Update of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis

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Kanwar, Yashpal S., Jun Wada, Sun Lin, Farhad R. Danesh, Sumant S. Chugh, Qiwei Yang, Tushar Banerjee, and Jon W. Lomasney. Update of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis. Am J Physiol Renal Physiol 286: F202–F215, 2004; 10.1152/ajprenal.00157.2003.—One of the hallmarks of mammalian nephrogenesis includes a mesenchymal-epithelial transition that is accomplished by intercalation of the ureteric bud, an epithelium-lined tubelike structure, into an undifferentiated mesenchyme, and the latter then undergoes an inductive transformation and differentiates into an epithelial phenotype. At the same time, the differentiating mesenchyme reciprocates by inducing branching morphogenesis of the ureteric bud, which forms a treelike structure with dichotomous iterations. These reciprocal inductive interactions lead to the development of a functioning nephron unit made up of a glomerulus and proximal and distal tubules. The inductive interactions and differentiation events are modulated by a number of transcription factors, protooncogenes, and growth factors and their receptors, which regulate the expression of target morphogenetic modulators including the ECM, integrin receptors, and cell adhesion molecules. These target macromolecules exhibit spatiotemporal and stage-specific developmental regulation in the metanephros. The ECM molecules expressed at the epithelial-mesenchymal interface are perhaps the most relevant and conducive to the paracrine-juxtacrine interactions in a scenario where the ligand is expressed in the mesenchyme while the receptor is located in the ureteric bud epithelium or vice versa. In addition, expression of the target ECM macromolecules is regulated by matrix metalloproteinases and their inhibitors to generate a concentration gradient at the interface to further propel epithelial-mesenchymal interactions so that nephrogenesis can proceed seamlessly. In this review, we discuss and update our current understanding of the role of the ECM and related macromolecules with respect to metanephric development.

renal development; integrins; metalloproteinases
cellular adhesion and cell-cell (homotypic or heterotypic) interactions and polarization of epithelia, are also important participants in the morphogenesis of various organs during cell-matrix interactions (16). Finally, critical to cell behavior during differentiation associated with organogenesis is the fundamental process of phosphorylation of various integrins and hormone or growth factor receptors that contain tyrosine or serine/threonine kinase intracellular catalytic domains (154, 167), whose catalytic activities are known to modulate the expression of various ECM proteins.

With the complexity of diverse groups of molecules participating in embryonic development taken into account, it seems that one of the essential predetermining factors would be the expression of a given molecule(s) at a particular stage of development of an organ system. This would mean that the macromolecules with restricted spatiotemporal genotypic or phenotypic expressions are relevant only to the morphogenesis of a particular organ system. Thus a morphogen may be defined as a molecule that expresses its concentration gradient in a given tissue and alters the fate of target cells in a dose-dependent manner. The concentration gradients of these macromolecules could also vary in various regions of the same developing tissue, and this would transduce a distinct set of specific cellular events, thereby adding further complexity to the differentiation processes that ultimately lead to regional specialization within an organ system. Moreover, the degree of expression of various morphogens during embryonic life would suggest that their function is specific for a given stage of a developing organ system. Although their expression may be stage and tissue specific, well-orchestrated coordination among the various other macromolecules, expressed during the same time frame, is crucial for morphogenesis to proceed successfully so that a particular visceral organ system can be sculpted with defined biological functions.

In recent years, a large body of literature has become available defining the role of various morphogenetic modulators. Much of the information has been derived from in vivo knockout experiments in mice as well as in vitro, whereby an isolated organ system, i.e., mammary (68), prostate (28), salivary glands (12), lung (178), and kidney (39, 153), has been subjected to various experimental manipulations under defined culture conditions. Interestingly, in the morphogenesis of all these organ systems, epithelial-mesenchymal or ligand-receptor interactions seem to be a common feature. Although morphogenesis of the metanephros represents a prototypical example of such interactions, certain unique features are distinctive in renal development in mammals (39, 153).

OVERVIEW OF THE DEVELOPMENT OF THE MAMMALIAN METANEPHEROS

Mammalian nephrogenesis commences with the successive appearance of the pronephros, mesonephros, and metanephros as a craniocaudal wave of cellular differentiation in the nephrogenic cord lying alongside the nephric or Wolffian duct (153). The pronephros is functional to a large extent in lower vertebrates only, e.g., fish, and the mesonephros is fully operational in amphibians. In mammals, the pronephros and mesonephros are vestiges of the nephrogenic cord, and their appearance is transitory; i.e., it is the metanephros that matures to form a functional multiunit organ system, i.e., the kidney. Nephrogenesis commences at days 10–11 in the mouse and at about week 5 of gestation in humans (153). During this process, an epithelium-lined tubular structure arising from the Wolffian duct, i.e., the ureteric bud, interacts with a loosely organized mesenchymal mass, i.e., the blastema, located on the lateral aspect of the aorta in the most caudal segment of the nephrogenic cord. This epithelial-mesenchymal interaction leads to a dramatic transformation of the mesenchyme into an epithelial phenotype, which reciprocates by inducing the ureteric bud to undergo arborization and generation of nascent nephrons. It is of interest that mesenchymal differentiation continues even after the removal of the inducer, i.e., the ureteric bud, and differentiation can be maintained by soluble factors such as embryonic extracts (10, 53). On the other hand, soluble factors/molecules, e.g., anti-GD3 antibodies, can interfere with the inductive interaction even if the mesenchyme and the ureteric bud are in close contact with one another (151). Along these lines, it is conceivable that the soluble factors released by the mesenchyme could influence the branching morphogenesis of the ureteric bud in in vitro systems (145). Nevertheless, epithelial-mesenchymal interaction(s) is still an accepted biological precept for in vivo renal development, based on which nascent nephrons are formed by undergoing the following developmental stages: condensate-vesicle, comma-shaped body, S-shaped body, and precapillary body (153). The precapillary bodies, on vascularization by the processes of vasculogenesis (in situ blood vessel formation) and angiogenesis (sprouting of preexisting capillaries), form the functioning mature glomeruli of the kidney (36, 136, 153), and these processes are modulated by various growth factors and their receptors and are described in recent reviews by Abrahamson and Gomez and their colleagues (52, 136).

