Role of transcriptional networks in coordinating early events during kidney development

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Boyle, Scott, and Mark de Caestecker. Role of transcriptional networks in coordinating early events during kidney development. Am J Physiol Renal Physiol 291: F1–F8, 2006; doi:10.1152/ajprenal.00447.2005.—Many of the signaling pathways that regulate tissue specification and coordinate cellular differentiation during embryogenesis have been identified over the last decade. These pathways are integrated at the transcriptional level, enabling activation of specific developmental programs in a temporally and spatially restricted fashion. Such developmental events are usually thought of in terms of hierarchical relationships, in which the expression of upstream factors leads to the sequential activation of a linear cascade of downstream genes. Whereas these models provide a simplistic approach to understand complex cellular events, genetic and biochemical studies in mice and other model organisms provide ample evidence that many of these factors interact at multiple levels in vivo and emphasize the importance of considering these linear events in context. The purpose of this review is to emphasize the complexity of these regulatory networks during the early phases of mammalian kidney development, outlining some of the limitations and alternative approaches that are being used to explore the complex nature of these networks in vivo. Before describing these networks in detail, we will provide a brief overview of the main structural changes and tissue interactions involved in mammalian kidney development, and go on to describe some of the limitations of our current approaches to evaluate the role of these developmental pathways in vivo.

transcription; Drosophila eye development

RENAL DEVELOPMENT IN MAMMALS

The kidney has received extensive attention from developmental biologists since the nature of its reciprocal tissue interactions was first described more than 50 years ago (10, 38). There are three kidney structures that arise during mammalian development (Fig. 1A). The pronephros and mesonephros develop first and are transient structures in mammals which serve as excretory organs during in utero development. Subsequently, the permanent kidney, or metanephros, is formed in the posterior aspect of the intermediate mesoderm, relying on similar tissue interactions involved in formation of the pro- and mesonephros (37). The first structural evidence of nephrogenesis arises around embryonic day 8 (E8) in the mouse with the formation of two parallel epithelial tubes known as the nephric ducts within the intermediate mesoderm, in between the lateral and somitic mesoderm (Fig. 1B). This occurs through mesenchyme-to-epithelial cell differentiation and is dependent on factors secreted by the overlying surface ectoderm and somites (23). Once formed, the nephric duct extends toward the posterior, or caudal, pole of the embryo, forming the core component of the developing urogenital system. The period between E8.5 and 9.5 is marked by formation of the pronephros and mesonephros, relying on many of the same genes required for metanephric development.

The earliest changes associated with development of the metanephric kidney begin around E10.5 as cells within the posterior region of the intermediate mesoderm aggregate to form a population of cells known as the metanephric mesenchyme (MM). This group of cells contains precursors for epithelial, stromal, and vascular elements of the adult kidney. Formation of this structure is associated with the expression of a distinct profile of transcription factors within the posterior portion of the intermediate mesoderm (Table 1). This culminates in the expression of the secreted growth factor Gdnf, which induces the outgrowth of the ureteric bud (UB) from the overlying nephric duct (Fig. 1C) (27). All subsequent developmental events within the metanephric kidney are dependent on cellular interactions that are established as a result of the invasion of the UB into the MM. Initially, the UB secretes factors that rescue the MM from apoptosis and stimulate early patterning of the MM. This is associated with condensation of a subset of MM cells into a mesenchyme structure that aggregates tightly around the UB tip and is distinguishable from the surrounding loosely packed stromal mesenchyme (Figs. 1C and 2A). These events are dependent on outgrowth of the UB into the MM, but the specific signaling pathways and transcriptional programs that promote formation of the cap mesenchyme are unknown. Furthermore, it is uncertain whether establishment of the stromal compartment reflects migration of exogenous cells or expansion of precursor cells within the MM. The cap mesenchyme itself gives rise to most of the epithelial cells in the mature nephron, whereas the stromal...
compartment plays an important role in support and patterning of these differentiating cells.

