndrome of the Finnish type (NPHS1) is a rare glomerular disorder inherited as an autosomal recessive trait (Holmberg et al., 1996). Patients that have NPHS1 suffer from loss of size-selectivity in the glomerular filtration barrier, which leads to massive proteinuria and death within the first months of life. Analysis of the GBM failed to reveal any major changes in protein and proteoglycan composition of affected kidneys (Van den Heuvel et al., 1992; Ljungberg et al., 1993). Kestilä et al. (1998) identified the gene that is defective in NPHS1; its product, nephrin, is a novel member of the immunoglobulin family of cell adhesion molecules. The function of nephrin is still unknown. It is synthesized by podocytes and localizes to the glomerular slit membranes (Kestilä et al., 1998; Sariola and Philipson, 1999). The subcellular localization of the nephrin protein indicates that it is not an integral constituent of the GBM. Sariola and Philipson (1999) have suggested that pairs of nephrin molecules form the glomerular filtration barrier between the foot processes of the podocytes. Alternatively, nephrin could function in the development or maintenance of the glomerular filtration barrier by interacting with components of the GBM.

Impaired glomerular filtration leading to progressive renal failure is a characteristic feature of other renal disorders, such as nail-patella syndrome (NPS), Denys-Drash syndrome (DDS) and Frasier syndrome (FS). In all these diseases, ultrastructural analysis has revealed abnormal GBM, glomerular and/or mesangial sclerosis and hyperplastic podocyte foot processes (Smeets et al., 1996). The disease-causing genes, \( LMX1B \) in NPS and \( WT-1 \) in DDS and FS, encode transcription factors of the LIM-homeodomain and zinc-finger families, respectively (Barbaux et al., 1997; Chen et al., 1998; Pelletier et al., 1991a). Both genes are highly expressed in glomeruli at all stages of differentiation (Chen et al., 1998; Pelletier et al., 1991b; Pritchard-Jones et al., 1990). It is currently not known how disturbances of \( LMX1B \) and \( WT-1 \) gene functions translate into aberrant production of GBM. The observed GBM abnormalities might be consequences of defective maturation and/or maintenance of podocyte cells. Alternatively, both genes might act in signaling processes that directly control the synthesis of specific GBM constituents. Identification of target genes for \( LMX1B \) and \( WT-1 \) in podocytes and analysis of the GBM composition in affected individuals will be necessary if we are to distinguish between these possibilities. The recently reported animal models for DDS and NPS (Dreyer et al., 1998; Patch et al., 1999) therefore represent invaluable tools for future studies.

**Disorders of tubular structures: familial juvenile nephronophthisis and polycystic kidney diseases**

Cystic kidney diseases are a heterogeneous group comprising hereditary, developmental and acquired disorders of renal tubular structures, and some forms might involve defects that perturb cell-cell and cell-matrix interactions (Gabow, 1993; Torres, 1998). The kidneys of affected individuals develop fluid-filled cysts surrounded by epithelial cells that have abnormal ECMs (Griffin et al., 1997; Sandford and Boulter, 1996). Cysts can develop from any segment of the nephron, but cysts of glomerular and distal-tubular origin are rare. Given that the major anatomical steps of nephrogenesis in kidneys of affected individual appear to occur normally, cyst development probably represents abnormal function or maintenance of tubular epithelial cells.

Familial juvenile nephronophthisis (NPH) or medullary
cystic kidney disease is an autosomal recessive, genetically heterogeneous disorder that is characterized by microscopic renal cysts and kidneys that do not become enlarged (Kleinkecht and Habib, 1992). About two-thirds of the patients that have NPH carry a large homozygous deletion at the NPH1 locus. A strong candidate gene for NPH1 has recently been identified (Hildebrandt et al., 1997; Saunier et al., 1997). The gene encodes a novel protein, NPHP1, that has an SH3 domain. The precise function of NPHP1 is still unresolved, but the structural motifs present suggest that it is involved in protein-protein interactions and functions in intracellular signaling. Characteristic features of NPH are morphological changes affecting the tubular basement membranes of all nephron segments (Cohen and Hoyer, 1986). The production of abnormal tubular basement membrane ultimately leads to its disruption and the development of cysts at the corticomedullary border of the kidney. It is tempting to speculate that NPHP1 is part of a signaling pathway ensuring balanced expression of ECM constituents.