For the precise delineation of various events governing metanephric development, several in vitro techniques, including organ or cell culture systems, have been successfully employed. For instance, Madin-Darby canine kidney (MDCK) cell culture has been useful in studying the branching morphogenesis of tubules and development of their epithelial junctions and polarity characteristics (121). In addition, the recent use of the three-dimensional Matrigel cell culture system has yielded deep insights into various processes that regulate tubulogenesis and the role of various growth factors and matrix-degrading enzymes in in vitro development of the mammalian metanephros (145, 164). Despite recent advances in culture techniques, the age-old in vitro metanephric organ culture transfelter technique established by Grobstein (56–58) is still in use and has substantive value in the study of the morphogenesis of the whole kidney, the glomerulus, and the tubules. In this culture system, various developmental stages, with the exception of vascularization of the metanephros, can be studied. The technique employs harvesting either uninduced (day 10.5) or induced (day 11.5) mouse metanephric mesenchyme, placing it on a microporous (~0.8 μm) filter, and maintaining it in culture for 7–10 days. Uninduced mesenchyme can be induced by gluing the ureteric bud to a heterologous inducer, e.g., embryonic neural tissue, to the bottom of the filter. In doing so, the metanephric mesenchyme receives inductive signals from the ureteric bud through the pores of the filter, perhaps by the establishment of transfelter pseudopodial cell-cell contacts (153) or from the ingrowth of neuronal processes in the case of the heterologous inducer (152), although induction by soluble
factors is also conceivable (78, 129). The induced mesenchyme then differentiates, leading to the formation of glomerular and tubular elements, while a part of it develops into stroma or interstitium of the kidney. These in vitro organ culture experiments emphasize that the inductive neotransformation of the mesenchyme is to a large extent modulated by the molecules expressed at the epithelial-mesenchymal interface or ligands expressed in the mesenchyme and receptors in the ureteric bud epithelium that exert paracrine-juxtacrine interactions (Fig. 1).

The concept of such epithelial-mesenchymal/juxtacrine-paracrine interactions in nephrogenesis was originally described for ECM proteins and their receptors (58). Current data suggest that these interactions are applicable to other macromolecules, including transcription factors, protooncogenes, acting as tyrosine kinase receptors, and growth factors and their receptors (71, 142). In the context of such interactions, these diverse sets of molecules exhibit a fair degree of interdependence in the regulation of growth and development of the kidney. The interdependence of growth factors and ECM proteins is reflected by the fact that the latter may act as storage depots for certain growth factors or may contain growth factor-like domains (142). In such a scenario, the ECM would be expected to modulate the biological activities of various growth factors, e.g., transforming growth factor (TGF)-β, whereas TGF-β in turn regulates the synthesis of ECM proteins (160). Similarly, c-ret, a protooncogene that regulates nephrogenesis (156) and ECM expression in metanephric tissues (100), serves as a receptor for glial-derived nerve growth factor (GDNF) (165), the latter apparently structurally related to members of the TGF-β superfamily and playing a vital role in embryonic organ development (113, 131, 148). Thus the actions of this group of diverse molecules are intricately linked, and this review addresses the role of various ECM proteins, their receptors, and the molecules relevant to ECM biology.

GENERAL DISCUSSION OF ECM, ECM-DEGRADING ENZYMES, INTEGRINS, AND CAMS

The role of ECM in metanephric development was conceptualized by the pioneering work of Grobstein and Saxen almost four decades ago (57, 58, 153). They postulated that the basement membrane (BM) of the ECM, interposed between the leading edge of the ureteric bud and the blastema, has a “paracrine” effect in facilitating the interactions among the two cell types that lead to conversion of the metanephric mesenchyme to an epithelial phenotype. The BM glycoproteins are constitutively expressed and are the cytoskeletal products of the native epithelium, as in salivary and breast glands (9, 68), or mesenchyme-derived epithelium, as in the metanephros (57, 153). Because the mesenchyme also induces epithelial branching morphogenesis and de novo synthesis of matrix molecules at the same time, it can be currently perceived as having a self-stimulatory or “autocrine” (?) effect on embryonic tissues during development. Such unique functional properties of ECM proteins were originally proposed on the basis of their expression in fetal tissues. However, in view of current knowledge, it can be argued that the expression of a given macromolecule in a particular tissue does not necessarily imply its direct relevance to a specific stage of embryonic development. Moreover, sometimes even in the face of their in vivo gene disruption, nephrogenesis proceeds normally. This may be related to redundancy in gene functions or compensation for functions by other genes. Also, the functions of some of the constitutively expressed genes/proteins remain elusive in vivo, whereas their role in metanephrogenesis can be readily demonstrated in the transfilter organ culture system (6, 14, 57, 153). Nevertheless, numerous investigators have conclusively demonstrated the morphogenetic role of some of the ECM and ECM-related macromolecules in renal development, both in vivo and in vitro, and it may be worth stressing here again that the impetus for these studies evolved after the initial detection of a given stage-specific, constitutively expressed glycoprotein in metanephric tissues.

ECM PROTEINS

A large number of ECM proteins are expressed during renal development (Table 1). Although they are constitutively expressed but their expression is largely modulated by the growth factors and their receptors, the latter are endowed at times with tyrosine kinase catalytic domains and belong to the family of protooncogenes. The ECM proteins expressed in the metanephric mesenchyme include interstitial collagens, tenascin, nidogen (entactin), fibrillins, osteopontin, and fibronectin; those associated with the BM include type IV collagen, laminin, proteoglycans (PGs), nephroectin, and tubulointerstitial antigen (TIN-Ag) (42, 71, 108, 109). The expression of these ECM proteins, especially mesenchymal proteins, is asynchr-
GBM, glomerular basement membrane; CS-PG, chondroitin sulfate-proteoglycan; TIN-Ag, tubulointerstitial nephritis antigen; BMP-7, bone morphogenetic protein-7; collagen (type-IV), laminins, perlecan, CS-PG, nidogen, and TIN-Ag are integral constituents of the basement membrane proteins.