Invasion of the UB initiates differentiation of the cap MM cells. This process is marked by the formation of small aggregates of cells contiguous with the ventral aspect of the cap structure called pretubular aggregates (PTA; Fig. 2A) (29). These cells undergo a burst of proliferation before they begin to express junctional and basement membrane markers, form-
Details morphometric analysis indicates that there is a second, temporally distinct phase of nephrogenesis after E16.5 (6). The iterative nature of these events suggests that there are likely to be populations of multipotent stem cells within the cap mesenchyme and UB bud tips that provide a continuous supply of epithelial progenitor cells as nephrogenesis progresses. These cells have yet to be positively identified. Detailed morphometric analysis indicates that there is a sec- ond, temporally distinct phase of nephrogenesis after E16.5 associated with a marked increase in tubular growth and patterning of nephron segments (Fig. 2C) (6). This gives rise to expansion of the renal cortex and patterning of medullary regions of the adult kidney. Apart from glomerular podocytes (14), remarkably little is known about the signaling pathways and transcriptional programs regulating specification and patterning of the remaining nephron segments in the mammalian kidney.

**TRANSCRIPTIONAL PROGRAMS INVOLVED IN EARLY METANEPHRIC KIDNEY DEVELOPMENT**

Much of our understanding of the role of transcription factors in renal development stems from global deletion of individual genes expressed within the developing urogenital system. These studies have a number of intrinsic limitations. There is significant duplication of gene function in mammalian development, so that some of these mutations result in incomplete phenotypes that are difficult to interpret. A good example of this are mutations in the paralogous *Hox11* gene cluster, where compound mutations of multiple components result in synergistic kidney phenotypes, whereas loss of individual genes has no effect on renal development (39). In addition, deletion of some genes, such as members of the cadherin family of junctional proteins (7), results in subtle defects that are only apparent through detailed analysis of nephron structure in adult mice. In practice, these studies are rarely performed if the mice are viable and have grossly normal kidneys. These issues are further complicated by the fact that genetic background can influence the phenotypic consequences of gene deletion. For example, deletion of Wnt4 on a pure genetic background demonstrated its requirement for all epithelial differentiation of the MM (33), whereas analysis of this mutation on a mixed background revealed that while kidneys were hypoplastic, they did contain differentiated nephrons (12).

The interpretation of observed renal phenotypes relies to a large extent on the analysis of downstream gene expression in the mouse (Fig. 2A) (2). Having established initial UB invasion, MM-derived signals provide for continued growth and symmetrical branching of the UB (Fig. 1C). This, in turn, promotes patterning and differentiation of the newly forming cap mesenchyme within the outer, nephrogenic zone of the developing kidney. At the same time, PTA structures undergo further polarization as they form renal vesicles lined with columnar epithelium. These vesicles undergo a series of invaginations to form first comma- and then S-shaped-bodies, fusing with the extending UB to form a continuous lumen (Fig. 2B). Distal elements elongate into the medulla, connecting the nephron to the UB-derived collecting system (Fig. 2C). These events reflect initiation of a program of proximal and distal patterning that gives rise to distinct tubular segments and glomeruli and are associated with recruitment of stromal and vascular cell components of the developing kidney.