The most prevalent form of inherited cystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD). This disorder is characterized by the presence of multiple expanding cysts of both kidneys, which ultimately destroy the renal parenchyma and thereby lead to renal failure (Gabow, 1993). The disease can be caused by mutations in three separate genes, termed PKD1, PKD2 and PKD3. The genetic loci for PKD1 and 2 have been identified. Mutations affecting PKD1 account for 85% of cases, and the gene encodes a large glycoprotein, polycystin 1, that contains multiple transmembrane segments and a C-terminal cytoplasmic domain (Hughes et al., 1995; International Polycystic Kidney Disease Consortium, 1995). The N-terminal extracellular region contains several structural motives found in cell adhesion molecules, including leucine-rich repeats, a C-type lectin domain, and 16 immunoglobulin-like PKD repeats. The extracellular domain also contains a region that has significant homology to the sea urchin receptor for egg jelly, an integral membrane protein whose function is coupled to calcium channels (Moy et al., 1996). The PKD2 gene product, polycystin 2, is also an integral membrane protein and shows homologies to the trp-type and voltage-dependent calcium channels (Mochizuki et al., 1996). Recent evidence from yeast two-hybrid studies suggests that polycystin 2 can assemble into homomultimeric complexes consistent with a possible function as an ion channel or pore (Qian et al., 1997; Tsiokas et al., 1997).

Polycystin 1 and polycystin 2 proteins interact through their C-terminal cytoplasmic domains, and they co-localize to the basolateral membranes of renal tubular epithelial cells, where they could interact with the basement membrane (Ibraghimov-Beskorovnaya et al., 1997; Ward et al., 1996; Wu et al., 1998). Interactions between polycystin 1 and polycystin 2 appear to be disrupted in naturally occurring mutations of PKD1 (Qian et al., 1997; Tsiokas et al., 1997). Therefore, PKD1 and PKD2 might be partners in a common process involved in tubular morphogenesis. This interpretation is reinforced by recent studies in mice (Lu et al., 1997, 1999; Wu et al., 1998). PKD1- and PKD2-deficient mice fail to establish normal renal-tubule architecture, develop renal cysts and die perinatally. During nephrogenesis the polycystins are required for elongation and maturation of renal-tubular structures, but not for nephrogenic condensation and epithelialization. In the adult, the PKD genes are essential for the maintenance of the mature differentiated nephrons: renal cysts arising in mice heterozygous for either PKD gene frequently have lost the capacity to produce the corresponding PKD gene product.

The processes leading to cyst formation are complex and involve enhanced cell proliferation, altered membrane polarity, secretion of fluid by the cystic epithelium, and synthesis of an abnormal basement membrane (Granath, 1996; Murcia et al., 1998; Torres, 1998). Polycystins could be involved in the regulation of any of these steps. For instance, they could control ion fluxes across epithelia into the lumen of the developing cysts. Polycystin 1, a possible modulator of channels, and polycystin 2, a putative ion-channel subunit, could be components of a protein complex that causes abnormal ion transport when mutated. Polycystin 1, either alone or in conjunction with polycystin 2, must also exert some sort of control on cell polarity and proliferation. This could be achieved through cross-talk with the WNT signaling pathway (Kim et al., 1999). Finally, the basement membrane of cystic epithelia exhibits alterations in the synthesis and composition of its components (Carone et al., 1992, 1993; Liu et al., 1992). These changes might reflect secondary effects of cyst formation. However, cell-matrix interactions are known to be important in the maintenance of epithelial cell polarity and the regulation of cell growth. Disruption of these interactions could therefore play a central role in promoting the development of renal cysts. The extracellular domain of polycystin 1 could function in protein-protein and protein-carbohydrate interactions with the basement membrane, and it might transduce extracellular adhesive events into alterations in ion transport through its association with polycystin 2. These exciting possibilities will have to be tested in the future.

CONCLUSIONS

The past decade has brought major progress in our understanding of the molecular mechanisms that regulate kidney development and underlying renal diseases. These findings have revealed that ECM constituents and their receptors might play instructive roles in development rather than serve just as adhesive glue. Matrix molecules and matrix receptors act at multiple steps during kidney development, from the onset of ureteric bud development, during the branching morphogenesis and during the formation of epithelial tubules from condensing mesenchymal cells. A major challenge in the future will be to decipher how these molecules regulate the activity of signal transduction pathways necessary for proper development of cell lineages and cell morphology within developing organs such as the kidney. It will also be important to develop therapeutic approaches that target defects in matrix molecules and matrix receptors.

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