A mesenchymal factor that allegedly seems to have a role in tubulogenesis, however, and no renal abnormalities are detected on tenascin gene disruption in mice (5, 144). Nidogen is involved in inhibiting in vitro nephrogenesis (151). Also, no nephric defects in mice lacking fibronectin have been described (49). Tenasin is another mesenchymal protein that interacts with fibronectin and PGs, and it appears transiently around the nascent nephron. Its expression decreases with the inhibition of tubulogenesis, however, and no renal abnormalities are detected on tenascin C gene disruption in mice (5, 144). Nidogen is a mesenchymal factor that allegedly seems to have a role in renal organogenesis. It is produced by mesenchymal cells and interacts with the \( \gamma_1 \)-chain of laminin-1 (Fig. 2). This interaction is apparently crucial for the assembly of the BM (41). Interestingly, disruption of the binding of the laminin-1 \( \gamma_1 \)-chain to nidogen by a blocking antibody leads to inhibition of nephrogenesis in embryonic renal explants in culture (40). Similarly, ablation of nidogen binding to the 56-amino acid module in laminin-1 \( \gamma_1 \) displays bilateral renal agenesis in a majority of mouse fetuses (181). However, it should be noted that gene inactivation of nidogen-1 by itself does not result in any obvious phenotype or abnormalities in renal development (117). It is conceivable that other isoforms, i.e., nidogen-2, may compensate for its functions (112). Other mesenchyme-derived proteins whose functions can be ascribed in vitro include fibrillin-1 and osteopontin. Fibrillin-1 interacts with \( \alpha_5 \beta_3 \)-integrins expressed in the ureteric bud epithelium. Inclusion of fibrillin-1 antisense oligo in metanephric culture has been shown to induce dysmorphogenesis of the kidney (76). Osteopontin is a secreted phosphoprotein that interacts with CD44, a cell-surface proteoglycan hyaluronic acid receptor, and \( \alpha_5 \beta_3 \) and \( \alpha_5 \beta_1 \)-integrin. Treatment of metanephric explants with anti-osteopontin blocking antibodies has been shown to induce their dysmorphogenesis (137). In contrast to
the limited functional data on mesenchymal proteins, the role of integral BM glycoproteins, e.g., laminin, PGs, and TIN-Ag and their receptors, is more well defined in embryonic development (74, 89, 93, 110). Type IV collagen exhibits restricted spatiotemporal distribution in the fetal metanephros; however, various gene deletion and insertional mutation studies suggest no definitive role during embryonic development. Nevertheless, mice with gene deletion of α5(IV) (26, 111) or mutation in α5(IV)- and α4(IV)-chains (102) develop an interesting phenotype at 3–4 wk after birth. They develop uremia, proteinuria, and glomerular BM (GBM) changes that are reminiscent of those seen in Alport syndrome. Last, although a minority of the integral BM proteins are expressed around the nephron progenitors, i.e., condensate, S-shaped bodies, and glomerular capillaries, and around the tubules (38), the expression of their individual polypeptide chains varies and even though exhibits stage-specific spatiotemporal distribution.

Laminins

An extensive amount of work carried out over the past two decades suggests a pivotal role of various forms of laminins, e.g., laminin-1,-5, and α5-chain, in development. A typical laminin is made up of α-, β-, and γ-subunits assembled into a cross-shaped structure. About 15 heterodimeric forms of laminins, made up of 5 α-, 4 β-, and 3 γ-subunits in various combinations, have been described (41, 127), and they are involved in a variety of biological processes, including cell adhesion, growth, and differentiation, by interacting with cellular receptors. These biological processes are in large part modulated by the interaction of a globular domain in the COOH terminus of the α-chain with other receptors. The G domain contains five repeating modules, known as LG1–LG5. The LG1–LG3 modules are connected to LG4–LG5 by a linker domain. Laminin-1 is a high-molecular-weight protein made up of α1-, β1-, and γ1-chains, and it is believed to participate in metanephric development, especially during the polarization of the epithelia (36). The expression of laminin chains is asynchronous during early development and is also species and stage specific (36). The mRNA expression of β1 and γ1 laminin chains is both seen in the nephric mesenchyme and detectable in vitro 24 h after the induction of nephrogenesis in mice. Although expression of the α1-chain appears after 2 days, coinciding with the appearance of polarized epithelia in the comma- and S-shaped bodies, its expression is gradually lost from the ureter, distal tubules, and GBM and becomes confined to the mesangium along with the laminin-2 (α1β1γ1) isotype in mature animals. Ekblom (36) demonstrated the role of laminin in nephrogenesis in an in vitro culture system by using elastase-generated peptides and chain-specific antibodies, i.e., against the COOH ends of α1 (E3, LG4–LG5 modules) and α1β1γ1 (E8, LG1–LG3 modules); the NH2 terminus of β1-chains (E4); and the central region of the whole laminin molecule containing the YIGSR-dependent laminin receptor binding sites (Fig. 2). Similarly, the antibodies against E3, the heparin binding domain and more recently found to bind to the dystroglycan complex (see ECM RECEPTORS, INTEGRINS, AND BINDING PROTEINS), and E8, the α1β1-integrin receptor binding site (159), were shown to inhibit the morphogenesis and formation of polarized epithelium (Fig. 2), whereas antibodies against the central region of the laminin molecule were incapable of perturbing renal development (36). During development, other roles of laminin include angiogenesis and integrin receptor-mediated attachment, with the activities confined to YIGSR sequences of laminin-β1 and RGD sequences of the α1-chain, respectively (41). In early nephron progenitors, i.e., the S-shaped body, laminin-1 (α1β1γ1) is seen in the vascular cleft, whereas in the intermediate capillary loop stage the expression switches to laminin-11 (α5β1γ1) (1). The expression of the α1-chain is confined to larger blood vessels (36), whereas that of the β2-chain is confined to capillaries of mature renal glomeruli. Such stage-specific isoforms or switch in their expression are also seen in other ECM proteins, type IV collagen chains, and PGs.

Among the other family members, the role of laminin-5 (α5β3γ1) has been recently demonstrated in vitro. Laminin-5 most likely exerts its effect via α5β1- and/or α5β2-integrin receptors (186). Addition of anti-laminin-5 blocking antibody to the culture medium perturbs the organogenesis of the embryonic kidney (186). Of great interest is the biology of a recently discovered novel α5-chain which is a component of laminin-10 (α5β2γ1) and -11 (α5β2γ1). The mice deficient in α5 usually die late in embryogenesis with no obvious renal phenotype. However, a minority of them lacked one or both kidneys (110). On closer inspection, glomerular development was found to be aberrant in that the mutant mice had constricting glomeruli with a disarray of podocytes and defective assemblage of GBMs. In addition to a deficiency in the α5-chain, its novel receptor, i.e., Lutheran blood group glycoprotein, was also lacking in mutant mice (115). The abnormalities in GBMs were attributed to an absence of laminin-11 in the α5 mutant mice, thus implying that the switch from the β1-chain of laminin-1 to the β2-chain of laminin-11 also plays a role in the macromolecular assemblage of GBMs. This notion is strengthened by the observations made in mutant mice lacking s-laminin/laminin-β2, which display heavy proteinuria although the GBMs appear ultrastructurally normal (122). Also, these mice do not reveal any molecular abnormalities in collagenous or noncollagenous components, which may suggest that the β1-chain compensates for the functions of β2, thus yielding normal morphology of the GBMs.