This process of UB branching and differentiation occurs throughout embryonic development, but 85% of the branching events that characterize mouse kidney development are complete by E16.5 (6). The iterative nature of these events suggests that there are likely to be populations of multipotent stem cells within the cap mesenchyme and UB bud tips that provide a continuous supply of epithelial progenitor cells as nephrogenesis progresses. These cells have yet to be positively identified. Detailed morphometric analysis indicates that there is a second, temporally distinct phase of nephrogenesis after E16.5 associated with a marked increase in tubular growth and patterning of nephron segments (Fig. 2C) (6). This gives rise to expansion of the renal cortex and patterning of medullary regions of the adult kidney. Apart from glomerular podocytes (14), remarkably little is known about the signaling pathways and transcriptional programs regulating specification and patterning of the remaining nephron segments in the mammalian kidney.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Early Expression</th>
<th>Kidney Phenotype</th>
<th>Molecular Phenotype</th>
<th>MM Competence*</th>
<th>Ref(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyia</td>
<td>E8.5 IM, E10.5 ND, U-MM</td>
<td>Agenesis, MM not specified from IM</td>
<td>Six1, Six2, Pax2, Gdnf lost†</td>
<td>No MM formed</td>
<td>(28, 39, 41)</td>
</tr>
<tr>
<td>FoxCl/2</td>
<td>E10.5 U-MM, E11.0 Stromal MM</td>
<td>Duplex kidney</td>
<td>Eya1, Gdnf expanded</td>
<td>Yes</td>
<td>(16)</td>
</tr>
<tr>
<td>Hoxa/d11</td>
<td>E10.5 U-MM, E11.0 Stromal MM</td>
<td>Agenesis (triple knockout)</td>
<td>Gdnf, Six2 absent</td>
<td>?</td>
<td>(38)</td>
</tr>
<tr>
<td>Pax2</td>
<td>E8.5 IM, ND, E10.5 U-MM, E11.0 UB, Cap MM</td>
<td>Agenesis, failure of UB outgrowth</td>
<td>Gdnf absent</td>
<td>No</td>
<td>(5, 9, 33)</td>
</tr>
<tr>
<td>Pbx1</td>
<td>E10.5 U-MM, E11.0 Cap and stromal MM</td>
<td>Expansion of cap MM, UB branching reduced, hyoplasia</td>
<td>Pax2, Wt1 expanded Bnl1, Pod1 (stroma) normal</td>
<td>Reduced</td>
<td>(30)</td>
</tr>
<tr>
<td>Rara/β2</td>
<td>E11.5 Stromal MM</td>
<td>Hypoplasia, agenesis</td>
<td>Pax2, Wt1, Gdnf, Bmp7 normal</td>
<td>Yes</td>
<td>(21)</td>
</tr>
<tr>
<td>Sall1</td>
<td>E9.5 MN, ND, E10.5 U-MM, E11.0 Cap MM</td>
<td>Agenesis (variable), failure of UB outgrowth</td>
<td>Pax2, Wt1, Eya1, Gdnf reduced‡</td>
<td>Yes</td>
<td>(22)</td>
</tr>
<tr>
<td>Six1</td>
<td>E10.5 U-MM, E11.0 Cap MM</td>
<td>Agenesis, failure of UB outgrowth</td>
<td>Pax2, Six2, Sall1 absent Eya1, Gdnf, Wt1 normal</td>
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<td>(40)</td>
</tr>
<tr>
<td>Wt1</td>
<td>E10.5 U-MM</td>
<td>Agenesis</td>
<td>Six2, Gdnf, Pax2 normal§</td>
<td>No</td>
<td>(8, 15)</td>
</tr>
</tbody>
</table>

*See text for a more comprehensive discussion on *Lim1*, *Pax2*, *Pax8*, *Eya1*, *Six1*, and *Wt1* IM, intermediate mesoderm; ND, nephric duct; MN, mesonephros; MM, metanephric mesenchyme; U-MM, uninduced MM; UB, ureteric bud. *Competence of isolated MM to differentiate in response to inductive signals (spinal cord or UB). †Perhaps a result of the failure of the MM to form; however, there is some evidence for direct regulation (34). ‡Likely a result of hypoplastic MM. §Pax2 mRNA is detectable; Pax2 protein is not.
expression by in situ hybridization. This is problematic, as the analysis of several mutant mice has demonstrated the importance of gene dosage in regulating these developmental events, a parameter that cannot be readily evaluated quantitatively using in situ hybridization techniques. Finally, failure to detect the expression of a putative downstream gene in a given mouse mutant does not necessarily indicate that it is directly regulated by the targeted gene, as the mutation may influence the survival and/or patterning of cell populations that normally express the gene of interest. This is further complicated by the fact that many of these developmental events are dependent on tissue interactions which are disorganized in mutant embryos. This has been a particular problem in regulating the early events in kidney development.

Subject to these limitations, this review will discuss the major transcriptional programs that have been shown to play a clear role in regulating three phases of renal development: the regulation of nephric duct formation, the formation of the MM and regulation of UB outgrowth, and the regulation of early epithelial differentiation of the MM. We will outline some of the difficulties in interpreting mouse genetic models that have been used to establish the functional role of these programs in nephrogenesis and describe some of the new approaches that are being used to address outstanding questions. The reader is referred to Table 1 for a more comprehensive list of transcription factors and their proposed function in the MM and to a number of recent reviews and papers describing subsequent specification and patterning of nephron segments (3, 14, 43), stroma (3, 17, 18, 43), and the regulation of UB branching (3, 25).

**Regulation of Nephric Duct Formation by Lim1, Pax2, and Pax8**

Lim1, also known as Lhx1, is a homeodomain DNA-binding transcription factor that is initially detected in the visceral endoderm during gastrulation and is differentially expressed in multiple tissues during organ development (11, 13). In the developing urogenital system, expression is initiated in the
lateral plate and intermediate mesoderm at E8.5. As the pro- and mesonephros are formed within the intermediate mesoderm, expression is lost in the lateral plate and becomes restricted to the mesonephric tubules and the nephric duct. Pax2 and Pax8 are two closely related members of a family of paired-box DNA-binding transcription factors that have largely overlapping expression domains in the developing kidney (4). Much like Lim1, Pax2 is expressed early in the intermediate mesoderm that gives rise to the renal anlage, and both Pax2 and Pax8 are expressed in the nephric duct as it extends posteriorly toward the cloaca (4, 9).

Global deletion of Lim1 is usually embryonically lethal due to a defect in formation of the placenta (32). The few mice that do survive until birth lack all intermediate mesoderm-derived structures, including the kidneys (36). More detailed analysis of these mutant mice indicates that Pax2 was initially expressed at low levels in the anterior portion of the intermediate mesoderm but failed to extend posteriorly into the nephrogenic region. Analysis of Hoxb6, which is normally expressed in the intermediate mesoderm and nephric duct, reveals markedly reduced expression in the Lim1 mutant mice in the region where the nephric duct, pro-, and mesonephric structures form. Expression of Hoxb6 is preserved, however, in the lateral plate. Taken together, these data suggest that Lim1 is specifically required for the differentiation of early nephrogenic structures within the intermediate mesoderm.

Like Lim1 mutants, deletion of Pax2 results in loss of the mesonephric tubules and the metanephric kidney (4, 34). In fact, marked kidney defects are observed in Pax2 heterozygotes, indicating that gene dosage is an important component of its function (34). Unlike the Lim1 mutants, however, the nephric duct forms in Pax2 mutants but fails to extend caudally to the level of metanephric development (34). Based on its wider expression in the intermediate mesoderm, it is surprising that the nephric duct forms at all in the absence of Pax2. The fact that Pax8 is also expressed in the nephric duct raises the possibility that it can functionally compensate for loss of Pax2 in this structure. Consistent with this, deletion of both Pax2 and Pax8 results in a complete failure of nephric duct formation (4). Interestingly, deletion of Pax8 alone does not disrupt formation of the meso- or metanephric kidney (20), suggesting that its function may be completely compensated for by Pax2 in the anterior intermediate mesoderm. The relationship between Lim1 and the Pax gene products during these early stages of kidney development is less clear. Most likely, the absence of Pax2 in the posterior intermediate mesoderm of Lim1 mutants reflects the failure of these differentiated structures to form and not direct regulation of Pax2 expression by Lim1 (36). However, our understanding of these early events in kidney development is limited to these descriptive studies, so that the precise relationship between these genes remains unclear.

**Specification of the MM and Regulation of UB Outgrowth by Eya1, Pax2, and Six1**

Aside from formation and extension of the nephric duct, the establishment of the interactions between the MM and the UB is initially dependent on specification of the MM from the intermediate mesoderm and, subsequently, on the stimulation of UB outgrowth by the established MM. Eya1, Pax2, and Six1 are part of an evolutionarily conserved network of transcriptional regulators that have been shown to play a critical role in regulating both of these events. Important insights into the functional and biochemical relationships between components of this network have been elucidated from studies of their ancestral orthologs in the fruit fly (35). These studies provide an example of how our concepts of transcriptional control in vivo have shifted from linear to more complex networks and provide the basis to interpret results from the less malleable genetic models in mice. We will therefore spend some time summarizing results from genetic studies of this network in *Drosophila* before discussing the functional role of the orthologous system in during nephrogenesis.

Transcriptional networks in *Drosophila* eye development. Specification of cells that give rise to the eye in *Drosophila* is dependent on a network of transcriptionally active proteins within the eye-antennal imaginal disc, deletion of any of which results in loss of the eye (reviewed in Ref. 31). These are ancestral orthologs of the mammalian Eya, Pax, and Six family proteins that are also involved in development of the mammalian eye and include 1) *eyes absent* (*eya*), the fly ortholog of mammalian Eya family proteins (*Eya1–4*); this is a non-DNA-binding transcriptional cofactor that contains an NH2-terminal transactivation domain and requires the activity of other factors to activate or repress transcriptional responses; 2) *sine oculis* (*so*), a DNA-binding, homeodomain containing transcription factor related to mammalian Six proteins (*Six1–6*); 3) *eyeless* (*ey*), *twin of eyeless* (*toy*), and *eyes gone* (*eyg*) encode DNA-binding Pax-like proteins, containing the signature paired box domains; and 4) *dachshund* (*dac*), a non-DNA-binding cofactor related to the mammalian Dach proteins (*Dach1/2*).