PGs

Several species of PGs have been described because of the original description of heparan sulfate (HS) as the integral constituent of renal BM or mesangial matrices (72, 73). They include perlecian, i.e., HS-PG, chondroitin sulfate PG (CS-PG), decorin, biglycans, agrin, leporecan, and type XVIII collagen (42). The latter is a nonfibrillar collagen belonging to the subfamily of multiplexins and has sulfated disaccharide chains attached to its polypeptide as in HS-PG (46). In addition, there are certain cell surface-associated PGs expressed in the kidney, including glypicans and syndecans (13, 54). Many of the PGs are believed to play a significant role in various developmental processes in several tissues (180). The classic PGs are made up of glycosaminoglycan (GAGs) chains that are bound by O-glycosidic linkage to the core peptide. Initially, at the time of induction, the sulfated PGs are highly concentrated at the “tips” of the ureteric bud branches, the epithelial-mesenchymal interface and the site where nascent nephrons are formed. Later, they are seen around the condensate and persist in the BMs throughout the fetal and neonatal periods (93). Inhibition
of the synthesis of sulfated PGs by puromycin or the addition of GAG chains onto the core peptide is associated with blunting of the tips of the ureteric bud branches and dysmorphogenesis of the kidney (93). Incidentally, the addition of heparin sulfate, and not HS, to the culture medium also inhibits nephrogenesis (133). Thus it appears that the biological actions of PGs are mediated via their GAG chains, and this notion has been supported by experiments with salivary glands in which enzymatic deletion of GAGs was shown to inhibit lobulogenesis (9). The GAG chains can interact with other matrix molecules, such as the E8 fragment of laminin (159), and they also act as reservoirs for various growth factors, e.g., basic FGF (142), and thus can influence morphogenesis by more than one mechanism. Also, the enzymes responsible for the sulfation of GAGs, e.g., HS-2-sulfotransferase (HS2ST), play a major role in modulating the effect of PGs on renal development. HS2ST is required for mesenchymal condensation around the ureteric bud and in the initiation of branching morphogenesis, and gene deletion of HS2ST results in bilateral agenesis (19). Interestingly, the perlecan null mice do not reveal any defective renal phenotype, but most of them die at midgestation due to bleeding diathesis (123). Those that survive appear to have normal BMs. Type XVIII collagen null mice do not appear have any renal abnormal phenotype, although ocular and vascular abnormalities are observed (46). These may be related to the dysfunction of the endostatin domain of type XVIII collagen. The latter interestingly has been shown to modulate branching morphogenesis of the ureteric bud in vitro (79).

Among the cell-surface PGs, the syndecans are more or less receptor forms of transmembrane HS-PGs. They are present in the mesenchyme and early differentiating epithelium, they gradually disappear with the maturation of the nephron (13), and no distinct role in renal organogenesis has been described in the literature. The glypicans (Gpcs) are HS-PGs that are linked to the cell surface via a glycosyl-phosphotyrosilinositol anchor and are composed of six family members (55). Gpc3 is expressed in the kidney, and its deficiency leads to enhanced ureteric bud branching, cortical collecting duct hyperplasia with reduced growth and apoptosis in medullary collecting duct cells, ultimately resulting in dysplastic kidneys, reminiscent of the phenotype observed in patients with Simpson-Golabi-Behmel syndrome (55). Along these lines, endostatin, a protein that binds to Gpc, has been shown to regulate branching morphogenesis of the ureteric bud in vitro (79). Interestingly, Gpc3 modulates bone morphogenetic protein (BMP) and FGF-mediated effects during renal branching morphogenesis (54), suggesting that the biology of PGs and growth factors is interlinked. A brief exemplary discussion of this intricate relationship is given below.

A number of growth factors and their receptors have been shown to modulate the expression of PGs and thus are believed to regulate morphogenesis of the kidney. They include insulin, IGF-I, and TGF-α and -β (97–99, 138–140, 176). Analogous to ECM ligand-integrin receptor interactions, epithelial-mesenchymal interactions are also amenable to growth factors and their receptors. For instance, ligands like insulin or IGF-I are expressed in the mesenchyme, whereas their receptors are in the ureteric bud epithelia, and both of them display restricted spatiotemporal distribution during metanephric development (97, 99, 176). The evidence for the linkage between growth factors and ECM proteins in renal development is derived from in vitro studies, in which the addition of IGF-I or insulin to the organ culture medium was found to result in a hypertrophic/hyperplastic response in metanephric explants, with parallel increased mRNA and protein expression of PGs (97, 98). Furthermore, in vitro experiments revealed that gene disruption of IGF-I or insulin receptors by addition of antisense oligonucleotides to the organ culture leads to atrophy of the explants, with markedly decreased expression of PGs, especially at the tips of ureteric bud branches, the site where epithelial-mesenchymal interactions take place (97, 176). Similarly, gene disruption of the GDNF receptor, i.e., c-ret protooncogene with a phosphotyrosine kinase catalytic domain (165), leads to a remarkable dysmorphogenesis of the kidney concomitant with a dramatic decrease in the expression of ECM proteins, in particular that of PGs (100). Conceivably, GDNF, a molecule related to the TGF-β superfamily, is expressed in the mesenchyme, whereas c-ret is in the ureteric bud epithelia, and in vivo gene disruption of either of them also leads to agenesis or hypogenesis of the kidney (148, 156).