Initial loss-of-function studies placed these proteins in a hierarchical arrangement initiated by *toy*, which goes on to activate a linear cascade of genes involved in eye development (Fig. 3A). In reality, coordination of components within this pathway is more complex. Evidence for this comes from studies in which these genes have been misexpressed in tissues...
that do not normally form eyes. For example, misexpression of eya or dac, which are thought to act downstream of toy in this linear model, leads to the induction of toy expression and the formation of ectopic eyes. Furthermore, ectopic eye formation is more robust when combinations of ey, dac, and so are misexpressed. These findings indicate that more complex interactions exist between components of this transcriptional network than originally predicted (Fig. 3A). Biochemical studies in mammalian cells support these observations and suggest potential mechanisms for some of these more complex circuits. For example, there is evidence that Six family proteins themselves interact with Eya1–3 in the cytoplasm and promote nuclear translocation of Eya-containing protein complexes (24). Furthermore, Dach1, the mammalian ortholog of dac, has been shown to repress Six1-dependent transcriptional responses, whereas Eya3 derepresses Dach1 by recruiting coactivators to the Six1/Dach1/DNA complexes (Fig. 3B) (19). These findings provide a theoretical framework from which to interpret genetic studies of conserved programs involved in the early phases of nephrogenesis.

**Specification of the MM.** Although the precise architecture of transcriptional networks in the MM is poorly understood, it is clear that Eya1 plays an essential role in regulating early events (28, 40, 42). Eya1 is one of four mammalian orthologs of Drosophila eyes absent (eya) and is expressed in the intermediate mesoderm from E8.5 and, more specifically, in the nephric duct and the uninduced MM at E10.5. As in the Drosophila eye, the expression domain of Eya1 in the MM overlaps with that of other members of the Eya/Six/Pax network. Mammalian so orthologs Six1 and Six2 are expressed in the MM before UB invasion, as are the toy and ey orthologs Pax2 and Pax8 (4, 41). The dac ortholog, Dach1, is expressed in early nephric epithelia of the developing kidney (1); however, there are no published data relating to its functional role in renal development. Initial studies indicated that Eya1 null mutant mice died at birth with renal agenesis (40, 42). More detailed analysis has demonstrated that these mice form normal nephric duct and mesonephric tissues but fail to form the distinct MM aggregate at E10.5 in the posterior region of the intermediate mesoderm (28). To date, this is the only mutant identified that results in the failure of the MM to be specified from the intermediate mesoderm. As outlined above, Eya1 is a non-DNA-binding transcriptional cofactor that must interact with other DNA-binding factors to activate or repress transcription. This suggests that Eya1 expression alone is unlikely to be sufficient to specify the MM. Given the biochemical data demonstrating functional and physical interactions between Eya and Six family proteins (19, 24), it is possible that Eya1 acts by modifying the transcriptional activity of Six family members in the MM. Indeed, Six1 is coexpressed with Eya1 in the MM and Six1 null mice also fail to form kidneys (41), suggesting that specification may require the assembly of Six1/Eya1 complexes in the MM.

**Stimulation of UB outgrowth from the nephric duct.** In many of the mouse mutants that lack kidneys, the UB fails to emerge from the nephric duct, usually due to the failure of the uninduced MM to express Gdnf (Table 1). Because of this, establishing the transcriptional network upstream of Gdnf has received considerable attention from the field in recent years. In vivo and in vitro studies clearly demonstrate a role for Pax2 in the regulation of Gdnf expression in the MM (5). In addition to the nephric duct, Pax2 is expressed in the MM before UB invasion (9), and the MM forms properly in Pax2 null mice but fails to express Gdnf (5). Pax2 binds and activates defined elements within the Gdnf promoter, suggesting that it may directly regulate Gdnf expression (5), but other components of this network up- and downstream of Pax2 are less clear. Based on their overlapping expression patterns and common phenotype, speculation has focused on other members of the Eya/Pax/Six network.