Another member of the TGF-β superfamily that seems to play a role in renal development is BMP-7. BMP-7 is involved in the mineralization of cartilage (66). Its spatiotemporal mRNA expression in the metanephros is unclear because it is reported to exhibit hypogenesis/agenesis of the kidney in metanephric development (105, 106). Whether a deficiency in BMP-7 leads to altered expression of PGs, which then results in aberrant metanephric development, remains to be investigated. Further evidence that TGF-β, a modulator of PG expression, plays a role in nephrogenesis is derived from cell culture studies, where it was found to perturb tubulogenesis of MDCK cells grown in collagen gels (149). The mechanism as to how TGF-β modulates the expression of PGs is rather complex. TGF-β seems to exert its influence via approximately nine binding proteins or receptors, and among them the first three are well characterized (8, 17). Type I and II receptors contain serine/threonine kinase domains. Subsequent to their heterodimerization, there is activation of the Mad (for Mothers against dpp) signaling pathway, leading to targeted gene expression. Type III receptors are made up of endoglin and betaglycan, and both are transmembrane PGs with serine and threonine residues likely to be phosphorylated by protein kinase C, which would be expected to influence the expression of the target genes. Other BMPs that play a role include BMP-2 and -4, because they are also expressed in the embryonic metanephros. The addition of BMP-4 to the culture medium is found to inhibit ureteric bud branching; however, notable renal abnormalities are observed in BMP-4 heterozygous null mutants (105, 106).
ECM RECEPTORS, INTEGRINS, AND BINDING PROTEINS

Of the known molecules that are expressed at the epithelial-mesenchymal or cell-matrix interface are the well-studied molecules known as integrins. Integrins are heterodimeric, noncovalently bound transmembrane glycoproteins and are made up of α- and β-chains. They are ubiquitously distributed and mediate diverse biological functions, including cell-cell and cell-matrix interactions, cell polarity, cell migration, and angiogenesis (30, 69). In the event there is disruption in cell-matrix interactions, the mesenchyme is likely to undergo apoptosis as observed in high-glucose ambience (75). To date, about 20 integrins have been discovered in various tissues, and they serve as receptors for a number of morphogenetic ECM proteins, including laminins, collagen, fibronectin, osteopontin, nephrogin, vitonectin, and tenasin (Table 2). Their interaction with ECM proteins is Ca^{2+} dependent and peptide sequence specific, and substrate specificity is largely determined by the β-subunit. The intracytoplasmic tail of the β-chain is responsible for its interactions with the cell cytoskeleton via binding to talin, vinculin, and α-actinin and may be also involved in transductional events, whereby β-subunits undergo tyrosine phosphorylation (30, 69). Like ECM proteins, integrins exhibit spatiotemporal expression in the mammalian metanephros (86). The β-subunit seems to have a wider distribution, whereas expression of the α-subunit is much more restricted. The αβ1- and αβ2-integrins are widely distributed in the uninduced mesenchyme. Like its ligand fibronectin, αβ1 disappears after induction. Expression of αβ1, αβ2, and αβ6 is at the interface between the basal lamina abutting the base of the foot processes of developing podocytes, endothelia, and tubular epithelium, respectively (83, 84). The α5-subunit, expressed in the cells of the condensing mesenchyme and polarized tubular epithelium, codistributes with the laminin α1-chain. αβ1-Integrin serves as a receptor for the E8 fragment of the α8-β1- and γ1-chains of laminin, and like the E8 laminin-1 fragment, anti-α8 antibodies also perturb the formation of polarized nephric epithelium and perturb tubulogenensis of the metanephros in vitro (36, 44, 186). Besides αβ1, αβ2, and αβ3 also bind to laminins, and α2-integrin is expressed in the distal tubules and α3 in the maturing glomerular podocytes (37). Although α2β1 binds to laminin, its affinity for type IV collagen is higher (130, 168). Because type IV collagen is present during the early stages of tubule formation and maturation of nephrons, it is likely that its interaction with α2β1 may be relevant to renal tubulogenesis.

The notion that integrins play a role in metanephric development is also supported by in vitro organ culture and in vivo knockout experiments. For instance, inclusion of anti-α8 or -β1, a receptor for laminin-10 and -11 causes inhibition of ureteric bud branching morphogenesis, whereas concomitant treatment with anti-α8 and -α8 induces a marked reduction in the size of the metanephris (186). It needs to be emphasized that α2 and α5 form heterodimers such as α2β1- and α5β1 integrins and share a common ligand, laminin-5; exposure of their blocking antibodies leads to aberrant metaneprogenesis (see above). Along these lines, in vitro gene disruption of α2β1-integrin has been shown to induce a failure in branching morphogenesis of tubules in the MDCK cell collagen gel culture system (143). Because the E8 fragment of laminin-1 also interacts with α5β1- and α2β1-integrins, this would suggest that their common β-subunit may also have a functional role in morphogenesis (83). The role of β1 in nephrogenesis has received limited attention (44), but its in vivo gene disruption is associated with peri-implantation lethality (161). Further support for its role in organogenesis is also derived from other in vitro model systems; i.e., anti-β1 antibodies have been shown to cause functional impairment in mammery epithelia and block the differentiation of colon carcinoma cells and the formation of glandular structures in three-dimensional collagen gels (36). Among the α- related integrins, αβ3 has been reported to be present on the endothelial cells of glomerular and extraglomerular capillaries (83). αβ3 is a promiscuous integrin receptor and binds to a number of ligands, including vitronectin, fibrinogen, von Willebrand factor, thrombospondin, fibronectin, osteopontin, bone sialoprotein, thrombin, laminin, and collagen type I and IV (51). In view of such a wide range of interactions, αβ3 would be expected to participate in diverse biological functions. Its role in angiogenesis has been well defined. Also, αβ3-related integrins have been shown to play a role in metanephric development, as assessed by in vitro gene disruption experiments (174). Interestingly, in vitro treatment of anti-αβ3 results in inhibited growth of the metanephros like that seen with the antibodies to its ligand osteopontin, with accentuated apoptosis of the mesenchyme (137).

Osteopontin, as indicated earlier, is a ligand for αβ1-integrin, and the most convincing role of integrins in metanephris development is attested to by knockout experiments (32, 116). The other known ligands of αβ1 include fibronectin, vitronectin, tenasin C, and preosteoblastic epithelial growth factor-like repeat protein with meprin. The latter is expressed in the kidney and may play a role in metanephric development (114). Expression of αβ1 occurs in the induced metanephric mesenchyme during the time frame when growing tips of the ureteric bud intercalate into the mesenchyme to exert epithelial-mesenchymal interactions. Another recently described ligand for αβ1 is nephrogin, an ECM protein with EGF-like repeats, a mucin region containing an RGD sequence, and COOH-terminal MAM domain expressed in the ureteric bud epithelium, thus making it amenable to suitable epithelial-mesenchymal interactions and for renal development to proceed normally (18). With gene disruption of the α8-subunit of αβ1-integrin, severe deficits in nephrogenesis are observed (116). More than 50% of αβ3 mutants are born without kidneys or ureters, and the remainder show variable renal and ureteric abnormalities. Besides αβ1 integrins, results after gene disrup-

Table 2. ECM proteins and their binding proteins/integrin receptors expressed during metanephris development

<table>
<thead>
<tr>
<th>ECM Protein</th>
<th>Integrin Receptor(s)/Binding Protein(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen IV (basal lamina)</td>
<td>αβ1, αβ2, αβ3, αβ1, αβ3</td>
</tr>
<tr>
<td>Laminin (basal lamina)</td>
<td>αβ1, αβ2, αβ3, αβ1, αβ3, αβ4, αβ1, αβ3, dystroglycan</td>
</tr>
<tr>
<td>Perlecán (basal lamina)</td>
<td>αβ1, αβ2, dystroglycan</td>
</tr>
<tr>
<td>Nidogen (basal lamina)</td>
<td>αβ1</td>
</tr>
<tr>
<td>TIN-Ag (basal lamina)</td>
<td>αβ1</td>
</tr>
<tr>
<td>Collagen IVa</td>
<td>αβ1, αβ2, αβ3</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>αβ1, αβ2, αβ1, αβ1, αβ3</td>
</tr>
<tr>
<td>Tenascin-C</td>
<td>αβ1, αβ3</td>
</tr>
<tr>
<td>Fibrillin-1</td>
<td>αβ1</td>
</tr>
<tr>
<td>Nephrogin</td>
<td>αβ1</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>αβ1, αβ3</td>
</tr>
</tbody>
</table>

AJP Renal Physiol • Vol. 286 • February 2004 • www.ajprenal.org
tion of other integrins are not encouraging. A target mutation in the integrin-α3 gene has been reported to have no abnormal phenotype, although mild growth retardation is observed (85). Akin to the integrin receptors is a unique transmembrane protein that also links the ECM glycoproteins to the intracellular filamentous network, and it is referred to as dystroglycan (22, 43). It is expressed in the embryonic metanephros and seems to be relevant to morphogenesis of the mammalian kidney. Dystroglycan is receptor for a number of BM proteins, including laminin-1 and -2, perlecian, and agrin (22, 43). Dystroglycan in its macromolecular complex is made up of several proteins that ultimately mediate transmembrane interactions between ECM proteins and intracellular cytoskeletal elements (22, 43). This complex includes six proteins, i.e., α- and β-dystroglycans, α-, β- and γ-sarcoglycans, and a 25-kDa protein. Data from various investigations collectively indicate that α-dystroglycan is extracellular, and it binds to E3-like G domains of laminin-2 in muscle and possibly interacts with laminin-1 as well. Transmembrane β-dystroglycan binds to the COOH terminus of intracellular dystrophin/utrophin, and the NH2 terminus of the latter has binding affinities with F-actin (34, 135). By in situ hybridization, it has been shown that dystroglycan and laminin α1-chains are coexpressed in epithelial components of the developing metanephros and both exhibit a restricted spatiotemporal distribution (35). Because extracellular α-dystroglycan interacts with the E3 domain of the laminin α1-chain that regulates morphogenesis of the kidney, it is possible that the dystroglycan complex plays a role in development (Fig. 2). Indeed, inclusion of monoclonal antibodies, which are known to block the binding of α-dystroglycan to laminin-1, in culture perturbs the morphogenesis of the embryonic metanephros, in particular that of the differentiating epithelium (35). It is interesting to note that dystroglycan, by interacting extracellularly with laminin and mesenchymal factor nidogen and intracellularly with epithelial utrophin or dystrophin and actin, exemplifies classic epithelial-mesenchymal or ligand-receptor interactions and reinforces the observations that are fundamental to the core biological precepts in the developmental biology of the metanephros (56).

CAMS AND SIALOGLYCOCONJUGATES

Two major families of proteins appear to play a major role in cell adhesion followed by epithelial differentiation and polarization. Like integrin receptors or the dystroglycan complex, some of these CAMs are integral plasma membrane glycoproteins, e.g., E-, N- and P-cadherin, belonging to class I cadherins, and interact with cytosolic proteins, e.g., α-, β-, and γ-catenin (plakoglobin), which in turn interact with the actin filament network. Conceivably, such an interaction may exert a substantial influence on metanephrine development (60). For instance, a β-catenin-E-cadherin complex may be involved in the increased adhesiveness of cells to form condensates around the ureteric bud branches during the comma- and S-shaped-body stage of metanephrine development. The cadherin superfamiliy includes >80 members, and ~16 subtypes of mammalian cadherins have been identified in mice that can be classified under classic cadherins, the latter containing 5 cadherin repeats in their extracellular domain and mediating Ca2+-dependent cell adhesion (185). E-cadherin (L-CAM or uvo- morulin), a prototype of class I cadherin and a glycosylated protein with adhesive properties confined to its 26-kDa fragment, appears subsequent to induction in the condensates and at lateral surfaces of the differentiated polarized tubular epithelium, coinciding with the expression of the laminin α1-chain. Initially, E-cadherin is expressed at the tips of the ureteric bud and later in the upper limb of the S-shaped body that is destined to become distal and collecting tubules (29). N-CAM, a heavily sialylated glycoprotein, is distributed in the uninucleated mesenchyme only. The role of both L-CAM and N-CAM is unclear because their antibodies do not inhibit the conversion of the mesenchyme to an epithelial phenotype of the metanephros (81, 170) or affect the morphogenesis of MDCK cells (59). Also, N-CAM-deficient mice do not exhibit any overt renal abnormalities (27). Incidentally, anti-uvomorulin antibodies perturb compaction at the 8–16 cell stage of primitive embryonic tissues, which may be due to interference with protein kinase C-dependent phosphorylation (157) and disruption of uvomorulin-actin interactions mediated via α- and β-catenins, which associate with the intracytoplasmic domain of uvomorulin (125).