Initial characterization of the Eya1 knockout mouse at E11.5 revealed that expression of Pax2, Six1, and Gdnf were lost in the area of the MM (42), and previous studies have shown that Eya1 expression is independent of Pax2 (41). Together, these data make it tempting to speculate that Eya1 acts upstream of Pax2 and Six1 in a transcriptional circuit driving Gdnf expression in the MM. However, as discussed above, the MM never forms in Eya1 null mice, making it unclear whether these effects are due to the direct regulation of this pathway by Eya1 or simply a consequence of losing the MM. Furthermore, Gdnf expression is maintained in the MM of Six1 null mice despite the failure of the UB to emerge from the nephric duct (41). It is unclear whether this is simply an effect of reduced Gdnf dosage that is not demonstrated by in situ analysis of mRNA expression or if another, parallel pathway is required for UB outgrowth. For example, deletion of the Hoxa11/Hoxc11/Hoxd11 gene cluster also results in loss of Gdnf expression in the MM and the subsequent failure of UB outgrowth, despite normal expression of Pax2 (39). It is unclear whether these genes regulate Gdnf expression through an independent, nonredundant pathway or if they are part of a common transcriptional network downstream of Pax2. Once again, studies in the developing Drosophila eye may provide insight into these mechanisms. Overexpression of the Hox ortholog antennapedia in the eye inhibits transcriptional responses mediated by the Pax ortholog eyeless by competitive inhibition of DNA binding (26). If a similar mechanism is operative in the mammalian kidney, these findings would suggest that the Hox11 paralogs could play a direct role in modifying the transcriptional activity of Pax2. This would account for the loss of Gdnf expression while Pax2 expression is maintained in the Hoxa11/Hoxc11/Hoxd11 compound mutant mouse.

**Early Epithelial Differentiation of the MM.**

The formation of early epithelial structures from the MM involves a host of coordinated cellular events that not only give rise to primitive nephrons but also replenish the MM for future rounds of differentiation. In vivo genetic studies of these events have been limited, as many of the factors that are expressed in the MM are also required for early specification and/or inductive events. Historically, this problem has been addressed in vitro by studying the ability of isolated MM structures from E11.5 mutant mouse embryos to undergo differentiation when exposed to potent inductive signals from spinal cord or wild-type UB isolates. This approach has enabled us to identify several so-called “competence” factors that are required for this process (Table 1), but it is unclear from these studies whether the apparent requirement for these factors in vitro actually reflects their functional role in the context of intact metanephroi. A good example of this problem comes...
from the analysis of Wt1 null mutant mice, in which there is complete loss of MM structures by E12.5 with no evidence of MM differentiation (15). Tissue recombination experiments demonstrated that wild-type spinal cord and UB structures are unable to rescue the Wt1 null MM phenotype in vitro (8, 15), suggesting that Wt1 could be a competence factor for MM differentiation. Loss of Wt1 is also associated with a marked increase in apoptosis of the uninduced MM over the 24-h period following its formation (15). The inappropriate death of MM cells in Wt1 null mice makes it impossible for tissue recombination experiments to determine whether the primary function of Wt1 is to act as a survival factor or whether it has an additional role in regulating MM differentiation.

Many of these issues can now be circumvented using Cre/LoxP technology to delete genes in a spatially and temporally controlled manner. A recent example of this approach in the developing kidney is the deletion of Lim1 exclusively in MM-derived structures, bypassing its requirement for the formation of early nephrogenic structures, using Rarb2Cre and Lim1loxP mutant mice (12). These studies demonstrated that in the absence of Lim1, the PTA and renal vesicles form normally but fail to differentiate further or express nephron segment-specific markers, Dll1 or Brn1. These findings indicate that Lim1 is not required for initial differentiation of PTAs but is essential for subsequent patterning of the renal vesicle into tubular structures of the nephron. An alternative approach to study the function of genes that are essential for early patterning is to study in vivo cellular function in mosaic mice generated by introducing mutant ES cells along with lineage-specific markers into wild-type blastocysts. This approach has recently been used to evaluate the functional role of Ret signaling in regulating UB branching morphogenesis (31). By limiting the dosage of mutant cells, it was possible to track and study mutant cell function while preserving early renal development programs. Together, these conditional deletion and mosaic studies should enable us to determine the functional relationship between other MM-specific transcription factors, including Wt1 and Pax2, in coordination of differentiation events.

CONCLUSION

Genetic studies have demonstrated that transcription factors regulate critical events in metanephric development including establishing the nephrogenic field, specifying cell fate, and regulating differentiation of nephron precursors. It is apparent that these factors are integrated into evolutionarily conserved networks that cooperate to direct developmental events. While the power of mouse genetics has uncovered many individual factors in recent years, one of the major challenges that lies ahead is to understand how these genes cooperate in context to form networks accessible at multiple levels. Studies using mosaic and conditional mutant mice will allow us to address some of these issues. However, advances in understanding the complexity of the transcriptional networks involved in coordinating these events will only occur as findings from these studies in mice are fully integrated with genetic and biochemical data from other model systems.

REFERENCES