Another CAM, a prototype of class II cadherin, is K-cadherin/cadherin-6. It is expressed in the lower and middle limbs of the S-shaped body, the progenitor of proximal tubules. It mediates Ca2+-dependent homophilic cell aggregation (184), and its role in nephrogenesis has been recently well documented in vivo using transgenic approaches (104). Cadherin-6 seems to modulate events regulating mesenchyme-to-epithelial conversion in the metanephros. Also, anti-cadherin-6 antibodies have been shown to inhibit aggregation of induced mesenchyme in vitro organ culture systems (24). Thus it seems that between E-cadherin and cadherin-6 there is a certain degree of parallelism between their expression and relevance to tubulogenesis in the metanephros. Another class I cadherin, R-cadherin, is expressed similarly to cadherins-6, and it may play a role in the transition of epithelialization of the mesenchyme (29). However, gene disruption of R-cadherin reveals delayed maturation of the kidneys, but the phenotype observed as a result of R-cadherin gene disruption did not reveal overt abnormalities in the genitourinary system, and the degree of phenotypic change is not appreciably different from that observed in cadherin-6-, P-cadherin-, or N-cadherin-deficient mice (29). Surprisingly, intercrossing of mice between different cadherin-deficient animals also did not result in any notable phenotype as far as genitourinary development was concerned, which again suggests a certain degree of redundancy in terms of functionality among various cadherins (29). In addition to E-,-N-, K-, and R-cadherin, KSP-cadherin, belonging to a subfamily of classic cadherins, is also expressed in the kidney along the entire length of the nephron. Although it is not involved in the directional morphogenesis of the early metanephros, it probably plays a role in the maintenance of the terminal differentiated tubular epithelial phenotype (158). Ten- sin is another recently described F-actin binding protein that is expressed in the kidney; however, it only regulates postnatal renal development (101). Thus it appears that the actin binding proteins, tensin or cadherins/catenins, play some role in metanephrine development, and whether they are essential to organogenesis of the embryonic kidney is debatable. Finally, L1 is another recently described CAM, which belongs to the Ig superfamiliy. It is expressed in the collecting ducts, and exposure of anti-L1 antibodies induces dysmorphogenesis of the
ureteric bud, suggesting its potential role in in vitro metanephric development (31).

Besides classic CAMs, there are several newer molecules that have been described in the literature that regulate cell-cell or cell-matrix interactions and are expressed in the kidney, and thus, conceivably, play a role in renal development. They include sialoconjugates (67, 90), e.g., podocalyxin (155), and sialylated glycosphingolipids, e.g., gangliosides (151), N-linked oligosaccharides (45), and galectins (11, 25). Podocalyxin is a major sialoglycoprotein that is expressed in the developing glomerular epithelium and is involved in podocyte-GBM interactions (155). However, its role in nephrogenesis remains to be established. The gangliosides are widely distributed in various tissues and display cell-cell and cell-substratum adhesive properties by modulating protein kinase C and tyrosine kinase activities (169). Among the various gangliosides, GD3 is expressed on the cell surfaces of podocytes, metanephric mesenchyme, and interstitial cells alongside the stalk of the ureteric bud. The anti-GD3 antibodies in vitro markedly inhibit tubulogenesis, suggesting that cell-cell contact in epithelial-mesenchymal interactions are crucial for metanephrhic development (151). The galectins are soluble β-galactoside binding lectins. To date, 14 galectins have been isolated and characterized, and 2 galectins, galectin-3 and -9, are expressed in the kidney (25). Galectin-3 and -9 have opposing properties, i.e., antiapoptotic vs. antiapoptotic (20, 172, 177). Galectin-3, being antiapoptotic, is involved in cell growth, adhesion, and neoplastic transformation. Inclusion of anti-galectin-3 antibodies in the culture medium inhibits ureteric bud branching, suggesting a potential role in renal morphogenesis during embryonic development (20).

ECM-DEGRADING ENZYMES

Besides ECM proteins, the biology of ECM-degrading enzymes is expectedly linked to metanephric development because they regulate expression of ECM protein by proteolytic processing and thus create an extracellular concentration in strategic locations with spatiotemporal distribution. As indicated above (see PGs), PGs, having a concentration gradient, the highest being at the tips of the ureteric bud branches or epithelial-mesenchymal interface, maintain branching morphogenesis of the kidney (93, 96). With perturbation of this gradient at the tips, there is a failure in the dichotomization of ureteric bud branches. Such a concentration gradient of PGs in salivary glands, i.e., cleft vs. the advancing tips, is also believed to be responsible for their lobulogenesis (9).

Other ECM glycoproteins that also exhibit such a concentration gradient include type III collagen, whose expression is highest in the cleft (119). These gradients may be due to their constitutive expression or to a relative local deficiency of degrading enzymes like collagenases or gelatinases. The gelatinases are collectively known as matrix metalloproteinases (MMPs) because their activity is dependent on the presence of metal ions, e.g., Zn2+ (95). To date, ~23 MMPs have been identified, and among them 7 are of the membrane type (MT1–MT7-MMP) and the remaining are of the soluble type (118, 141). The latter are made up of a propeptide, a furin cleavage site, a catalytic domain containing Zn2+ and Ca2+ binding sites, and a hemopexin domain. The MT-MMPs have either a transmembrane domain (MT1–MT3, MT5) or they are glycosylphosphatidylinositol anchored (MT4, MT6) (64). At times, MT-MMPs activate soluble forms of MMPs; e.g., MT1-MMP activates pro-MMP2 to MMP2. In general, the MMPs can degrade a broad range of substrates, including collagens, proteoglycans, fibronectin, tenascin, entactin, elastin, fibrin, and fibrinogen (118, 141). Interestingly, their activity is inhibited by tissue inhibitors of metalloproteinases (TIMP-1–4), α2-macroglobulin, and reversion-inducing cysteine-rich protein with Kazal motifs (7). Conceivably, these three groups of molecules, i.e., MMPs, MT-MMPs and TIMPs, as a trimolecular complex exert influence on a wide variety of biological processes, including branching morphogenesis of the kidney (77, 124) and possibly of the trachea, lung, and mammary gland (3). Their role in organotypical epithelial-mesenchymal interaction, as seen in nephrogenesis, was originally proposed more than three decades ago (58, 179), whereas the direct evidence implicating a differential gradient of collagen fragments initiating cleft formation and lobulogenesis was provided by Nakanishi and colleagues (47, 62). Their studies suggested that treatment with bacterial collagenase leads to branching dysmorphogenesis of the salivary glands (47, 62), whereas, on the other hand, collagenase inhibitors stimulated branching morphogenesis of the salivary glands (120). In lungs, enhanced gelatinase-A (MMP-2) activity in response to TGF-α and EGF has been reported to inhibit arborization of the pulmonary alveoli (48). Conversely, increased expression of stromelysin-1 (MMP-3) in transgenic mice correlates with the accentuated lobulation of the mammary gland (162, 182). Along these lines, Ota and colleagues (124) noted increasing mRNA expression of MMP-2 in stromal cells and of MT-1-MMP in ureteric bud epithelia, respectively, during embryonic life. Such a distribution of MMP-2 and MT-1-MMP would be conducive to epithelial-mesenchymal interactions. Interestingly, their levels decreased during the postnatal period, suggesting that their potential role in organogenesis of the kidney in vitro is confined to the embryonic period (94). In subsequent studies using antisense techniques, Ota et al. (77) elucidated the roles of MMP-2 and MT-1-MMP in morphogenesis and suggested that MT-1-MMP, MMP-2, and TIMP-2, in a trimolecular complex, influence organogenesis of the embryonic kidney. However, it should be noted that anti-MMP-2 antibodies alone fail to inhibit organogenesis of the kidney (94). The failure to perturb renal morphogenesis by the latter may be due to the fact that anti-MMP-2 antibodies may not be of the blocking type. Nevertheless, MMP-9, expressed in the metanephros, has been shown to play a role in the organogenesis of the kidney in vitro (94). The role of MMP-2, MMP-9, and MT1-MMP has been suggested in tubular segmentation because they are expressed in specific segments of the nephron. The role of MMPs in organogenesis can be also extrapolated from studies of mouse inner medullary collecting duct cell (mIMCD3) culture systems. In these cell culture systems, Nigam and colleagues (146, 147) have been able to demonstrate a parallel rise or fall in the mRNA expression of MMP-2 with stimulation or inhibition of branching morphogenesis under the influence of TGF-β or ligands of the EGF receptor, e.g., TGF-α. Using a similar three-dimensional culture system, they also showed that metanephric mesenchyme cell-derived conditioned medium alters the transcription of MMP-2, -3, -9, MT1-MMP, and TIMP-2 along with the changes in ureteric bud branching morphogenesis (134). Despite the convincing
data in in vitro studies, they had been difficult to reconcile with the results of in vivo studies. In this regard, MMP-9-deficient mice do not reveal any renal abnormalities (4). Moreover, intercrossing them with Col4a3-deficient mice, a model of Alport syndrome, did not modify the progression of disease or integrity of the extracellular matrices. It is conceivable that the MMP-9 activity may have been compensated for by other MMPs (92).

Besides MMPs, there are certain other serine proteases that display ECM glycoprotein substrate specificities and are expressed in the fetal kidney and thus are believed to play a role in nephrogenesis, although the data are not so convincing. One of the important members of this family includes tissue-type plasminogen activator (t-PA), which has been localized to S-shaped bodies and glomeruli. Another one is urokinase type (u-PA), which is heavily expressed in renal tubular epithelia (150). Both t-PA and u-PA can induce proteolytic cleavage of glycoproteins either directly or indirectly by first activating latent MMPs. Using a three-dimensional collagen culture system, Orci and colleagues (128) showed that in vitro tubulogenesis of MDCK cells can be modulated by inhibitors of serine proteases, whose enzymatic activities and expression are regulated by hepatocyte growth factor (128). Hepatocyte growth factor is a morphogen, and its role in branching morphogenesis has been well documented in an in vitro nephric organ culture system (183). Although these matrix-degrading serine proteases, i.e., t-PA or u-PA, are expressed in the embryonic metanephros as well as in renal tubules and modulate branching morphogenesis in vitro, both t-PA- and u-PA-deficient mice do not develop any aberrant renal phenotype (23).

CONCLUSIONS

During renal organogenesis, there is remarkable cellular activity that is associated with inductive transformation of majority of the undifferentiated mesenchyme into an epithelial phenotype, followed by establishment of polarized epithelia with specialized junctions, and formation of a defined sculpted tissue mass in the form of tubules, which serve a number of absorptive and transport functions. At about the same time, a segment of the epithelial-mesenchymal mass undergoes vascularization, which then matures into a capillarized structure known as the glomerulus, an organ with diverse biological functions, most importantly, the filtration of various macromolecules. To achieve such a high degree of complex organization of the functioning nephron, a diverse set of macromolecules exert a marked influence in a tightly regulated manner during the entire embryonic development of the metanephros.

The role of these macromolecules has been heavily scrutinized over three last three decades, and a wealth of information has been generated, which has given deep insights into the cellular events that modulate renal organogenesis. The modulation seems to be an interplay among various ECM glycoproteins, ECM-degrading enzymes and their inhibitors, CAMs, integrins, growth factors and their receptors, and proteoconogenes, all essential for the proper epithelial-mesenchymal interactions to take place so that nephrogenesis can proceed normally. Besides many of the known molecules that regulate branching morphogenesis/organogenesis, many new molecules have been added to the list during the last few years, and there are others that are expressed during embryogenesis whose functions are still to be clearly defined. They include crystallins (neuroectodermal lens proteins) (70), epimorphins (mesenchymal cell-surface proteins) (65), small PGs (15, 63), osteogenin (61), and matrillin (187). Also, there are other proteins, such as paclitaxin (166), an intracellular cytoskeletal protein, ADAMTS, and extracellular proteases (163), whose roles need to be delineated during epithelial-mesenchymal interactions and renal metanephrogenesis. The understudied area seems to be that of the oligosaccharides (45), which are involved in posttranslational modifications and thus conceivably would modulate morphogenesis by cell-cell or cell-matrix interactions. The galectins (25) are other hexose binding proteins that are not fully investigated as to their role in balancing growth vs. apoptotic potential, an essentiality in development. Finally, there are several other novel genes encoding mesenchymal or plasmalemmal proteins that are yet to be discovered in the fetal kidney, and they can be readily identified, first, by inducing dysmorphogenesis with various pathophysiological stimuli, such as glucose (75), followed by subjecting the embryonic metanephros to various subtraction hybridization analyses (87, 173, 175).

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REFERENCES

invited review

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